WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues

Steven H. Swerdlow, Elias Campo, Nancy Lee Harris, Elaine S. Jaffe, Stefano A. Pileri, Harald Stein, Jürgen Thiele, Daniel A. Arber, Robert P. Hasserjian, Michelle M. Le Beau, Attilio Orazi, Reiner Siebert
Myeloid proliferations associated with Down syndrome 169
Transient abnormal myelopoiesis associated with Down syndrome 169
Myeloid leukaemia associated with Down syndrome 170

9 Blastic plasmacytoid dendritic cell neoplasm 173

10 Acute leukaemias of ambiguous lineage 179
Acute undifferentiated leukaemia 182
Mixed-phenotype acute leukaemia with t(9;22)(q34.1;q11.2); BCR-ABL1 182
Mixed-phenotype acute leukaemia with t(v;11q23.3); KMT2A-rearranged 183
Mixed-phenotype acute leukaemia, B/myeloid, not otherwise specified 184
Mixed-phenotype acute leukaemia, T/myeloid, not otherwise specified 185
Mixed-phenotype acute leukaemia, not otherwise specified, rare types 186
Acute leukaemias of ambiguous lineage, not otherwise specified 187

11 Introduction and overview of the classification of the lymphoid neoplasms 189

12 Precursor lymphoid neoplasms 199
B-lymphoblastic leukaemia/lymphoma, not otherwise specified 200
B-lymphoblastic leukaemia/lymphoma with recurrent genetic abnormalities 203
B-lymphoblastic leukaemia/lymphoma with t(9;22)(q34.1;q11.2); BCR-ABL1 203
B-lymphoblastic leukaemia/lymphoma with t(v;11q23.3); KMT2A-rearranged 203
B-lymphoblastic leukaemia/lymphoma with t(12;21)(p13.2;q22.1); ETV6-RUNX1 204
B-lymphoblastic leukaemia/lymphoma with hyperdiploidy 205
B-lymphoblastic leukaemia/lymphoma with hypodiploidy 206
B-lymphoblastic leukaemia/lymphoma with t(5;14)(q31.1;q32.1); IGHI/IL3 206
B-lymphoblastic leukaemia/lymphoma with t(1;19)(q23;p13.3); TCF3-PBX1 207
B-lymphoblastic leukaemia/lymphoma, BCR-ABL1-like 208
B-lymphoblastic leukaemia/lymphoma with iAMP21 208
T-lymphoblastic leukaemia/lymphoma 209
Early T-cell precursor lymphoblastic leukaemia 212
NK-lymphoblastic leukaemia/lymphoma 213

13 Mature B-cell neoplasms 215
Chronic lymphocytic leukaemia/ small lymphocytic lymphoma 216
Monoclonal B-cell lymphocytosis 220
B-cell prolymphocytic leukaemia 222
Splenic marginal zone lymphoma 223
Hairy cell leukaemia 226
Splenic B-cell lymphoma/leukaemia, unclassifiable 229
Splenic diffuse red pulp small B-cell lymphoma 229
Hairy cell leukaemia variant 230
Lymphoplasmacytic lymphoma 232
IgM Monoclonal gammopathy of undetermined significance 236

Heavy chain diseases 237
Mu heavy chain disease 237
Gamma heavy chain disease 238
Alpha heavy chain disease 240
Plasma cell neoplasms 241
Non-IgM monoclonal gammopathy of undetermined significance 241
Plasma cell myeloma 243
Plasma cell myeloma variants 249
Smouldering (asymptomatic) plasma cell myeloma 249
Non-secretory myeloma 250
Plasma cell leukaemia 250
Plasmacytoma 250
Solitary plasmacytoma of bone 250
Extraosseous plasmacytoma 251
Monoclonal immunoglobulin deposition diseases 254
Primary amyloidosis 254
Light chain and heavy chain deposition diseases 255
Plasma cell neoplasms with associated paraneoplastic syndrome 256
POEMS syndrome 256
TEMPI syndrome 257
Extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma) 259
Nodal marginal zone lymphoma 263
Paediatric nodal marginal zone lymphoma 264
Follicular lymphoma 266
Testicular follicular lymphoma 268
In situ follicular neoplasia 274
Duodenal-type follicular lymphoma 276
Paediatric-type follicular lymphoma 278
Large B-cell lymphoma with ALK4 rearrangement 280
Primary cutaneous follicle centre lymphoma 282
Mantle cell lymphoma 285
Leukaemic non-nodal mantle cell lymphoma 290
In situ mantle cell neoplasia 290
Diffuse large B-cell lymphoma (DLBCL), NOS 291
T-cell/histiocyte-rich large B-cell lymphoma 298
Primary diffuse large B-cell lymphoma of the CNS 300
Primary cutaneous diffuse large B-cell lymphoma, leg type 303
EBV-positive diffuse large B-cell lymphoma, NOS 304
EBV-positive mucocutaneous ulcer 307
Diffuse large B-cell lymphoma associated with chronic inflammation 309
Fibrosarcoma-associated diffuse large B-cell lymphoma 311
Lymphomatoid granulomatosis 312
Primary mediastinal (thymic) large B-cell lymphoma 314
Intravascular large B-cell lymphoma 317
ALK-positive large B-cell lymphoma 319
Plasmablastic lymphoma 321
Primary effusion lymphoma 323
HHV8-associated lymphoproliferative disorders 325
Multicentric Castleman disease 325
HHV8-positive diffuse large B-cell lymphoma, NOS 327
HHV8-positive germinotropic lymphoproliferative disorder 328
Burkitt lymphoma 330
Burkitt-like lymphoma with 11q aberration 334
High-grade B-cell lymphoma 335
High-grade B-cell lymphoma with MYC and BCL2 and/or BCL6 rearrangements 335
High-grade B-cell lymphoma, NOS 340
B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and classic Hodgkin lymphoma 342

14 Mature T- and NK-cell neoplasms 345
T-cell prolymphocytic leukaemia 346
T-cell large granular lymphocytic leukaemia 348
Chronic lymphoproliferative disorder of NK cells 351
Aggressive NK-cell leukaemia 353
EBV-positive T-cell and NK-cell lymphoproliferative diseases of childhood 355
Systemic EBV+ T-cell lymphoma of childhood 355
Chronic active EBV infection of T- and NK-cell type, systemic form 358
Hydroa vacciniforme-like lymphoproliferative disorder 360
Severe mosquito bite allergy 362
Adult T-cell leukaemia/lymphoma 363
Extranodal NK/T-cell lymphoma, nasal type 368
Intestinal T-cell lymphoma 372
Enteropathy-associated T-cell lymphoma 372
Monomorphic epitheliotropic intestinal T-cell lymphoma 377
Intestinal T-cell lymphoma, NOS 378
Indolent T-cell lymphoproliferative disorder of the gastrointestinal tract 379
Hepatosplenic T-cell lymphoma 381
Subcutaneous panniculitis-like T-cell lymphoma 383
Mycosis fungoides 385
Sézary syndrome 390
Primary cutaneous CD30-positive T-cell lymphoproliferative disorders 392
Lymphomatoid papulosis 392
Primary cutaneous anaplastic large cell lymphoma 395
Primary cutaneous peripheral T-cell lymphomas, rare subtypes 397
Introduction 397
Primary cutaneous gamma delta T-cell lymphoma 397
Primary cutaneous CD8-positive aggressive epidermotropic cytotoxic T-cell lymphoma 399
Primary cutaneous acral CD8-positive T-cell lymphoma 400
Primary cutaneous CD4+ small/medium T-cell lymphoproliferative disorder 401
Peripheral T-cell lymphoma, NOS 403
Anoimmunoblastic T-cell lymphoma and other nodal lymphomas of T follicular helper (TFH) cell origin 407
Anoimmunoblastic T-cell lymphoma 408
Follicular T-cell lymphoma 411
Nodal peripheral T-cell lymphoma with TFH phenotype 412
Anaplastic large cell lymphoma, ALK-positive 413
Anaplastic large cell lymphoma, ALK-negative 418
Breast implant-associated anaplastic large cell lymphoma 421

15 Hodgkin lymphomas 423
Introduction 424
Nodular lymphocyte predominant Hodgkin lymphoma 431
Classic Hodgkin lymphoma 435
Nodular sclerosis classic Hodgkin lymphoma 435
Lymphocyte-rich classic Hodgkin lymphoma 438
Mixed-cellularity classic Hodgkin lymphoma 440
Lymphocyte depleted classic Hodgkin lymphoma 441

16 Immunodeficiency-associated lymphoproliferative disorders 443
Lymphoproliferative diseases associated with primary immune disorders 444
Lymphomas associated with HIV infection 449
Post-transplant lymphoproliferative disorders (PTLD) 453
Non-destructive PTLD 456
Polymorphic PTLD 457
Monomorphic PTLD (B- and T/NK-cell types) 459
Monomorphic B-cell PTLD 459
Monomorphic T/NK-cell PTLD 461
Classic Hodgkin lymphoma PTLD 462
Other iatrogenic immunodeficiency-associated lymphoproliferative disorders 462

17 Histiocytic and dendritic cell neoplasms 465
Introduction 466
Histiocytic sarcoma 468
Tumours derived from Langerhans cells 470
Langerhans cell histiocytosis 470
Langerhans cell sarcoma 473
Indeterminate dendritic cell tumour 474
Interdigitating dendritic cell sarcoma 475
Follicular dendritic cell sarcoma 476
Inflammatory pseudotumour-like follicular/fibroblastic dendritic cell sarcoma 478
Fibroblastic reticular cell tumour 479
Disseminated juvenile xanthogranuloma 480
Erdheim-Chester disease 481

Contributors 484
Declaration of interests 493
Clinical Advisory Committees 494
IARC/WHO Committee for ICD-O 496
Sources of figures and tables 497
References 504
Subject index 577
List of abbreviations 586
<table>
<thead>
<tr>
<th>WHO classification of tumours of haematopoietic and lymphoid tissues</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Myeloproliferative neoplasms</strong></td>
</tr>
<tr>
<td>Chronic myeloid leukaemia, <em>BCR-ABL1</em>-positive</td>
</tr>
<tr>
<td>Chronic neutrophilic leukaemia</td>
</tr>
<tr>
<td>Polycythaemia vera</td>
</tr>
<tr>
<td>Primary myelofibrosis</td>
</tr>
<tr>
<td>Essential thrombocytopenia</td>
</tr>
<tr>
<td>Chronic eosinophilic leukaemia, NOS</td>
</tr>
<tr>
<td>Myeloproliferative neoplasm, unclassifiable</td>
</tr>
<tr>
<td><strong>Mastocytosis</strong></td>
</tr>
<tr>
<td>Cutaneous mastocytosis</td>
</tr>
<tr>
<td>Indolent systemic mastocytosis</td>
</tr>
<tr>
<td>Systemic mastocytosis with an associated haematological neoplasm</td>
</tr>
<tr>
<td>Aggressive systemic mastocytosis</td>
</tr>
<tr>
<td>Mast cell leukaemia</td>
</tr>
<tr>
<td>Mast cell sarcoma</td>
</tr>
<tr>
<td><strong>Myeloid/lymphoid neoplasms with eosinophilia and gene rearrangement</strong></td>
</tr>
<tr>
<td>Myeloid/lymphoid neoplasms with <em>PDGFR</em> rearrangement</td>
</tr>
<tr>
<td>Myeloid/lymphoid neoplasms with <em>PDGFRB</em> rearrangement</td>
</tr>
<tr>
<td>Myeloid/lymphoid neoplasms with <em>FGFR1</em> rearrangement</td>
</tr>
<tr>
<td>Myeloid/lymphoid neoplasms with <em>PCM1-JAK2</em></td>
</tr>
<tr>
<td><strong>Myelodysplastic/myeloproliferative neoplasms</strong></td>
</tr>
<tr>
<td>Chronic myelomonocytic leukaemia</td>
</tr>
<tr>
<td>Atypical chronic myeloid leukaemia, <em>BCR-ABL1</em>-negative</td>
</tr>
<tr>
<td>Juvenile myelomonocytic leukaemia</td>
</tr>
<tr>
<td>Myelodysplastic/myeloproliferative neoplasm with ring sideroblasts and thrombocytosis</td>
</tr>
<tr>
<td>Myelodysplastic/myeloproliferative neoplasm, unclassifiable</td>
</tr>
<tr>
<td><strong>Myelodysplastic syndromes</strong></td>
</tr>
<tr>
<td>Myelodysplastic syndrome with single lineage dysplasia</td>
</tr>
<tr>
<td>Myelodysplastic syndrome with ring sideroblasts and single lineage dysplasia</td>
</tr>
<tr>
<td>Myelodysplastic syndrome with ring sideroblasts and multilineage dysplasia</td>
</tr>
<tr>
<td>Myelodysplastic syndrome with multilineage dysplasia</td>
</tr>
<tr>
<td>Myelodysplastic syndrome with excess blasts</td>
</tr>
<tr>
<td>Myelodysplastic syndrome with isolated del(5q)</td>
</tr>
<tr>
<td>Myelodysplastic syndrome, unclassifiable</td>
</tr>
<tr>
<td>Refractory cytopenia of childhood</td>
</tr>
<tr>
<td><strong>Myeloid proliferations associated with Down syndrome</strong></td>
</tr>
<tr>
<td>Transient abnormal myelopoiesis associated with Down syndrome</td>
</tr>
<tr>
<td>Myeloid leukaemia associated with Down syndrome</td>
</tr>
<tr>
<td><strong>Myeloid neoplasms with germline predisposition</strong></td>
</tr>
<tr>
<td>Acute myeloid leukaemia with germline <em>CGBP</em> mutation</td>
</tr>
<tr>
<td>Myeloid neoplasms with germline <em>DDX41</em> mutation</td>
</tr>
<tr>
<td>Myeloid neoplasms with germline <em>ANKRD26</em> mutation</td>
</tr>
<tr>
<td>Myeloid neoplasms with germline <em>ETV6</em> mutation</td>
</tr>
<tr>
<td>Myeloid neoplasms with germline <em>GATA2</em> mutation</td>
</tr>
<tr>
<td><strong>Acute myeloid leukaemia (AML) and related precursor neoplasms</strong></td>
</tr>
<tr>
<td>AML with recurrent genetic abnormalities</td>
</tr>
<tr>
<td>AML with t(8;21)(q22;q22.1); <em>RUNX1-RUNX1T1</em></td>
</tr>
<tr>
<td>AML with inv(16)(p13.1;q22) or t(16;16)(p13.1;q22); <em>CBFB-MYH11</em></td>
</tr>
<tr>
<td>AML with <em>PML-RARA</em></td>
</tr>
<tr>
<td>AML with t(9;11)(p21.3;q23.3); <em>KMT2A-MLLT3</em></td>
</tr>
<tr>
<td>AML with t(6;9)(q23;p34.1); <em>DEK-NUP214</em></td>
</tr>
<tr>
<td>AML with inv(3)(q21.3;q26.2) or t(3;3)(q21.3;q26.2); <em>GATA2, MECOM</em></td>
</tr>
<tr>
<td>AML (megakaryoblastic) with t(1;22)(p13.3;q13.1); <em>RBM15-MKL1</em></td>
</tr>
<tr>
<td>AML with <em>BCR-ABL</em></td>
</tr>
<tr>
<td>AML with mutated <em>NPM1</em></td>
</tr>
<tr>
<td>AML with biallelic mutation of <em>CGBP</em></td>
</tr>
<tr>
<td>AML with mutated <em>RUNX1</em></td>
</tr>
<tr>
<td><strong>AML with myelodysplasia-related changes</strong></td>
</tr>
<tr>
<td>9895/3</td>
</tr>
<tr>
<td><strong>Therapy-related myeloid neoplasms</strong></td>
</tr>
<tr>
<td>9920/3</td>
</tr>
<tr>
<td><strong>Acute myeloid leukaemia, NOS</strong></td>
</tr>
<tr>
<td>9861/3</td>
</tr>
<tr>
<td>AML with minimal differentiation</td>
</tr>
<tr>
<td>AML without maturation</td>
</tr>
<tr>
<td>AML with maturation</td>
</tr>
<tr>
<td>Acute myelomonocytic leukaemia</td>
</tr>
<tr>
<td>Acute monoblastic and monocytic leukaemia</td>
</tr>
<tr>
<td>Pure erythroid leukaemia</td>
</tr>
<tr>
<td>Acute megakaryoblastic leukaemia</td>
</tr>
<tr>
<td>Acute basophilic leukaemia</td>
</tr>
<tr>
<td>Acute panmyelosis with myelofibrosis</td>
</tr>
<tr>
<td><strong>Myeloid sarcoma</strong></td>
</tr>
<tr>
<td>9930/3</td>
</tr>
<tr>
<td><strong>Myeloid proliferations associated with Down syndrome</strong></td>
</tr>
<tr>
<td>Transient abnormal myelopoiesis associated with Down syndrome</td>
</tr>
<tr>
<td>Myeloid leukaemia associated with Down syndrome</td>
</tr>
</tbody>
</table>
### Blastic plasmacytoid dendritic cell neoplasm

9727/3

### Acute leukaemias of ambiguous lineage

**Acute undifferentiated leukaemia**

9801/3

**Mixed-phenotype acute leukaemia with t(9;22)(q34.1;q11.2); BCR-ABL1**

9806/3

**Mixed-phenotype acute leukaemia with t(v;11q23.3); KMT2A-rearranged**

9807/3

**Mixed-phenotype acute leukaemia, B/myeloid, NOS**

9808/3

**Mixed-phenotype acute leukaemia, T/myeloid, NOS**

9809/3

**Mixed-phenotype acute leukaemia, NOS, rare types**

**Acute leukaemias of ambiguous lineage, NOS**

**Precursor lymphoid neoplasms**

**B-lymphoblastic leukaemia/lymphoma, NOS**

9811/3

**B-lymphoblastic leukaemia/lymphoma with t(9;22)(q34.1;q11.2); BCR-ABL1**

9812/3

**B-lymphoblastic leukaemia/lymphoma with t(v;12;21)(p13.2;q22.1); ETV6-RUNX1**

9814/3

**B-lymphoblastic leukaemia/lymphoma with hyperdiploidy**

9815/3

**B-lymphoblastic leukaemia/lymphoma with hypodiploidy (hypodiploid ALL)**

9816/3

**B-lymphoblastic leukaemia/lymphoma with t(5;14)(q31.1;q32.1); IGH/IL3**

9817/3

**B-lymphoblastic leukaemia/lymphoma with t(12;19)(q23;p13.3); TCF3-PBX1**

9818/3

**B-lymphoblastic leukaemia/lymphoma, BCR-ABL1-like**

9819/3*

**B-lymphoblastic leukaemia/lymphoma with iAMP21**

9811/3

**T-lymphoblastic leukaemia/lymphoma**

9837/3

**Early T-cell precursor lymphoblastic leukaemia**

9837/3

**NK-lymphoblastic leukaemia/lymphoma**

**Mature B-cell neoplasms**

**Chronic lymphocytic leukaemia (CLL)/small lymphocytic lymphoma**

9823/3

**Monoclonal B-cell lymphocytosis, CLL-type**

9823/1*

**Monoclonal B-cell lymphocytosis, non-CLL-type**

9591/1*

**B-cell prolymphocytic leukaemia**

9833/3

**Splenic marginal zone lymphoma**

9689/3

**Hairy cell leukaemia**

9940/3

**Splenic B-cell lymphoma/leukaemia, unclassifiable**

9591/3

**Splenic diffuse red pulp small B-cell lymphoma**

9591/3

**Hairy cell leukaemia variant**

9591/3

**Lymphoplasmacytic lymphoma**

9671/3

**Waldenström macroglobulinemia**

9761/3

**IgM monoclonal gammopathy of undetermined significance**

9761/1*

**Heavy chain diseases**

**Mu heavy chain disease**

9762/3

**Gamma heavy chain disease**

9762/3

**Alpha heavy chain disease**

9762/3

**Plasma cell neoplasms**

**Non-IgM monoclonal gammopathy of undetermined significance**

9765/1

**Plasma cell myeloma**

9732/3

**Solitary plasmacytoma of bone**

9731/3

**Extraosseous plasmacytoma**

9734/3

**Monoclonal immunoglobulin deposition diseases**

**Primary amyloidosis**

9769/1

**Light chain and heavy chain deposition diseases**

9769/1

**Extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma)**

9699/3

**Nodal marginal zone lymphoma**

9699/3

**Paediatric nodal marginal zone lymphoma**

9699/3

**Follicular lymphoma**

9690/3

**In situ follicular neoplasia**

9695/1*

**Duodenal-type follicular lymphoma**

9695/3

**Testicular follicular lymphoma**

9690/3

**Paediatric-type follicular lymphoma**

9690/3

**Large B-cell lymphoma with IRF4 rearrangement**

9698/3

**Primary cutaneous follicle centre lymphoma**

9597/3

**Mantle cell lymphoma**

9673/3

**In situ mantle cell neoplasia**

9673/1*

**Diffuse large B-cell lymphoma (DLBCL), NOS**

9680/3

**Germinal centre B-cell subtype**

9680/3

**Activated B-cell subtype**

9680/3

**T-cell/histiocyte-rich large B-cell lymphoma**

9688/3

**Primary DLBCL of the CNS**

9680/3

**Primary cutaneous DLBCL, leg type**

9680/3

**EBV-positive DLBCL, NOS**

9680/3

**EBV-positive mucocutaneous ulcer**

9680/1*

**DLBCL associated with chronic inflammation**

9680/3

**Fibrin-associated diffuse large B-cell lymphoma**

9766/1

**Lymphomatoid granulomatosis, grade 1, 2**

9766/3

**Lymphomatoid granulomatosis, grade 3**

9766/3*

**Primary mediastinal (thymic) large B-cell lymphoma**

9679/3

**Intravascular large B-cell lymphoma**

9712/3

**ALK-positive large B-cell lymphoma**

9737/3

**Plasmablastic lymphoma**

9735/3

**Primary effusion lymphoma**

9678/3

**Multicentric Castleman disease**

9738/3

**HHV8-positive DLBCL, NOS**

9738/3

**HHV8-positive germinal tropic lymphoproliferative disorder**

9738/1*

**Burkitt lymphoma**

9687/3

**Burkitt-like lymphoma with 11q aberration**

9687/3*

**High-grade B-cell lymphoma**

9683/3

**High-grade B-cell lymphoma with MYC and BCL2 and/or BCL6 rearrangements**

9683/3

**High-grade B-cell lymphoma, NOS**

9680/3

**B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and classic Hodgkin lymphoma**

9596/3

**WHO classification**

9
### Mature T- and NK-cell neoplasms

<table>
<thead>
<tr>
<th>Condition</th>
<th>Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>T-cell prolymphocytic leukaemia</td>
<td>9834/3</td>
</tr>
<tr>
<td>T-cell large granular lymphocytic leukaemia</td>
<td>9831/3</td>
</tr>
<tr>
<td><strong>Chronic lymphoproliferative disorder of NK cells</strong></td>
<td>9831/3</td>
</tr>
<tr>
<td>Aggressive NK-cell leukaemia</td>
<td>9948/3</td>
</tr>
<tr>
<td>Systemic EBV-positive T-cell lymphoma of childhood</td>
<td>9724/3</td>
</tr>
<tr>
<td>Chronic active EBV infection of T- and NK-cell type, systemic form</td>
<td>9725/1*</td>
</tr>
<tr>
<td>Hydroa vacciniforme-like lymphoproliferative disorder</td>
<td>9725/1*</td>
</tr>
<tr>
<td>Severe mosquito bite allergy</td>
<td>9827/3</td>
</tr>
<tr>
<td>Adult T-cell leukaemia/lymphoma</td>
<td>9719/3</td>
</tr>
<tr>
<td>Extranodal NK/T-cell lymphoma, nasal type</td>
<td>9719/3</td>
</tr>
<tr>
<td>Enteropathy-associated T-cell lymphoma</td>
<td>9717/3</td>
</tr>
<tr>
<td>Monomorphic epitheliotropic intestinal T-cell lymphoma</td>
<td>9717/3</td>
</tr>
<tr>
<td>Intestinal T-cell lymphoma, NOS</td>
<td>9717/3</td>
</tr>
<tr>
<td><strong>Indolent T-cell lymphoproliferative disorder of the gastrointestinal tract</strong></td>
<td>9702/1*</td>
</tr>
<tr>
<td>Hepatosplenic T-cell lymphoma</td>
<td>9716/3</td>
</tr>
<tr>
<td>Subcutaneous panniculitis-like T-cell lymphoma</td>
<td>9708/3</td>
</tr>
<tr>
<td>Mycosis fungoides</td>
<td>9700/3</td>
</tr>
<tr>
<td>Sézary syndrome</td>
<td>9701/3</td>
</tr>
<tr>
<td>Primary cutaneous CD30-positive T-cell lymphoproliferative disorders</td>
<td></td>
</tr>
<tr>
<td>Lymphomatoid papulosis</td>
<td>9718/1*</td>
</tr>
<tr>
<td>Primary cutaneous anaplastic large cell lymphoma</td>
<td>9718/3</td>
</tr>
<tr>
<td>Primary cutaneous gamma delta T-cell lymphoma</td>
<td>9726/3</td>
</tr>
<tr>
<td><strong>Primary cutaneous CD8-positive aggressive epidermotropic cytotoxic T-cell lymphoma</strong></td>
<td>9709/3</td>
</tr>
<tr>
<td><strong>Primary cutaneous acral CD8-positive T-cell lymphoma</strong></td>
<td>9709/3*</td>
</tr>
<tr>
<td><strong>Primary cutaneous CD4-positive small/medium T-cell lymphoproliferative disorder</strong></td>
<td>9709/1</td>
</tr>
<tr>
<td>Peripheral T-cell lymphoma, NOS</td>
<td>9702/3</td>
</tr>
<tr>
<td>Angioimmunoblastic T-cell lymphoma</td>
<td>9705/3</td>
</tr>
<tr>
<td>Follicular T-cell lymphoma</td>
<td>9702/3</td>
</tr>
<tr>
<td>Nodal peripheral T-cell lymphoma with T follicular helper phenotype</td>
<td>9702/3</td>
</tr>
<tr>
<td>Anaplastic large cell lymphoma, ALK-positive</td>
<td>9714/3</td>
</tr>
<tr>
<td>Anaplastic large cell lymphoma, ALK-negative</td>
<td>9715/3*</td>
</tr>
<tr>
<td><strong>Breast implant-associated anaplastic large cell lymphoma</strong></td>
<td>9715/3*</td>
</tr>
</tbody>
</table>

### Hodgkin lymphomas

<table>
<thead>
<tr>
<th>Condition</th>
<th>Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nodular lymphocyte predominant Hodgkin lymphoma</td>
<td>9659/3</td>
</tr>
<tr>
<td>Classic Hodgkin lymphoma</td>
<td>9650/3</td>
</tr>
<tr>
<td>Nodular sclerosis classic Hodgkin lymphoma</td>
<td>9663/3</td>
</tr>
<tr>
<td>Lymphocyte-rich classic Hodgkin lymphoma</td>
<td>9651/3</td>
</tr>
<tr>
<td>Mixed cellularity classic Hodgkin lymphoma</td>
<td>9652/3</td>
</tr>
<tr>
<td>Lymphocyte-depleted classic Hodgkin lymphoma</td>
<td>9653/3</td>
</tr>
</tbody>
</table>

### Immunodeficiency-associated lymphoproliferative disorders

- Post-transplant lymphoproliferative disorders (PTLD)
  - Non-destructive PTLD
  - Plasmacytic hyperplasia PTLD
  - Infectious mononucleosis PTLD
  - Florid follicular hyperplasia
  - Polymorphic PTLD
  - Monomorphic PTLD
  - Classic Hodgkin Lymphoma PTLD
  - Other iatrogenic immunodeficiency-associated lymphoproliferative disorders

### Histiocytic and dendritic cell neoplasms

<table>
<thead>
<tr>
<th>Condition</th>
<th>Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histiocytic sarcoma</td>
<td>9755/3</td>
</tr>
<tr>
<td>Langerhans cell histiocytosis, NOS</td>
<td>9751/1</td>
</tr>
<tr>
<td>Langerhans cell histiocytosis, monostotic</td>
<td>9751/1</td>
</tr>
<tr>
<td>Langerhans cell histiocytosis, polyostotic</td>
<td>9751/1</td>
</tr>
<tr>
<td>Langerhans cell histiocytosis, disseminated</td>
<td>9751/3</td>
</tr>
<tr>
<td>Langerhans cell sarcoma</td>
<td>9756/3</td>
</tr>
<tr>
<td>Indeterminate dendritic cell tumour</td>
<td>9757/3</td>
</tr>
<tr>
<td>Intergenerating dendritic cell sarcoma</td>
<td>9757/3</td>
</tr>
<tr>
<td>Follicular dendritic cell sarcoma</td>
<td>9758/3</td>
</tr>
<tr>
<td>Fibroblastic reticular cell tumour</td>
<td>9759/3</td>
</tr>
<tr>
<td>Disseminated juvenile xanthogranuloma</td>
<td></td>
</tr>
<tr>
<td>Erdheim–Chester disease</td>
<td>9749/3</td>
</tr>
</tbody>
</table>

The morphology codes are from the International Classification of Diseases for Oncology (ICD-O) [1257A1]. Behaviour is coded /0 for benign tumours; /1 for unspecified, borderline, or uncertain behaviour; /2 for carcinoma in situ and grade III intraepithelial neoplasia; and /3 for malignant tumours. The classification is modified from the previous WHO classification, taking into account changes in our understanding of these lesions.

* These new codes were approved by the IARC/WHO Committee for ICD-O.
** These lesions are classified according to the lymphoma to which they correspond, and are assigned the respective ICD-O code.

Italics: Provisional tumour entities.
Introduction to the WHO classification of tumours of haematopoietic and lymphoid tissues

Why classify? Classification is the language of medicine; diseases must be described, defined, and named before they can be diagnosed, treated, and studied. A consensus on definitions and terminology is essential for both clinical practice and investigation. A classification should contain diseases that are clearly defined, clinically distinctive, and non-overlapping (i.e., mutually exclusive), and that together constitute all known entities (i.e., are collectively exhaustive). A classification should provide a basis for future investigation and should be able to incorporate new information as it becomes available. Disease classification involves two distinct processes: class discovery (the process of identifying categories of diseases) and class prediction (the process of determining to which category individual cases belong). The work of pathologists is essential for both processes.

The 2008 WHO classification of tumours of the haematopoietic and lymphoid tissues (4th edition) [3848] was a collaborative project of the European Association for Haematopathology and the Society for Haematopathology. It was a revision and update of the 3rd edition (1820), which was the first true worldwide consensus classification of haematological malignancies. The 4th edition had an eight-member steering committee composed of members of both societies. Through a series of meetings and discussions, with input from both societies, the steering committee agreed on a proposed list of diseases and chapters and chose authors. As was done for the 3rd edition, the advice of clinical haematologists and oncologists was obtained to ensure that the classification would be clinically useful [1556]. Two clinical advisory committees were convened: one for myeloid neoplasms and another for acute leukaemias and one for lymphoid neoplasms. The meetings were organized around a series of questions, which addressed topics such as disease definitions, nomenclature, grading, and clinical relevance. The committees were able to reach consensus on most of the questions posed, and much of the input from the committees was incorporated into the classification. More than 130 pathologists and haematologists from around the world were involved in writing the chapters.

It has now been more than 8 years since the publication of the 4th edition, and numerous basic and clinical investigations have since led to many advances in the field that warrant an update to the classification. Important contributions have been made through the application of high-throughput genetic technologies such as gene expression profiling and next-generation sequencing. These technologies have led to new diagnostic tools and have revealed new mechanisms of tumorigenesis and new potential therapeutic targets. Because the 4th edition of the WHO classification of tumours series is not yet complete (with several volumes yet to be released), the 5th edition cannot yet be started, so the editors and authors have instead undertaken a major update to the existing 4th edition of the WHO classification of tumours of the haematopoietic and lymphoid tissues. This process has involved many of the original editors as well as an additional three senior advisors specializing in myeloid neoplasms and two senior advisors with expertise in molecular and cytogenetic issues. Clinical advisory committee meetings were held regarding both myeloid and lymphoid neoplasms, as was done for prior editions. The key features of this revision have been summarized in recent review articles [129A,3848A].

The WHO classification of tumours of haematopoietic and lymphoid tissues is based on the principles initially defined in the Revised European-American classification of lymphoid neoplasms (REAL), proposed by the International Lymphoma Study Group (ILSG) [1557]. In the WHO classification, these principles have also been applied to the classification of myeloid and histiocytic neoplasms. The guiding principle of both the REAL and the WHO classification is the importance of defining real diseases that can be recognized by pathologists using the available techniques, and that appear to be distinct clinical entities. There are three important components of this process.

The first component is the recognition that the underlying causes of these neoplasms are often unknown and may vary. Therefore, the WHO approach to classification incorporates all available information – morphology, immunophenotype, genetic features, and clinical features – to define the diseases. The relative importance of each of these features varies by disease, depending on the current state of knowledge; there is no single gold standard by which all diseases are defined.

The second important component of this classification process is the recognition that the complexity of the field makes it impossible for any single expert or small group of experts to be completely authoritative; for a classification to be widely accepted, broad agreement is necessary. Therefore, the WHO approach to classification relies on building a consensus on the definitions and nomenclature of the diseases among as many experts as possible. We recognize that compromise is essential for establishing a consensus, but we believe that even an imperfect single classification is better than multiple competing classifications.

The final important component of this classification process is the understanding that although pathologists must take primary responsibility for developing a classification, the involvement of clinicians is also essential, to ensure the classification's usefulness and acceptance in daily practice [1556]. When the 3rd edition of the WHO classification was published, previous proponents of other classifications of haematological neoplasms agreed to accept and use the new classification, ending decades of controversy over the classification of these tumours [338A,339,340,1165,1330A,1643A,2412A,2836,3310A]. As stated above, there is no single gold standard by which all diseases are defined in the WHO classification. Morphology is always important; many diseases have characteristic or even diagnostic morphological features. Immunophenotype and genetic features are also important aspects of the definition of tumours of haematopoietic and lymphoid tissues, and the availability of this...
Introduction to the WHO classification of tumours of haematopoietic and lymphoid tissues

14

The WHO classification classifies neo¬plasms primarily according to lineage — myeloid, lymphoid, or histiocytic/dendritic — and a normal counterpart is postulated for each neoplasm. Although the goal is to define the lineage of each neoplasm, lineage plasticity can occur in precursor or immature neoplasms, and has also been identified in some mature haemato¬lymphoid neoplasms. In addition, genetic abnormalities such as rearrangements in FGFR1, PDGFA, and PDGFRB, or POM1-JAK2 fusion, can give rise to neoplasms of either myeloid or lymphoid lineage associated with eosinophilia; these disorders are recognized as a separate group. Precursor neoplasms (i.e., acute myeloid leukaemias, lymphoblastic leukaemias/lymphomas, acute leukaemias of ambiguous lineage, and blastic plasmacytoid dendritic cell neoplasm) are discussed separately from more-mature neoplasms (i.e., myeloproliferative neoplasms, mastocytosis, myelodysplastic/myeloproliferative neoplasms, myelodysplastic syndromes, mature [peripheral] B-cell and T/NK-cell neoplasms, Hodgkin lymphomas, and histiocytic and dendritic cell neoplasms). The mature myeloid neoplasms are classified according to their biological features (i.e., myeloproliferative neoplasms with effective haematopoesis vs myelodysplastic neoplasms with ineffective haematopoesis), as well as by their genetic features. Within the category of mature lymphoid neoplasms, the diseases are generally listed according to their clinical presentation (i.e., disseminated often leukemic, extranodal, indolent, or aggressive), and to some extent according to the stage of differen¬tiation when this can be postulated. However, the order in which the diseases are listed is in part arbitrary, and is not an integral aspect of the classification. This revised 4th edition of the WHO classi¬fication incorporates new information that has emerged since the publication of the original 4th edition. It includes some changes in terminology related to our improved understanding of certain disease entities and presents revised defining criteria for some neoplasms. In addition, a number of previously provisional enti¬ties have now been accepted as definite entities, and new provisional entities have been added — some defined by genetic criteria (particularly among the myeloid neoplasms) and others by a combination of morphology, immunophenotype, and clinical features. The frequent application of immunophenotyping and genetic stud¬ies using peripheral blood, bone marrow, and lymph node samples has led to the detection of small clonal populations in asymptomatic individuals. These clonal populations include small clones of cells with the BCR-ABL1 translocation seen in chronic myeloid leukaemia, small clones of cells with IGH/BCL2 rearrangement, and small populations of cells that have the immunophenotype of chronic lympho¬cytic leukaemia or follicular lymphoma (i.e., monoclonal B-cell lymphocytosis, in situ follicular and mantle cell neoplasia, paediatric follicular hyperplasia with monoclonal B cells, and more recently, mutations in haematopoietic cells in older individuals, without evidence of a haematological malig¬nancy — so-called clonal haematopoesis of indeterminate potential [3772]). It is not always clear whether these clonal prolif¬erations constitute early involvement by a neoplasm, a precursor lesion, or an incon¬sequential finding. These situations are somewhat analogous to the identification of small monoclonal immunoglobulin com¬ponents in serum (i.e., monoclonal gam¬mopathy of undetermined significance). The chapters on these neoplasms include updated recommendations for dealing with these situations. The recommendations of international consensus groups have also been updated, with regard to criteria for the diagnosis of chronic lymphocytic leu¬kaemia and plasma cell myeloma.

Any classification of diseases must be periodically reviewed and updated to incor¬porate new information. The Society for Hematopathology and the European Asso¬ciation for Haematopathology now have a record of nearly two decades of collabora¬tion and cooperation in this effort. The soci¬eties are committed to updating and revis¬ing the classification as needed, with input from clinicians and in collaboration with the International Agency for Research on Cancer (IARC) and WHO. The process of developing and updating the WHO classi¬fication has generated a new and exciting degree of cooperation and communication among pathologists and oncologists from around the world, which will facilitate our continued progress in the understanding and treatment of haematological malig¬nancies. The multiparameter classifica¬tion approach that has been adopted by the WHO classification, with its emphasis on defining real disease entities, has been shown in international studies to be reproducible; the diseases defined are clinically distinctive, and the uniform definitions and terminology used facilitate the interpreta¬tion of clinical and translational studies (1. 148). In addition, the accurate and precise classification of disease entities has facili¬tated the discovery of the genetic basis of myeloid and lymphoid neoplasms in the basic science laboratory.
CHAPTER 1

Introduction and overview of the classification of myeloid neoplasms
Introduction and overview of the classification of myeloid neoplasms

The 2001 WHO Classification of Tumours: Pathology and Genetics of Tumours of Haematopoietic and Lymphoid Tissues (3rd edition) reflected a paradigm shift in the approach to the classification of myeloid neoplasms [1820]. For the first time, genetic information was incorporated into diagnostic algorithms provided for the various entities. The publication was prefaced with a comment predicting future revisions necessitated by rapidly emerging genetic information relevant to the diagnosis and classification of myeloid malignancies. The 4th edition (published in 2008) and the current 4th edition revision reflect the significant new molecular insights that have become available since the publication of the 2001 edition.

The first entity described in this volume, chronic myeloid leukaemia, remains the prototype for the identification and classification of myeloid neoplasms. This leukaemia is recognized by its clinical and morphological features, and its natural progression is characterized by an increase in blasts of myeloid, lymphoid, or mixed myeloid–lymphoid immunophenotype. It is always associated with the BCR-ABL1 fusion gene, which results in the production of an abnormal protein tyrosine kinase with enhanced enzymatic activity. This oncoprotein is sufficient to cause the disease and is also a target for protein tyrosine kinase inhibitor therapy, which has prolonged the lives of thousands of patients with this previously fatal illness [1040]. This successful integration of clinical, morphological, and genetic information embodies the goal of the WHO classification scheme.

In this revision, the combination of clinical, morphological, immunophenotypic, and genetic features continues to be used in an attempt to define disease entities, such as chronic myeloid leukemia, that are biologically homogeneous and clinically relevant – the same approach used in the 3rd and 4th editions of the classification. The previous classification schemes opened the door to including genetic abnormalities as criteria for classifying myeloid neoplasms, and the current revision explicitly acknowledges that recurrent genetic abnormalities not only provide objective criteria for recognition of specific entities but are also vital for the identification of abnormal gene products and pathways that are potential therapeutic targets. Several disease subgroups and sets of defining criteria have been expanded to include not only neoplasms associated with chromosomal abnormalities recognizable by conventional karyotyping, but also those with gene mutations with or without a cytogenetic correlate. However, the importance of careful clinical, morphological, and immunophenotypic characterization of every myeloid neoplasm, and correlation with the genetic findings, cannot be overemphasized. The discoveries of activating JAK2 mutations and mutations in CALR and MPL have revolutionized the diagnostic approach to myeloproliferative neoplasms (MPNs) [299,1831,2014, 2037,2099,2290,2823]. However, these mutations are not specific for any single clinical or morphological MPN phenotype, and some are also reported in certain cases of myelodysplastic syndromes (MDSs), myelodysplastic/myeloproliferative neoplasms (MDS/MPNs), and acute myeloid leukaemia (AML). Therefore, an integrated, multimodality approach is necessary for the classification of all myeloid neoplasms. It is also critical to elucidate how molecular testing can be used to inform the diagnosis and treatment of myeloid malignancies, and to articulate how these tests should be incorporated into clinical practice, on the basis of current and evolving scientific evidence.

With so much yet to be learned, there may be some missteps as traditional approaches to categorization are fused with more molecularly oriented classification schemes. But the authors, senior advisors and editors of this revision of the WHO classification, as well as the clinicians who served as members of its clinical advisory committees, have worked diligently to develop an updated, evidence-based classification that can be used in daily practice for therapeutic decision-making and that also provides a flexible framework for integration of new data.

Prerequisites for the classification of myeloid neoplasms by WHO criteria

The WHO classification of myeloid neoplasms relies on the morphological, cytochemical, and immunophenotypic features of the neoplastic cells to establish their lineage and degree of maturation, and to determine whether the cellular appearance is cytologically normal, dysplastic, or otherwise morphologically abnormal. The classification is based on criteria applied strictly to initial specimens obtained prior to any therapy. Blast percentages in the peripheral blood, bone marrow, and other involved tissues remain of practical importance for categorizing myeloid neoplasms and determining their progression. Cytogenetic and molecular genetic studies are required at the time of diagnosis not only for recognition of specific genetically defined entities, but also for establishing a baseline against which follow-up studies can be interpreted to assess disease progression. Given the integrated, multimodality approach required for diagnosing and classifying these neoplasms, it is recommended that the various diagnostic studies be correlated with the clinical findings and communicated in a single integrated report. If a definitive classification cannot be determined, the report should indicate why and provide guidance for additional studies that may clarify the diagnosis.

For the purpose of achieving consistency, the following guidelines are recommended for the evaluation of specimens when a myeloid neoplasm is suspected. In this context, a standardized approach to the processing, documentation, and reporting of bone marrow findings is emphasized [2253]. It is assumed that the evaluation will be performed with full knowledge of the clinical history and pertinent laboratory data.
Morphology

Peripheral blood

A peripheral blood smear should be examined and correlated with the results of a complete blood count. Freshly made smears should be stained with May-Grunwald-Giemsa or Wright-Giemsa stain and examined for white blood cell, red blood cell, and platelet abnormalities. It is important to ensure that smears are well stained. Evaluation of neutrophil granularity is important when a myeloid disorder is suspected; the designation of neutrophils as abnormal on the basis of hypogranular cytoplasm alone should not be considered unless the stain is well controlled. Manual 200-cell leukocyte differential counts are recommended as part of the peripheral blood smear evaluation in patients with a myeloid neoplasm when the white blood cell count permits. The presence of abnormal erythrocytes (e.g. tear-drop cells) as well as platelet size and granularity should also be taken into account.

Bone marrow aspiration

Aspirate smears should be stained with May-Grunwald-Giemsa or Wright-Giemsa stain for optimal visualization of cytoplasmic granules and nuclear chromatin. Because the WHO classification relies on percentages of blasts and other specific cells to categorize some entities, it is recommended that 500 nucleated bone marrow cells be counted on cellular aspirate smears in an area as close to the particle and as undiluted with blood as possible. Counting from multiple smears may reduce sampling error due to irregular distribution of cells. The cells to be counted include blasts and promonocytes (as defined below), promyelocytes, myelocytes, metamyelocytes, band neutrophils, segmented neutrophils, eosinophils, basophils, monocytes, lymphocytes, plasma cells, erythroid precursors, and mast cells. Megakaryocytes (including dysplastic forms) should not be counted. If a concomitant non-myeloid neoplasm (e.g. plasma cell myeloma) is present, it is reasonable to exclude those neoplastic cells from the count for the purpose of classifying the myeloid neoplasm. If an aspirate cannot be obtained due to fibrosis or cellular packing, touch preparations of the biopsy may yield valuable cytological information, but differential counts from touch preparations may not be representative. When performing touch preparations, care must be taken to avoid crush artefact or damage to the core biopsy. The differential counts obtained from marrow aspirates should be compared with an estimate of the proportions of cells observed in available corresponding biopsy sections.

Bone marrow trephine biopsy

The importance of adequate bone marrow biopsy sections for the diagnosis of myeloid neoplasms cannot be overstated. The bone marrow biopsy provides information regarding overall (age-matched) cellularity, histotopography, and the proportion and maturation of haematopoietic cells and also enables evaluation of bone marrow stroma and cancellous bone structure. The biopsy also provides material for immunohistochemical studies that may be of diagnostic and prognostic importance. A biopsy is essential whenever there is myelofibrosis, and the classification of some entities, in particular MPNs, relies heavily on histology sections. The specimen must be adequate, be taken at a right angle from the cortical bone, and be ≥1.5 cm in length (to enable evaluation of ≥10 partially preserved intertrabecular areas [2253]). It should be well fixed, thinly sectioned (at 3–4 μm), and stained with H&E and/or a stain such as Giemsa that allows for detailed morphological evaluation. A silver impregnation method (including reticulin and collagen assessment) is recommended for evaluation of marrow fibrosis, which should be graded according to the European consensus scoring system [2148,3975]. Periodic acid–Schiff (PAS) staining may facilitate the detection of megakaryocytes. Immunohistochemical study of the biopsy (discussed below) can be very useful in the evaluation of myeloid neoplasms.

Blasts

The percentage of myeloid blasts is very important for the diagnosis and classification of myeloid neoplasms. In the peripheral blood, the blast percentage should be determined from a 200-cell leukocyte differential count and in the bone marrow, from a 500-cell count using cellular bone marrow aspirate smears as described above. The blast percentage determined from the bone marrow aspirate should correlate with an estimate of

Fig. 1.01 Myelodysplastic syndrome. Bone marrow biopsies should be well fixed, and thin (3–4 μm) sections should be stained with H&E and/or Giemsa stain to enable optimal evaluation of histological details.

Fig. 1.02 Bone marrow trephine biopsies of suspected myeloid neoplasms should be ≥1.5 cm in length and obtained at right angles to the cortical bone.
Myeloblasts, monoblasts, and megakaryoblasts are included in the blast count. Myeloblasts range from slightly larger than mature lymphocytes to the size of monocytes or larger, with scant to abundant dark-blue to bluish-grey cytoplasm. The nuclei are round to oval, with finely granular chromatin and usually several nucleoli; in some, nuclear irregularities are prominent. The cytoplasm may contain a few azurophilic granules. Monoblasts are large cells with abundant cytoplasm that can be light grey to deep blue and may show pseudopod formation. Their nuclei are usually round, with delicate, lacy chromatin and one or more large, prominent nucleoli. They are usually strongly positive for non-specific esterase (NSE), but have no or only weak myeloperoxidase (MPO) activity. Promonocytes have a delicately convoluted, folded, or grooved nucleus with finely dispersed chromatin; a small, indistinct, or absent nucleolus; and finely granulated cytoplasm. Most promonocytes express NSE and have MPO activity. Promonocytes are considered to be monoblast equivalents when the requisite percentage of blasts is tallied for the diagnosis of acute monoblastic, acute monocytic, or acute myelomonocytic leukaemia and in subclassifying chronic myelomonocytic leukaemia. Distinguishing between monoblasts and promonocytes is often difficult, but because both cell types are regarded as monoblasts for the purpose of rendering a diagnosis of AML, the distinction between a monoblast and a promonocyte is not always critical. Distinguishing promonocytes from more mature but abnormal leukaemic monocytes can also be difficult, but is critical, because the designation of a case as acute monocytic or acute myelomonocytic leukaemia versus chronic myelomonocytic leukaemia often hinges on this distinction. Abnormal monocytes have more clumped chromatin than promonocytes, variably indented folded nuclei, and grey cytoplasm with more abundant lilac-coloured granules. Nucleoli are usually absent or indistinct. Abnormal monocytes are not considered to be monoblast equivalents. Megakaryoblasts are usually small to medium-sized, with a round, indented, or irregular nucleus with fine reticular chromatin and 1–3 nucleoli. The cytoplasm is basophilic, is usually agranular, and may show cytoplasmic blebs (see Acute megakaryoblastic leukaemia, p. 162). Small dysplastic megakaryocytes and micromegakaryocytes are not blasts. In acute promyelocytic leukaemia, the blast equivalent is the abnormal promyelocyte. Early erythroid precursors (proerythroblasts) are not included in the blast count, except in the rare setting of pure erythroid leukaemia.

Cytochemistry and other special stains
Cytochemical studies are useful in determining the lineage of blasts, although in some laboratories they have been supplanted by immunological studies using flow cytometry and/or immunohistochemistry. Cytochemical studies are usually performed on peripheral blood and bone marrow aspirate smears, but some can be performed on histological sections of bone marrow or other tissues. Detection of MPO indicates myeloid differentiation, but its absence does not exclude a myeloid lineage, because early myeloblasts as well as monoblasts can lack MPO. The MPO activity in myeloblasts is usually granular and is often concentrated in the Golgi region, whereas monoblasts (although usually MPO-negative) may show fine, scattered MPO-positive granules, a pattern that becomes more pronounced in promonocytes. Erythroid blasts, megakaryoblasts, and lymphoblasts are also MPO-negative. Sudan Black B staining parallels MPO staining but is less specific. In the occasional cases of lymphoblastic leukaemia that exhibit Sudan Black B positivity, light-grey granules are seen rather than the black granules that characterize myeloblasts. The NSEs (alpha-naphthyl butyrate esterase and alpha-naphthyl acetate esterase) show diffuse cytoplasmic activity in monoblasts and monocytes. Lympho-
blasts may have focal punctate activity with NSEs, but neutrophils are usually negative. Megakaryoblasts and erythroid blasts may have some multifocal, punctate alpha-naphthyl acetate positivity, but the reactivity is partially resistant to sodium fluoride inhibition, whereas monocyte reactivity is totally inhibited by sodium fluoride. The combined use of an NSE and the specific esterase naphthol AS-D chloroacetate esterase (CAE), which primarily stains cells of the neutrophil lineage and mast cells, enables identification of monocytes and immature and mature neutrophils simultaneously. Some cells, particularly in myelomonocytic leukemias, may exhibit simultaneous activity with NSEs and CAE. Although normal eosinophils lack CAE, it may be expressed by neoplastic eosinophils. CAE staining can be performed on tissue sections (providing the sections are not acid de-calcified) as well as on peripheral blood or bone marrow aspirate smears. In pure erythroid leukaemia, periodic acid–Schiff (PAS) staining may be helpful because the cytoplasm of the leukaemic pro-erythroblasts may show large globules of PAS positivity. Well-controlled iron stains should always be performed on the bone marrow aspirate to detect iron stores, normal sideroblasts, and ring sideroblasts; ring sideroblasts are defined as erythroid

Prerequisites for the classification of myeloid neoplasms by WHO criteria
precursors with ≥5 granules of iron, encircling one third or more of the nucleus.

Immunophenotype
Immunophenotypic analysis by either multiparameter flow cytometry or immunohistochemistry is an essential tool in the characterization of myeloid neoplasms. Differentiation antigens that appear at various stages of haematopoietic development and in corresponding myeloid neoplasms are illustrated in Fig. 1.05, and a thorough description of the lineage assignment criteria is provided in the sections on mixed-phenotype acute leukaemia (See Acute leukaemias of ambiguous lineage, p. 179). The techniques used and the antigens analysed vary according to the myeloid neoplasm suspected and the information required to best characterize it, as well as by the tissue available. Immunophenotyping is often important in the diagnosis of any haematological neoplasm; in myeloid neoplasms, it is most commonly required to identify mixed-phenotype acute leukaemia, to distinguish between AML with minimal differentiation and lymphoblastic leukaemia, to detect monocytic differentiation in AML, and in determining the phenotype of blasts at the time of transformation of chronic myeloid leukaemia, MDS, MDS/MPN, and MPN. Multiparameter flow cytometry is the preferred method of immunophenotypic analysis in AML due to the ability to analyse large numbers of cells in a relatively short period of time with simultaneous recording of information about several antigens for each individual cell. Extensive panels of monoclonal antibodies directed against leukocyte differentiation antigens are usually applied, due to the limited utility of individual markers in identifying the commitment of leukaemic cells to the various haematopoietic lineages. Evaluation of the expression patterns of several antigens, both membrane and cytoplasmic, is necessary for determining lineage, identifying mixed-phenotype acute leukaemia, and detecting aberrant phenotypes that will facilitate the evaluation of follow-up specimens for minimal residual disease.

Immunophenotypic analysis has a central role in distinguishing between AML with minimal differentiation and lymphoblastic leukaemia, and in chronic myeloid leukaemia in distinguishing the myeloid blast phase from the lymphoid blast phase. Among the AMLs with recurrent genetic abnormalities, several have characteristic phenotypes. These patterns, described in the respective sections, can facilitate the planning of molecular cytogenetic (FISH) and molecular genetic investigations for individual patients. The immunophenotypic features of the other AML categories are extremely heterogeneous, probably due to high genetic diversity. It has been suggested that the expression of certain antigens (e.g. CD7, CD9, CD11b, CD14, CD56, and CD34) could be associated with an adverse prognosis in AML, but their independent prognostic value is still controversial, and cytogenetic and molecular genetic abnormalities are generally more reliable prognostic markers than is immunophenotype. With 8- or 10-colour flow cytometry, aberrant or unusual immunophenotypes have been found in as many as 90% of cases of AML; these aberrancies include cross-lineage antigen expression, maturational asynchronous expression of antigens, antigen overexpression, and the reduction or absence of antigen expression. Similar aberrancies have been reported in MDS, and their presence can be used to support the diagnosis in early or morphologically difficult cases; however, aberrant flow cytometry immunophenotypes should not be used to diagnose MDS in the absence of standard diagnostic criteria.

Immunophenotyping by immunohistochemistry on bone marrow biopsy sections can be performed, provided that appropriate methods for fixation and decalcification have been applied. Antibodies reactive with paraffin-embedded bone marrow biopsy tissue are available for many lineage-associated markers (e.g. MPO, KIT [CD117], CD33, CD68R, CD14, lysozyme, glycophorin A and C, CD71, CD61, CD42b, CD19, CD3, PAX5, CD79a, and t ypase). As noted previously, CD34 staining of the biopsy can facilitate the detection of blasts and enable assessment of their distribution (provided the blasts express CD34) [2989]. For cases rich in megakaryoblastoid erythroblasts, immunohistochemistry for glycophorin, CD71, E-cadherin, or haemoglobin may be helpful in distinguishing those cells from myeloblasts in MDS with excess blasts or pure erythroid leukaemia, and CD61 or CD42b staining often facilitates the identification of abnormal megakaryocytes and megakaryoblasts.

Genetics
The WHO classification includes a number of entities defined in part by specific genetic abnormalities, including gene rearrangements due to chromosomal translocations, deletions, and specific gene mutations; therefore, the determination of genetic features of the neoplastic cells is of critical importance for a comprehensive clinicopathological evaluation. A complete cytogenetic analysis of bone marrow by conventional karyotyping should be performed at the time of initial evaluation to establish the cytogenetic profile and at regular intervals thereafter to detect evidence of genetic evolution. Additional diagnostic genetic studies should be guided by the diagnosis suspected on the basis of clinical, morphological, and immunophenotypic studies. In cases with variants of typical cytogenetic abnormalities and cases in which the abnormality is cryptic (e.g. the FIP1L1-PDGFRα fusion in myeloid neoplasms associated with eosinophilia), RT-PCR and/or FISH may detect gene rearrangements that are not apparent in the initial chromosomal analysis. Depending on the abnormality, quantitative PCR and/or RT-PCR performed at the time of diagnosis may also provide a baseline against which the response to therapy can be monitored. In addition, the use of array-based and next-generation sequencing technologies enables the sensitive and accurate detection of many common gene rearrangements and has emerged as an alternative to RT-PCR and FISH for the detection of pathogenic fusion genes in haematological malignancies.

A rapidly increasing number of somatic gene mutations detected by gene sequencing, allele-specific PCR, and other techniques have emerged as important diagnostic and prognostic markers for all categories of myeloid neoplasms. Mutations in JAK2, MPL, CALR, N Ras, N F1, PTPN11, ASXL1, and KIT in MPN and MDS/MPN; TP53, SF3B1, ASXL1, RUNX1, EZH2, and ETV6 (among others) in MDS; and NPM1, CEBPA, FLT3, RUNX1, IDH1, IDH2, ASXL1, and KIT (among others) in AML are important for diagnosis and prognosis. In particular, JAK2, MPL, CALR, CSF3R, SF3B1, FLT3, NPM1, RUNX1, and CEBPA figure prominently in this revised classification. In addition, many recently characterized somatic disease alleles (e.g. recurrent mutations in TET2, ASXL1, and DNMT3A)
can serve as definitive markers of clonal haematopoiesis, which can be used as an adjunct to the diagnosis of myeloid malignancies, despite the fact that these alleles are neither specific for a particular disease nor sufficient to diagnose a myeloid neoplasm. These mutations and others seen in myeloid malignancies can also be observed in healthy individuals with clonal haematopoiesis, which appears to constitute a premalignant, clonal state with a variable risk of progression to overt, clinical disease, and has important implications for interpreting genetic profiling in the context of clinical, laboratory, and pathological evaluation to make a specific diagnosis.

Next-generation sequencing continues to emerge as a standard technology for mutational profiling; it is therefore critical to establish methods for identifying alleles with diagnostic, prognostic, and therapeutic relevance, and to use best practices (including informational annotation and paired sequencing of tumour and normal material when possible) to ascertain which alleles are present as acquired mutations and which are present in the germline. In particular, given the likelihood of tumour-derived contamination of paired normal material collected at diagnosis and the frequent presence of antecedent, premalignant clonal haematopoiesis at the time of clinical remission, the choice and timing of collection of non-haematopoietic reference DNA are of critical importance for best-practice genomic profiling. The current approaches to genomic profiling include focused, gene-specific tests for a small set of genes tailored to a specific disease and/or clinical scenario, as well as panel-based assays that query all genes implicated in the pathogenesis of myeloid malignancies, or even more broadly, of all haematological malignancies. Both approaches have clinical value in the current context, but we expect the use of panel-based assays and whole-genome/exome sequencing to increase as the cost and throughput of clinical genomic profiling continue to improve.

Gene over- and underexpression, as well as loss of heterozygosity (LOH) and copy number variants detected by array-based approaches, are only now being recognized as important abnormalities, and may influence diagnostic and prognostic models in the near future [2770]. It will be critical to develop gene-specific and panel-based assays to query for differential expression of specific biomarkers and to assess for copy number and zygosity alterations at specific loci with diagnostic, prognostic, and therapeutic relevance.

Revised WHO classification of myeloid neoplasms

Myeloproliferative neoplasms

The major subgroups of MPNs are listed in the WHO classification table at the beginning of this volume (p. 10). Note that the name of the entity previously called ‘chronic myelogenous leukaemia, BCR-ABL1 positive’ has been changed to ‘chronic myeloid leukaemia, BCR-ABL1-positive’.

The MPNs are clonal haematopoietic stem cell disorders characterized by the proliferation of cells of one or more of the myeloid lineages (i.e., granulocytic, erythroid, and megakaryocytic). They primarily occur in adults, with incidence peaking in the fifth to seventh decades of life, but some subtypes are also reported in children. The annual incidence of all subtypes combined is 6 cases per 100,000 population [1375, 1864, 2764, 4010].

Most MPNs are initially characterized by varying degrees of age-matched hypercellularity of the bone marrow, with effective haematopoietic maturation and increased numbers of granulocytes, red blood cells, and/or platelets in the peripheral blood. Splenomegaly and hepatomegaly, caused by sequestration of excess blood cells and/or proliferation

Revised WHO classification of myeloid neoplasms

Myeloproliferative neoplasms

The major subgroups of MPNs are listed in the WHO classification table at the beginning of this volume (p. 10). Note that the name of the entity previously called ‘chronic myelogenous leukaemia, BCR-ABL1 positive’ has been changed to ‘chronic myeloid leukaemia, BCR-ABL1-positive’.

The MPNs are clonal haematopoietic stem cell disorders characterized by the proliferation of cells of one or more of the myeloid lineages (i.e., granulocytic, erythroid, and megakaryocytic). They primarily occur in adults, with incidence peaking in the fifth to seventh decades of life, but some subtypes are also reported in children. The annual incidence of all subtypes combined is 6 cases per 100,000 population [1375, 1864, 2764, 4010].

Most MPNs are initially characterized by varying degrees of age-matched hypercellularity of the bone marrow, with effective haematopoietic maturation and increased numbers of granulocytes, red blood cells, and/or platelets in the peripheral blood. Splenomegaly and hepatomegaly, caused by sequestration of excess blood cells and/or proliferation

Revised WHO classification of myeloid neoplasms

Myeloproliferative neoplasms

The major subgroups of MPNs are listed in the WHO classification table at the beginning of this volume (p. 10). Note that the name of the entity previously called ‘chronic myelogenous leukaemia, BCR-ABL1 positive’ has been changed to ‘chronic myeloid leukaemia, BCR-ABL1-positive’.

The MPNs are clonal haematopoietic stem cell disorders characterized by the proliferation of cells of one or more of the myeloid lineages (i.e., granulocytic, erythroid, and megakaryocytic). They primarily occur in adults, with incidence peaking in the fifth to seventh decades of life, but some subtypes are also reported in children. The annual incidence of all subtypes combined is 6 cases per 100,000 population [1375, 1864, 2764, 4010].

Most MPNs are initially characterized by varying degrees of age-matched hypercellularity of the bone marrow, with effective haematopoietic maturation and increased numbers of granulocytes, red blood cells, and/or platelets in the peripheral blood. Splenomegaly and hepatomegaly, caused by sequestration of excess blood cells and/or proliferation

Revised WHO classification of myeloid neoplasms

Myeloproliferative neoplasms

The major subgroups of MPNs are listed in the WHO classification table at the beginning of this volume (p. 10). Note that the name of the entity previously called ‘chronic myelogenous leukaemia, BCR-ABL1 positive’ has been changed to ‘chronic myeloid leukaemia, BCR-ABL1-positive’.

The MPNs are clonal haematopoietic stem cell disorders characterized by the proliferation of cells of one or more of the myeloid lineages (i.e., granulocytic, erythroid, and megakaryocytic). They primarily occur in adults, with incidence peaking in the fifth to seventh decades of life, but some subtypes are also reported in children. The annual incidence of all subtypes combined is 6 cases per 100,000 population [1375, 1864, 2764, 4010].

Most MPNs are initially characterized by varying degrees of age-matched hypercellularity of the bone marrow, with effective haematopoietic maturation and increased numbers of granulocytes, red blood cells, and/or platelets in the peripheral blood. Splenomegaly and hepatomegaly, caused by sequestration of excess blood cells and/or proliferation
of abnormal haematopoietic progenitor cells, are common. Despite an insidious onset, each MPN has the potential to undergo a stepwise progression that terminates in marrow failure due to myelofibrosis, ineffective haematopoiesis, or transformation to an acute blast phase. Evidence of genetic evolution usually heralds disease progression, as may increasing organomegaly, increasing or decreasing blood counts, myelofibrosis, and the onset of myelodysplasia. The finding of 10-19% blasts in the peripheral blood or bone marrow generally signifies accelerated disease, and a proportion of ≥20% is sufficient for the diagnosis of blast phase.

Rationale for and problems with diagnosis and classification of myeloproliferative neoplasms

The revisions to the 2008 criteria for the classification of MPNs have been influenced by three main factors (258):

1. The recent discovery of genetic abnormalities has provided diagnostic and prognostic markers and novel insights into the pathobiology of BCR-ABL1-negative MPNs (3920,3932).
2. Improved characterization and standardization of morphological features aiding in histological pattern recognition and differentiation of disease groups has increased the reliability and reproducibility of diagnosis (257,1361,1379,2433,3977).
3. A number of clinicopathological studies have now validated the WHO postulate of an integrated approach that includes haematological, morphological, and molecular genetic findings (251,266,1363,1379,1380,2433,3977).

Reports of controversial aspects have mainly focused on subjectivity and lack of interobserver reproducibility regarding the morphological criteria, especially their validity in distinguishing essential thrombocythaemia (ET) from prefibrotic/eary phases of primary myelofibrosis (pre-PMF) and polycythaemia vera (PV).

A critical evaluation of these studies suggests that the failure to use a standardized approach to recognizing the distinctive bone marrow features of these disorders resulted in incorrect histological pattern recognition (255,257,3977). However, several studies on large cohorts of patients have reported consensus rates for the correct diagnosis of MPNs of 76-88%, which are significantly depend-ent on study design; for example, inclusion of all subtypes of MPN as opposed to restriction to ET vs pre-PMF, inclusion of control cases with reactive changes, and blinded morphological evaluation vs evaluation together with clinical data as recommended by the WHO diagnostic guidelines (257,1361,1379,2433). In this context, the learning effects of a workshop exercise including interobserver consensus among six haematopathologists included an increase in consensus from 49% to 72% and an agreement rate of 83% between blinded histological and clinical diagnoses (2434). A number of problems and pitfalls associated with assessing the fibrous matrix of the bone marrow, including the differentiation between reticulin and collagen fibres and the grading of osteosclerosis, must be taken into account (2148). A multicentre study that compared the results of fibre grading between local pathologists and a panel of experts showed an overall agreement rate of only 56%, supporting the concept of central pathology review for clinical studies (3228).

Most (if not all) MPNs are associated with clonal abnormalities either involving genes that encode cytoplasmic or receptor protein tyrosine kinases (resulting in the constitutive activation of oncogenic signalling pathways) or occurring in regulators of these pathways (resulting in similar biological consequences). The abnormalities described to date include translocations, insertions, deletions, and point mutations of genes resulting in abnormal, constitutively activated protein tyrosine kinases that activate signal transduction pathways, leading to abnormal proliferation. In some cases, these genetic abnormalities (e.g. the BCR-ABL1 fusion gene in chronic myeloid leukaemia) are associated with consistent clinical, laboratory, and morphological findings, which enables their use as major criteria for classification; other genetic abnormalities provide proof that the myeloid proliferation is neoplastic rather than reactive.

Acquired somatic mutations in JAK2, at chromosome band 9p24, have been shown to play a pivotal role in the pathogenesis of many cases of BCR-ABL1-negative MPNs (1831,2045,2099,2289,2290). The most common mutation, JAK2 V617F, results in a constitutively active cytoplasmic JAK2, which activates STAT, MAPK, and PI3K signalling pathways to promote transformation and proliferation of haematopoietic progenitors. The JAK2 V617F mutation is found in almost all patients with PV and in nearly half of those with PMF and ET. In the few patients with PV who lack the JAK2 V617F mutation, activating JAK2 exon 12 mutations may be found; these can be missense or insertion/deletion mutations that are not always detectable by standard JAK2 mutation assays. In a small proportion of cases of PMF and ET, an activating MPL W515L or W515K mutation is seen, and somatic mutations in CALR are found in most ET and PMF cases with wildtype JAK2 and MPL, CALR and MPL mutations are therefore important diagnostic criteria for JAK2-wildtype ET and PMF. It is important to note that JAK2 V617F is not specific for any MPN, nor does its absence exclude MPN. The mutation can also be found in some cases of MDS/MPN and in rare cases of de novo AML and MDS, and can occur in combination with other well-defined genetic abnormalities, such as BCR-ABL1 (1872). Therefore, the diagnostic algorithms for PV, ET, and PMF have been updated to take into account the mutational status of JAK2, MPL, and CALR, as well as to summarize the additional laboratory and histological findings required to accurately classify cases, regardless of whether a mutation is present.

Due to its unique clinical and pathological features, mastocytosis (which ranges from indolent cutaneous disease to aggressive systemic disease) is no longer considered a subgroup of the MPNs. It is now a separate disease category in the WHO classification.
Myeloid/lymphoid neoplasms with eosinophilia and gene rearrangement

This category of the classification remains largely unchanged, except for the addition of the provisional entity of myeloid/lymphoid neoplasms with t(8;9) (p22;p24.1) resulting in PCM1-JAK2 [230,3108]. The finding of a rearrangement of PDGFRα, PDGFRB, or FGFR1, or of PCM1-JAK2 places a case in this category regardless of the morphological classification; eosinophilia is absent in a subset of cases. Myeloid neoplasms with eosinophilia that lack all of these abnormalities and that meet the criteria for chronic eosinophilic leukaemia, not otherwise specified (NOS), should be placed in that MPN subgroup. Other JAK2-rearranged neoplasms, such as those with t(9;12)(p24.1;p13.2), resulting in ETV6-JAK2, and t(9;22)(p24.1;q11.2), resulting in BCR-JAK2, may have similar features, but are uncommon and are not included as formal entities in this classification. Many cases with BCR-JAK2 present primarily as B-lymphoblastic leukaemia, and these are best classified as B-lymphoblastic leukaemia/lymphoma, BCR-ABL1-like (a new provisional category of B-lymphoblastic leukaemia/lymphoma) [230].

Myelodysplastic/myeloproliferative neoplasms

The MDS/MPNs include clonal myeloid neoplasms that at the time of initial presentation are associated with some findings that support the diagnosis of an MDS and other findings more consistent with an MPN [2987]. These neoplasms are usually characterized by hypercellularity of the bone marrow due to proliferation in one or more of the myeloid lineages. Often, the proliferation is effective in some lineages, with increased numbers of circulating cells that may be morphologically and/or functionally dysplastic. Simultaneously, one or more of the other lineages may exhibit ineffective proliferation, so that cytopenia may be present as well. The blast percentage in the bone marrow and blood is always <20%. Although hepatosplenomegaly is common, the clinical and laboratory findings vary along a continuum between those usually associated with MDSs and those usually associated with MPNs. Cases of well-defined MPNs in which dysplasia and ineffective haematopoiesis develop as part of the natural history of the disease are often chemotherapy should not be placed in this category. Rarely, MPNs may present as accelerated phase in which the chronic phase was not recognized, and may have findings that suggest they belong to the MDS/MPN group. In such cases, if clinical and laboratory studies fail to reveal the nature of the underlying process, the designation 'MDS/MPN unclassifiable' may be appropriate. Cases with BCR-ABL1, with rearrangement of PDGFRα, PDGFRB, or FGFR1, or with PCM1-JAK2 should not be categorized as MDS/MPNs. Mutations in non-kinase genes, including in epigenetic regulators such as TET2 and ASXL1, are very common in MDS/MPNs; they can be used to establish clonality, but are neither diagnostic nor specific for this disease subset [1795,2779].

Rationale for diagnosis and classification of myelodysplastic/myeloproliferative neoplasms

A diagnosis of chronic myelomonocytic leukaemia (CMML) requires both the presence of persistent peripheral blood monocytes (monocyte count ≥1 x 10^9/L) and monocytes accounting for ≥10% of white blood cells on the differential count. As a result of the discovery of molecular and clinical differences between the so-called proliferative type (white blood cell count ≥13 x 10^9/L) and the dysplastic type (white blood cell count <13 x 10^9/L) [620,3349,3827], these cases have been separated into two subtypes, myelodysplastic and myeloproliferative, in this classification update. Cases of CMML with eosinophilia associated with PDGFRB rearrangement are excluded, but rare cases of CMML with eosinophilia that do not exhibit such rearrangement are included in this category. The category 'CMML-0' has also been added, for cases with low peripheral blood and bone marrow blast cell counts [2978,3587,3805].

In juvenile myelomonocytic leukaemia, nearly 80% of cases demonstrate mutually exclusive mutations of PTPN11, NRAS or KRAS, or NF1 [2382,3796,3906], all of which encode components of RAS-dependent pathways; approximately 30–40% of cases of CMML and atypical chronic myeloid leukaemia, BCR-ABL1-negative, exhibit NRAS mutations [3053,4152,4329]. Given the lack of any specific genetic abnormality to suggest that these entities should be relocated to another myeloid subgroup, they remain in this mixed category, which acknowledges the overlap that may occur between MDS and MPN.

In the original version of the 4th edition of the WHO classification, refractory anaemia with ring sideroblasts associated with marked thrombocytosis was proposed as a provisional entity to encompass cases with the clinical and morphological features of MDS with ring sideroblasts but also with thrombocytosis associated with abnormal megakaryocytes similar to those observed in BCR-ABL1-negative MPNs. More recently, and in particular after the discovery of a strong association with SF3B1 and concurrent JAK2 V617F, MPL, or CALR mutations, MDS/MPN with ring sideroblasts and thrombocytosis, the new term for the former refractory anaemia with ring sideroblasts associated with marked thrombocytosis category, has become a distinct, well-characterized MDS/MPN overlap entity [2460,2461,3102].

The classification of myeloid neoplasms that carry an isolated isochromosome of 17q and that have <20% blasts in the peripheral blood and bone marrow has proven difficult [1913]. Some authors suggest that this cytogenetic defect defines a unique disorder characterized by mixed MDS and MPN features associated with prominent pseudo–Pelger–Huët anomaly of the neutrophils, a low bone marrow blast count, and a rapidly progressive clinical course. A proportion of cases are reported to have prominent monocytes that meets the criteria for CMML, but in some, the peripheral blood monocyte count does not reach the threshold for that diagnosis [1220,2594]. For cases that do not fulfil the criteria for CMML or another well-defined myeloid neoplasm category, designation as MDS/MPN, unclassifiable, with isolated isochromosome 17q abnormality is most appropriate [1912].

Myelodysplastic syndromes

These neoplasms are characterized by the simultaneous proliferation and apoptosis of haematopoietic cells that result in a normocellular or hypercellular bone marrow and peripheral blood cytopenia. MDSs are among the most diagnostically challenging of the myeloid neoplasms, both in terms of their distinction from the numerous other (often non-neoplastic) causes of cytopenia and in terms of the...
Table 1.01 Diagnostic approach to myeloid neoplasms in which erythroid precursors constitute ≥50% of the nucleated bone marrow (BM) cells

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>≥ 50%</td>
<td>n/a</td>
<td>yes</td>
<td>n/a</td>
<td>n/a</td>
<td>Therapy-related myeloid neoplasm</td>
<td>Therapy-related myeloid neoplasm</td>
</tr>
<tr>
<td>≥ 50%</td>
<td>≥ 20%</td>
<td>no</td>
<td>yes</td>
<td>n/a</td>
<td>AML with recurrent genetic abnormality</td>
<td>AML with recurrent genetic abnormality</td>
</tr>
<tr>
<td>≥ 50%</td>
<td>≥ 20%</td>
<td>no</td>
<td>no</td>
<td>AML with myelodysplasia-related changes</td>
<td>AML with myelodysplasia-related changes</td>
<td></td>
</tr>
<tr>
<td>≥ 50%</td>
<td>≥ 20%</td>
<td>no</td>
<td>no</td>
<td>AML, NOS; acute erythroid leukaemia (erythroid/myeloid subtype)</td>
<td>AML, NOS (a non-erythroid subtype)</td>
<td></td>
</tr>
<tr>
<td>≥ 50%</td>
<td>&lt; 20%, but ≥ 20% of non-erythroid cells</td>
<td>no</td>
<td>no^</td>
<td>n/a</td>
<td>AML, NOS; acute erythroid leukaemia (erythroid/myeloid subtype)</td>
<td>MDS^b</td>
</tr>
<tr>
<td>≥ 50%</td>
<td>&lt; 20%, and &lt; 20% of non-erythroid cells</td>
<td>no</td>
<td>no^</td>
<td>n/a</td>
<td>MDS^b</td>
<td>MDS^b</td>
</tr>
<tr>
<td>&gt; 80% immature erythroid precursors with &gt; 30% proerythroblasts</td>
<td>≥ 20%</td>
<td>no</td>
<td>no^</td>
<td>n/a</td>
<td>AML, NOS; acute erythroid leukaemia (pure erythroid subtype)</td>
<td>AML, NOS; pure erythroid leukaemia</td>
</tr>
</tbody>
</table>

| AML, acute myeloid leukaemia; BM, bone marrow; MDS, myelodysplastic syndrome; n/a, not applicable; NOS, not otherwise specified; PB, peripheral blood. |

a Cases of AML with t(8;21)(q22;q22.1) resulting in the RUNX1-RUNX1T1 fusion protein, AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22) resulting in the CBFB-MYH11 fusion protein, or acute promyelocytic leukaemia with the PML-RARA fusion protein may rarely occur in this setting with <20% blasts, and those diagnoses take precedence over the diagnosis of either AML, NOS or MDS.

b Classify according to the myeloblast percentage of all BM cells and PB leukocytes, along with other MDS criteria.

proper classification to guide the clinical approach. The general features of MDS, as well as specific guidelines for their diagnosis and classification, are outlined in Chapter 6, *Myelodysplastic syndromes: Overview* (p. 98).

In this revised WHO classification, new terminology has been introduced. In the original 4th edition, MDS disease names included references to cytopenia or specific types of cytopenia (e.g. refractory anaemia). Although cytopenia is a sine qua non of any MDS diagnosis, the WHO classification relies mainly on the degree of dysplasia and blast percentages for MDS classification; specific cytopenias have only a minor impact on classification. Moreover, the lineage(s) manifesting significant morphological dysplasia often do not correlate with the specific cytopenias seen in individual MDS cases. For these reasons, the updated MDS names do not refer to cytopenia. All diagnostic entity names start with ‘myelodysplastic syndrome’, with further qualifiers specified: single lineage versus multilineage dysplasia, ring sideroblasts, excess blasts, or the defining del(5q) cytogenetic abnormality. No new disease entities have been introduced, but the diagnostic criteria for some entities have been refined, as detailed in Table 6.01 (p. 101) in the *Myelodysplastic syndromes* chapter and in the sections on each MDS entity. MDS cases with multilineage dysplasia, ring sideroblasts, and no excess of blasts or isolated del(5q) cytogenetic abnormality are now categorized as a subgroup of MDS with ring sideroblasts rather than being grouped with MDS with multilineage dysplasia lacking ring sideroblasts as in the original 4th edition. MDS in children has features that differ from those of most MDS in adults, and the provisional entity refractory cytopenia of childhood remains in this updated classification. Although this entity is still provisional, its morphological features and distinction from severe aplastic anaemia are now better defined. An important change in this revision that affects MDS diagnosis is in the diagnostic criteria for myeloid neoplasms in which ≥50% of the bone marrow cells are erythroid precursors. In the original 4th edition WHO classification, erythroid/myeloid-type acute erythroid leukaemia (erythroleukaemia) was diagnosed if blasts accounted for ≥20% of the non-erythroid cells in the bone marrow; if blasts accounted for <20% of the non-erythroid cells, the case was considered to be MDS and subclassified on the basis of the blast count among all nucleated bone marrow cells. Due to the apparent close biological relationship of erythroleukaemia to MDS and the poor reproducibility and potential liability of non-erythroid blast counts, and in an attempt to achieve consistency in expressing blast percentages across all myeloid neoplasms, non-erythroid blast counting has been eliminated from the diagnostic criteria for all myeloid neoplasms. For all cases (even those with ≥50% bone marrow erythroid cells), the bone mar-
row blast percentage is now expressed as a percentage of all nucleated marrow cells. This will result in most cases that previously would have been classified as erythroleukaemia (i.e. those in which blasts constitute <20% of all nucleated marrow cells) now being classified as MDS with excess blasts, rather than as a subtype of AML. The diagnostic approach to dealing with myeloid proliferations with increased numbers of erythroid cells is summarized in Table 1.01. The revised classification of MDS is shown in the WHO classification table at the beginning of this volume (p. 10); the rationale for the changes is provided in the sections on each MDS entity.

Acute myeloid leukaemia
AML results from the clonal expansion of myeloid blasts in the peripheral blood, bone marrow, or other tissue. It is a heterogeneous disease clinically, morphologically, and genetically and can involve a single or all myeloid lineages. Worldwide, the annual incidence is approximately 2.5–3 cases per 100,000 population per year, and is reportedly highest in Australia, western Europe, and the USA. The median patient age at diagnosis is 65 years, and there is a slight male predominance in most countries. In children aged <15 years, AML constitutes 15–20% of all cases of acute leukaemia, with peak incidence in the first 3–4 years of life [960,4409].

The requisite blast percentage for a diagnosis of AML is ≥20% myeloblasts, monoblasts/promonocytes, and/or megakaryoblasts in the peripheral blood or bone marrow. The diagnosis of myeloid sarcoma is synonymous with AML regardless of the number of blasts in the peripheral blood or bone marrow. If there is a prior history of MPN or MDS/MPN, myeloid sarcoma is evidence of acute transformation (blast phase). A diagnosis of AML can also be made when the blast percentage in the peripheral blood and/or bone marrow is <20% if there is an associated t(8;21)(q22;q22), inv(16)(p13.1q22), or t(16;16)(p13.1;q22) chromosomal abnormality or PML-RARA fusion. Although the line between AML and MDS when other recurrent cytogenetic abnormalities are present is increasingly blurred, such cases continue to be classified on the basis of peripheral blood and bone marrow blast cell counts. The revised classification also continues to place a high proportion of cases into the AML, NOS category, for which the prognosis is variable. This is particularly true in paediatric AML [3503], but studies seeking additional prognostic markers in all age groups are probably warranted. Although the diagnosis of AML according to the above guidelines is operationally useful by indicating an underlying defect in myeloid maturation, the diagnosis does not necessarily confer a mandate to treat the patient for AML; clinical factors, including the pace of progression of the disease, must always be taken into consideration when choosing therapy.

Rationale for the diagnosis and classification of acute myeloid leukaemia
The 3rd edition of the WHO classification ushered in the era of formal incorporation of genetic abnormalities in the diagnostic algorithms for AML. The abnormalities included were mainly chromosomal translocations involving transcription factors and associated with characteristic clinical, morphological, and immunophenotypic features that defined a clinicopathological and genetic entity. As our knowledge about leukaemogenesis has increased, so has the acceptance that the genetic abnormalities leading to leukaemia are not only heterogeneous, but also complex; multiple aberrations often contribute in a multistep process to initiate the complete leukaemia phenotype. Experimental evidence suggests that in cases with rearrangements or mutations in genes (e.g. RUNX1, CBFB, and RARA) that encode transcription factors implicated in myeloid differentiation, an additional genetic abnormality is necessary to promote proliferation or survival of the neoplastic clone [1984]. Often, this additional abnormality is a mutation of a gene (e.g. FLT3 or KIT) that encodes proteins that activate signal transduction pathways to promote proliferation/survival. A similar multistep process is also evident in AML that evolves from MDS or that has myelodysplasia-related features, often characterized by loss of genetic material and haploinsufficiency of genes. Within the past few years, novel genetic mutations have also been identified in essentially all types of AML [545,2774], and our approach to and understanding of gene mutations in AML has evolved (see Table 1.02, p. 26). Some of the mutations, such as those of CEBPA and perhaps NPM1, involve transcription factors; others, including those of FLT3, NRRAS, and KRAS, affect signal transduction. An emerging class of mutations in epigenetic regulators, including TET2, IDH1, IDH2, ASXL1, DNMT3A, and cohesin complex family members, are seen in nearly half of all AML cases. These discoveries have improved our understanding of the pathogenesis of AML and suggest that many cases are driven by mutations in ≥3 distinct biological pathways, which act in concert to induce progression from normal haematopoietic stem/progenitor cells to clonal, preleukaemic stem/progenitor cells, to overtly transformed leukaemic cells. Not only have these mutations informed our understanding of leukaemogenesis in cytogenetically normal AML, they have also proved to be powerful prognostic factors [2774]. Genetic abnormalities in AML elucidate the pathogenesis of the neoplasm, provide the most reliable prognostic information, and will likely lead to the development of more-successful targeted therapies. Therefore, the use of genomic profiling is a critical aspect of the evaluation and risk stratification of AML in the clinical context.

One of the challenges in this revision and in the original 4th edition has been how to incorporate important and/or recently acquired genetic information into a classification scheme for AML and yet adhere to the WHO principle of defining homogeneous, biologically relevant entities based not only on genetic studies or their prognostic value, but also on clinical, morphological, and/or immunophenotypic studies. This was particularly problematic with the most frequent and prognostically important mutations in cytogenetically normal AML: mutations in FLT3, NPM1, RUNX1, and CEBPA. Cases with these mutations have few or variably consistent morphological, immunophenotypic, and clinical features reported to date, and the mutations are not mutually exclusive. For the most part, the framework established in the 3rd edition and
Table 1.02: Functional complementation groups of genetic alterations in acute myeloid leukaemia

<table>
<thead>
<tr>
<th>Period</th>
<th>Analysis</th>
<th>2008–2012</th>
<th>From 2013</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cytogenetic and molecular genetic analysis</td>
<td></td>
<td>The Cancer Genome Atlas (TCGA) project (545)</td>
</tr>
<tr>
<td></td>
<td>Next-generation sequencing approaches</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Functional groups</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Class I</td>
<td>Activated signalling</td>
<td>e.g. FLT3, KIT, and RAS mutations</td>
<td>Class 1 – Transcription factor fusions</td>
</tr>
<tr>
<td></td>
<td>Class II</td>
<td>Transcription and differentiation</td>
<td>e.g. t(8:21), inv(16), t(15:17), CEBPA and RUNX1 mutations</td>
</tr>
<tr>
<td></td>
<td>Class II</td>
<td>Transcription and differentiation</td>
<td>e.g. t(8:21), inv(16), t(15:17), CEBPA and RUNX1 mutations</td>
</tr>
<tr>
<td></td>
<td>Epigenetic modifiers</td>
<td>(so-called ‘Class III’)</td>
<td>e.g. TET2, DNMT3A, and ASXL1 mutations</td>
</tr>
<tr>
<td></td>
<td>Class 5</td>
<td>Activated signalling genes</td>
<td>e.g. FLT3, KIT, RAS mutations</td>
</tr>
<tr>
<td></td>
<td>Class 6</td>
<td>Chromatin-modifying genes</td>
<td>e.g. ASXL1 and EZH2 mutations, KMT2A fusions, KMT2A-PTD</td>
</tr>
<tr>
<td></td>
<td>Class 7</td>
<td>Myeloid transcription factor genes</td>
<td>e.g. CEBPA, RUNX1 mutations</td>
</tr>
<tr>
<td></td>
<td>Class 8</td>
<td>Cohesin complex genes</td>
<td>e.g. STAG2, RAD21, SMC1, SMC2 mutations</td>
</tr>
<tr>
<td></td>
<td>Class 9</td>
<td>Spliceosome-complex genes</td>
<td>e.g. SRSF2, U2AF1, ZRSR2 mutations</td>
</tr>
</tbody>
</table>

Studies have shown no difference in de novo cases with and without this finding [211,975,1145]. Lastly, the provisional category of AML with mutated RUNX1 has been added for de novo cases with this mutation that are not associated with MDS-related cytogenetic abnormalities. This provisional category appears to represent a biologically distinct form of AML (1274,2627,3576,3897). AML with mutated FLT3 is not included as a separate entity, because FLT3 mutation occurs across multiple AML subtypes; however, the significance of this mutation should not be underestimated, and it should be tested for in essentially all cases, including those with NPM1 or CEBPA mutation or other recurrent genetic abnormalities. Broader gene panels are becoming increasingly available and are probably indicated in most, if not all, types of AML. Modifications have been made to the AML with myelodysplasia-related changes subgroup. Cases should still be assigned to this category if they evolve from previously documented MDS, have specific myelodysplasia-related cytogenetic abnormalities, or exhibit morphological multilineage dysplasia. However, these features do not supersede therapy-related disease or the defined cytogenetic categories of AML. As mentioned above, de novo cases with NPM1 or biallelic CEBPA mutation with no MDS-related cytogenetic abnormalities, but with multilineage dysplasia, are now classified as either AML with mutated NPM1 or AML with biallelic mutation of CEBPA. The cytogenetic abnormalities that define MDS-associated disease have also been modified: del(9q), which does not appear to have prognostic significance in the setting of NPM1 or biallelic CEBPA mutation, has been removed from the list (1511,3562), as has monosomy 5; del(5q) and unbalanced translocation involving 5q remain (1559,4209).
Therapy-related myeloid neoplasms (i.e. therapy-related AML, MDS, and MDS/MPN) remain in the revised classification as a distinct subgroup. Most patients who develop therapy-related neoplasms have received therapy with both alkylating agents and topoisomerase II inhibitors, so division according to type of therapy remains impractical. It has been argued that ≥90% of cases of therapy-related AML have cytogenetic abnormalities similar to those seen in AML with recurrent genetic abnormalities or AML with myelodysplasia-related changes, and could be assigned to those categories. However, in most reported series, therapy-related myeloid neoplasms – except therapy-related AML with inv(16) (p13.1q22), t(16;16)(p13.1;q22), or PML-RARA – have a significantly worse clinical outcome than do their de novo counterparts with the same genetic abnormalities [94,392,3435,3699,3709], suggesting some biological differences between the two groups. The study of therapy-related neoplasms may provide valuable insight into the pathogenesis of de novo disease by providing clues as to why certain patients develop leukaemia whereas most patients treated with the same therapies do not. Therefore, cases of therapy-related neoplasms should always be designated as such, and any specific genetic abnormality should also be listed as part of the diagnosis; for example, therapy-related AML with t(9;11) (p21.3;q23.3).

The category ‘acute myeloid leukaemia, NOS’ encompasses the cases that do not fulfill the specific criteria for any of the other entities. This group currently accounts for 25–30% of all AML cases. If cases of AML with mutated NPM1 or biallelic mutation of CEBPA are removed from this group as is advocated in this revised classification, the subtypes of AML, NOS, no longer have prognostic significance (4233). The number of cases that fall into the AML, NOS, category will continue to diminish as more genetic subgroups are identified. As mentioned above, the category ‘acute erythroid leukaemia (erythroid/myeloid)’ has been removed from the classification, and most of these cases are now classified as MDS. Myeloid sarcoma, an extramedullary tumour mass consisting of myeloid blasts, is included in the classification as a distinct pathological entity. However, when myeloid sarcoma occurs de novo, the diagnosis is equivalent to a diagnosis of AML, and further evaluation (including genetic analysis) is necessary to determine the appropriate classification of the leukaemia [3177]. When the peripheral blood and/or bone marrow are concurrently involved by AML, these specimens can be used for analysis and further classification. However, when the myeloid sarcoma precedes evidence of peripheral blood or bone marrow involvement, the immunophenotype should be ascertained by flow cytometry and/or immunohistochemistry, and the genotype determined by cytogenetic analysis or (in the absence of fresh tissue) by FISH or molecular analysis for recurrent genetic abnormalities. Myeloid sarcoma may also be the initial indication of relapse in a patient previously diagnosed with AML, or may indicate disease progression to AML or to the blast phase in patients with a prior diagnosis of MDS, MDS/MPN, or MPN.

As in the original 4th edition, the unique features of myeloid proliferations associated with Down syndrome are addressed in a separate category, which encompasses transient abnormal myelopoiesis associated with Down syndrome and myeloid leukaemia associated with Down syndrome.

A section on myeloid neoplasms with germline predisposition [1390,4301] has been added to the classification to address cases of AML, MDS, and MDS/MPN that have germline genetic abnormalities. The recognition of such cases should lead to screening of family members, which may enable earlier disease detection in affected individuals.
CHAPTER 2

Myeloproliferative neoplasms
Chronic myeloid leukaemia, \textit{BCR-ABL1}-positive
Chronic neutrophilic leukaemia
Polycythaemia vera
Primary myelofibrosis
Essential thrombocythaemia
Chronic eosinophilic leukaemia, NOS
Myeloproliferative neoplasm, unclassifiable
Chronic myeloid leukaemia, BCR-ABL1-positive

Definition
Chronic myeloid leukaemia (CML), BCR-ABL1-positive, is a myeloproliferative neoplasm (MPN) in which granulocytes are the major proliferative component. It arises in a haematopoietic stem cell and is characterized by the chromosomal translocation t(9;22)(q34.1;q11.2), which results in the formation of the Philadelphia (Ph) chromosome, containing the BCR-ABL1 fusion gene {282,2620,2905,3433}. In CML, BCR-ABL1 is found in all myeloid lineages and in some lymphoid and endothelial cells [1210,1496]. The natural history of untreated CML is biphasic or triphasic: an initial indolent chronic phase (CP) is followed by an accelerated phase (AP), a blast phase (BP), or both. The diagnosis requires detection of the Ph chromosome and/or BCR-ABL1 in the appropriate clinical and laboratory settings.

ICD-O code 9875/3

Synonyms
Chronic myelogenous leukaemia, BCR-ABL1-positive; chronic granulocytic leukaemia, SC/T-ASL/-positive (9863/3); chronic myelogenous leukaemia, Philadelphia chromosome-positive (Ph-i); chronic myelogenous leukaemia, t(9;22)(q34;q11); chronic granulocytic leukaemia, Philadelphia chromosome-positive (Ph-f); chronic granulocytic leukaemia, BCR/ABL1; chronic myeloid leukaemia (9863/3)

Epidemiology
Worldwide, CML has an annual incidence of 1–2 cases per 100,000 population, with a slight male predominance. The annual incidence increases with age, from <0.1 cases per 100,000 children to ≥2.5 cases per 100,000 elderly individuals [1662,1749]. Significant ethnic or geographical variations in incidence have not been reported, but an earlier patient age at onset has been reported in areas where socioeconomic status is lower [2626]. Due to the success of tyrosine kinase inhibitor (TKI) therapy in reducing mortality rates (down to only 2–3% per year), the prevalence of CML is expected to increase considerably [1725].

Etiology
The predisposing factors for CML are largely unknown. Acute radiation exposure has been implicated, largely due to the reported increased incidence of CML among atomic bomb survivors [387,815]. Unlike other MPNs, there is slight, if any, inherited predisposition [2209,3306].

Localization
In CP, the leukaemia cells are minimally invasive and mostly confined to the blood, bone marrow, spleen, and liver, in BP, the blasts can infiltrate any extramedullary site, with a predilection for spleen, liver, lymph nodes, skin, and soft tissue [822,1810,2776].

Clinical features
Most patients with CML are diagnosed in CP, which usually has an insidious onset. Nearly 50% of newly diagnosed cases are asymptomatic and discovered when a white blood cell (WBC) count performed as part of a routine medical examination is found to be abnormal [822,1809]. Common findings at presentation include fatigue, malaise, weight loss, night sweats, and anaemia, and about 50% of patients have palpable splenomegaly [822,1662,1809,3534]. Atypical presentations include marked thrombocytosis without leukocytosis that mimics essential thrombocythaemia or other types of MPN [512,618,3732]. About 5% of cases are diagnosed in AP or BP without a recognized CP [1662,3534]. Without effective therapy, most cases of CML progress from CP to BP (directly or via AP) within 3–5 years after diagnosis [822,1602]. These transformed stages are characterized clinically by declining performance status, constitutional signs such as fever and weight loss, and symptoms related to severe anaemia, thrombocytopenia, increased WBC count, splenic enlargement, and in BP, a dismal outcome [1601,1809]. With targeted TKI therapy and careful disease monitoring, the incidence of AP and BP has decreased, and the 10-year overall survival rate for CML is 80–90% [207,573,1602,1904].

Fig. 2.01 Splenomegaly in chronic myeloid leukaemia, BCR-ABL1-positive. A The gross appearance of the spleen is solid and uniformly deep red, although areas of infarction may appear as lighter-coloured regions. B The red pulp distribution of the infiltrate usually compresses and obliterates the white pulp. C The leukaemic cells are present in the splenic cords and sinuses. In this case the cells are shifted towards immature forms; care must be taken to assess the maturity of the cells in splenectomy specimen, because enlarging spleens, particularly in the face of TKI therapy, may be associated with disease progression.

Vardiman J.W.
Melo J.V.
Baccarani M.
Radich J.P.
Kvasnicka H.M.

Myeloproliferative neoplasms
Microscopy

Chronic phase

In CP, the peripheral blood shows leukocytosis (12–1000 × 10⁹/L; median: ~80 × 10⁹/L) due to neutrophils in various stages of maturation, with peaks in the proportions of myelocytes and segmented neutrophils (1662,3534,3753); children often have higher WBC counts than adults (median: ~250 × 10⁹/L) (1374,2665,3836). Significant granulocytic dysplasia is absent. Blasts typically account for <2% of the WBCs. Absolute basophilia and eosinophilia are common (1662,3753). Absolute monocytes may be present, but the proportion of monocytes is usually <3% (339), except in rare cases with the p190 BCR-ABL1 isoform, which often mimic chronic myelomonocytic leukaemia (2623). Platelet counts are normal or increased to ≥1000 × 10⁹/L; marked thrombocytopenia is uncommon in CP (1662).

Most cases of CML can be diagnosed on the basis of peripheral blood findings combined with detection of the Ph chromosome and/or BCR-ABL1 by cytogenetic and molecular genetic techniques. However, bone marrow aspiration is essential to ensure sufficient material for a complete karyotype and for morphological evaluation to confirm the phase of disease. Bone marrow biopsy is not required to diagnose CML in most cases, but should be done if the findings in the peripheral blood are atypical or if a cellular aspirate cannot be obtained (1662, 2911).

In CP, bone marrow specimens are hypercellular, with marked granulocytic proliferation and a maturation pattern similar to that in the blood, including expansion at the myelocyte stage (822). There is no significant dysplasia. Blasts usually account for <5% of the marrow cells; ≥10% suggests advanced disease (817). The proportion of erythroid precursors is usually significantly decreased. Megakaryocytes may be normal or slightly decreased in number, but 40–50% of cases exhibit moderate to marked megakaryocytic proliferation (495,3976,3982). In CP, the megakaryocytes are smaller than normal and have hyposegmented nuclei; they are referred to as ‘dwarf’ megakaryocytes, but are not true micromegakaryocytes (5–10 cells in thickness) seen in myelodysplastic syndromes (853,3982). Eosinophils and basophils are usually increased in number, and pseudo-Gaucher cells are common. These features are mirrored in marrow biopsy sections, in which a layer of immature granulocytes (5–10 cells in thickness) is common around the bone trabeculae, in contrast to the normal thickness of 2–3 cells (853).
Moderate to marked reticulin fibrosis, which correlates with increased numbers of megakaryocytes and may be associated with an enlarged spleen, has been reported in 30–40% of biopsies at diagnosis (490,495,1329,3982). Although the presence of fibrosis at the time of diagnosis was reported to be associated with a worse outcome in the pre-TKI era, it reportedly has no substantial impact on prognosis in patients treated with TKIs (926,1932).

Splenomegaly in CP is due to infiltration of the red pulp cords by mature and immature granulocytes. A similar infiltrate can be seen in hepatic sinuses and portal areas.

### Disease phases

#### Disease progression: accelerated phase and blast phase

Recognition of disease progression is important for treatment and prognostic purposes, but the clinical and morphological boundaries between CP, AP, and BP are not always sharp, and the parameters used to define them differ between investigators. These categories were of substantial prognostic importance in the pre-TKI era, when effective treatment without allogeneic transplant was not available, but the effectiveness of TKIs has further blurred the lines between these phases of CML. For example, some studies show that newly diagnosed patients who initially present in AP may have similar outcomes, when treated with TKIs, as those of patients with newly diagnosed CP (2939,3324). In contrast, AP disease that develops during TKI therapy has a poor outcome. Furthermore, gene expression studies of CP, AP, and BP suggest that progression of CP to AP and/or BP is more consistent with a two-step process, with new gene expression profiles occurring early in AP (or late in CP), before the accumulation of blasts and other features often used to define AP (3277). BP continues to have a very poor outcome, even with TKI therapy (1601). Death occurs due to bleeding or infectious complications, as normal haematopoiesis is increasingly disrupted by the malignant cells. In BP, the increase in blasts not only indicates a loss of response to therapy, but also signifies that the disease has acquired characteristics of acute leukaemia.

#### Accelerated phase

In the original 4th edition of the classification, it was recommended that the diagnosis of AP be made if any of the following parameters were present:
1. a persistent or increasing high WBC count (>10 × 10⁹/L) and/or persistent or increasing splenomegaly, unresponsive to therapy;
2. persistent thrombocytosis (>1000 × 10⁹/L), unresponsive to therapy;
3. persistent thrombocytopenia (<100 × 10⁹/L), unrelated to therapy;
4. evidence of clonal cytogenetic evolution, defined by cells harbouring the Ph chromosome and additional cytogenetic changes;
5. ≥20% basophils in the peripheral blood; and
6. 10–19% blasts in the peripheral blood and/or bone marrow. In addition, large clusters or sheets of small, abnormal megakaryocytes associated with marked reticulin or collagen fibrosis were considered to be presumptive evidence of AP, particularly if accompanied by any of the haematological parameters listed above. Although other defining criteria for AP have been
suggested [817], these clinical, haematological, morphological, and genetic parameters are evidence of disease progression (Table 2.01).

When defined as above, however, AP includes a very heterogeneous group of cases, so these parameters alone are insufficient for prognostic purposes. However, their utility may be increased by the consideration of additional response-defined parameters [1535]. Response to therapy (e.g. TKIs or allogeneic transplant) is linked to the phase of disease. For example, the rare cases defined at diagnosis as AP solely on the basis of additional cytogenetic changes [1124] but who do not have an increase in blasts respond to therapy similar to patients with CP disease, whereas patients with newly diagnosed AP disease with additional cytogenetic abnormalities and increased blasts do appreciably worse [3324]. Moreover, cases in clinical and morphological CP that develop resistant BCR-ABL1 mutations have gene expression patterns similar to those seen in advanced disease [2605,3277]. Therefore, response-to-therapy parameters are now included as provisional criteria for AP, pending verification of their validity. According to these provisional criteria, CP cases can be considered to be functionally in AP (with poor rates of long-term, progression-free survival) if there is (1) haematological resistance to the first TKI, (2) any grade of resistance to two sequential TKIs, or (3) occurrence of two or more BCR-ABL1 mutations (Table 2.01).

In AP, bone marrow specimens are often hypercellular, and dysplastic changes may be seen in any of the myeloid lineages [2776,4395]. Clusters of small megakaryocytes (including true micromegakaryocytes similar to those seen in myelodysplastic syndromes) may be present and may be associated with significant reticulin and/or collagen fibrosis, which is best assessed in biopsy sections. The increased proportion of blasts in AP (10–19%) may be highlighted with immunohistochemical staining for CD34 [2989,3960]. In most cases, the blasts have a myeloid phenotype [1131]. Lymphoid blasts may be seen, but some data suggest that the finding of any bona fide lymphoblasts in the blood or bone marrow (even if <10%) should prompt concern that lymphoblastic transformation may be imminent, and warrants further clinical and genetic investigation.

**Blast phase**

The criteria for BP include (1) >20% blasts in the blood or bone marrow or (2) the presence of an extramedullary proliferation of blasts. Some investigators and clinical trials have instead used a threshold of >30% blasts in the blood and/or bone marrow to define BP, but most patients in BP have a very poor prognosis regardless of which cut-off point is used [817,1601]. In most BP cases, the blast lineage is myeloid, and may include neutrophilic, monocyctic, megakaryocytic, basophilic, eosinophilic, or erythroid blasts, or any combination thereof [1131,1601,1997, 2806]. In approximately 20–30% of BP

---

**Table 2.01 Defining criteria for the accelerated phase (AP) of chronic myeloid leukaemia (CML)**

**CML-AP** is defined by the presence of ≥1 of the following haematological/cytogenetic criteria or provisional criteria concerning response to tyrosine kinase inhibitor (TKI) therapy:

<table>
<thead>
<tr>
<th>Haematological/cytogenetic criteria³</th>
<th>Provisional response-to-TKI criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Persistent or increasing high white blood cell count (&gt; 10 × 10⁹/L), unresponsive to therapy</td>
<td>- Haematological resistance (or failure to achieve a complete haematological response) to the first TKI</td>
</tr>
<tr>
<td>Persistent or increasing splenomegaly, unresponsive to therapy</td>
<td>- Any haematological, cytogenetic, or molecular indications of resistance to two sequential TKIs</td>
</tr>
<tr>
<td>Persistent thrombocytosis (&gt; 1000 × 10⁹/L), unresponsive to therapy</td>
<td>- Occurrence of two or more mutations in the BCR-ABL1 fusion gene during TKI therapy</td>
</tr>
<tr>
<td>Persistent thrombocytopenia (&lt; 100 × 10⁹/L), unrelated to therapy</td>
<td>- Any new clonal chromosomal abnormality in Ph+ cells that occurs during therapy</td>
</tr>
<tr>
<td>≥ 20% basophils in the peripheral blood</td>
<td></td>
</tr>
<tr>
<td>10–19% blasts in the peripheral blood and/or bone marrow</td>
<td></td>
</tr>
<tr>
<td>Additional clonal chromosomal abnormalities in Philadelphia (Ph) chromosome-positive (Ph+) cells at diagnosis, including so-called major route abnormalities (a second Ph chromosome, trisomy 8, isochromosome 17q, trisomy 19), complex karyotype, and abnormalities of 3q26.2</td>
<td></td>
</tr>
</tbody>
</table>

³ Large clusters or sheets of small, abnormal megakaryocytes associated with marked reticulin or collagen fibrosis in biopsy specimens may be considered presumptive evidence of AP, although these findings are usually associated with one or more of the criteria listed above.

² The finding of bona fide lymphoblasts in the peripheral blood or bone marrow (even if <10%) should prompt concern that lymphoblastic transformation may be imminent, and warrants further clinical and genetic investigation.

- ≥20% blasts in the peripheral blood or bone marrow, or an infiltrative proliferation of blasts in an extramedullary site, is diagnostic of the blast phase of CML.
- Complete haematological response is defined as white blood cell count < 10 × 10⁹/L, platelet count < 450 × 10⁹/L, no immature granulocytes in the differential, and spleen not palpable.

---

Fig. 2.06 Chronic myeloid leukaemia, BCR-ABL1-positive (lymphoid blast phase). A Peripheral blood smear with numerous lymphoid-morphology blasts among leukaemic myelocytes. B Bone marrow biopsy of the same case. C Marrow aspirate smear. By flow cytometry, the blasts coexpressed CD19, CD10, CD22, terminal deoxynucleotidyl transferase (TdT), and CD13, but there was no expression of MPO.
cases, the blasts are lymphoblasts (usually of B-cell origin, although cases of T-lymphoblastic and NK-cell transformation have been reported) [682,2015, 4261]. Sequential lymphoblastic and myeloblastic crises have also been reported. In BP, the blast lineage may be morphologically obvious, but the blasts are often primitive and/or heterogeneous, and expression of antigens of more than one lineage is common (1997, 3335); therefore, cytochemical and immunophenotypic analysis of the blasts is recommended. Extramedullary blast proliferations are most common in the skin, lymph nodes, bone, and CNS, but can occur anywhere; they may be of myeloid, lymphoid, or mixed-lineage phenotype [682,1810]. In bone marrow biopsy specimens, sheets of blasts that occupy focal but substantial areas of the bone marrow (e.g. an entire intertrabecular space or more) can be considered presumptive evidence of BP even if the rest of the marrow shows CP.

Immunophenotype

Immunophenotypic data on CML-CP suggest that the expression of CD7 on CD34+ cells has adverse prognostic significance, whereas a normal CD34+ stem-cell population, lacking expression of abnormal markers (e.g. CD7, CD56, or CD11b), predicts a better response to TKI therapy [2089,3393,4427]. However, the main role of immunophenotyping in CML is analysis of the blasts in BP. In myeloid BP, the blasts have strong, weak, or no MPO activity but express one or more antigens associated with granulocytic, monocyctic, megakaryocytic, and/or erythroid differentiation, such as CD33, CD13, CD14, CD11b, CD11c, KIT (CD117), CD15, CD41, CD61, and glycoporphin A and C. However, in many myeloid BP cases, the blasts also express one or more lymphoid-related antigens (1997,3335,3480). Most lymphoblastic BP blasts are of precursor-B-cell origin and express terminal deoxynucleotidyl transferase (TdT) in addition to B-cell–related antigens (e.g. CD19, CD10, CD79a, PAX5, and CD20), but a minority of lymphoblastic BP blasts are of T-cell origin and express T-cell–related antigens (e.g. CD3, CD2, CD5, CD4, CD8, and CD7). Expression of one or more myeloid-related antigens by the blasts is common in both B- and T-cell–derived blast transformations (2015,4261). Bilineage cases (i.e. with distinct populations of myeloid and lymphoid blasts) also occur [682, 3480], and sequential lymphoblastic BP and myeloblastic BP have been reported [525]. A recent study showed a higher frequency of unusual blast types and immunophenotypes (e.g. basophil blasts or megakaryoblasts) after the introduction of TKI therapy [3647]. Flow cytometry is the preferred technique for phenotypic analysis in order to detect mixed phenotypes, but immunohistochemical stains can also be applied if a marrow aspirate cannot be obtained and there are insufficient numbers of blasts in the blood.

Cell of origin

CML originates from an abnormal pluripotent bone marrow stem cell. However, disease progression may originate in more committed precursors than previously supposed, given that myeloid BP has been reported to involve the granulocyte–macrophage progenitor pool rather than the haematopoietic stem cell pool [2655]. It is unknown whether this is also the case with lymphoid BP. Recent data have shown that the quiescent leukaemic stem cells do not rely on BCR-ABL1 for survival, and are refractory to TKI therapy [808,1529].
Genetic profile

At diagnosis, 90–95% of cases of CML have the characteristic t(9;22) (q34.1;q11.2) reciprocal translocation that results in the Ph chromosome, der(22) (t(9;22)) [3433]. This translocation fuses sequences of the BCR gene on chromosome 22 with regions of ABL1 on chromosome 9 [282]. The remaining cases have either variant translocations that involve a third or even a fourth chromosome in addition to chromosomes 9 and 22, or a cryptic translocation of 9q34.1 and 22q11.2 that cannot be identified by routine cytogenetic analysis. In such cases, the BCR-ABL1 fusion gene is present and can be detected by FISH analysis and/or RT-PCR [2619].

The site of the breakpoint in chromosome 22 may influence the phenotype of the disease [2619]. In CML, the breakpoint cluster region (BCR) is almost always in the major BCR (M-BCR), spanning exons 12-16 (previously known as b1-b5) and an abnormal fusion protein, p210, is formed, which has increased tyrosine kinase activity. Rarely, the breakpoint in the BCR gene occurs in the u-BCR region, spanning exons 17-20 (previously known as c1-c4), and a larger fusion protein, p230, is encoded. Patients with this fusion may demonstrate prominent neutrophilic maturation and/or conspicuous thrombocytosis [2619,3048]. Although breaks in the minor breakpoint region, m-BCR (exons 1-2) lead to a short fusion proteins (p190) and is most frequently associated with Ph chromosome-positive ALL, small amounts of the p190 transcript can be detected in more than 90% of patients with CML as well, due to alternative splicing of the BCR gene [4140]. However, this breakpoint may also be seen in rare cases of CML associated with increased numbers of monocytes, which can resemble chronic myelomonocytic leukaemia [2623].

It is generally accepted that the increased tyrosine kinase activity of BCR-ABL1 is necessary and sufficient to cause CML-CP through the constitutive activation of proteins in several signal transduction pathways. Among the many pathways affected are the JAK/STAT

---

**Fig. 2.09** Chronic myeloid leukaemia, BCR-ABL1-positive (chronic phase): incidence of reported mutations within the kinase domain by percentage of the total. The seven most frequent mutations are depicted in red and the next eight in blue. The mutations shown in green have been reported in <2% of clinical resistance cases. Specific regions of the kinase domain are indicated as a P-loop or ATP-binding site (P), an imatinib-binding site (B), a catalytic domain (C), and an activation loop (A). The contact regions with SH2 and SH3 domain-containing proteins are also shown. Based on data from Apperley JF [122] and Soverini S et al. [3743A].

---

**Fig. 2.10** Chronic myeloid leukaemia, BCR-ABL1-positive. A Schematic representation of the t(9;22) chromosomal translocation, the fusion mRNA transcripts encoded by the BCR-ABL1 fusion gene generated in the changed chromosome 22 and the Philadelphia (Ph) chromosome, and the translated BCR-ABL1 fusion protein (p210) — whose oncogenic properties are due primarily to its constitutively activated tyrosine kinase, encoded by the SH1 domain (indicated in red). Some of the other important functional domains contributed by the BCR and ABL1 portions of the oncoprotein are also shown: the dimerization domain (DD); Y177, which is the autophosphorylation site crucial for binding to GRB2; the phospho-serine/threonine (P-S/T) SH2-binding domain; a region homologous to Rho guanine nucleotide exchange factors (rho-GEF); the ABL1 regulatory SH3 and SH2 domains; Y412, which is the major site of autophosphorylation within the SH1 kinase domain; nuclear localization signals (NLS); and the DNA-binding and actin-binding domains. B Mechanism of action of BCR-ABL1 tyrosine kinase inhibitors. The physiological binding of ATP to its pocket allows BCR-ABL1 to phosphorylate selected tyrosine residues on its substrates (left panel); a synthetic ATP mimic such as imatinib fits the pocket (right panel), but does not provide the essential phosphate group to be transferred to the substrate. The downstream chain of reactions is then halted because, with its tyrosines in the unphosphorylated form, the substrate does not assume the necessary conformation to ensure association with its effector.
(cell growth and survival), PI3K/AKT (cell growth, cell survival, and inhibition of apoptosis), and RAS/MEK (activation of transcription factors, including NF-κB) pathways [689,3771]. The understanding of the abnormal signalling in CML cells led to the design and synthesis of small molecules that target the tyrosine kinase activity of BCR-ABL1, of which imatinib mesylate was the first to be successfully used to treat CML [1041,1042]. Imatinib competes with ATP for binding to the BCR-ABL1 kinase domain, thus preventing phosphorylation of tyrosine residues on its substrates. Interruption of the oncogenic signal in this way is effective for control of the disease, particularly when used early in CP. However, the emergence of subclones of leukemic progenitor cells that have BCR-ABL1 point mutations that alter amino acids and prevent the binding of the inhibitor to the BCR-ABL1 kinase domain can lead to drug resistance, particularly in AP and BP [122]. The second and third generations of TKIs (i.e. nilotinib, dasatinib, bosutinib, and ponatinib) can circumvent this form of drug failure in the presence of most kinase domain mutations [4281]. The molecular basis of transformation is still largely unknown [2620]. Progression is usually associated with clonal evolution; at the time of transformation to AP or BP, 80% of cases demonstrate cytogenetic changes in addition to the Ph chromosome, including an extra Ph chromosome, isochromosome 17q, and gain of chromosome 8 or 19, the so-called major route karyotypic abnormalities. The presence of any of these abnormalities in the karyotype at the time of initial diagnosis of CML has been reported to be an adverse prognostic finding, and places a case in the AP category [1161]. Genes reported to be altered in the transformed stages include TP53, RB1, MYC, CDKN2A (also called p16INK4a), NRAS, Kras, RUNX1 (also called AML1), MECOM (also called EVI1), TET2, CBL, ASXL1, IDH1, and IDH2, but their exact role (if any) in the transformation is unknown [2453,3743]. The introduction of genome-wide expression profiling by microarray technology has revealed other candidate genes associated with the advanced stages, and has also revealed similar gene expression patterns in AP and BP, suggesting that the genetic events leading to transformation in AP and BP occur in late CP or early AP [4426]. These studies have also shown that progression to advanced-phase disease often shares biological features associated with resistance to TKI therapy [3277].

Prognosis and predictive factors
In the current era of TKI therapy, the most important prognostic indicator is response to treatment at the haematological, cytogenetic, and molecular level. However, the risk scores based on clinical and haematological findings (the Sokal score [3716] and EUTOS score [1582]) are also valid, with low-risk patients responding to TKIs significantly better than high-risk patients [207]. Overall, the complete cytogenetic response rate to first-line TKI is 70–90%, with 5-year progression-free and overall survival rates of 80–95%. A major molecular response (BCR-ABL1 <0.1% on the international scale) and a deeper molecular response (BCR-ABL1 ≤0.01%) are achieved faster and more frequently with second-generation TKIs, predicting that a condition of treatment-free remission will be achieved in more patients than with imatinib. However, the progression-free survival rate is only marginally improved, and overall survival is the same irrespective of the TKI used as first-line therapy. Today, few patients die from leukaemia, and the overall survival is similar to that of the non-leukaemic population [207,1602].
Chronic neutrophilic leukaemia

Definition
Chronic neutrophilic leukaemia is a rare myeloproliferative neoplasm characterized by sustained peripheral blood neutrophilia, bone marrow hypercellularity due to neutrophilic granulocyte proliferation, and hepatosplenomegaly. There is no Philadelphia (Ph) chromosome or BCR-ABL1 fusion gene. The diagnosis requires exclusion of reactive neutrophilia and other myeloproliferative and myelodysplastic/myeloproliferative neoplasms (Table 2.02, p.38).

ICD-O code 9963/3

Epidemiology
The true incidence of chronic neutrophilic leukaemia is unknown; >200 cases have been reported, but only about 150 of these meet the current diagnostic criteria [231]. In one study of 660 cases of chronic leukaemias of myeloid origin, not a single case of chronic neutrophilic leukaemia was observed [3642]. Chronic neutrophilic leukaemia generally affects older adults, but has also been reported rarely in adolescents and young adults [1588,4441,4501]. In 116 patients whose age and sex were reported, the median age at presentation was 66 years and the male-to-female ratio was 1.6:1 [231].

Etiology
The cause of chronic neutrophilic leukaemia is unknown. Some cases have followed cytotoxic chemotherapy [1095]. Its occurrence in a father and son has been reported [2072]. In about a quarter of reported cases of chronic neutrophilic leukaemia or an apparently similar condition, the neutrophilia was associated with an underlying neoplasm, most often multiple myeloma or monoclonal gammopathy of undetermined significance. The majority of such cases constitute a neutrophilic leukaemoid reaction resulting from synthesis of granulocyte colony-stimulating factor by neoplastic plasma cells. A very small number of patients appear to have had both a plasma cell neoplasm and true chronic neutrophilic leukaemia [391,2845], but there is no reason to postulate a relationship between the two conditions. Acute myeloid leukaemia has supervened in 3 cases of a chronic neutrophilic leukaemia-like condition associated with a plasma cell neoplasm, but all 3 patients had been exposed to leukaemogenic drugs before the emergence of acute myeloid leukaemia [995,2294,4079]. The association of a condition resembling chronic neutrophilic leukaemia with other neoplasms is also likely to reflect a leukaemoid reaction.

Localization
The peripheral blood and bone marrow are always involved, and the spleen and liver usually show leukaemic infiltrates. However, any tissue can be infiltrated [4035,4441,4501].

Clinical features
The most constant clinical feature reported is splenomegaly, which may be symptomatic. Hepatomegaly is usually present as well [4441,4501]. There may be bruising and purpura. A history of bleeding from mucocutaneous surfaces or from the gastrointestinal tract is reported in 25-30% of cases [1588,4501]. Gout and pruritus are other possible features [4501]. Serum vitamin B12 and uric acid are often elevated.

Imaging
Imaging may show enlargement of the liver or spleen.

Microscopy
The peripheral blood shows neutrophilia, with a white blood cell count ≥25 x 10^9/L. The neutrophils are usually segmented, but there may also be a substantial increase in band forms. In almost all cases, neutrophil precursors (promyelocytes, myelocytes, and metamyelocytes) account for <5% of the white blood cells, but occasionally they may account for as much as 10% [515,1094,1096A,1588,4441,4501]. Myeloblasts are almost
never observed in the blood. The neutrophils often show toxic granulation and Döhle bodies, but they may also appear normal. However, it should be noted that toxic granulation and Döhle bodies appear to be more consistently present in plasma cell-associated leukaemoid reactions than in chronic neutrophilic leukaemia [231]. Neutrophil dysplasia is not present. Red blood cell and platelet morphology is usually normal. Bone marrow biopsy shows hypercellularity, with neutrophilic proliferation. The myeloid-to-erythroid ratio may reach ≥20:1. Myeloblasts and promyelocytes are not increased in percentage at the time of diagnosis, but the proportion of myelocytes and mature neutrophils is increased. Erythroid and megakaryocytic proliferation may also occur [515, 4441]. Megakaryocytes may be cytologically normal or there may be increased smaller forms. Significant dysplasia is not present in any of the cell lineages; therefore, if it is found, another diagnosis, such as atypical chronic myeloid leukaemia (CML), BCR-ABL1-negative, should be considered. Reticulin fibrosis is uncommon [515, 1094, 4441, 4501].

Given the frequency of neutrophilic leukaemoid reaction in association with multiple myeloma and monoclonal gammapathy of undetermined significance, the bone marrow should be examined for evidence of a plasma cell neoplasm [231, 595, 995, 3764, 3765]. If plasma cell abnormalities are present, clonality of the neutrophil lineage should be demonstrated by cytogentic or molecular techniques before a diagnosis of chronic neutrophilic leukaemia is made. Splenomegaly and hepatomegaly result from tissue infiltration by neutrophils. In the spleen, the infiltrate is mainly confined to the red pulp; in the liver, infiltration may affect the sinusoids, portal areas or both (4441, 4501).

Cytochemistry
The neutrophil alkaline phosphatase score is usually elevated, but is occasionally normal or even low [231]. However, because the score is also usually elevated in neutrophilic leukaemoid reactions, this is not a diagnostically useful test.

The distinction of chronic neutrophilic leukaemia from the neutrophilic variant of BCR-ABL1-positive CML should rely on molecular analysis. No other cytochemical abnormality has been reported [1588, 4501].

Cell of origin
A haematopoietic stem cell, which may have limited lineage potential [1259, 4417]

Genetic profile
Cytogenetic studies are normal in nearly 90% of cases. In the remaining cases, reported clonal karyotypic abnormalities include gains of chromosomes 8, 9, and 21; del(7q); del(20q) (the most frequently observed abnormality); del(11q); del(12p); nullisomy 17; a complex karyotype; and several non-recurrent translocations [735, 846, 970, 1588, 2630]. ASXL1 mutation is associated with a worse prognosis [1095]. CSF3R mutation was reported in 8 of 20 patients with a diagnosis of atypical CML, BCR-ABL1-negative, in an initial study [2588], but this high prevalence was not confirmed in subsequent investigations [3057, 4248]. JAK2 V617F has been reported in at least a dozen patients [231, 2591] and is sometimes homozygous [1902]; this appears to be an alternative mechanism of leukaemogenesis. Complete cytogenetic remission with imatinib was reported in a patient with chronic neutrophilic leukaemia and t(15;19)(q13;p13.3), suggesting the possibility of an unidentified fusion gene in some cases [735].

Prognosis and predictive factors
Although generally considered a slowly progressive disorder, chronic neutrophilic leukaemia is associated with variable survival, ranging from 6 months to >20 years. The neutrophilia is usually progressive, and anaemia and thrombocytopenia may follow. The development of myelodysplastic features may signal a transformation of the disease to acute myeloid leukaemia [1588, 4501]. Transformation has been reported following cytotoxic therapy, but also in its absence.

Table 2.02 Diagnostic criteria for chronic neutrophilic leukaemia

| 1. - Peripheral blood white blood cell count ≥25 \times 10^9/L |
| - Segmented neutrophils plus band neutrophils constitute ≥80% of the white blood cells |
| - Neutrophil precursors (promyelocytes, myelocytes, and metamyelocytes) constitute <10% of the white blood cells |
| - Myeloblasts rarely observed |
| - Monocyte count <1 \times 10^9/L |
| - No dysgranulopoiesis |
| 2. - Hypercellular bone marrow |
| - Neutrophil granulocytes increased in percentage and number |
| - Neutrophil maturation appears normal |
| - Myeloblasts constitute <5% of the nucleated cells |
| 3. - Not meeting WHO criteria for BCR-ABL1-positive chronic myeloid leukaemia, polycythaemia vera, essential thrombocythaemia, or primary myelofibrosis |
| 4. - No rearrangement of PDGFRA, PDGFRB, or FGFR1, and no PCM1-JAK2 fusion |
| 5. - CSF3R T618I or another activating CSF3R mutation or Persistent neutrophilia (≥3 months), splenomegaly, and no identifiable cause of reactive neutrophilia including absence of a plasma cell neoplasm or, if a plasma cell neoplasm is present, demonstration of clonality of myeloid cells by cytogenetic or molecular studies |

846, 1096A, 2226, 2588, 2630. Coexisting ASXL1 mutation is associated with a worse prognosis [1095]. CSF3R mutation was reported in 8 of 20 patients with a diagnosis of atypical CML, BCR-ABL1-negative, in an initial study [2588], but this high prevalence was not confirmed in subsequent investigations [3057, 4248]. JAK2 V617F has been reported in at least a dozen patients [231, 2591] and is sometimes homozygous [1902]; this appears to be an alternative mechanism of leukaemogenesis. Complete cytogenetic remission with imatinib was reported in a patient with chronic neutrophilic leukaemia and t(15;19)(q13;p13.3), suggesting the possibility of an unidentified fusion gene in some cases [735].
Polycythaemia vera

Definition
Polycythaemia vera (PV) is a chronic myeloproliferative neoplasm (MPN) characterized by increased red blood cell (RBC) production independent of the mechanisms that normally regulate erythropoiesis. Virtually all patients carry the somatic JAK2 V617F gain-of-function mutation or another functionally similar JAK2 mutation that results in proliferation not only of the erythroid lineage but also of granulocytes and megakaryocytes (i.e. pancytosis). Generally, two phases of PV are recognized: a polycythaemic phase, associated with elevated haemoglobin level, elevated haematocrit, and increased RBC mass, and a spent phase or post-polycythaemic myelofibrosis phase (post-PV myelofibrosis), in which cytopenias, including anaemia, are associated with ineffective haematopoiesis, bone marrow fibrosis, extramedullary haematopoiesis, and hypersplenism. The natural progression of PV also includes a low incidence of evolution to a myelodysplastic/pre-leukaemic phase and/or blast phase (i.e. acute leukaemia). All causes of secondary erythrocytosis, heritable polycythaemia, and other MPNs must be excluded. The diagnosis requires integration of clinical, laboratory, and bone marrow histological features, as outlined in Table 2.03.

ICD-O code 9950/3

Synonyms
Polycythaemia rubra vera; proliferative polycythaemia (no longer recommended); chronic erythraemia (obsolete)

Epidemiology
The reported annual incidence of PV increases with advanced age and ranges from 0.01 to 2.8 cases per 100,000 population in Europe and North America; the lowest incidence rates are reported in Japan and Israel [1864, 2764, 4010]. Most reports indicate a slight male predominance, with a male-to-female ratio of 1:2:1 [2500, 3209]. The median patient age at diagnosis is 60 years [2500, 3209, 3929]; cases in patients aged <20 years are rarely reported [1375, 3087]. The reported worldwide annual incidence of MPNs is 0.44–5.87 cases per 100,000 population, with annual rates of 0.84, 1.03, and 0.47 cases per 100,000 population for PV, essential thrombocythaemia, and primary myelofibrosis, respectively [1864].

Etiology
The underlying cause is unknown in most cases. A genetic predisposition has been reported in some families [1411, 1677, 3457]. Ionizing radiation and occupational exposure to toxins have been suggested as possible causes in some cases [526, 3606].

Localization
The blood and bone marrow are the major sites of involvement, but the spleen and liver are also affected and are the major sites of extramedullary haematopoiesis in later stages. However, any organ can be damaged as a result of the vascular consequences of the increased RBC mass.

Clinical features
The major symptoms of PV are related to hypertension or vascular abnormalities caused by the increased RBC mass (i.e. increased viscosity of blood). In nearly 20% of cases, an episode of venous or arterial thrombosis (such as deep vein thrombosis, myocardial ischaemia, or stroke) is documented in the medical history, and may be the first manifestation of PV [1103, 2500, 3209, 3929]. Mesenteric, portal, or splenic vein thrombosis or Budd-Chiari syndrome should always lead to consideration of PV as an underlying cause, and may precede the onset of overt polycythaemic disease manifestations [1023, 1362, 2012, 3240, 3702]. Headache, dizziness, visual disturbances, and paraesthesias are major symptoms; pruritus, erythromelalgia, and gout are also common. In PV, physical findings usually include plethora and palpable splenomegaly [2603], and may also include hepatomegaly depending on the diagnostic criteria used [2602, 3758, 3917, 3929]. There is debate as to which of the three RBC variables (haemoglobin, haematocrit, or RBC mass) should be used to define the diagnostic hallmark of PV [256]. In the original 4th edition of the WHO classification, the first major diagnostic criterion for PV was haemoglobin concentration >18.5 g/dL in men or >16.5 g/dL in women; haemoglobin concentration >18.5 g/dL in men or >16.5 g/dL in women, haematocrit values of >55.5% in men or >49.5% in women, or if major criterion 3 and the minor criterion are present. However, initial myelofibrosis (present in as many as 20% of patients) can only be detected by bone marrow biopsy, and this finding may predict a more rapid progression to overt myelofibrosis (post-PV myelofibrosis) [253].

| Major criterion 2 (bone marrow biopsy) may not be required in patients with sustained absolute erythrocytosis (haemoglobin concentrations of > 18.5 g/dL in men or > 16.5 g/dL in women and haematocrit values of > 55.5% in men or > 49.5% in women), if major criterion 3 and the minor criterion are present. However, initial myelofibrosis (present in as many as 20% of patients) can only be detected by bone marrow biopsy, and this finding may predict a more rapid progression to overt myelofibrosis (post-PV myelofibrosis) [253]. |  

Table 2.03 Diagnostic criteria for polycythaemia vera

The diagnosis of polycythaemia vera requires either all 3 major criteria or the first 2 major criteria plus the minor criterion a.

| Major criteria |  
|---|---  
| 1. Elevated haemoglobin concentration (>16.5 g/dL in men; > 16.0 g/dL in women) or Elevated haematocrit (>49% in men; > 48% in women) or Increased red blood cell mass (>25% above mean normal predicted value) |  
| 2. Bone marrow biopsy showing age-adjusted hypercellularity with trilineage growth (panmyelosclerosis), including prominent erythroid, granulocytic, and megakaryocytic proliferation with pleomorphic, mature megakaryocytes (differences in size) |  
| 3. Presence of JAK2 V617F or JAK2 exon 12 mutation |  

**a** Major criterion 2 (bone marrow biopsy) may not be required in patients with sustained absolute erythrocytosis (haemoglobin concentrations of >18.5 g/dL in men or >16.5 g/dL in women and haematocrit values of >55.5% in men or >49.5% in women), if major criterion 3 and the minor criterion are present. However, initial myelofibrosis (present in as many as 20% of patients) can only be detected by bone marrow biopsy, and this finding may predict a more rapid progression to overt myelofibrosis (post-PV myelofibrosis) [253].
Fig. 2.13 Polycythaemia vera, so-called masked/prodromal presentation. A Mildly hypercellular bone marrow showing a predominance of large megakaryocytes in the bone marrow biopsy section. B Many large and giant hypersegmented (essential thrombocythaemia-like) megakaryocytes in a case clinically mimicking ET, because of a platelet count >1000 × 10^9/L. Note the only mildly increased granulo- and erythropoiesis (panmyelosis), better demonstrated by naphthol-AS-D-chloroacetate esterase (CAE) reaction.

Fig. 2.14 Polycythaemia vera, overt polycythaemic presentation. A Bone marrow biopsy shows a characteristically hypercellular marrow characterizing the overt polycythaemic presentation (panmyelosis). B Megakaryocytes revealing different sizes are always a prominent feature. The cells are more easily evaluated by using the periodic acid–Schiff (PAS) staining reaction. C Panmyelosis (trilineage proliferation) as demonstrated by the naphthol-ASD-chloroacetate (CAE) stain which, by accurately identifying the granulocytic cells (red reaction product), helps in the assessment of the relative proportion of the two major marrow lineages (erythropoietic and granulopoietic). D At this disease stage, the megakaryocytes show increased pleomorphism, with significant differences in size but no relevant maturation defects such as nuclear or cytoplasmic differentiation, as shown with PAS staining.

>15 g/dL in women associated with a sustained increase of ≥2 g/dL from baseline that cannot be attributed to correction of iron deficiency, or RBC mass >25% above the mean normal predicted value, but with haemoglobin levels and haematocrit below the cut-off points (2602, 3677, 3758). For this reason, the term ‘masked PV’ was introduced (82, 251) for JAK2-mutated cases in patients with latent (initial, occult pre-polycthyaeamic) disease manifestations who have persistently elevated haemoglobin concentrations (>16.5 g/dL in men; >16.0 g/dL in women) or that fulfill the diagnostic criteria for PV proposed in the 2007 British Committee for Standards in Haematology (BCSH) guidelines (248, 2603). Cut-off points were determined to be the optimal thresholds for distinguishing JAK2-mutated essential thrombocythaemia from PV (249, 252). Consequently, these values have been included in the revised WHO criteria (3932) (see Table 2.03, p. 39).
Clinical laboratory studies that facilitate the diagnosis of PV include detection of \textit{JAK2} V617F or functionally similar mutations (e.g. \textit{JAK2} exon 12 mutations) \cite{2603,23056,3929,3931,3932} and subnormal erythropoietin levels \cite{251,2602,2760,3677,3929}. Endogenous erythroid colony growth is no longer included as a minor diagnostic criterion, due to its limited practicality \cite{3932}; it is time-consuming, unstandardized, restricted to specialized institutions, and costly.

Some cases (in particular cases in \textit{JAK2} V617F-mutated patients with prominent thrombocytosis and initially low haemoglobin levels and/or haematocrit) can clinically mimic essential thrombocythaemia at onset \cite{249}, despite showing bone marrow histopathology characteristic of PV and later increases in the RBC parameters \cite{1364,2152,3972}. Therefore, determination of \textit{JAK2} and \textit{CALR} mutation status alone (without morphological examination) is not sufficient to differentiate PV from \textit{JAK2}-mutated essential thrombocythaemia \cite{3920}.

Microscopy
In PV, the major features in the peripheral blood and bone marrow are attributable to effective proliferation in the erythroid, granulocytic, and megakaryocytic lineages; i.e. there is a panmyelosis. The peripheral blood shows a mild to overt excess of normochromic, normocytic RBCs. If there is iron deficiency due to bleeding, the RBCs may be hypochromic and microcytic. Neutrophilia and rarely basophilia may be present. Occasional immature granulocytes may be detectable, but circulating blasts are generally not observed.

Bone marrow cellularity is usually increased \cite{3975}. Hypercellularity is especially noteworthy in the subcortical marrow space, which is normally hypocellular \cite{1329,3964}. Panmyelosis accounts for the increased haematopoietic cellularity, but increased numbers of erythroid precursors and megakaryocytes are often most prominent \cite{1098,1329,3964,3978}. Using standardized prominent bone marrow features \cite{3969,3973}, several groups have succeeded in identifying histological patterns that are characteristic of WHO-defined PV, including so-called
Table 2.04  Relative incidence of discriminating features according to standardized WHO morphological criteria generating histological patterns in initially performed bone marrow biopsy specimens. Modified and adapted from Thiele J. and Kvasnicka H.M. (3969)

<table>
<thead>
<tr>
<th>Bone marrow morphology features</th>
<th>Relative frequency of features</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PV</td>
</tr>
<tr>
<td><strong>Cellularity</strong></td>
<td></td>
</tr>
<tr>
<td>Increased in quantity</td>
<td>&gt; 80%</td>
</tr>
<tr>
<td><strong>Granulopoiesis</strong></td>
<td></td>
</tr>
<tr>
<td>Increased in quantity</td>
<td>&gt; 80%</td>
</tr>
<tr>
<td>Left-shifted</td>
<td>&gt; 80%</td>
</tr>
<tr>
<td><strong>Erythropoiesis</strong></td>
<td></td>
</tr>
<tr>
<td>Increased in quantity</td>
<td>&gt; 80%</td>
</tr>
<tr>
<td>Left-shifted</td>
<td>&gt; 80%</td>
</tr>
<tr>
<td><strong>Megakaryopoiesis</strong></td>
<td></td>
</tr>
<tr>
<td>Increased in quantity</td>
<td>50–80%</td>
</tr>
<tr>
<td><strong>Size of cells</strong></td>
<td></td>
</tr>
<tr>
<td>Small</td>
<td>20–49%</td>
</tr>
<tr>
<td>Medium</td>
<td>20–49%</td>
</tr>
<tr>
<td>Large</td>
<td>20–49%</td>
</tr>
<tr>
<td>Giant</td>
<td>10–19%</td>
</tr>
<tr>
<td><strong>Histotopography</strong></td>
<td></td>
</tr>
<tr>
<td>Endosteal translocation</td>
<td>10–19%</td>
</tr>
<tr>
<td>Small clusters (&gt;3 cells)</td>
<td>10–19%</td>
</tr>
<tr>
<td>Large clusters (&gt;7 cells)</td>
<td>&lt; 10%</td>
</tr>
<tr>
<td>Dense clusters</td>
<td>&lt; 10%</td>
</tr>
<tr>
<td>Loose clusters</td>
<td>20–49%</td>
</tr>
<tr>
<td><strong>Nuclear features</strong></td>
<td></td>
</tr>
<tr>
<td>Hypolobulation (bulbous)</td>
<td>10–19%</td>
</tr>
<tr>
<td>Hyperlobulation (staghorn-like)</td>
<td>50–80%</td>
</tr>
<tr>
<td><strong>Fibrosis</strong></td>
<td></td>
</tr>
<tr>
<td>Increased reticulin</td>
<td>10–19%</td>
</tr>
<tr>
<td>Increased collagen</td>
<td>0%</td>
</tr>
<tr>
<td><strong>Stroma</strong></td>
<td></td>
</tr>
<tr>
<td>Iron deposits</td>
<td>0%</td>
</tr>
<tr>
<td>Lymphoid nodules</td>
<td>10–19%</td>
</tr>
</tbody>
</table>

PV, polycythaemia vera; ET, essential thrombocythaemia; pre-PMF, prefibrotic/early primary myelofibrosis; Overt PMF, overt primary myelofibrosis

masked PV [251,1361,1364,3677,3964,3972,3978,3983]. Erythropoiesis is usually normoblastic, erythroid precursors form large islets or sheets, and granulopoiesis is morphologically normal. The proportion of myeloblasts is not increased. Megakaryocytes are increased in number (particularly in cases with an excess of platelets) and frequently display hypersegmented nuclei. Cases with high platelet counts and low haemoglobin or haematocrit values may mimic essential thrombocythaemia at onset [1364, 2152,3972]. Megakaryocytes tend to form loose clusters or lie close to the bone trabeculae, and often show a significant degree of pleomorphism, resulting in a mixture of sizes. Most of the megakaryocytes exhibit normally folded or deeply lobed nuclei, and they usually lack significant atypia, although a minority may show bulbous nuclei and other nuclear abnormalities, particularly when associated with a minor increase in reticulin [3964]. Marrow sinuses are dilated and often densely filled by erythrocytes. It is usually possible to distinguish PV from essential thrombocythaemia, primary myelofibrosis, and reactive erythrocytosis and thrombocythaemia [1361,1380,2147,2433,3964,3983] on the basis of the characteristic histological pattern of PV (Table 2.04) [3969]. Therefore, WHO has adopted bone marrow morphology as one of the major diagnostic criteria for PV [257,3932]. Reticulin staining reveals a normal reticulin fibre network in about 80% of cases, but the remainder may display increased reticulin and even mild increases of disease progression is post-PV myelofibrosis, underscoring the value of bone marrow biopsy [11,253]. Reactive nodular lymphoid aggregates are found in as many as 20% of cases [3964,3969]. In >95% of cases, stainable iron is absent in bone marrow aspirate and biopsy specimens [1098,3978,3983].

Spent phase and post-polycythaemic myelofibrosis (post-PV MF)

During the later phases of PV, erythropoiesis progressively decreases. As a result, the RBC mass normalizes and then decreases, and the spleen further enlarges. Persistent leukocytosis occurring at or around the time of progression to post-PV MF has been reported to be associated with an overall more aggressive course of disease [405]. These changes are usually accompanied by corresponding bone marrow alterations [407,1098,3511,3758]. The most common pattern of disease progression is post-PV MF.
accompanied by myeloid metaplasia, which is characterized by a leukoerythroblastic peripheral blood smear, poikilocytosis with teardrop-shaped RBCs, and splenomegaly due to extramedullary haematopoiesis, as defined in Table 2.05, p.38 (265). In two large series of patients with WHO-defined myelofibrosis, post-PV MF was found to account for about 20% of the cases (3920). The morphological hallmark of this disease stage is overt reticulin (3983) and collagen fibrosis (407,1098,3511,3964) of the bone marrow. Cellularity varies in this terminal stage, but hypocellular specimens are common. Clusters of megakaryocytes, often with hyperchromatic and very dysmorphic nuclei, are prominent; however, cases retaining PV-like features have also been described (407). Erythropoiesis and granulopoiesis are decreased in quantity; RBCs, granulocytes, and megakaryocytes are sometimes found lying within dilated marrow sinusoids (3511,3964). Bone marrow fibrosis is usually of grade 2–3 on a 0–3 scale (2148,3975) or grade 3–4 on a 0–4 scale (2146). Osteosclerosis may occur (1098,3511). There is overt reticulin (3983) and collagen fibrosis (407,1098,3511,3964) of the bone marrow. Cellularity varies in this terminal stage, but hypocellular specimens are common. Clusters of megakaryocytes, often with hyperchromatic and very dysmorphic nuclei, are prominent; however, cases retaining PV-like features have also been described (407). Erythropoiesis and granulopoiesis are decreased in quantity; RBCs, granulocytes, and megakaryocytes are sometimes found lying within dilated marrow sinusoids (3511,3964). Bone marrow fibrosis is usually of grade 2–3 on a 0–3 scale (2148,3975) or grade 3–4 on a 0–4 scale (2146). Osteosclerosis may occur (1098,3511). There is disagreement about the degree to which advanced post-PV MF resembles overt primary myelofibrosis in terms of clinical and morphological features (407,3511). The splenic enlargement is a consequence of extramedullary haematopoiesis, which is characterized by the presence of erythroid, granulocytic, and megakaryocytic elements in the splenic sinuses and cords of Billroth. An increase in the number of immature cells may be observed in these stages, but the finding of ≥10% blasts in the peripheral blood or bone marrow or the presence of substantial myelodysplasia is unusual, and most likely indicates transformation to an accelerated phase. Cases with ≥20% blasts are considered to be blast-phase post-PV MF, formerly called acute leukaemia (3089,3758,3964).

Cell of origin
The postulated cell of origin is a haematopoietic stem cell.

Genetic profile
The most common genetic abnormality in PV is the somatic gain-of-function mutation JAK2 V617F (2099). Although this mutation occurs in >95% of patients with PV (3915,3929), it is not specific for this entity; it is found in other MPNs (3915,3920) and is also seen in a small subset (<5%) of cases of acute myeloid leukaemia, myelodysplastic syndromes, chronic myelomonocytic leukaemia, and other myeloid malignancies (3775). Rare cases of JAK2-mutant PV acquire a BCR-ABL1 rearrangement; however, the clinical significance of this is uncertain. Due to this additional phenotypic mutation, a morphological and haematological shift capable of producing a chronic myeloid leukaemia–like evolution may occur (1740,3190). Functionally similar mutations in exon 12 of JAK2 are found in about 3% of cases, which generally show a predominant erythroid haematopoiesis (2194,3056,3604). Mutations similar to those described in MPNs (3915) have also been found at very low frequencies in elderly patients with no haematological malignancy (1326,1830,4386). At diagnosis, cytogenetic abnormalities are detectable in as many as 20% of cases (3920). The most common recurrent abnormalities are gain of chromosome 8 or 9, del(20q), del(13q), and del(9p); gains of chromosomes 8 and 9 are sometimes found together (102,4307). There is no Philadelphia (Ph) chromosome or BCR-ABL1 fusion. The frequency of chromosomal abnormalities increases with disease progression; they are seen in nearly 80–90% of cases of post-PV myelofibrosis (102).

Prognosis and predictive factors
With currently available treatment, median survival times > 10 years are commonly reported (2150,3090,3209,3758). Recent studies using WHO criteria have found median survivals of > 13 years overall and about 24 years in patients aged <60 years (3920,3929). Prognostic factors other than older age remain controversial (2150,2500,3090). It has been shown that survival is adversely affected by leuко cytosis and abnormal karyotype (405,3929). Most patients die from thrombotic complications or second malignancies, but as many as 20% succumb to myelodysplastic syndrome or blast phase / acute myeloid leukaemia (2500,3209,3758). In large series of cases defined per the WHO criteria, 3–7% of cases were found to be in the blast phase (3920).

The factors that predict the risk of thrombosis or haemorrhage are not well defined, but age and previous thrombosis have been shown to indicate a higher thrombotic risk (247,2500,2501,3087,3758). The incidence of myelodysplastic syndrome and the blast phase is only 2–3% in patients who have not been treated with cytotoxic agents, but increases to ≥10% following certain types of chemotherapy (1216,2500,3087,3089,3758,3929).
Primary myelofibrosis

Definition
Primary myelofibrosis (PMF) is a clonal myeloproliferative neoplasm (MPN) characterized by a proliferation of predominantly abnormal megakaryocytes and granulocytes in the bone marrow, which in fully developed disease is associated with reactive deposition of fibrous connective tissue and with extramedullary haematopoiesis.

There is a stepwise evolution from an initial prefibrotic/early stage, characterized by hypercellular bone marrow with absent or minimal reticulin fibrosis, to an overt fibrotic stage with marked reticulin or collagen fibrosis in the bone marrow, and often osteosclerosis. The fibrotic stage of PMF is clinically characterized by leukoerythroblastosis in the blood (with teardrop-shaped red blood cells), hepatomegaly, and splenomegaly.

The diagnostic criteria for prefibrotic/early PMF (pre-PMF) are summarized in Table 2.06 and those of overt (classic) PMF in Table 2.07.

ICD-O codes
Primary myelofibrosis 9961/3
Prefibrotic/early stage 9961/3
Primary myelofibrosis, overt fibrotic stage 9961/3

Synonyms
Chronic idiopathic myelofibrosis; myelofibrosis/sclerosis with myeloid metaplasia; agnogenic myeloid metaplasia; megakaryocytic myelosclerosis; idiopathic myelofibrosis; myelofibrosis with myeloid metaplasia; myelofibrosis as a result of myeloproliferative disease

Epidemiology
The estimated annual incidence of overt PMF is 0.5–1.5 cases per 100 000 population [2613,2764,3916,4010]. Valid data on the incidence of pre-PMF are not available, but data from reference centres suggest that the prefibrotic/early stage accounts for 30–50% of all PMF cases. There are few reliable estimates of prevalence [2764,4010]. The prevalence of PMF is probably increasing, due to earlier diagnosis (i.e. of pre-PMF) and prolonged survival [615]. PMF affects men and women nearly equally [3916]. It occurs most commonly in the sixth to seventh decades of life, and only about 10% of overt PMF cases are diagnosed in patients aged <40 years [614]. Children are rarely affected [81,614].

Etiology
Exposure to benzene or ionizing radiation has been documented in some cases [998]. Rare familial cases of bone marrow fibrosis in young children have been reported; how many of these constitute MPNs is unknown, but at least some cases appear to constitute an autosomal recessive inherited condition [3457]. In other families, with a somewhat older patient age at onset, the features have been consistent with an MPN, suggesting a familial predisposition to PMF [617,3418].

Localization
The blood and bone marrow are always involved. In the later stages of the disease, extramedullary haematopoiesis (also known as myeloid metaplasia) becomes prominent, particularly in the spleen [267], which harbours neoplastic stem cells [4255]. In the initial stages, the number of randomly distributed CD34+ progenitors is slightly increased in the bone marrow, but not in the peripheral blood. The frequency of bone marrow CD34+ cells is inversely related to the number of circulating CD34+ cells [2684,3971]; only in the later stages do they appear in large numbers in the peripheral blood. This increase in the number of circulating CD34+ cells is a phenomenon largely restricted to overt PMF; it is not seen in non-fibrotic polycythaemia vera.
or essential thrombocythaemia (101,268,3091). It has been postulated that extramedullary haematopoiesis is a consequence of the unique ability of the spleen to sequester the numerous circulating CD34+ cells (3970,4255). Other possible sites of extramedullary haematopoiesis are the liver, lymph nodes, kidneys, adrenal glands, dura mater, gastrointestinal tract, lungs and pleura, breasts, skin, and soft tissue (3916).

**Clinical features**

As many as 30% of cases are asymptomatic at the time of diagnosis and are discovered by detection of splenomegaly during a routine physical examination or when a routine blood count reveals anaemia, leukocytosis, and/or thrombocytosis. Less commonly, the diagnosis results from discovery of unexplained leukoerythroblastosis or an increased lactate dehydrogenase level (613,3916,3931). In the initial prefibrotic stage of PMF, the only finding may be marked thrombocytosis mimicking essential thrombocythaemia, because the other clinical features may be either within the normal range or only borderline abnormal (254,266,3931,3962,3966,3977). Therefore, neither sustained thrombocytosis nor positive mutation status alone can distinguish prefibrotic PMF from essential thrombocythaemia; careful analysis of bone marrow morphology is necessary (see Table 2.04, p. 42, and Table 2.08, p. 47) (254,257,263,1366,1380,3933,3965).

More than 50% of patients with PMF experience constitutional symptoms, including fatigue, dyspnoea, weight loss, night sweats, low-grade fever, and cachexia (2643). These symptoms, which reflect the biological activity of the disease, compromise quality of life and are associated with prognosis (616). Gouty arthritis and renal stones due to hyperuricaemia can also occur. Splenomegaly of various degrees is detected in as many as 90% of patients, and can be massive. Nearly 50% of patients have hepatomegaly, depending on the stage of disease (264,613,614,3916).

In WHO-defined PMF, JAK2 V617F mutation is found in 50–60% of early-stage cases (254) (as well as in advanced stages); CALR mutations are found in about 24% of PMF cases and MPL mutations in 8%. About 12% of cases are triple-negative for mutations in JAK2, CALR, and MPL (3920,3932,3933). Although these mutations are helpful in distinguishing PMF from reactive conditions that can result in bone marrow fibrosis, they are not specific for PMF; mutations in these genes can also be found in essential thrombocythaemia, and JAK2 V617F can be found in polycythaemia vera (3920).

### Table 2.07 Diagnostic criteria for primary myelofibrosis, overt fibrotic stage

The diagnosis of overt primary myelofibrosis requires that all 3 major criteria and at least 1 minor criterion are met.

**Major criteria**

1. Megakaryocytic proliferation and atypia, accompanied by reticulin and/or collagen fibrosis grades 2 or 3

2. WHO criteria for essential thrombocythaemia, polycythaemia vera, BCR-ABL1-positive chronic myeloid leukaemia, myelodysplastic syndrome, or other myeloid neoplasms are not met

3. JAK2, CALR, or MPL mutation

   - Presence of another clonal marker

   - Absence of reactive myelofibrosis

**Minor criteria**

- Anaemia not attributed to a comorbid condition
- Leukocytosis $>11 \times 10^9/L$
- Palpable splenomegaly
- Lactate dehydrogenase level above the upper limit of the institutional reference range
- Leukoerythroblastosis

- See Table 2.09 (p. 47).

- Myeloproliferative neoplasms can be associated with monocytosis or they can develop it during the course of the disease; these cases may mimic chronic myelomonocytic leukaemia (CMML); in these rare instances, a history of MPN excludes CMML, whereas the presence of MPN features in the bone marrow and/or MPN-associated mutations (in JAK2, CALR, or MPL) tend to support the diagnosis of MPN with monocytosis rather than CMML.

- In the absence of any of the 3 major clonal mutations, a search for other mutations associated with myeloid neoplasms (e.g. ASXL1, EZH2, TET2, IDH1, IDH2, SRSF2, and SF3B1 mutations) may be of help in determining the clonal nature of the disease.

- Bone marrow fibrosis secondary to infection, autoimmune disorder or another chronic inflammatory condition, hairy cell leukaemia or another lymphoid neoplasm, metastatic malignancy, or toxic (chronic) myelopathy.

**Microscopy**

The classic picture of overt (advanced) PMF includes a peripheral blood smear that shows leukoerythroblastosis and anisopoikilocytosis (typically with teardrop-shaped red blood cells) associated with a hypocellular bone marrow with marked reticulin and/or collagen fibrosis and organomegaly caused by extramedullary...
Fig. 2.19 Primary myelofibrosis (PMF). A Bone marrow biopsy in this prefibrotic/early-stage case shows megakaryocytic and granulocytic proliferation. Naphthol AS-D chloroacetate esterase (CAE) staining identifies the granulocytic component (red reaction product); megakaryocytes show extensive clustering and condensed nuclei with conspicuously clumped chromatin and abnormal nuclear:cytoplasmic ratios (N:C). B Megakaryocytic abnormalities are the key finding in diagnosing PMF and in its distinction from other myeloproliferative and reactive disorders; in this prefibrotic/early-stage case, note the abnormalities of megakaryopoiesis, including anisocytosis, abnormal N:C ratios, abnormal chromatin clumping with hyperchromatic nuclei, plump lobation of some nuclei (cloud-like nuclei), and clustering. C Immunohistochemistry for CD61 highlights abnormal megakaryocytes, including small forms.

Fig. 2.20 Primary myelofibrosis: bone marrow fibrosis (MF) grading in silver-stained bone marrow biopsy sections. A MF-0, with no increase in reticulin. B MF-1, with a very loose network of reticulin fibres. C MF-2, showing a more diffuse and dense increase in reticulin fibres and some coarse collagen fibres. D MF-3, with coarse bundles of collagen fibres intermingled with dense reticulin, accompanied by initial osteosclerosis.

Prefibrotic/early primary myelofibrosis
No registry-based prevalence or incidence data are available for this prodromal stage of PMF, but series from reference centres show that 30–50% of PMF cases are first detected in the prefibrotic/early stage (263,496,3963,3965), with no significant increase in reticulin and/or collagen fibres (i.e. fibrosis grades 0 and 1) (2148,3975). In these cases, the bone marrow biopsy usually shows hypercei-
ularity, with an increase in the number of neutrophils and atypical megakaryocytes. There may be a minor left shift in granulopoiesis, but metamyelocytes, bands, and segmented neutrophils usually predominate. Myeloblasts are not increased in percentage, and conspicuous clusters of blasts are not observed; however, the number of randomly distributed CD34+ progenitors is slightly increased (3965, 3971). In most cases, erythropoiesis is reduced but the erythrocytes show no dysplastic features (3963, 3965). The megakaryocytes are markedly abnormal, and their morphological atypia and topographical distribution within the bone marrow are critical for the recognition of pre-PMF. The megakaryocytes often form dense clusters, which are frequently adjacent to the bone marrow vascular sinuses and bone trabeculae (496, 2147, 2152, 3965, 3969, 3978). Most megakaryocytes are enlarged, but small megakaryocytes may also be seen; their detection is facilitated by immunohistochemistry with antibodies reactive with megakaryocytic antigens (3962, 3965). Deviations from the normal N:C ratio (an indication of defective maturation), abnormal patterns of chromatin clumping (including hyperchromatic forms and bulbous, cloud-like, or balloon-shaped nuclei), and the frequent occurrence of bare (naked) megakaryocytic nuclei are typical findings. Overall, in pre-PMF, the megakaryocytes are more atypical than in any other type of MPN (Table 2.04, p.42, and Table 2.08). Vascular proliferation is only mildly increased in the bone marrow, with no gross abnormalities of vessel shape (2149). Lymphoid nodules are found in as many as 20% of cases (3962, 3965).

Careful morphological examination of the bone marrow is particularly crucial for distinguishing pre-PMF with accompanying thrombocytopaenia from essential thrombocythaemia, as has been demonstrated by a number of groups (254, 1227, 1361, 1366, 1379, 1380, 2105, 2147, 2433, 3962, 3966, 3977). No single morphological feature is pathognomonic of a specific subtype, but the identification of characteristic morphological patterns is key for the differential diagnosis between pre-PMF and essential thrombocythaemia (Table 2.04, p.42, and Table 2.08). Most cases of pre-PMF eventually transform into overt fibrotic/sclerotic myelofibrosis associated with extramedullary haematopoiesis (494, 2105, 2152, 3965, 3980).

### Table 2.08 Morphological features helpful in distinguishing essential thrombocythaemia (ET) from prefibrotic/early primary myelofibrosis (pre-PMF)\(^a\)

<table>
<thead>
<tr>
<th>Morphological feature</th>
<th>ET</th>
<th>Pre-PMF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cellularity (age-adjusted)</td>
<td>Normal</td>
<td>Increased</td>
</tr>
<tr>
<td>Myeloid-to-erythroid ratio</td>
<td>Normal</td>
<td>Increased</td>
</tr>
<tr>
<td>Dense megakaryocyte clusters</td>
<td>Rare</td>
<td>Frequent</td>
</tr>
<tr>
<td>Megakaryocyte size</td>
<td>Large/giant</td>
<td>Variable</td>
</tr>
<tr>
<td>Megakaryocyte nuclear lobulation</td>
<td>Hyperlobulated</td>
<td>Bulbous/hypolobulated</td>
</tr>
<tr>
<td>Reticulin fibrosis, grade 1(^b)</td>
<td>Very rare</td>
<td>More frequent</td>
</tr>
</tbody>
</table>

\(^a\) On the basis of a representative, artifact-free bone marrow biopsy (>1.5 cm) taken at a right angle (90\(^\circ\)) from the cortical bone.

\(^b\) According to WHO grading (3975); see Table 2.09.

### Table 2.09 Semiquantitative bone marrow fibrosis (MF) grading system proposed by Thiele J et al. (3975), with minor modifications concerning collagen and osteosclerosis\(^a\)

<table>
<thead>
<tr>
<th>Grade</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>MF-0</td>
<td>Scattered linear reticulin with no intersections (cross-overs), corresponding to normal bone marrow</td>
</tr>
<tr>
<td>MF-1</td>
<td>Loose network of reticulin with many intersections, especially in perivascular areas</td>
</tr>
<tr>
<td>MF-2</td>
<td>Diffuse and dense increase in reticulin with extensive intersections, occasionally with focal bundles of thick fibres mostly consistent with collagen and/or associated with focal osteosclerosis(^b)</td>
</tr>
<tr>
<td>MF-3</td>
<td>Diffuse and dense increase in reticulin with extensive intersections and coarse bundles of thick fibres consistent with collagen, usually associated with osteosclerosis(^b)</td>
</tr>
</tbody>
</table>

\(^a\) Fibre density should be assessed only in haematopoietic areas; if the pattern is heterogeneous, the final grade is determined by the highest grade present in \(\geq 30\%\) of the marrow area.

\(^b\) In grades MF-2 and MF-3, an additional trichome stain is recommended (Table 2.10).

### Table 2.10 Semiquantitative grading of collagen\(^a\)

<table>
<thead>
<tr>
<th>Grade</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Perivascular collagen only (normal)</td>
</tr>
<tr>
<td>1</td>
<td>Focal paratrabecular or central collagen deposition with no connecting meshwork</td>
</tr>
<tr>
<td>2</td>
<td>Paratrabecular or central deposition of collagen with focally connecting meshwork or generalized paratrabecular apposition of collagen</td>
</tr>
<tr>
<td>3</td>
<td>Diffuse (complete) connecting meshwork of collagen in (\geq 30%) of marrow spaces</td>
</tr>
</tbody>
</table>

\(^a\) If the pattern is heterogeneous, the final grade is determined by the highest grade present in \(\geq 30\%\) of the marrow area.

### Table 2.11 Semiquantitative grading of osteosclerosis\(^a\)\(^b\)

<table>
<thead>
<tr>
<th>Grade</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Regular bone trabeculae (distinct paratrabecular borders)</td>
</tr>
<tr>
<td>1</td>
<td>Focal budding, hooks, spikes, or parablastic apposition of new bone</td>
</tr>
<tr>
<td>2</td>
<td>Diffuse paratrabecular formation of new bone with thickening of trabeculae, occasionally with focal interconnections</td>
</tr>
<tr>
<td>3</td>
<td>Extensive interconnecting meshwork of new bone with overall effacement of marrow spaces</td>
</tr>
</tbody>
</table>

\(^a\) If the pattern is heterogeneous, the final grade is determined by the highest grade present in \(\geq 30\%\) of the marrow area.

\(^b\) Bone marrow core biopsy of sufficient length, taken at a right angle (90\(^\circ\)) from the cortical bone, without fragmentation, is mandatory for the grading of osteosclerosis.
Overt primary myelofibrosis

Most cases of PMF are initially diagnosed in the overt fibrotic stage [264,613,3916]. In this stage, the bone marrow biopsy shows clear-cut reticulin or collagen fibrosis (i.e., fibrosis grades 2 and 3) [2148, 3975], often associated with various degrees of collagen fibrosis and osteosclerosis (see Table 2.10 and Table 2.11, p. 47). The bone marrow may still be focally hypercellular, but is more often normocellular or hypocellular, with patches of active haematopoiesis alternating with hypocellular regions of loose connective tissue and/or fat. Foci of immature cells may be more prominent, although myeloblasts account for <10% of the bone marrow cells [3965]. Atypical megakaryocytes are often the most conspicuous finding; they occur in large clusters or sheets, often within dilated vascular sinuses [494,3965]. In some cases, the bone marrow is almost devoid of haematopoietic cells, showing mainly dense reticulin or collagen fibrosis, with small islands of haematopoietic precursors situated mostly within the vascular sinuses. Associated with the development of myelofibrosis is a significant proliferation of vessels showing marked tortuosity and luminal distension, often also associated with conspicuous intrasinusoidal haematopoiesis [438,2149,2642,2863]. Osteoid seams or appositional new bone formation in bud-like endophytic plaques may be observed [3965]. In this osteosclerotic phase, the bone may form broad, irregular trabeculae that can occupy >50% of the bone marrow space.

The development of overt fibrosis in PMF is related to disease progression [494, 3979,3980], and is not significantly influenced by standard cytoreductive treatment modalities (with the exception of allogeneic stem cell transplantation [2125,2864,3974]). However, it has been reported that interferon [744,3204] and JAK1/JAK2 inhibitor [1563A,1798,1800, 1833,2543A,4316] therapy may delay or even reverse bone marrow fibrosis progression across all aspects of the fibrotic process (i.e., reticulin fibrosis, collagen deposition, and osteosclerosis).

The development of monocytosis in PMF may indicate disease progression [404]. In patients with a previously established diagnosis of PMF, the finding of 10–19% blasts in the peripheral blood and/or bone marrow and the detection by immunohistochemistry of an increased number of CD34+ cells with cluster formation and/or an abnormal endosteal location in the bone marrow [3965,3971] indicate an accelerated phase of the disease, whereas the finding of ≥20% blasts is diagnostic of blast transformation. Patients with PMF may rarely present initially in the accelerated phase or blast phase.
sis, and cirrhosis of the liver may occur of platelets. Hepatic sinuses also show cords may exhibit fibrosis and pooling topoiesis in general, the possibility of a prominent extramedullary haematopoiesis should be considered and carefully excluded through immuno- myeloid sarcoma. In any advanced-stage disease with In the presence of nodular lesions the identification of neoangiogenesis KIT (CD117) (3970,4255). The red pulp nohistochemical studies with CD34 and can be facilitated by immunohistochemistry (2918,3959), which also facilitates the identification of neoangiogenesis (267). Megakaryocytes are often the most conspicuous haematopoietic element in the marrow; the cells often appear to stream through the marrow, due to the underlying fibrosis. Megakaryocytes are often the most conspicuous haematopoietic C diagnostic) of PMF with myeloid metaplasia. C Megakaryocytes are often the most conspicuous haematopoietic cells growing cohesively can produce macroscopically evident tumoural lesions. In the presence of nodular lesions and in any advanced-stage disease with large amounts of extramedullary haematopoiesis in general, the possibility of a myeloid sarcoma should be considered and carefully excluded through immunohistochemical studies with CD34 and KIT (CD117) (3970,4255). The red pulp cords may exhibit fibrosis and pooling of platelets. Hepatic sinuses also show prominent extramedullary haematopoiesis, and cirrhosis of the liver may occur (3916).

**Extramedullary haematopoiesis**

The most common site of extramedullary haematopoiesis is the spleen, followed by the liver. The spleen shows an expansion of the red pulp by erythroid, granulocytic, and megakaryocytic cells (3232). The identification of these cells can be facilitated by immunohistochemistry (2918,3959), which also facilitates the identification of neoangiogenesis (267). Megakaryocytes are often the most conspicuous component of the extramedullary haematopoiesis. Occasionally, large aggregates of megakaryocytes growing cohesively can produce macroscopically evident tumoural lesions. In the presence of nodular lesions and in any advanced-stage disease with large amounts of extramedullary haematopoiesis in general, the possibility of a myeloid sarcoma should be considered and carefully excluded through immunohistochemical studies with CD34 and KIT (CD117) (3970,4255). The red pulp cords may exhibit fibrosis and pooling of platelets. Hepatic sinuses also show prominent extramedullary haematopoiesis, and cirrhosis of the liver may occur (3916).

**Cell of origin**

The postulated cell of origin is a haematopoietic stem cell.

**Genetic profile**

No genetic defect specific for PMF has been identified. Approximately 50–60% of WHO-defined PMF cases carry JAK2 V617F or a functionally similar mutation, about 30% of cases have a mutation in CALR and 8% in MPL, and about 12% of cases are triple-negative for these mutations (3915,3920,3933). A subset of triple-negative cases have been found to have gain-of-function mutations (e.g. MPL S204P and MPL Y591N) through whole-exome sequencing or other sensitive molecular techniques (2666). This finding is consistent with the assumption that JAK2/CALR/MPL-wild-type PMF is not a homogeneous entity and that cases with polyclonal haematopoiesis probably constitute a hereditary disorder (2666). Although the presence of the JAK2 mutation confirms the clonality of the proliferation, the mutation is also found in polycythaemia vera and essential thrombocythaemia, and therefore does not distinguish PMF from these MPNs (3920,3933). CALR mutation has been reported to have a favourable im- pact on survival (3920,3924,3934), in contrast to the negative prognostic value of the triple-negative mutation status (i.e. JAK2, CALR, and MPL wild-type) (3920, 3924) and other, less frequent mutations (1486,3921,4149). Mutations similar to those described in MPNs (3915) have also been found at very low frequencies in elderly patients with no haematological malignancy (1326,1830,4386). Very rarely, cases of PMF acquire a BCRABL1 fusion gene. The presence of either del(13)(q12-22) or der(6)(1;6)(q21-23,p21.3) is strongly suggestive (but not diagnostic) of PMF (3996). The most common recurrent abnormalities are del(20q) and partial trisomy 1q; gains of chromosomes 9 and/or 8 have also been reported (3336,3926). Deletions affecting the long arms of chromosomes 7 and 5 occur as well, but may be associated with prior cytotoxic therapy used to treat the myeloproliferative process.

**Prognosis and predictive factors**

The time of survival with PMF ranges from months to decades. Overall prognosis depends on the stage at which the neoplasm is initially diagnosed (2150, 3965) and the corresponding risk status, which can be determined using several prognostic scoring systems (616,1290, 3086,3923). The median overall survival time for patients diagnosed in the overt fibrotic stage (myelofibrosis with myeloid metaplasia) is approximately 3–7 years (264,613,3914), whereas diagnosis in the prefibrotic/early stage is associated with 10-year and 15-year relative survival rates of 72% and 59%, respectively (254, 2150,3965,3967,4173). The widely used refined Dynamic International Prognostic Scoring System (DIPSS Plus) includes eight predictors of inferior survival: patient age >65 years, haemoglobin concentration <10 g/dL, leukocytes >25 x 10⁹/L, circulating blasts ≥1%, constitutional symptoms, red blood cell transfusion dependency, platelet count <100 x 10⁹/L, and unfavourable karyotype (i.e. a complex karyotype or 1–2 of the following...
abnormalities: gain of chromosome 8, loss of chromosome 7/7q, isochromosome 17q, inv(3), loss of chromosome 5/5q or 12p, or 11q23 rearrangement). Risk status is defined by the number of adverse prognostic factors present: 0 (low risk), 1 (intermediate-1 risk), 2 or 3 (intermediate-2 risk), or ≥4 (high risk), with respective median survival times of approximately 15.4, 6.5, 2.9, and 1.3 years (1290,3916). High-risk disease is also defined by a CALR-negative and ASXL1-positive mutation status (1486,3920,3921,3924). In the context of these risk models, the prognostic value of bone marrow fibrosis reflecting the stage of disease (pre-PMF vs overt PMF) is emphasized (2150,3923,3967,4173). The findings of a study investigating the relationship between DIPSS score (3086) and marrow fibrosis grading (3975) in patients with PMF suggested that better prognostication could be achieved by considering morphological parameters in addition to clinical and mutation data (1365). Major causes of morbidity and mortality are bone marrow failure (infection, haemorrhage), thromboembolic events, portal hypertension, cardiac failure, and blast-phase disease (i.e. secondary acute myeloid leukaemia). The reported frequency of the blast phase is 5–30% [264,613,3914,3916,3920]. Although some blast-phase cases are related to prior cytotoxic therapy, many have been reported in patients who have never been treated, confirming that blast transformation is part of the natural history of PMF.

**Essential thrombocythaemia**

**Definition**

Essential thrombocythaemia (ET) is a chronic myeloproliferative neoplasm (MPN) that primarily involves the megakaryocytic lineage. It is characterized by sustained thrombocytosis (platelet count ≥450 × 10⁹/L) in the peripheral blood and increased numbers of large, mature megakaryocytes in the bone marrow and clinically by the occurrence of thrombosis and/or haemorrhage. Because there is no known genetic or biological marker specific for ET, other causes of thrombocytosis must be excluded, including other MPNs, inflammatory and infectious disorders, haemorrhage, and other types of haematopoietic and non-haematopoietic neoplasms. The presence of BCR-ABL1 gene fusion excludes the diagnosis of ET. The diagnostic criteria for ET are listed in Table 2.12.

**ICD-O code**

9962/3

**Synonyms**

Idiopathic thrombocythaemia/ thrombocytosis; essential haemorrhagic thrombocythaemia; idiopathic haemorrhagic thrombocythaemia; idiopathic thrombocythaemia

<table>
<thead>
<tr>
<th>Table 2.12 Diagnostic criteria for essential thrombocythaemia</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>The diagnosis of essential thrombocythaemia requires that either all major criteria or the first 3 major criteria plus the minor criterion are met.</strong></td>
</tr>
<tr>
<td><strong>Major criteria</strong></td>
</tr>
<tr>
<td>1. Platelet count ≥450 × 10⁹/L</td>
</tr>
<tr>
<td>2. Bone marrow biopsy showing proliferation mainly of the megakaryocytic lineage, with increased numbers of enlarged, mature megakaryocytes with hyperlobulated nuclei; no significant increase or left shift in neutrophil granulopoiesis or erythropoiesis; very rarely a minor (grade 1*) increase in reticulin fibres</td>
</tr>
<tr>
<td>3. WHO criteria for BCR-ABL1-positive chronic myeloid leukaemia, polycythaemia vera, primary myelofibrosis, or other myeloid neoplasms are not met</td>
</tr>
<tr>
<td>4. JAK2, CALR, or MPL mutation</td>
</tr>
<tr>
<td><strong>Minor criterion</strong></td>
</tr>
<tr>
<td>Presence of a clonal marker or</td>
</tr>
<tr>
<td>Absence of evidence of reactive thrombocytois</td>
</tr>
<tr>
<td>a See Table 2.09 (p. 47).</td>
</tr>
</tbody>
</table>

**Epidemiology**

The true overall incidence of ET is unknown, but the annual incidence in Europe and the USA of cases diagnosed per the guidelines of the Polycythemia Vera Study Group (PVSG) [2791] is estimated to be 0.2–2.3 cases per 100,000 population [2613,2764,4010]. Most cases occur in patients aged 50–60 years, and a slight female predilection was found in a series of strictly WHO-defined cases [254,3933,3977]. There is a second peak in incidence among patients (in particular women) aged about 30 years [1217,1563]. ET also occurs (infrequently) in children, in whom it must be distinguished from rare cases of hereditary thrombocytois [1375,3302].

**Etiology**

In most patients, the etiology of ET is unknown. However, germline mutations in
JAK2 and mutations of the gelsolin gene (GSN) were recently reported in several pedigrees of hereditary thrombocytosis (1677,3457).

Localization
The bone marrow and blood are the principal sites of involvement. The spleen does not show significant extramedullary haematopoiesis at the time of onset, but is a sequestration site for platelets (1217,1563).

Clinical features
More than half of all cases are asymptomatic at the time of diagnosis, discovered incidentally when an elevated platelet count is found on a routine peripheral blood count (254,1217,1378,1563). The other half present with some manifestation of vascular occlusion or haemorrhage (566,1096,1215,3088,3933). Microvascular occlusion can lead to transient ischaemic attacks, digital ischaemia with paraesthesias, and gangrene (566,3088). Thrombosis of major arteries and veins can also occur, and ET can be a cause of splenic or hepatic vein thrombosis as seen in Budd–Chiari syndrome (1022,2012,3703). Bleeding occurs most commonly from mucosal surfaces, such as in the gastrointestinal tract and upper airway passages (1096,1215,1378). In PVSG-defined ET, mild splenomegaly is present in approximately 50% of cases at diagnosis and hepatomegaly in 15–20% (1563,2791,3927). When the WHO criteria, which exclude cases with thrombocytosis associated with prefibrotic/early primary myelofibrosis (pre-PMF), are used, minor palpable splenomegaly is seen in only 15–20% of ET cases (254,3917,3933,3977). In three studies including almost 1500 WHO-defined cases of ET from various centres, normal platelet counts (adjusted for sex and race) (2265,3452,3469,3931). This threshold value has also been adopted by the British Committee for Standards in Haematology (BCSH) (1562). Although this lowered threshold will encompass more cases of ET, it will also include more cases of conditions that mimic ET (257,1380); therefore, it is essential that all diagnostic criteria for ET (see Table 2.12) are met in order to exclude other neoplastic and non-neoplastic causes of thrombocytosis (1380,3931,3932). Bone marrow biopsy is particularly helpful in excluding other myeloid neoplasms associated with high platelet counts, such as myelodysplastic syndromes associated with isolated del(5q), myelodys-
plastic/myeloproliferative neoplasm with ring sideroblasts and thrombocytosis, and in particular pre-PMF. Although most WHO-defined ET cases harbour a phenotypic driver mutation in \textit{JAK2} (present in 50–60\% of cases), CALR (in \textasciitilde 30\%), or \textit{MPL} (in \textasciitilde 3\%), about 12\% of cases are triple-negative for these mutations. None of these mutations is specific for ET, but their presence does exclude reactive thrombocytosis (3920,3933). Similarly, in vitro endogenous erythroid and/or megakaryocytic colony formation, although not specific for ET, also excludes reactive thrombocytosis (1011).

\section*{Microscopy}

The major abnormality seen in the peripheral blood is marked thrombocytosis. The platelets often display anisocytosis, ranging from tiny forms to atypical large or giant platelets. Bizarre shapes, pseudopods, and agranular platelets may be seen, but are not common. In WHO-defined ET, the white blood cell count and leukocyte differential count are usually normal, although a borderline elevation in the white blood cell count may occur (254,1378,3920,3933,3977). The red blood cells are usually normocytic and normochromic, unless recurrent haemorrhage has caused iron deficiency, in which case they may be microcytic and hypochromic. Leukoerythroblastosis and teardrop-shaped red blood cells are not seen in ET (254,3977).

Haematopoietic cellularity is normal in most cases (3975), but a small proportion of cases show a hypercellular marrow (Table 2.04, p.42) (3969,3972). The most striking abnormality is a marked proliferation of megakaryocytes, with a predominance of large to giant forms displaying abundant, mature cytoplasm and deeply lobed and hypersegmented (staghorn-like) nuclei. The megakaryocytes are typically dispersed throughout the bone marrow, but may occur in loose clusters. Unlike in pre-PMF and overt primary myelofibrosis, bizarre, highly atypical megakaryocytes or large dense clusters are very rarely found in ET; if they are present, the diagnosis of ET should be reconsidered [1227,1366,3961,3966,3977]. Proliferation of erythroid precursors is seen in some cases (most commonly when the patient has experienced recurrent major haemorrhages or has been pretreated with hydroxycarbamide), but granulocytic proliferation is highly unusual; if present, the increase in granulopoiesis is usually only slight. There is no increase in myeloblasts and no myelodysplasia. The network of reticulin fibres is usually normal, or is very rarely (in <5\% of cases) minimally increased (but never to more than WHO grade 1 (3975)) (254,2105,3981); infrequently, reticulin fibrosis may increase in sequential bone marrow biopsy examinations (2105,3981). The finding of significant reticulin fibrosis or any collagen fibrosis at presentation excludes the diagnosis of ET (1227,1329,1366,2105,3961,3966,3969). Bone marrow aspirate smears also reveal markedly increased numbers of large megakaryocytes with hyperlobulated nuclei, as well as large sheets of platelets in the background. Emperipolesis of bone marrow elements is frequently observed in ET, but is not a specific finding. Stainable iron may be present in aspirated bone marrow specimens at diagnosis (2791).

The morphological findings, i.e. the characteristic histological patterns in the bone marrow biopsy (Table 2.04, p.42), are essential for distinguishing ET from other MPNs [1380] and myeloid disorders or reactive conditions that present with sustained thrombocytosis. The finding of even a low degree of combined granulocytic and erythroid proliferation should raise suspicion of the prodromal stage of polycythaemia vera [1364,2152,2147,3962,3984]. Significant dyserythropoiesis or dysgranulopoiesis suggests a diagnosis of myelodysplastic syndrome rather than ET. The large megakaryocytes with hypersegmented nuclei seen in ET contrast with the medium-sized non-lobated megakaryocytes seen in myelodysplastic syndrome with isolated del(5q) and with the small, dysplastic megakaryocytes seen in acute myeloid leukaemia or myelodysplastic syndrome with inv(3)(q21q26.2) or t(3;3)(q21;q26.2). Some cases of chronic myeloid leukaemia initially present with thrombocytosis without leukocytosis, and can mimic ET clinically. The large megakaryocytes of ET can be easily distinguished from the small (dwarf) megakaryocytes of chronic myeloid leukaemia, but cytogenetic and/or molecular genetic analysis to exclude \textit{BCR-ABL1} fusion is recommended for all patients in whom the diagnosis of ET is considered (3350).

\section*{Cell of origin}

The postulated cell of origin is a haematopoietic stem cell.
Genetic profile

No molecular genetic or cytogentic abnormality specific for ET is known. Approximately 50–60% of WHO-defined ET cases carry JAK2 V617F or a functionally similar mutation, about 30% of cases have a mutation in CALR and 3% MPL, and about 12% of cases are triple-negative for these mutations (3915,3920, 3933,3935). A subset of triple-negative cases have been found to have gain-of-function mutations (e.g. MPL S204P and MPL Y591N) through whole-exome sequencing or other sensitive molecular techniques (517,2666). This finding is consistent with the assumptions that JAK2/CALR/MPL-wildtype ET is not a homogeneous entity and that cases with polycythaemia haematoepoiesis probably constitute a hereditary disorder (2666). These mutations are not specific for ET; they are found in polycythaemia vera and primary myelofibrosis as well. But none of these mutations have been reported in cases of reactive thrombocytosis (3920,3933). Mutations similar to those described in MPNs (3915) have also been found at very low frequencies in elderly patients with no haematological malignancy (1326,1830,4386). Very rarely, cases of ET acquire a BCR-ABL1 rearrangement; however, the clinical significance of this is uncertain. Due to this additional phenotypic mutation, a morphological and haematological shift capable of producing a chronic myeloid leukaemia-like evolution may occur (1740).

An abnormal karyotype is found in only 5–10% of ET cases diagnosed according to the previous PVSG criteria (2791) and in 7.7% of WHO-defined cases (3920). There is no consistent abnormality, but reported abnormalities include gain of chromosome 8, abnormalities 9q, and del(20q) (1641,3045). Isolated instances of del(5q) have also been reported in ET, and careful morphological examination is needed to distinguish such cases from myelodysplastic syndromes associated with this abnormality (3045).

Prognosis and predictive factors

ET is an indolent disorder characterized by long symptom-free intervals interrupted by occasional life-threatening thromboembolic or haemorrhagic events (566, 1096,1215,1217,1378,1563,2791,3088, 3927). After many years, a few patients with ET develop bone marrow fibrosis of grade 2–3 on a 0–3 scale (2148,3975) or grade 3–4 on a 0–4 scale (2146), associated with myeloid metaplasia (extramedullary haematoepoiesis), but such progression is uncommon (254,612,717,1217,1377,1378,1380,3511,3927), occurring in only about 10% of cases in large, strictly WHO-defined cohorts (3920). The diagnostic criteria for post-ET myelofibrosis are listed in Table 2.13. Strict adherence to these and other WHO criteria (265,3931,3932) is necessary to avoid diagnostic confusion associated with pre-PF accompanied by thrombocytosis (263,1380). Clear-cut differentiation of ET from pre-PF is crucial, because these entities differ significantly in terms of complications and survival (11,254, 1086,1380,2150,3961,3977). In large series of WHO-defined cases, the relative incidence rates of post-ET myelofibrosis were found to be approximately half the rates of post-polycythaemia vera myelofibrosis (254,3920). Transformation of ET to the blast phase (i.e. acute myeloid leukaemia) or myelodysplastic syndrome occurs in <5% of cases, and is likely related to previous cytotoxic therapy (1217, 1563); the risk of transformation is lower among strictly WHO-defined cases (254, 1378,3920). Median survival times of 10–15 years are commonly reported. Because ET usually occurs late in middle age, life expectancy is near normal for many patients (254,717,1377,2150,3090, 4348). However, most clinical studies are based on older diagnostic guidelines (2791), which fail to differentiate clearly between pre-PF with accompanying thrombocytosis and ET according to the current WHO classification (263). A substantial difference in overall prognosis has been reported depending on which classification system is applied (2150). For patients with strictly WHO-defined ET, the observed and relative survival was similar to that of the general European population (254,2150), and transformation to overt myelofibrosis and the blast phase appeared to be relatively rare (254,3933). In contrast, the survival of patients with WHO-defined ET was found to be inferior to that of a sex- and age-matched United States population at one centre (3920), whereas the observed rates of fibrotic and blast transformation were comparable with those found in a previous Italian study (254). The rates of conversion of ET to overt polycythaemia vera in JAK2-mutated cases reported in some studies (540,3458) depend on the diagnostic criteria applied; when the WHO criteria are used, the incidence of transformation appears to be <5% (250).

Table 2.13 Diagnostic criteria for post-essential thrombocythaemia (ET) myelofibrosis (265)

<table>
<thead>
<tr>
<th>Required criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Documentation of a previous diagnosis of WHO-defined ET</td>
</tr>
<tr>
<td>2. Bone marrow fibrosis of grade 2–3 on a 0–3 scale (3975) or grade 3–4 on a 0–4 scale (2146)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Additional criteria (2 are required)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Anaemia (i.e. below the reference range given age, sex, and altitude considerations) and a &gt;2 g/dL decrease from baseline haemoglobin concentration</td>
</tr>
<tr>
<td>2. Leukoerythroblasticosis</td>
</tr>
<tr>
<td>3. Increasing splenomegaly, defined as either an increase in palpable splenomegaly of &gt;5 cm from baseline (distance from the left costal margin) or the development of a newly palpable splenomegaly</td>
</tr>
<tr>
<td>4. Elevated lactate dehydrogenase level (above the reference range)</td>
</tr>
<tr>
<td>5. Development of any 2 (or all 3) of the following constitutional symptoms: &gt;10% weight loss in 6 months, night sweats, unexplained fever (&gt;37.5 °C)</td>
</tr>
</tbody>
</table>

Chronic eosinophilic leukaemia, NOS

Definition
Chronic eosinophilic leukaemia (CEL) not otherwise specified (NOS), is a myeloproliferative neoplasm (MPN) in which an autonomous, clonal proliferation of eosinophil precursors results in persistently increased numbers of eosinophils in the peripheral blood, bone marrow, and peripheral tissues, with eosinophilia being the dominant haematological abnormality. Organ damage occurs as a result of leukemic infiltration or of the release of cytokines, enzymes, or other proteins by the eosinophils.

CEL, NOS excludes cases with a Philadelphia (Ph) chromosome; BCR-ABL1 fusion; rearrangement of PDGFRα, PDGFRα, or FGFR1; or PCM1-JAK2, ETV6-JAK2, or BCR-JAK2 fusion.

In CEL, NOS the eosinophil count is $>1.5 \times 10^9/L$ in the blood, and there are <20% blasts in the peripheral blood and bone marrow. For a diagnosis of CEL, NOS to be made, there should be evidence for clonality of myeloid cells or an increase in myeloblasts in the peripheral blood or bone marrow. However, in many cases it is impossible to prove clonality; in such cases, providing there is no increase in blast cells, the diagnosis of idiopathic hypereosinophilic syndrome (HES) is made. It is clinically important to clearly distinguish between CEL, NOS and idiopathic HES. Idiopathic HES is defined as eosinophilia (eosinophil count ≥1.5 × 10^9/L) persisting for ≥6 months for which no underlying cause can be found, associated with signs of organ involvement and dysfunction (759,4291); there is no evidence of eosinophil clonality. It is a diagnosis of exclusion, and may include some cases of true eosinophilic leukaemia that cannot currently be recognized, as well as cases of cytokine-driven eosinophilia due to the abnormal release of eosinophil growth factors (e.g. IL2, IL3, and IL5) for unknown reasons (226,759,3581,3759,4291). If there is a similar unexplained hypereosinophilia but with no evidence of tissue damage, the designation ‘idiopathic hypereosinophilia’ is appropriate. As outlined in Table 2.14, diagnosis of CEL, NOS requires integration of clinical, laboratory, and molecular features.

ICD-O code 9964/3

Epidemiology
Due to the previous difficulty in distinguishing CEL from idiopathic HES, the true incidence of these rare diseases is unknown. CEL, NOS appears to be more common in men, with a reported median age of occurrence in the seventh decade of life (42498,1606). The epidemiological features of idiopathic cases of HES remain undefined.

Localization
CEL, NOS is a multisystemic disorder. The peripheral blood and bone marrow are always involved. Tissue infiltration by eosinophils and the release of cytokines
Fig. 2.27 Reactive eosinophilia in lymphoblastic leukaemia. A The elevation of the white blood cell count seen in this peripheral blood smear is due primarily to eosinophils, with only an occasional lymphoblast. B Lymphoblasts (arrows) are clearly appreciable in this blood smear.

Fig. 2.28 Chronic eosinophilic leukaemia, NOS. Peripheral blood smear from a patient with a history of persistent eosinophilia. Immature and mature eosinophils are present. The cytogenetic analysis showed trisomy 10.

Fig. 2.29 Idiopathic hypereosinophilic syndrome. Blood smear from a patient with cardiac failure, leukocytosis, and hypereosinophilia.

and humoral factors from the eosinophil granules lead to tissue damage in a number of organs; the heart, lungs, central nervous system (CNS), skin, and gastrointestinal tract are commonly involved. Splenic and hepatic involvement is also common [1606].

Clinical features
Eosinophilia is sometimes detected incidentally in patients who are otherwise asymptomatic. In other cases, patients experience constitutional symptoms such as weight loss, night sweats, fever, fatigue, cough, angio-oedema, muscle pain, pruritus, and diarrhoea. The most serious clinical findings relate to endomyocardial fibrosis, with ensuing restrictive cardiomegaly. Scarring of the mitral and tricuspid valves leads to valvular regurgitation and the formation of intracardiac thrombi, which can embolise to the brain or elsewhere. Cardiac failure can occur. Peripheral neuropathy, CNS dysfunction, pulmonary symptoms due to lung infiltration, and rheumatological findings are frequent manifestations [1606].

Microscopy
In CEL, NOS the most striking feature in the peripheral blood is eosinophilia, mainly of mature eosinophils, with only small numbers of eosinophilic myelocytes and promyelocytes [2127]. There may be a range of eosinophil abnormalities, including sparse granulation (with clear areas of cytoplasm), cytoplasmic vacuolation, nuclear hypersegmentation or hyposegmentation, and increased size. Because these changes can be seen in both reactive and neoplastic eosinophilia, they are not very helpful in determining whether a case is likely to be CEL, NOS [226]. Occasional patients with CEL, NOS have cytologically normal eosinophils, but the absence of eosinophil dysplasia generally favours reactive eosinophilia [4249B]. Significant dysplasia in cells of other myeloid lineages supports the diagnosis of CEL, NOS [4249B]. Neutrophilia often accompanies the eosinophilia, and some cases show mild monocytosis, but do not meet all the criteria for chronic myelomonocytic leukaemia. Mild basophilia has been reported. Blast cells may be present but account for <20% of the cells.

The bone marrow is hypercellular, due in part to eosinophil proliferation [481, 1606, 2127]. In most cases, eosinophil maturation is orderly (i.e. without a disproportionate increase in myeloblasts). Charcot-Leyden crystals are often present. Erythropoiesis and megakaryocyteopoiesis are usually normal. Increased proportions of myeloblasts (>2% in peripheral blood or 5–19% in bone marrow) support the diagnosis of CEL, NOS, as do dysplastic features in other cell lineages [4249B]. Marrow fibrosis is seen in about one third of cases, although severe fibrosis is rare [481,4249B]. Any tissue can show eosinophilic infiltration, and Charcot-Leyden crystals are often present. Fibrosis is common, caused
by the degranulation of the eosinophils and the resulting release of eosinophil basic proteins and eosinophil cathelicidin.

**Cytochemistry**

Cytochemical stains can be used to identify eosinophils but are not necessary for diagnosis. Partial degranulation can lead to eosinophils having reduced peroxidase content and can render automated leukocyte counts unreliable.

**Differential diagnosis**

Diagnosis requires positive evidence of the leukemic nature of the condition and exclusion of myeloid neoplasms with rearrangement of PDGFRα, PDGFRβ or FGFR1, or with PCM1-JAK2, ETV6-JAK2, or BCR-JAK2 fusion. The diagnostic process often starts with exclusion of reactive eosinophilia. A detailed history, physical examination, blood count and examination of the blood smear are essential. Conditions to be excluded include parasitic infection, allergies, pulmonary diseases such as Löffler syndrome, cyclical eosinophilia, skin diseases such as angiitis, Hodgkin lymphoma, systemic mastocytosis, lymphoblastic leukaemia and other MPNs can be associated with abnormal release of IL2, IL3, IL5, or granulocyte-macrophage colony-stimulating factor and a secondary eosinophilia that mimics CEL, NOS {226,2063,2043,3510}. In addition, a number of neoplastic disorders, such as T-cell lymphoma, Hodgkin lymphoma, systemic mastocytosis, lymphoblastic leukaemia and other MNPs can be associated with abnormal release of IL2, IL3, IL5, or granulocyte-macrophage colony-stimulating factor and a secondary eosinophilia that mimics CEL, NOS. Cases with rearrangement of PDGFRα, PDGFRβ or FGFR1, or with PCM1-JAK2, ETV6-JAK2, or BCR-JAK2 fusion. The diagnostic process often starts with exclusion of reactive eosinophilia. A detailed history, physical examination, blood count and examination of the blood smear are essential. Conditions to be excluded include parasitic infection, allergies, pulmonary diseases such as Löffler syndrome, cyclical eosinophilia, skin diseases such as angiitis, Hodgkin lymphoma, systemic mastocytosis, lymphoblastic leukaemia and other MPNs can be associated with abnormal release of IL2, IL3, IL5, or granulocyte-macrophage colony-stimulating factor and a secondary eosinophilia that mimics CEL, NOS.

**Immunochemistry**

No specific immunophenotypic abnormality has been reported in CEL, NOS. However, immunophenotyping is relevant to the diagnosis of T lymphocyte-driven eosinophilia.

**Cell of origin**

The cell of origin is a haematopoietic stem cell, but the lineage potential of the affected cell may be variable. T-lymphoblastic transformation has been reported, so it appears that some cases arise from a pluripotent lymphoid-myeloid stem cell (1606).

**Genetic profile**

No single or specific cytogenetic or molecular genetic abnormality has been identified in CEL, NOS. Cases with rearrangement of PDGFRα, PDGFRβ or FGFR1, or with PCM1-JAK2 or variants are specifically excluded. The presence of a Ph chromosome or BCR-ABL1 fusion indicates one of the rare cases of chronic myeloid leukaemia with dominant eosinophilia, rather than CEL, NOS. Even when eosinophilia occurs in conjunction with a chromosomal abnormality that is usually myeloid neoplasm-associated, it may be difficult to determine whether the eosinophils are part of the clonal process, because reactive eosinophilia can occur in patients with myeloid neoplasms (1226). However, the finding of a recurrent karyotypic abnormality that is usually observed in myeloid disorders (e.g. gain of chromosome 8, loss of chromosome 7, or isochromosome 17q) does support the diagnosis of CEL, NOS (226,3068), as does the presence of a translocation. Occasional cases have a JAK2 mutation, but mutations in ASXL1, TET2 and EZH2 appear to be more common (1872,4294). Four patients with a somatic activating KIT M541L mutation, whose disease was responsive to low-dose imatinib, have been reported (1799). Further studies to establish the frequency of this mutation are needed. X-linked polymorphism analysis of the AR (also called HUMARA) or PGK genes have been used in female patients to demonstrate clonality (652,2418). In the appropriate context, the finding of somatic mutations in genes that are frequently mutated in other myeloid neoplasms can support the diagnosis of CEL, NOS. However, mutations in genes such as TET2, ASXL1 and DNMT3A are sometimes detected by DNA sequencing in elderly people without neoplasms (1326,4386), so their presence should not be considered definitive proof that eosinophilia results from a neoplastic rather than reactive process.
Myeloproliferative neoplasm, unclassifiable

Definition
The designation of myeloproliferative neoplasm, unclassifiable (MPN-U) should be applied only to cases that have definite clinical, laboratory, molecular, and morphological features of a myeloproliferative neoplasm (MPN) but fail to meet the diagnostic criteria for any of the specific MPN entities, or that present with features that overlap between two or more of the MPN categories (Table 2.15, p. 54). Most cases of MPN-U fall into one of three groups:

1. A subset of cases with so-called masked/pre-polycythaemic presentation of polycythaemia vera, prefibrotic/early primary myelofibrosis, or early-phase essential thrombocythaemia in which the characteristic features are not yet fully developed [1380,2147,2152] – a proportion of cases presenting with portal or splanchnic vein thrombosis that fail to meet the diagnostic criteria for any of the specific MPN entities may also be considered to belong in this group [1103,1362];

2. Advanced-stage MPN, in which pronounced myelofibrosis, osteosclerosis, or transformation to a more aggressive stage with increased blast counts and/or myelodysplastic changes obscures the underlying disorder [404,405,2151,3968,3978,3983]; or

3. Cases with convincing evidence of an MPN in which a coexisting neoplastic or inflammatory disorder obscures some of the usual diagnostic clinical and/or morphological features [2147].

ICD-O code 9975/3

Epidemiology
The exact incidence of MPN-U is unknown, but some reports indicate that unclassifiable cases account for as many as 10–15% of all MPNs [3983]. The relative frequency varies significantly according to the experience of the diagnostician and the specific classification system and criteria used [1361,1361A,1362,1363,2151,2348A,3978]. Careful evaluation of clinical, morphological, and molecular features reduces the frequency of unclassifiable cases to <5% [257,1086,1361A,1799A,2147,2433].

Localization
The blood and bone marrow are the major sites of involvement, but in advanced stages the spleen and liver (i.e. the major sites of extramedullary haematopoiesis) may be also affected.

Clinical features
The clinical features of MPN-U are similar to those of other MPNs. In early unclassifiable disease, organomegaly may be minimal or absent, but splenomegaly and hepatomegaly can be massive in advanced cases in which bone marrow specimens are characterized by marked myelofibrosis and/or increased numbers of blasts [3978,3983]. The haematological values are also variable, ranging from mild leukocytosis to moderate or marked thrombocytosis, with or without accompanying anaemia. Prominent cytopenia or myelodysplastic features should always prompt the definitive exclusion of MDS/MPN and myelodysplastic syndrome (MDS) [257,2147]. Discrepancies between morphological and clinical features are particularly common in cases presenting with otherwise unexplained portal or splanchnic vein thrombosis [1103,1362].

Exclusionary criteria
The presence of BCR-ABL1 fusion; rearrangement of PDGERA, PDGFRB, or FGFR1; or PCM1-JAK2 fusion excludes the diagnosis of MPN-U. This diagnosis is also inappropriate if clinical data sufficient for proper classification are not available, if the bone marrow specimen is of inadequate quality or size for accurate evaluation [2147,2151,2152,3978,3983], or if the...
particularly when myelodysplastic features are encountered in routine practice, particularly when myelodysplastic features are observed (1361). There may be significant discrepancies between morphological and clinical features (1362). It is often preferable to describe the morphological findings and to recommend additional clinical and laboratory procedures (e.g., adequate peripheral blood smears and bone marrow biopsy and aspirate specimens, as well as extended molecular testing) to further classify the process. When a diagnosis of MPN-U is made, the report should summarize the reason for the difficulty in reaching a more specific diagnosis, and if possible should specify which of the MPN subtypes can be excluded from consideration.

Differential diagnosis

If a case does not have the features of one of the well-defined entities, the possibility that it is not an MPN must be strongly considered (2147,2152). A reactive bone marrow response to infection; an inflammatory response; the effects of toxins; and the results of administration of chemotherapy, growth factors, cytokines, or immunosuppressive agents can closely mimic MPN and must be definitively excluded (1363). Furthermore, other haematopoietic and non-haematopoietic neoplasms, such as lymphoma or metastatic carcinoma, can infiltrate the marrow and cause reactive changes (including dense fibrosis and osteosclerosis) that can be misconstrued as MPN (3968).

The detection of characteristic JAK2, CALR, or MPL driver mutations separates an MPN from reactive conditions, although not all cases of MPN-U express one of these major clonal markers (2019, 3924,3932). In the absence of mutations in any of these genes, clonality should be confirmed whenever possible (2666). It has been shown that non-canonical mutations of MPL and JAK2 (i.e., those outside the exons usually investigated in routine diagnostic tests) are present in about 15% of triple-negative (i.e., apparently JAK2/CALR/MPL-wild-type) MPN cases (2666); therefore, extended molecular testing can help to establish the diagnosis of an MPN. The identification of other mutations frequently associated with myeloid neoplasms (e.g., ASXL1, EZH2, TET2, IDH1, IDH2, SRSF2, and SF3B1 mutations) can be of help in confirming the presence of clonal haemopoiesis (3924,3932). However, acquired clonal mutations (including some of those mentioned above) may also occur in the haematopoietic cells of apparently healthy elderly individuals with no myeloid neoplasm (1326,1830,3772). Therefore, caution is advised. Cytogenetic abnormalities occur in as many as 30% of cases (3920,3924) and can be useful in confirming clonality.

Cases with prominent cytopenia and/or significant accompanying myelodysplastic changes may be better categorized as myelodysplastic/myeloproliferative neoplasm (MDS/MPN) (994,1517,1748,2987).

The defining characteristics of each MPN must be considered with the understanding that (as with any biological process) variations do occur, and that the clinical and morphological manifestations can change over time as the disease progresses through various stages (257, 2147,2150,2151,2152).

Microscopy

Many cases that are diagnosed as MPN-U constitute very early stage disease in which the differentiation between essential thrombocythaemia, primary myelofibrosis, and polycythaemia vera may be very difficult (2147,2151,2152). In such cases, peripheral blood smears often reveal thrombocytosis and variable neutrophilia (2152). The haemoglobin concentration may be normal, mildly decreased, or borderline increased (3966, 3969,3972). Bone marrow biopsy specimens frequently show hypercellularity and prominent megakaryocytic proliferation, with variable amounts of granulocytic and erythroid proliferation (2147,2152, 3978,3984,3985). Careful application of the diagnostic guidelines recommended in this volume for each specific MPN, with close attention paid to the megakaryocyte morphology and histotopography, will enable the classification of most MPNs as a specific subtype (1361, 2433); the remainder are best classified as MPN-U until careful follow-up or additional laboratory studies provide sufficient evidence for a more precise diagnosis (2147,2152).

In late-stage disease, bone marrow specimens may show dense fibrosis and/or osteomyelosclerosis, indicating a terminal burnt-out stage; if no previous history or histology is available, it may be impossible to distinguish between the post-polycythaemic stage of polycythaemia vera (post-polycythaemia vera myelofibrosis) or, rarely, essential thrombocythaemia (post-essential thrombocythaemia myelofibrosis) and the overt fibrotic/osteosclerotic stage of primary myelofibrosis (407,3964,3965, 3978,3983). Differential diagnosis with MDS (or MDS/MPN, unclassifiable) associated with severe bone marrow fibrosis may be challenging in cases with multilineage dysplasia, due to the overlapping clinical and morphological features (218, 941,942,2986). In these cases, meticulous investigation with respect to splenomegaly, blood cell count, peripheral blood and bone marrow findings, presence or absence of MPN-associated mutations (in JAK2, MPL, or CALR), and karyotypic findings may facilitate diagnosis.
Although chronic myeloid leukaemia may also be accompanied by marked myelofibrosis, the small size of the megakaryocytes indicates the correct diagnosis (3978, 3983), and cytogenetic and molecular genetic demonstration of the Philadelphia (Ph) chromosome or BCR-ABL1 fusion confirms the diagnosis of chronic myeloid leukaemia.

The presence of ≥10% blasts in the peripheral blood or bone marrow and/or the finding of significant myelodysplasia generally indicate a transition to a more aggressive, often terminal phase of the disease (404, 405). Cases initially diagnosed as MPN-U in which 10–19% blasts are found in the peripheral blood or bone marrow are considered to be in the accelerated phase. Blast percentages of ≥20% in the peripheral blood or bone marrow indicate the blast phase (i.e. acute leukaemic transformation) of previously diagnosed MPN-U. In most of these advanced-stage cases, fibrosis can cause dilution of bone marrow aspirates; therefore, immunohistochemical staining of the bone marrow biopsy sections for CD34 provides diagnostic value by demonstrating increased numbers and/or clusters or aggregates of blasts (3978, 3983). Prominent myelodysplastic features may appear during the natural progression of an MPN even without prior cytoreductive therapy (404). However, if an initial, untreated case demonstrates significant myelodysplasia, the diagnostic alternatives of MDS/MPN or MDS, unclassifiable, should be considered (225, 994, 1520, 1748, 2987, 3919, 3930). In such cases, additional molecular testing and cytogenetic analysis may be necessary for better diagnostic characterization (1517, 3930, 4502).

**Cell of origin**

The postulated cell of origin is a haematopoietic stem cell.

**Genetic profile**

There is no cytogenetic or molecular genetic finding specific for this group. There is no BCR-ABL1 fusion; no rearrangement of PDGFRα, PDGFRβ, or FGFR1; and no PCM1-JAK2 fusion. The presence of a phenotypic driver mutation in JAK2, CALR, or MPL supports the diagnosis of an MPN. Cases that do not meet the clinical and/or morphological criteria for a specific MPN subtype or any other specific disease category are best categorized as MPN-U.

**Prognosis and predictive factors**

In patients with MPNs that are initially unclassifiable, follow-up studies performed at intervals of 6–12 months can often provide sufficient information for a more precise classification (257, 2147, 2152, 3978, 3983). In the early stages of disease, such patients have a prognosis similar to patients with the neoplasms into which their disease eventually evolves (2150). Patients with advanced disease in whom the initial process is no longer recognizable due to bone marrow fibrosis or blastic infiltration are expected to have a poor prognosis (404, 405). Selective JAK1/JAK2 inhibitor therapy has been shown to rapidly reduce splenomegaly, markedly improve myelofibrosis-associated symptoms, and prolong overall survival in primary and secondary myelofibrosis (i.e. primary myelofibrosis, post-polycythaemia vera myelofibrosis, and post–essential thrombocythaemia myelofibrosis) (1798, 1833, 4316). However, the same therapeutic approach was not found to be effective in MDS/MPN or MDS associated with bone marrow fibrosis. Therefore, the identification of MPNs (1261, 2962), even if they are unclassifiable, is of importance for clinical decision-making and overall prognosis (941, 942).
CHAPTER 3

Mastocytosis
Cutaneous mastocytosis
Systemic mastocytosis
Mast cell sarcoma
Mastocytosis

Definition
Mastocytosis occurs due to a clonal, neoplastic proliferation of mast cells that accumulate in one or more organ systems. It is characterized by an abnormal mast cell infiltrate, which often contains multifocal compact clusters or cohesive aggregates. The disorder is heterogeneous, with manifestations ranging from skin lesions that can spontaneously regress to highly aggressive neoplasms associated with multiorgan failure and poor survival. Mastocytosis variants (Table 3.01) are recognized mainly by pathology investigations, distribution of the disease, and clinical manifestations. In cutaneous mastocytosis, the mast cell infiltrate remains confined to the skin, whereas systemic mastocytosis is characterized by the involvement of at least one extracutaneous organ, with or without evidence of skin lesions. Mastocytosis should be strictly distinguished from mast cell hyperplasia and mast cell activation states in which the morphological and molecular abnormalities that characterize the neoplastic proliferation of mast cells are absent. The diagnostic criteria for cutaneous and systemic mastocytosis are listed in Table 3.02.

ICD-O codes
Cutaneous mastocytosis 9740/1
Indolent systemic mastocytosis 9741/1
Systemic mastocytosis with an associated haematological neoplasm (AHN) 9741/3
Aggressive systemic mastocytosis (ASM) 9741/3
Mast cell leukaemia 9742/3
Mast cell sarcoma 9740/3

Epidemiology
Mastocytosis can occur at any age. Cutaneous mastocytosis is most common in children and can be present at birth. About 50% of affected children develop typical skin lesions before the age of 6 months. Cutaneous mastocytosis is much less common in adults than in children [576,1568,4105,4353], and a slight male predominance has been reported. Systemic mastocytosis is generally diagnosed after the second decade of life, and reported male-to-female ratios range from 1:1 to 1:1.5 (2646,3074).

Clinical features
There are three main forms of cutaneous mastocytosis, which constitute distinct clinicopathological entities: urticaria pigmentosa/maculopapular cutaneous mastocytosis, diffuse cutaneous mastocytosis, and mastocytoma of skin. The lesions of all forms may urticate when stroked (Darier’s sign), and most show intraepidermal accumulation of melanin pigment. The term ‘urticaria pigmentosa’ describes these two clinical features macroscopically. Blistering is usually seen in patients aged <3 years, and may be observed in all forms of paediatric cutaneous mastocytosis [1568,4353]. However, blistering does not indicate a separate subtype of cutaneous mastocytosis. The presenting symptoms of systemic mastocytosis have been grouped into four categories: constitutional symptoms (e.g. fatigue, weight loss, fever, diaphoresis), skin manifestations (e.g. pruritus, urticaria, dermatographism, flushing), mediator-related systemic events (e.g. abdominal pain, gastrointestinal distress, syncope, headache, hypotension, tachycardia, respiratory symptoms) and musculoskeletal symptoms (e.g. bone pain, osteopenia/osteoporosis, fractures, arthralgias, myalgias) [4107]. These disease manifestations can range from mild

Table 3.01 Classification of mastocytosis variants

<table>
<thead>
<tr>
<th>Variant</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cutaneous mastocytosis</strong></td>
</tr>
<tr>
<td>Urticaria pigmentosa/maculopapular cutaneous mastocytosis</td>
</tr>
<tr>
<td>Diffuse cutaneous mastocytosis</td>
</tr>
<tr>
<td>Mastocytoma of skin</td>
</tr>
<tr>
<td><strong>Systemic mastocytosis</strong></td>
</tr>
<tr>
<td>Indolent systemic mastocytosis (including the bone marrow mastocytosis subtype)</td>
</tr>
<tr>
<td>Smouldering systemic mastocytosis</td>
</tr>
<tr>
<td>Systemic mastocytosis with an associated haematological neoplasm</td>
</tr>
<tr>
<td>Aggressive systemic mastocytosis</td>
</tr>
<tr>
<td>Mast cell leukaemia</td>
</tr>
<tr>
<td><strong>Mast cell sarcoma</strong></td>
</tr>
</tbody>
</table>

* The complete diagnosis of these variants requires information regarding B and C findings (Table 3.04, p. 66), all of which may not be available at the time of initial tissue diagnosis.

* This variant is equivalent to the previously described entity ‘systemic mastocytosis with an associated clonal haematological non—mast cell lineage disease’, and the terms can be used synonymously.
### Table 3.02 Diagnostic criteria for cutaneous and systemic mastocytosis

<table>
<thead>
<tr>
<th>Cutaneous mastocytosis</th>
<th>Systemic mastocytosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skin lesions demonstrating the typical findings of urticaria pigmentosa/maculopapular cutaneous mastocytosis, diffuse cutaneous mastocytosis or solitary mastocytoma, and typical histological infiltrates of mast cells in a multifocal or diffuse pattern in an adequate skin biopsy. In addition, features/criteria sufficient to establish the diagnosis of systemic mastocytosis must be absent (1567,4105,4107). There are three variants of cutaneous mastocytosis (see Table 3.01).</td>
<td>The diagnosis of systemic mastocytosis can be made when the major criterion and at least 1 minor criterion are present, or when ≥ 3 minor criteria are present.</td>
</tr>
<tr>
<td><strong>Major criterion</strong></td>
<td><strong>Major criterion</strong></td>
</tr>
<tr>
<td>Multifocal dense infiltrates of mast cells (≥ 15 mast cells in aggregates) detected in sections of bone marrow and/or other extracutaneous organ(s)</td>
<td>Detection of an activating point mutation at codon 816 of KIT in the bone marrow, blood or another extracutaneous organ</td>
</tr>
<tr>
<td><strong>Minor criteria</strong></td>
<td><strong>Minor criteria</strong></td>
</tr>
<tr>
<td>1. In biopsy sections of bone marrow or other extracutaneous organs, &gt; 25% of the mast cells in the infiltrate are spindle-shaped or have atypical morphology or &gt; 25% of all mast cells in bone marrow aspirate smears are immature or atypical.</td>
<td>1. Mast cells in bone marrow, blood or another extracutaneous organ express CD25, with or without CD2, in addition to normal mast cell markers.</td>
</tr>
<tr>
<td>2. Detection of an activating point mutation at codon 816 of KIT in the bone marrow, blood or another extracutaneous organ</td>
<td>2. Serum total tryptase is persistently &gt; 20 ng/mL, unless there is an associated myeloid neoplasm, or when ≥ 3 minor criteria are present.</td>
</tr>
<tr>
<td>3. Mast cells in bone marrow, blood or another extracutaneous organ express CD25, with or without CD2, in addition to normal mast cell markers.</td>
<td>3. In biopsy sections of bone marrow or other extracutaneous organs, &gt; 25% of all mast cells in bone marrow aspirate smears are immature or atypical.</td>
</tr>
<tr>
<td>4. Serum total tryptase is persistently &gt; 20 ng/mL, unless there is an associated myeloid neoplasm, in which case this parameter is not valid.</td>
<td>4. Mast cells in bone marrow, blood or another extracutaneous organ express CD25, with or without CD2, in addition to normal mast cell markers.</td>
</tr>
</tbody>
</table>

---

**Fig. 3.01 Cutaneous mastocytosis.** A Numerous typical macular and maculopapular pigmented lesions of urticaria pigmentosa in a young child. B The skin lesions of all forms of cutaneous mastocytosis may uricate when stroked (Darier's sign). A palpable wheal appears a few moments after the physical stimulation, due to the release of histamine from the mast cells. C Thickened, reddish, peau chagrine lesions characteristic of diffuse cutaneous mastocytosis, which occur almost exclusively in children.
haematological neoplasm, the clinical symptoms, disease course and prognosis relate both to systemic mastocytosis and to the associated haematological disorder [2329,3751,4107]; in many cases, the clinical outcome is determined primarily by the associated haematological neoplasm [570,3928]. Therefore, both the type of systemic mastocytosis and the type of associated haematological neoplasm should be classified according to the WHO criteria in all cases. Serum tryptase levels are used in the evaluation and monitoring of patients with mastocytosis. Serum total tryptase persistently >20 ng/mL suggests systemic mastocytosis, and is a minor criterion for diagnosis (unless there is an associated myeloid neoplasm, in which case this parameter is not valid). In most patients with cutaneous mastocytosis, serum tryptase levels are normal to slightly elevated [3591,4107].

Microscopy
The diagnosis of mastocytosis is usually based on the demonstration of multifocal clusters or cohesive aggregates/infiltrates of mast cells in adequate biopsy specimens (Table 3.02, p.63). The histological pattern of the mast cell infiltrate can vary depending on the tissue sampled [1568, 1698,4107]. A diffuse interstitial infiltration pattern is defined as loosely scattered mast cells in the absence of compact aggregates. However, this pattern is also observed in reactive mast cell hyperplasia and in some myeloproliferative neoplasms, including cases in which elevated numbers of immature atypical mast cells are found but the criteria for systemic mastocytosis or mast cell leukaemia are not met [1696]. In patients with the diffuse infiltration pattern, it is therefore impossible to establish the diagnosis of mastocytosis without additional studies, including the demonstration of an aberrant immunophenotype and/or detection of an activating point mutation in KIT [1698,1699,2122,3735,3736]. In contrast, the presence of multifocal compact mast cell infiltrates or a diffuse–compact mast cell infiltration pattern is highly compatible with the diagnosis of mastocytosis. However, additional immunohistochemical and molecular studies are strongly recommended even in these cases.

In tissue sections stained with haematoxylin and eosin, normal/reactive mast cells are usually loosely scattered throughout the sample and display round to oval nuclei with clumped chromatin, a low nuclear:cytoplasmic ratio, and absent or indistinct nucleoli. The mast cell cytoplasm is abundant and usually filled with small, faintly visible granules. Dense aggregates of mast cells are only very exceptionally detected in reactive states [2268,3074,4107].

In smear preparations, mast cells are readily visible with Romanowsky staining as medium-sized round or oval cells with plentiful cytoplasm, containing densely packed metachromatic granules and round or oval nuclei. In normal/reactive states, mast cells are easily distinguished from basophils, which have segmented nuclei and larger and fewer granules. Enzyme cytochemistry shows that mast cells react strongly with naphthol AS-D chloroacetate esterase (CAE) but do not express myeloperoxidase. In mastocytosis, the cytology of mast cells varies, but abnormal cytological features (including marked spindling and hypogranularity) are almost always present [3750,4107].

In high-grade mastocytosis lesions, cytological atypia is pronounced, and the occurrence of metachromatic blast cells is a typical feature of mast cell leukaemia [4107,4108]. The finding of frequent mast cells with bilobated or multilobated nuclei (called promastocytes) usually indicates an aggressive mast cell proliferation, although these cells may also be seen at lower frequency in other subtypes of the disease. Mitotic figures do occur in mast cells, but are infrequent even in the aggressive and leukaemic variants of systemic mastocytosis. In a few patients with systemic mastocytosis, the mast cells are mature and well granulated, without atypia or aberrant CD25 expression; such cases have been referred to as well-differentiated systemic mastocytosis. In most of these cases, no KIT mutation at codon 816 is found, and the mast cells usually respond to KIT tyrosine kinase inhibitors, including imatinib [42, 83]. However, well-differentiated morphology of mast cells can be present in any variant of systemic mastocytosis. Therefore, well-differentiated systemic mastocytosis is not considered a distinct category of systemic mastocytosis.

The number of mast cells can be assessed by conventional staining procedures, using Giemsa or toluidine blue staining to detect the metachromatic mast cell granules; CAE is also helpful [1698]. However, the most specific methods for identifying immature or atypical mast cells in tissue sections involve immunohistochemical staining for tryptase/chymase and KIT (CD117), and, for identifying neoplastic mast cells, CD25 or less commonly CD2. In a subset of cases, mast-cells also express CD30 [2729,3734]. Aberrant expression of surface markers, including
CD2 and CD25, can also be detected by flow cytometry [1114]. The morphological and clinical features of the common forms of mastocytosis are described in the following sections on each variant.

### Cutaneous mastocytosis

**Definition**
The diagnosis of cutaneous mastocytosis (CM) requires the demonstration of typical clinical findings and histological proof of abnormal mast cell infiltration of the dermis. In cutaneous mastocytosis, there is no evidence of systemic involvement in the bone marrow or any other organ. In addition, the diagnostic criteria for systemic mastocytosis are not fulfilled. However, patients with cutaneous mastocytosis may present with one or two of the minor diagnostic criteria for systemic mastocytosis, such as an elevated serum tryptase level or abnormal morphology of mast cells in the bone marrow (Table 3.02, p.63).

Three major variants of cutaneous mastocytosis are recognized: urticaria pigmentosa/maculopapular cutaneous mastocytosis, diffuse cutaneous mastocytosis and mastocytoma of skin [4105,4107].

**ICD-O code** 9740/1

**Synonyms**
Maculopapular cutaneous mastocytosis; diffuse cutaneous mastocytosis; solitary mastocytoma of skin; urticaria pigmentosa

**Urticaria pigmentosa/maculopapular cutaneous mastocytosis**

This is the most common form of cutaneous mastocytosis. In children, the lesions of urticaria pigmentosa tend to be larger, fewer, and more papular than the skin lesions seen in adults with systemic mastocytosis. Recent data suggest that monomorphic small lesions detected in early childhood are more likely to persist into adulthood than are larger polymorphic lesions [569,1567]. Histopathology typically reveals aggregates of spindle-shaped mast cells filling the papillary dermis and extending as sheets and aggregates into the reticular dermis (4353). A subset of cases, usually occurring in young children, present as non-pigmented plaque-forming lesions.

In adults, the lesions are disseminated and they tend to be red or brownish red and macular or maculopapular. Histopathology of urticaria pigmentosa in adults typically reveals fewer mast cells than are seen in children. However, as mentioned above, cutaneous mastocytosis is very rare in adults; upon thorough bone marrow examination, most adult patients with skin lesions are found to have systemic mastocytosis. The number of lesional mast cells can sometimes overlap with the upper range of mast cell counts found in normal or inflamed skin. In some cases, examination of multiple biopsies and immunohistochemical analysis may be necessary to establish the diagnosis of cutaneous mastocytosis (4105,4353). Various KIT mutations, including D816V, have been detected in lesional skin in childhood cutaneous mastocytosis.

**Diffuse cutaneous mastocytosis**

This clinically remarkable variant of cutaneous mastocytosis is much less common than urticaria pigmentosa and presents almost exclusively in childhood. The skin is diffusely thickened and may have a grain leather (peau chagrine) or orange peel (peau d'orange) appearance. There are no individual lesions. In patients with clinically less obvious infiltration of the skin, the biopsy usually shows a band-like infiltrate of mast cells in the papillary and upper reticular dermis. In massively infiltrated skin, the histology may be the same as that seen in mastocytoma of skin [1568,4353].

**Mastocytoma of skin**

This variant typically occurs as a single lesion, almost exclusively in children, and without predilection for site [576,4353]. In some cases, two or three lesions occur. Histology shows sheets of mature-looking, highly metachromatic mast cells with abundant cytoplasm, which densely infiltrate the papillary and reticular dermis. These mast cell infiltrates may extend into the subcutaneous tissues. Cytological
Table 3.03 Diagnostic criteria for the variants of systemic mastocytosis

**Indolent systemic mastocytosis**
Meets the general criteria for systemic mastocytosis
- No C findings
- No evidence of an associated haematological neoplasm
- Low mast cell burden
- Skin lesions are almost invariably present

**Bone marrow mastocytosis**
As above (indolent systemic mastocytosis), but with bone marrow involvement and no skin lesions

**Smouldering systemic mastocytosis**
Meets the general criteria for systemic mastocytosis
- ≥ 2 B findings; no C findings
- No evidence of an associated haematological neoplasm
- High mast cell burden
- Does not meet the criteria for mast cell leukaemia

**Systemic mastocytosis with an associated haematological neoplasm**
Meets the general criteria for systemic mastocytosis
Meets the criteria for an associated haematological neoplasm (i.e. a myelodysplastic syndrome, myeloproliferative neoplasm, acute myeloid leukaemia, lymphoma or another haematological neoplasm classified as a distinct entity in the WHO classification)

**Aggressive systemic mastocytosis**
Meets the general criteria for systemic mastocytosis
- ≥ 1 C finding
- Does not meet the criteria for mast cell leukaemia
- Skin lesions are usually absent.

**Mast cell leukaemia**
Meets the general criteria for systemic mastocytosis
Bone marrow biopsy shows diffuse infiltration (usually dense) by atypical, immature mast cells.
Bone marrow aspirate smears show >20% mast cells.
In classic cases, mast cells account for >10% of the peripheral blood white blood cells, but the aleukaemic variant (in which mast cells account for <10%) is more common.
Skin lesions are usually absent.

Table 3.03 summarizes the specific diagnostic criteria for each variant of systemic mastocytosis.

**Indolent systemic mastocytosis**
In indolent systemic mastocytosis (ISM), the mast cell burden is usually low and skin lesions are found in most patients. For cases that fulfil the criteria for indolent systemic mastocytosis and also present with one B finding (Table 3.04), the diagnosis remains indolent systemic mastocytosis. However, if two or more B findings are detected, the diagnosis changes to smouldering systemic mastocytosis. The **KIT** gene should be sequenced if possible, because other **KIT** mutations have been found in some patients.

**ICD-O code**
9741/1

---

**Systemic mastocytosis**

**Definition**
Consensus criteria for the diagnosis of systemic mastocytosis have been established (Table 3.02, p.63), and five variants are recognized: indolent systemic mastocytosis, smouldering systemic mastocytosis, systemic mastocytosis with an associated haematological neoplasm, aggressive systemic mastocytosis and mast cell leukaemia. Table 3.03 summarizes the specific diagnostic criteria for each variant of systemic mastocytosis.

**Indolent systemic mastocytosis**
In indolent systemic mastocytosis (ISM), the mast cell burden is usually low and skin lesions are found in most patients. For cases that fulfil the criteria for indolent systemic mastocytosis and also present with one B finding (Table 3.04), the diagnosis remains indolent systemic mastocytosis. However, if two or more B findings are detected, the diagnosis changes to smouldering systemic mastocytosis. The **KIT** D816V mutation is present in the vast majority (>90%) of typical indolent systemic mastocytosis cases. If this mutation is not found but there remains high suspicion of systemic mastocytosis (e.g. in patients with well-differentiated mast cell morphology or advanced infiltration of the bone marrow), the **KIT** gene should be sequenced if possible, because other **KIT** mutations have been found in some patients.

**ICD-O code**
9741/1

---

**Fig. 3.05** Systemic mastocytosis. Skeletal lesions are common in systemic mastocytosis. This X-ray shows patchy osteosclerosis, osteoporosis and multiple lytic lesions in the femur.
Bone marrow mastocytosis
In the bone marrow mastocytosis subtype of indolent systemic mastocytosis, the burden of neoplastic mast cells is usually low, and serum tryptase levels are often normal or nearly normal.

Smouldering systemic mastocytosis
In smouldering systemic mastocytosis, the mast cell burden is high, organomegaly is often found, and multilineage involvement is typically present. Although the clinical course is often stable for many years, progression to aggressive systemic mastocytosis or mast cell leukaemia can occur. Skin lesions are found in most patients and the KIT D816V mutation is almost invariably present; unlike in typical indolent systemic mastocytosis, the mutation is usually detectable in several myeloid lineages and sometimes even in lymphocytes, reflecting multilineage involvement by the neoplastic process.
despite a lack of morphological evidence of an associated haematological neoplasm.

Systemic mastocytosis with an associated haematological neoplasm
Systemic mastocytosis with an associated haematological neoplasm (AHN) fulfils the general criteria for systemic mastocytosis as well as the criteria for an AHN. In most cases, a myeloid disease of non-mast cell lineage is detected, such as a myelodysplastic syndrome, myeloproliferative neoplasm, myelodysplastic/myeloproliferative neoplasm or acute myeloid leukaemia [4107]. The AHN should usually be considered a secondary neoplasm with clinical and prognostic implications. The most commonly detected AHN is chronic myelomonocytic leukaemia. Lymphoid neoplasms, such as multiple myeloma and lymphoma, are rare. The activating Kit D816V mutation is found in most cases of systemic mastocytosis with an AHN, and in many cases is detectable not only in the systemic mastocytosis compartment but also in the AHN cells (e.g. acute myeloid leukaemia blasts or chronic myelomonocytic leukaemia monocytes). Depending on the type of AHN, additional mutations in other genes (e.g. TET2, SRSF2, ASXL1, CBL, RUNX1 and the RAS family of oncogenes) may also be detected, and the accumulation of such mutations appears to be of prognostic significance.

Fig. 3.09 Systemic mastocytosis, lymph node biopsy. A This biopsy is diffusely infiltrated by neoplastic mast cells; only a remnant of a normal follicle can be seen. B The infiltrate is often paraffollicular in distribution; it is seen here as a monotonous population of cells with abundant, lightly staining cytoplasm. C Immunohistochemical staining for mast cell tryptase highlights the paraffollicular distribution of the mast cell infiltrate.

Fig. 3.10 Mast cell leukaemia. A Immunohistochemical staining for of the bone marrow biopsy mast cell tryptase. B Peripheral blood; note the bilobated nuclei and granulated cytoplasm often seen in this aggressive form of mastocytosis. C This image demonstrates the so-called clear-cell appearance and the folded, somewhat monocytoid nuclei that are typical of immature mast cells in mast cell leukaemia. D The bone marrow biopsy is diffusely infiltrated by the neoplastic mast cells.
Mast cell sarcoma

**Definition**
Mast cell sarcoma (MCS) is an extremely rare entity characterized by localized destructive growth of highly atypical mast cells, which can be identified only through the application of appropriate immunohistochemical markers, such as antibodies specific for tryptase and KIT (CD117). Although the disease is initially localized, distant spread followed by a terminal phase resembling mast cell leukemia occurs after a short interval of several months. Mast cell sarcomas have been reported occurring in the larynx, large bowel, meninges, bone and skin [745,1693,2073,3461].

**Synonym**
Systemic tissue mast cell disease

**Mast cell sarcoma**

**Mast cell leukaemia**
Mast cell leukaemia (MCL) is the leukaemic variant of systemic mastocytosis, in which bone marrow aspirate smears contain ≥20% mast cells [4107]. These mast cells are usually immature and atypical. Unlike in indolent systemic mastocytosis, the mast cells are often round rather than spindle-shaped. In classic mast cell leukaemia, mast cells account for ≥10% of the peripheral white blood cells, but the aleukaemic variant (the definition of which differs only in that the mast cells account for <10% of peripheral blood white blood cells) is more common [4107,4108]. In most patients with mast cell leukaemia, no skin lesions are detectable. Bone marrow biopsy shows a diffuse, dense infiltration with atypical, immature mast cells. C findings (Table 3.04, p.66), indicative of organ damage caused by the malignant mast cell infiltration, are usually present at diagnosis [4107], although rare cases present without them. Such cases, in which the mast cells are often mature and the clinical course less aggressive, constitute chronic mast cell leukaemia [4108]. In general, however, the prognosis of mast cell leukaemia is poor, with a survival of <1 year in most patients. Unlike indolent systemic mastocytosis, mast cell leukaemia may harbour atypical KIT mutations, such as non-D816V codon 816 mutations or non-codon 816 mutations. Therefore, if a case of mast cell leukaemia is negative for the KIT D816V mutation, KIT should be sequenced if possible. Patients with mast cell leukaemia may also accumulate mutations in other genes, such as TET2, SRSF2 and CBL.
CHAPTER 4

Myeloid/lymphoid neoplasms with eosinophilia and gene rearrangement

Myeloid/lymphoid neoplasms with PDGFRα rearrangement
Myeloid/lymphoid neoplasms with PDGFRβ rearrangement
Myeloid/lymphoid neoplasms with FGFR1 rearrangement
Myeloid/lymphoid neoplasms with PCM1-JAK2
The category 'myeloid/lymphoid neoplasms with eosinophilia and rearrangement of PDGFRA, PDGFRB or FGFR1, or with PCM1-JAK2' contains three specific rare disease groups and a provisional entity. Within this category, some features are shared and others differ, but all the neoplasms result from the formation of a fusion gene, or (rarely) from a mutation, resulting in the expression of an aberrant tyrosine kinase. Eosinophilia is characteristic but not invariable. In at least some cases in each group, the cell of origin is a mutated pluripotent (lymphoid–myeloid) stem cell.

These disorders can present as chronic myeloproliferative neoplasms (MPNs), but the frequency of manifestation as lymphoid neoplasms or acute myeloid leukaemia varies. The clinical and haematological features are also influenced by the partner gene involved. PDGFRA-related disorders usually present as chronic eosinophilic leukaemia with prominent involvement of the mast cell lineage and sometimes the neutrophil lineage. Less often, they present as acute myeloid leukaemia or T-lymphoblastic leukaemia/lymphoma, with accompanying eosinophilia in either case. Uncommonly, there is B-lymphoblastic transformation [4050]. In the setting of PDGFB-related disease, the features of the MPN are more variable, but are often those of chronic myelomonocytic leukaemia with eosinophilia. The proliferation of aberrant mast cells can also be a feature. Acute transformation is usually myeloid, but there have been reports of at least two cases of T-lymphoblastic transformation [726, 2977] and one case of unspecified lymphoblastic transformation [2649]. In the setting of FGFR1-related disease, lymphomatous presentations are common, in particular T-lymphoblastic leukaemia/lymphoma with accompanying eosinophilia. Other presentations include chronic eosinophilic leukaemia, B-lymphoblastic leukaemia/lymphoma, and acute myeloid leukaemia. PCM1-JAK2-related cases can undergo myeloblastic or B-lymphoblastic transformation [230].

Recognizing these disorders is important, because the aberrant tyrosine kinase activity can make the disease responsive to tyrosine kinase inhibitors. This therapeutic approach has already proven successful for the treatment of cases of PDGFRA- and PDGFRB-related disease, which are responsive to imatinib and some related tyrosine kinase inhibitors, and to some extent for the treatment of PCM1-JAK2-related disease, which is responsive to ruxolitinib. Related cases with ETV6-JAK2 or BCR-JAK2 may respond to JAK2 inhibitors. A similar specific therapy has not yet been developed for FGFR1-related disease. The relevant cytogenetic analysis and/or molecular genetic analysis should be carried out in all cases in which MPN with eosinophilia is suspected, as well as in cases presenting with an acute leukaemia or lymphoblastic lymphoma with eosinophilia. The identification of PDGFRA-related disease usually requires molecular genetic analysis (because most cases result from a cryptic deletion), whereas cytogenetic
analysis can reveal the causative abnormality in cases related to PDGFRB, FGFR1, or JAK2.

**Myeloid/lymphoid neoplasms with PDGFRα rearrangement**

**Definition**
The most common myeloproliferative neoplasm associated with PDGFRα rearrangement is that associated with FIP1L1-PDGFRA gene fusion, which occurs as a result of a cryptic deletion at 4q12 [798] (see Table 4.01). These neoplasms generally present as chronic eosinophilic leukaemia (CEL), but can also present as acute myeloid leukaemia, T-lymphoblastic leukaemia/lymphoma, or both simultaneously [2650]. Acute transformation can follow presentation as CEL. Organ damage occurs as a result of leukemic infiltration or the release of cytokines, enzymes or other proteins by eosinophils and possibly also by mast cells. The peripheral blood eosinophil count is usually markedly elevated, although it should be noted that, in some series of cases, investigation was limited to cases with eosinophilia. A small number of cases that lacked eosinophilia have been reported (1829,3450). There is no Philadelphia (Ph) chromosome or BCR-ABL1 fusion gene. Except when there is transformation to acute leukemia, there are <20% blasts in the peripheral blood and bone marrow.

**Variants**
Several possible molecular variants of FIP1L1-PDGFRA-associated CEL have been recognized in which other fusion genes incorporate part of PDGFRα. A male patient with imatinib-responsive CEL was found to have a KIF5B-PDGFRA fusion gene associated with a complex chromosomal abnormality involving chromosomes 3, 4 and 10 [3598], and a female patient had a CDK5RAP2-PDGFRA fusion gene associated with ins(9;4)(q33;q12q25) [4235]. One case in a male patient with t(2;4)(p24;q12) and STRN-PDGFRA fusion [849] and another case in a male patient with t(4;12)(q12;p13.2) and an ETV6-PDGFRA fusion gene, both with the haematological features of CEL, responded to low-dose imatinib [849]. A case with FIP1L1-PDGFRA had the features of an atypical myeloproliferative neoplasm without eosinophilia (2900).

Neoplasms with t(4;22)(q12;q11.2) and a BCR-PDGFRα fusion gene, at least 9 cases of which have been described, have disease characteristics intermediate between those of FIP1L1-PDGFRA-associated eosinophilic leukaemia and those of BCR-ABL1-positive chronic myeloid leukaemia; eosinophilia may or may not be prominent [298,1312,3471,4050]. Accelerated phase, T-lymphoblastic transformation and B-lymphoblastic transformation [298,4050] have been reported. The disease is imatinib-sensitive [3471,4050] and a tyrosine kinase inhibitor would normally be included in the treatment regime. One case has been reported of imatinib-sensitive CEL associated with t(4;10)(q12;q23.3) and TNKS2-PDGFRA [637]. CEL can also result from an activating point mutation in PDGFRα [1093].

**ICD-O code** 9965/3

**Synonyms**
Myeloid and lymphoid neoplasms with PDGFRα rearrangement; myeloid and lymphoid neoplasms associated with PDGFRα rearrangement

**Epidemiology**
The FIP1L1-PDGFRA syndrome is rare. It is considerably more common in men than in women, with a male-to-female ratio of about 17:1. The peak incidence is 25 and 55 years, with a median age at onset in the late 40s (range: 7-77 years) [233].

**Etiology**
The cause is unknown, although several cases with FIP1L1-PDGFRα have occurred following cytotoxic chemotherapy [2946,3892], as did a case with features resembling those of chronic myeloid leukaemia with a BCR-PDGFRα fusion gene [3471].

**Localization**
CEL associated with FIP1L1-PDGFRα is a multisystem disorder. The peripheral blood and bone marrow are always involved. Tissue infiltration by eosinophils and the release of cytokines and humoral factors from the eosinophil granules result in tissue damage in a number of organs; the heart, lungs, central and peripheral nervous system, skin and gastrointestinal tract are commonly involved.

**Clinical features**
Patients usually present with fatigue or pruritus, or with respiratory, cardiac or gastrointestinal symptoms [798,2508,4147]. Some patients are asymptomatic at diagnosis [1605], but most have splenomegaly and some have hepatomegaly. The most serious clinical findings relate to endomyocardial fibrosis, with ensuing restrictive cardiomyopathy. Scarring of the mitral and/or tricuspid valves leads to valvular regurgitation and the formation of intracardiac thrombi, which may embolize. Venous thromboembolism and arterial thromboses can also occur. Pulmonary disease is restrictive and related to fibrosis; symptoms include dyspnoea and cough, and there may also be an obstructive element. Serum tryptase is elevated (>12 ng/mL), usually to a lesser extent than in mast cell disease but with some overlap.
Serum vitamin B12 is markedly elevated \cite{4147}. \textit{FIP1L1-PDGFR}\alpha-
related CEL is very responsive to imatinib; the \textit{FIP1L1}-
\textit{PDGFR}\alpha fusion protein is 100 times as
sensitive as BCR-ABL1 \cite{798}.

**Microscopy**

The most striking feature in the peripheral blood is eosinophilia. Most of the eosinophils are mature, with only small numbers of eosinophil myelocytes or

promyelocytes. A range of eosinophil abnormalities can be present, including sparse granulation with clear areas of cytoplasm, cytoplasmic vacuolation, granules that are smaller than normal, immature granules that are purplish on Romanowsky staining, nuclear hyper- or hyposegmentation, and increased eosinophil size \cite{798,4147}. However, these abnormalities can also be seen in cases of reactive eosinophilia \cite{226} and in some cases of \textit{FIP1L1-PDGFR}\alpha-associated CEL the eosinophil morphology is close to normal. Only a minority of patients have any increase in the number of peripheral blast cells \cite{4147}. Neutrophils may be increased, whereas basophil and monocyte counts are usually normal \cite{3378}. Anaemia and thrombocytopenia are sometimes present.

Any tissue may show eosinophilic infiltration and Charcot–Leyden crystals may be present. The bone marrow is hypercellular, with markedly increased numbers of eosinophils and precursors. In most cases eosinophil maturation is orderly (without a disproportionate increase in blasts), but in some cases the proportion of blast cells is increased. There may be necrosis, particularly when the disease is becoming more acute \cite{798}. The number of bone marrow mast cells seen on trephine biopsy is often increased \cite{2045,3055}, and mast cell proliferation should be recognized as a feature of \textit{FIP1L1-PDGFR}\alpha-associated myeloproliferative neoplasm. The mast cells may be scattered, in loose non-cohesive clusters, or in cohesive clusters \cite{2045,3055}. Many cases show a marked increase in spindle-shaped atypical mast cells, and in some cases the morphological features resemble those of systemic mastocytosis. Reticulin is increased \cite{2045}.

Patients presenting with acute myeloid leukaemia or T-lymphoblastic leukaemia/lymphoma have been reported to have coexisting eosinophilia (i.e. peripheral blood counts of 1.4–17.2 \texttimes 10^9/L); in most cases, pre-existing eosinophilia was also documented \cite{2650}.

**Cytochemistry**

Cytochemical stains are not necessary for diagnosis. The reduced granule content of the eosinophils can result in reduced peroxidase content and inaccurate automated eosinophil counts.
Immunophenotype
The eosinophils in this syndrome may show immunophenotypic evidence of activation, such as expression of CD23, CD25 and CD69 [2045]. The mast cells are usually CD2-negative and CD25-positive [2044], but in some cases they are negative for both [2650] and in occasional cases they are positive for both [2650]. In comparison, the mast cells of systemic mastocytosis are CD25-positive in almost all cases and CD2-negative in about two thirds of cases.

Cell of origin
The cell of origin is a pluripotent hematopoietic stem cell that can give rise to eosinophils and (at least in some patients) neutrophils, monocytes, mast cells, T cells and B cells [3378]. The detection of the fusion gene in a given lineage does not necessarily correlate with morphological evidence of involvement of that lineage. For example, lymphocytosis is not typical, even in cases with apparent involvement of the B-cell or T-cell lineage [3378]. In chronic-phase disease, involvement is predominantly of eosinophils and to a lesser extent mast cells and neutrophils. Acute-phase disease can be myeloid, T-lymphoblastic [2650], or (rarely) B-lymphoblastic [4050].

Genetic profile
Cytogenetic findings are usually normal, with the $FIP1L1$-$PDGFRA$ fusion gene resulting from a cryptic del(4)(q12). In some patients, there is a chromosomal rearrangement with a 4q12 breakpoint, such as t(1;4)(q44;q12) [798] or t(4;10) (q12;p11.1-p11.2) [3907]. In other patients, there is an unrelated cytogenetic abnormality (e.g. trisomy 8), which is likely to represent disease evolution. The fusion gene can be detected by reverse transcriptase-polymerase chain reaction (RT-PCR), with nested RT-PCR often being required [798]. The causative deletion can also be detected by FISH analysis, often using a probe for the CHIC2 gene (which is uniformly deleted) or using a break-apart probe that encompasses $FIP1L1$ and $PDGFRA$. Because most patients do not have increased blast cells or any abnormality on conventional cytogenetic analysis, it is usually the detection of the $FIP1L1$-$PDGFRA$ fusion gene that enables the definitive diagnosis of this neoplasm. Cytogenetic abnormalities appear to be more common when evolution to acute myeloid leukaemia has occurred [2650].

Prognosis and predictive factors
Because $FIP1L1$-$PDGFRA$-associated CEL and its responsiveness to imatinib were not recognized until 2003 [798], the long-term prognosis is still unknown. However, the prognosis appears to be favourable if cardiac damage has not yet occurred and imatinib treatment is available. Imatinib resistance can develop (e.g. as a result of a T674I mutation, which is equivalent to the T315I mutation that can occur in the $BCR-ABL1$ gene) [798,1461]. Alternative tyrosine kinase inhibitors such as midostaurin (PKC412) and sorafenib may be effective in these patients [800,2326,3806]. Patients presenting with acute myeloid leukaemia or T-lymphoblastic leukaemia can achieve sustained complete molecular remission with imatinib [2650].

### Myeloid/lymphoid neoplasms with PDGFRB rearrangement

#### Definition
A distinct type of myeloid neoplasm occurs in association with rearrangement of $PDGFRB$ at 5q32 (see Table 4.02). Usually there is t(5;12)(q32;p13.2) with formation of an $ETV6$-$PDGFRB$ fusion gene [1398,1980]. In uncommon variants, other translocations with a 5q32 breakpoint lead to the formation of other fusion genes, also incorporating part of $PDGFRB$ (see Table 4.03, p. 77). However, fusion genes typically associated only with $BCR-ABL1$-like B-lymphoblastic leukaemia are specifically excluded from this category; these include $EBF1$-$PDGFRB$, $SSBP2$-$PDGFRB$, $TNIP1$-$PDGFRB$, $ZEB2$-$PDGFRB$ and $ATF7IP1$-$PDGFRB$ [2059,3370]. Cases with $ETV6$-$PDGFRB$ that present as $BCR-ABL1$-like B-lymphoblastic leukaemia may be more appropriately assigned to that category. In cases with t(5;12) and in the variant translocations, there is synthesis of an aberrant, constitutively activated tyrosine kinase. The haematological features are most often those of chronic myelomonocytic leukaemia (usually with eosinophilia), but some cases have been characterized as atypical chronic myeloid leukaemia, $BCR-ABL1$-negative (usually with eosinophilia), chronic eosinophilic leukaemia or myeloproliferative neoplasm (MPN) with eosinophilia [233,3776]. Single cases have been reported of acute myeloid leukaemia, probably superimposed on primary myelofibrosis [4013], and of juvenile myelomonocytic leukaemia [2728], the latter associated with a variant fusion gene. Eosinophilia is typical but not invariable [3776]. Acute transformation can occur, often in a relatively short period of time. MPNs with $PDGFRB$ rearrangement are sensitive to tyrosine kinase inhibitors such as imatinib [123].

#### Variants
A number of molecular variants of MPNs with $ETV6$-$PDGFRB$ fusion have been reported [233,3776]. In addition, a patient who developed eosinophilia at relapse of acute myeloid leukaemia was found to have acquired t(5;14)(q32;2.1), with a $TRIP11$-$PDGFRB$ fusion gene. Other patients have rearrangement of $PDGFRB$ with an unknown partner gene. Complex rearrangements appear to be common (e.g. a small inversion as well as translocation) [3776]. Because of the therapeutic implications of $PDGFRB$ rearrangement, FISH (break-apart FISH with a $PDGFRB$ probe) is indicated in all patients with a presumptive diagnosis of MPN with a 5q31-33 breakpoint, in particular if there is eosinophilia. However, FISH analysis does not always demonstrate rearrangement of $PDGFRB$, even when such rearrangement is detectable on Southern blot analysis [3776]. Molecular analysis

### Table 4.02 Diagnostic criteria for myeloid/lymphoid neoplasms associated with $ETV6$-$PDGFRB$ or other rearrangement of $PDGFRB$\(^a\)

<table>
<thead>
<tr>
<th>Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>A myeloid or lymphoid neoplasm, often with prominent eosinophilia and sometimes with neutrophilia or monocytosis AND Presence of t(5;12)(q32;p13.2) or a variant translocation(^b) or demonstration of $ETV6$-$PDGFRB$ fusion gene or other rearrangement of $PDGFRB$</td>
</tr>
</tbody>
</table>

\(^a\) Cases with fusion genes typically associated only with $BCR-ABL1$-like B-lymphoblastic leukaemia are specifically excluded (see text);
\(^b\) Because t(5;12)(q32;p13.2) does not always result in $ETV6$-$PDGFRB$ fusion, molecular confirmation is highly desirable; if molecular analysis is not possible, this diagnosis should be suspected if there is a myeloproliferative neoplasm associated with eosinophilia, with no Philadelphia (Ph) chromosome and with a translocation with a 5q32 breakpoint.

---
Fig. 4.04 Myeloid neoplasm with eosinophilia and rearrangement of PDGFRB. A Bone marrow trephine biopsy section from a patient with t(5;12) shows a marked increase in eosinophils. B Peripheral blood smear from a patient with t(5;12) shows numerous abnormal eosinophils, at lower (B) and higher (C) magnification. Eosinophils accounted for 40% of the leukocytes.

is not indicated when no 5q31-33 breakpoint is found by conventional cytogenetic analysis, because almost all cases reported to date in which 20 metaphases were available for examination have had a cytogenetically detectable abnormality.

ICD-O code 9966/3

Synonyms
Chronic myelomonocytic leukaemia with eosinophilia associated with t(5;12); myeloid neoplasms with PDGFRB rearrangement; myeloid neoplasms associated with PDGFRB rearrangement

Epidemiology
This neoplasm is considerably more common in men than in women (male-to-female ratio: 2:1) and occurs over a wide age range (8–72 years), with peak incidence in middle-aged adults and a median age of onset in the late 40s [3776].

Localization
MPN associated with t(5;12)(q32;p13.2) is a multisystem disorder. The peripheral blood and bone marrow are always involved. The spleen is enlarged in most cases. Tissue infiltration by eosinophils and the release of cytokines, humoral factors or granule contents by eosinophils can contribute to tissue damage in several organs.

Clinical features
Most patients have splenomegaly and some have hepatomegaly. Some patients have skin infiltration and some have cardiac damage leading to cardiac failure. Serum tryptase may be mildly or moderately elevated. The vast majority of patients who have been treated with imatinib have been found to be responsive.

Microscopy
The white blood cell count is increased. There may be anaemia and thrombocytopenia. There is a variable increase in neutrophils, eosinophils, monocytes and eosinophil and neutrophil precursors. Rarely, there is a marked increase in basophils [4236].

The bone marrow is hypercellular as a result of active granulopoiesis (neutrophilic and eosinophilic). Bone marrow trephine biopsy may show an increase in mast cells, which may be spindle-shaped

Cytochemistry
The eosinophils, neutrophils and monocytes show the cytochemical reactions expected for cells of these lineages.

Immunophenotype
Immunophenotypic analysis of the mast cells has shown expression of CD2 and CD25, which is also found in most cases of mast cell disease [4236].

Cell of origin
The postulated cell of origin is a pluripotent haematopoietic stem cell that can give rise to neutrophils, monocytes, eosinophils, probably mast cells and (in some patients) B-cell lineage lymphoblasts.

Genetic profile
Cytogenetic analysis usually shows t(5;12)(q32;p13.2), with the translocation resulting in ETV6-PDGFRB gene fusion [1398] (previously called TEL-PDGFRB). In one patient, ETV6-PDGFRB fusion resulted from a four-way translocation; t(1;12;5;12)(p36;p13.2;q32;q24) [835]; in another, the fusion occurred in association with ins(2;12)(p21;q13;q22) [883]. The 5q breakpoint is sometimes assigned to 5q31 and sometimes to 5q33, although the gene map locus of PDGFRB is 5q32.

Not all translocations characterized as t(5;12)(q31-33;p12) lead to ETV6-PDGFRB fusion. Cases without a fusion gene are not assigned to this category of MPN and, importantly, are not likely to respond to imatinib; in such cases, an alternative leukaemogenic mechanism is upregulation of interleukin 3 (IL3) [799]. Therefore, RT-PCR using primers suitable for all known breakpoints is recommended for confirmation of ETV6-PDGFRB [850], but if molecular analysis is not available, a trial of imatinib is justified in patients with an MPN associated with t(5;12). Due to the large number of potential partner genes, molecular analysis to demonstrate variant fusion genes is only feasible after a translocation has been shown by cytogenetic analysis. If subsequent monitoring of treatment response is planned, the fusion gene must be identified to ensure appropriate therapy.

76 Myeloid/lymphoid neoplasms with eosinophilia and gene rearrangement
gene should be characterized at diagnosis by RT-PCR or RNA sequencing.

**Prognosis and predictive factors**

Before the introduction of imatinib therapy, the median survival was <2 years. Reliable survival data are not yet available for imatinib-treated patients, but in a small series (10 cases) the median survival was 65 months [883]. Median survival is likely to improve as patients are increasingly identified and started on appropriate treatment at the time of diagnosis rather than after cardiac damage or transformation has already occurred.

### Myeloid/lymphoid neoplasms with FGFR1 rearrangement

**Definition**

Haematological neoplasms with *FGFR1* rearrangement are heterogeneous. They are derived from a pluripotent haematopoietic stem cell; however, in different patients or at different stages of disease, the neoplastic cells may be precursor cells or mature cells. Cases can present as a myeloproliferative neoplasm or (in transformation) as acute myeloid leukaemia, T- or B-lymphoblastic leukaemia/lymphoma or mixed-phenotype acute leukaemia (see Table 4.04, p. 78).

In one reported case, there was coexisting atypical chronic myeloid leukaemia, *BCR-ABL1*-negative, with *t*(8;19) (p11.2;q13.1) and *KIT* D816V–positive systemic mastocytosis (1051). Such cases, which are rare, can be classified as systemic mastocytosis with an associated haematological neoplasm.

**ICD-O code**

9967/3

**Synonyms**

8p11 myeloproliferative syndrome; 8p11 stem cell syndrome; 8p11 stem cell leukaemia/lymphoma syndrome; haematopoietic stem cell neoplasm with *FGFR1* abnormalities; myeloid and lymphoid neoplasms with *FGFR1* abnormalities

**Epidemiology**

This neoplasm occurs across a wide age range (3–84 years), but most patients are young, with a median age at onset of about 32 years [2424]. Unlike in myeloid/lymphoid neoplasms with *PDGFRα* or *PDGFRβ* rearrangement, there is only a moderate male predominance, with a male-to-female ratio of 1.5:1.

**Localization**

The tissues primarily involved are the bone marrow, peripheral blood, lymph nodes, liver and spleen. Lymphadenopathy occurs as a result of infiltration by either lymphoblasts or myeloid cells.

**Clinical features**

Some cases present as lymphoma with mainly lymph node involvement or with a mediastinal mass; others present with myeloproliferative features, such as splenomegaly and hypermetabolism, or with features of acute myeloid leukaemia or myeloid sarcoma [15,1760,2424].
Table 4.04 Diagnostic criteria for myeloid/lymphoid neoplasms with FGFR1 rearrangement

A myeloproliferative or myelodysplastic/myeloproliferative neoplasm with prominent eosinophilia and sometimes with neutrophilia or monocytosis

OR

Acute myeloid leukaemia, T- or B-lymphoblastic leukaemia/lymphoma, or mixed-phenotype acute leukaemia (usually associated with peripheral blood or bone marrow eosinophilia)

AND

The presence of t(8;13)(p11.2;q12) or a variant translocation leading to FGFR1 rearrangement, demonstrated in myeloid cells, lymphoblasts or both

4167]. Systemic symptoms such as fever, weight loss and night sweats are often present [233].

Microscopy

Cases can present as chronic eosinophilic leukaemia, acute myeloid leukaemia, T-lymphoblastic leukaemia/lymphoma, or (least often) B-lymphoblastic leukaemia/lymphoma or mixed-phenotype acute leukaemia. In cases that present with chronic eosinophilic leukaemia, there may be subsequent transformation to acute myeloid leukaemia (including myeloid sarcoma), T- or B-lymphoblastic leukaemia/lymphoma, or mixed-phenotype acute leukaemia. Lymphoblastic leukaemia appears to be more common in patients with t(8;13) than in those with variant translocations [2424]. Patients who present in the chronic phase usually have eosinophilia and neutrophilia and occasionally have monocytosis. Those who present in acute transformation are also often found to have eosinophilia. Overall, about 90% of patients have peripheral blood or bone marrow eosinophilia [2424]. The eosinophils belong to the neoplastic clone, as do the lymphoblasts and myeloblasts in cases in acute transformation. Basophilia is not typical, but may be more common in cases with BCR-FGFR1 fusion [3429], and has also been observed in association with t(1;8) (q31.1;p11.2) and TPR-FGFR1 [2298]. An association with polycythaemia vera has been reported in 3 cases with t(6;8)/FGFR1OP-FGFR1 fusion [3217, 4207].

T-lymphoblastic leukaemia/lymphoma characteristically shows eosinophilic infiltration within the lymphoma, which can be a clue to this diagnosis.

Table 4.05 Cytogenetics (chromosomal rearrangements) and molecular genetics (fusion genes) reported in myeloid/lymphoid neoplasms with FGFR1 rearrangement. Modified from Bain BJ and Fletcher SH [233], Macdonald D et al. [2424]

<table>
<thead>
<tr>
<th>Cytogenetics</th>
<th>Molecular genetics</th>
</tr>
</thead>
<tbody>
<tr>
<td>t(8;13)(p11.2;q12.1)</td>
<td>ZMYM2-FGFR1</td>
</tr>
<tr>
<td>t(8;9)(p11.2;q33.2)</td>
<td>CNTRL-FGFR1</td>
</tr>
<tr>
<td>t(6;8)(q27;p11.2)</td>
<td>FGFR10P-FGFR1</td>
</tr>
<tr>
<td>t(8;22)(p11.2;q11.2)</td>
<td>BCR-FGFR1</td>
</tr>
<tr>
<td>t(7;8)(q33;p11.2)</td>
<td>TRIM24-FGFR1</td>
</tr>
<tr>
<td>t(8;17)(p11.2;q11.2)</td>
<td>MYO18A-FGFR1</td>
</tr>
<tr>
<td>t(6;19)(p11.2;q13.3)</td>
<td>HERVK-FGFR1</td>
</tr>
<tr>
<td>ins(12;8)(p11.2;p11.2;q22)</td>
<td>FGFR10P2-FGFR1</td>
</tr>
<tr>
<td>t(1;8)(q51.1;p11.2)</td>
<td>TPR-FGFR1</td>
</tr>
<tr>
<td>t(2;8)(q13;p11.2)</td>
<td>RANBP2-FGFR1</td>
</tr>
<tr>
<td>t(28;3)(q37.3;p11.2)</td>
<td>LRRFIP1-FGFR1</td>
</tr>
<tr>
<td>t(7;8)(q22.1;p11.2)</td>
<td>CUX1-FGFR1</td>
</tr>
<tr>
<td>t(8;12)(p11.2;q15)</td>
<td>CPSF6-FGFR1</td>
</tr>
</tbody>
</table>

FGFR1 rearrangement has also been found in association with t(8;12)(p11.2;q15) and t(8;17)(p11.2;q25), but the suspected involvement of FGFR1 in t(8;11)(p11.1-p11.2;p15) was not confirmed.

Genetic profile

A variety of translocations with an 8p11 breakpoint can underlie this syndrome. Secondary cytogenetic abnormalities (most commonly trisomy 21) also occur. Depending on the partner chromosome, a variety of fusion genes incorporating part of FGFR1 can be formed. All such fusion genes encode an aberrant tyrosine kinase (see Table 4.05).

Prognosis and predictive factors

Due to the high incidence of transformation, the prognosis is poor, even for patients presenting in the chronic phase. There is no established tyrosine kinase inhibitor therapy for myeloproliferative neoplasms with FGFR1 rearrangement, although midostaurin (PKC412) was effective in one case [673]. Interferon has induced a cytogenetic response in several cases [2424, 2532]. Until a specific therapy has been developed, haematopoietic stem cell transplantation should be considered even for patients who present in the chronic phase.

Myeloid/lymphoid neoplasms with PCM1-JAK2

Definition

Myeloid and lymphoid neoplasms associated with t(8;9)(p22;p24.1) [3799] and PCM1-JAK2 [3337] share characteristic features that justify the recognition of this group as a provisional entity [230]. The haematological features may be those characteristic of a myeloproliferative neoplasm (e.g. chronic eosinophilic leukaemia or primary myelofibrosis) or those characteristic of a myelodysplastic/myeloproliferative neoplasm (e.g. atypical chronic myeloid leukaemia, BCR-ABL1-negative), often with eosinophilia. Acute myeloid transformation can occur; in addition, one patient presented with B-lymphoblastic leukaemia [3337], two patients experienced B-lymphoblastic transformation [1656, 3108] and one patient presented with T-lymphoblastic lymphoma [25]. Another patient developed a constellation of T-cell lineage neoplasms [1084].

Variants

Cases with translocations resulting in a fusion gene between JAK2 and an alternative partner – specifically, t(9;12) (p24.1;p13.2) resulting in ETV6-JAK2 and
(9;22)(p24.1;q11.2) resulting in BCR-JAK2 may be considered variants of this provisional entity [230]. Both of these groups of disorders are more heterogeneous than are cases with PCM1-JAK2. Among the small number of reported cases with ETV6-JAK2, B- and T-lymphoblastic leukaemia/lymphoma have been common, but myeloid neoplasms (including myelodysplastic syndromes) have also been reported; eosinophilia has not been commonly observed [230]. Cases of B-lymphoblastic leukaemia/lymphoma associated with ETV6-JAK2 may have the features of BCR-ABL1–like lymphoblastic leukaemia [3370]. Most of the few reported cases with BCR-JAK2 have been myeloid neoplasms (most commonly atypical chronic myeloid leukaemia, BCR-ABL1–negative), but there have also been 3 reported cases of B-lymphoblastic leukaemia/lymphoma [230,3370], which may also have the features of BCR-ABL1–like lymphoblastic leukaemia [3370].

ICD-O code 9968/3

Epidemiology
There is a marked male predominance, with a male-to-female ratio of 27:5, and a wide age range (12–75 years), with a median age of 47 years [230].

Localization
The peripheral blood and bone marrow are involved.

Clinical features
Patients often have hepatosplenomegaly.

Microscopy
The haematological features often include eosinophilia, and neutrophil precursors may be present in the peripheral blood. Some cases have the haematological features of chronic eosinophilic leukaemia. Monocytosis is uncommon, and an increase in basophils is only occasionally observed. There may be dyserythropoiesis (which is often prominent) and dysgranulopoiesis. Erythropoiesis may be considerably increased.

There may be sheets of proerythroblasts, as seen in acute leukaemia. There have been reports of several cases with features similar to those of primary myeloﬁbrosis, and other patients have shown bone marrow fibrosis.

Immunophenotype
Immunophenotypic analysis is useful in characterizing any lymphoid component and in cases with acute myeloid transformation.

Cell of origin
For PCM1-JAK2–related cases, the postulated cell of origin is a pluripotent haematopoietic stem cell that can give rise to neutrophils, eosinophils, and T- and B-lineage cells. Cases with variant fusion genes are also thought to arise from a pluripotent haematopoietic stem cell.

Genetic profile
More than 30 cases with t(8;9)(p22;p24.1) and resulting PCM1-JAK2 fusion have been reported [230]. To date, smaller numbers of cases with other fusion genes involving JAK2 have been reported: 8 cases with t(9;12)(p24.1;p13.2) and ETV6-JAK2 and 11 cases with t(9;22)(p24.1;q11.2) and BCR-JAK2 [230]; some of these cases are BCR-ABL1–like B-lymphoblastic leukaemia/lymphoma and may be best categorized with other cases of BCR-ABL1–like B-lymphoblastic leukaemia/lymphoma.

Prognosis and predictive factors
Survival is highly variable; for patients presenting in the chronic phase, it ranges from a matter of days to many years. The prognosis is also quite variable for cases presenting as acute leukaemia or with acute transformation, ranging from a few weeks to >5 years (or longer when haematopoietic stem cell transplantation is possible). Other than acute phase disease, no predictive factors are known.

Fig. 4.05 Myeloid/lymphoid neoplasm with PCM1-JAK2. Trephine biopsy section. Hypercellular bone marrow with increased granulopoiesis and numerous eosinophilic forms, associated with a sheet-like proliferation of proerythroblasts (at far right).
CHAPTER 5

Myelodysplastic/myeloproliferative neoplasms

Chronic myelomonocytic leukaemia
Atypical chronic myeloid leukaemia, *BCR-ABL1*-negative
Juvenile myelomonocytic leukaemia
Myelodysplastic/myeloproliferative neoplasm with ring sideroblasts and thrombocytosis
Myelodysplastic/myeloproliferative neoplasm, unclassifiable
Chronic myelomonocytic leukaemia

Definition
Chronic myelomonocytic leukaemia (CMML) is a clonal haematopoietic malignancy with features of both a myeloproliferative neoplasm (MPN) and a myelodysplastic syndrome (MDS). The diagnostic criteria for this entity are listed in Table 5.01.

On the basis of the percentage of blasts and promonocytes in the blood and bone marrow (see Microscopy), CMML cases can be further divided into three subcategories: CMML-0, CMML-1 and CMML-2. The clinical, haematological, and morphological features of CMML are heterogeneous, varying along a spectrum from predominantly myelodysplastic to mainly myeloproliferative in nature. Unlike BCR-ABL1-negative MPN, the JAK2V617F mutation is uncommon in CMML [1863, 3775].

Rarely, cases previously diagnosed as MDS or MPN show evolution to a CMML-like phenotype [404, 4245]; because this evolution constitutes disease progression, such cases should not be classified as CMML. Therapy-related CMML is discussed separately (see Therapy-related myeloid neoplasms, p. 153).

ICD-O codes
Chronic myelomonocytic leukaemia 9945/3
Chronic myelomonocytic leukaemia-0 9945/3
Chronic myelomonocytic leukaemia-1 9945/3
Chronic myelomonocytic leukaemia-2 9945/3

Synonyms
Chronic myelomonocytic leukaemia, type I; chronic myelomonocytic leukaemia, type II; chronic myelomonocytic leukaemia in transformation (obsolete); chronic myelomonocytic leukaemia, NOS

Epidemiology
There are few reliable incidence data for CMML; in some epidemiological surveys, CMML has been grouped with chronic myeloid leukaemias and in others it has been considered an MDS subtype. In one study, in which CMML accounted for 31% of the cases of MDS, the annual incidence of MDS was estimated to be approximately 12.8 cases per 100 000 population (4327). An epidemiological report on a well-defined population, with centralized morphology review, reported a much lower age-standardized annual incidence (0.41 cases per 100 000 population) and a crude prevalence of 1.05–1.94 cases per 100 000 population [2854]. This incidence rate was confirmed by a recent study that found an annual incidence in 2008–2010 of 0.4 cases per 100 000 population (999); in this study, the annual incidence of CMML was highest in patients aged >80 years (3.8 cases per 100 000 population) and higher in males (0.5 cases per 100 000 males; 95% CI: 0.4–0.6) than in females (0.2 cases per 100 000 females; 95% CI: 0.2–0.3). The median patient age at diagnosis is 65–75 years, with a male-to-female ratio of 1.5–3.1 [1334, 3719, 3805].

Table 5.01 Diagnostic criteria for chronic myelomonocytic leukaemia (CMML)

1. Persistent peripheral blood monocytosis (≥1 x 10^9/L) with monocytes accounting for ≥10% of the leucocytes
2. WHO criteria for BCR-ABL1–positive chronic myeloid leukaemia, primary myelofibrosis, polycythaemia vera and essential thrombocythaemia are not met
3. No rearrangement of PDGFRα, PDGFRβ or FGFR1 and no PCM1-JAK2 (which should be specifically excluded in cases with eosinophilia)
4. Blasts constitute <20% of the cells in the peripheral blood and bone marrow
5. Dysplasia involving ≥1 myeloid lineages

or

If myelodysplasia is absent or minimal, criteria 1–4 are met and:
- an acquired, clonal cytogenetic or molecular genetic abnormality is present in haematopoietic cells
- the monocytosis has persisted for ≥3 months and all other causes of monocytosis (e.g. malignancy, infection, and inflammation) have been excluded

Myeloproliferative neoplasms (MPN) can be associated with monocytosis or it can develop during the course of the disease; such cases can mimic CMML. In these rare instances, a documented history of MPN excludes CMML, whereas the presence of MPN features in the bone marrow and/or MPN-associated mutations (in JAK2, CALR or MPL) tends to support MPN with monocytosis rather than CMML.

Blasts and blast equivalents include myeloblasts, monoblasts and promonocytes. Promonocytes are monocytic precursors with abundant light-grey or slightly basophilic cytoplasm with a few scattered fine lilac-coloured granules, finely distributed stippled nuclear chromatin, variably prominent nucleoli and delicate nuclear folding or creasing. Abnormal monocytes, which can be present in both the peripheral blood and the bone marrow, are excluded from the blast count (see Introduction and overview of the classification of myeloid neoplasms, Fig. 1.04, p. 18).

In the appropriate clinical context, mutations in genes often associated with CMML (e.g. TET2, SRSF2, ASXL1 and SETBP1) support the diagnosis. However, some of these mutations can be age-related or present in other neoplasms; therefore, these genetic findings must be interpreted with caution.

References
- Bennett J.M., Germing U., Brunning R.D., Orazi A., Cazzola M., Foucar K., Bain B.J.

Localization
The blood and bone marrow are always involved. The spleen, liver, skin and lymph nodes are the most common sites of extramedullary leukaemic infiltration [1334, 4206], but other organs can also be involved [3819].

Clinical features
Half or more of all cases present with an increased white blood cell (WBC) count. In the remaining cases, the WBC count is normal or slightly decreased (with variable neutropenia), and the disease resembles MDS. Although once consid-
Microscopy
Peripheral blood monocytosis is the hallmark of CMML. By definition, the monocyte count is always ≥ 1 x 10^9/L; it is usually 2–5 x 10^9/L, but can exceed 80 x 10^9/L [2517,2653]. Monocytes should account for ≥ 10% of the leukocytes [339]. In general, the monocytes are mature and have unremarkable morphology, but they can exhibit unusual nuclear segmentation or chromatin patterns and abnormal granulation [2092]. Those with abnormal granulation are best termed abnormal monocytes, a designation used to describe monocytes that are immature but have denser chromatin, more nuclear convolutions and folds, and more abundant greyish cytoplasm than do promonocytes and monoblasts (see Introduction and overview of the classification of myeloid neoplasms, Fig. 1.04, p. 18). Blasts and promonocytes may also be seen, but if they account for ≥ 20% of the leukocytes, considered to be controversial, the subdivision of CMML into dysplastic (WBC count < 13 x 10^9/L) and proliferative (WBC count ≥ 13 x 10^9/L) groups appears to be justified [620,3349,3587,3827]. The incidence of constitutional symptoms (e.g. weight loss, fever, and night sweats) is higher with the proliferative type, whereas consequences of haematopoietic insufficiency (e.g. fatigue, infection, and bleeding due to thrombocytopenia) are more common with the dysplastic type [1334,3719,3805]. Splenomegaly and hepatomegaly can be present in either type, but are more frequent (occurring in as many as 50% of cases) in patients with leukocytosis [1334]. In rare cases, life-threatening hyperleukocytosis can occur [197].

Fig. 5.01 Chronic myelomonocytic leukaemia-1. A With Wright-Giemsa staining in this bone marrow aspirate smear, the dysplastic granulocytic component is obvious, but the monocytic component is more difficult to identify. B The monocytic component can be highlighted with special staining: naphthol AS-D chloroacetate esterase (CAE) reaction combined with alpha-naphthyl butyrate esterase stains monocytes brown, neutrophils blue and myelomonocytic cells a mixture of blue and brown. C CD163 immunostaining of a bone marrow biopsy section shows positivity in scattered monocytic cells and strong staining of the bone marrow macrophages. Immunohistochemistry can be used to identify monocytes in tissue sections, but is less sensitive than cytochemistry applied to bone marrow aspirate smears.

Fig. 5.02 Chronic myelomonocytic leukaemia-1. The degree of leukocytosis, neutrophilia and dysplasia is variable. Often, the granulocytic component is most obvious in the biopsy specimen, and monocytes may not be readily appreciable. This specimen shows hypercellular bone marrow with prominent granulocytic and megakaryocytic proliferation.

Fig. 5.03 Chronic myelomonocytic leukaemia-1. The degree of leukocytosis, neutrophilia and dysplasia is variable. A The white blood cell count in this case is elevated, with minimal dysplasia in the neutrophil series. B The white blood cell count in this case is normal, with absolute monocytosis, neutropenia and dysgranulopoiesis. C In this biopsy section, monocytes (with their characteristic folded nuclei and delicate nuclear chromatin) can be seen among the granulocytes. D Bone marrow biopsy section immunostain shows that the monocytic cells in this case are strongly positive for CD14.
the diagnosis is acute myeloid leukaemia (AML; acute myelomonocytic leukaemia or acute monocytic leukaemia) rather than CMML. Other changes in the blood are variable. The WBC count may be normal or slightly decreased with neutropenia, but in half or more of all cases it is increased due to both monocytosis and neutrophilia [1334,2517,2978]. Neutrophil precursors (promyelocytes and myelocytes) usually account for < 10% of the leukocytes [339]. Dysgranulopoiesis, including the formation of neutrophils with hyposegmented or abnormally segmented nuclei or abnormal cytoplasmic granulation, is present in most cases, but may be less prominent in patients with leukocytosis than in those with a normal or low WBC count [2092,2517]. In some cases, it may be difficult to distinguish between hypogranular neutrophils and dysplastic monocytes. Mild basophilia is sometimes present. Eosinophils are usually normal or slightly increased in number, but in some cases eosinophilia is striking. CMML with eosinophilia can be diagnosed when the criteria for CMML are met and the eosinophil count in the peripheral blood is $\geq 1.5 \times 10^9/L$. Patients with this diagnosis may have complications related to the degranulation of the eosinophils. These hypereosinophilic cases of CMML can closely resemble cases of myeloid neoplasms with eosinophilia associated with specific genetic abnormalities, which are classified and discussed separately from CMML (see Myeloid/lymphoid neoplasms with PDGFRB rearrangement, p. 75). Mild anaemia, often normocytic but sometimes macrocytic, is common. Platelet counts vary, but moderate thrombocytopenia is often present. Atypical, large platelets and nucleated red blood cell precursors may be seen [1338,2517]. The bone marrow is hypercellular in >75% of cases, but normocellular specimens are also seen [2653,2986,3805]. Hypocellularity is very rare. One recent study on the histological assessment of marrow found that <5% of CMML cases were hypocellular and 84% were hypercellular [3554]. Dysgranulopoiesis, similar to that in the blood, is present in the bone marrow of most patients with CMML. Dyserythropoiesis (e.g. megaloblastoid changes, other abnormal nuclear features and ring sideroblasts) may also be observed, but is usually mild [1334,2517,2653]. Micromegakaryocytes and/or megakaryocytes with hyposegmented nuclei are found in as many as 80% of cases [1338,2517,2653]. Unlike in chronic myeloid leukaemia, pseudo-Gaucher cells are usually absent. A mild to moderate increase in the number of reticulin fibres is seen in the bone marrow in nearly 30% of cases [2538]. Careful attention to morphological features and other disease-specific clinicopathological findings may be needed to distinguish CMML from MPN associated with monocytosis [404,1097]. The presence of one of the characteristic MPN driver mutations (in JAK2, CALR or...
MPL) is helpful for identifying MPN associated with monocytosis.

Nodules composed of mature plasmacytoid dendritic cells in the bone marrow biopsy have been reported in 20% of cases [2985]. The cells have round nuclei, finely dispersed chromatin, inconspicuous nucleoli, and a rim of eosinophilic cytoplasm. The cytoplasmic membrane is usually distinct with well-defined cytoplasmic borders, imparting a cohesive appearance to the infiltrating cells. Apoptotic bodies, often within starry-sky histiocytoses, are frequently present. The relationship of the plasmacytoid dendritic cell proliferation to the leukemic cells was previously uncertain [216,1127,1555,1691], but there is now evidence that the proliferation is neoplastic in nature and is clonally related to the associated CMML [1126].

The splenic enlargement seen in CMML is usually due to infiltration of the red pulp by leukemic cells. Lymphadenopathy is uncommon, but when it occurs it may indicate transformation to a more acute phase, and the lymph node may show diffuse infiltration by myeloid blasts. There is sometimes lymph node (and less commonly splenic) involvement by a diffuse infiltration of plasmacytoid dendritic cells. In some cases, generalized lymphadenopathy due to tumoral proliferations of plasmacytoid dendritic cells is the presenting manifestation of CMML. Blast cells and promonocytes usually account for <5% of the peripheral blood leukocytes and <10% of the nucleated marrow cells at the time of diagnosis. A higher proportion may indicate poor prognosis or higher risk of rapid transformation to acute leukemia [1180,1339,1442,3805,3922,4365]. The previously used category of CMML-1 (defined by blasts including promonocytes accounting for <5% of the leukocytes in the peripheral blood and <10% in the bone marrow) has now been split into two new categories: CMML-0 and CMML-1. It is currently recommended [2978,3587,3805] that CMML be subdivided into three categories, defined by the percentage of blasts and promonocytes in the peripheral blood and bone marrow:

- **CMML-0:** <2% blasts in the blood and <5% in the bone marrow; no Auer rods.
- **CMML-1:** 2–4% blasts in the blood or 5–9% in the bone marrow; <5% blasts in the blood, <10% blasts in the bone marrow, and no Auer rods.
- **CMML-2:** 5–19% blasts in the blood, 10–19% in the bone marrow or Auer rods are present; <20% blasts in the bone marrow and blood.

### Cytochemistry

Cytochemical studies are strongly recommended whenever the diagnosis of CMML is considered [2985]. Alpha-naphthyl butyrate esterase or alpha-naphthyl acetate esterase (with fluoride inhibition) staining of blood and bone marrow aspirate smears, alone or in combination with naphthol AS-D chloroacetate esterase (CAE) staining, is extremely useful for assessing the monocytic component, and in some cases may facilitate distinguishing monocytes from monoblasts and promonocytes (blast equivalents) and from non-monocytic cells.

### Immunophenotype

The blood and marrow cells usually express typical myelomonocytic antigens (e.g., CD33 and CD13) and variably express CD14, CD68 and CD64 [1993,2482,3640,4364]. The blood and marrow monocytes often have aberrant phenotypes, with two or more aberrant features shown by flow cytometric analysis [4396]. Decreased CD14 expression may reflect relative monocyte immaturity [3618]. Other aberrant characteristics include: overexpression of CD56; aberrant expression of CD2; and decreased expression of HLA-DR, CD13, CD11c, CD15, CD16, CD64 and CD36 [2554,3618,3714,4396]. An increased proportion of CD14+/CD16- monocytes has recently been described [3618]. Maturing myeloid cells may also have aberrant immunophenotypic features, and neutrophils may show aberrant light-scattering properties. An increased proportion of CD34+ cells and an emerging blast population with an aberrant immunophenotype have been associated with early transformation to acute leukemia [1063,4365,4396].

For the identification of monocytic cells, immunohistochemistry on tissue sections is less sensitive than cytochemistry or flow cytometry. The most reliable marker is CD14 [3257]. CD68R and CD163 can also be helpful [2985]. Lysozyme used in conjunction with cytochemistry for CAE can facilitate the identification of monocytic cells, which are lysozyme positive but CAE negative (in contrast to the granulocyte precursor cells, which are positive for both). An increased proportion of CD34+ cells detected by immunohistochemistry has been associated with transformation to acute leukemia [2985]. The mature plasmacytoid dendritic cells associated with CMML have a characteristic immunophenotype. They are positive for antigens normally expressed...
by reactive cells of this lineage, such as CD123, CD2AP, CD4, CD43, CD45RA, CD68/CD68R, CD303, BCL11A and granzyme B {406,870,2497}. Rarely, they also express some of the following antigens: CD2, CD5, CD7, CD10, CD13, CD14, CD15 and CD33 and, very rarely, CD56. TIA1 and perforin are usually negative. The Ki-67 (MIB1) proliferation index is usually low.

Cell of origin
A haematopoietic stem cell

Genetic profile
Clonal cytogenetic abnormalities are found in 20–40% of CMML cases, but none are specific {1182,1334,3105,3805,3826,3896,3922}. The most common recurrent abnormalities include gain of chromosome 8 and loss of chromosome 7 or del(7q). In a large study of 414 patients with CMML, 73% of the patients had a normal karyotype, 7% had trisomy 8, 4% had loss of Y chromosome, 3% had a complex karyotype, 1.5% had an abnormality of chromosome 7 and 10% had another aberration {3826}. Some myeloid neoplasms with isolated isochromosome 17q have haematological features of CMML {1220,1912,2594}, whereas others are better diagnosed as myelodysplastic/myeloproliferative neoplasm, unclassifiable {1912,1913}. Abnormalities of 11q23 are uncommon in CMML and suggest an alternate diagnosis of acute leukaemia. Several of the prognostic systems for CMML currently in use include cytogenetic features {3827,3896}.

Myeloid neoplasms with eosinophilia associated with t(5;12)(q31-33;p12) and ETV6-PDGFRA fusion were formerly included in the CMML category, but are now considered to be a distinct entity (see Myeloid/lymphoid neoplasms with PDGFRB rearrangement. p.75). Cases of chronic myeloid leukaemia that express the p190 BCR-ABL1 isoform can mimic CMML. Therefore, even if t(9;22)(q34.1;q11.2) is not detected by conventional karyotyping, PCR analysis for the presence of p210 and p190 and/or FISH analysis for the BCR-ABL1 fusion gene must be performed.

Many patients with CMML harbour somatic mutations, most frequently in ASXL1 (found in 40% of cases), TET2 (in 58%), SRSF2 (in 46%), RUNX1 (in 15%), NRAS (in 11%) and CBL (in 10%); many other genes can also be mutated (in < 10% of cases) {1795,2779}. Studies that confirmed the frequency of mutations in TET2, SRSF2, SETBP1 and ASXL1 {1170,2611,3100} have shown that at least one of these four genes is mutated in most CMML cases. Recently proposed prognostic models include mutation analysis {3025,3100,3105,4264}. NPM1 mutations are uncommon in CMML (occurring in <5% of cases); if they are found, the alternative diagnosis of AML with monocytic differentiation associated with NPM1 mutation should be carefully excluded. Confirmed cases of CMML with NPM1 mutation (in particular cases with a high mutation burden) appear to have a higher probability of progressing to AML and may require aggressive clinical intervention {3129}.

Prognosis and predictive factors
The reported survival times of patients with CMML range from 1 month to > 100 months; the median survival time in most series is 20–40 months {198,1180,1334,1336,1442,3587,3719,3805,3922,4365}. Progression to AML occurs in 15–30% of cases. Clinical and haematological parameters including lactate dehydrogenase level, splenomegaly, anaemia, thrombocytopenia and lymphocytosis {2978} have been reported as important factors for predicting the course of the disease. However, in virtually all studies, the percentage of blood and bone marrow blasts is the most important factor determining survival, together with karyotype, WBC count, and haematopoietic function {816,1180,1334,1339,1442,3719,3805,3922,4365}. These factors are included in a CMML-specific prognostic scoring system {3827}. Somatic ASXL1 mutation has also been incorporated in a clinical prognostic scoring system {1795}.

AML as transformation of CMML is an aggressive disease that belongs in the group of AML with myelodysplasia-related changes; the blasts often exhibit morphological features of acute myelomonocytic leukaemia or acute monocytic leukaemia, i.e. French–American–British (FAB) classification M4 or M5 {827}. 
Atypical chronic myeloid leukaemia, BCR-ABL1-negative

Definition
Atypical chronic myeloid leukaemia, BCR-ABL1-negative (aCML) is a leukemic disorder with myelodysplastic as well as myeloproliferative features present at the time of initial diagnosis (Table 5.02). It is characterized by principal involvement of the neutrophil lineage, with leukocytosis resulting from an increase of morphologically dysplastic neutrophils and their precursors. However, multilineage dysplasia is common, and reflects the stem-cell origin of this entity. The neoplastic cells do not have BCR-ABL1 fusion; rearrangement of PDGFRα, PDGFRβ or FGFR1, or PCM1-JAK2. Therapy-related cases with features of aCML are discussed separately (see Therapy-related myeloid neoplasms, p. 153).

ICD-O code
9876/3

Synonyms
Atypical chronic myeloid leukaemia, Philadelphia chromosome-negative (Ph1-); atypical chronic myeloid leukaemia, BCR/ABL1-negative

Epidemiology
The exact incidence of aCML is unknown, but it is estimated that there are only 1–2 aCML cases for every 100 cases of BCR-ABL1-positive chronic myeloid leukaemia [2987,4248]. Patients with aCML tend to be elderly. In the few series reported to date, the median patient age at diagnosis is in the seventh or eighth decade of life, although the disease has also been reported in teenagers [447,1621,2143,2517,4248]. The reported male-to-female ratio varies, but in larger series it is approximately 1:1 [447,1621,2143,2517,4248].

Localisation
The peripheral blood and bone marrow are always involved; splenic and hepatic involvement is also common.

Clinical features
There are only a few reports of the clinical features of aCML. Patients usually have symptoms related to anaemia or sometimes to thrombocytopenia; for other patients, the primary symptoms are related to splenomegaly [447,1621,2143,2517].

Microscopy
The white blood cell count is always ≥ 13 × 10^9/L [339], but median values of 24–96 × 10^9/L have been reported, and some patients have white blood cell counts of > 300 × 10^9/L [447,1621,2143,2517,4248]. Blasts are usually < 5% and always < 20% of peripheral blood leukocytes. Neutrophil precursors (promyelocytes, myelocytes, and metamyelocytes) constitute ≥ 10% of the leukocytes. The absolute monocyte count may be

Table 5.02 Diagnostic criteria for atypical chronic myeloid leukaemia, BCR-ABL1-negative (aCML)
- Peripheral blood leukocytosis ≥ 13 × 10^9/L, due to increased numbers of neutrophils and their precursors (i.e. promyelocytes, myelocytes and metamyelocytes), with neutrophil precursors constituting ≥ 10% of the leukocytes
- Dysgranulopoiesis, which may include abnormal chromatin clumping
- No or minimal absolute basophilia; basophils constitute < 2% of the peripheral blood leukocytes
- No or minimal absolute monocytosis; monocytes constitute < 10% of the peripheral blood leukocytes
- Hypercellular bone marrow with granulocytic proliferation and granulocytic dysplasia, with or without dysplasia in the erythroid and megakaryocytic lineages
- < 20% blasts in the blood and bone marrow
- No evidence of PDGFRα, PDGFRβ or FGFR1 rearrangement, or of PCM1-JAK2
- WHO criteria for BCR-ABL1-positive chronic myeloid leukaemia, primary myelofibrosis, polycythaemia vera, or essential thrombocythaemia are not met

*Myeloproliferative neoplasms (MPNs), in particular those in accelerated phase and/or in post-polycythaemia vera or post-essential thrombocythaemia myelofibrosis, if neutrophilic, may simulate aCML. A history of MPN, the presence of MPN features in the bone marrow, and/or MPN-associated mutations (in JAK2, CALR or MPL) tend to exclude the diagnosis of aCML; conversely, the diagnosis is supported by the presence of SETBP1 and/or ETNK1 mutations. CSF3R mutation is uncommon and, if detected, should prompt careful morphological review to exclude an alternative diagnosis of chronic neutrophilic leukaemia or another myeloid neoplasm.
increased, but the percentage of peripheral blood monocytes is < 10%. Basophilia may be observed but is not prominent [339,447,1621,2517,4248]. One of the major features that characterize aCML is dysgranulopoiesis, which may be pronounced. Acquired Pelger–Huët anomaly or other nuclear abnormalities, such as hypersegmentation with abnormally clumped nuclear chromatin or bizarrely segmented nuclei, abnormal cytoplasmic granularity (usually hypogranularity) and multiple nuclear projections, may be observed in the neutrophils. Moderate anaemia is common, and the red blood cells may show changes indicative of dyserythropoiesis. The platelet count is variable, but thrombocytopenia is common [339,1621,2517]. Careful morphological examination of the peripheral blood is crucial for distinguishing this entity from chronic neutrophilic leukaemia [4248], which lacks dysplastic features in the neutrophils and has < 10% circulating immature myeloid cells. There are also differences in the frequency of associated mutations (see Genetic profile). The bone marrow is hypercellular due to an increase of neutrophils and their precursors. The myeloid-to-erythroid ratio is usually > 10:1, reflecting the increased granulopoiesis and the decreased erythropoiesis, but in some cases erythroid precursors account for > 30% of the marrow cells. Dyserythropoiesis is present in about 40% of cases [339,447,4248]. Dysgranulopoiesis is invariably present, and the changes in the neutrophil lineage observed in the bone marrow are similar to those in the blood. Megakaryopoiesis is usually quantitatively normal or increased, but is sometimes decreased; there is often evidence of dysmegakaryopoiesis, including micromegakaryocytes and small megakaryocytes with hypolobated nuclei [447,1621]. Blasts may be moderately increased in number, but are always < 20%; large sheets or clusters of blasts are not present. Marrow fibrosis (usually mild) is seen in some cases at the time of diagnosis; in others, it appears later in the course of the disease.

Variant
Most cases reported as the syndrome of abnormal chromatin clumping can in fact be considered a variant of aCML [460,1178,1766]. These cases are characterized in the peripheral blood and bone marrow by a high percentage of neutrophils and precursors that exhibit exaggerated clumping of the nuclear chromatin.

Cytochemistry
No specific cytochemical abnormalities have been reported to date, although the use of esterase stains or immunohistochemistry to exclude a significant monocytic component can facilitate the exclusion of chronic myelomonocytic leukaemia. Neutrophil alkaline phosphatase scoring is rarely done; the scores can be low, normal, or high, and are therefore not useful for distinguishing this entity from BCR-ABL1–positive chronic myeloid leukaemia [2143,2517].

Immunophenotype
No specific immunophenotypic characteristics have been reported to date. Like esterase cytochemistry, immunohistochemistry for CD14 or CD68R on biopsy sections may facilitate the identification of monocytes; the finding of significant marrow monocytosis should call into question a diagnosis of aCML. In some cases with decreased megakaryocytes, CD61 or CD42b immunohistochemistry may facilitate the identification of dysmegakaryopoiesis. CD34 can facilitate the identification of blasts.

Cell of origin
A bone marrow haematopoietic stem cell

Genetic profile
Karyotypic abnormalities are reported in as many as 80% of cases. The most common abnormalities are gain of chromosome 8 and del(20q), but abnormalities of chromosomes 13, 14, 17, 19 and 12 are also common [447,1621,2517]. Rarely, cases is which the neoplastic cells have an isolated isochromosome 17q have features of aCML, although most fulfill the criteria for chronic myelomonocytic leukaemia [2594]. There is no BCR-ABL1 fusion. Cases with rearrangement of PDGFRα, PDGFRβ or FGFR1, or with PCM1-JAK2, are also specifically excluded. In the past, some cases of t(8;9) (p22;p24) with PCM1-JAK2 fusion were diagnosed as aCML [436,3337], but such cases are now grouped with other eosinophilic neoplasms associated with specific chromosomal rearrangements, and are discussed separately (see Myeloid/lymphoid neoplasms with PCM1-
JAK2, p.78). JAK2 V617F mutation has only rarely been reported in patients with aCML (1184,2288,4248); therefore, the typical myeloproliferative neoplasm-associated mutations (in JAK2, CALR and MPL) tend to exclude the diagnosis of aCML (1184). Recent data indicate that SETBP1 and ETNK1 mutations are relatively common in aCML (1286,2610,2779,3167), whereas CSR?/? mutation is present in <10% of cases (2779). Because this mutation is found in a considerably larger proportion of chronic neutrophilic leukaemia cases (1414,2588,3057,3918), it is helpful in distinguishing between the two neoplasms.

Prognosis and predictive factors
Patients with aCML fare poorly. Among the small numbers of patients included in reported series to date, the median survival time is 14–29 months (447,1621,2143,4248). Age >65 years, female sex, white blood cell count >50 x 10^9/L, thrombocytopenia and haemoglobin level <10 g/dL have been reported to be adverse prognostic findings (447,1621). However, patients who receive a bone marrow transplant may have an improved outcome (2077). In 30–40% of patients, aCML evolves to acute myeloid leukaemia (4248); most of the remaining patients die of marrow failure (447,2143).

Juvenile myelomonocytic leukaemia

Baumann I.
Bennett J.M.
Niemeyer C.M
Thiele J.

Definition
Juvenile myelomonocytic leukaemia (JMML) is a clonal haematopoietic disorder of childhood characterized by a proliferation principally of the granulocytic and monocytic lineages. Blasts and promonocytes account for <20% of the white blood cells in the peripheral blood and bone marrow. Erythroid and megakaryocytic abnormalities are often present (88,507,2662). BCR-ABL1 fusion is absent, whereas mutations involving genes of the RAS pathway are characteristic. The diagnostic criteria are listed in Table 5.03.

ICD-O code 9946/3

Synonym
Juvenile chronic myelomonocytic leukaemia

Epidemiology
The annual incidence of JMML is estimated to be approximately 0.13 cases per 100 000 children aged 0–14 years. It accounts for <2–3% of all leukaemias in children, but for 20–30% of all cases of myelodysplastic and myeloproliferative diseases in patients aged <14 years (1585,3092). Patient age at diagnosis ranges from 1 month to early adolescence, but 75% of cases occur in children aged <3 years (2415,2872). Boys are affected nearly twice as frequently as girls. Approximately 15% of cases occur in infants with Noonan syndrome-like disorder (which is caused by a CBL mutation) (2381), and 10% occur in children with neurofibromatosis type 1 (NF1) (2872).

Etiology
The cause of JMML is unknown. Rare cases have been reported in identical twins (3093). The association between NF1 and JMML has long been established (581,2872,3804). In children with NF1 (unlike in adults with the disorder), the risk of developing myeloid malignancy (mainly JMML) is reported to be 200–500 times that in the general paediatric population (2872). Occasionally, infants with Noonan syndrome develop a JMML-like disorder, which resolves

Table 5.03 Diagnostic criteria for juvenile myelomonocytic leukaemia; modified from Locatelli F and Niemeyer CM (2377)

Clinical and haematological criteria (all 4 criteria are required)
- Peripheral blood monocyte count ≥1 x 10^9/L
- Blast percentage in peripheral blood and bone marrow of <20%
- Splenomegaly
- No Philadelphia (Ph) chromosome or BCR-ABL1 fusion

Genetic criteria (any 1 criterion is sufficient)
- Somatic mutation in PTPN11, KRAS or NRAS
- Clinical diagnosis of neurofibromatosis type 1 or NF1 mutation
- Germline CBL mutation and loss of heterozygosity of CBL

Other criteria
Cases that do not meet any of the genetic criteria above must meet the following criteria in addition to the clinical and haematological criteria above:
- Monosomy 7 or any other chromosomal abnormality
- ≥2 of the following:
  - Increased haemoglobin F for age
  - Myeloid or erythroid precursor on peripheral blood smear
  - Granulocyte-macrophage colony-stimulating factor (also called CSF2) hypersensitivity in colony assay
  - Hyperphosphorylation of STAT5

- If a mutation is found in PTPN11, KRAS or NRAS it is essential to consider that it might be a germline mutation and the diagnosis of transient abnormal myeloipoiesis of Noonan syndrome must be considered.
- Occasional cases have heterozygous splice-site mutations.
without treatment in some cases and behaves more aggressively in others [217]. These children carry germline mutations in PTPN11 (the gene encoding the protein tyrosine phosphatase SHP2 [3906]) or KRAS [3586].

Localization
The peripheral blood and bone marrow always show evidence of myelomonocytic proliferation. Leukaemic infiltration of the liver and spleen is found in virtually all cases. The lymph nodes, skin, respiratory system, and gut are other common sites of involvement, although any tissue can be infiltrated [2415,2872].

Clinical features
Most patients present with constitutional symptoms or evidence of infection [2415,2872]. There is generally marked hepatosplenomegaly. Occasionally, spleen size is normal at diagnosis but rapidly increases thereafter. About half of all patients have lymphadenopathy, and leukaemic infiltrates may give rise to markedly enlarged tonsils. Dry cough, tachypnoea and interstitial infiltrates on chest X-ray are signs of pulmonary infiltration. Gut infiltration may predispose patients to diarrhoea and gastrointestinal infections. Signs of bleeding are common, and about a quarter of all patients have skin rashes (eczematous eruptions or indurations with central clearing). Café-au-lait spots might be indicative of an underlying germline condition such as NF1 or Noonan syndrome–like disorder [2528,2874]. JMML rarely involves the central nervous system (CNS), although a small number of patients with CNS myeloid sarcoma and ocular infiltrates have been described [2872]. A notable feature of many JMML cases is markedly increased synthesis of haemoglobin F, particularly in cases with a normal karyotype [2872]. Other features include polyclonal hypergammaglobulinemia and the presence of autoantibodies [2872]. In vitro hypersensitivity of JMML myeloid progenitors to granulocyte–macrophage colony-stimulating factor (also called CSF2) [1100] is a hallmark of the disease, and served as an important diagnostic tool before the discovery of the five canonical RAS pathway mutations (in PTPN11, NRAS, KRAS, NF1 and CBL), which now allow molecular diagnosis in approximately 85% of all JMML cases, greatly facilitating the diagnosis. In RAS pathway mutation-negative cases, disorders with a clinical and haematological picture mimicking that of JMML, such as infection [2481], Wiskott–Aldrich syndrome (eczema-thrombocytopenia–immunodeficiency syndrome) [4436] and malignant infantile osteopetrosis [3811], must be excluded.

Microscopy
The peripheral blood is the most important specimen for diagnosis. It typically shows leukocytosis and thrombocytopenia, and often anaemia [2415,2872]. The median reported white blood cell counts are 25–30 x 10^9/L. The leukaemia consists mainly of neutrophils, with some immature cells (e.g. promyelocytes and myelocytes) and monocytes. Blasts (including promonocytes) usually account for <5% of the white blood cells, and always <20%. Eosinophilia and basophilia are observed in a minority of cases. Nucleated red blood cells are often seen. Red blood cell changes include macrocytosis (particularly in patients with monosomy 7), but normocytic red blood cells are more common; microcytosis due to iron deficiency or acquired thalassaemia phenotype [1680] may be seen as well. Platelet counts vary, but thrombocytopenia is typical and may be severe [2415,2872,3093]. Bone marrow findings alone are not diagnostic. The bone marrow aspirate and biopsy are hypercellular with granulocytic proliferation, although in some patients erythroid precursors may predominate [2872,3093]. Monocytes in the bone marrow are often less prominent than in the peripheral blood, generally accounting for 5–10% of the bone marrow cells. Blasts (including promonocytes) account for <20% of the bone marrow cells, and Auer rods are never present. Dysplasia is usually minimal; however, dysgranulo-
lipoiesis (including pseudo–Peiger–Huët neutrophils and hypogranularity) may be noted in some cases, and erythroid precursors may be enlarged. Megakaryocytes are often reduced in number, but marked megakaryocytic dysplasia is unusual (2872,3093).

Leukaemic infiltrates are common in the skin, where myelomonocytic cells infiltrate the papillary and reticular dermis. In the lung, leukaemic cells spread from the capillaries of the alveolar septa into alveoli; in the spleen, they infiltrate the red pulp and have a predilection for trabecular and central arteries; in the liver, the sinusoids and portal tracts are infiltrated.

Cytochemistry

No specific cytochemical abnormalities have been reported. In bone marrow aspirate smears, cytochemical staining for alpha-naphthyl acetate esterase or alpha-naphthyl butyrate esterase, alone or in combination with staining for naphthol AS-D chloroacetate esterase (CAE), may be helpful in identifying the monocytic component. Neutrophil alkaline phosphatase scores are reported to be elevated in about 50% of cases, but this test is not helpful in establishing the diagnosis (2415).

Immunophenotype

No specific immunophenotypic abnormalities have been reported in JMML. In extramedullary tissues, the monocytic component is best identified using immunohistochemical techniques that detect lysozyme and CD68R. However, individual cases may show infiltration almost exclusively by MPO-positive granulopoietic precursor cells. Flow cytometry, which enables simultaneous analysis of cell phenotype and cell signalling, shows that JMML cells exhibit an aberrant response of phospho-STAT5A to subsaturating doses of granulocyte–macrophage colony-stimulating factor (1578,2090).

Cell of origin

A haematopoietic stem cell

Genetic profile

Karyotyping studies reveal monosomy 7 in about 25% of patients, other abnormalities in 10% and a normal karyotype in 65% (2872). The Philadelphia (Ph) chromosome and the BCR-ABL1 fusion gene are absent. JMML occurs, at least in part, due to aberrant signal transduction of the RAS signalling pathway. As many as 85% of patients harbour driving molecular alteration in one of five particular genes (PTPN11, NRAS, KRAS, CBL and NF1), which encode proteins that when mutated are predicted to activate RAS effector pathways. Heterozygous somatic gain-of-function mutations in PTPN11 are the most frequent alterations, occurring in approximately 35% of patients (2382, 3906). Typical oncogenic heterozygous somatic NRAS and KRAS mutations in codons 12, 13, and 61 account for 20–25% of JMML cases (3484,3801,3906). Approximately 15% of children with JMML harbour germline CBL mutations (2874, 3131), commonly missense alterations in the linker region or ring finger domain (exons 8 and 9), with JMML cells showing duplication of the mutant CBL through acquired uniparental disomy (587,2381, 2788). Occasionally, heterozygous germline splice-site CBL mutations are noted in CBL-associated JMML (2381,2528, 3817). Germline mutations in NF1 are present in approximately 10% of children with JMML (587,2872,3484). Because the NF1 gene product (neurofibromin) is a negative modulator of RAS function, loss of heterozygosity (LOH) with loss of the normal NF1 allele in leukaemic cells is associated with RAS hyperactivity. Despite the central role of RAS pathway mutation, a small subset (approximately 15%) of cases remain RAS pathway mutation negative (3484,3801). JMML is characterized by a paucity of additional genetic abnormalities (3484). Secondary abnormalities (in addition to the canonical RAS pathway mutation) are present in fewer than half of all cases, and include second hits in one of the other RAS pathway genes (so-called RAS double mutants) as well as mutations in SETBP1, JAK3, SH2B3, the genes of the polycomb repressor complex, and ASXL1 (587,3484,3801). Secondary mutations are often subclonal and may be involved in disease progression rather than initiation of leukaemia (587,3484,3802).

Genetic susceptibility

The RASopathies constitute a class of autosomal dominant developmental disorders caused by germline mutations in genes that encode components of the RAS pathway. These disorders' major features include facial dysmorphism, cardiac defects, reduced growth, variable cognitive deficits, ectodermal and skeletal anomalies, and susceptibility to...
malignancies (including JMML) [2102, 2871, 3316];
NF1, the first syndrome found to be associated with a germline mutation in the RAS pathway, can manifest in early childhood with cafe-au-lait spots, JMLM, plexiform neurofibromas, optic pathway tumours, and bone lesions [3314]. In children with NF1, the risk of developing JMLM is estimated to be 200–350 times the risk in children without the syndrome [3804]. For about half of the patients with JMLM and NF1, a positive family history is known. In most affected children, the clinical diagnosis of NF1 can be made at the time of leukaemic presentation; JMLM may be the first manifestation of NF1 in some of these infants [3801]. Patients with Noonan syndrome–like disorder exhibit a variable Noonan syndrome–like phenotype, with a high frequency of neurological features and pigmented skin lesions [2528, 2874, 3817]. Susceptibility for JMLM in children with germline CBL mutation is high, although the small number of patients precludes more precise risk estimation [2528]. Noonan syndrome is the most common RASopathy, with an incidence of 1 case per 1000–2500 births [3317]. It is characterized by a typical facial appearance, heart defects, and a variety of abnormalities in other organs. Heterozygous germline mutations in PTPN11, SOS1, RAF1, KRAS, NRAS and other components of the RAS pathway [811] are recognized, with PTPN11 mutations accounting for about half of the cases [3317]. As many as 10% of children with Noonan syndrome develop a transient myeloproliferative disorder in early infancy [217, 2871]. The vast majority of patients with Noonan syndrome/myeloproliferative disorder harbour germline PTPN11 mutations predicted to result in a weaker gain-of-function effect than the somatic PTPN11 mutations found in children with JMLM [2103]. The abnormal myelopoiesis in Noonan syndrome/myeloproliferative disorder is benign in most infants, but about 10% of these children acquire clonal chromosomal abnormalities and develop JMLM [217, 2871].

Prognosis and predictive factors
JMLM with somatic PTPN11 mutation or occurring in children with NF1 is invariably rapidly fatal if left untreated. The median survival time without allogeneic haematopoietic stem cell transplantation is about 1 year. Low platelet count, patient age >2 years at diagnosis and high haemoglobin F levels at diagnosis are the main clinical predictors of short survival [2872, 3093]. JMLM with KRAS or NRAS mutation generally has an aggressive course, with early haematopoietic stem cell transplantation needed. In a few infants with KRAS or NRAS alterations, long-term survival in the absence of therapy has been observed; these children had low haemoglobin F levels, normal or moderately decreased platelet counts, and no subclonal mutations [2377, 3801]. There are similarities between JMLM with KRAS or NRAS mutation and RAS-associated autoimmune leukoproliferative disorder (RALD) [530]: JMLM and RALD show overlapping clinical and laboratory features (with the exception of the leukaemia seen in RALD). However, long-term follow-up suggests that RALD has an indolent clinical course, unlike most cases of JMLM with RAS mutations [530]. Most children with JMLM and germline CBL mutations experience spontaneous regression of JMLM with persistence of uniparental disomy of the CBL locus in haematopoietic cells. Occasionally, secondary genetic alterations occur that result in an aggressive clinical course.
**Myelodysplastic/myeloproliferative neoplasm with ring sideroblasts and thrombocytosis**

**Definition**
Myelodysplastic/myeloproliferative neoplasm (MDS/MPN) with ring sideroblasts and thrombocytosis (MDS/MPN-RS-T) is a subtype of MDS/MPN characterized by the presence of thrombocytosis (≥450 × 10^9/L) and <1% blasts in the peripheral blood and associated with ring sideroblasts accounting for ≥15% of erythroblasts, dyserythropoiesis and <5% blasts in the bone marrow [1499, 1890,3104,3862].

In the original 4th edition of the WHO classification, refractory anaemia with ring sideroblasts associated with marked thrombocytosis (RARS-T) was proposed as a provisional entity to encompass cases with the clinical and morphological features of myelodysplastic syndrome with ring sideroblasts (MDS-RS) but also with thrombocytosis associated with abnormal megakaryocytes similar to those seen in BCR-ABL1-negative myeloproliferative neoplasms [1499,1890]. More recently, MDS/MPN-RS-T has become a well-characterized, distinct MDS/MPN overlap entity, particularly following the discovery of a strong association with SF3B1 mutations, which are often concurrent with the JAK2 V617F mutation and less commonly with MPL or CALR mutation [409,594,1310,2461, 2464,3103,3339,3344,3569,3575,3773, 3862,4249].

Cases that fulfil the diagnostic criteria for MDS with isolated del(5q) or that have t(3;3)(q21.3;q26.2) or inv(3)(q21.3;q26.2) cytogenetic abnormalities are excluded from this category, as are cases with a BCR-ABL1 fusion gene. If there has been a prior diagnosis of an MPN without ring sideroblasts, or if there is evidence that the ring sideroblasts might be a consequence of therapy or might reflect disease progression of a case that meets the criteria for another well-defined MPN, this designation should not be used. It is unclear how to best categorize the rare cases that initially present as MDS-RS and later evolve to MDS/MPN-RS-T upon acquisition of a JAK2 V617F mutation [2461] or other MPN-associated mutations. By convention, these cases would be considered to constitute disease progression of MDS, and would therefore be excluded from the MDS/MPN category (see Table 5.04); however, given the lack of prognostic difference, it might be most appropriate to group these cases with the rest of the cases of MDS/MPN-RS-T. Therapy-related cases with features of MDS/MPN-RS-T are diagnosed as therapy-related myeloid neoplasms and are discussed separately (see Therapy-related myeloid neoplasms, p. 153).

**ICD-O code** 9982/3

**Synonym** Refractory anaemia with ring sideroblasts associated with marked thrombocytosis

**Epidemiology**
A median patient age at the time of diagnosis of 74 years has been reported, which is higher than that observed in MPN such as essential thrombocythaemia. A slight female prevalence has been consistently reported across studies [462,463].

**Localization**
The peripheral blood and bone marrow are always involved. Splenomegaly has been reported in about 40% of cases, and hepatomegaly can also occur [3861].

**Clinical features**
The clinical features of MDS/MPN-RS-T greatly overlap those seen in MDS-RS and the BCR-ABL1-negative MPN categories, in particular essential thrombocythaemia. Anaemia is always present, but at the time of clinical presentation, patients with MDS/MPN-RS-T tend to have higher haemoglobin levels, white blood cell (WBC) counts, and platelet counts than do patients with MDS-RS, with similar mean corpuscular volumes. In contrast, patients with MDS/MPN-RS-T have lower haemoglobin levels, WBC counts, and platelet counts, but higher mean corpuscular volumes than do patients with essential thrombocythaemia (463).

**Microscopy**
The peripheral blood typically shows normochromic macrocytic or normocytic anaemia. The red blood cells in the blood smear may show anisocytosis, often with a dimorphic pattern. Circulating blasts are absent or rare (accounting for <1% of the cells). Thrombocytosis (≥450 × 10^9/L) is one of the defining features. The platelets often display anisocytosis, ranging from tiny forms to atypical large or giant platelets. Bizarrely shaped or agranular platelets may be seen, but are uncommon. The WBC count and leukocyte differential count are usually normal, although a borderline elevation in the WBC count can occur.

---

Table 5.04 Diagnostic criteria for myelodysplastic/myeloproliferative neoplasm with ring sideroblasts and thrombocytosis

- Anaemia associated with erythroid-lineage dysplasia, with or without multilineage dysplasia;
- ≥15% ring sideroblasts, <1% blasts in the peripheral blood and <5% blasts in the bone marrow
- Persistent thrombocytosis, with platelet count ≥450 × 10^9/L
- SF3B1 mutation or, in the absence of SF3B1 mutation, no history of recent cytotoxic or growth factor therapy that could explain the myelodysplastic/myeloproliferative features
- No BCR-ABL1 fusion; no rearrangement of PDGFRα, PDGFRβ or FGFR1; no PCM1-JAK2 and no t(3;3)(q21.3;q26.2), inv(3)(q21.3;q26.2), or del(5q)
- No history of myelodysplastic/myeloproliferative neoplasm with ring sideroblasts, or other myelodysplastic/myeloproliferative neoplasm

---

Orazi A.
Hasserjian R.P.
Cazzola M.
Thiele J.
Malcovati L.
The bone marrow shows increased erythroid proliferation, with megaloblastoid and/or other dyserythropoietic features of the erythroid precursors associated with ≥15% ring sideroblasts present on iron staining. Multilineage dysplasia, similar to that seen in MDS-RS and multilineage dysplasia, occurs in some cases [3569, 3570]. Megakaryocytes are increased and usually have morphological features similar to those observed in the BCR-ABL1-negative MPN. A proportion of patients have marrow fibrosis [3569,3570].

**Cell of origin**
A haematopoietic stem cell

**Genetic profile**
Cytogenetic abnormalities have been reported in about 10% of patients [2461]. Many cases (60–90%) harbour the SF3B1 mutation [2460]. The mutant allele burden is comparable to that seen in other WHO categories with ring sideroblasts. The SF3B1 mutation is often (in >60% of cases) found in association with the JAK2 V617F mutation, and much less commonly (in <10% of cases) in association with the CALR or MPL W515 mutation. The presence of these MPN-associated mutations may account for the proliferative aspects of MDS/MPN-RS-T and would seem to confirm its true hybrid nature [2461,2464,3103,3570,4202]. Therefore, although studies for SF3B1, JAK2 V617F, CALR and MPL W515 mutations are not required for the diagnosis of MDS/MPN-RS-T, the presence of these mutations supports the diagnosis and appears to have prognostic significance.

**Prognosis and predictive factors**
A median overall survival of 76–128 months has been reported in patients with MDS/MPN-RS-T [462,463]. A retrospective study including a total of 200 cases from 16 centres in six European countries showed that sex- and age-standardized survival in patients with MDS/MPN-RS-T is significantly shorter than that of patients with essential thrombocythaemia, but longer than that of patients with MDS-RS and single lineage dysplasia (P<0.001) [463,4249]. Patient age, JAK2 V617F and SF3B1 mutation have been reported as independent prognostic factors. In one study of MDS/MPN-RS-T, SF3B1 mutation was associated with a significantly longer median overall survival (6.9 years with SF3B1 mutation vs 3.3 years with SF3B1 wildtype, P = 0.003); JAK2 V617F mutation was also associated with a more favourable outcome compared with JAK2 wildtype (P = 0.019) [462].

The existing scientific literature does not support any therapy specific for patients with MDS/MPN-RS-T; the treatments available for MDS and MPN are typically adopted for these patients, depending on the clinical picture [2460].
Myelodysplastic/myeloproliferative neoplasm, unclassifiable

Definition
Myelodysplastic/myeloproliferative neoplasm (MDS/MPN), unclassifiable (MDS/MPN-U) meets the criteria for the MDS/MPN category in that at the time of initial presentation, it has clinical, laboratory, and morphological features that overlap with both myelodysplastic syndrome (MDS) and myeloproliferative neoplasm (MPN) categories (1748, 2987, 4152) (Table 5.05). Cases diagnosed as MDS/MPN-U do not meet the criteria for chronic myelomonocytic leukaemia, juvenile myelomonocytic leukaemia, BCR-ABL1-negative atypical chronic myeloid leukaemia, or MDS/MPN with ring sideroblasts and thrombocytosis (MDS/MPN-RS-T). The removal of MDS/MPN-RS-T from the category of MDS/MPN-U is a change from the original 4th edition of the WHO classification. The finding of BCR-ABL1 fusion; rearrangement of PDGFRA, PDGFRB, or FGFR1; or PCM1-JAK2 also rules out the diagnosis of MDS/MPN-U.

Table 5.05 Diagnostic criteria for myelodysplastic/myeloproliferative neoplasm, unclassifiable

<table>
<thead>
<tr>
<th>Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myeloid neoplasm with mixed myeloproliferative and myelodysplastic features at onset, not meeting the WHO criteria for any other myelodysplastic/myeloproliferative neoplasm, myelodysplastic syndrome or myeloproliferative neoplasm</td>
</tr>
<tr>
<td>- &lt;20% blasts in the peripheral blood and bone marrow</td>
</tr>
<tr>
<td>- Clinical and morphological features of one of the categories of myelodysplastic syndrome²</td>
</tr>
<tr>
<td>- Clinical and morphological myeloproliferative features manifesting as a platelet count of ≥450 x 10^9/L associated with bone marrow megakaryocytic proliferation and/or a white blood cell count of ≥13 x 10^9/L²</td>
</tr>
<tr>
<td>- No history of recent cytotoxic or growth factor therapy that could explain the myelodysplastic/myeloproliferative features</td>
</tr>
<tr>
<td>- No PDGFRA, PDGFRB, or FGFR1 rearrangement and no PCM1-JAK2</td>
</tr>
</tbody>
</table>

² Cases that meet criteria for myelodysplastic syndrome with isolated del(5q) are excluded irrespective of the presence of thrombocytosis or leukocytosis.

Synonyms
Chronic myelodysplastic/myeloproliferative disease (no longer used); mixed myeloproliferative/myelodysplastic syndrome, unclassifiable; overlap syndrome, unclassifiable

Localization
The bone marrow and peripheral blood are always involved. The spleen, liver, and other extramedullary tissues may be involved.

Clinical features
The clinical features of MDS/MPN-U overlap with those of entities in the MDS and MPN categories (227, 2857, 4152).

Microscopy
MDS/MPN-U is characterized by ineffective and/or dysplastic proliferation of one or more myeloid lineages and simultaneously by effective proliferation (with or without dysplasia) of another myeloid lineage or lineages. Laboratory features usually include anaemia of variable severity, with or without macrocytosis. There is evidence of effective proliferation in one or more lineages: either thrombocytosis (platelet count ≥ 450 x 10^9/L) or leucocytosis (white blood cell count ≥ 13 x 10^9/L). In a recent study, the median white blood cell count was lower in MDS/MPN-U (19.4 x 10^9/L) than in atypical chronic myeloid leukaemia, BCR-ABL1-negative, (40.8 x 10^9/L) [4248]. Neutrophils may show dysplastic features, but dysgranulopoiesis is seen in only about 50% of cases [4248]. There may be giant or hypogranular platelets. Dysmegakaryopoiesis with megakaryocytes resembling those seen in MDS is found in more than half of cases. In the remaining cases, there is a mixture of MDS-like and MPN-like megakaryocytes or (rarely) a predominance of MPN-like megakaryocytes. Dysmegakaryopoiesis is absent in <10% of cases [4248]. Blasts account for <20% of leukocytes in the peripheral blood and <20% of nucleated cells in the bone marrow. The bone marrow biopsy specimen is hypercellular and may show proliferation in any or all of the myeloid lineages; however, significant (≥ 10%) dysplastic features are simultaneously present in at least one cell line. Although cases of MDS/MPN-RS-T are classified separately, rare cases of MDS/MPN with ≥15% ring sideroblasts and ≥1% peripheral blood blasts or ≥5% bone marrow blasts belong in the MDS/MPN-U group.

Cytochemistry
The cytochemical findings may be similar to those seen in MDS or MPN.

Immunophenotype
The immunophenotype may be similar to that of MDS or MPN.
Cell of origin  
The postulated cell of origin is a haematopoietic stem cell.

Genetic profile  
There are no cytogenetic or molecular genetic findings specific for this group. The Philadelphia (Ph) chromosome and the BCR-ABL1 fusion gene should always be excluded prior to making the diagnosis of MDS/MPN-U. Cases with rearrangements of PDGFRα, PDGFRβ, or FGFR1 or with PCM1-JAK2, are excluded from this category as well. The appropriate categorization of cases associated with isolated isochromosome 17q is uncertain. A proportion of cases meet the criteria for chronic myelomonocytic leukaemia, BCR-ABL1-negative atypical chronic myeloid leukaemia, MPN in accelerated or blast phase, MDS or acute myeloid leukaemia, but other cases may be appropriately categorized as MDS/MPN-U (1220,1912,2594). Relatively high frequencies of TET2, NRAS, RUNX1, CBL, SETBP1 and ASXL1 mutations have been reported in several studies (2610,4248,4502). In diagnostically difficult cases, the presence of one or more of these mutations in the appropriate clinicopathological context may help to confirm a suspected diagnosis. SF3B1 mutation should prompt careful exclusion of MDS/MPN-RS-T, including rare instances of disease progression from MDS with ring sideroblasts. Although cases that meet the criteria for MDS with isolated del(5q) are excluded, a small proportion of such cases with a combined del(5q) and JAK2/V617F mutation have been reported to have proliferative features in the bone marrow associated with higher median platelet counts (1759). However, it is unclear whether their clinical presentation or prognosis is any different from that of the MDS with isolated del(5q) and wildtype JAK2 (3101,3717). Until more evidence is published, it is recommended that cases with combined del(5q) and JAK2 V617F mutation be classified as MDS with isolated del(5q) rather than included in the MDS/MPN-U category.

Cases with MDS/MPN features that harbour one of the types of driver mutations seen in classic MPN (i.e, JAK2, MPL or CALR mutations) most likely constitute MPN with features of disease progression. However, if a chronic phase has not been previously detected or cannot be documented and therefore the underlying MPN cannot be confirmed, then the designation of MDS/MPN-U is justified.

Prognosis and predictive factors  
There is very limited information available about this rare subgroup. In a recent study, patients with MDS/MPN-U had a median overall survival of 21.8 months and leukaemia-free survival of 18.9 months (4248). Prognosis is variable, with its uncertainty further compounded by inadequate representation of the subgroup in commonly used prognostic scoring systems, including the International Prognostic Scoring System (IPSS) and Revised IPSS (IPSS-R) (4011). As with other MDS/MPN overlap disorders, the treatment for patients with MDS/MPN-U is based on therapies used for MDS or MPN and is guided by symptoms and/or cytopenias (4011). Growth factors (erythropoiesis- and granulopoiesis-stimulating agents) can alleviate cytopenias, whereas leukocytosis can be managed with cyto reducive therapies.
CHAPTER 6

Myelodysplastic syndromes
Myelodysplastic syndrome with single lineage dysplasia
Myelodysplastic syndrome with ring sideroblasts
Myelodysplastic syndrome with multilineage dysplasia
Myelodysplastic syndrome with excess blasts
Myelodysplastic syndrome with isolated del(5q)
Myelodysplastic syndrome, unclassifiable
Childhood myelodysplastic syndrome
Refractory cytopenia of childhood
Myelodysplastic syndromes: Overview

Definition
The myelodysplastic syndromes (MDS) are a group of clonal haematopoietic stem cell diseases characterized by cytopenia, dysplasia in one or more of the major myeloid lineages, ineffective haematopoiesis, recurrent genetic abnormalities and increased risk of developing acute myeloid leukaemia (AML) [340, 592, 4151]. There is an increased degree of apoptosis within the bone marrow progenitors, which contributes to the cytopenias [439]. Cytopenia in at least one haematopoietic lineage is required for a diagnosis of MDS. The recommended thresholds for cytopenias established in the original International Prognostic Scoring System (IPSS) for risk stratification [haemoglobin concentration <10g/dL, platelet count <100 x 10⁶/L, and absolute neutrophil count <1.8 x 10⁹/L (1442, 1442A)], have traditionally been used to define cytopenias for MDS diagnosis and most MDS patients will have a cytopenia below at least one of these thresholds. However, a diagnosis of MDS may still be made in patients with milder degrees of anaemia (haemoglobin <13g/dL in men or <12g/dL in women) or thrombocytopenia (platelets <150 x 10⁶/L) if definitive morphologic and/or cytogenetic findings are present [1444A, 4179]. In determining whether a patient is cytopenic, it is important to be cognizant of each laboratory's lower reference range and to take into account conditional variants of these values, such as due to ethnicity and sex. These are particularly important considerations in patients with a borderline low neutrophil count [229]. Persistent neutrophilia, monocytosis, erythrocytosis or thrombocytosis in a patient with cytopenias and dysplastic morphology generally warrants classification as a myelodysplastic/myeloproliferative neoplasm (MDS/MPN) or myeloproliferative neoplasm rather than MDS. However, thrombocytosis (platelet count ≥450 x 10⁹/L) is allowed in MDS with isolated del(5q) or with inv(3) (q21.3;q26.2) or t(3;3)(q21.3;q26.2).

Fig. 6.01 Parvovirus B19 infection. Bone marrow smear shows marked erythroid hypoplasia, with occasional giant erythroblasts with dispersed chromatin and fine cytoplasmic vacuoles.

The morphological hallmark of MDS is dysplasia in one or more myeloid lineages. Dysplasia may be accompanied by an increase in myeloblasts in the peripheral blood and/or bone marrow, but the blast percentage is always <20%, which is the requisite threshold recommended for the diagnosis of AML. It is important to recognize that the threshold of 20% blasts distinguishing AML from MDS does not reflect a therapeutic mandate to treat cases with >20% blasts as acute leukaemia. Recurrent cytogenetic abnormalities are present in 40–50% of cases, whereas acquired somatic gene mutations are seen in the vast majority of MDS cases at diagnosis.

The MDS category encompasses several distinct subtypes, which are defined by the number of cytopenias at presentation, the number of myeloid lineages manifesting dysplasia, the presence of ring sideroblasts, and the blast percentages in the blood and bone marrow. In the current classification, only one cytogenetic abnormality, del(5q), is used in the definition of a specific MDS subtype. Mutation of one gene, SF3B1, is closely associated with MDS with ring sideroblasts as well as with one of the MDS/MPN subtypes: MDS/MPN with ring sideroblasts and thrombocytosis.

Although progression to AML is the natural course in many cases of MDS, the percentage of patients who progress varies substantially across the subtypes, with a higher probability of progression in subtypes with increased myeloblasts.

Fig. 6.02 Arsenic poisoning. Bone marrow smear from a 47-year-old man with pancytopenia being chronically exposed to arsenic; there is marked dyserythropoiesis.

Fig. 6.03 Antifolate chemotherapy effect. Bone marrow smear from a 57-year-old woman who received several chemotherapeutic agents for breast carcinoma, including folic acid antagonists, showing transient marked dyserythropoiesis and megaloblastic changes.
Most subtypes are characterized by progressive bone marrow failure, but the biological course of some subtypes is prolonged and indolent, with a very low incidence of evolution to AML.

**Epidemiology**

MDS occurs principally in older adults (median patient age: 70 years), with a male predominance. The annual incidence is 3–5 cases per 100 000 population overall (non–age-corrected) and is at least 20 cases per 100 000 individuals aged >70 years. Due to underreporting of MDS in most cancer registries, the true annual incidence in patients aged >65 years may be closer to 75 cases per 100 000 population [199,777,1342]. Approximately 10 000 new cases of MDS are diagnosed annually in the USA, according to 2003–2004 data from the Surveillance, Epidemiology, and End Results (SEER) Program and the North American Association of Central Cancer Registries (NAACCR), but estimates based on Medicare claims for the same time period are as high as 45 000 cases diagnosed in individuals aged >65 years annually [1394,2421,3397]. Therapy-related myeloid neoplasms are discussed separately (see Therapy-related myeloid neoplasms, p. 153). MDS affecting children is rare and has unique characteristics and diagnostic criteria that differ from those of MDS in adults; therefore, childhood cases are also discussed separately (p. 116).

**Etiology**

Primary or de novo MDS occurs without a known history of chemotherapy or radiation exposure. Possible etiologies for primary MDS include benzene exposure (at levels well above the minimum allowed by most government agencies), cigarette smoking (at least in part also due to benzene in cigarette smoke), exposure to agricultural chemicals or solvents, and family history of haematopoietic neoplasms [3815]. Some inherited haematological disorders, such as Fanconi anaemia, dyskeratosis congenita, Shwachman–Diamond syndrome and Diamond–Blackfan anaemia, are also associated with an increased risk of MDS; familial syndromes predisposing to MDS and AML are discussed separately (see Myeloid neoplasms with germline predisposition, p. 121). Acquired aplastic anaemia is also associated with increased risk of development of MDS [222].

**Clinical features**

The majority of patients present with symptoms related to cytopenia. Most patients are anaemic, whereas neutropenia and/or thrombocytopenia are less common; about one third of patients are dependent on red blood cell transfusions at diagnosis [1444,2511]. Organomegaly is infrequently observed.

**Microscopy**

The morphological classification of MDS is principally based on the percentage of blasts in the bone marrow and peripheral blood, the type and degree of dysplasia, and the percentage of ring sideroblasts (Table 6.01, p. 101). The myeloid lineages affected by cytopenias are not necessarily those that manifest dysplasia [1338,2423,4179]. To determine blast percentage in the bone marrow and blood, a 500-cell differential count of all nucleated cells in a smear or trephine biopsy imprint is recommended for the bone marrow and a 200-leukocyte differential count for the peripheral blood. In patients with severe cytopenia, buffy coat smears of peripheral blood may facilitate the differential count. An accurate blast count in the peripheral blood is important, because patients with higher blast percentages in the blood than in the bone marrow (seen in ~13% of MDS cases) appear to have more aggressive disease [89]. The blast count in myeloid neoplasms is expressed as a percentage of all nucleated cells (always including nucleated erythroid cells) in the bone marrow and as a percentage of the leukocytes (excluding nucleated erythroid cells) in the peripheral blood.

The number of dysplastic lineages (i.e. single lineage vs multilineage dysplasia) is relevant for distinguishing between the types of MDS (see Table 6.01, p. 101) and may be important for predicting disease behaviour [947,4179]. Assessment of the degree of dysplasia may be problematic, depending on the quality of smear preparations and the stain. Poor-quality smears may result in misinterpretation of the presence or absence of dysplasia, particularly in assessing neutrophil granulocyte. Given the critical importance of recognizing dysplasia, the need for high-quality slide preparations for the diagnosis of MDS cannot be overemphasized. Slides for the assessment of dysplasia should be made from freshly obtained specimens; specimens exposed to anticoagulants for >2 hours are unsatisfactory. It should be noted that the determination of whether significant dysplasia is present (particularly in the erythroid lineage) and the distinction between single lineage and multilineage dysplasia have been found in some studies to be subject to significant interobserver variability [1233,3622]. This interobserver variability is more problematic for cases in which the degree of dysplasia is near the requisite 10% threshold, and some authors have reported individual lineage dysplasia exceeding the 10% threshold in non-cytopenic controls [947,3067,3297]; consequently, it is essential to apply strict criteria for dysplasia and to evaluate high-quality and well-stained material.

![Fig. 6.04](image1.png) **Fig. 6.04** Congenital dyserythropoietic anaemia, type III. Bone marrow smear shows marked dyserythropoiesis.

![Fig. 6.05](image2.png) **Fig. 6.05** Granulocyte colony-stimulating factor (G-CSF) therapy effect. A Blood smear from a patient on G-CSF, showing a neutrophil with a bilobed nucleus and increased azurophilic granulation and a myeloblast (B).
As a general precaution, no patient should be diagnosed with MDS if the clinical and drug history is unknown, and no case of MDS should be reclassified while the patient is on growth factor therapy, including erythropoietin. Certain drugs, infections, metabolic deficiencies and immune disorders can cause both cytopenias and morphological dysplasia; these possible secondary etiologies must be carefully considered prior to rendering a diagnosis of MDS (see Differential diagnosis). Unexplained, persistent cytopenia in the absence of dysplasia should not be interpreted as MDS unless certain specific cytogenetic abnormalities are present (see Genetic profile below and Table 6.03, p. 104). Persistent cytopenia without dysplasia and without one of the specific cytogenetic abnormalities should be diagnosed as idiopathic cytopenia of undetermined significance, and the patient's haematological and cytogenetic status should be carefully monitored [4106,4336]. Patients with MDS-associated clonal gene mutations identified in haematopoietic cells but without significant dysplasia on bone marrow examination should not be diagnosed with MDS either; this condition has been termed 'clonal haematopoiesis of indeterminate potential' [3772]. Cases of MDS without an increase in blasts are recognized as manifesting either single lineage dysplasia or multilineage dysplasia. In most cases of MDS with single lineage dysplasia, the dysplasia is confined to the erythroid lineage. Single lineage dysplasia can also affect the granulocytic lineage or megakaryocytes, but this is much less common than dysplasia isolated to erythroid cells [450,2423]. In MDS with multilineage dysplasia, significant dysplastic features are recognized in two or more lineages. The recommended requisite percentage of erythroid and granulocytic cells manifesting dysplasia to be considered significant is ≥10% [3404]. Significant megakaryocyte dysplasia is defined as ≥10% dysplastic megakaryocytes based on evaluation of ≥30 megakaryocytes in smears or sections; however, some studies suggest that a 30–40% threshold for megakaryocyte dysplasia may provide greater specificity [947,1309,2567]. Micromegakaryocytes and multinucleated megakaryocytes with separated nuclei are the most reliable dysplastic findings in the megakaryocyte series [947,2567, 4179].

Characteristics of dysplasia
Dyserythropoiesis manifests principally as nuclear alterations, including budding, internuclear bridging, karyorrhexis and multinuclearity. Megaloblastoid changes are often present in MDS, but alone they are insufficiently specific to firmly establish dyserythropoiesis. Cytoplasmic features include formation of ring sideroblasts, vacuolization and aberrant perinuclear acid–Schiff (PAS) positivity (either diffuse or granular). Dysgranulopoiesis is characterized primarily by nuclear hyposegmentation (pseudo–Pelger–Huët anomaly) or hypersegmentation, cytoplasmic hypogranularity, pseudo–Chêdiak–Higashi granules and small size [1387]. Megakaryocyte dysplasia is characterized by micromegakaryocytes, non-lobated nuclei in megakaryocytes of all sizes, and multiple widely separated nuclei [1388]; however, the finding of multiple widely separated nuclei is of limited specificity for MDS, unless the nuclei are rounded and roughly similar in size. Megakaryocytic dysplasia is readily apparent in bone marrow sections, and both biopsy and aspirate specimens should be evaluated. The morphological manifestations of dysplasia in each lineage are summarized in Table 6.02. Auer rods are considered to be evidence of MDS with excess blasts regardless of the blast percentage. Cases of MDS with <5% blasts in the bone marrow and <1% in the peripheral blood may rarely have Auer rods, and such cases are associated with an adverse prognosis [4328].
Table 6.01 Diagnostic criteria for myelodysplastic syndrome (MDS) entities

<table>
<thead>
<tr>
<th>Entity name</th>
<th>Number of dysplastic lineages</th>
<th>Number of cytopenias</th>
<th>Ring sideroblasts as percentage of marrow erythroid elements</th>
<th>Bone marrow (BM) and peripheral blood (PB) blasts</th>
<th>Cytogenetics by conventional karyotype analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDS-SLD</td>
<td>1</td>
<td>1-2</td>
<td>&lt;15% / &lt;5%&lt;sup&gt;b&lt;/sup&gt;</td>
<td>BM &lt;5%, PB &lt;1%, no Auer rods</td>
<td>Any, unless fulfils all criteria for MDS with isolated del(5q)</td>
</tr>
<tr>
<td>MDS-MLD</td>
<td>2-3</td>
<td>1-3</td>
<td>&lt;15% / &lt;5%&lt;sup&gt;b&lt;/sup&gt;</td>
<td>BM &lt;5%, PB &lt;1%, no Auer rods</td>
<td>Any, unless fulfils all criteria for MDS with isolated del(5q)</td>
</tr>
<tr>
<td>MDS-RS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Any, unless fulfils all criteria for MDS with isolated del(5q)</td>
</tr>
<tr>
<td>MDS-RS-SLD</td>
<td>1</td>
<td>1-2</td>
<td>≥15% / ≥5%&lt;sup&gt;b&lt;/sup&gt;</td>
<td>BM &lt;5%, PB &lt;1%, no Auer rods</td>
<td>Any, unless fulfils all criteria for MDS with isolated del(5q)</td>
</tr>
<tr>
<td>MDS-RS-MLD</td>
<td>2-3</td>
<td>1-3</td>
<td>≥15% / ≥5%&lt;sup&gt;b&lt;/sup&gt;</td>
<td>BM &lt;5%, PB &lt;1%, no Auer rods</td>
<td>Any, unless fulfils all criteria for MDS with isolated del(5q)</td>
</tr>
<tr>
<td>MDS with isolated del(5q)</td>
<td>1-3</td>
<td>1-2</td>
<td>None or any</td>
<td>BM &lt;5%, PB &lt;1%, no Auer rods</td>
<td>del(5q) alone or with 1 additional abnormality, except loss of chromosome 7 or del(7q)</td>
</tr>
<tr>
<td>MDS-EB</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Any</td>
</tr>
<tr>
<td>MDS-EB-1</td>
<td>1-3</td>
<td>1-3</td>
<td>None or any</td>
<td>BM 5-9% or PB 2-4%, BM &lt;10% and PB &lt;5%, no Auer rods</td>
<td>Any</td>
</tr>
<tr>
<td>MDS-EB-2</td>
<td>1-3</td>
<td>1-3</td>
<td>None or any</td>
<td>BM 10-19% or PB 5-19% or Auer rods, BM and PB &lt;20%</td>
<td>Any</td>
</tr>
<tr>
<td>MDS-U</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>with 1% blood blasts</td>
<td>1-3</td>
<td>1-3</td>
<td>None or any</td>
<td>BM &lt;5%, PB = 1%, no Auer rods</td>
<td>Any</td>
</tr>
<tr>
<td>with SLD and pancytopenia</td>
<td>1</td>
<td>3</td>
<td>None or any</td>
<td>BM &lt;5%, PB &lt;1%, no Auer rods</td>
<td>Any</td>
</tr>
<tr>
<td>based on defining cytogenetic abnormality</td>
<td>0</td>
<td>1-3</td>
<td>&lt;15%&lt;sup&gt;d&lt;/sup&gt;</td>
<td>BM &lt;5%, PB &lt;1%, no Auer rods</td>
<td>MDS-defining abnormality&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

MDS-EB, MDS with excess blasts; MDS-MLD, MDS with multilineage dysplasia; MDS-RS, MDS with ring sideroblasts; MDS-MLD, MDS with ring sideroblasts and multilineage dysplasia; MDS-RS-SLD, MDS with ring sideroblasts and single lineage dysplasia; MDS-SLD, MDS with single lineage dysplasia; MDS-U, MDS, unclassifiable; SLD, single lineage dysplasia.

<sup>a</sup> Cytopenias defined as haemoglobin concentration <10 g/dL, platelet count <100 x 10^6/L and absolute neutrophil count <1.8 x 10^10/L, although MDS can present with mild anaemia or thrombocytopenia above these levels; PB monocytes must be <1 x 10^9/L.

<sup>b</sup> If SF3B1 mutation is present.

<sup>c</sup> 1% PB blasts must be recorded on ≥2 separate occasions.

<sup>d</sup> Cases with ≥15% ring sideroblasts by definition have significant erythroid dysplasia and are classified as MDS-RS-SLD.

<sup>e</sup> See Table 6.03, p. 104.

**Differential diagnosis**

A major difficulty in the diagnosis of MDS is the determination of whether the presence of morphological dysplasia and cytopenia is due to a clonal disorder or is the result of another factor. Dysplasia, even if prominent, is not in itself definitive evidence of a clonal process. Some dysplastic features, such as micromegakaryocytes, are strongly associated with MDS (947), but several nutritional, toxic and other factors can also cause myelodysplastic changes in any of the haematopoietic lineages. These factors include vitamin B12 and folic acid deficiency, essential element deficiencies (such as copper deficiency), exposure to heavy metals (in particular arsenic, lead and toxic levels of zinc) and exposure to several commonly used drugs and biological agents (439). Isoniazole treatment in the absence of vitamin B6 supplementation causes ring sideroblast formation. The antibiotic cotrimoxazole and the immunosuppressants tacrolimus and mycophenolate mofetil can cause marked neutrophil hyposegmentation, often indistinguishable from the changes seen in MDS. In some patients on multiple drugs or with multiple comorbidities, it may be difficult to identify the cause of dysplastic changes (1991,3867). Dysplastic changes can also be encountered in otherwise
normal marrow (3067), such as in individuals with the hereditary autosomal dominant Pelger–Huët anomaly resulting from mutations in the LBR gene (encoding lamin B receptor) (1658). Therefore, it is extremely important to correlate the morphological findings with the clinical presentation and any pertinent family history. Congenital haematological disorders such as congenital dyserythropoietic anaemia must also be considered as a possible cause of isolated dyserythropoiesis. Parvovirus B19 infection may be associated with erythroleukemia with giant pronormoblasts; the immunosuppressive agent mycophenolate mofetil may also be associated with erythroleukemia. Chemotherapeutic agents may result in transient marked dysplasia of all myeloid lineages. Granulocyte colony-stimulating factor therapy causes morphological alterations in the neutrophils, including a substantial left shift, marked hypergranularity and nuclear hyposegmentation (3572). Additionally, blasts (usually <5%) may be observed transiently in the peripheral blood; the bone marrow blast percentage is generally normal in such cases, but is transiently increased in some cases. Hypothyroidism, infections, autoimmune disorders, paroxysmal nocturnal haemoglobinuria and bone marrow lymphomatous involvement (in particular large granular lymphocytic leukaemia and hairy cell leukaemia) may clinically mimic MDS.

Given all these possibilities, it is extremely important to be aware of the clinical history (including exposure to drugs or chemicals) and to always consider nonclonal disorders as possible etiologies of morphological dysplasia in haematopoietic cells, particularly in cases with no increase in blasts. Haematological follow-up over a period of several months, possibly including repeated bone marrow sampling, may be necessary for difficult cases.

Microscopy
The value of bone marrow biopsy in MDS is well established (2981). It increases the diagnostic accuracy compared with examination of the aspirate smear alone and provides additional information about blast percentage and distribution within the marrow space (4180). Bone marrow cellularity, megakaryocyte morphology and stromal fibrosis are important features revealed by the biopsy. The bone marrow in MDS is usually hypercellular and less commonly normocellular or hypocellular for age; cytopenias result from ineffective haematopoiesis despite the typically increased cellularity. Histologically, aggressive MDS subtypes can be characterized by the presence of aggregates (3–5 cells) or clusters (>5 cells) of immature myeloid cells in bone marrow biopsies, usually localized in the central portion of the bone marrow away from the vascular structures and endosteal surfaces of the bone trabeculae (so-called abnormal localization of immature precursors) (942). Immunohistochemistry with an antibody to CD34 (an antigen expressed in the blasts in most MDS cases) can be used to confirm the blast nature of immature myeloid cells in the biopsy sections (3724,3895A). Immunohistochemical analysis with CD34 is especially useful for assessing blast percentage in cases of MDS with fibrosis or a hypocellular marrow, in which blast percentages are often underestimated in the smear preparations. CD34 is positive in megakaryocytes in some cases of MDS, but may also stain megakaryocytes in megakaryocytic anaemia (1761). KIT (CD117) staining can be informative in MDS cases with CD34-negative blasts. However, KIT is expressed not only by myeloblasts but also by proerythroblasts, promyelocytes and mast cells. Megakaryocyte markers (e.g. CD42b and CD61) can facilitate identification of small megakaryocytes and micromegakaryocytes, although apoptotic megakaryocytes may superficially mimic micromegakaryocytes in the immunostained sections. Immunostaining for p53 can be useful (2983,2986), because it correlates well with TP53 mutation status and has important prognostic significance (769,3472).

Hypoplastic myelodysplastic syndrome
In approximately 10% of MDS cases, the bone marrow is hypocellular for age. These cases have been referred to as hypoplastic MDS. This group does not constitute a specific MDS subtype in this classification. It can present with or without increased bone marrow blasts; some studies have suggested that hypopcellularity may be an independent favourable prognostic variable in MDS (1723,4453). Hypocellularity in MDS may lead to difficulties in the differential diagnosis with aplastic anaemia (1445,2982); significant dysplasia (most often micromegakaryocytes), increased blasts identified by CD34 staining of bone marrow biopsy sections, and an abnormal karyotype (excluding trisomy 8, which may be seen

---

**Table 6.02 Morphological manifestations of dysplasia**

<table>
<thead>
<tr>
<th>Category</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dyserythropoiesis</td>
<td>Nuclear</td>
</tr>
<tr>
<td></td>
<td>Nuclear budding</td>
</tr>
<tr>
<td></td>
<td>Internuclear bridging</td>
</tr>
<tr>
<td></td>
<td>Karyorrhexis</td>
</tr>
<tr>
<td></td>
<td>Multinuclearity</td>
</tr>
<tr>
<td></td>
<td>Megablastic changes</td>
</tr>
<tr>
<td></td>
<td>Cytoplasmic</td>
</tr>
<tr>
<td></td>
<td>Ring sideroblasts</td>
</tr>
<tr>
<td></td>
<td>Vacuolization</td>
</tr>
<tr>
<td></td>
<td>Periodic acid–Schiff (PAS) positivity</td>
</tr>
<tr>
<td>Dysgranulopoiesis</td>
<td>Small or unusually large size</td>
</tr>
<tr>
<td></td>
<td>Nuclear hyposegmentation</td>
</tr>
<tr>
<td></td>
<td>(pseudo–Pelger–Huët)</td>
</tr>
<tr>
<td></td>
<td>Nuclear hypersegmentation</td>
</tr>
<tr>
<td></td>
<td>Decreased granules; agranularity</td>
</tr>
<tr>
<td></td>
<td>Pseudo–Chédiak–Higashi granules</td>
</tr>
<tr>
<td></td>
<td>Döhle bodies</td>
</tr>
<tr>
<td></td>
<td>Auer rods</td>
</tr>
<tr>
<td>Dysmegakaryopoiesis</td>
<td>Micromegakaryocytes</td>
</tr>
<tr>
<td></td>
<td>Nuclear hypoblobalisation</td>
</tr>
<tr>
<td></td>
<td>Multinucleation (normal megakaryocytes are</td>
</tr>
<tr>
<td></td>
<td>uninuclear with lobated nuclei)</td>
</tr>
</tbody>
</table>

---

**Fig. 6.08** Myelodysplastic syndrome with multilineage dysplasia and complex cytogenetic abnormalities, including del(17p) and del(5q). Dyserythropoiesis is evident on this bone marrow smear from an adult male patient.

**Fig. 6.09** Dysplastic megakaryocytes. Bone marrow aspirate smear from a 37-year-old man with pan-cytopenia, showing hypolobated megakaryocytes and micromegakaryocytes.
in some cases of aplastic anaemia) are helpful in this distinction [342,945,947]. MDS-associated somatic mutations have been reported to occur in as many as one third of patients with aplastic anaemia [4440]. Immunosuppressive therapies used to treat aplastic anaemia have been used with some degree of success in this MDS subgroup [439,2334,3698,4447,4448]. When considering the diagnosis of hypoplastic MDS, it is important to exclude acute marrow injury due to a toxin, infection or an autoimmune disorder.

**Myelodysplastic syndrome with fibrosis**

Significant degrees of myelofibrosis (i.e., corresponding to grade 2 or 3 of the WHO grading scheme) [3975] are observed in 10–15% of MDS cases, and these cases have been referred to as MDS with fibrosis (MDS-F) [2201]. Significant fibrosis does not define a specific MDS subtype in this classification. However, many of the cases with fibrosis have an excess of blasts, and significant fibrosis is associated with an aggressive clinical course in MDS, independent of the blast count [942,1261]. MDS-F cases with excess blasts may erroneously be diagnosed as low-grade MDS based only on the blast count determined from the bone marrow aspirate, which is usually diluted with peripheral blood. In this fibrotic group, as in other cases of MDS with inadequate aspirates, accurate blast determination requires a bone marrow biopsy, and immunohistochemical studies for CD34 may prove invaluable. Unlike the myeloproliferative neoplasm entity primary myelofibrosis, MDS-F is usually not associated with spleenomegaly, leukoerythroblastosis or intrasinusoidal haematopoiesis and typically exhibits MDS-type megakaryocyte morphology (i.e., micromegakaryocytes), other dysplastic changes and often increased blasts as revealed by CD34 immunostaining [947].

**Immunophenotype**

The immunophenotypic abnormalities that have been described in MDS haematopoietic cells compared with normal haematopoiesis are abnormal quantity and aberrant phenotypes of progenitor cells; aberrant immunophenotypic profiles of maturing granulocytic, erythroid and monocytic cells; and a decrease of haematogones [63,1856,2555,2935,3895]. Abnormal myeloid maturation patterns include asynchrony of CD15 and CD16 on granulocytes; altered expression of CD13 in relation to CD11b or CD16; and aberrant expression of CD56 and/or CD7 on progenitors, granulocytes or monocytes. Decreased side-scatter of granulocytes can also be seen. In erythroid cells, an increased coefficient of variation and decreased intensity of CD71 or CD117 may be noted, immunohistochemistry of the bone marrow biopsy section and flow cytometry (CD34+ cells) [1994]. However, in some cases there may be significant discordance due to marrow fibrosis or haemodiluted samples; therefore, percentages of CD34+ cells as determined by flow cytometry cannot replace the morphological differential count. Nevertheless, the finding of CD34+ myeloid progenitors accounting for >2% of nucleated cells has been reported to be of adverse prognostic significance in MDS [2555A,2556]. Flow cytometry findings alone are not sufficient to establish a primary diagnosis of MDS in the absence of definitive morphological and/or cytogenetic findings. A series of consensus guidelines has been published by the European LeukemiaNet (ELN) MDS working group regarding the use of flow cytometry in the diagnostic work-up of patients with MDS [3223,4117,4118,4305], including a summary of the reported aberrations associated with MDS and how to report the results [2463,3223,4119]. Aberrant findings in at least three tested features and at least two cell compartments have been reported to be highly associated with an MDS or MDS/MPN diagnosis in several studies [3221,3223,4063,4119]. More limited screening panels have also been applied [4,259,945,1856,2933,3287], but may be less sensitive and less specific than larger panels.

**Cell of origin**

The postulated cell of origin is a haematopoietic stem cell.

**Genetic profile**

Cytogenetic studies play a major role in the evaluation of patients with MDS in regard to prognosis, determination of clonality [1442,3551,2971] and recognition of cytogenetic correlates with morphological and clinical features. MDS with isolated del(5q), i.e. either with a del(5q) alone or with one additional abnormality other than loss of chromosome 7 or del(7q), is a specific MDS subtype in this classification. It occurs more often in women and is characterized by megakaryocytes with nonlobated or hypolobated nuclei, macrocytic anaemia, normal or increased platelet count, and a favourable clinical course. Loss of 17p is associated with MDS or AML with pseudo–Pelger–Huët anomaly, small vacuolated neutrophils, TP53 mutation and an unfavourable clinical course; it is most common in therapy-related MDS [2187]. Complex karyotypes (≥3 abnormalities) typically include abnormalities of chromosomes 5 and/or 7, such as del(5q), loss of 5q, loss of chromosome 7 and del(7q); these are generally associated with an unfavourable clinical course. Several other cytogenetic findings appear to be associated with characteristic morphological abnormalities; for example, isolated del(20q) is associated with dysmegakaryopoiesis and thrombocytopenia, and inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2) is associated with abnormal megakaryocytes and may be associated with thrombocytosis. [446,1500,2142,3392].
Certain clonal cytogenetic abnormalities that often occur in MDS, i.e. loss of Y chromosome, gain of chromosome 8, and del(20q), have also been described in non-neoplastic conditions; when these occur as a sole abnormality in the absence of defining morphological criteria, they are not considered definitive evidence of MDS. In cases with refractory, unexplained cytopenia but no morphological evidence of dysplasia or increased blasts, the other cytogenetic abnormalities listed in Table 6.03 are considered presumptive evidence of MDS, and such cases are included in the category of MDS, unclassifiable. It is recommended that these patients be followed carefully for emerging morphological evidence of a more specific MDS subtype. The presence of MDS-type cytogenetic abnormalities may be used to support a diagnosis of MDS-EB in rare cases associated with excess blasts without clear-cut evidence of dysplasia.

In addition to recurrent cytogenetic abnormalities identified by conventional karyotyping, which are present in about 50% of MDS cases, recurrent somatic mutations in more than 50 genes have been identified in 80–90% of MDS cases. The genes found to be mutated in at least 5% of MDS cases are listed in Table 6.04. The most commonly mutated genes in MDS encode proteins that control RNA splicing (SF3B1, SRSF2, U2AF1 and ZRSR2) in aggregate mutated in >50% of cases) or epigenetic regulation of gene expression via DNA methylation (TET2, DNMT3A, IDH1 and IDH2) or histone modification (ASXL1 and EZH2). Other commonly mutated genes are those encoding transcription factors (RUNX1, NRAS, BCOR), signaling proteins (CBL), the tumour suppressor p53 (TP53), and the cohesin complex (STAG2), which controls the cohesion of sister chromatids [1513,3050]. As with cytogenetic abnormalities, specific mutations have been associated with specific morphological features in MDS. For example, SF3B1 mutation is associated with ring sideroblasts and mutations in ASXL1, RUNX1, TP53 and SRSF2 are associated with severe granulocytic dysplasia [947].

The mutational landscape of MDS is complex and dynamic. Multiple mutations can be present (most often in a spliceosome gene plus an epigenetic regulator); distinct mutation profiles can be present in two or more subclones; and the relative proportions of these subclones can shift over the course of treatment and disease progression [4232]. Acquired clonal mutations identical to those seen in MDS (affecting genes such as ASXL1, TP53, JAK2, SF3B1, TET2 and DNMT3A) can also occur in the haematopoietic cells of apparently healthy older individuals without MDS [1326,1830,3772]. Therefore, MDS-associated somatic mutations alone are not considered diagnostic of MDS in this classification, even in patients with unexplained cytopenia. Rare cases of familial MDS are associated with germline mutations, which can be investigated by sequencing non-MDS tissue (e.g. normal lymphocytes). These cases and their associated mutations are discussed separately (see Myeloid neoplasms with germline predisposition, p. 121). In the current classification, SF3B1 mutation is the only genetic abnormality that influences MDS subtype assignment, as part of the diagnostic criteria for MDS with ring sideroblasts.

### Table 6.03 Recurrent chromosomal abnormalities and their frequencies in myelodysplastic syndrome (MDS) at diagnosis

<table>
<thead>
<tr>
<th>Chromosomal abnormality</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MDS overall</td>
</tr>
<tr>
<td><strong>Unbalanced</strong></td>
<td></td>
</tr>
<tr>
<td>Gain of chromosome 8</td>
<td>10%</td>
</tr>
<tr>
<td>Loss of chromosome 7 or del(7q)</td>
<td>10%</td>
</tr>
<tr>
<td>del(5q)</td>
<td>10%</td>
</tr>
<tr>
<td>del(20q)</td>
<td>5–8%</td>
</tr>
<tr>
<td>Loss of Y chromosome</td>
<td>5%</td>
</tr>
<tr>
<td>Isochromosome 17q or t(17p)</td>
<td>3–5%</td>
</tr>
<tr>
<td>Loss of chromosome 13 or del(13q)</td>
<td>3%</td>
</tr>
<tr>
<td>del(11q)</td>
<td>3%</td>
</tr>
<tr>
<td>del(12p) or t(12p)</td>
<td>3%</td>
</tr>
<tr>
<td>del(9q)</td>
<td>1–2%</td>
</tr>
<tr>
<td>idic(X)(q13)</td>
<td>1–2%</td>
</tr>
<tr>
<td><strong>Balanced</strong></td>
<td></td>
</tr>
<tr>
<td>t(11;16)(p23.3;p13.3)</td>
<td>3%</td>
</tr>
<tr>
<td>t(3;21)(q26.2;q22.1)</td>
<td>2%</td>
</tr>
<tr>
<td>t(1;3)(p36.3;q21.2)</td>
<td>1%</td>
</tr>
<tr>
<td>t(2;11)(p21;q23.3)</td>
<td>1%</td>
</tr>
<tr>
<td>inv(3)(q21.3;q26.2)/t(3;3)(q21.3;q26.2)</td>
<td>1%</td>
</tr>
<tr>
<td>t(6;9)(p23;q34.1)</td>
<td>1%</td>
</tr>
</tbody>
</table>

| As a sole cytogenetic abnormality in the absence of morphological criteria, gain of chromosome 8, del(20q) and loss of Y chromosome are not considered definitive evidence of MDS; in the setting of persistent cytopenia of undetermined origin, the other abnormalities shown in this table are considered presumptive evidence of MDS, even in the absence of definitive morphological features. |

**Prognosis and predictive factors**

The subtypes of MDS included in this classification can be generally categorized into three risk groups on the basis of survival time and incidence of evolution to AML. The low-risk group contains MDS with single lineage dysplasia, MDS with ring sideroblasts and single lineage dysplasia, and MDS with isolated del(5q). The intermediate-risk group contains MDS with multilineage dysplasia and MDS with ring sideroblasts and multilineage dysplasia. The high-risk group consists of MDS with excess blasts that the category of MDS, unclassifiable, encompasses cases with heterogeneous clinical behaviour. Patients with bicytopenia despite single lineage dysplasia have been reported to have shorter survival times than patients with one cytopenia; conversely, patients with one cytopenia and multilineage dysplasia have longer survival times than patients with bicytopenia [4179].

The importance of cytogenetic features as prognostic indicators in MDS was codified by the International MDS Risk Analysis Workshop in 1997 [1442], and this cytogenetic risk categorization was updated in 2012 [3551]. The current Comprehensive Cytogenetic Scoring System (CCSS) for MDS contains five prognostic subgroups (Table 6.05). The original IPSS risk stratification scheme for MDS [1442] was also updated in 2012. The Revised IPSS (IPSS-R) [1444] incorporates the percentage of bone marrow blasts, CCSS cytogenetic risk group, and degree of cytopenia in each lineage to predict survival and risk of evolution to AML (Table 6.06). The blast percentage thresholds used in the IPSS-R differ from those in the current WHO classification, and include a 0–2% blast category that is not included in this classification; therefore, it is important to note the actual
Table 6.04 Common gene mutations in myelodysplastic syndromes (i.e. found in at least 5% of cases) 
{311,312,1513,3050,3988,4478}

<table>
<thead>
<tr>
<th>Gene mutated</th>
<th>Pathway</th>
<th>Frequency</th>
<th>Prognostic impact</th>
</tr>
</thead>
<tbody>
<tr>
<td>SF3B1^</td>
<td>RNA splicing</td>
<td>20-30%</td>
<td>Favourable</td>
</tr>
<tr>
<td>TET2^</td>
<td>DNA methylation</td>
<td>20-30%</td>
<td>See footnote b</td>
</tr>
<tr>
<td>ASXL1^</td>
<td>Histone modification</td>
<td>15-20%</td>
<td>Adverse</td>
</tr>
<tr>
<td>SRSF2^</td>
<td>RNA splicing</td>
<td>~15%</td>
<td>Adverse</td>
</tr>
<tr>
<td>DNMT3A^</td>
<td>DNA methylation</td>
<td>~10%</td>
<td>Adverse</td>
</tr>
<tr>
<td>RUNX1</td>
<td>Transcription factor</td>
<td>~10%</td>
<td>Adverse</td>
</tr>
<tr>
<td>U2AF1^</td>
<td>RNA splicing</td>
<td>5-10%</td>
<td>Adverse</td>
</tr>
<tr>
<td>TP53^</td>
<td>Tumour suppressor</td>
<td>5-10%</td>
<td>Adverse</td>
</tr>
<tr>
<td>EZH2</td>
<td>Histone modification</td>
<td>5-10%</td>
<td>Adverse</td>
</tr>
<tr>
<td>ZRSR2</td>
<td>RNA splicing</td>
<td>5-10%</td>
<td>See footnote b</td>
</tr>
<tr>
<td>STAG2</td>
<td>Cohesin complex</td>
<td>5-7%</td>
<td>Adverse</td>
</tr>
<tr>
<td>IDH1/IDH2</td>
<td>DNA methylation</td>
<td>~5%</td>
<td>See footnote b</td>
</tr>
<tr>
<td>CBL^</td>
<td>Signalling</td>
<td>~5%</td>
<td>Adverse</td>
</tr>
<tr>
<td>NRAS</td>
<td>Transcription factor</td>
<td>~5%</td>
<td>Adverse</td>
</tr>
<tr>
<td>BCOR</td>
<td>Transcription factor</td>
<td>~5%</td>
<td>Adverse</td>
</tr>
</tbody>
</table>

^ These genes are also reported to be mutated in clonal haematopoietic cells in a subset of healthy individuals (clonal haematopoiesis of indeterminate potential).

^ Either neutral prognostic impact or conflicting data.

Table 6.05 The Comprehensive Cytogenetic Scoring System (CCSS) for myelodysplastic syndromes. 
From: Schanz J, et al. (3551)

<table>
<thead>
<tr>
<th>Prognostic subgroup</th>
<th>Defining cytogenetic abnormalities</th>
</tr>
</thead>
<tbody>
<tr>
<td>Very good</td>
<td>Loss of Y chromosome del(11q)</td>
</tr>
<tr>
<td>Good</td>
<td>Normal del(5q), del(12p), del(20q)</td>
</tr>
<tr>
<td>Intermediate</td>
<td>del(7q), Gain of chromosome 8, Gain of chromosome 19, Isochromosome 17q, Single or double abnormalities not specified in other subgroups, Two or more independent non-complex clones</td>
</tr>
<tr>
<td>Poor</td>
<td>Loss of chromosome 7, inv(3), t(3q) or del(3q), Double including loss of chromosome 7 or del(7q), Complex (3 abnormalities)</td>
</tr>
<tr>
<td>Very poor</td>
<td>Complex (&gt; 3 abnormalities)</td>
</tr>
</tbody>
</table>

Bone marrow blast percentage in all MDS diagnoses, so that the IPSS-R can be applied. Five IPSS-R risk groups are defined, on the basis of the total score of the parameters listed in Table 6.06: very low, low, intermediate, high and very high. The IPSS-R is significantly better at predicting survival and evolution to AML than the original IPSS (4219). Consideration of patient age further improves survival prediction in the IPSS-R (1444). Another risk-stratification scheme used to predict outcome in MDS is the WHO Classification-based Prognostic Scoring System (WPSS), which incorporates additional variables of transfusion requirement and morphological dysplasia (single lineage vs multilineage) that are not included in the IPSS-R. The WPSS may be particularly useful when applied to lower-risk cases and at time points after the initial diagnosis (2462). Accumulating data indicate that both number and type of individual gene mutations are strongly associated with disease outcome in MDS. The addition of mutation data improves the ability of existing risk-stratification schemes such as the IPSS to predict prognosis in MDS (311,312). Many commonly mutated...
genes have been associated with an unfavourable prognosis in MDS; whereas mutation in SF3B1 is associated with a more favourable prognosis (Table 6.04, p. 105). Certain mutations may also be associated with responses to specific therapies. For example, TET2 and DNMT3A mutations appeared to affect the therapeutic response of patients with MDS to hypomethylating agents in one study [4041], and TP53 mutation in MDS with del(5q) may predict a poorer response to lenalidomide (2474), TP53 mutation in MDS with del(5q) is associated with very aggressive disease, and predicts shorter survival in patients undergoing stem cell transplantation (769,313). Sensitive sequencing techniques should optimally be applied in MDS mutation analysis for prognosis, because even small subclones present at the initial diagnosis may show mutations in relevant genes, such as TP53, and can later expand to confer therapeutic resistance (1894,4232).

### Table 6.06 The Revised International Prognostic Scoring System (IPSS-R) score values for myelodysplastic syndromes. From: Greenberg PL, et al. (1444)

<table>
<thead>
<tr>
<th>Prognostic variable</th>
<th>Score values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Karyotype (CCSS group)</td>
<td>Very good</td>
</tr>
<tr>
<td>Bone marrow blast percentage</td>
<td>≤2%</td>
</tr>
<tr>
<td>Haemoglobin concentration (g/dL)</td>
<td>≥10</td>
</tr>
<tr>
<td>Platelets (x 10^9/L)</td>
<td>≥100</td>
</tr>
<tr>
<td>Absolute neutrophil count (x 10^9/L)</td>
<td>≥0.8</td>
</tr>
</tbody>
</table>

Five risk groups are defined, on the basis of the total score of the parameters listed above:

- Very low: ≤1.5
- Low: >1.5 to 3
- Intermediate: >3 to 4.5
- High: >4.5 to 6
- Very high: >6

— Indicates not applicable

The Comprehensive Cytogenetic Scoring System (CCSS) group definitions are listed in Table 6.05, p. 105.

---

**Myelodysplastic syndromes**

### Myelodysplastic syndrome with single lineage dysplasia

Brunning R.D., Hasserjian R.P., Porwit A., Bennett J.M., Orazi A.

**Definition**

The category of myelodysplastic syndrome (MDS) with single lineage dysplasia (MDS-SLD) encompasses the MDS cases that present with unexplained cytopenia or bicytopenia, with ≥10% dysplastic cells in one myeloid lineage. Most patients present with persistent unexplained anaemia or bicytopenia; some present with persistent unexplained neutropenia or thrombocytopenia [2423]. In the 2008 edition of this classification, MDS-SLD was called refractory cytopenia with unilineage dysplasia, and was divided into three subtypes: refractory anaemia, refractory neutropenia and refractory thrombocytopenia. This subclassification has been controversial; some studies have demonstrated no clear correlation between lineage cytopenia and lineage dysplasia and no significant differences in survival between the three subtypes [1503,2423,2568,4179]. However, other studies have found some survival differences [449,2511]. Given these conflicting findings and the inconsistencies between lineage cytopenia and lineage dysplasia, we recommend that cases of MDS presenting with single lineage cytopenia or bicytopenia and unilineage dysplasia be classified as MDS-SLD, without additional subclassification. The presenting lineage dysplasia and cytopenia(s) should be noted in the diagnostic conclusion. The defining feature of this type of MDS is ≥10% dysplastic cells in one myeloid lineage. Cases with erythroid dysplasia only and ≥15% ring sideroblasts (or ≥5% ring sideroblasts in the presence of SF3B1 mutation) are classified as MDS with ring sideroblasts and single lineage dysplasia (MDS-RS-SLD). If SF3B1 mutation status is unknown, it is recommended that cases with 5-14% ring sideroblasts and single lineage dysplasia be classified as MDS-SLD. As in the 2008 classification, it is recommended that cases with single lineage dysplasia and pancytopenia be categorized as MDS, unclassifiable.

As noted in the Overview section (p. 98), the recommended thresholds for defining cytopenias are haemoglobin concentration <10 g/dL, absolute neutrophil count <1.8 x 10^9/L and platelet count <100 x 10^9/L, as per the risk...
stratification guidelines of the original International Prognostic Scoring System (IPSS) [1442,1444]. Ethnicity and sex should be taken into consideration when assessing cytopenias. Presentation with milder cytopenias above the IPSS levels does not exclude a diagnosis of MDS if definitive morphological and/or cytogenetic evidence of MDS is present. The type of cytopenia may correspond to the type of lineage dysplasia (e.g. anaemia and erythroid dysplasia), or there may be discordance between the cytopenia and the dysplastic lineage. If there is no clonal cytogenetic abnormality, it is recommended that the patient be observed for ≥6 months before a definitive diagnosis of MDS-SLD is established, unless more definitive morphological and/or cytogenetic evidence emerges during the observation period.

All potential non-clonal causes of the dysplasia must be excluded before the diagnosis of MDS is established [1503], including drug and toxin exposure, growth factor therapy, viral infections, immunological disorders, congenital disorders, vitamin deficiencies and essential element deficiencies (e.g. copper deficiency) [1449]. Excessive zinc supplementation has also been reported to be associated with severe cytopenia and dysplastic changes [1778].

The presence of blasts in the peripheral blood essentially excludes a diagnosis of MDS-SLD, although in an occasional case a rare blast may be identified; cases with the characteristics of MDS-SLD and 1% blasts in the peripheral blood on two successive evaluations and <5% blasts in the bone marrow should be categorized as MDS, unclassifiable, due to the more aggressive clinical course reported for such cases [2423]. The number of these patients is very low; they should be carefully monitored for increasing bone marrow blast percentage and, if appropriate, reclassified. Cases in which there are 2–4% blasts in the peripheral blood and <5% blasts in the bone marrow should be classified as MDS with excess blasts 1 if other criteria for MDS are present.

MDS-SLD should not be equated with idiopathic cytopenia of undetermined significance, which lacks the minimal morphological criteria requisite for a diagnosis of MDS [4336]. Given the evidence of MDS-associated mutations in healthy older individuals, referred to as clonal haematopoiesis of indeterminate potential, the presence of mutations alone, even in a patient with cytopenia, is not sufficient for a diagnosis of MDS-SLD in the absence of ≥10% dysplastic cells in one myeloid lineage [3772].

**Differential diagnosis**

The major features of MDS-SLD are cytopenia and ≥10% dysplastic cells in one myeloid cell line. As emphasized in the Overview section (p. 98), it is essential to exclude all possible non-clonal etiologies for the abnormalities. In the age group in which MDS-SLD most commonly occurs, this can be particularly problematic because of the frequency of anaemia due to a number of comorbid conditions, including nutritional deficiencies, impaired renal function, inflammatory responses and non-haematopoietic neoplasms.

In patients presenting with anaemia, the evaluation should include a detailed clinical history and laboratory studies, with particular emphasis on possible vitamin B12 or folic acid deficiency. Less common causes (e.g. paroxysmal nocturnal haemoglobinuria) must also be excluded. The possibility of toxic exposure should always be considered. Arsenic poisoning, either accidental or intentional, may cause cytopenias with substantial dysplastic features.

Persistent unexplained neutropenia and thrombocytopenia as specific entities are much less common. Detailed evaluation should exclude the possibility of familial occurrence and comorbid conditions such as immune disorders, T-cell large granular lymphocytic leukaemia, viral infections and medications [1503]. Cytogenetic studies will facilitate the diagnosis in some cases with insufficient or borderline dysplasia.

**ICD-O code**

9980/3

**Synonyms**

Refractory neutropenia (no longer recommended); refractory cytopenia with unilineage dysplasia; refractory anaemia (no longer recommended); refractory thrombocytopenia (no longer recommended)

**Epidemiology**

MDS-SLD accounts for 7–20% of all cases of MDS [947,1338,1341,2467]. It is primarily a disease of older adults; the median patient age at onset is 65–70 years. There is no significant sex predilection. The vast majority of cases present with refractory anaemia or bicytopenia. Presentations with persistent unexplained isolated neutropenia or thrombocytopenia are uncommon and extreme caution.
should be used in making a diagnosis of MDS-SLD in these settings [1503,2423]. The frequency of MDS-SLD appears to be higher in the Japanese population than in Germans [2566].

Localization
The peripheral blood and bone marrow are the principal sites of involvement.

Clinical features
The presenting symptoms are generally related to the type of cytopenia. The cytopenias are unresponsive to haematologic therapy but may respond to growth factors [1707].

Microscopy
In the peripheral blood, the red blood cells are usually normochromic and normocytic or normochromic and macrocytic. Unusually, there is anisocytosis or dimorphism with populations of both normochromic and hypochromic red blood cells; this finding is more common in MDS with ring sideroblasts. Anisocytosis and poikilocytosis range from absent to marked.

As noted, circulating blasts are rarely seen, and if present, account for <1% of the peripheral blood leukocytes. The presence of any blasts should call into question the diagnosis of MDS-SLD; as previously stated, the presence of 1% blasts on two successive examinations warrants categorization of the case as MDS, unclassifiable.

In cases of MDS-SLD with dyserythropoiesis, the erythroid precursors in the bone marrow can vary from markedly decreased to markedly increased. Dyserythropoiesis varies in degree, but is always present in >10% of the nucleated erythroid precursors. There may be a shift to more immature cells. The primary manifestation of dyserythropoiesis is in the nucleus. There may be nuclear budding, internuclear chromatin bridging, multinuclearity, megaloblastoid changes or karyorrhexis. Megaloblastoid nuclear changes are best evaluated in the polychromatic and orthochromatic stages of development because of the normally fine chromatin pattern in the nuclei of proerythroblasts and basophilic erythroblasts. Dysplastic cytoplasmic features include impaired haemoglobinization, vacuolization, and periodic acid–Schiff (PAS) positivity (either diffuse or granular). Ring sideroblasts may be present, but account for <15% of the erythroid precursors. If the proportion of ring sideroblasts is >5% but <15% and there is SF3B1 mutation, the case should be classified as MDS-RS-SLD. If the SF3B1 mutation status is unknown, the case should be classified as MDS-SLD.

Neutrophil dysplasia can manifest as small size, dense chromatin, variable degrees of nuclear hyposegmentation, pseudo–Pelger–Huet changes, and abnormalities of granulation (either agranularity or hypogranularity). Less commonly, large granules resembling those found in Chédiak-Higashi syndrome may be seen. Nuclear hypersegmentation may also be noted.

The principal manifestations of megakaryocytic dysplasia include hypolobation or bilobed nuclei, multiple separated nuclei, and micromegakaryocytes; micromegakaryocytes are considered to be the most reliable and reproducible evidence of megakaryocytic dysplasia [1338,2568,4179]. Megakaryocytic dysplasia may be more apparent in bone marrow sections than smears, and its conspicuousness is increased by immunohistochemical reactions and PAS stain. The assessment of megakaryocytic dysplasia should be based on examination of >30 megakaryocytes. A threshold of >10% dysplastic megakaryocytes is recommended for the diagnosis of megakaryocytic dysplasia. However, some experts have found thresholds of 30–40% dysplastic megakaryocytes to be more reliable for distinguishing normal from dysplastic bone marrow [1338,2568,947]. The bone marrow is typically normocellular or hypercellular; hypocellularity is observed in a subset of cases [1338,2566].

Immunophenotype
The immunophenotyping principles described in the Overview section (p. 98) should be followed. In MDS-SLD with erythroid dysplasia, aberrant immunophenotypic features of erythropoietic precursors can be found by flow cytometry analysis [944,1994,3223]. In a fraction of these cases, flow cytometry also detects aberrant immunophenotypic features in the myelomonocytic compartment or in precursors [259,946,1994,3223]. These patients should be reassessed and closely observed for development of multilineage dysplasia [3223].

Cell of origin
A haematopoietic stem cell

Genetic profile
Cytogenetic abnormalities may be observed in as many as 50% of cases [1341,2423]. Several acquired clonal chromosomal abnormalities may be observed, but although useful for establishing a diagnosis of MDS, they are not specific. The abnormalities generally associated with MDS-SLD include del(20q), gain of chromosome 8, and abnormalities of chromosomes 5 and 7; del(20q) has been reported in patients with MDS-SLD (as well as MDS-MLD) presenting with thrombocytopenia [446,1500,3526]. This finding may be useful in distinguishing MDS from immune-mediated thrombocytopenia.

Somatic driver mutations have been identified in 60–70% of cases of MDS-SLD. The underlying mutations affect a haematopoietic stem cell and are present in all lineages despite the limitation of dysplastic findings to one lineage [4354]. TET2 and ASXL1 appear to be the most commonly mutated genes in MDS-SLD [1513]. However, mutations in other DNA methyltransfer enzymes, splice factors, RAS pathway genes, cohesion complex genes and RUNX1 are less common than in MDS-MLD and MDS with excess blasts [2465]. SF3B1 mutation is rare. Cases with features of MDS-SLD, an SF3B1 mutation, and <5% ring sideroblasts should be diagnosed as MDS-SLD, whereas cases with features of MDS-SLD, an SF3B1 mutation, and >5% ring sideroblasts should be
diagnosed as MDS-RS-SLD. In the absence of diagnostic dysplasia or a defining cytogenetic abnormality, somatic mutations or copy number abnormalities are not sufficient to establish a diagnosis of MDS-SLD in a patient with cytopenia, and are considered to be within the spectrum of clonal haematopoesis of indeterminate potential [3772].

Prognosis and predictive factors
The clinical course is usually protracted. The median overall survival of patients with MDS-SLD is approximately 66 months, and the rate of progression to acute myeloid leukaemia at 5 years is 10% [1341,2423,2467]. In one study, the median survival of patients aged ≥70 years with MDS-SLD was not significantly different from that of the non-affected population [2467]. Approximately 90–95% of patients with MDS-SLD have a low or intermediate 1 IPSS risk score [1442,2423]. A similar percentage of patients have a very low or low WHO Classification–based Prognostic Scoring System (WPSS) risk score [948,2423,2467]. Approximately 85% of patients have good or very good cytogenetic profiles. Most patients with MDS-SLD presenting with thrombocytopenia have low IPSS risk scores, and 90% of the patients live more than 2 years [3526]. However, one study reported shorter survival for patients presenting with thrombocytopenia as a result of haemorrhagic complications [450].

Myelodysplastic syndrome with ring sideroblasts
Hasserjian R.P.
Gattermann N.
Bennett J.M.
Brunning R.D.
Malcovati L.
Thiele J.

Definition
Myelodysplastic syndrome (MDS) with ring sideroblasts (MDS-RS) is an MDS characterized by cytopenias, morphological dysplasia and ring sideroblasts usually constituting ≥15% of the bone marrow erythroid precursors; secondary causes of ring sideroblasts must be excluded. There is associated SF3B1 mutation in most cases, and in the presence of such mutation, the diagnosis can be made with ≥5% marrow ring sideroblasts. Myeloblasts account for <5% of the nucleated bone marrow cells and <1% of peripheral blood leukocytes. Auer rods are absent, and the diagnostic criteria for MDS with isolated del(5q) are not fulfilled. Two categories of MDS-RS are recognized. In MDS with ring sideroblasts and single lineage dysplasia (MDS-RS-SLD), patients present with anaemia, and dysplasia is limited to the erythroid lineage. In MDS with ring sideroblasts and multilineage dysplasia (MDS-RS-MLD), patients present with any number of cytopenias, and significant dysplasia is present in two or three haematopoietic lineages.

ICD-O codes
Myelodysplastic syndrome with ring sideroblasts and single lineage dysplasia 9982/3
Myelodysplastic syndrome with ring sideroblasts and multilineage dysplasia 9993/3

Synonyms
Refractory anaemia with ring sideroblasts; refractory cytopenia with multilineage dysplasia and ring sideroblasts

Epidemiology
MDS-RS-SLD accounts for 3–11% of all MDS cases. It occurs primarily in older individuals, with a median patient age of 60–73 years, and has a similar frequency in males and females [448,1341,2467]. MDS-RS-MLD appears to be more common, accounting for about 13% of MDS cases, and has an age distribution similar to that of MDS-RS-SLD [1341].

Etiology
Ring sideroblasts are erythroid precursors with abnormal accumulation of iron within mitochondria, including some iron deposited as mitochondrial ferritin [591,1425]. Stem cells from patients with MDS-RS display poor erythroid colony formation in vitro and manifest abnormal iron deposition at a very early stage of erythroid development [761,3936], as well as deregulated expression of genes encoding mitochondrial and iron metabolism [930,1344]. MDS-RS is closely associated with heterozygous mutations in SF3B1, which encodes a core component of the U2 snRNP spliceosome that is critical for RNA splicing; less often, other genes that control RNA splicing are mutated in MDS-RS [3049,4433]. Recent data suggest that haploinsufficiency of SF3B1 is associated with a globally altered and distinct gene expression profile [1344]. The effects include altered splicing of the mitochondrial iron transporter gene ABCB7 and other mitochondrial metabolism genes, which may lead to the ineffective erythropoiesis and ring sideroblasts that characterize MDS-RS [1019,2881,4201]. However, data have been conflicting as to the association of SF3B1 haploinsufficiency with an MDS-RS disease phenotype in mouse models [4201,4203,4239].

Localization
The peripheral blood and bone marrow are the principal sites of involvement. The liver and spleen may show evidence of iron overload.

Clinical features
The presenting symptoms are usually related to anaemia; a minority of patients with MDS-RS-SLD may additionally have thrombocytopenia or neutropenia, whereas bicytopenia occurs in a higher proportion of patients with MDS-RS-MLD [1341]. There may be symptoms related to progressive iron overload. Most patients with MDS-RS-SLD (64% and 34%, respectively) fall into the low or very low Revised International Prognostic Scoring System (IPSS-R) risk groups, whereas patients with MDS-RS-MLD more frequently have a higher IPSS-R risk score [3104].

Microscopy
Patients typically present with normochromic macrocytic or normochromic normocytic anaemia. The red blood cells in the peripheral blood smear may show a dimorphic pattern, with a major population of normochromic red blood cells and a minor population of hypochromic cells. Blasts in the peripheral blood are absent or very rare (accounting for <1% of the leukocytes). In MDS-RS-SLD, the bone marrow aspirate smear shows an increase in erythroid precursors with erythroid lineage dysplasia, including nuclear segmentation and megaloblastoid features. Granulocytes and megakaryocytes show no significant dysplasia (<10% dysplastic forms). Hae-mosiderin-laden macrophages are often abundant. Myeloblasts constitute <5% of the nucleated bone marrow cells. On iron-
stained aspirate smears, ≥15% (or ≥5% if \textit{SF3B1} mutation has been documented) of the red blood cell precursors are ring sideroblasts, as defined by ≥5 iron granules encircling one third or more of the nucleus (2778). The bone marrow biopsy specimen is normocellular to markedly hypercellular, usually with marked erythroid proliferation. Megakaryocytes are normal in number and morphology. In MDS-RS-MLD, in addition to ring sideroblasts and erythroid lineage dysplasia, there is significant dysplasia (≥10% dysplastic forms) in one or two non-erythroid lineages. Aside from the presence of ring sideroblasts, the morphological features of MDS-RS-MLD are generally similar to those of MDS with multilineage dysplasia.

Ring sideroblasts are often observed in other types of MDS and can also be seen in other myeloid neoplasms, including acute myeloid leukaemia (1333, 1891). For example, cases of MDS with ring sideroblasts that have excess blasts in the peripheral blood or bone marrow are classified as MDS with excess blasts and, cases that fulfill the criteria for MDS with isolated del(5q) should be classified as such, even if ring sideroblasts and/or \textit{SF3B1} mutation are present. Non-neoplastic causes of ring sideroblasts, including alcohol, toxins (e.g. lead and benzene), drugs (e.g. isoniazid), copper deficiency (which may be induced by zinc administration), and congenital sideroblastic anaemia must be excluded (55). Unlike in MDS-RS, patients with congenital sideroblastic anaemia tend to present at a much younger age and with microcytic (rather than macrocytic) anaemia (2940).

**Immunophenotype**

In MDS-RS, aberrant immunophenotypic features of erythropoietic precursors may be found by flow cytometry analysis (944).
es progress to acute myeloid leukaemia (448,1335,1341). In MDS-RS, adverse prognostic features include poor-risk karyotype, the presence of multilineage dysplasia (MDS-RS-MLD), thrombocytopenia and absence of SF3B1 mutation; however, data are conflicting as to whether the influence of SF3B1 mutation on prognosis is independent of multilineage dysplasia (845,2464,3103). Among SF3B1-mutant MDS-RS cases, RUNX1 mutation appears to be associated with shorter survival [2464].

Myelodysplastic syndrome with multilineage dysplasia

Bennett J.M. Cazzola M.
Matutes E. Thiele J.
Orazi A. Hasserjian R.P.

Definition
Myelodysplastic syndrome (MDS) with multilineage dysplasia (MDS-MLD), called refractory cytopenia with multilineage dysplasia in the 2008 edition of this classification, is an MDS characterized by one or more cytopenias and dysplastic changes in two or more of the myeloid lineages (erythroid, granulocytic, and megakaryocytic) [3404].

ICD-0 code
9985/3

Synonym
Refractory cytopenia with multilineage dysplasia

The blast percentage is <1% in the peripheral blood and <5% in the bone marrow. Auer rods are absent, and the monocytic count in the peripheral blood is <1 x 109/L. The recommended values for defining cytopenias are those suggested in the International Prognostic Scoring System (IPSS); haemoglobin concentration <10 g/dL, platelet count <100 x 10^9/L and absolute neutrophil count <1.8 x 10^9/L [1442,1442A]. Ethnicity and sex should be taken into consideration when assessing cytopenias. Milder cytopenias in excess of these thresholds do not exclude a diagnosis of MDS if definitive morphological and/or cytogenetic findings are consistent with the diagnosis.

The thresholds for dysplasia are ≥10% in each of the affected cell lineages. For assessing dysplasia, it is recommended that 200 erythroid precursors and 200 neutrophils and precursors be evaluated in bone marrow smear and/or trephine biopsy imprint preparations. Neutrophil dysplasia may also be evaluated in peripheral blood smears. At least 30 megakaryocytes should be evaluated for dysplasia in bone marrow smears, imprint preparations or sections. In particular, the presence of micromegakaryocytes should be noted, because this is considered by most experts to be the most significant feature of megakaryocytic dysplasia. Some experts have found thresholds for megakaryocytic dysplasia of 30-40% to be more reliable for distinguishing normal marrow from dysplastic marrow [1338, 2567,4179]. In some cases, dysplastic megakaryocytes may be more readily identified in sections than on smears, in particular with immunohistochemistry using antibodies such as anti-CD61. The presence of 1% blasts in the peripheral blood excludes a diagnosis of MDS-MLD; cases that otherwise fulfill the criteria for MDS-MLD but have 1% blasts in the blood on two separate occasions should be diagnosed as MDS, unclassifiable, because of the more aggressive course associated with this finding. Cases with multilineage dysplasia, 2-4% blasts in the peripheral blood, and no Auer rods should be classified as MDS with excess blasts 1, even with <5% bone marrow blasts. Cases that otherwise have the features of MDS-MLD and have 5-19% blasts in the peripheral blood and/or Auer rods should be classified as MDS with excess blasts 2, even with <5% bone marrow blasts. Cases with multilineage dysplasia and ≥15% ring sideroblasts (or ≥5% ring sideroblasts and SF3B1 mutation) should be classified as MDS with ring sideroblasts and multilineage dysplasia (MDS-RS-MLD). Cases with multilineage dysplasia and ≥5% but <15% ring sideroblasts with unknown SF3B1 mutation status should be classified as MDS-MLD.

Epidemiology
MDS-MLD occurs in older individuals; the median patient age is 67-70 years. There is a higher incidence in males. The peak incidence in males is 70-74 years and in females is 75-79 years [1338, 1341,2423,2467]. MDS-MLD accounts for about 30% of all cases of MDS and 48% of cases of MDS without excess blasts. Combined, MDS-MLD and MDS-RS-MLD account for approximately 65% of all cases of MDS without excess blasts [2423].

Etiology
The etiology of de novo MDS-MLD is unknown. Exposure to toxic environmental factors probably increases risk. Given that the vast majority of cases occur in older individuals, haematopoietic stem cell mutations (which occur with ageing) may underlie the age-associated risk [1326,1830].

Fig. 6.18 Myelodysplastic syndrome with multilineage dysplasia and complex cytogenetic abnormalities. Bone marrow section from a 37-year-old man with pancytopenia showing markedly increased megakaryocytes, many with dysplastic features.
Localization
The blood and bone marrow are involved.

Clinical features
Patients usually present with evidence of bone marrow failure. In most patients, there is unicytopenia or bicytopenia. Some patients present with pancytopenia or milder cytopenias above the threshold levels in the IPSS [2423].

Microscopy
The erythrocytes frequently show anisopoikilocytosis with macrocytosis. The major dysplastic changes in neutrophils are nuclear clumping and hyposegmentation or lack of lobation (pseudo–Pelger–Huët anomaly) and cytoplasmic hypogranularity or agranularity. Infrequently, abnormal granular clumping resembling the findings in Chédiak–Higashi syndrome is present. Blasts are not usually identified in the peripheral blood; however, the presence of <1% peripheral blood blasts with <5% blasts in the bone marrow does not alter the classification, because the prognosis for cases with rare (<1%) circulating blasts is essentially the same as that for cases without this finding [2423].

The bone marrow is usually normocellular or hypercellular, but is hypocellular in a small subset of cases [2423]. The erythroid precursors are usually increased in number; uncommonly there is erythroid hypoplasia. Erythroid precursors may show cytoplasmic vacuoles and marked nuclear irregularity, including intranuclear chromatin bridging, multilobation, nuclear budding, multinucleation and megaloblastoid nuclei. The latter are better evaluated in the polychromatic and orthochromatic erythroblasts, due to the fine nuclear chromatin pattern normally present in proerythroblasts and early basophilic erythroblasts. The cytoplasmic vacuoles are usually poorly defined and dissimilar to the sharply demarcated vacuoles observed in copper deficiency or alcoholism. The vacuoles may give a positive periodic acid–Schiff (PAS) reaction; there may also be diffuse cytoplasmic PAS positivity. Variable numbers of ring sideroblasts or erythroid precursors may be identified, but they must account for <15% of the erythroblasts. If ≥15% ring sideroblasts or ≥5% ring sideroblasts and SF3B1 mutation are present, the case should be classified as MDS-RS-MLD. The percentage of neutrophils and precursors in the bone marrow varies; a relative increase in granulocytes is present in approximately 25% of cases [2423]. There may be a left shift in maturation. Neutrophil dysplasia is characterized principally by nuclear cytoplastic asynchrony, hypogranulation, and/or nuclear hyposegmentation with marked clumping of the nuclear chromatin (pseudo–Pelger–Huët nuclei). Nuclear hyposegmentation may occur as two clumped nuclear lobes connected by a thin chromatin strand (pince-nez type) or non-lobated nuclei with markedly clumped chromatin. Myeloblasts account for <5% of the bone marrow cells. Megakaryocyte abnormalities that may be observed include non-lobated or hypolobated nuclei, binucleation or multinucleation, and micromegakaryocytes. A micromegakaryocyte is a megakaryocyte that is approximately the size of a promyelocyte or smaller, with a non-lobated or bi-lobated nucleus; this morphological finding is considered by most experts to be the most reliable and reproducible dysplastic feature in the megakaryocyte series [1341,2567,4179]. In some cases, bone marrow sections may be more reliable than smears for evaluating megakaryocyte dysplasia, in particular with the addition of immunohistochemical reactions with appropriate antibodies such as CD61 and CD41. PAS staining may also be helpful. Several studies have documented the various morphological abnormalities that constitute evidence of dysplasia [947,1341,2567,4179].

In a study of marrow fibrosis in patients with MDS, 16% of cases classified as MDS-MLD had a significant degree of marrow fibrosis [491]. In general, marrow fibrosis in MDS correlated with multilineage dysplasia, more severe thrombocytopenia, higher probability of clonal karyotype, higher percentage of blasts in the peripheral blood and shorter survival than for patients without fibrosis [491].

Immunophenotype
Flow cytometry usually reveals immunophenotypic abnormalities in various cell populations, including aberrations in the immature progenitor compartment and abnormal maturation in granulopoiesis, the monocytic compartment and erythropoiesis [3221,3223,4119]. Immunophenotypic abnormalities in the progenitor compartment are reported to be of prognostic significance and can be used to guide therapy and for follow-up [64,4304]. CD34+ cells are typically <5%, but the blast percentage determination for classification purposes should be based on a microscopic differential of the aspirate smear, not on the flow cytometry blast percentage. Details concerning the overall use of flow cytometry analysis in MDS are given in the Overview section (p. 98).

Cell of origin
A haematopoietic stem cell

Genetic profile
Clonal cytogenetic abnormalities, including trisomy 8, monosomy 7, del(7q),...
monosomy 5, del(5q) and del(20q), as well as complex karyotypes, are found in as many as 50% of patients with MDS-MLD [2423]. Whole-genome sequencing has shown that more than half of all cases of MDS-MLD carry mutations in genes that are also mutated in MDS with excess blasts and acute myeloid leukaemia. These include genes from the cohesion family (STAG2), chromatin modifiers (ASXL1), spliceosome genes (SRSF2), transcription factors (RUNX1), signalling molecules (CB1), tumour suppressors (TP53) and DNA modifiers (TET2) [1513, 3050,4392]. Mutations in SF3B1 are present in a minority (1–5%) of patients with 0–1% ring sideroblasts and as high as 15% of cases with 3–9% ring sideroblasts [2464]; cases with ≥5% ring sideroblasts should be classified as MDS- RS-MLD if SF3B1 mutation is present.

**Prognosis and predictive factors**

The clinical course varies [948,2423, 2462,2467]. Prognostic factors relate to the karyotype and the degree of cytopenia and dysplasia. In a very large study, approximately 40% of patients with MDS-MLD or MDS-RS-MLD had low IPSS risk scores, and approximately 50% had an intermediate 1 risk score [2423]. Approximately 50% had a low WHO Classification–based Prognostic Scoring System (WPSS) risk score, 20% had an intermediate risk score, and 20% had a high risk score; no patients had a very low risk score. Approximately 75% were in the good karyotype risk group, 17% were in the poor risk group, and 8% were in the intermediate risk group [948, 2423,2462]. Although gene mutations may have a prognostic impact on overall survival and progression-free survival for MDS in general, there is no definitive evidence that mutations influence the prognosis within the MDS-MLD group specifically [4392].

In a database of 1010 patients with MDS-MLD, the frequency of acute leukaemia evolution was approximately 15% at 2 years and 28% at 5 years and the median overall survival was 36 months (data from the Düsseldorf MDS registry, September 2015). Patients with complex karyotypes have survivals similar to those of patients with MDS with excess blasts [2423].

**Myelodysplastic syndrome with excess blasts**

**Orazi A.**

**Bruning R.D.**

**Hasseriyan R.P.**

**German U.**

**Thiele J.**

**Definition**

Myelodysplastic syndrome (MDS) with excess blasts (MDS-EB) is an MDS characterized by 5–19% myeloblasts in the bone marrow or 2–19% blasts in the peripheral blood (but <20% blasts in both bone marrow and blood). Two subcategories, with differences in survival and incidence of evolution to acute myeloid leukaemia (AML), have been defined. MDS with excess blasts 1 (MDS-EB-1) is defined by 5–9% blasts in the bone marrow or 2–4% blasts in the peripheral blood (but <10% blasts in the bone marrow and <5% blasts in the blood), and MDS with excess blasts 2 (MDS-EB-2) is defined by 10–19% blasts in the bone marrow or 5–19% blasts in the peripheral blood [1442]. The presence of Auer rods in blasts designates any MDS case as MDS-EB-2 irrespective of the blast percentage [1340].

**ICD-O code** 9983/3

**Synonym** Refractory anaemia with excess blasts

**Epidemiology**

This disease affects primarily individuals aged >50 years. It accounts for approximately 40% of all cases of MDS.

**Etiology**

The etiology is unknown. Exposure to environmental toxins, including pesticides, petroleum derivatives, and some heavy metals, increases risk, as does cigarette smoking [3816].

**Localization**

The blood and bone marrow are affected.

**Clinical features**

Most patients initially present with clinical features related to cytopenias, including anaemia, thrombocytopenia, and neutropenia.

**Microscopy**

Peripheral blood smears frequently show abnormalities in all three myeloid cell lines, including anisopoikilocytosis and macrocytosis; large, giant or hypogranular platelets; and abnormal cytoplasmic granularity and nuclear segmentation of the neutrophils. Pseudo–Pelger–Huet nuclei and hypogranulated forms are usually detected. Blasts are commonly present. The bone marrow is usually hypercellular. The degree of dysplasia varies. Erythroid precursors may show dyserythropoiesis, including the presence of abnormally lobated nuclei and internuclear bridging. Granulopoiesis is variable in quantity and usually shows dysplasia, which is characterized primarily by neutrophils with nuclear hyposegmentation (pseudo–Pelger–Huet nucleus) or hypersegmented nuclei, cytoplasmic hypogranularity, and/or pseudo–Chédiak–Higashi granules. Megakaryopoiesis is variable in quantity but is frequently normal to increased. Dysmegakaryopoiesis is almost invariably present and is usually characterized by abnormal forms that are predominantly small, including micromegakaryocytes [3987]. However, megakaryocytes of all sizes, as well as forms with multiple widely separated nuclei, can also occur. The megakaryocytes may show a tendency to

---

**Fig. 6.20** Myelodysplastic syndrome with excess blasts 1. On the bone marrow aspirate smear, the mature neutrophils in this case show nuclear hyposegmentation (pseudo–Pelger–Huet nuclei) and cytoplasmic hypogranularity.
Myelodysplastic syndrome with excess blasts and fibrosis

In about 15% of cases of MDS, the bone marrow shows a significant degree of reticulin fibrosis (grade 2 or 3 according to the WHO grading system). Such cases have been termed MDS with fibrosis (MDS-F) [2201,2538], and most belong to the MDS-EB category (MDS-EB-F).

The presence of fibrosis is an independent prognostic parameter in MDS [942,1261]. Marrow fibrosis can also be seen in cases of therapy-related myeloid neoplasms, myeloproliferative neoplasms, lymphoid neoplasms and various reactive conditions, including infections and autoimmune disorders [4182]. These conditions must be ruled out. Bone marrow smears are often inadequate. The presence of excess of blasts in cases of MDS-EB-F can usually be confirmed by immunohistochemistry, in particular for CD34. A characteristic finding in MDS-F is an increased number of megakaryocytes with a high degree of dysplasia, including small forms and micromegakaryocytes [2201]. MDS-EB-F may overlap morphologically with acute panmyelosis with myelofibrosis; however, acute panmyelosis with myelofibrosis is distinguished by its abrupt onset with fever and bone pain, as well as by its higher blast count [2991,3986].

Immunophenotype

In MDS-EB, flow cytometry often shows increased numbers of cells positive for the precursor cell-associated antigens CD34 and/or KIT (CD117). These cells are usually positive for CD38, HLA-DR and the myeloid-associated antigens CD13 and/or CD33. Asynchronous expression of the granulocytic maturation antigens CD15, CD11b and/or CD65 can be seen in the blast population. Aberrant expression of CD7 on blast cells is seen in 20% of cases, and CD56 is present in 10% of cases; expression of other lymphoid markers is rare [2936,3724]. Expression of CD7 has been reported to correlate with worse prognosis [2936].

In tissue sections, CD34 immunohistochemistry can be used to confirm the presence of an increased number of blasts; it highlights their arrangement into clusters or aggregates, a characteristic finding seen in most cases of MDS-EB [942,2201,3724]. Antibodies such as CD61 and CD42b can facilitate the identification of micromegakaryocytes and other small dysplastic megakaryocytes, which are often particularly numerous in MDS-EB-F [2201,3987].

Cell of origin

A haematopoietic stem cell

Genetic profile

A variable percentage (30–50%) of cases of MDS-EB have clonal cytogenetic abnormalities, including gain of chromosome 8, loss of chromosome 5, del(5q), loss of chromosome 7, del(7q), and del(20q). Complex karyotypes may also be observed [1341].

Mutations affecting mRNA splicing genes are common in MDS-EB. SRSF2 mutations are present in both the MDS-EB-1 and MDS-EB-2 subtypes. Splicing mutations are mutually exclusive and less likely to occur in patients with complex cytogenetics or TP53 mutations [209,864]. Other mutations relatively common in MDS-EB include mutations in IDH1 and IDH2 [3098], ASXL1 and CBL [3380], as well as mutations in RUNX1, cohesin complex family genes and RAS pathway genes [2465].

Excess blasts appear to define a distinct disease phenotype that is independent of mutation status, underscoring the importance of blast count for risk-stratifying MDS, irrespective of the mutation profile [2465]. Both FLT3 and NPM1 mutations are found primarily in AML and occur very rarely in MDS-EB [880,3380]; when present, these mutations are associated with more rapid progression to AML [234]. Therefore, an alternative diagnosis of AML must be excluded in such cases by careful verification of the bone marrow and blood blast counts and close clinical follow-up.

Prognosis and predictive factors

MDS-EB is usually characterized by progressive bone marrow failure, with increasing cytopenias. Approximately 25% of cases of MDS-EB-1 and 33% of cases of MDS-EB-2 progress to AML; the remainder of patients succumb to the sequelae of bone marrow failure. The median survival times are approximately 16 months for MDS-EB-1 and 9 months for MDS-EB-2 [1341,3818]. Patients with MDS-EB-2 with 5–19% blasts in the peripheral blood have a median survival of 3–8 months [89], whereas patients with MDS-EB-2 based only on the presence of Auer rods have a median survival of about 12 months [4328].
Myelodysplastic syndrome with isolated del(5q)

Hasserjian R.P.
Le Beau M.M.
List A.F.
Bennett J.M.
Brunning R.D.
Thiele J.

Definition
Myelodysplastic syndrome (MDS) with isolated del(5q) is an MDS characterized by anaemia (with or without other cytopenias and/or thrombocytosis) and in which the cytogenetic abnormality del(5q) occurs either in isolation or with one other cytogenetic abnormality, other than monosomy 7 or del(7q). Myeloblasts constitute <5% of the nucleated bone marrow cells and <1% of the peripheral blood leukocytes. Auer rods are absent.

ICD-O code 9986/3

Synonyms
Myelodysplastic syndrome with 5q deletion; 5q minus syndrome

Epidemiology
MDS with isolated del(5q) occurs more often in women, with a median age of 67 years.

Etiology
The presumed etiology is loss of a tumour suppressor gene or genes in the minimally deleted region (5q33.1) (433, 2370). Haploinsufficiency of RPS14, which encodes a ribosomal structural protein, appears to contribute to the disease phenotype, possibly through p53 pathway activation (261,1078A,3574A). Haploinsufficiency of miR-145 and miR-146a in the deleted region may contribute to the megakaryocyte abnormalities and thrombocytosis (3767). Haploinsufficiency of CSNK1A1 (encoding casein kinase 1A1) leading to WNT/beta-catenin pathway deregulation has been implicated in proliferation of the del(5q) clone (3574). Haploinsufficiency of additional genes on 5q, such as APC (another WNT pathway regulator) and EGR1, may also contribute to disease pathogenesis (1885).

Localization
The blood and bone marrow are affected.

Clinical features
The most common symptoms are related to anaemia, which is often severe and usually macrocytic. Thrombocytosis is present in one third to one half of cases, whereas thrombocytopenia is uncommon (1358,2561). Pancytopenia is rare (2423); it is recommended that cases otherwise fulfilling the criteria for MDS with isolated del(5q), but with pancytopenia (haemoglobin concentration <10g/dL, absolute neutrophil count <1.8 x 10⁹/L and platelet count <100 x 10⁹/L) be categorized as MDS, unclassifiable, because their clinical behaviour is uncertain.

Microscopy
The bone marrow is usually hypercellular or normocellular and frequently exhibits erythroid hypoplasia (4263). Megakaryocytes are increased in number and are normal to slightly decreased in size, with conspicuously non-lobated and hypolobated nuclei. In contrast, dysplasia in the erythroid lineage is less pronounced (434,1358). Significant granulocytic dysplasia is uncommon. The blast percentage is <5% in the bone marrow and <1% in the peripheral blood. Ring sideroblasts may be present and do not exclude the diagnosis of MDS with isolated del(5q), provided the other criteria are fulfilled.

Cell of origin
The cell of origin is a haematopoietic stem cell. FISH analysis has demonstrated the presence of the del(5q) abnormality in differentiating erythroid, myeloid and megakaryocytic cells, but generally not in mature lymphoid cells (96,376). The del(5q) abnormality is the dominant clone in most cases and is present in the stem cell compartment, consistent with an early or initiating event in disease pathogenesis (4354).

Genetic profile
The defining cytogenetic abnormality involves an interstitial deletion of chromosome 5; the size of the deletion and the breakpoints vary, but bands q31-q33 are invariably deleted. Cases with one additional cytogenetic abnormality, with the exception of monosomy 7 or del(7q), have similar outcome as cases in which del(5q) is the sole abnormality, and are included in this category (1337,2473,3551). A small subset of patients with isolated del(5q) show concomitant JAK2 V617F or MPL W515L mutation, which does not appear to alter the disease phenotype or prognosis (1759,3101); in some of these cases, the JAK2 mutation and del(5q) have been found in different clones (3717). A subset of cases have SF3B1 mutation (2464,2466).

Fig. 6.21 Myelodysplastic syndrome with isolated del(5q). A Bone marrow section showing numerous megakaryocytes of various sizes, several with non-lobated nuclei. B Bone marrow aspirate smear showing two megakaryocytes with non-lobated, rounded nuclei.
Prognosis and predictive factors
This disease is associated with a median survival of 66–145 months, with transformation to acute myeloid leukaemia occurring in <10% of cases (1358,2473, 3101). Cases with del(5q) associated with loss of chromosome 7, del(7q), two or more additional chromosomal abnormalities, or excess blasts have an inferior survival and are excluded from this diagnosis. Significant granulocytic dysplasia has been associated with additional cytogenetic abnormalities and an inferior prognosis (671,1350).

The thalidomide analogue lenalidomide has been shown to benefit patients with MDS with isolated del(5q) or del(5q) with additional cytogenetic abnormalities, most likely by targeting caspase-3 and suppressing the abnormal clone (1359,2361). TP53 mutation is present in a significant subset of cases, and is associated with increased risk of leukaemic transformation, inferior response to lenalidomide, and shorter survival (1894,2129A,2474). Therefore, in MDS with isolated del(5q), it is recommended that TP53 mutation status be determined by sequencing or p53 immunohistochemistry to identify high-risk cases (3472).

**Myelodysplastic syndrome, unclassifiable**

Orazi A.

Brunning R.D.

Baumann I.

Hasserjian R.P.

Germing U.

**Definition**
The category of myelodysplastic syndrome (MDS), unclassifiable (MDS-U) encompasses the cases of MDS that initially lack appropriate findings for classification into any other MDS category.

**ICD-O code**
9989/3

**Synonyms**
Myelodysplastic syndrome, NOS; preleukaemia (obsolete); preleukaemic syndrome (obsolete)

**Epidemiology**
The exact incidence is unknown. In one study of 2032 patients, MDS-U accounted for 6.3% of cases of MDS with a bone marrow blast count of <5% (2423). A higher incidence of MDS-U has been reported among Japanese patients, in particular those with single lineage dysplasia and pancytopenia (1746,2566).

**Localization**
The peripheral blood and bone marrow are the principal sites of involvement.

**Clinical features**
Patients present with symptoms similar to those seen in the other MDS.

**Microscopy**
There are no specific morphological findings. The diagnosis of MDS-U can be made in any of the following settings:
1. There are findings that would otherwise suggest classification as MDS with single lineage dysplasia, MDS with multilineage dysplasia, MDS with ring sideroblasts and single lineage dysplasia, MDS with ring sideroblasts and multilineage dysplasia, or MDS with isolated del(5q), but with 1% blasts in the peripheral blood measured on at least two separate occasions (2052).
2. There are findings that would otherwise suggest classification as MDS with single lineage dysplasia, MDS with ring sideroblasts and single lineage dysplasia, or MDS with isolated del(5q) associated with pancytopenia. In contrast, pancytopenia is allowed in both MDS with multilineage dysplasia and MDS with ring sideroblasts and multilineage dysplasia.
3. There is persistent cytopenia with <2% blasts in the blood and <5% in the bone marrow, no significant (<10%) unequivocal dysplasia (Table 6.02, p. 102) in any myeloid lineage, and the presence of a cytogenetic abnormality considered presumptive evidence of MDS (Table 6.03, p. 104) (3774). Patients with MDS-U should be carefully followed for evidence of disease evolution to a more specific MDS type.

**Cell of origin**
A haematopoietic stem cell

**Genetic profile**
See Table 6.03 (p. 104).

**Prognosis and predictive factors**
If characteristics of a specific subtype of MDS develop later in the course of the disease, the case should be reclassified accordingly.

In a recent study, cases otherwise meeting the criteria for MDS with single lineage dysplasia, but with 1% blasts in the peripheral blood or with pancytopenia, were shown to have a prognosis similar to that of MDS with multilineage dysplasia cases (2423). In that study, patients with MDS-U and 1% peripheral blood blasts had a median survival of 35 months and a 14% 5-year cumulative risk of acute myeloid leukaemia progression, whereas patients with MDS-U and pancytopenia had a median survival of 30 months and an 18% 5-year cumulative risk of acute myeloid leukaemia progression (2423). The prognosis for MDS-U defined by cytogenetic abnormalities is unknown.

**Childhood myelodysplastic syndrome**

Baumann I.

Niemeyer C.M.

Bennett J.M.

Myelodysplastic syndrome (MDS) is very uncommon in children, accounting for <5% of all haematopoietic neoplasms in patients aged less than 14 years (1586, 2873). Some cases of MDS in children are secondary to cytotoxic therapy, inherited bone marrow failure disorders, or acquired severe aplastic anaemia. Other cases are generally categorized as primary MDS, but it is reasonable to assume that most of these are in fact secondary to a known or as-yet-unknown genetic predisposition (see Myeloid neoplasms with germline predisposition, p.121). GATA2 germline mutation is present in 7% of all primary MDS cases in children, but absent in children with secondary MDS (4342). The pre-leukaemic and leukaemic phase of myeloid leukaemia associated with Down syndrome is a unique disease entity of early childhood characterized by acquired GATA1 mutations (see Myeloid proliferations associated with Down syndrome, p.169), but little is known about the pathophysiology of MDS in older children with Down syndrome (3369). Many of the morphological, immunophenotypic and genetic features observed in MDS in
adults are also seen in childhood forms of the disease, but there are some significant differences reported, particularly in patients who do not have increased blasts in their peripheral blood or bone marrow. For example, MDS with ring sideroblasts and MDS with isolated del(5q) are exceedingly rare in children [2873]. Isolated anaemia, which is the major presenting manifestation of lower-grade MDS affecting adults, is uncommon in children, who are more likely to present with neutropenia and thrombocytopenia [1587,1940]. In addition, hypocellularity of the bone marrow is more commonly observed in childhood MDS than in older patients. Therefore, some childhood cases do not readily fit into the typical lower-grade MDS categories. To address these differences, a provisional entity, refractory cytopenia of childhood, is recognized and defined below. For childhood cases of MDS with 2–19% blasts in the peripheral blood or 5–19% blasts in the bone marrow, the same criteria should be applied as for adult cases of MDS with excess blasts. Unlike in adult MDS, there are no data indicating that a distinction between MDS with excess blasts 1 and MDS with excess blasts 2 is of prognostic relevance in children [2009,3808]. Children with MDS with excess blasts generally have relatively stable peripheral blood counts for weeks or months. Some cases diagnosed in children as acute myeloid leukaemia with 20–29% blasts in the peripheral blood and/or bone marrow that have myelodysplasia-related changes, including cases with myelodysplasia-related cytogenetic abnormalities (see Acute myeloid leukaemia with myelodysplasia-related changes, p. 150) may also be slowly progressive disease. These cases, categorized by the French–American–British (FAB) classification as refractory anaemia with excess blasts in transformation, may lack the clinical features of acute leukaemia and may behave more like MDS than acute myeloid leukaemia (1587); therefore, follow-up peripheral blood and bone marrow studies are often necessary to determine the pace of the disease in such cases. Children who present with a peripheral blood and/or bone marrow disorder associated with one of the core-binding factor rearrangements – t(8;21) (q22;q22.1); RUNX1-RUNX1T1; inv(16) (p13.1q22); CBFβ-MYH11, or t(16;16) (p13.1;q22); CBFβ-MYH11 – or with PML-RARA rearrangement should be considered to have acute myeloid leukaemia regardless of the blast count. The mutational landscape differs between adult and paediatric MDS. In children, most of the somatic mutations identified alter genes of the RAS pathway, transcription factors and epigenetic modifiers [2096].

Refractory cytopenia of childhood

Definition
Refractory cytopenia of childhood (RCC) is a provisional MDS entity characterized by persistent cytopenia, with <5% blasts in the bone marrow and <2% blasts in the peripheral blood [1587]. Although the presence of dysplasia is required for the diagnosis, the cytological finding of dysplasia constitutes only one aspect of the morphological diagnosis of RCC. The evaluation of an adequate bone marrow trephine biopsy specimen is essential for diagnosis. About 80% of children with RCC show considerable hypocellularity of the bone marrow [2873]. Therefore, it may be very challenging to differentiate hypocellular RCC from other bone marrow failure disorders, in particular acquired aplastic anaemia and inherited bone marrow failure disorders.

ICD-O code 9985/3

Epidemiology
RCC is the most common subtype of MDS in childhood, accounting for about 50% of all cases [3092,3246]. It is diagnosed in all age groups and affects boys and girls with equal frequency [1940].

Etiology
The etiology is unknown in most cases. In some cases, it is related to an underlying germline mutation.

Localization
The blood and bone marrow are always affected. Generally, the spleen, liver and lymph nodes are not sites of initial manifestation.

Clinical features
The most common symptoms are malaise, bleeding, fever and infection [1940]. Lymphadenopathy secondary to local or systemic infection may be present, but hepatosplenomegaly is generally not a feature of RCC. In as many as 20% of patients, no clinical signs or symptoms are reported [1940]. Congenital abnormalities of different organ systems may be present.

Three quarters of patients have a platelet count <150 × 10^9/L, and anaemia with a haemoglobin concentration of <10 g/dL is noted in about half of all affected children [1940]. Age-specific macrocytosis of red blood cells is seen in most patients. The white blood cell count is generally decreased, with severe neutropenia noted in about 25% of cases [1940].

Microscopy
The classic picture of RCC is a peripheral blood smear that shows anisopoikilocy-
Anisocytosis and macrocytosis. Anisochromasia and polychromasia may be present. Platelets often display anisocytosis, and giant platelets can occasionally be detected. Neutropenia, with pseudo–Pelger–Huët nuclei and/or hypogranularity or agranularity of neutrophil cytoplasm, may be noted. Blasts are absent or account for <2% of the white blood cells.

On bone marrow aspirate smears, dysplastic changes should be present in two myeloid cell lineages or account for at least 10% in one cell line (Table 6.07). Erythroid abnormalities include nuclear budding, multinuclearity, karyorrhexis, internuclear bridging, cytoplasmic granules and megaloblastoid changes. Cells of the granulocytic lineage may exhibit nuclear hyposegmentation (with pseudo–Pelger–Huët nuclei), hypogranularity or agranularity of the cytoplasm, macrocytic (giant) bands and asynchronous nuclear–cytoplasmic maturation. Megakaryocytes account for <5% of the bone marrow cells. Megakaryocytes are usually absent or very few. The detection of micromegakaryocytes is a strong indicator of RCC. Ring sideroblasts are not found. In cases of RCC with normocellular or hypercellular bone marrow, there is a slight to moderate increase in erythropoiesis, with accumulation of immature precursor cells (mainly proerythroblasts). Increased numbers of mitoses are present, indicating ineffective erythropoiesis. Granulopoiesis appears slightly to moderately decreased and cells of the granulocytic lineage are often loosely scattered. Blasts account for <5% of the bone marrow cells. Megakaryocytes may be normal, decreased or increased in number, and display dysplasia with small non-lobated nuclei, abnormally separated nuclear lobes, and (infrequently) characteristic micromegakaryocytes. There is no increase in reticulin fibres.

About 80% of patients with RCC show a marked decrease of bone marrow cellularity, as low as 5–10% of the normal value [2873]. The morphological findings in these cases are similar to those observed in normocellular or hypercellular cases. Immature erythroid precursors form one or several islands consisting of ≥20 cells. This patchy pattern of erythropoiesis is usually accompanied by sparsely distributed granulopoiesis. Megakaryocytes are significantly decreased or absent. Although micromegakaryocytes may be rare or not always found, they should be searched for carefully because they are important for establishing the diagnosis. Immunohistochemistry to identify micromegakaryocytes is obligatory. Multiple sections prepared from the biopsy may facilitate the identification of megakaryocytes and erythroid aggregates. Fatty tissue between the areas of haematopoiesis can mimic aplastic anaemia (Table 6.08). Therefore, at least two biopsies ≥2 weeks apart or two biopsies from two different locations of the pelvis are recommended to facilitate the detection of representative bone marrow spaces containing foci of erythropoiesis.

**Differential diagnosis**

In children, a variety of non-haematological disorders (e.g. viral infections, nutritional deficiencies and metabolic diseases) can give rise to secondary dysplastic morphology mimicking RCC (Table 6.09). In the absence of a cytogenetic marker, the clinical course in cases suspected of RCC must be carefully evaluated before a clear-cut diagnosis can be made. The haematological differential diagnosis includes acquired aplastic anaemia, inherited bone marrow failure diseases, and paroxysmal nocturnal haemoglobinuria. Unlike RCC, acquired aplastic anaemia presents with adipocytosis of the bone marrow spaces or with sparsely scattered

---

**Fig. 6.23** Refractory cytopenia of childhood. On bone marrow biopsy, CD61 immunohistochemistry shows dysplasia of megakaryocytes, with small, non-lobated nuclei or separated nuclei.

**Fig. 6.24** Aplastic anaemia. Bone marrow biopsy shows adipocytosis of bone marrow spaces; the few scattered cells are mainly lymphocytes, plasmatic cells, macrophages, mast cells and occasional mature myeloid cells.

**Fig. 6.25** Refractory cytopenia of childhood (RCC). A Bone marrow aspirate smear showing abnormal nuclear segmentation of an erythropoietic precursor cell and a small megakaryocyte with a bilobed nucleus. B Bone marrow biopsy showing a cluster of proerythroblasts without maturation.
myeloid cells. There are no significant erythroblast islands, no increased immaturity of erythroblasts, and no granulocytic or megakaryocytic dysplasia, in particular no micromegakaryocytes (Table 6.08). Contrary to what is sometimes reported in adults with aplastic anaemia, acquired aplastic anaemia in children does not have megaloblastoid features at presentation. The vast majority of cases of RCC and severe aplastic anaemia can be reliably differentiated by histomorphological means alone (296), and flow cytometry can be a relevant addition (3). However, after immunosuppressive therapy, the histological pattern of acquired aplastic anaemia can no longer be distinguished from that observed in RCC. The inherited bone marrow failure disorders (e.g. Fanconi anaemia, dyskeratosis congenita, Shwachman-Diamond syndrome, and artemegakaryocytic thrombocytopenia or panmyelohipoiesis with radiulnar synostosis) as well as some DNA repair deficiency disorders (e.g. LIG4 syndrome) show morphological features overlapping those of RCC (1942,4437,4481). These entities must be excluded by medical history, physical examination and the appropriate laboratory and molecular studies before a definite diagnosis of RCC can be made. The clinical picture of paroxysmal nocturnal haemoglobinuria is rare in childhood, although paroxysmal nocturnal haemoglobinuria clones in the absence of haemolysis or thrombosis may be observed in children with RCC (5). The association between RCC with two or more dysplastic lineages and MDS with multilineage dysplasia has not been fully investigated (4418). It is currently recommended that cases that would otherwise fulfill the criteria for MDS with multilineage dysplasia be considered as RCC until further studies clarify whether the number of lineages involved is an important prognostic discriminator in childhood MDS.

Immunophenotype
Micromegakaryocytes can easily be missed in H&E-stained bone marrow trephine biopsy sections, but are more readily apparent with immunostaining for platelet glycoproteins such as CD61 (also called glycoprotein IIb/IIIa), CD41 (also called glycoprotein IIb/IIIa) or von Willebrand factor. Myeloblasts are <5% of the bone marrow cells; detection of 5% blasts cells that are positive for myeloperoxidase (MPO), lysozyme, and/or

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Erythropoiesis</th>
<th>Granulopoiesis</th>
<th>Megakaryopoiesis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone marrow</td>
<td>Dysplastic changes(^a) and/or</td>
<td>Dysplastic changes(^b) in granulocytic</td>
<td>Unequivocal micromegakaryocytes; other dysplastic changes(^c) in variable numbers</td>
</tr>
<tr>
<td>aspirate biopsy</td>
<td>megakaryoblastoid changes</td>
<td>precursors and neutrophils; &lt;5% blasts</td>
<td></td>
</tr>
<tr>
<td></td>
<td>A few clusters of (\geq 20) erythroid</td>
<td>No minimal diagnostic criteria</td>
<td></td>
</tr>
<tr>
<td></td>
<td>precursors. Arrest in maturation, with</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>increased number of proerythroblasts.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Increased number of mitoses.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peripheral blood</td>
<td></td>
<td>Dysplastic changes(^b) in neutrophils</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) Erythroid dysplasia: abnormal nuclear segmentation, multinucleated cells, nuclear bridges.
\(^b\) Granulocytic dysplasia: pseudo-Pelger-Huet cells, hypogranularity or agranularity, giant bands (in cases with severe neutropenia, this criterion may not be fulfilled).
\(^c\) Megakaryocytic dysplasia: variable size with separated nuclei or round nuclei; the absence of megakaryocytes does not rule out refractory cytopenia of childhood.

<p>| Table 6.07 Minimal diagnostic criteria for refractory cytopenia of childhood. The criteria of dysplasia must be fulfilled in (\geq 10)% of cells in (\geq 1) lineage; in some cases, lesser degrees of dysplasia are present in 2 or 3 lineages. |
|-------------------|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|</p>
<table>
<thead>
<tr>
<th>Specimen</th>
<th>Erythropoiesis</th>
<th>Granulopoiesis</th>
<th>Megakaryopoiesis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone marrow</td>
<td>Dysplastic changes(^a) and/or</td>
<td>Dysplastic changes(^b) in granulocytic</td>
<td>Unequivocal micromegakaryocytes; other dysplastic changes(^c) in variable numbers</td>
</tr>
<tr>
<td>aspirate biopsy</td>
<td>megakaryoblastoid changes</td>
<td>precursors and neutrophils; &lt;5% blasts</td>
<td></td>
</tr>
<tr>
<td></td>
<td>A few clusters of (\geq 20) erythroid</td>
<td>No minimal diagnostic criteria</td>
<td></td>
</tr>
<tr>
<td></td>
<td>precursors. Arrest in maturation, with</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>increased number of proerythroblasts.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Increased number of mitoses.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peripheral blood</td>
<td></td>
<td>Dysplastic changes(^b) in neutrophils</td>
<td></td>
</tr>
</tbody>
</table>

<p>| Table 6.08 Comparison of the morphological criteria for hypoplastic refractory cytopenia of childhood and aplastic anaemia in children. | Refractory cytopenia of childhood | Aplastic anaemia in children |
|-------------------------------------------|----------------------------------|----------------------------------|-----------------------------|</p>
<table>
<thead>
<tr>
<th>Criterion</th>
<th>Bone marrow biopsy</th>
<th>Bone marrow aspirate cytology</th>
<th>Bone marrow biopsy</th>
<th>Bone marrow aspirate cytology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erythropoiesis</td>
<td>Patchy distribution</td>
<td>Nuclear segmentation</td>
<td>Absent or single small focus; &lt;10 cells with maturation</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Left shift</td>
<td>Multinularity</td>
<td></td>
<td>Absent or very few cells, without dysplasia or megaloblastoid change</td>
</tr>
<tr>
<td></td>
<td>Increased mitosis</td>
<td>Megakaryoblastoid changes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Granulopoiesis</td>
<td>Marked decrease</td>
<td>Pseudo-Pelger-Huet anomaly</td>
<td>Absent or markedly decreased; with very few small foci with maturation</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Left shift</td>
<td>Agranularity of cytoplasm</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hypogranularity of cytoplasm</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Nuclear–cytoplasmic maturation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Megakaryopoiesis</td>
<td>Marked decrease or aplasia</td>
<td>Micromegakaryocytes</td>
<td>Absent or very few non-dysplastic megakaryocytes</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Dysplastic changes</td>
<td>Multiple separated nuclei</td>
<td></td>
<td>Absent or few non-dysplastic megakaryocytes</td>
</tr>
<tr>
<td></td>
<td>Micromegakaryocytes</td>
<td>Small round nuclei</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>May be increased focally or dispersed</td>
<td>May be increased</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD34+ precursor cells</td>
<td>No increase</td>
<td>No increase</td>
<td></td>
<td></td>
</tr>
<tr>
<td>KIT+ (CD117+) precursor cells</td>
<td>No increase</td>
<td>No increase</td>
<td></td>
<td></td>
</tr>
<tr>
<td>KIT+ (CD117+) mast cells</td>
<td>Slightly increased</td>
<td>Slightly increased</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 6.09 Disorders that can present with morphological features indistinguishable from those of refractory cytopenia of childhood

- Infection (e.g. cytomegalovirus, herpesvirus, parvovirus B19, visceral leishmaniasis)
- Vitamin deficiency (e.g. deficiency of vitamin B12, folate, vitamin E)
- Metabolic disorders (e.g. mevalonate kinase deficiency)
- Rheumatological disease
- Systemic lupus erythematosus
- Autoimmune lymphoproliferative disorders (e.g. FAS deficiency)
- Mitochondrial DNA deletions (e.g. Pearson syndrome)
- Inherited bone marrow failure disorders (e.g. Fanconi anaemia, dyskeratosis congenita, Shwachman–Diamond syndrome, megalakaryocytic thrombocytopenia, thrombocytopenia with absent radii, radioulnar synostosis, Suckel syndrome)
- Paroxysmal nocturnal haemoglobinuria
- Acquired aplastic anaemia during haematological recovery during or after immunosuppression

KIT (CD117) may indicate progression to higher-grade MDS. CD34 staining is useful for identifying myeloblasts, but an increase of haematogones positive for CD34 and CD79a should be excluded. Clusters of myeloblasts are not seen in RCC. In most cases, flow cytometric immunophenotyping indicates a greatly reduced myeloid compartment, but not as severely reduced as in children with aplastic anaemia [3].

**Cell of origin**
A haematopoietic stem cell with multilineage potential

**Genetic profile**
The genetic changes that predispose individuals to MDS in childhood remain largely obscure. The presumed underlying mechanism may also give rise to subtle phenotypic abnormalities noted in many children with MDS. GATA2 deficiency was identified as germline predisposition in 5% of consecutively diagnosed children with RCC and can be associated with monosomy 7 or trisomy 8 [4342]. Monosomy 7 is the most common cytogenetic abnormality in RCC [1504,1940,2749,2873]. In one study, patients with normocellular or hypercellular bone marrow showed a normal karyotype, monosomy 7 or other aberrations in 61%, 19% and 12% of cases, respectively [2873]. In hypocellular RCC, the incidence of monosomy 7 and other aberrations is approximately 20%.

**Prognosis and predictive factors**
Karyotype is the most important factor predicting progression to advanced MDS. Patients with monosomy 7 have a significantly higher probability of progression than do patients with other chromosomal abnormalities or a normal karyotype [1940]. Spontaneous disappearance of cytopenia with monosomy 7 and del (7q) has been reported in some infants, but remains a rare event [3065]. Unlike with monosomy 7, patients with trisomy 8 or a normal karyotype may experience a long, stable course of disease. Currently, haematopoietic stem cell transplantation is the only curative therapy available, and is the treatment of choice for patients with monosomy 7 or complex karyotypes early in the course of their disease. Given the low rate of transplant-related mortality, haematopoietic stem cell transplantation can also be recommended for patients with other karyotypes if a suitable donor is available [3807].

An expectant approach, with careful observation, is reasonable in the absence of transfusion requirement, severe cytopenia and infections [1579]. Because early bone marrow failure can be mediated at least in part by T-cell immunosuppression of haematopoesis, and because T-cell receptor V beta skewing with expansion of effector cytotoxic T cells is noted in approximately 40% of RCC cases, immunosuppressive therapy can be an effective therapeutic strategy in select patients [1580,4438]; however, the long-term risk of relapse or clonal evolution remains.
CHAPTER 7

Myeloid neoplasms with germline predisposition
Myeloid neoplasms with germline predisposition

Definition
Myelodysplastic syndromes (MDSs) and acute myeloid leukaemia (AML) present primarily as sporadic diseases and typically occur in older adults. However, it has become increasingly apparent that some cases of myeloid neoplasms, in particular MDS and AML, occur in association with inherited or de novo germline mutations characterized by specific genetic and clinical findings. The most established of these disorders are those that occur in the setting of well-defined inherited syndromes that exhibit additional non-haematological findings and often present in childhood, such as the bone marrow failure syndromes (including Fanconi anaemia) and the telomere biology disorders (e.g. dyskeratosis congenita, see Table 7.01) [2866,3613,3800]. However, we have become increasingly aware of additional autosomal dominant disorders with predisposition to MDS/AML. Some patients with these disorders initially present with a myeloid neoplasm, whereas others have a pre-existing disorder or organ dysfunction. Some of the first cases to be described were AML with germline-mutated CEBPA and MDS/AML with germline mutations in RUNX1 and a pre-existing familial platelet disorder, but the list of these disorders is expanding (Table 7.01) [854,1390,3015,4301]. Myeloid neoplasms associated with predisposing mutations are generally considered to be rare, but as recognition grows, they may be found to be more common than is currently realized. Because the hereditary basis for these neoplasms is only beginning to be understood, it is likely that more disorders will be identified and that many of the currently recognized disorders will become more clearly defined.

The recognition and diagnosis of myeloid malignancies that arise from a germline mutation is critical for the proper clinical management and long-term follow-up of affected individuals. Even if a patient has not developed malignancy, the presence of additional organ dysfunction and abnormalities in platelet number and function warrant clinical management. For example, patients with germline RUNX1, ANKRD26, or ETV6 mutations can bleed out of proportion to their platelet counts and may require anticipatory transfusion of normal platelets prior to invasive procedures or childbirth. Individuals in these families benefit greatly from genetic counselling from counsellors with training in familial haematopoietic disorders. Because the clinical management of patients with malignant myeloid disorders often involves allogeneic haematopoietic stem cell transplantation, careful donor selection is critical in these families, to avoid re-introduction of the deleterious mutation. Inadvertent use of affected donor stem cells has resulted in poor or failed stem cell engraftment, poor graft function, and donor-derived leukaemias. Therefore, it is critical to distinguish myeloid neoplasms that arise as a consequence of germline predisposition from those that arise spontaneously or are secondary to environmental or chemical exposures.

This chapter discusses the major myeloid neoplasms with germline predisposition. The discussion focuses on myeloid neoplasms, but lymphoid neoplasms and solid tumours also occasionally occur in these same pedigrees, and are mentioned when relevant. There is increasing evidence to support germline mutations with predisposition to lymphoblastic leukaemia [1734,3134,3630]; this is discussed in the sections on T- and B-lymphoblastic leukaemias/lymphomas.

The familial myeloid neoplasms included in this chapter are categorized into three groups (Table 7.01). The first group includes myeloid neoplasms associated with germline mutations of CEBPA or DDX41, with a clinical picture dominated by either MDS or AML and with no other significant organ dysfunction or pre-existing disorder. The second group consists of myeloid neoplasms associated with germline mutations of RUNX1, ANKRD26, or ETV6 in which affected patients have a pre-existing platelet disorder. The third category includes myeloid neoplasms with predisposing mutations...
in which affected patients frequently exhibit additional non-haematological phenotypic abnormalities; the entities in this group are neoplasms with germline GATA2 mutation, the telomer biology disorders, and the inherited bone marrow failure disorders that often present in childhood (Table 7.01). Myeloid proliferations associated with Down syndrome, neurofibromatosis, and Noonan syndrome are also germline predisposition disorders, but are discussed elsewhere in this volume.

**Synonyms**
Familial myeloid neoplasms; familial myelodysplastic syndromes/acute leukemias

**Epidemiology**
The frequency of myeloid neoplasms associated with genetic predisposition is unknown, but they are considered rare. Some germline mutations are associated with pre-existing non-neoplastic haematological disorders or organ dysfunction that may present during childhood or in young adults. The neoplasms can present in any age group, often in children and young adults but also in older individuals, depending on the specific gene mutation.

**Etiology**
The etiology of these neoplasms is related to the underlying germline mutations. In some instances, the development of the myeloid neoplasm has been found to be associated with additional molecular and/or cytogenetic events.

**Clinical features**
There are no clinical features specific to myeloid neoplasms with a predisposing germline mutation. However, many of the germline mutations are associated with non-neoplastic haematological disorders, organ dysfunction, or inherited syndromic disorders that correlate with the specific gene involved and often manifest before the myeloid neoplasm develops.

Recognition of the presence of a germline predisposition syndrome requires familiarity with the currently defined syndromes and their clinical features. Guidelines recommend conducting a complete family history in the context of a comprehensive medical history and physical examination of all patients diagnosed with a haematological malignancy. It is particularly important to include detailed questioning about personal and family history including bleeding history as described in Table 7.02; documentation may be facilitated by genetic counselling with a counsellor familiar with inherited haematological malignancies (757).

**Microscopy and immunophenotype**
The morphology of the neoplasm depends on its subtype (MDS or AML). In many instances, the morphology has not been defined in detail or the number of cases described is limited. There is no known morphological finding specific for any neoplasm with an underlying germline mutation, but some findings (e.g. bone marrow hypocellularity, dysplasia, and the presence of Auer rods) characteristic of certain mutations are described here. The neoplasms have a myeloid immunophenotype. Some of these disorders exhibit distinctive blood and bone marrow morphology at baseline, before the development of overt neoplasia. The presence of a genetic predisposition does not in itself place a case into the category of a myeloid neoplasm until the appearance of standard diagnostic features of MDS, AML, or other myeloid malignancy. In general, the diagnostic criteria for the germline predisposition disorders are the same as those for sporadic cases; however, the diagnosis of MDS may be challenging in some cases. For example, some cases exhibit early dysplastic features that are stable and may not progress to MDS or AML for decades. These include the familial disorders in which dysmegakaryopoiesis accompanies thrombocytopenia secondary to germline ANKRD26 or ETv6 mutations. It is recommended that these cases not be considered neoplastic unless additional signs of neoplasia develop (e.g. increased blasts, increasing marrow cellularity in the presence of persisting cytopenias, increasing cytopenias, and/or the presence of additional cytogenetic or molecular genetic abnormalities to suggest progression to MDS/AML). Diagnosis of the childhood syndromic disorders can also be challenging. For example, patients with Fanconi anaemia often have a clinical course with fluctuating clonal haematopoiesis that can be difficult to distinguish from overt MDS (78,79). Close collaboration of pathologists and clinicians with clinical geneticists and/or certified genetic counsellors is essential for addressing these challenges. Another challenge is that the genetic predisposition may not be known or identified at the time of diagnosis of the first myeloid neoplasm within a family, which is often classified as a sporadic neoplasm. When the germline mutation becomes known, the diagnosis can be modified. For example, after a germline mutation is identified, a diagnosis of AML could be modified to AML with germline CEBPA mutation. Similarly, myeloid neoplasms

<table>
<thead>
<tr>
<th>Table 7.01</th>
<th>Classification of myeloid neoplasms with germline predisposition</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Myeloid neoplasms with germline predisposition without a pre-existing disorder or organ dysfunction</strong></td>
<td></td>
</tr>
<tr>
<td>Acute myeloid leukaemia with germline CEBPA mutation</td>
<td></td>
</tr>
<tr>
<td>Myeloid neoplasms with germline DDX41 mutation</td>
<td></td>
</tr>
<tr>
<td><strong>Myeloid neoplasms with germline predisposition and pre-existing platelet disorders</strong></td>
<td></td>
</tr>
<tr>
<td>Myeloid neoplasms with germline RUNX1 mutation</td>
<td></td>
</tr>
<tr>
<td>Myeloid neoplasms with germline ANKRD26 mutation</td>
<td></td>
</tr>
<tr>
<td>Myeloid neoplasms with germline ETv6 mutation</td>
<td></td>
</tr>
<tr>
<td><strong>Myeloid neoplasms with germline predisposition and other organ dysfunction</strong></td>
<td></td>
</tr>
<tr>
<td>Myeloid neoplasms with germline GATA2 mutation</td>
<td></td>
</tr>
<tr>
<td>Myeloid neoplasms associated with bone marrow failure syndromes</td>
<td></td>
</tr>
<tr>
<td>Myeloid neoplasms associated with telomere biology disorders</td>
<td></td>
</tr>
<tr>
<td>Juvenile myelomonocytic leukaemia associated with neurofibromatosis, Noonan syndrome, or Noonan syndrome–like disorders</td>
<td></td>
</tr>
<tr>
<td>Myeloid neoplasms associated with Down syndrome</td>
<td></td>
</tr>
</tbody>
</table>

a Lymphoid neoplasms have also been reported.
b See Table 7.03 (p. 127) for specific genes.
c See Juvenile myelomonocytic leukaemia, p. 89.
d See Myeloid proliferations associated with Down syndrome, p. 169.
occuring in association with the syndromic disorders often presenting in childhood should be classified according to the genetic abnormality (e.g. AML with germline SBDS mutation). If the affected gene has not been determined in a given patient, the neoplasm can be classified as AML associated with Shwachman-Diamond syndrome.

### Genetic profile

Each of these neoplasms exhibits a specific germline predisposition mutation (described below) identified by molecular genetic testing (including gene sequencing). Routine cytogenetic analysis may be normal or may reveal non-defining karyotypic alterations. Because many of the genes that are mutated in the germline can also be mutated as acquired events in MDS/AML, it is critical to perform germline testing on constitutional DNA. Growth of skin fibroblasts is the gold standard for obtaining germline DNA, but DNA from nails or hair can also be used. Because blood and bone marrow are both affected by haematological tumours, they should be used cautiously as a source of germline DNA. Saliva and buccal swabs are often contaminated with blood cells and should not be considered to be purely germline material. Once true germline DNA is obtained, it can be sent for molecular genetic testing. Panel-based testing for all known predisposition genes and testing for individual genes is available at academic and commercial laboratories (for availability details, consult https://www.genetests.org). Because many individuals and families have unique familial mutations, many variants are initially classified as variants of uncertain significance and require functional testing to determine whether they are deleterious.

The results of molecular testing of myeloid neoplasms must be interpreted carefully, with these syndromes in mind. Standard testing for prognostication of AML includes CEBPA mutation testing; in about 10% of cases with biallelic CEBPA mutations, one of the mutations is a germline event. Because many mutations in familial syndromes can also be acquired events, panel-based mutation tests must be interpreted with caution. For example, if a RUNX1 or ETV6 mutation is found by mutation testing of affected blood and/or bone marrow, consideration should be given as to whether the mutation is actually germline.

### Genetic susceptibility

When a patient’s personal or family history suggests familial predisposition to myeloid malignancies, clinical testing for mutations known to confer increased risk of development of myeloid malignancies is often negative, suggesting that additional predisposition alleles exist and await discovery. Therefore, the number of recognized germline predisposition syndromes is likely to increase. For example, recently described germline mutations of SRP72 (1390,2033) and germline duplications of ATG2B and GSKIP (3496) may emerge as myeloid neoplasm predisposition syndromes as more information becomes available.

### Myeloid neoplasms with germline predisposition without a pre-existing disorder or organ dysfunction

#### Acute myeloid leukaemia with germline CEBPA mutation

This familial AML syndrome is due to the inheritance of a single copy of mutated CEBPA, which encodes a granulocyte differentiation factor on chromosome band 19q13.1 (3708). The AML is associated with biallelic CEBPA mutations, with the germline mutation usually found in the 5' end of the gene and a somatic mutation at the 3' end of the other allele acquired at the time of progression to AML (3015,3023). Acquired GATA2 mutations are also common in this setting (1430). This disorder appears to have near-complete penetrance for development of AML (3015,3792), but the prevalence is unknown. Both monoallelic and biallelic mutations occur in sporadic AML, but only biallelic mutations are associated with a good prognosis (3023,3908). In the current WHO classification, only cases with biallelic CEBPA mutations are recognized as a specific subtype of AML. Because some of these cases may constitute AML with genetic predisposition, the identification of biallelic CEBPA mutations within leukemic cells should prompt evaluation for germline inheritance of one of the alleles. Patients with AML with germline mutations of CEBPA typically present with AML as children or young adults; in one report of 10 affected families, 24 patients with AML presented at a median age of 24.5 years (range: 1.75–46 years) (3909). AML is the primary presenting feature, with no preceding blood count abnormalities. The familial forms have morphological and immunophenotypic features similar to those of sporadic AML with CEBPA mutations, including a predominance of AML with or without maturation, the presence of Auer rods, frequent aberrant CD7 expression in the blast population, and a normal karyotype (3015,3708). Overall, AML with germline CEBPA mutation has a favourable prognosis. In one series, the 10-year overall survival rate was 67%; although multiple relapses were reported (3909). The somatic CEBPA mutations were found to be unstable throughout the disease course, with different mutations identified at recurrence, suggesting that...
apparent relapses may in fact represent novel, independent clones rather than being true relapses (3909).

Myeloid neoplasms with germline DDX41 mutation
This is a recently described autosomal dominant familial MDS/AML syndrome [3208] characterized by inherited mutations in the gene on chromosome 5 encoding the DEAD box RNA helicase DDX41. As with CEBPA, there is a significant subset of cases in which the DDX41 mutation is biallelic, with one mutation being germline. The prevalence of this germline DDX41 mutation is unknown. However, DDX41 mutations have been found in about 1.5% of myeloid neoplasms, and half of these patients had germline mutations (114,2291, 3208). Although the number of described pedigrees with MDS/AML with germline DDX41 mutation is limited, this disorder appears to be associated with long latency (with a mean patient age of 62 years at haematological malignancy onset) and development of high-grade myeloid neoplasms. The neoplasms reported are mainly MDS – MDS with multilineage dysplasia, MDS with excess blasts, and MDS with isolated del(5q) – and AML. Chronic myeloid leukaemia, chronic myelomonocytic leukaemia (CMML), and Hodgkin and non-Hodgkin lymphomas have also been reported. The penetrance of the disease is not fully established, but appears to be high. Patients with germline DDX41 mutation who develop MDS/AML usually present with leukaemia (with or without other cytopenias or macrocytosis), hypoplastic bone marrow with prominent erythroid dysplasia, and a normal karyotype, often leading to erythroleukaemia. The prognosis is generally poor. Early data suggest that patients may respond to lenalidomide, but this observation is based on a limited number of patients (3208).

Myeloid neoplasms with germline predisposition and pre-existing platelet disorders

Myeloid neoplasms with germline RUNX1 mutation
Familial platelet disorder with predisposition to AML is an autosomal dominant syndrome characterized by abnormalities in platelet number and function and enhanced risk of developing MDS/AML at a young age (2866,3015). Patients with this disorder have germline monoallelic mutations in RUNX1, a gene on chromosome band 21q22 encoding one subunit of the core binding transcription factor that regulates expression of several genes essential for haematopoiesis. Somatic RUNX1 alterations also occur in sporadic myeloid neoplasms, including participation as a partner in the RUNXI-RUNX1T1 fusion associated with t(8;21) (q22;q22.1) in AML and in the newly recognized WHO provisional entity AML with mutated RUNX1. The prevalence of germline RUNX1 mutations has not been determined. The clinical presentation is variable, even within the same family. Most affected individuals have a mild to moderate bleeding tendency, usually evident from childhood, but some have no bleeding history. Platelet counts are normal or mildly reduced, with normal platelet morphology and variable degrees of platelet dysfunction. Most patients exhibit impaired platelet aggregation with collagen and epinephrine, as well as a dense granule storage pool deficiency (3015,3729).

Distinct families with germline RUNX1 mutations exhibit varying risks of development of myeloid neoplasms, with 11–100% (median: 44%) of family members affected. The median patient age at onset of MDS/AML is 33 years, younger than for sporadic MDS/AML (4301). MDS and AML are the most common haematological neoplasms with germline RUNX1 mutations, but CMML, T-lymphoblastic leukaemia/lymphoma, and (rarely) B-cell neoplasms (including hairy cell leukaemia) have also been reported (1390). There are limited data on the morphology of MDS and AML, but the AMLs are reported to typically be AML with or without maturation, and Auer rods are common (3014). Anticipation appears to occur in many of the reported pedigrees, with children sometimes presenting before family members of older generations. Long-term data on the outcomes of patients treated for myeloid neoplasms with germline RUNX1 mutation are limited, making the determination of prognosis difficult (1390). The causative germline RUNX1 mutations include nonsense mutations, frameshift mutations, duplications, deletions, and missense mutations. Some of the mutations appear to act by haploinsufficiency and have dominant negative effects. Progression to MDS/AML likely requires additional mutations, which may account for some of the variation in penetrance of MDS/AML as well as the variable neoplasms that develop (4301). Acquisition of a mutation of the second RUNX1 allele appears to be a common second hit, but it is not required (3235). Other additional acquired abnormalities, including a CBL mutation in an individual who developed CMML and a mutation of ASXL1 in addition to loss of NF1 in a case of T lymphoblastic leukaemia, have also been reported (1390,4038,4301). For cases suspected to harbour RUNX1 mutations in which standard sequencing fails to reveal a point mutation, it is recommended that germline testing include testing for gene deletions, duplications, and rearrangements (1390).

Myeloid neoplasms with germline ANKRD26 mutation
Thrombocytopenia 2 (germline ANKRD26 mutation) is an autosomal dominant disorder characterized by moderate thrombocytopenia and increased risk of developing MDS/AML. This disorder is characterized by germline mutations in ANKRD26, located on chromosome band 10p12.1 (2894). Most such mutations occur within the 5’ untranslated region of the gene and disrupt the assembly of RUNX1 and FLI1 on the ANKRD26 promoter, ultimately resulting in increased gene transcription and signalling through the MPL pathway. This leads to impaired proplatelet formation by megakaryocytes. It has been shown that inhibition of EPHB2/MAPK (also called ERK) reverses the proplatelet defect in vitro, which implicates the MAPK pathway in the pathogenesis of the thrombocytopenia 2 platelet defect [1390]. Notably, one missense mutation (D158G) has been identified within a family [45]. The incidence of this disorder is unknown, but more than 20 affected families have been reported. Platelet count is variable, but the thrombocytopenia is usually moderate, with normal platelet size and volume. Most patients have glycoprotein la and alpha-granule deficiency, whereas in vitro platelet aggregation studies are often normal. Thrombopoietin levels in these patients are elevated. Bleeding tendencies in affected patients are usually mild.
Evidence of dysmegakaryopoiesis has been observed in the small number of patients without leukaemia who have undergone bone marrow biopsies: megakaryocytes are increased in number and small, have hyposegmented nuclei or two nuclei, and include micromegakaryocytes. A small subset of patients have elevated haemoglobin concentrations and leucocyte counts [2894]. Although the number of reported families with this disorder is limited, the prevalence of the development of myeloid neoplasms in these families is estimated to be approximately 30 times higher than that in the general population. Most of the reported cases are AML or MDS, but the number of reported cases is low [2893]. A smaller number of patients had chronic myeloid leukaemia, CMML [3132], or chronic lymphocytic leukaemia [2894].

Myeloid neoplasms with germline ETV6 mutation
Thrombocytopenia 5 (germline ETV6 mutation) is a recently described disorder characterized by autosomal dominant familial thrombocytopenia and haematological neoplasms. Affected patients have variable thrombocytopenia with normal-sized platelets, and a mild to moderate bleeding tendency, occasionally presenting in infancy. The limited number of bone marrow biopsies from affected individuals without leukaemia have shown small hyposegmented megakaryocytes. Mild dyserythropoiesis has also been reported. The haematological malignancies reported in these individuals are diverse, including MDS, AML, CMML, B lymphoblastic leukaemia, and plasma cell myeloma. Non-haematological neoplasms, including colorectal adenocarcinoma, have been also reported in these families. The missense mutations identified to date have a dominant negative effect, resulting in disrupted nuclear localization of the ETV6 transcription factor and reduced expression of platelet-associated genes [2887, 4024, 4480].

Myeloid neoplasms with germline predisposition associated with other organ dysfunction

Myeloid neoplasms with germline GATA2 mutation
Germline GATA2 gene mutations were originally identified as four separate syndromes:
- MonoMAC syndrome, characterized by monocytopenia and non-tuberculous mycobacterial infection [1715, 4197]
- Dendritic cell, monocyte, B- and NK-lymphoid (DCML) deficiency with vulnerability to viral infections [375, 977, 1518]
- Familial MDS/AML [1293, 1518]
- Emberger syndrome, characterized by primary lymphoedema, warts and a predisposition to MDS/AML [2490, 3002]

GATA2 mutations were also recognized in a minority of cases of congenital neutropenia and aplastic anaemia. Considering the overlapping features present in these disorders, they are now recognized as a single genetic disorder with protean manifestations [784, 978, 1292, 3755].

GATA2 is a zinc-finger transcription factor regulating haematopoiesis, autoimmunity, and inflammatory and developmental processes. Germline GATA2 mutations have been identified in both coding and non-coding regions; they are monoallelic and broadly classified as missense, null, and regulatory mutations. Germline GATA2 mutations result in loss of function of the mutated allele, resulting in haploinsufficiency [3755]. No significant association between genotype and clinical manifestations has been identified, with the exception of lymphoedema and severe infections that are seen preferentially in patients with null mutations [1289]. GATA2 haploinsufficiency is diagnosed by full gene sequencing and large rearrangement testing. The incidence of GATA2 haploinsufficiency is unknown.

The clinical presentation of germline GATA2 mutation is heterogeneous. In a study of 57 GATA2-mutated cases, the median patient age at presentation was 20 years (range: 5 months to 78 years), with 64% of cases presenting with infection, 21% with MDS/AML, and 9% with lymphoedema [3755]. A small subset of cases present with AML, but many
<table>
<thead>
<tr>
<th>Syndrome</th>
<th>Inheritance patterns and genes</th>
<th>Characteristic haematological neoplasms</th>
<th>Risk of myeloid neoplasm</th>
<th>Other phenotypic findings</th>
<th>Diagnostic testing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fanconi anaemia</td>
<td>AR: FANCA</td>
<td>MDS, AML</td>
<td>MDS: 7%</td>
<td>Bone marrow failure, low birth weight, short stature, radial anomalies, congenital heart disease, microphthalmia, ear anomalies, deafness, renal malformations, hypogonadism, café-au-lait spots, solid tumours</td>
<td>Screening: chromosomal breakage analysis, Gene sequencing for relevant mutations</td>
</tr>
<tr>
<td></td>
<td>XLR: FANCB, FANCC, BRCA2 (FANCD1), FANCD2, FANCE, FANCF, FANCQ, FANCI, BRIP1 (FANCJ), FANCL, FANCQ, PALB2 (FANCN), RAD51C, SLX4 (BTBD12)</td>
<td></td>
<td>AML: 9%</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Severe congenital neutropenia</td>
<td>AD: ELANE, CSF3R, GFI1</td>
<td>MDS, AML</td>
<td>21-40%</td>
<td>HAX1: neurodevelopmental G6PC3: cardiac and other</td>
<td>Gene sequencing for relevant mutations</td>
</tr>
<tr>
<td></td>
<td>AR: HAX1, G6PC3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>XLR: WAS</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shwachman–Diamond syndrome</td>
<td>AR: SBDS</td>
<td>MDS, AML, ALL</td>
<td>5-24%</td>
<td>Preceding isolated neutropenia, pancreatic insufficiency, short stature, skeletal abnormalities including metaphyseal dysostosis</td>
<td>Gene sequencing for SBDS mutations</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diamond–Blackfan anaemia</td>
<td>AD: RPS19, RPS17, RPS24, RPL35A, RPL5, RPL11, RPS7, RPS26, RPS10</td>
<td>MDS, AML, ALL</td>
<td>5%</td>
<td>Small stature, congenital anomalies (e.g. craniofacial, cardiac, skeletal, genitourinary)</td>
<td>Screening: elevated erythrocyte adenosine deaminase and haemoglobin F</td>
</tr>
<tr>
<td></td>
<td>XLR: GATA1</td>
<td></td>
<td></td>
<td></td>
<td>Gene sequencing for relevant mutations</td>
</tr>
<tr>
<td>Telomere biology disorders</td>
<td>XLR: DKC1</td>
<td>MDS, AML</td>
<td>2-30%</td>
<td>Nail dystrophy, abnormal skin and pigmentation, oral leukoplakia, pulmonary fibrosis, hepatic fibrosis, squamous cell carcinoma</td>
<td>Telomere length measurement by flow-FISH, If abnormal, gene sequencing for relevant mutations</td>
</tr>
<tr>
<td>including dyskeratosis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>congenita and syndromes due</td>
<td>AD: TERT, TERC, TINF2, RTEL1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>to TERCl or TERT mutation</td>
<td>AR: NOP10, NHP2, WRAP53, RTEL1, TERT, CTC1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

AD, autosomal dominant; ALL, lymphoblastic leukaemia/lymphoma; AML, acute myeloid leukaemia; AR, autosomal recessive; CMML, chronic myelomonocytic leukaemia; MDS, myelodysplastic syndrome; XLR, X-linked recessive.

* Because phenotypes are variable, some cases may show additional features or lack the key features listed.

patents develop MDS with a high risk of evolution to AML or development of CMML [3755]. Concurrent ASXL1 mutations have been reported in many patients with monosomy 7, some of whom have a germline GATA2 mutation (514,2656,4303). MDS/AML develops in approximately 70% of affected individuals, at a median patient age of 29 years [2656]. The clinical history of immunodeficiency associated with reduced monocytes, B cells, and NK cells; lymphoedema; and/or other clinical manifestations of GATA2 mutation may point to the diagnosis. However, some patients with germline GATA2 mutation present with MDS/AML without these clues. In MDS in children and adolescents, GATA2 mutation accounts for 15% of advanced MDS cases and 7% of all MDS cases (4342). It is highly prevalent among patients with monosomy 7, with 37% of such patients harbouring GATA2 germline mutations, and peak incidence in adolescence. In most children with germline GATA2 mutation, MDS appears to be sporadic, without a family history of myeloid leukaemia or other GATA2-related symptoms [4342]. GATA2-mutant MDS can be heralded by anaemia, neutropenia, or thrombocytopenia. Characteristic morphological features are bone marrow hypocellularity and multilineage dysplasia (most prominent in the megakaryocyte lineage), including micromegakaryocytes...
and megakaryocytes with separated and peripheralized nuclear lobes. Increased reticulin fibrosis is also a feature. Flow cytometric immunophenotyping shows abnormal granulocytic maturation, monocytopenia, and reduced numbers of bone marrow NK cells and B cells. Plasma cells are present, but are often abnormal (e.g. CD56+). Increased T-cell large granular lymphocyte populations are common [531]. The most common cytogenetic abnormalities are monosomy 7 and trisomy 8. Based on the limited numbers of cases reported in the literature, affected patients appear to have a poor prognosis. However, improved clinical outcomes have been reported with haematopoietic stem cell transplantation in patients with MDS [4303].

**Myeloid neoplasms with germline predisposition associated with inherited bone failure syndromes and telomere biology disorders**

The remaining cases of familial MDS/AML associated with other organ dysfunction include the well-known classic disorders often diagnosed in childhood and known as the inherited bone marrow failure syndromes and telomere biology disorders. The main features of these disorders are listed in Table 7.03 (p. 127), and the reader is referred to excellent reviews of this topic [1390,2866, 3613,3800]. It should be noted that the phenotypes of these disorders are highly variable, in some cases key findings may be absent, and patients may not be diagnosed until adulthood [3658]. The inherited bone marrow failure syndromes are a heterogeneous group of disorders including Fanconi anaemia, Shwachman–Diamond syndrome, Diamond–Blackfan anaemia, and severe congenital neutropenia [1390,3613]. Although lymphoblastic leukaemias have been described in these syndromes, MDS and AML are the most common haematological neoplasms [2471]. Patients are typically diagnosed in childhood due to bone marrow failure or systemic manifestations such as limb abnormalities in Fanconi anaemia or pancreatic dysfunction in Shwachman–Diamond syndrome, but some cases may not be recognized until adulthood [3658]. One of the classic disorders in this group, Fanconi anaemia, lacks the characteristic physical features of short stature and radial anomalies in about 25% of cases. Because there is a 600- to 800-fold increased risk of MDS/AML, development of a haematological malignancy may be the presenting feature of the disease [4301]. Also within the spectrum of inherited bone marrow failure syndromes are the telomere biology disorders associated with abnormal telomere maintenance and predisposition to MDS and AML. Dyskeratosis congenita is a prototypical disorder in this group, with the classic triad of nail dystrophy, abnormal reticular skin pigmentation, and oral leukoplakia, and a high risk of developing MDS/AML. Telomere biology disorders result from mutations in one of several genes, and inheritance patterns are diverse (Table 7.03, p. 127). The clinical presentations of telomere biology disorders are heterogeneous, and not all patients exhibit the classic features. The involvement of at least two genes (TERC, which encodes the telomerase RNA components, and TERT, which encodes the telomerase reverse transcriptase component) can cause clinical presentations that completely lack the characteristic mucocutaneous findings. These disorders predispose patients not only to MDS/AML, but also to a variety of solid tumours [1390].
CHAPTER 8

Acute myeloid leukaemia and related precursor neoplasms

Acute myeloid leukaemia with recurrent genetic abnormalities
Acute myeloid leukaemia with myelodysplasia-related changes
Therapy-related myeloid neoplasms
Acute myeloid leukaemia, NOS
Myeloid sarcoma
Myeloid proliferations associated with Down syndrome
Acute myeloid leukaemia with recurrent genetic abnormalities

Introduction

Acute myeloid leukaemia with balanced translocations/inversions

The recurrent genetic abnormalities in acute myeloid leukaemia (AML) are associated with distinctive clinicopathological features and have prognostic significance. Those most commonly identified are balanced abnormalities: t(8;21)(q22;q22.1), inv(16)(p13.1q22) or t(16;16)(p13.1;q22), t(15;17)(q24.1;q21.2), and t(9;11)(p21.3;q32.3) [511,1236,1465, 1466,3701]. Most of these structural chromosomal rearrangements create a fusion gene encoding a chimeric protein that is required, but usually not sufficient, for leukaemogenesis [3746]. Many of these disease groups have characteristic morphological and immunophenotypic features [126]. Many other balanced translocations and inversions also recur in AML, but are uncommon. Several of these occur more commonly in paediatric patients, and are summarized in Table 8.01. AML with t(8;21)(q22;q22.1), AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22), and acute promyelocytic leukaemia with PML-RARA are considered to be acute leukaemias without regard to blast cell count. It is controversial whether all cases with t(9;11)(p21.3;q23.3), t(6;9) (p23;q34.1), inv(3)(q21.3q26.2), t(3;3) (q21.3;q26.2), or t(1;22)(p13.3;q13.1) as well as AML with the BCR-ABL1 fusion should be categorized as AML when the blast cell count is <20%. Therapy-related myeloid neoplasms may also have the balanced translocations and inversions described in this section, but these should be diagnosed as therapy-related myeloid neoplasms, with the associated genetic abnormality noted.

Acute myeloid leukaemia with gene mutations

It is now understood that, in addition to translocations and inversions, gene mutations are also common in AML [9,545, 1074,3095,3651]. The Cancer Genome Atlas (TCGA) Research Network evaluation of 200 AML cases found an average of 13 mutations per case of AML, with at least 23 recurrent mutations identified [545]. These discoveries and others have been identified at least eight distinct categories of mutations in AML, which are discussed in more detail in Chapter 1 (Introduction and overview of the classification of the myeloid neoplasms, p. 15). The current WHO classification recognizes AML with mutated NPM1 and AML with biallelic mutation of CEBPA as specific AML classification categories (entities), and AML with mutated RUNX1 as a provisional entity. However, many other mutations also occur in AML, and some that have diagnostic significance, such as FLT3 internal tandem duplication (FLT3-ITD) and KIT mutations, may be mutated in specific AML types. Next-generation sequencing panels are now available to screen for a large number of mutations in AML. Table 8.02 (p. 146) summarizes the more common gene mutations in AML. It is beyond the scope of this classification to discuss each prognostically significant mutation in AML individually, and the significance of some mutations is still unclear. Some combinations of gene mutations (e.g. NPM1 mutation and FLT3-ITD in normal-karyotype AML) appear to cluster within certain disease categories [9,545]; the prognostic significance of these mutations and combinations of mutations is discussed within the various sections throughout this volume.

Acute myeloid leukaemia with t(8;21)(q22;q22.1); RUNX1-RUNX1T1

Definition

Acute myeloid leukaemia (AML) with t(8;21)(q22;q22.1) resulting in RUNX1-RUNX1T1 is an AML showing predominantly neutrophilic maturation. The bone marrow and peripheral blood show large myeloblasts with abundant basophilic cytoplasm, often containing azurophilic granules. This type of AML is associated with a high rate of complete remission and favourable long-term outcome.

ICD-O code

9896/3

Synonyms

Acute myeloid leukaemia, t(8;21)(q22;q22); acute myeloid leukaemia, AML(1)(CBF-alpha)/ETO, acute myeloid leukaemia with t(8;21)(q22;q22), RUNX1-RUNX1T1

Epidemiology

The t(8;21)(q22;q22.1) is found in 1–5% of cases of AML, usually in younger patients and in cases with features of AML with granulocytic maturation.

Clinical features

Tumour manifestations, such as myeloid sarcoma, may be present at presentation. In such cases the initial bone marrow aspiration may show a low number of blast cells.

Microscopy

The common morphological features include the presence of large blasts with abundant basophilic cytoplasm, often containing numerous azurophilic granules and perinuclear clearing (hofs). In many cases, a few blasts show very large granules (pseudo-Chédiak-Higashi granules), suggesting abnormal fusion. Auer rods are frequently found and appear as a single long and sharp rod with tapered ends; they may be detected in mature neutrophils. In addition to the large blast cells, some smaller blasts, predominantly in the peripheral blood, may be found. Promyelocytes, myelocytes, and mature neutrophils with variable dysplasia are present in the bone marrow. These cells may show abnormal nuclear segmentation (e.g. pseudo-Pelger–Huët nuclei) and/or cytoplasmic staining abnormalities, including homogeneous pink cytoplasm in neutrophils. However, dysplasia of other cell lines is uncommon; erythroblasts and megakaryocytes usually have normal morphology. A mononuclear component is usually minimal or absent. Eosino-
Table 8.01  Chromosomal translocations with higher prevalence in paediatric acute myeloid leukaemia than in adult cases

<table>
<thead>
<tr>
<th>Translocation</th>
<th>Gene fusions</th>
<th>Frequency in children and adults, respectively</th>
<th>Age group predilection</th>
<th>Comments and Prognosis</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>t(1;22)(p13.3;q13.1)</td>
<td>RBM15-MKL1</td>
<td>0.8% 0%</td>
<td>Infants</td>
<td>Acute megakaryoblastic leukaemia (FAB M7) Intermediate</td>
<td>(2422,2637)</td>
</tr>
<tr>
<td>t(7;12)(q36.3;p13.2)</td>
<td>MNX1-ETV6</td>
<td>0.8% &lt;0.5%</td>
<td>Infants</td>
<td>Gain of chromosome 19 considered secondary abnormality Adverse</td>
<td>(364A,1559,1641A, 4208A)</td>
</tr>
<tr>
<td>t(8;16)(p11.2;p13.3)</td>
<td>KAT6A-CREBBP</td>
<td>0.5% &lt;0.5%</td>
<td>Infants and children</td>
<td>Can spontaneously remit in infancy Intermediate prognosis in later childhood</td>
<td>(776A)</td>
</tr>
<tr>
<td>t(6;9)(p23;q34.1)</td>
<td>DEK-NUP214</td>
<td>1.7% 1%</td>
<td>Older children; rare in infants</td>
<td>Adverse 65% with FLT3-ITD</td>
<td>(3502A,3905)</td>
</tr>
<tr>
<td>t(11;q23.3)</td>
<td>KMT2A translocated</td>
<td>25% 5-10%</td>
<td>Infants (50%)</td>
<td>Prognosis dependent on the partner gene</td>
<td>(1177A,2104A)</td>
</tr>
<tr>
<td>t(9;11)(p21.3;q23.3)</td>
<td>KMT2A-MLLT3</td>
<td>9.5% 2%</td>
<td>Children</td>
<td>Intermediate</td>
<td>(239C)</td>
</tr>
<tr>
<td>t(10;11)(p12;q23.3)</td>
<td>KMT2A-MLLT10</td>
<td>3.5% 1%</td>
<td>Children</td>
<td>Includes subtle and cryptic KMT2A rearrangements Adverse</td>
<td>(239C)</td>
</tr>
<tr>
<td>t(6;11)(q27;q23.3)</td>
<td>KMT2A-AFDN</td>
<td>2% &lt;0.5%</td>
<td>Children</td>
<td>Adverse</td>
<td>(239C)</td>
</tr>
<tr>
<td>t(1;11)(q21;q23.3)</td>
<td>KMT2A-MLLT11</td>
<td>1% &lt;0.5%</td>
<td>Children</td>
<td>Favourable</td>
<td>(239C)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Cryptic chromosomal translocations</th>
</tr>
</thead>
<tbody>
<tr>
<td>t(5;11)(q35.3;p15.5)</td>
</tr>
<tr>
<td>inv(16)(p13.3;q24.3)</td>
</tr>
<tr>
<td>t(11;12)(p15.5;p13.5)</td>
</tr>
</tbody>
</table>

FAB, French–American–British classification; FLT3-ITD, FLT3 internal tandem duplication.
Data tabulated by Betsy Hirsch, Susana Raimondi, Soheil Meschinchi, Nyla Heerema and Andrew J. Carroll.

Immunophenotype
Most cases of AML with t(8;21) display a characteristic immunophenotype, with a subpopulation of blast cells showing high-intensity expression of CD34, HLA-DR, MPO, and CD13, but relatively weak expression of CD33 [2002,3224]. There is reported in some cases; mast cell infiltrates may be masked by the acute leukaemia infiltration of the bone marrow at diagnosis [1868]. Rare cases with a bone marrow blast percentage <20% occur; these should be classified as AML rather than myelodysplastic syndrome.
are usually signs of neutrophilic differentiation, with subpopulations of cells showing neutrophilic maturation demonstrated by CD15 and/or CD65 expression. Populations of blasts showing maturation asynchrony (e.g. coexpressing CD34 and CD15) are sometimes present. These leukaemias frequently express the lymphoid markers CD19 and PAX5, and may express cytoplasmic CD79a (1738, 2034,3996). In t(8;21) AML, PAX5 is not directly activated by RUNX1-RUNX1T1, but expression requires constitutive MAPK signalling (3322). Some cases are TdT-positive, but TdT expression is generally weak. CD56 is expressed in a fraction of cases and may have adverse prognostic significance (221,1777). The adverse prognostic significance of CD56 may be due to higher CD56 expression in cases with KIT mutations [894].

Postulated normal counterpart
A haematopoietic progenitor cell with the potential to differentiate along granulocytic and monocytic lineages.

Genetic profile
The genes for both heterodimeric components of core-binding factor (CBF), RUNX1 (also called AML1 and CBFA) and CBFB, are involved in rearrangements associated with acute leukaemias [3746]. The t(8;21)(q22;q22.1) involves RUNX1, which encodes the alpha subunit of CBF, and RUNX1T1 (ETO) [1032,2321, 3149]. The RUNX1-RUNX1T1 fusion transcript is consistently detected in patients with t(8;21)(q22;q22.1) AML. The CBF transcription factor is essential for haematopoiesis; transformation by RUNX1-RUNX1T1 likely results from transcriptional repression of normal RUNX1 target genes via aberrant recruitment of nuclear transcripational co-repressor complexes.

More than 70% of cases show additional chromosome abnormalities, such as loss of a sex chromosome or del(9q) with loss of 9q22. KIT mutations occur in 20–30% of cases (3078). Secondary cooperating mutations of KRAS or NRAS are common, occurring in 30% of paediatric and 10–20% of adult CBF-associated leukaemias [1392,3077]. ASXL1 mutations occur in approximately 10% of patients, mostly adults; ASXL2 mutations occur in 20–25% of patients of all ages (2657).

Prognosis and predictive factors
AML with t(8;21)(q22;q22.1) is usually associated with a high rate of complete remission and long-term disease-free survival when treated with intensive consolidation therapy (e.g. high-dose cytarabine) [393,1466]. Some factors appear to adversely affect prognosis, including the presence of KIT mutations in adults and CD56 expression [221,3078]. Therapeutic trials investigating mutant KIT in this AML type are under way.

**Acute myeloid leukaemia with inv(16)(p13.1q22) or t(16;16) (p13.1;q22); CBFB-MYH11**

**Definition**
Acute myeloid leukaemia (AML) with inv(16)(p13.1q22) or t(16;16)(p13.1;q22) resulting in CBFB-MYH11 is an AML that usually shows monocytic and granulocytic differentiation and characteristically an abnormal eosinophil component in the bone marrow [2236,2513,3746].

**ICD-O code**
9871/3

**Synonyms**
Acute myeloid leukaemia, t(16;16)(p13;q11); acute myeloid leukaemia, CBF-betamyelomonocytic leukaemia with abnormal eosinophils; French–American–British (FAB) classification M4Eo; acute myeloid leukaemia, inv(16)(p13;q22)

**Epidemiology**
Either inv(16)(p13.1q22) or t(16;16) (p13.1;q22) is found in 5–8% of younger patients with AML; the frequency is lower in older adults.

**Clinical features**
Myeloid sarcoma may be present at initial diagnosis or at relapse and may constitute the only evidence of relapse in some patients. The white blood cell count at diagnosis is significantly higher in AML with inv(16)(p13.1q22) or t(16;16) (p13.1;q22) than in cases with t(8;21) (q22;q22.1) [2502,3559].

**Microscopy**
In AML with inv(16)(p13.1q22) or t(16;16) (p13.1;q22), in addition to the usual morphological features of acute myelomonocytic leukaemia, the bone marrow shows a variable number of eosinophils (usually increased, but sometimes <5%) at all stages of maturation, without significant maturation arrest. The most striking abnormalities involve the immature eosinophilic granules, mainly evident at the late promyelocyte and myelocyte stages. The abnormalities are usually not present at later stages of eosinophil maturation. The eosinophilic granules are often larger than those normally present in immature eosinophils, are purple-violet in colour, and in some cells are so dense that they obscure the cell morphology. The mature eosinophils occasionally show nuclear hyposegmentation. Auer rods may be observed in myeloblasts. Neutrophils in the bone marrow are usually sparse, with a decreased number of
Acute myeloid leukaemia with recurrent genetic abnormalities

The peripheral blood is not different from that in other cases of acute myelomonocytic leukaemia; eosinophils are not usually increased, but an occasional case has been reported with abnormal and increased eosinophils in the peripheral blood. Most cases with inv(16)(p13.1q22) have abnormal eosinophils, but in some cases they are rare and difficult to find. Occasional cases with this genetic abnormality lack eosinophilia, show only granulocytic maturation without a monocytic component, or show only monocytic differentiation. In some cases, the blast percentage is at the 20% threshold or occasionally lower. Cases with inv(16)(p13.1q22) or t(16;16) (p13.1;q22) and <20% bone marrow blasts should be diagnosed as AML.

Cytochemistry
The naphthol AS-D chloroacetate esterase (CAE) reaction, which is normally negative in eosinophils, is characteristically faintly positive in the abnormal eosinophils. Such a reaction is not seen in eosinophils of AML with t(8;21)q22;q22.1. At least 3% of the blasts show MPO reactivity. The monoblasts and promonocytes usually show non-specific esterase reactivity, although it may be weaker than expected or even absent in some cases.

Immunophenotype
Most of these leukaemias are characterized by a complex immunophenotype, with the presence of multiple blast populations: immature blasts with high CD34 and KIT (CD117) expression and populations differentiating towards the granulocytic lineage (positive for CD13, CD33, CD15, CD65, and MPO) and the monocytic lineage (positive for CD14, CD4, CD11b, CD11c, CD64, CD36, and lysozyme). Maturation asynchrony is often seen. Coexpression of CD2 with myeloid markers has been frequently documented, but it is not specific for this diagnosis.

Postulated normal counterpart
A haematopoietic progenitor cell with the potential to differentiate along granulocytic and monocytic lineages.

Genetic profile
The inv(16)(p13.1q22) found in the vast majority of this subtype and the less common t(16;16)(p13.1;q22) both result in the fusion of CBFB at 16q22 to MYH11 at 16p13.1 [3650]. MYH11 codes for a smooth muscle myosin heavy chain [871]. CBFB codes for the beta subunit of core-binding factor (CBFB), a heterodimeric transcription factor known to bind the enhancers of the T-cell receptor, cytokine genes, and other genes. The CBFB subunit heterodimerizes with RUNX1 (CBFA2), the gene product of RUNX1, which is one of the genes involved in AML with t(8;21) (q22;q22.1). Occasionally, cytological features of AML with abnormal eosinophils may be present without karyotypic evidence of a chromosome 16 abnormality, but with CBFB-MYH11 nevertheless demonstrated by molecular genetic studies [2775,3432]. By conventional cytogenetic analysis, inv(16)(p13.1q22) is a subtle rearrangement that may be overlooked when metaphase preparations are suboptimal. Therefore FISH and RT-PCR methods may be necessary at diagnosis to document the genetic alteration. Secondary cytogenetic abnormalities occur in approximately 40% of cases, with gains of chromosomes 22 and 8 (each occurring in 10–15% of cases), del(7q), and gain of chromosome 21 (in ~5% of cases) most commonly observed [2502]. Trisomy 22 is fairly specific for inv(16)(p13.1q22) cases, being rarely detected with other primary aberrations in AML, whereas gain of chromosome 8 is commonly seen in patients with other primary aberrations. Rare cases of AML and chronic myeloid leukaemia with both inv(16)(p13.1q22) and t(9;22) (q34.1;q11.2) have been reported, and this finding in chronic myeloid leukaemia is usually associated with the accelerated or blast phase of the disease [4379]. Secondary gene mutations are very common in this AML type; present in >90% of cases. Mutations of KIT (most commonly in exons 8 and 17) occur in 30–40% of cases [3078]. Mutations of NRAS (in 45% of cases), KRAS (in 13%), and FLT3 (in 14%) have also been reported [3076]. ASXL2 mutations, although common in AML with t(8;21), are uncommon in AML with inv(16) or t(16;16) [2657].

Prognosis and predictive factors
Like AML with t(8;21)(q22;q22.1), AML with inv(16)(p13.1q22) or t(16;16) (p13.1;q22) is associated with a high rate of complete remission and favourable overall survival when treated with intensive consolidation therapy (e.g. high-dose cytarabine) [1466,2772].

---

Fig. 8.04 Acute myeloid leukaemia with inv(16)(p13.1q22). A The inversion results from the breakage and rejoining of bands 16p13.1 and 16q22; G-banded normal chromosome 16 (nl) and inv(16) are shown. B Dual-colour FISH, with the 5' region of CBFB labelled in red and the 3' region in green; in a normal chromosome 16, the 5' and 3' regions are contiguous, resulting in a single yellow or overlapping red/green signals; the inv(16) splits the CBFB locus, resulting in separate red and green signals; both interphase cells have one normal chromosome 16 and one inv(16).
Acute promyelocytic leukaemia (APL) are frequently associated with dissemi¬
frequency in elderly patients (3766). The and KIT trisomy 8 are associated with a worse
outcome (3076, 3077). Clinical therapeutic¬
tic investigations testing mutant KIT and
FLT3 in this AML type are under way.

**Acute promyelocytic leukaemia with PML-RARA**

**Definition**
Acute promyelocytic leukaemia (APL) with PML-RARA is an acute myeloid leu¬
kaemia (AML) in which abnormal promye¬
locytes predominate. Both hypergranular (so-called typical) APL and microgranu¬
lar (hypogranular) types exist.

**ICD-O code**
9866/3

**Synonyms**
Acute promyelocytic leukaemia, t(15;17) (q22;q11-12), PML-RARA; acute myeloid leukaemia, t(15;17)(q22;q11-12), PML¬
RARA; acute promyelocytic leukaemia, M3; acute myeloid leukaemia,
PML/RARA-alpha; acute promyelo¬
cytic leukaemia, PML-RARA-alpha

**Epidemiology**
APL accounts for 5–8% of AML cases in younger patients, with a lower relative fre¬
quency in elderly patients (3766). The disease can occur at any age, but most pa¬
patients are middle-aged adults. The annual incidence rate is 0.08 cases per
100 000 population (3113).

**Clinical features**
Both hypergranular and microgranular APL are frequently associated with dis¬
seminated intravascular coagulation and increased fibrinolysis (454, 3519). Co¬
agulopathy is associated with significant early death rates in APL patients (3282).
In microgranular APL, unlike hypergranu¬
lar APL, the leukocyte count is very high, with a short doubling time.

**Microscopy**
The nuclear size and shape in the ab¬
normal promyelocytes of hypergranular
APL are irregular and greatly variable; the abnormal promyelocyte nuclei are often kidney-shaped or bilobed. The cyto¬
plasm is marked by densely packed or even coalescent large granules, staining bright pink, red, or purple on Romanowsky staining. The cytoplasmic granules may be so large and/or numerous that they totally obscure the nuclear–cyto¬
plasmic margin. In some cells, the cyto¬
plasm is filled with fine dust-like granules. Characteristic cells containing bundles of
Auer rods randomly distributed within the cytoplasm are present in most cases. My¬
eloblasts with single Auer rods may also be observed. Auer rods in hypergranu¬
lar APL are usually larger than those in other types of AML, and they may have a characteristic morphology at the ultrastruc¬
tural level, with a hexagonal arrange¬
ment of tubular structures with a specific periodicity of approximately 250 nm, in contrast to the 6–20 laminar periodicity of Auer rods in other types of AML. Only occasional obvious leukaemic promye¬
locytes may be observed in the peripheral blood, especially in hypergranular APL, in which the white blood cell count is of¬
ten very low.

Cases of microgranular (hypogranular) APL are characterized by distinct morphological features such as an appar¬
ant paucity or absence of granules, and predominantly bilobed nuclei (1397). The hypogranular appearance of the cyto¬
plasm is due to the submicroscop¬
ic size of the azurophilic granules. This may cause confusion with acute mono¬
cytic leukaemia on Romanowsky-stained preparations; however, a small number of abnormal promyelocytes with clearly vis¬
ible granules and/or bundles of Auer rods can be identified in many cases. The leu¬
ocyte count is frequently markedly ele¬
vated in the microgranular variant of APL, with numerous abnormal microgranular promyelocytes, in contrast to hypergranu¬
lar APL. Abnormal promyelocytes with deeply basophilic cytoplasm have been described mainly in the relapse phase in patients who have been previously treated with tretinoin. The bone marrow is usually hypercellular. The abnormal promyelocytes have relatively abundant cytoplasm with numerous granules; occasionally, Auer rods may be identified in well-prepared specimens. The nuclei are often convoluted.

**Cytochemistry**
The MPO reaction is always strongly positive in all the leukaemic promyelo¬
cytes, with the reaction product covering the entire cytoplasm and often the nucleus. The non-specific esterase reaction is weakly positive in approximately 25% of cases. In cases of microgranular (hypogranular) APL, the MPO reaction is strongly positive in the leukaemic cells, contrasting with the weak or negative reaction in monocytes.

**Immunophenotype**
APL with PML-RARA (hypergranular variant) is characterized by low or absent expression of HLA-DR, CD34, and the leukocyte integrins CD11a, CD11b, and CD18. It shows homogeneous bright expression of CD33 and heterogeneous expression of CD13. Most cases show expression of KIT (CD117), although this is sometimes weak. The granulocytic dif-

---

**Fig. 8.05** Acute promyelocytic leukaemia. A Hypergranular type; in bone marrow smear, there are several abnormal promyelocytes with intense azurophilic granulation; bundles of numerous Auer rods are seen in some of the promyelocytes. B Microgranular variant; in peripheral blood smear, there are several abnormal promyelocytes with lobed, almost cerebriform nuclei; the cytoplasm contains numerous small azurophilic granules; other cells appear sparsely granular.
Differentiation markers CD15 and CD65 are negative or only weakly expressed [3028, 4490], and CD64 expression is common. In cases with microgranular morphology or the bcr3 transcript of the PML-RARA fusion gene, there is frequently expression of CD34 and CD2 by at least some cells [1123]. CD11c can also be expressed in some cells. CD2 expression in APL has been associated with FLT3-JKD [3881]. Expression of CD11b and CD11c can be upregulated after retinoic treatment [1687]. Approximately 10% of APL cases express CD56, which has been associated with a worse outcome [451, 2704]. On immunocytochemistry, antibodies against the PML gene product show a characteristic nuclear multigranular pattern with nucleolar exclusion, in contrast to the speckled, relatively large nuclear bodies seen in normal promyelocytes or the blasts in other types of AML [1142].

Postulated normal counterpart
A myeloid progenitor cell with the potential to differentiate along a granulocytic lineage

Genetic profile
The sensitivity of APL cells to retinoic acid (also called all-trans retinoic acid) has led to the discovery that the RARA gene on 17q21.2 fuses with a nuclear regulatory factor gene on 15q24.1 (PML), giving rise to a PML-RARA fusion gene product [871, 911, 2618]. Rare cases of APL lacking the classic t(15;17)(q21.2;q21.2) on routine cytogenetic studies have been described with complex variant translocations involving chromosomes 15 and 17, with an additional chromosome or with submicroscopic insertion of RARA into PML [3482]. Variants of the RARA translocation in acute leukaemia

A subset of cases, often with morphological features resembling those of APL, show variant translocations involving RARA. The variant fusion partners include ZBTB16 (previously called PLZF) at 11q23.2, NUMA1 at 11q13.4, NPM1 at 5q35.1, and STAT5B at 17q21.2 [4464]. Some cases with variant translocations were initially reported as having APL morphology [3482]. However, the subgroup of cases with t(11;17)(q23.2;q21.2) resulting in ZBTB16-RARA shows some morphological differences, with a predominance of cells with regular nuclei, many granules, usually an absence of Auer rods, an increased number of pelgeroid neutrophils, and strong MPO activity [3482]. The initial cases of APL associated with t(5;17)(q35.1;q21.2) had a predominant population of hypergranular promyelocytes and a minor population of hypogranular promyelocytes; Auer rods

Variant RARA translocations in acute leukaemia

A subset of cases, often with morphological features resembling those of APL, show variant translocations involving RARA. The variant fusion partners include ZBTB16 (previously called PLZF) at 11q23.2, NUMA1 at 11q13.4, NPM1 at 5q35.1, and STAT5B at 17q21.2 [4464]. Some cases with variant translocations were initially reported as having APL morphology [3482]. However, the subgroup of cases with t(11;17)(q23.2;q21.2) resulting in ZBTB16-RARA shows some morphological differences, with a predominance of cells with regular nuclei, many granules, usually an absence of Auer rods, an increased number of pelgeroid neutrophils, and strong MPO activity [3482]. The initial cases of APL associated with t(5;17)(q35.1;q21.2) had a predominant population of hypergranular promyelocytes and a minor population of hypogranular promyelocytes; Auer rods
were not identified by light microscopy (812). Some APL variants, including those with ZBTB16-RARA and STAT5B-RARA fusions, are resistant to tretinoin (2618). APL with t(5;17)(q35.1;q21.2) appears to respond to tretinoin (2618). Cases with these variant translocations should be diagnosed as APL with a variant RARA translocation.

Prognosis and predictive factors
APL has a particular sensitivity to treatment with tretinoin and arsenic trioxide, which act as differentiating agents (574, 3641, 3885). The prognosis for APL treated optimally with tretinoin and an anthracycline was more favourable than that for any other AML cytogenetic subtype. More recently, however, the combination of tretinoin and arsenic trioxide therapy has become the standard therapeutic approach for most patients with an excellent outcome, with anthracycline added for high-risk patients (505, 1116, 2376). Previously reported adverse prognostic factors include hyperleukocytosis, CD56 expression, FLT3-ITD mutation, and older patient age (451, 879, 2704, 3113), but the significance of these features with current therapy is unclear.

Acute myeloid leukaemia with t(9;11)(p21.3;q23.3); KMT2A-MLLT3

Definition
Acute myeloid leukaemia (AML) with t(9;11)(p21.3;q23.3) resulting in KMT2A-MLLT3 fusion is usually associated with monocytic features.

ICD-O code 9897/3

Synonym
Acute myeloid leukaemia with t(9;11)(p22;q23) resulting in KMT2A-MLLT3

Epidemiology
The t(9;11)(p21.3;q23.3) can occur at any age, but is more common in children; it is present in 9–12% of paediatric and 2% of adult AML cases (511, 1236).

Clinical features
Patients may present with disseminated intravascular coagulation. They may have extramedullary myeloid sarcoma and/or tissue infiltration (gingiva, skin).

Microscopy
Cytochemistry
Monoblasts and promonocytes usually show strongly positive non-specific esterase reactions. The monoblasts often lack MPO reactivity.

Immunophenotype
Cases of AML with t(9;11)(p21.3;q23.3) in children are associated with strong expression of CD33, CD65, CD4, and HLA-DR, whereas expression of CD13, CD34, and CD14 is usually low (837). Most AML cases with 11q23.3 abnormalities express the NG2 homologue encoded by CSPG4, a chondroitin sulfate molecule reacting with the anti-7.1 monoclonal antibody (4380). Most adult AML cases with 11q23.3 abnormalities express some markers of monocytic differentiation, including CD14, CD4, CD11b, CD11c, CD64, CD36, and lysozyme, whereas variable expression of markers of immaturity, such as CD34, KIT (CD117), and CD56 has been reported (2783).

Postulated normal counterpart
A haematopoietic progenitor cell of probable haematopoietic stem cell or granulocyte-macrophage progenitor origin (2120).

Genetic profile
Molecular studies have identified a human homologue of the Drosophila trithorax gene designated KMT2A (previously called MLL and HRX) that results in a fusion gene in translocations involving 11q23.3 (203). The KMT2A protein is a histone methyltransferase that assembles in protein complexes that regulate gene transcription via chromatin remodelling. The t(9;11)(p21.3;q23.3), involving MLLT3 (AF9), is the most common KMT2A translocation in AML and appears to define a distinct entity. Secondary cytogenetic abnormalities are common with t(9;11)(p21.3;q23.3), with gain of chromosome 8 most frequently observed (usually MECOM-negative), but do not appear to influence survival (511, 2773). Overexpression of MECOM (also called EVI1) is reported in 40% of cases of AML with t(9;11) (1482). There is evidence that MECOM-positive KMT2A-rearranged AMLs differ genetically, molecularly, morphologically, and immunophenotypically from MECOM-negative KMT2A-rearranged leukaemias (381, 1482).
Variant KMT2A translocations in acute leukaemia

More than 120 different translocations involving KMT2A (previously called MLL) have been described in adult and paediatric acute leukaemia, with 79 translocation partner genes now characterized (2651, 3653). Translocations involving AFF1 (MLL2, AF4), resulting predominantly in lymphoblastic leukaemia, and MLLT3 (AF9), resulting predominantly in AML, are the most common. Other KMT2A translocations that commonly result in AML have MLLT1 (ENL), MLLT10 (AF10), AFDN (MLLT4, AF6), or ELL as partner genes. Other than the KMT2A-ELL fusion resulting from t(11;19)(q23.3;p13.1), which is strongly associated with AML, these fusions occur predominantly in AML but can also be seen in lymphoblastic leukaemia. Some KMT2A translocations in AML are subtle; FISH or other molecular studies may be necessary to identify these variant translocations (3653). MECOM overexpression is common in this group of AMLs, with the highest expression associated with an AFDN (MLLT4) translocation (1482). AML cases with these fusions usually have myelomonocytic or monoblastic morphological and immunophenotypic features. In the past, all of these translocations were encompassed by the category of AML with 11q23.3 abnormalities, but the diagnosis should now include the specific abnormality and should be limited to cases of de novo AML and with 11q23.3 balanced translocations involving KMT2A. For example, a case of AML with KMT2A-MLLT1 fusion should be diagnosed as AML with t(11;19)(q23.3;p13.3). AML that is associated with prior therapy and has a KMT2A translocation, such as t(2;11)(p21;q23.3), should be classified as therapy-related myeloid neoplasm with KMT2A rearrangement. Similarly, AML with myelodysplasia-related changes and a KMT2A translocation, such as t(11;16)(q23.3;p13.3), should be diagnosed as AML with myelodysplasia-related changes.

Prognosis and predictive factors

AML with t(9;11)(p21.3;q23.3) has an intermediate survival, superior to that of AML with other 11q23.3 translocations (2773,3448). Overexpression of MECOM has been reported to be associated with a poor prognosis (1482). Cases with t(9;11) and <20% blasts are not currently classified as AML (although this is controversial), but they may be treated as such if clinically appropriate.

Acute myeloid leukaemia with t(6;9)(p23;q34.1); DEK-NUP214

Definition

Acute myeloid leukaemia (AML) with t(6;9)(p23;q34.1) resulting in DEK-NUP214 is an AML with ≥20% peripheral blood or bone marrow blasts with or without monocytic features. It is often associated with basophilia and multilineage dysplasia (3116,3700).

ICD-O code 9865/3

Epidemiology

The t(6;9)(p23;q34.1) is detected in 0.7–1.8% of AML cases, and occurs in both children and adults, with a median patient age of 13 years in childhood and 35–44 years in studies of younger adults (511,1464,3700,3701,3905).

Clinical features

AML with t(6;9)(p23;q34.1) usually presents with anaemia and thrombocytopenia, and often with pancytopenia. In adults, the presenting white blood cell count is generally lower than in other AML types, with a median white blood cell count of 12 × 10^9/L (3700).

Microscopy

The bone marrow blasts of AML with t(6;9)(p23;q34.1) may have morphological and cytochemical features similar to those of many subtypes of AML (other than acute promyelocytic leukaemia and acute megakaryoblastic leukaemia), most commonly AML with maturation and acute myelomonocytic leukaemia (76,3020,3700). Auer rods are present in approximately one third of cases. Therefore, there are no features specific to the blast cell population in this entity. Marrow and peripheral blood basophilia, defined as ≥2% basophils, is generally uncommon in AML, but is seen in 44–62% of cases of AML with t(6;9)(p23;q34.1). Most cases show evidence of granulocytic and erythroid dysplasia. Ring sideroblasts are present in some cases.

Cytochemistry

Blasts are positive for MPO and can be positive or negative for non-specific esterase.

Immunophenotype

The blasts have a non-specific myeloid immunophenotype, with consistent expression of MPO, CD9, CD13, CD33, CD38, CD123, and HLA-DR (76,707,2191,3020,3700). Most cases also express KIT (CD117), CD34, and CD15; some cases express the monocye-associated marker CD64; and approximately half are TdT-positive. Other lymphoid antigen expression is uncommon. Basophils can be seen as separate clusters of cells positive for CD123, CD33, and CD38 but negative for HLA-DR.

Postulated normal counterpart

A haematopoietic progenitor cell with multilineage potential

Genetic profile

The t(6;9)(p23;q34.1) results in a fusion of DEK on chromosome 6 with NUP214 (also called CAN) on chromosome 9. The resulting nucleoporin fusion protein acts as an aberrant transcription factor and alters nuclear transport by binding to soluble transport factors (3544). The t(6;9) is the sole clonal karyotypic abnormality in the vast majority of cases, but some patients have t(6;9)(p23;q34.1) in association with a complex karyotype (3700). FLT3-ITD mutations are very common in AML with t(6;9)(p23;q34.1), occurring in 69% of paediatric and 78% of adult cases (3020,3700,3905). FLT3-TKD mutation appears to be uncommon in this entity.

Prognosis and predictive factors

In both adults and children, AML with t(6;9)(p23;q34.1) has a generally poor prognosis. Elevated white blood cell
Acute myeloid leukaemia with inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2); GATA2, MECOM

Definition
Acute myeloid leukaemia (AML) with inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2) resulting in deregulated MECOM (also called EVI1) and GATA2 expression is an AML with ≥20% peripheral blood or bone marrow blasts. It is often associated with normal or elevated platelet counts and has increased dysplastic megakaryocytes with unlobed or bilobed nuclei and multilineage dysplasia in the bone marrow [386,3608,3844].

ICD-O code 9869/3

Epidemiology
AML with inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2) accounts for 1–2% of all AML [511,3701]. It occurs most commonly in adults, with no sex predilection.

Clinical features
Patients most commonly present with anaemia and a normal platelet count, but marked thrombocytopenia occurs in 7–22% of cases [1462,3608]. Some patients present with hepatosplenomegaly, but lymphadenopathy is uncommon [3608,3644,3948].

Microscopy
Peripheral blood changes may include hypogranular neutrophils with a pseudo-Pelger–Huet anomaly, with or without associated peripheral blasts. Red blood cell abnormalities are usually mild, without teardrop cells. Giant and hypogranular platelets are common, and bare megakaryocyte nuclei may be present [386]. The bone marrow blasts of AML with inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2) have variable morphological and cytological features; the morphologies of AML without maturation, acute myelomonocytic leukaemia, and acute megakaryoblastic leukaemia are most common [1228,3608]. Multilineage dysplasia of non-blast bone marrow elements is a frequent finding, with dysplastic megakaryocytes being most common [1228,1852,3608]. Megakaryocytes may be normal or increased in number with many small non-lobated or bilobed forms, but other dysplastic megakaryocytic forms may also occur. Dysplasia of maturing erythroid cells and neutrophils is also common. Marrow eosinophils, basophils, and/or mast cells may be increased. The bone marrow biopsy shows increased small non-lobated or bilobed megakaryocytes and sometimes other dysplastic forms. Bone marrow cellularity is variable, with some cases presenting as hypocellular AML. Marrow fibrosis is also variable.

Immunophenotype
Flow cytometry studies show blasts that are positive for CD34, CD33, CD13, KIT (CD117), and HLA-DR; most are CD38-positive, with aberrant CD7 expression frequently observed [2606]. High CD34 expression is more common with inv(3) than with t(3;3) [3323]. A subset of cases may express megakaryocytic markers such as CD41 and CD61. Aberrant expression of lymphoid markers other than CD7 appears to be uncommon [3644].

Postulated normal counterpart
A haematopoietic progenitor cell with multilineage potential

Genetic profile
A variety of abnormalities of the long arm of chromosome 3 occur in myeloid malignancies, with inv(3)(q21.3q26.2) and t(3;3)(q21.3;q26.2) being the most common [2410]. The abnormalities involve the oncogene MECOM at 3q26.2. The inv(3) or t(3;3) repositions a distal GATA2 enhancer to activate MECOM expression, and simultaneously confers GATA2 haploinsufficiency [1480,4413]. MECOM overexpression is not limited to leukaemias with inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2) [2186]. Other cytogenetic aberrations involving 3q26.2, such as t(3;21)(q26.2;q22.1), resulting in a MECOM-RUNX1 fusion and usually seen in therapy-related disease, are not included in this disease category. Secondary karyotypic abnormalities are common with inv(3)(q21.3q26.2) and t(3;3)(q21.3;q26.2); monosomy 7 is most frequent, occurring in more than half of all cases, followed by 5q deletions and complex karyotypes [2410,3608]. Secondary gene mutations are found in virtually all cases of AML with inv(3) or t(3;3). Mutations of genes activating RAS/receptor tyrosine kinase signalling pathways are reported in 98% of cases, with the most common of these mutations affecting NRAS (mutated in 27% of cases), PTPN11 (in 20%), FLT3 (in 13%), KRAS (in 11%), NF1 (in 9%), CBL (in 7%), and KIT (in 2%). Other commonly mutated genes are GATA2 (mutated in 15% of cases), RUNX1 (in 12%), and SF3B1 (in 27%, often with GATA2) [1481,2907]. Patients with BCR-ABL1-positive chronic myeloid leukaemia may acquire inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2), and such a finding indicates an accelerated or blast phase of disease. Cases with both t(9;22)(q34.1;q11.2) and inv(3)(q21.3q26.2) or t(3;3)(q21.3q26.2) at presentation are best considered an aggressive phase of chronic myeloid leukaemia, rather than AML with inv(3) or t(3;3).

Prognosis and predictive factors
AML with inv(3)(q21.3q26.2) or t(3;3)(q21.3q26.2) is an aggressive disease with short survival [1228,2410,3338,3392,3608]. The outcomes for patients...
Acute myeloid leukaemia (megakaryoblastic) with t(1;22) (p13.3;q13.1); RBM15-MKL1

Definition
Acute myeloid leukaemia (AML) with t(1;22)(p13.3;q13.1) resulting in RBM15-MKL1 fusion is an AML generally showing maturation in the megakaryocyte lineage.

ICD-O code 9911/3

Epidemiology
The t(1;22)(p13.3;q13.1) is an uncommon abnormality in AML, occurring in <1% of all cases. It occurs most commonly in infants without trisomy 21 (Down syndrome), with a female predominance. Some cases are congenital [232].

Clinical features
Most of the balanced translocations and inversions discussed in this chapter are more common in adult AML than in pediatric cases. However, AML with t(1;22)(p13.3;q13.1) is a de novo AML restricted to infants and young children (aged ≤3 years), with most cases occurring in the first 6 months of life (median patient age: 4 months). The vast majority of cases present with marked organomegaly, most commonly hepatosplenomegaly. Patients also have anaemia and usually have thrombocytopenia and a moderately elevated white blood cell count.

Microscopy
The peripheral blood and bone marrow blasts of AML with t(1;22)(p13.3;q13.1) are similar to those of acute megakaryoblastic leukaemia (one of the subtypes of AML, NOS). Small and large megakaryoblasts may be present and they may be admixed with more morphologically undifferentiated blast cells with a high N:C ratio, resembling lymphoblasts. The megakaryoblasts are usually medium-sized to large blasts (12–18 μm) with a round, slightly irregular, or indented nucleus with fine reticular chromatin and 1–3 nucleoli. The cytoplasm is basophilic, often agranular, and may show distinct blebs or pseudopod formation. Micromegakaryocytes are common, but dysplastic features of granulocytic and erythroid cells are not usually present. The bone marrow is usually normocellular to hypercellular, with reticulin and collagenous fibrosis usually present. Due to the often dense fibrosis, the pattern of bone marrow infiltration may mimic that of a metastatic tumour [357,567]. The presence of fibrosis may cause difficulties in establishing the presence of ≥20% blast cells in the bone marrow based on the aspirate; correlation with bone marrow biopsy findings may be crucial.

Cytochemistry
Cytochemical staining for Sudan Black B and MPO is consistently negative in the megakaryoblasts.

Immunophenotype
The megakaryoblasts express one or more of the platelet glycoproteins: CD41 (glycoprotein IIb/IIIa), CD61 (glycoprotein IIa), and CD42b (glycoprotein Ib). The myeloid-associated markers CD13 and CD33 may also be positive. CD34, CD45, and HLA-DR are often negative; CD36 is characteristically positive but not specific. Blasts are negative with MPO antibodies. Lymphoid markers and TdT are not expressed. Cytoplasmic expression of CD41 or CD61 is more specific and sensitive than is surface staining.

Postulated normal counterpart
A myeloid progenitor cell with predominant megakaryocytic differentiation

Genetic profile
Cases should show karyotypic evidence of t(1;22)(p13.3;q13.1) or molecular genetic evidence of RBM15-MKL1 fusion. In most cases, t(1;22)(p13.3;q13.1) is the sole karyotypic abnormality. This translo-

with <20% or ≥20% blasts are similarly poor. Cases with inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2) and <20% blasts are not currently classified as AML (although this is controversial), but they may be treated as such if clinically appropriate. Complex karyotype and additional monosomy 7, regardless of blast percentage, are associated with an even worse prognosis in this already poor-prognosis disease [2410,3392].
Acute myeloid leukaemia with BCR-ABL1

Definition
Acute myeloid leukaemia (AML) with BCR-ABL1 (a provisional entity in the current classification) is a de novo AML in which patients show no evidence (either before or after therapy) of chronic myeloid leukaemia (CML). Cases that meet the criteria for mixed-phenotype acute leukaemia, therapy-related myeloid neoplasms, or other AML types with recurrent genetic abnormalities are excluded from this category.

ICD-O code 9912/3

Epidemiology
AML with BCR-ABL1 accounts for <1% of all AMLs and <10% of all BCR-ABL1-positive acute and chronic leukaemias. It occurs primarily in adults, with a possible male predominance [2082,3029,3740].

Clinical features
Patients most commonly present with leukocytosis with a blast predominance and variable presence of anaemia and thrombocytopenia. Compared with patients with myeloid blast transformation of CML, patients with AML with BCR-ABL1 have less frequent splenomegaly and lower peripheral blood basophilia (usually <2% basophils) [2082,3740].

Microscopy
The morphological features of AML with BCR-ABL1 are non-specific; they demonstrate the presence of bone marrow and peripheral blood myeloblasts, with features ranging from those of minimal differentiation to those of granulocytic maturation. Average bone marrow cellularity is reported to be less than that typically seen in blast transformation of CML (80% versus 95–100% in blast crisis), and dwarf megakaryocytes are reported to be less common in AML with BCR-ABL1 than in blast transformation of CML. The non-blast cell myeloid-to-erythroid ratio is reported to be relatively normal compared with the more elevated ratio associated with blast transformation of CML [2082,3029,3740].

Immunophenotype
The limited number of immunophenotypic studies of AML with BCR-ABL1 have demonstrated expression of myeloid antigens (CD13 and CD33) and CD34. Aberrant expression of CD19, CD7, and TdT appears to be common. However, cases meeting the criteria for a mixed phenotype should be diagnosed as mixed-phenotype acute leukaemia with BCR-ABL1 [3029,3740].

Postulated normal counterpart
A haematopoietic progenitor cell with multilineage potential

Genetic profile
All cases demonstrate t(9;22) (q34.1;q11.2) or molecular genetic evidence of BCR-ABL1 fusion. Most cases demonstrate the p210 fusion, with b2a2 and b3a2 fusions being next most common. A minority of reported cases have demonstrated p190 transcripts. In most cases, cytogenetic abnormalities, such as loss of chromosome 7, gain of chromosome 8, and complex karyotypes, are present in addition to t(9;22)(q34.1;q11.2) [2082,3029,3740].

AML-associated mutations, in particular NPM1 and FLT3-ITD, have been reported to be restricted to AML with BCR-ABL1, not occurring in blast transformation of CML, but these mutations are relatively infrequent [2082]. A recent study reported frequent loss of IKZF1 and CDKN2A in AML with BCR-ABL1, as well as cryptic deletions within the IGH and TRG genes. These deletions are also reported in B-lymphoblastic leukaemia with BCR-ABL1, but they do not appear to occur in myeloid blast transformation of CML; if these results are confirmed, such testing may be a useful means of distinguishing between these disorders in the future [2801].

Although some recurrent genetic abnormalities, in particular inv(16)(p13.1q22), have been reported to be acquired in CML at the time of blast transformation [4379], these and other additional genetic abnormalities are reported to occur in de novo AML with BCR-ABL1. These genetic abnormalities include CEBPA and NPM1 mutations, inv(16)(p13.1q22), and inv(3) (q21.3q26.2) [520,1533,2082,3421], all of which, if present at diagnosis, define entities in the category of AML with recurrent genetic abnormalities, which would take precedence over a diagnosis of AML with BCR-ABL1. Late acquisition of BCR-ABL1 fusion in a pre-existing AML has also been reported, and is not considered sufficient for a diagnosis of AML with BCR-ABL1 [3273,3629,4401]. However, despite the ultimate classification of the disorder, therapy targeting the BCR-ABL1 fusion is indicated in cases with this acquired abnormality.

Prognosis and predictive factors
AML with BCR-ABL1 appears to be an aggressive disease, with poor response to traditional AML therapy or tyrosine kinase inhibitor therapy alone [3029,3740]. Recent reports suggest improved survival with tyrosine kinase inhibitor therapy followed by allogeneic haematopoietic cell transplantation [368,520,1171].
Acute myeloid leukaemia with gene mutations

Acute myeloid leukaemia with mutated NPM1

Definition
Acute myeloid leukaemia (AML) with mutated NPM1 carries mutations that usually involve exon 12 of NPM1. Aberrant cytoplasmic expression of NPM1 is a surrogate marker of such mutations [1149]. This AML type frequently has myelomonocytic or monocytic features and typically presents de novo in adults with a normal karyotype.

ICD-O code 9877/3

Synonym
Acute myeloid leukaemia with cytoplasmic nucleophosmin

Epidemiology
NPM1 mutation is one of the most common recurrent genetic lesions in AML [470,1075,1149,1150,3958,4185], and is relatively specific for AML: it occurs in 2–8% of childhood cases and 27–35% of adult cases overall, as well as in 45–64% of adult cases with a normal karyotype [470,590,749,1149,3958,4185]. There is a female predominance.

Clinical features
Patients with AML with mutated NPM1 often have anaemia and thrombocytopenia, and often have higher white blood cell and platelet counts than seen with other AML types [1075]. Cases may show extramedullary involvement; the most frequently affected sites are gingiva, lymph nodes, and skin.

Microscopy
There is a strong association between both acute myelomonocytic and acute monocytic leukaemia and NPM1 mutation [1149,1150]; notably, 80–90% of acute monocytic leukaemias show NPM1 mutation. However, NPM1 mutations are also detected in AML with and without maturation and in pure erythroid leukaemia.

The diagnosis relies on the identification of the genetic lesion by molecular techniques and/or immunohistochemical detection in paraffin sections of aberrant cytoplasmic expression of NPM1 [1150]. Immunostaining with anti-NPM1 antibodies reveals involvement of two or more bone marrow lineages (myeloid, monocytic, erythroid, megakaryocytic) in the vast majority of cases [3083]. The variability of bone marrow cell types showing NPM1 mutation accounts for the wide morphological spectrum of this leukaemia. Multilineage dysplasia, as seen in many cases of AML with myelodysplasia-related changes (AML-MRC), is observed in almost a quarter of cases of de novo AML with mutated NPM1. These cases usually have a normal karyotype, and the blast cells are CD34-negative. The bone marrow is usually markedly hypocellular. In the setting of NPM1 mutation, this type of dysplasia does not result in a worse prognosis, and its presence does not eliminate a diagnosis of AML with mutated NPM1 [975,1145].

Immunophenotype
AML with mutated NPM1 is characterized by high CD33 expression and variable (often low) CD13 expression. KIT (CD117), CD123, and CD110 expression are common [2889]. HLA-DR is often negative. Two major subgroups of...
AML with mutated NPM1 have been described: one with an immature myeloid immunophenotypic profile and one with a monocytic (CD36+, CD64+, CD14+) immunophenotypic profile [2375]. CD34 is negative in most cases but CD34+ cases do occur and have been associated with an adverse prognosis [669,866]. A very small fraction of cells with the immunophenotype of leukaemic stem cells (CD34+, CD38-, CD123+) is detectable by flow cytometry in most patients with AML with mutated NPM1 (2515). The presence of a CD34+/CD25+/CD123+/CD99+ population is reportedly associated with FLT3-ITD mutations [105]. On paraffin sections, immunohistochemical staining for NPM1 shows the characteristic aberrant expression of the protein in the cytoplasm of leukaemic cells [1149]. In contrast, positivity for another major nucleolar protein, nucleolin (also called C23), is restricted to the nucleus of leukaemic cells. Immunohistochemical detection of cytoplasmic NPM1 is predictive of NPM1 mutations [1146], because the mutations cause critical changes (i.e., loss of the nucleolar localization signal and addition of a nuclear export signal) in the structure of native NPM1 protein (characteristically located in the nucleolus), leading to its increased export from the nucleus and aberrant accumulation in the cytoplasm [1140].

Postulated normal counterpart
A haematopoietic progenitor cell

Genetic profile
AML with mutated NPM1 is usually associated with a normal karyotype; however, 5–15% of cases show chromosomal aberrations [1149,1150,1151], including gain of chromosome 8 and del(9q) [3958]. In most AMLs, del(9q) is considered a myelodysplasia-associated abnormality, and was previously used to define AML-MRC. However, this does not appear to be true when NPM1 is mutated, and such cases should be diagnosed as AML with mutated NPM1 [1151]. Other myelodysplasia-associated cytogenetic abnormalities seen in AML-MRC are uncommon when NPM1 is mutated [1151], and such rare cases should continue to be diagnosed as AML-MRC. NPM1 mutations are usually mutually exclusive of the other AMLs with recurrent genetic abnormalities [1148]. Secondary mutations are common in AML with mutated NPM1 and most frequently involve FLT3 and DNMT3A, but mutations of IDH1, KRAS, N-RAS, and cohesion-complex genes are also relatively common [545,1149]. Although NPM1 mutation is a class-defining lesion, it is frequently a later event in leukaemogenesis, commonly secondary to mutations in epigenetic modifiers such as DNMT3A, TET2, IDH1, and IDH2 [809,2126,3665]. NPM1 mutations appear to precede FLT3-ITD [749,3958]. AML with mutated NPM1 shows a distinct gene expression profile (characterized by up-regulation of HOX genes [54,4185]) that differs from that of other AML types, including AML with KMT2A rearrangement [2782]. AML with mutated NPM1 is also characterized by a unique microRNA signature [1304].

Prognosis and predictive factors
AML with mutated NPM1 typically shows a good response to induction therapy [1149]. Cases with a normal karyotype, in the absence of FLT3-ITD mutation, have a characteristically favourable prognosis [470,1075,3560,3577,3958,4185]. Younger patients with a normal karyotype and no FLT3-ITD have a prognosis comparable to that of patients with AML with t(8;21)(q22;q22) or AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22), and may be exempted from allogeneic haematopoietic cell transplantation in first complete remission [1075]. At least in younger adult patients, the coexistence of FLT3-ITD mutations is associated with a poorer prognosis, but these patients still appear to have a better prognosis than do patients with AML with FLT3-ITD and wildtype NPM1, especially when the allelic ratio of FLT3-ITD is low [1278,3233,3561]. The negative prognostic impact of a concomitant FLT3-ITD mutation in older patients (in particular those aged ≥70 years) with AML with mutated NPM1 is less clear [310]. Co-occurrence of NPM1, FLT3-ITD, and DNMT3A mutations has been associated with a particularly poor outcome [2380]. It is unknown whether a NPM1-mutated, FLT3-ITD- negative genotype also confers a favourable prognosis in the rare cases of AML with a chromosomal aberration.

Acute myeloid leukaemia with biallelic mutation of CEBPA

Definition
Acute myeloid leukaemia (AML) with biallelic mutation of CEBPA usually meets the criteria for AML with maturation or AML without maturation, but some cases show myelomonocytic or monoblastic features. This leukaemia usually presents de novo.

ICD-O code 9878/3
First-identified NPM1 mutations (mutations A to F) are shown \(1149\); mutation A is the most frequent, accounting for 70–80% of cases; all mutations result in common changes at the C-terminus (COOH) of the wildtype NPM1 (NPM) protein; these changes (asterisk) consist of replacement of tryptophan(s) at positions 288 and 290 and creation of a new nuclear export signal (NES) motif; both changes are responsible for the increased nuclear export and aberrant protein; these changes (asterisk) consist of replacement of tryptophan(s) at positions 288 and 290 and creation of a new nuclear export signal (NES) motif; both changes are responsible for the increased nuclear export and aberrant cytoplasmic accumulation of mutant NPM1.

**Fig. 8.17** Acute myeloid leukaemia with mutated NPM1. Mutations usually occur at exon 12 of the NPM1 gene; the first-identified NPM1 mutations (mutations A to F) are shown \(1149\); mutation A is the most frequent, accounting for 70–80% of cases; all mutations result in common changes at the C-terminus (COOH) of the wildtype NPM1 (NPM) protein; these changes (asterisk) consist of replacement of tryptophan(s) at positions 288 and 290 and creation of a new nuclear export signal (NES) motif; both changes are responsible for the increased nuclear export and aberrant cytoplasmic accumulation of mutant NPM1.

**Fig. 8.18** Prognosis of acute myeloid leukaemia (AML) according to NPM1, FLT3, and CEBPA mutations. The genotypes NPM1\(^{mut}\)/FLT3-ITD\(^{neg}\) and CEBPA\(^{mut}\) are favourable prognostic markers (A and B). For univariate donor versus no-donor analysis on relapse-free survival for AML with normal karyotype in first complete remission, according to genotype, the donor group was defined by the availability of an HLA-matched related donor. As evidenced by the results of this analysis in cases with the favourable genotype NPM1\(^{mut}\)/FLT3-ITD\(^{neg}\) (C) and those with the adverse genotypes FLT3-ITD\(^{pos}\) and NPM1\(^{mut}\)/CEBPA\(^{mut}\)/FLT3-ITD\(^{neg}\) (D), only cases with adverse genotypes (D) benefit from allogeneic stem cell transplantation. From Schlenk R F et al. \(3560\).

## Epidemiology
Biallelic mutations of CEBPA are reported in 4–9% of children and young adults with AML \(1429,1668,1705,3022,4368\), with a frequency in normal-karyotype AML similar to the overall incidence \(1052,3908\). However, the frequency in older patients is probably lower.

## Clinical features
AML with biallelic mutation of CEBPA tends to be associated with higher haemoglobin levels, lower platelet counts, and lower lactate dehydrogenase levels than does CEBPA-wildtype AML \(1052,1705,3908\). It may also be associated with a lower frequency of lymphadenopathy and myeloid sarcoma \(372,1260\). The diagnosis of AML with biallelic mutation of CEBPA (especially in younger patients) should raise concern and prompt investigation for the possibility of a germline mutation with predisposition to develop AML (see Myeloid neoplasms with germline predisposition, p. 121).

## Microscopy
AML with biallelic mutation of CEBPA has no distinctive morphological features, but the vast majority of cases have features of AML either with or without maturation \(1052,1429,1668\). Multilineage dysplasia is reportedly present in 26% of cases of de novo AML with mutated CEBPA (similar to in AML with mutated NPM1), with no adverse prognostic significance \(211\). Therefore, in the setting of biallelic CEBPA mutations, this type of dysplasia no longer excludes a case from this category.

## Immunophenotype
In earlier studies that did not distinguish between single and double mutations in CEBPA, leukaemic blasts were reported to usually express one or more of the myeloid-associated antigens (CD13, CD33, CD65, CD11b, and CD15). There was usually expression of HLA-DR and CD34 by most blasts. CD7 was present in 50–73% of cases, whereas expression of CD56 or other lymphoid antigens was uncommon \(372,1776,2341\). In contrast, in cases with biallelic mutation of CEBPA, the expression of HLA-DR, CD7, and CD15, was reported to be more frequent than in AML with a single mutation \(1705,2341\). Monocytic markers such as CD14 and CD64 are usually absent.
Postulated normal counterpart
A hematopoietic progenitor cell

Genetic profile
The favourable prognosis associated with CEBPA mutation in AML is now known to be related to biallelic mutations only; therefore, biallelic mutation is now required for assignment to this category [1429,1668,1705,3022,4368]. The biallelic mutation is associated with a specific gene expression profile that is not associated with the single mutation [3908, 4368]. More than 70% of cases of AML with biallelic mutation of CEBPA have a normal karyotype. FLT3-ITD mutations are found in 5–9% of cases [1052,3562, 3908]. GATA2 zinc finger 1 mutations are also associated with biallelic CEBPA mutation, and occur in approximately 39% of cases [1450]. A subset of cases of AML with biallelic mutation of CEBPA have an abnormal karyotype. Similarly to in AML with mutated NPM1, del(9q) is common among this group and does not appear to influence prognosis [3562]. Therefore, detection of biallelic mutation of CEBPA and del(9q) should not place a case into the category of AML with myelodysplasia-related changes. Other myelodysplasia-related cytogenetic abnormalities are less common, although del(11q) in association with biallelic CEBPA mutation has been reported [3562]. Until more data are available, such rare cases should continue to be diagnosed as AML with myelodysplasia-related changes.

Patients with biallelic CEBPA mutations should be evaluated for a familial syndrome (see Myeloid neoplasms with germline predisposition, p. 121).

Prognosis and predictive factors
AML with biallelic mutation of CEBPA is associated with a favourable prognosis, similar to that of AML with inv(16) (p13.1q22) or t(8;21)(q22;q22.1). The influence of FLT3-ITD and GATA2 mutations on prognosis in this group is currently unclear.

Acute myeloid leukaemia with mutated RUNX1

Definition
Acute myeloid leukaemia (AML) with mutated RUNX1 (a provisional entity in the current classification) is a de novo leukaemia with ≥20% bone marrow or peripheral blood blast cells that may have morphological features of most AML, NOS, categories and has a higher frequency among cases with minimal differentiation. The diagnosis of AML with mutated RUNX1 should not be made for cases that fulfill the criteria for other specific AML subtypes in the categories of AML with recurrent genetic abnormalities, therapy-related myeloid neoplasms, or AML with myelodysplasia-related changes.

ICD-O code
9879/3

Epidemiology
RUNX1 mutations are reported to occur in 4–16% of AML cases. Most studies find a higher frequency in older adults (aged >60 years). One study reported a male predominance [3897], but most have not identified a sex predilection [192,1274, 2627,3236,3576,3897]. RUNX1 mutations in AML and myelodysplastic syndrome (MDS) are associated with radiation exposure [1546] and prior alkylating agent chemotherapy [751,1546]; cases associated with alkylating agent chemotherapy are also associated with monosomy 7 or del(7q). Such cases are considered to be therapy-related AML rather than de novo disease. Patients with Fanconi anaemia or congenital neutropenia who develop AML or MDS also frequently carry RUNX1 mutations [3261,3694].

Clinical features
Patients with AML with mutated RUNX1 may have lower haemoglobin and lactate dehydrogenase levels and lower white blood cell and peripheral blood blast cell counts than patients with wildtype RUNX1 [2627,3897]. Cases with a history of prior therapy (in particular radiation therapy), MDS, or a myelodysplastic/myeloproliferative neoplasm may also harbour RUNX1 mutations [1546,2050], but are excluded from this category.

Microscopy
There are no morphological features specific for AML with mutated RUNX1. Although 15–65% of cases of minimally
differentiated AML demonstrate this mutation, most reported cases have other morphological features, including AML with maturation and AML with monocytic or myelomonocytic features [1274,3576,3897].

Immunophenotype
The leukaemic blasts usually express CD13, CD34, and HLA-DR, with variable expression of CD33, monocytic markers, and MPO [3576,3897].

Postulated normal counterpart
A haematopoietic progenitor cell with multilineage potential

Genetic profile
Most RUNX1 mutations are monoallelic, involving the runt homology domain (RHD), spanning exons 3–5, and the transactivation domain (TAD), spanning exons 6–8; they are most commonly frameshift or missense mutations [1274, 2627,3576,3897]. Mutations may occur with karyotypic abnormalities, most commonly trisomies 8 and 13 [976, 1274,3576]. Cases with myelodysplasia-related cytogenetic abnormalities should be classified as AML with myelodysplasia-related changes. The recurrent cytogenetic abnormalities described in this chapter for other specific WHO categories are not commonly associated with RUNX1 mutation, and the presence of such abnormalities takes diagnostic precedence over this provisional entity. Cooperating mutations are common with RUNX1 mutation, commonly involving ASXL1, KMT2A partial tandem duplication (KMT2A-PTD), FLT3-ITD, IDH1 R132, and IDH2 R140 and R172; however, some of these mutations do not appear to be increased versus in RUNX1-wildtype cases. Studies in MDS with mutated RUNX1 find a characteristic mutation signature involving SRSF2, EZH2, STAG2, and ASXL1 [1513,3050], which appears to be similar in AML with mutated RUNX1. Mutations of NPM1, CEBPA, and JAK2 are uncommon in this group [1274,2627,3576,3897]. Cases with both RUNX1 and NPM1 or RUNX1 and biallelic CEBPA mutations should be classified as AML with mutated NPM1 or AML with biallelic mutation of CEBPA, respectively.

A subset of these patients have germline mutations of RUNX1; when RUNX1 mutation is detected, germline studies should be performed or careful family histories obtained. Affected family members may have autosomal dominant thrombocytopenia and dense granule platelet storage pool deficiency, as well as an increased risk of development of AML or MDS (see Myeloid neoplasms with germline predisposition, p. 121) [1291,1390].

Prognosis and predictive factors
In some studies, RUNX1 mutations in AML have been associated with worse overall survival in multivariate analysis [1274,2627,3576,3897]. The combination of RUNX1 and ASXL1 mutations is reported to be associated with an adverse prognosis [3079]. It is unclear whether other cooperating mutations influence the prognosis of this disease group. Improved survival in patients treated with allogeneic haematopoietic cell transplantation has been reported [1274,3897].
<table>
<thead>
<tr>
<th>Molecular genetic alteration</th>
<th>Cytogenetic group</th>
<th>Prognostic significance</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>KIT mutation</strong></td>
<td>t(8;21)(q22;q22.1)</td>
<td>Significantly shorter DFS [3063A, RFS [408A], EFS [408A,2016A,3576B], and OS [408A,523A,2016A,3063A,3095,3576B] and higher CIR [3078] and RI [523A] for patients with KIT mutation (especially in exon 17) than for patients with wildtype KIT in series from the USA [3207A] and Taiwan, China [3653A], no significant difference in CRR [3207A], DFS [3207A], EFS [3653A], RR [3207A,3653A], or OS [3207A,3653A] between paediatric patients with and without KIT mutation; in a Japanese study, significantly shorter DFS and OS and higher RR for paediatric patients with KIT mutation (especially in exon 17) than for those with wildtype KIT [3653A].</td>
</tr>
<tr>
<td><strong>FLT3-ITD</strong></td>
<td>inv(16)(p13.1q22)/t(16;16)(p13.1;q22)</td>
<td>In most studies, no significant difference in R1 [523A], RFS [408A], PFS [1876A], EFS [408A], or OS [408A,523A,1876A,3076,3095] between patients with and without KIT mutation, or in EFS [2016A] or OS [2016A] between patients with and without KIT mutation in exon 17 at codon D816.</td>
</tr>
<tr>
<td><strong>FLT3-ITD</strong></td>
<td>Various abnormal and normal karyotypes combined</td>
<td>Among younger patients (aged &lt;60 years), significantly shorter OS for patients with FLT3-ITD than for patients without FLT3-ITD [3095].</td>
</tr>
<tr>
<td><strong>FLT3-ITD mutant level</strong></td>
<td>Various abnormal and normal karyotypes combined</td>
<td>Increasingly worse RR and OS (but not CRR) with increasing FLT3-ITD mutant level (i.e. the proportion of total FLT3 alleles accounted for by FLT3-ITD) in a comparison of mutant levels in 4 subsets of patients: without FLT3-ITD, with low (1–24%) FLT3-ITD mutant level, with intermediate (25–50%) mutant level, and with high (&gt;50%) mutant level [1278].</td>
</tr>
<tr>
<td><strong>Biallelic CEBPA mutation</strong></td>
<td>Normal karyotype</td>
<td>Significantly higher CRR [3308] and DFS [1705] and longer RFS [3908], and OS [1052,1705,3908] for patients with double CEBPA mutation than for patients with wildtype CEBPA.</td>
</tr>
<tr>
<td><strong>Biallelic CEBPA mutation</strong></td>
<td>Various abnormal and normal karyotypes combined</td>
<td>Significantly longer DFS [1705,3022], EFS [4368], and OS [1429,1705,3022,4368] for patients with double CEBPA mutation than for patients with wildtype CEBPA or single CEBPA mutation.</td>
</tr>
<tr>
<td><strong>Single CEBPA mutation</strong></td>
<td>Normal karyotype</td>
<td>Significantly lower CRR [3908] and shorter DFS [1705] and OS [1705] for patients with single CEBPA mutation than for patients with double CEBPA mutation.</td>
</tr>
<tr>
<td><strong>NPM1 mutation</strong></td>
<td>Normal karyotype</td>
<td>In some studies, significantly higher CRR [3577] and longer DFS [3576], RFS [1075], and EFS [3577] for patients with NPM1 mutation than for patients with wildtype NPM1; in other studies, no significant difference in CRR [258A,408C], RFS [258A,408C,3577], or EFS [258A,408C] between patients with and without NPM1 mutation No consistently observed significant difference in OS between patients with and without NPM1 mutation [258A,408C,1075,3577,3598]. Among older patients (aged ≥60 years), significantly better CRR, DFS, and OS for patients with NPM1 mutation than for patients with wildtype NPM1 [510].</td>
</tr>
<tr>
<td><strong>NPM1 mutation &amp; FLT3-ITD</strong></td>
<td>Normal karyotype</td>
<td>Significantly better CRR [3560], EFS [3577], RFS [1075,3560], and OS [1075,3560,3577,3598] for patients with NPM1 mutation and FLT3-ITD than for patients with NPM1 mutation and FLT3-ITD or with wildtype NPM1 with or without FLT3-ITD.</td>
</tr>
<tr>
<td><strong>RUNX1 mutation</strong></td>
<td>Normal karyotype</td>
<td>Significantly lower CRR [2627]; higher resistant disease rate (1274); and shorter DFS [2627,3897], EFS [1274,2627,3576], and OS [2627,3576,3897] for patients with RUNX1 mutation than for patients with wildtype RUNX1.</td>
</tr>
<tr>
<td>Molecular genetic alteration</td>
<td>Cytogenetic group</td>
<td>Prognostic significance*</td>
</tr>
<tr>
<td>----------------------------</td>
<td>------------------</td>
<td>--------------------------</td>
</tr>
<tr>
<td><strong>RUNX1 mutation</strong></td>
<td>Various abnormal and normal karyotypes combined</td>
<td>Significantly lower CRR (3897), higher resistant disease rate (1274), and shorter DFS (3897), RFS (1274), EFS (1274), and OS (1274,3897) for patients with RUNX1 mutation than for patients with wildtype RUNX1</td>
</tr>
<tr>
<td><strong>RUNX1 mutation</strong></td>
<td>Non-complex karyotype (i.e. 1 or 2 abnormalities and a normal karyotype combined)</td>
<td>Significantly shorter EFS and OS for patients with RUNX1 mutation than for patients with wildtype RUNX1 (3576)</td>
</tr>
<tr>
<td><strong>KMT2A-PJD</strong></td>
<td>Normal karyotype</td>
<td>No difference in CRR, DFS, or OS between patients with and without KMT2A-PJD, either among younger patients (aged &lt;60 years) receiving intensive treatment including autologous stem cell transplantation (4307D) or among older patients (aged ≥60 years) (4307B)</td>
</tr>
<tr>
<td></td>
<td>Various abnormal and normal karyotypes combined</td>
<td>Among younger patients (aged &lt;60 years), significantly shorter OS for patients with KMT2A-PJD than for patients without KMT2A-PJD (3095)</td>
</tr>
<tr>
<td><strong>WT1 mutation</strong></td>
<td>Normal karyotype</td>
<td>Significantly lower CRR (4197A); higher resistant disease rates (4197A), RR (3343B), and CIR (4197A); and shorter DFS (3078A), RFS (4197A), EFS (2104B), and OS (3078A) for patients with WT1 mutation than for patients with wildtype WT1</td>
</tr>
<tr>
<td></td>
<td>Various abnormal and normal karyotypes combined</td>
<td>Significantly worse CRD (but not CRR or OS) (526A,1075A) and higher RR or risk of death during CR (3560) for patients with KMT2A-PJD than for patients without KMT2A-PJD</td>
</tr>
<tr>
<td><strong>TET2 mutation</strong></td>
<td>Normal karyotype</td>
<td>No significant difference in CRR, DFS, or OS between patients with and without TET2 mutation; reported higher CRR for younger patients (aged ≤60 years) with TET2 mutations classified in the ELN intermediate-1 genetic group (but not those in the favourable group) than for those with wildtype TET2</td>
</tr>
<tr>
<td></td>
<td>Various abnormal and normal karyotypes combined</td>
<td>Significantly worse CRR (2648C), DFS (2648C), RR (4290A), EFS (2648C,4290A), and OS (2648C) for patients with TET2 mutations classified in the ELN intermediate-1 genetic group than for those with wildtype TET2</td>
</tr>
<tr>
<td><strong>ASXL1 mutation</strong></td>
<td>Normal karyotype</td>
<td>Significantly worse CRR (2648A), DFS (2648A), EFS (2648A,3576A), and OS (2648A,3576A) for patients with ASXL1 mutation than for patients with wildtype ASXL1</td>
</tr>
<tr>
<td></td>
<td>Various abnormal and normal karyotypes combined</td>
<td>Among older patients (aged ≥60 years), significantly worse CRR, DFS, EFS, and OS for patients with ASXL1 mutation classified in the ELN favourable genetic group than for patients with wildtype ASXL1</td>
</tr>
<tr>
<td><strong>ASXL1 mutation</strong></td>
<td>Intermediate-risk karyotype</td>
<td>Significantly shorter EFS and OS for patients with ASXL1 mutation than for patients with wildtype ASXL1 (3576A)</td>
</tr>
</tbody>
</table>

*Genetic alterations affecting clinical outcome*
### Molecular genetic alteration  
**DNMT3A** mutation

- **Normal karyotype**
  - Among younger patients (aged \( \leq 60 \) years), significantly shorter DFS and OS for patients with **DNMT3A** mutation (mainly **R882**) compared to patients with wildtype **DNMT3A** (2501E).

### Cytogenetic group

- **Normal karyotype**
  - Among older patients (aged \( \geq 60 \) years), significantly shorter DFS and OS for patients with **DNMT3A** R882 mutation compared to patients with wildtype **DNMT3A** (2501E).

- **Non-R882 DNMT3A mutation**
  - Among younger patients (aged \( < 60 \) years), significantly shorter DFS for patients with non-R882 **DNMT3A** mutation compared to patients with wildtype **DNMT3A** (2501E).

- **DNMT3A mutation**
  - Among younger patients (aged \( \leq 60 \) years), significantly higher CRR but no significant difference in RFS, EFS, or OS for patients with **DNMT3A** mutation (R882 and non-R882 combined) compared to patients with wildtype **DNMT3A** (1274C).

- **IDH1 mutation**
  - Among patients with **IDH1** mutation without **FLT3-ITD**, significantly shorter DFS for patients with **IDH1** R132 mutation compared to patients with wildtype **IDH1** and **IDH2**.

- **IDH2** mutation
  - Among patients with **IDH2** mutation without **FLT3-ITD**, significantly shorter DFS for patients with **IDH2** R132 mutation compared to patients with wildtype **IDH1** and **IDH2**.

- **IDH2 R172 mutation**
  - Significantly worse CRR (2501D), RR (2501D), and OS (2501D) compared to patients with wildtype **IDH2**.

- **IDH2 R140 mutation**
  - Among younger patients (aged \( \leq 60 \) years), significantly longer OS for patients with **IDH2** R140Q mutation compared to patients with wildtype **IDH2**.

### Prognostic significance

- **IDH1 and IDH2 mutations combined**
  - Significantly shorter DFS and OS for patients with **IDH1** or **IDH2** mutation compared to patients with wildtype **IDH1 and IDH2** (3078B).

- **IDH1 and IDH2 mutations combined**
  - Among younger patients (aged \( \leq 60 \) years), no significant difference in CRR, DFS, or OS between patients with WT **IDH1** or **IDH2** and patients with either **IDH1** or **IDH2** mutation or with both **IDH1** and **IDH2** (2889A).

- **TP53 alteration (mutation or loss)**
  - Complex karyotype (\( \geq 3 \) abnormalities)
  - Significantly shorter RFS, EFS, and OS for patients with **TP53** alteration or loss compared to patients with wildtype **TP53** (3462A).

- **TP53 mutation**
  - Complex karyotype (\( \geq 5 \) abnormalities)
  - No significant difference in CRR, DFS, or OS between patients with and without **TP53** mutation (439A).

- **TP53 mutation**
  - Abnormalities of chromosome 5, 7, or 17 and/or complex karyotype (\( \geq 5 \) abnormalities)
  - Significantly shorter OS for patients with **TP53** mutation compared to patients with wildtype **TP53** (439A).
<table>
<thead>
<tr>
<th>Molecular genetic alteration</th>
<th>Cytogenetic group</th>
<th>Prognostic significance®</th>
</tr>
</thead>
<tbody>
<tr>
<td>BAALC expression</td>
<td>Normal karyotype</td>
<td>Significantly worse CRR (239B,3594C), primary resistant disease rates (239B), DFS (239A,372,3594C), EFS (239A), RR (239B), CIR (239B), and OS (239A,239B,372,3594C) for patients with high BAALC expression in blood than for patients with low expression. No significant difference in CIR or EFS between paediatric patients with high and low BAALC expression, whereas OS was significantly shorter for patients with high expression in univariate but not multivariate analysis (1620A).</td>
</tr>
<tr>
<td>BAALC expression</td>
<td>Various abnormal and normal karyotypes combined</td>
<td>No significant difference in CIR (1620A), EFS (1620A), or OS (1620A,3763A) between paediatric patients with high and low BAALC expression; in one study (3763A), high expression was associated with significantly shorter CIR.</td>
</tr>
<tr>
<td>ERG expression</td>
<td>Normal karyotype</td>
<td>Significantly worse CRR (2501C,2648B), DFS (2648B), EFS (2501C), CIR (2501A), and OS (2501A,2648B,3594C) for patients with high ERG expression in blood (2501A,2501C) or bone marrow (2648B) than for patients with low expression. No significant difference in CIR, EFS, or OS between paediatric patients with high and low ERG expression (1620A).</td>
</tr>
<tr>
<td>ERG expression</td>
<td>Various abnormal and normal karyotypes combined</td>
<td>No significant difference in CIR, EFS, or OS between paediatric patients with high and low ERG expression (1620A).</td>
</tr>
<tr>
<td>MN1 expression</td>
<td>Normal karyotype</td>
<td>Significantly lower CRR (2217A,3594B); higher RR (1631A); and shorter RFS (1631A), EFS (3594B), and OS (1631A,2217A) for patients with high MN1 expression than for patients with low expression.</td>
</tr>
<tr>
<td>DNMT3B expression</td>
<td>Normal karyotype</td>
<td>Among older patients (aged ≥60 years), significantly lower CRR and shorter DFS and OS for patients with high DNMT3B expression than for patients with low expression (2870A).</td>
</tr>
<tr>
<td>SPARC expression</td>
<td>Normal karyotype</td>
<td>Among younger patients (aged &lt;60 years), significantly lower CRR and shorter DFS and OS for patients with high SPARC expression than for patients with low expression (50A).</td>
</tr>
<tr>
<td>MECOM expression</td>
<td>Normal karyotype</td>
<td>Among younger patients (aged ≤60 years), significantly shorter EFS for patients with high MECOM (EV1) expression than for patients with low expression (1479A).</td>
</tr>
<tr>
<td>MECOM expression</td>
<td>Various abnormal and normal karyotypes combined</td>
<td>Among younger patients (aged ≤60 years), significantly lower CRR and shorter RFS and EFS for patients with high MECOM (EV1) expression than for patients with low expression (1479A).</td>
</tr>
<tr>
<td>MECOM expression</td>
<td>Intermediate-risk karyotype®</td>
<td>Among younger patients (aged ≤60 years), significantly shorter RFS and EFS for patients with high MECOM (EV1) expression than for patients with low expression (1479A).</td>
</tr>
<tr>
<td>MIR181A expression</td>
<td>Normal karyotype</td>
<td>Among younger patients (aged &lt;60 years), significantly better CRR and OS for patients with high MIR181A expression than for patients with low expression (3594A).</td>
</tr>
<tr>
<td>MIR3151 expression</td>
<td>Normal karyotype</td>
<td>Among older patients (aged ≥60 years), significantly shorter DFS and OS for patients with high MIR3151 expression than for patients with low expression (1085A).</td>
</tr>
<tr>
<td>MIR3151 expression</td>
<td>Intermediate-risk karyotype®</td>
<td>Significantly shorter DFS and OS and higher CIR for patients with high MIR3151 expression than for patients with low expression (974B).</td>
</tr>
<tr>
<td>MIR155 expression</td>
<td>Normal karyotype</td>
<td>Significantly lower CRR and shorter DFS and OS for patients with high MIR155 expression than for patients with low expression (2501B).</td>
</tr>
</tbody>
</table>

© The data presented pertain to adult patients, unless otherwise indicated.

® According to the refined UK Medical Research Council criteria (1464).

CIR, cumulative incidence of relapse; CR, complete remission; CRO, complete remission duration; CRR, complete remission rate; DFS, disease-free survival; EFS, event-free survival; ELN, European LeukemiaNet; FLT3-ITD, FLT3 internal tandem duplication; KMT2A-PTD, KMT2A (MLL) partial tandem duplication; OS, overall survival; PFS, progression-free survival; RFS, relapse-free survival; RI, relapse incidence; RR, risk of relapse.

Intermediate-risk karyotype: defined by ELN as ≥3 chromosome abnormalities in the absence of the WHO-designated recurring translocations or inversions, i.e. t(8;21), inv(16) or t(16;16), t(15;17), t(9;11), t(v;11)(v;q23.3), t(6;9), inv(3), or t(3;3). Table prepared by Krzysztof Mrózek.
Acute myeloid leukaemia with myelodysplasia-related changes

Definition
Acute myeloid leukaemia with myelodysplasia-related changes (AML-MRC) is an acute leukaemia with ≥20% peripheral blood or bone marrow blasts with morphological features of myelodysplasia, or occurring in patients with a prior history of a myelodysplastic syndrome (MDS) or myelodysplastic/myeloproliferative neoplasm (MDS/MPN), with MDS-related cytogenetic abnormalities; the specific genetic abnormalities characteristic of acute myeloid leukaemia (AML) with recurrent genetic abnormalities are absent. Patients should not have a history of prior cytotoxic or radiation therapy for an unrelated disease. Therefore, there are three possible reasons for classifying a case as this subtype (Table 8.03): AML arising from previous MDS or MDS/MPN, AML with an MDS-related cytogenetic abnormality and AML with multilineage dysplasia. A given case may be classified as this subtype for one, two or all three of these reasons.

ICD-O code 9895/3

Synonyms
Acute myeloid leukaemia with multilineage dysplasia; acute myeloid leukaemia with prior myelodysplastic syndrome

Epidemiology
AML-MRC occurs mainly in elderly patients, and is rare in children [1595,2261]. Although the definitions of multilineage dysplasia in the literature vary, this category appears to account for 24 to 35% of all cases of AML [132,885,1514,2682,4276,4416].

Clinical features
AML-MRC often presents with severe pancytopenia. Some cases with 20–29% blasts, especially cases arising from MDS or in childhood, may be slowly progressive. Such cases, with relatively stable peripheral blood counts for weeks or months, are categorized by the French-American-British (FAB) classification as refractory anaemia with excess blasts in transformation, and may have clinical behaviour more similar to that of MDS than that of AML [1590]. Some authors recommended categorizing such cases as a favourable prognostic subtype of AML [1590]. However, this recommendation is controversial. The National Comprehensive Cancer Network (NCCN) Clinical Practice Guidelines for Myelodysplastic Syndromes, which endorse using the WHO classification system, recommend that cases with 20–29% marrow blasts and a stable clinical course for ≥2 months be considered as either MDS or AML, in order for such cases to be eligible for treatment as either entity [1443]. Several important findings support this recommendation: (1) patients with 20–29% marrow blasts have previously been included in and benefited from major therapeutic trials for MDS [1183,1796]; (2) survival and disease evolution were similar in the MDS patient groups with 10–19% and 20–29% marrow blasts [1444]; and (3) molecular and cytogenetic features have been reported to be similar between these two patient groups [210]. Molecular genetic factors (rather than strictly morphological or historical factors) have helped refine the diagnosis of MDS-type AML; a set of gene mutations defines a subset of de novo AML cases, including those that evolved from MDS, with clinical features and therapeutic responses highly specific for MDS [2351].

Microscopy
Most cases in this category of AML have morphological evidence of multilineage dysplasia, which must be assessed on well-stained smears of peripheral blood and bone marrow. For an AML to be classified as having myelodysplasia-related changes based on morphology, dysplasia must be present in ≥50% of the cells in at least two haematopoietic cell lines. Dysgranulopoiesis is characterized by neutrophils with hypogranular cytoplasm, hyposegmented nuclei (pseudo–Pelger–Huet anomaly) or bizarrely segmented nuclei. In some cases, these features can be more readily identified on peripheral blood than bone marrow smears. Dyserythropoiesis is characterized by megaloblastosis, karyorrhexis, and nuclear irregularity, fragmentation or multinucleation. Ring sideroblasts, cytoplasmic vacuoles and periodic acid–Schiff (PAS) positivity are additional features of dyserythropoiesis. Dysmegakaryopoiesis is characterized by micromegakaryocytes and normal-sized or large megakaryocytes with non-lobated or multiple nuclei. Dysplastic megakaryocytes may be more readily appreciated in sections than on smears [132,1270]. Some cases lack sufficient non-blast bone marrow elements to adequately assess for multilineage dysplasia; others have sufficient non-blast cells but do not meet the criteria described above for

Table 8.03 Diagnostic criteria for acute myeloid leukaemia with myelodysplasia-related changes (AML-MRC)

<table>
<thead>
<tr>
<th>Diagnostic criteria for acute myeloid leukaemia with myelodysplasia-related changes (AML-MRC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>The diagnosis of AML-MRC requires that the following 3 criteria are met.</td>
</tr>
<tr>
<td>1. ≥20% blood or marrow blasts</td>
</tr>
<tr>
<td>2. Any of the following:</td>
</tr>
<tr>
<td>- History of myelodysplastic syndrome or myelodysplastic/myeloproliferative neoplasm</td>
</tr>
<tr>
<td>- Myelodysplastic syndrome-related cytogenetic abnormality (Table 8.04)</td>
</tr>
<tr>
<td>- Multilineage dysplasia</td>
</tr>
<tr>
<td>3. Absence of both of the following:</td>
</tr>
<tr>
<td>- Prior cytotoxic or radiation therapy for an unrelated disease</td>
</tr>
<tr>
<td>- Recurrent cytogenetic abnormality as described in Acute myeloid leukaemia with recurrent genetic abnormalities (p. 130)</td>
</tr>
</tbody>
</table>

* Multilineage dysplasia alone is insufficient for a diagnosis of AML-MRC in a de novo case of AML with mutated NPM1 or biallelic mutation of CEBPA (see text for details).
A morphological diagnosis of AML with multilineage dysplasia. These cases are diagnosed as AML-MRC on the basis of detection of MDS-related cytogenetic abnormalities and/or a history of MDS or MDS/MPN.

Differential diagnosis
The principal differential diagnoses are MDS with excess blasts, pure erythroid leukemia, acute megakaryoblastic leukemia and the other categories of AML, not otherwise specified (NOS). Careful blast cell counts, adherence to the diagnostic criteria for morphological dysplasia and evaluation for MDS-related cytogenetic abnormalities should resolve most cases, with this category taking priority over the purely morphological categories of AML, NOS. For example, a case with ≥20% bone marrow megakaryoblasts and multilineage dysplasia should be considered AML-MRC (megakaryoblastic type) if AML with t(1;22)(p13.3;q13,1) is considered AML-MRC (megakaryoblastic type) if AML with t(1;22)(p13.3;q13,1) and multilineage dysplasia should be associated with Down syndrome.

Immunophenotype
Immunophenotyping results are variable due to the heterogeneity of the underlying genetic changes, but an increase in CD11b expression on blasts cells has been reported (4276) and is related to a poor prognosis (739). An increased frequency of CD11b expression has been noted in patients with high-risk and monosomy karyotypes (677,1899,2309). Decreased expression of HLA-DR, KIT (CD117), FLT3 (CD135) and CD38 and increased expression of lactoferrin are reported to be associated with the presence of multilineage dysplasia (2658). In cases with aberrations of chromosomes 5 and 7, a high incidence of CD34, TdT, and CD7 expression has been reported (4172). In cases with antecedent MDS, CD34+ cells frequently constitute only a subpopulation of blasts and may have a stem-cell immunophenotype, with low expression of CD38 and/or HLA-DR. An increase in the fraction of cells with this stem-cell immunophenotype has been correlated with high-risk cytogenetics and poor outcome (3415). Blasts often express panmyeloid markers (CD10, CD33), but aberrantly high or low expression of these markers is common. There is frequently aberrant expression of CD56 and/or CD7 (2934, 2936). The maturing myeloid cells may show patterns of antigen expression differing from those seen in normal myeloid development, and there may be alterations in the light-scattering properties of maturing cells (in particular neutrophils), similar to those described in MDS (see Myelodysplastic syndromes: Overview, p. 98). There is an increased incidence of expression of the multidrug resistance glycoprotein ABCB1 (also called MDR1) in the blast cells (2141,2260,2261).

Postulated normal counterpart
A multipotent haematopoietic stem cell

Genetic profile
The chromosome abnormalities are similar to those found in MDS; they often involve gain or loss of major segments of certain chromosomes, with complex karyotypes, loss of chromosome 7/del(7q), del(5q) and unbalanced translocations involving 5q being most common (2261, 2772,2929). Additional abnormalities that are considered sufficient to include a case in this category are listed in Table 8.04. Although trisomy 8 and del(20q) are also common in MDS, these findings are not considered to be disease-specific and are not by themselves sufficient to classify a case as AML-MRC. Similarly, loss of Y chromosome is a non-specific finding in older men and should not be considered sufficient cytogenetic evidence of this disease category.

Balanced translocations are less common in this disorder, but when they occur they often involve 5q32-33. The presence of t(3;5)(q25.3;q35.1) is associated with multilineage dysplasia and a younger patient age at presentation compared with most other cases in this disease group (127). In addition, AML with inv(3) (q21.3q26.2) or t(3;3)(q21.3;q26.2) and AML with t(6;9)(p23;q34.1) may show evidence of multilineage dysplasia, but these are recognized as distinct entities within the AML with recurrent genetic abnormalities group, and should be classified as such. However, cases with the specific 11q23.3 rearrangements t(11;16) (p13.3;q23.3) and t(2;11)(p21;q23.3), if not associated with prior cytotoxic therapy, should be classified in this group rather than as AML with a variant translocation of 11q23.3.

Cases of AML with multilineage dysplasia may carry NPM1 and/or FLT3 mutations, or mutations of CEBPA (4237). Most NPM1-mutated or CEBPA-double-mutated cases have a normal karyotype and no history of prior MDS (3664) and have a prognosis similar to that of cases

<table>
<thead>
<tr>
<th>Balanced abnormalities</th>
</tr>
</thead>
<tbody>
<tr>
<td>t(11;16)(q23.3;p13.3)</td>
</tr>
<tr>
<td>t(3;21)(q26.2;q22.1)</td>
</tr>
<tr>
<td>t(1;3)(p36.3;q21.2)</td>
</tr>
<tr>
<td>t(2;11)(p21;q23.3)</td>
</tr>
<tr>
<td>t(5;12)(p32;q13.2)</td>
</tr>
<tr>
<td>t(5;7)(q32;q11.2)</td>
</tr>
<tr>
<td>t(5;17)(q32;p13.2)</td>
</tr>
<tr>
<td>t(5;10)(q32;p21)</td>
</tr>
<tr>
<td>t(3;5)(q25.3;q35.1)</td>
</tr>
</tbody>
</table>

Unbalanced abnormalities
Loss of chromosome 7 or del(7q)
del(5q) or (5q)
Isochromosome 17q or t(17p)
Loss of chromosome 13 or del(13q)
del(11q)
del(12p) or t(12p)
idic(X)(q13)

<table>
<thead>
<tr>
<th>Complex karyotype (≥3 abnormalities)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unbalanced abnormalities</td>
</tr>
<tr>
<td>Loss of chromosome 7 or del(7q)</td>
</tr>
<tr>
<td>del(5q) or (5q)</td>
</tr>
<tr>
<td>Isochromosome 17q or t(17p)</td>
</tr>
<tr>
<td>Loss of chromosome 13 or del(13q)</td>
</tr>
<tr>
<td>del(11q)</td>
</tr>
<tr>
<td>del(12p) or t(12p)</td>
</tr>
<tr>
<td>idic(X)(q13)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Balanced abnormalities</th>
</tr>
</thead>
<tbody>
<tr>
<td>t(11;16)(q23.3;p13.3)</td>
</tr>
<tr>
<td>t(3;21)(q26.2;q22.1)</td>
</tr>
<tr>
<td>t(1;3)(p36.3;q21.2)</td>
</tr>
<tr>
<td>t(2;11)(p21;q23.3)</td>
</tr>
<tr>
<td>t(5;12)(p32;q13.2)</td>
</tr>
<tr>
<td>t(5;7)(q32;q11.2)</td>
</tr>
<tr>
<td>t(5;17)(q32;p13.2)</td>
</tr>
<tr>
<td>t(5;10)(q32;p21)</td>
</tr>
<tr>
<td>t(3;5)(q25.3;q35.1)</td>
</tr>
</tbody>
</table>

Table 8.04 Cytogenetic abnormalities sufficient for the diagnosis of acute myeloid leukaemia with myelodysplasia-related changes when ≥20% peripheral blood or bone marrow blasts are present and prior therapy has been excluded

Acute myeloid leukaemia with myelodysplasia-related changes (multilineage dysplasia). A The marrow aspirate shows numerous agranular blasts admixed with hypogranular neutrophils with clumped nuclear chromatin and erythroid precursors with irregular nuclear contours; a small, hypolobated megakaryocyte is present at the bottom of the field. B The marrow aspirate shows numerous agranular blasts admixed with hypogranular neutrophils with clumped nuclear chromatin and erythroid precursors with irregular nuclear contours.

<table>
<thead>
<tr>
<th>Chromosomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 and 7</td>
</tr>
<tr>
<td>12 and 20</td>
</tr>
<tr>
<td>15 and 17</td>
</tr>
<tr>
<td>16 and 17</td>
</tr>
<tr>
<td>22 and 11</td>
</tr>
<tr>
<td>3 and 5</td>
</tr>
<tr>
<td>8 and 20</td>
</tr>
<tr>
<td>21 and 12</td>
</tr>
<tr>
<td>23 and 11</td>
</tr>
</tbody>
</table>

Acute myeloid leukaemia with myelodysplasia-related changes (multilineage dysplasia). A The marrow aspirate shows numerous agranular blasts admixed with hypogranular neutrophils with clumped nuclear chromatin and erythroid precursors with irregular nuclear contours; a small, hypolobated megakaryocyte is present at the bottom of the field. B The marrow aspirate shows numerous agranular blasts admixed with hypogranular neutrophils with clumped nuclear chromatin and erythroid precursors with irregular nuclear contours.

<table>
<thead>
<tr>
<th>Genetic profile</th>
</tr>
</thead>
</table>
| The chromosome abnormalities are similar to those found in MDS; they often involve gain or loss of major segments of certain chromosomes, with complex karyotypes, loss of chromosome 7/del(7q), del(5q) and unbalanced translocations involving 5q being most common (2261, 2772,2929). Additional abnormalities that are considered sufficient to include a case in this category are listed in Table 8.04. Although trisomy 8 and del(20q) are also common in MDS, these findings are not considered to be disease-specific and are not by themselves sufficient to classify a case as AML-MRC. Similarly, loss of Y chromosome is a non-specific finding in older men and should not be considered sufficient cytogenetic evidence of this disease category.

Balanced translocations are less common in this disorder, but when they occur they often involve 5q32-33. The presence of t(3;5)(q25.3;q35.1) is associated with multilineage dysplasia and a younger patient age at presentation compared with most other cases in this disease group (127). In addition, AML with inv(3) (q21.3q26.2) or t(3;3)(q21.3;q26.2) and AML with t(6;9)(p23;q34.1) may show evidence of multilineage dysplasia, but these are recognized as distinct entities within the AML with recurrent genetic abnormalities group, and should be classified as such. However, cases with the specific 11q23.3 rearrangements t(11;16) (p13.3;q23.3) and t(2;11)(p21;q23.3), if not associated with prior cytotoxic therapy, should be classified in this group rather than as AML with a variant translocation of 11q23.3.

Cases of AML with multilineage dysplasia may carry NPM1 and/or FLT3 mutations, or mutations of CEBPA (4237). Most NPM1-mutated or CEBPA-double-mutated cases have a normal karyotype and no history of prior MDS (3664) and have a prognosis similar to that of cases.
without multilineage dysplasia [211,1145]. Therefore, such cases are now considered to be AML with mutated NPM1 or AML with biallelic mutation of CEBPA, respectively, rather than AML-MRC. In the absence of NPM1 mutation or biallelic CEBPA mutation, the presence of multilineage dysplasia in the absence of prior MDS or an MDS-related cytogenetic abnormality appears to remain a significantly poor prognostic indicator in adults [975,3442,4276]. However, the presence of an MDS-associated karyotypic abnormality takes diagnostic precedence over the detection of an NPM1 mutation or biallelic CEBPA mutation for classification purposes. Although del(9q) was previously accepted as an MDS-related cytogenetic abnormality, its presence as a sole abnormality in patients with mutation of NPM1 or biallelic mutation of CEBPA does not appear to have prognostic significance [1511,3562], and it is no longer considered diagnostic of AML-MRC. However, the other MDS-related cytogenetic abnormalities are very uncommon in association with NPM1 or biallelic CEBPA mutation, and are diagnostic of AML-MRC even when these mutations are present.

NPM1 mutation and biallelic mutation of CEBPA are fairly uncommon in AML-MRC, but other mutations are reported with variable frequency. These include a variety of MDS-related mutations, such as mutation of U2AF1 and mutations of ASXL1 and TP53, which are more frequent in this entity than in AML, NOS. TP53 mutations are almost always associated with a complex karyotype, but may suggest an even worse prognosis in this generally poor prognostic group [963,964,2943].

**Prognosis and predictive factors**

AML-MRC generally has a poor prognosis, with a lower rate of complete remission than in other AML subtypes [132, 1270,2682,4276,4416]. Although there are no overall prognostic differences between cases with and without prior MDS [132], cases with prior MDS and relatively low blast counts may constitute less clinically aggressive disease. Some cases with prior MDS and 20–29% bone marrow blasts, considered refractory anaemia with excess blasts in transformation in the FAB classification, may behave in a manner more similar to MDS than to other AMLs [1590]. These cases, as well as cases with myelodysplasia and slightly less than 20% blasts, require regular monitoring of peripheral blood counts and bone marrow morphology for changes suggesting disease progression to overt AML.

Although AML-MRC is generally associated with a poor prognosis, several studies have not found morphology to be a significant parameter when using multivariable analysis that also incorporates the results of cytogenetics, with high-risk cytogenetic abnormalities being more significantly associated with prognosis [1514,4237,4416]. Therefore, multilineage dysplasia should be considered a possible indicator of high-risk cytogenetic abnormalities. In the absence of karyotype abnormalities diagnostic of AML-MRC or a history of MDS or MDS/MPN, investigation for mutation of NPM1 and biallelic mutation of CEBPA is essential to exclude these specific AML disease groups that have a more favourable prognosis. In the absence of CEBPA double mutation or NPM1 mutation, the detection of multilineage dysplasia still appears to confer a poor prognosis, although not as poor as the prognosis for cases with high-risk cytogenetic abnormalities [975,3442,4276]. The increasing use of gene mutation panels may be of value in this group to evaluate for other gene mutations with prognostic significance. In one study, ASXL1 mutations were reported in almost half of such cases, and they may be associated with a worse prognosis [963,964].
Therapy-related myeloid neoplasms

Definition
Therapy-related myeloid neoplasms (t-MNs) include therapy-related cases of acute myeloid leukaemia (t-AML), myelodysplastic syndromes (t-MDS), and myelodysplastic/myeloproliferative neoplasms (t-MDS/MPN) that occur as a late complication of cytotoxic chemotherapy and/or radiation therapy administered for a prior neoplastic or non-neoplastic disorder. Although cases may be diagnosed morphologically as t-MDS, t-MDS/MPN, or t-AML according to the number of blasts in the blood and/or bone marrow, these t-MNs are best considered together as a unique clinical syndrome distinguished by prior iatrogenic exposure to mutagenic agents [756,2221,3435,3709]. Excluded from this category are progression of myeloproliferative neoplasms (MPNs) and evolution of primary MDS or primary MDS/MPN to AML (so-called ‘secondary’ AML); in each of these latter cases evolution to AML is part of the natural history of the primary disease and it may be impossible to distinguish natural progression from therapy-induced changes.

ICD-O code 9920/3

Synonyms
Therapy-related acute myeloid leukaemia, alkylating agent–related; therapy-related acute myeloid leukaemia, epipodophyllotoxin-related; therapy-related acute myeloid leukaemia, NOS

Epidemiology
T-MNs account for 10–20% of all cases of AML, MDS and MDS/MPN [1420,1976,2757]. The incidence of t-MN among patients treated with cytotoxic agents varies depending on the underlying disease and the treatment strategy. Most patients have been treated for a previous malignancy; of these, recently reported data suggest that about 70% have been treated for a solid tumour and 30% for a haematological neoplasm, with breast cancer and non-Hodgkin lymphoma accounting for the largest numbers of cases, respectively, within these two subgroups. However, 5–20% of all cases of t-MN occur following therapy for a non-neoplastic disorder [1211,1976,2757], and a similar proportion of cases occur following high-dose chemotherapy and autologous haematopoietic cell transplantation for a previously treated, non-myeloid neoplasm [367,756,1211,1976,3709]. Any age group can be affected, but the risk associated with alkylating agents or radiation therapy generally increases with age, whereas the risk associated with topoisomerase II inhibitors is similar across all ages [3709]. The incidence increases with increasing age proportionately to the increased prevalence of cancer in older adults. As the number of cancer survivors increases due to improved outcomes for the primary malignancy, the incidence of t-MN is expected to increase.

Etiology
Therapy-related neoplasms are thought to be the consequence of mutation events [367,1813,2351,4360]. The fact that only a small proportion of patients treated with identical protocols develop t-MN suggests that some individuals may have a heritable predisposition due to mutations in DNA damage–sensing or repair genes (e.g. BRCA1/2 or TP53) or polymorphisms in genes that affect drug metabolism, drug transport or DNA-repair mechanisms [69,758,1489,2224,2356]. However, for most cases the underlying pathogenesis remains uncertain. The cytotoxic agents commonly implicated in t-MN are listed in Table 8.05. Other therapies, such as hydroxy carbamide (hydroxyurea), radioisotopes, L-asparaginase, purine analogues and mycophenolate mofetil, have also been suggested to be leukaemogenic, but their primary role in t-MN, if any, is unclear. Adjunctive use of haematopoietic growth factors during chemotherapy reportedly increases the risk [756]. Characteristic clinical, morphological, and genetic features are often related to the therapy previously received, although the common use of multiple classes of agents concurrently and combined modality therapy (i.e. chemotherapy plus radiation therapy) result in overlapping features [1976,3709].

Table 8.05 Cytotoxic agents implicated in therapy-related myeloid neoplasms

<table>
<thead>
<tr>
<th>Alkylating agents</th>
<th>Ionizing radiation therapya</th>
</tr>
</thead>
<tbody>
<tr>
<td>Melphalan, cyclophosphamide, nitrogen mustard, chlorambucil, busulfan, carboplatin, cisplatin, dacarbazine, procarbazine, camustine, mitomycin C, thiopeta, lomustine</td>
<td></td>
</tr>
<tr>
<td>Large fields containing active bone marrow</td>
<td></td>
</tr>
<tr>
<td>Topoisomerase II inhibitorsb</td>
<td>Others</td>
</tr>
<tr>
<td>Etoposide, teniposide, doxorubicin, daunorubicin, mitoxantrone, amsacrine, actinomycin</td>
<td>Anti-metabolites: thiopurines, mycophenolate mofetil, fludarabine</td>
</tr>
<tr>
<td></td>
<td>Antibulin agents (usually in combination with other agents): vincristine, vinblastine, vindesine, paclitaxel, docetaxel</td>
</tr>
</tbody>
</table>

a The incidence of therapy-related acute myeloid leukaemia due to the genetic effects of modern limited-field radiation therapy is unknown [2824];
b Topoisomerase II inhibitors may also be associated with therapy-related lymphoblastic leukaemia.
Localization

The blood and bone marrow are the principal sites of involvement, but initial presentation as an extramedullary myeloid sarcoma has also been reported [369, 3122].

Clinical features

Two subsets of t-MNs are generally recognized clinically [1976,2221,3119,3435,3709]. The more common occurs 5–10 years after exposure to alkylating agents and/or ionizing radiation. These cases often present with an MDS with marrow failure and one or more cytopenias, although a minority present with t-MDS/MPN or overt t-AML. This subset is commonly associated with unbalanced loss of genetic material, often involving chromosomes 5 and/or 7, as well as complex karyotypes and mutations or loss of TP53. The second t-MN subset accounts for 20–30% of cases, has a shorter latent period (1–5 years), and follows treatment with agents that interact with DNA topoisomerase II (topoisomerase II inhibitors). Most cases in this subset do not have a myelodysplastic phase but present with overt acute leukaemia, often associated with a balanced chromosomal translocation. Although it may be useful to consider t-MN cases as being either alkylating agent/radiation-related or topoisomerase II inhibitor-related, many patients have received multiple types of therapy, and the boundaries between these two categories are therefore not always clear [1976]. Most cases of t-MN present within 10 years of the most recent exposure, and the origin of AML in cases with very long latent periods may be unrelated to therapy [1420].

Microscopy

Most patients present with an MDS or acute leukaemia associated with multilineage dysplasia [130,2654,3709,4154,4479]. There is commonly an elicited history of prior therapy with alkylating agents and/or radiation therapy, and cytogenetic studies often reveal abnormalities of chromosomes 5 and/or 7 or a complex karyotype, often associated with mutated TP53 [2351,3253,3652]. The peripheral blood shows one or more cytopenias. Anaemia is almost always present and red blood cell morphology is usually characterized by macrocytosis and poikilocytosis. Dysplastic changes in the neutrophils include abnormal nuclear segmentation and hypgranular cytoplasm. Basophilia is frequently present. The bone marrow may be hypercellular, normocellular, or hypocellular, and reticulin fibrosis is common [852,2654]. Dysgranulopoiesis and dyserythropoiesis are usually present. Most cases show dysplastic megakaryocytes with nonlobated or hypolobated nuclei or widely separated nuclear lobes. Cases presenting with myelodysplasia and cytopenias may be designated as t-MDS or t-AML depending on the blast percentage, but whether the percentage of blasts is prognostically significant in this group with MDS-related features is unclear [208,1211,2124,3686,4479]. About half of the cases that present with a myelodysplastic phase have <5% bone marrow blasts, but they often exhibit poor-risk cytogenetics [2654,3686]. The diagnosis of t-MDS, t-AML, or t-MDS/MPN should include the associated cytogenetic abnormalities.

In 20–30% of cases, the first manifestation of a t-MN is overt acute leukaemia without a preceding myelodysplastic phase [2654,3435,3709]. These cases often occur following topoisomerase II inhibitor therapy, and most are associated with recurrent balanced chromosomal translocations, which frequently involve 11q23 (KMT2A, previously called MLL) or 21q22.1 (RUNX1) and have a morphology resembling that of de novo acute leukaemia associated with these same chromosomal abnormalities, although a few such cases present as MDS or...
have myelodysplastic features as well \(130,852,3435\). Many of these cases have monoblastic or myelomonocytic morphology, but cases morphologically and cytogenetically identical to those observed in any of the subtypes of de novo AML with recurrent cytogenetic abnormalities have been described, including therapy-related acute promyelocytic leukaemia with PML-RARA. Such cases should be designated t-AML with the appropriate cytogenetic abnormality; for example, t-AML with t(9;11)(p21.3;q23.3) \(94,392,2223\).

**Genetic profile**

The leukaemic cells of \(> 90\%\) of patients with t-MN show an abnormal karyotype \(1211,2587,3435,3709\). The cytogenetic abnormalities often correlate with the latent period between the initial therapy and the onset of the leukaemic disorder, as well as with the particular cytotoxic agent(s). Approximately \(70\%\) of patients have an apparently normal karyotype; for example, t-AML with t(9;11)(p21.3;q23.3) \(94,392,2223\).

**Immunophenotype**

There are no specific immunophenotypic findings in t-MN. Immunophenotyping studies of t-MNs reflect the heterogeneity of the underlying morphology and show changes similar to their de novo counterparts \(130,2984\). Blasts are generally CD34 positive \(2984\) and express pan-myeloid antigens such as CD13, CD33, and MPO, although MPO expression has been reported to be downregulated in the neoplastic cells of some patients with t-MN \(3252,4153\). The maturing myeloid cells may show patterns of antigen expression that differ from those seen in normal myeloid development, and there may be alterations in the light-scattering properties of maturing cells (in particular neutrophils) when studied by flow cytometry. Immunohistochemical staining of p53-positive cells in bone marrow biopsies has been demonstrated to correlate well with TP53 mutations and with a poor prognosis \(769,2983\).

**Cell of origin**

An abnormal haematopoietic stem cell

**Prognosis and predictive factors**

The prognosis of t-MN is generally poor, although it is strongly influenced by the associated karyotypic abnormality as well as the comorbidity of the underlying malignancy or illness for which the cytotoxic therapy was administered \(1211,1976,3119,3709\). Overall 5-year survival rates of \(< 10\%\) are commonly reported. Cases associated with abnormalities of chromosomes 5 and/or 7, TP53 mutations, and a complex karyotype have a particularly poor outcome, with a median survival time of \(< 1\) year, regardless of presentation as overt t-AML or as t-MDS \(769,1211,1976,1995,3709\). Cases with balanced chromosomal translocations generally have a better prognosis; however, such cases, except those with t(15;17) and inv(16) or t(16;16), have shorter median survival times than do their de novo counterparts. Patients with therapy-related acute promyelocytic leukaemia (APL) with PML-RARA should be managed with the same degree of urgency as de novo APL \(94,2222,2223,3119\). Occasional cases assigned to the category of t-MN on the basis of the medical history may in fact constitute coincidental disease, and would consequently be expected to behave like de novo disease.
Acute myeloid leukaemia, NOS

Introduction
The category of acute myeloid leukaemia (AML), NOS, encompasses the cases that do not fulfill the criteria for inclusion in one of the previously described groups (i.e. AML with recurrent genetic abnormalities, myelodysplasia-related changes or therapy-related AML). With the possible exception of pure erythroid leukaemia, the subgroups of AML, NOS, are not prognostically significant [132, 3886] when AML with mutated NPM1 and CEBPA are excluded [4233]. Most of the subgroups are nevertheless retained in this classification, because they define criteria for the diagnosis of AML across a diverse morphological spectrum and include the specific diagnostic criteria for pure erythroid leukaemia. Mutation analysis and cytogenetic studies are required before a case can be placed into this category, and such studies offer key prognostic information that appears to be independent of the morphological subtypes. Cytochemical studies are often useful in the subtyping of AML, NOS, but they are not considered essential for diagnosis, given the lack of prognostic significance of the subgroups.

The primary basis for subclassification within this category is the morphological and cytochemical/immunophenotypic features of the leukaemic cells, which indicate the major lineages involved and their degree of maturation. The defining criterion for AML is the presence of ≥20% myeloid blasts in the peripheral blood or bone marrow; the promonocytes in AML with monocytic differentiation are considered blast equivalents. The classification of pure erythroid leukaemia is unique and is based on the percentage of abnormal, immature erythroblasts; by definition, such cases cannot be classified as AML with myelodysplasia-related changes because they have <20% myeloid blasts, but they should be classified as therapy-related myeloid neoplasms or AML with recurrent genetic abnormalities if they fulfill the criteria for one of those entities. The previously recognized subtype of acute erythroid leukaemia termed erythroid/myeloid type, has been eliminated from AML, NOS; cases with myeloblasts accounting for <20% of total bone marrow and peripheral blood cells are now classified as myelodysplastic syndrome, whereas cases with ≥20% myeloblasts continue to be classified according to standard AML criteria. It is recommended that the blast percentage in the bone marrow be determined from a 500-cell differential count using an appropriate Romanowsky stain (e.g. Wright-Giemsa stain). In the peripheral blood, the differential should include 200 leukocytes; if there is marked leukopenia, buffy coat films can be used. If an aspirate film is not obtainable due to bone marrow fibrosis and if the blasts express CD34, immunohistochemical detection of CD34 on biopsy sections may enable the diagnosis of AML if the 20% blast threshold is met. The major criteria required for this diagnosis are based on examination of bone marrow aspirates, peripheral blood smear, and bone marrow biopsy sections. The recommendations for classification apply only to specimens obtained prior to chemotherapy. Please note that the epidemiological data cited for each AML, NOS, subtype have been gathered from studies using the previously described groups (i.e. AML with recurrent genetic abnormalities, AML with myelodysplasia-related changes, or therapy-related AML).

ICD-O code 9861/3

Synonyms
Acute non-lymphocytic leukaemia; acute granulocytic leukaemia; acute myelogenenous leukaemia; acute myelocytic leukaemia

Acute myeloid leukaemia with minimal differentiation

Definition
Acute myeloid leukaemia (AML) with minimal differentiation is an AML with no morphological or cytochemical evidence of myeloid differentiation. The myeloid nature of the blasts is demonstrated by immunological markers, which are essential for distinguishing this entity from lymphoblastic leukaemia. AML with minimal differentiation does not fulfill the criteria for inclusion in any of the previously described groups (i.e. AML with recurrent genetic abnormalities, AML with myelodysplasia-related changes, or therapy-related AML).

ICD-O code 9872/3

Synonyms
Acute myeloblastic leukaemia; French-American-British (FAB) classification M0

Epidemiology
AML with minimal differentiation accounts for <5% of AML cases. It can occur at...
any age, but most patients are either infants or older adults.

**Clinical features**

Patients present with evidence of bone marrow failure, with anaemia, thrombocytopenia, and neutropenia. Some patients present with leukocytosis and numerous circulating blasts.

**Microscopy**

The blasts are usually medium-sized, with dispersed nuclear chromatin and round or slightly indented nuclei with one or two nucleoli. The cytoplasm is agranular, with a variable degree of basophilia. Less frequently, the blasts are small, with more condensed chromatin, inconspicuous nucleoli, and scant cytoplasm resembling that of lymphoblasts. Cytochemical staining for/with MPO, Sudan Black B, and naphthol AS-D chloroacetate esterase (CAE) is negative (i.e. <3% of blasts are positive); alpha-naphthyl acetate esterase and alpha-naphthyl butyrate esterase are either negative or show a non-specific weak or focal reaction distinct from that of monocytic cells [330,1903,3430]. Sensitive ultrastructural studies may reveal MPO and CAE activity in small cytoplasmic granules, endoplasmic reticulum, the Golgi region, and/or nuclear membranes. In some unusual cases, there is a residual normal population of maturing neutrophils; these cases may resemble AML with maturation, but are distinguished by the absence of MPO or Sudan Black B positivity in the blasts and the absence of Auer rods. The bone marrow is usually markedly hypercellular, with poorly differentiated blasts.

**Differential diagnosis**

The differential diagnosis includes lymphoblastic leukaemia, acute megakaryoblastic leukaemia, mixed-phenotype acute leukaemia, acute undifferentiated leukaemia, and (more rarely), the leukemic phase of large cell lymphoma. Immunophenotyping is essential for distinguishing these conditions.

**Immunophenotype**

Most cases express early haematopoietic-associated antigens (e.g. CD34, CD38, and HLA-DR) and lack antigens associated with myeloid and monocytic maturation, such as CD11b, CD15, CD14, and CD65. Blast cells express at least two myeloid-associated markers, usually CD13 and KIT (CD117), and approximately 60% of cases express CD33. CD38 and/or HLA-DR expression may be decreased. There are no signs of monocytic differentiation, such as coexpression of CD64 and CD33. Blasts are negative for the B-cell and T-cell cytoplasmic lymphoid markers cCD3, cCD79a, and cCD22. MPO is negative by cytochemistry, but may be positive in some blasts by flow cytometry or immunohistochemistry. Nuclear TdT is positive in approximately 50% of cases and has been suggested to be of favourable prognostic significance [3096]. CD7 expression has been reported in approximately 40% of cases, but expression of other lymphoid-associated membrane markers is rare. Expression of a single, relatively non-specific myeloid-associated antigen (e.g. CD13 or CD33), especially on only some blasts and along with other markers of primitive cells (e.g. CD7, CD34, and HLA-DR) is more typical of acute undifferentiated leukaemia than of AML with minimal differentiation.

**Cell of origin**

A haematopoietic stem cell

**Genetic profile**

No unique chromosomal abnormality has been identified in AML with minimal differentiation. The most common abnormalities reported are complex karyotypes and unbalanced abnormalities, such as loss of chromosome 5 or del(5q), loss of chromosome 7 or del(7q), gain of chromosome 8, and del(11q), but the presence of some of these abnormalities would place the case in the category of AML with myelodysplasia-related changes. Mutations of **RUNX1** (also called **AML1**) occur in 27% of cases, and 16–22% of cases have **FLT3** mutations, but *de novo* cases with **RUNX1** mutations are now classified as the provisional entity of AML with mutated **RUNX1**.

**ICD-O code**

9873/3

**Synonym**

French–American–British (FAB) classification M1

**Epidemiology**

AML without maturation accounts for 5–10% of AML cases. It can occur at any age, but most patients are adults; the median patient age is about 46 years.

**Clinical features**

Patients usually present with evidence of bone marrow failure, with anaemia, thrombocytopenia, and neutropenia. There may be leukocytosis with markedly increased blasts.

**Acute myeloid leukaemia without maturation**

**Definition**

Acute myeloid leukaemia (AML) without maturation is characterized by a high percentage of bone marrow blasts without significant evidence of maturation to more mature neutrophils; maturing cells of the granulocytic lineage constitute <10% of the nucleated bone marrow cells. The myeloid nature of the blasts is demonstrated by positivity for MPO or Sudan Black B staining (in >3% of blasts) and/or the presence of Auer rods. AML without maturation does not fulfill the criteria for inclusion in any of the previously described groups (i.e. AML with recurrent genetic abnormalities, AML with myelodysplasia-related changes, or therapy-related AML).

**Fig. 8.25 Acute myeloid leukaemia without maturation.**

A On bone marrow smear, the cells are predominantly myeloblasts; occasional myeloblasts contain azurophilic granules or Auer rods; there is no evidence of maturation beyond the myeloblast stage. B MPO reaction reveals numerous myeloblasts with strong peroxidase reactivity; there are several peroxidase-negative erythroid precursors in the centre.
Microscopy
Some cases are characterized by myeloblasts with azurophilic granules and/or Auer rods. In other cases, the blasts resemble lymphoblasts and lack azurophilic granules. Bone marrow biopsy sections are usually markedly hypercellular, although normocellular or hypocellular cases also occur.

Cytochemistry
MPO and Sudan Black B positivity is present in a variable proportion of blasts, but always ≥3%.

Differential diagnosis
The differential diagnosis includes lymphoblastic leukaemia in cases with blast cells lacking granules or that have a low percentage of MPO-positive blasts, and AML with maturation in cases with a higher percentage of MPO-positive blasts.

Immunophenotype
AML without maturation usually presents with a population of blasts expressing MPO and one or more myeloid-associated antigens, such as CD13, CD33, and KIT (CD117). CD34 and HLA-DR are positive in approximately 70% of cases. There is generally no expression of markers associated with granulocytic maturation (e.g., CD15 and CD65) or monocytic markers (e.g., CD14 and CD64). CD11b is expressed in some cases. Blasts are negative for the B-cell and T-cell cytoplasmic lymphoid markers CD3, CD79a, and CD22. CD7 is found in about 30% of cases, and expression of other lymphoid-associated membrane markers (e.g., CD2, CD4, CD19, and CD56) has been described in 10–20% of cases.

Cell of origin
The postulated cell or origin is a haematopoietic stem cell.

Genetic profile
There is no demonstrated association between AML without maturation and specific recurrent chromosomal abnormalities.

Acute myeloid leukaemia with maturation

Definition
Acute myeloid leukaemia (AML) with maturation is characterized by the presence of ≥20% blasts in the bone marrow or peripheral blood and evidence of maturation (with ≥10% of maturing cells of granulocytic lineage) in the bone marrow; cells of monocyte lineage constitute <20% of bone marrow cells. AML with maturation does not fulfill the criteria for inclusion in any of the previously described groups (i.e., AML with recurrent genetic abnormalities, AML with myelodysplasia-related changes, or therapy-related AML).

ICD-O code
9874/3

Synonym
French–American–British (FAB) classification M2, NOS

Epidemiology
AML with maturation accounts for approximately 10% of AML cases (132). It occurs in all age groups; 20% of patients are aged <25 years and 40% are aged ≥60 years (3766).

Clinical features
Patients often present with symptoms related to anaemia, thrombocytopenia, and neutropenia. The white blood cell count is variable, as is the number of blasts.

Microscopy
Blasts with and without azurophilic granulation are present. Auer rods are frequently present. Promyelocytes, myelocytes, and mature neutrophils constitute ≥10% of bone marrow cells; variable degrees of dysplasia are frequently present, but ≤50% of cells in two lineages are dysplastic. Eosinophil precursors are frequently increased, but do not exhibit the cytological or cytochemical abnormalities characteristic of the abnormal eosinophils in acute myelomonocytic leukaemia associated with inv(16)(p13.1q22) or t(16;16)(p13.1;q22). Basophils and/or mast cells are sometimes increased. Bone marrow biopsy sections usually show hypercellularity.

Differential diagnosis
The differential diagnosis includes myelodysplastic syndrome with excess blasts (for cases with a low blast percentage), AML without maturation (when the blast percentage is high), and acute myelomonocytic leukaemia (for cases with increased numbers of monocytes). AML with t(8;21)(q22;q22.1) usually has histological features of AML with maturation, but should be classified according to its genetic abnormality.
Acute myelomonocytic leukaemia

Definition
Acute myelomonocytic leukaemia is an acute leukaemia characterized by the proliferation of both neutrophil and monocytic precursors. The peripheral blood or bone marrow has ≥20% blasts (including promonocytes); neutrophils and their precursors and monocytes and their precursors each constitute ≥20% of bone marrow cells. This conventional minimal limit of 20% monocytes and their precursors distinguishes acute myelomonocytic leukaemia from cases of acute myeloid leukaemia (AML) with or without maturation, in which some monocytes may be present. A high number of monocytic cells may be present in the peripheral blood. Acute myelomonocytic leukaemia does not fulfil the criteria for inclusion in any of the previously described groups (i.e. AML with recurrent genetic abnormalities, AML with myelodysplasia-related changes, or therapy-related AML).

ICD-O code 9867/3

Synonym
French-American-British (FAB) classification M4

Epidemiology
Acute myelomonocytic leukaemia accounts for 5–10% of AML cases. It occurs in all age groups, but is more common in older individuals; the median patient age is 50 years. The male-to-female ratio is 1.4:1 (3766).

Clinical features
Patients typically present with anaemia, thrombocytopenia, fever, and fatigue. The white blood cell count may be high, with numerous blasts and promonocytes.

Microscopy
The monoblasts are large cells with abundant cytoplasm that can be moderately to intensely basophilic and may show pseudopod formation. Scattered fine azurophilic granules, vacuoles, and Auer rods may be present. The monoblasts usually have round nuclei with delicate lacy chromatin and one or more large prominent nucleoli. Promonocytes have a more irregular and delicately convoluted nuclear configuration; the cytoplasm is usually less basophilic and sometimes more obviously granulated, with occasional large azurophilic granules and vacuoles. Monocytes and promonocytes are not always readily distinguishable from maturing myeloid cells in routinely stained bone marrow smears; cytochemistry may be useful in such cases. The peripheral blood typically shows an increase in monocytes, which are often more mature than those in the bone marrow. The monocytic component may be more evident in the peripheral blood than in the bone marrow.

Cytochemistry
At least 3% of the blasts should show MPO positivity. The monoblasts, promonocytes, and monocytes are typically positive for non-specific esterase, although reactivity can be weak or absent in some cases. If the cells meet the morphological criteria for monocytes, absence of non-specific esterase does not exclude this diagnosis. Double staining for non-specific esterase and naphthol AS-D chloroacetate esterase (CAE) or MPO may reveal dual-positive cells.

Differential diagnosis
The major differential diagnoses include AML with maturation, acute monocytic leukaemia, and chronic myelomonocytic leukaemia. Distinction from the other AML types is based on the cytochemical findings and percentage of monocytic cells. The differential diagnosis with chronic myelomonocytic leukaemia is critical; it relies on the proper identification of blasts and promonocytes, which may be increased only in the bone marrow. In some cases, reliance on peripheral blood only may lead to a misdiagnosis of chronic myelomonocytic leukaemia instead of AML.

Immunophenotype
Acute myelomonocytic leukaemia generally shows several populations of blasts variably expressing the myeloid antigens CD13, CD33, CD65, and CD15. One of the blast populations is usually also posi-
Acute monoblastic and monocytic leukaemia

Definition
Acute monoblastic leukaemia and acute monocytic leukaemia are myeloid leukemias in which the peripheral blood or bone marrow has ≥20% blasts (including promonocytes) and in which ≥80% of the leukaemic cells are of monocytic lineage, including monoblasts, promonocytes, and monocytes; a minor neutrophil component (<20%) may be present. Acute monoblastic leukaemia and acute monocytic leukaemia are distinguished by the relative proportions of monoblasts and promonocytes. In acute monoblastic leukaemia, most (≥80%) of the monocytic cells are monoblasts. In acute monocytic leukaemia, most of the monocytic cells are promonocytes or monocytes. Acute monoblastic and monocytic leukaemia does not fulfil the criteria for inclusion in any of the previously described groups; i.e. acute myeloid leukaemia (AML) with recurrent genetic abnormalities, AML with myelodysplasia-related changes, or therapy-related AML.

ICD-O code: 9891/3

Synonyms
Acute monocytic leukaemia; acute monoblastic leukaemia; French-American-British (FAB) classification M5; monoclastic leukaemia, NOS

Epidemiology
Acute monoblastic leukaemia accounts for <5% of cases of AML. It can occur at any age, but is most common in young individuals. Extramedullary lesions can occur.

Acute monocytic leukaemia accounts for <5% of cases of AML. It is more common in adults; the median patient age is 49 years (3766). The male-to-female ratio is 1.8:1.

Clinical features
Patients commonly present with bleeding disorders. Extramedullary masses, cutaneous and gingival infiltration, and CNS involvement are common.

Microscopy
The monoblasts are large cells with abundant cytoplasm that can be moderately to intensely basophilic and may show pseudopod formation. Scattered fine azurophilic granules and vacuoles may be present. The monoblasts usually have round nuclei with delicate lacy chromatin and one or more large prominent nucleoli. Promonocytes have a more irregular and delicately convoluted nuclear configuration; the cytoplasm is usually less basophilic and sometimes more obviously granulated, with occasional large azurophilic granules and vacuoles. Auer rods are rare; when present, they are usually in cells identifiable as myeloblasts. Haemophagocytosis (erythrophagocytosis) may be observed and is often associated with t(8;16)(p11.2;p13.3) [972,1512,3768]. Haemophagocytosis with associated t(8;16)(p11.2;p13.3) may also be observed in acute myelomonocytic leukaemia and some cases of AML with maturation. The bone marrow in acute monoblastic leukaemia is usually hypercellular, with a predominant population of large, poorly differentiated blasts with abundant cytoplasm. Nucleoli may be prominent. The promonocytes in acute monocytic leukaemia show nuclear segmentation. The extramedullary lesion may be composed predominantly of monoblasts or promonocytes or an admixture of two cell types.

Cytochemistry
In most cases, the monoblasts and promonocytes show intense non-specific esterase activity. In as many as 10–20% of cases of acute monoblastic leukaemia, the non-specific esterase reaction is negative or only very weakly positive. In cases in which non-specific esterase is negative, immunophenotyping may be necessary for establishing monocytic differentiation. Monoblasts are typically MPO-negative; promonocytes may show some scattered MPO positivity.

Differential diagnosis
The major differential diagnoses of acute monoblastic leukaemia include AML without maturation, AML with minimal differentiation, AML with t(9;11)(p21.3;q23.3), and acute megakaryoblastic leukaemia. Extramedullary myeloid (monoblastic) sarcoma may be confused with malig-
nant lymphoma or soft tissue sarcomas. Occasional cases resemble prolymphocytic leukaemia; they are readily distinguished by immunophenotyping and cytochemistry. The major differential diagnoses of acute monocytic leukaemia include chronic myelomonocytic leukaemia, acute myelomonocytic leukaemia, and microgranular acute promyelocytic leukaemia. These can be distinguished by careful examination of well-stained smears. The differential diagnosis with chronic myelomonocytic leukaemia is critical; it relies on the proper identification of promonocytes and their inclusion as blast equivalents. The abnormal promyelocytes in acute promyelocytic leukaemia show intense MPO and naphthol AS-D chloroacetate esterase (CAE) positivity, whereas the monocytes are weakly reactive or negative.

Immunophenotype
Flow cytometry shows that these leukaemias variably express the myeloid antigens CD13, CD33 (often very bright), CD15, and CD65. There is generally expression of at least two markers characteristic of monocytic differentiation, such as CD14, CD4, CD11b, CD11c, CD64 (bright), CD68, CD36 (bright), and lysozyme. CD34 is positive in only 30% of cases, whereas KIT (CD117) is more often expressed. Most cases are positive for HLA-DR. MPO can be expressed in acute monocytic leukaemia and less often in monoblastic leukaemia. Aberrant expression of CD7 and/or CD56 is found in 25-40% of cases. By immunohistochemistry in paraffin-embedded bone marrow biopsy specimens and in extramedullary myeloid (monoblastic) sarcomas, MPO and CAE are typically negative but can be weakly positive. Lysozyme is often positive, but is also expressed in AML lacking monocytic differentiation. CD68 (PGM1) and CD163 are often positive.

Cell of origin
The postulated cell of origin is a haematopoietic stem cell.

Genetic profile
Myeloid-associated, non-specific cytogenetic abnormalities are present in most cases. The t(8;16)(p11.2;p13.3) can be associated with acute monocytic leukaemia or acute myelomonocytic leukaemia and in most cases is associated with haemophagocytosis (in particular erythropagocytosis) by leukaemic cells and with coagulopathy [972,3768].

**Pure erythroid leukaemia**

**Definition**
Pure erythroid leukaemia is a neoplastic proliferation of immature cells (undifferentiated or proerythroblastic in appearance) committed exclusively to the erythroid lineage (>80% of the bone marrow cells are erythroid, with ≥30% proerythroblasts), with no evidence of a significant myeloblastic component [2095,2281,2371]. Cases previously classified as erythroblastoid leukaemia (erythroid/myeloid type) on the basis of counting myeloblasts as a percentage of non-erythroid cells when erythroid precursor cells constituted ≥50% of the marrow cells are now classified on the basis of the total bone marrow or peripheral blood blast cell count. Such cases are classified as myelodysplastic syndrome with excess blasts if blasts constitute <20% of all marrow or blood cells, and usually as acute myeloid leukaemia (AML) with myelodysplasia-related changes if blasts constitute ≥20% of the cells, irrespective of the erythroid precursor cell count.

**ICD-O code** 9840/3

**Synonyms**
Acute myeloid leukaemia, M6 type; acute erythroid leukaemia; erythroleukaemia; pure erythroid leukaemia (M6B); erythroleukaemia; French–American–British (FAB) classification M6; erythraemic myelosis, NOS; acute erythraemia; Di Guglielmo disease; acute erythraemic myelosis

**Epidemiology**
Pure erythroid leukaemia is extremely rare. It can occur at any age, including in childhood. It can occur de novo, but more often occurs as progression of a prior myelodysplastic syndrome or as therapy-related disease [2371,4247]. Therapy-related cases should be diagnosed as therapy-related myeloid neoplasms.

**Clinical features**
The clinical features are not unique, but profound anaemia and circulating erythroblasts are common.

**Microscopy**
Pure erythroid leukaemia is characterized by the presence of medium-sized to large erythroblasts, usually with round nuclei, fine chromatin, and one or more nucleoli (proerythroblasts); the cytoplasm is deeply basophilic and agranular and frequently contains vacuoles, which often give a positive periodic acid–Schiff (PAS) reaction. Occasionally, the blasts are smaller, with scanty cytoplasm, and can resemble the lymphoblasts of lymphoblastic leukaemia. The cells are negative for MPO and Sudan Black B staining; they show reactivity with alpha-naphthyl acetate esterase, acid phosphatase, and PAS (with PAS, usually in a block-like staining pattern). In bone marrow biopsy sections of pure erythroid leukaemia, the cells appear undifferentiated and

![Fig. 8.31 Acute monocytic leukaemia, testicular infiltration. Cytoplasm and very dispersed chromatin.](image)

![Fig. 8.32 Pure erythroid leukaemia. Bone marrow smear with numerous very immature erythroid precursors; these cells have cytoplasmic vacuoles, which occasionally coalesce.](image)
The cytoplasm of the proerythroblasts shows intense globular periodic acid-Schiff (PAS) staining. They are large, with finely dispersed chromatin, prominent nucleoli, and cytoplasmic vacuoles, some of which are coalescent.

The immature erythroid precursors and mitotic figures show positivity for glycophorin A.

**Differential diagnosis**

Pure erythroid leukaemia without morphological evidence of erythroid maturation can be difficult to distinguish from other types of AML (in particular acute megakaryoblastic leukaemia). It can also be difficult to distinguish from lymphoblastic leukaemia, lymphoma, and (occasionally) metastatic tumours. Distinction from megakaryoblastic leukaemia is the most difficult; if the immunophenotype is characteristic of erythroid precursors, a diagnosis can be established, but some cases are ambiguous and there may be cases with concurrent erythroid and megakaryocytic differentiation [2942, 4247].

Erythroid leukaemia with some morphological evidence of maturation must be distinguished from reactive erythroid hyperplasia, including hyperplasia secondary to erythroid growth factor administration or megaloblastic anaemia. Pure erythroid leukaemia should not be diagnosed as AML with myelodysplasia-related changes, even if there is a history of prior myeloid neoplasm, significant dysplasia of two lineages, or a defining cytogenetic abnormality: the diagnosis of AML with myelodysplasia-related changes requires ≥20% myeloblasts, whereas the neoplastic cells in pure erythroid leukaemia are erythroblasts.

**Immunophenotype**

The erythroblasts usually express glycophorin and haemoglobin A, as well as the less lineage-specific marker CD71. However, glycophorin and/or haemoglobin can be negative in the presence of poorly differentiated erythroblasts. E-cadherin, which stains early erythroid forms, is positive in the vast majority of cases and is specific for erythroid differentiation [2371, 2942]. The blasts are often positive for CD36 and negative for HLA-DR and CD34. CD36 is positive in most cases, but is not specific for erythroblasts and can be expressed by monocytes and megakaryocytes.

**Prognosis and predictive factors**

Pure erythroid leukaemia is usually associated with a rapid clinical course; the median survival is only 3 months [2371].

**Acute megakaryoblastic leukaemia**

**Definition**

Acute megakaryoblastic leukaemia is an acute leukaemia with ≥20% blasts, of which ≥50% are of megakaryocyte lineage; however, this category excludes cases of acute myeloid leukaemia with myelodysplasia-related changes (AML-MRC), therapy-related acute myeloid leukaemia (AML), and AML with recurrent genetic abnormalities, such as AML associated with t(1;22)(p13.3;q13.1), inv(3)q(q21.3q26.2), or t(3;3)(q21.3;q26.2). Acute megakaryoblastic leukaemia occurring in a patient with Down syndrome should be classified as myeloid leukaemia associated with Down syndrome.

**ICD-O code** 9910/3

**Synonyms**

Megakaryocytic leukaemia; French-American-British (FAB) classification M7

**Epidemiology**

Acute megakaryoblastic leukaemia is an uncommon disease, accounting for <5% of cases of AML. It occurs in both adults and children.

**Clinical features**

Patients present with cytopenias (often thrombocytopenia), although some may have thrombocytosis. Dysplastic features...
in the neutrophils, erythroid precursors, platelets, and megakaryocytes may be present, but the criteria for AML-MRC are not met. An association between acute megakaryoblastic leukaemia and mediastinal germ cell tumours has been observed in young adult males (2865).

**Microscopy**

The megakaryoblasts are usually medium-sized to large blasts (12–18 μm) with a round, slightly irregular, or indented nucleus with fine reticular chromatin and 1–3 nucleoli. The cytoplasm is basophilic, often agranular, and may show distinct blebs or pseudopod formation. In some cases, the blasts are predominantly small with a high N:C ratio, resembling lymphoblasts; large and small blasts may be present in the same patient. Occasionally, the blasts occur in small clusters. Circulating micromegakaryocytes, megakaryoblast fragments, dysplastic large platelets, and hypogranular neutrophils may be present. Micromegakaryocytes should not be counted as blasts. In some patients, because of extensive bone marrow fibrosis resulting in so-called dry tap, the percentage of bone marrow blasts is estimated from the bone marrow biopsy. Imprints of the biopsy may also be useful. Although acute megakaryoblastic leukaemia can be associated with extensive fibrosis, this is not an invariable finding. The histopathology of the biopsy specimen varies; some cases have a uniform population of poorly differentiated blasts and others have a mixture of poorly differentiated blasts and maturing dysplastic megakaryocytes. Various degrees of reticulin fibrosis can be present.

**Cytochemistry**

Cytochemical staining with/s for Sudan Black B, naphthol AS-D chloroacetate esterase (CAE), and MPO is consistently negative in the megakaryoblasts; the blasts may give a positive periodic acid–Schiff (PAS) reaction, show reactivity for acid phosphatase, and show punctate or focal non-specific esterase reactivity.

**Differential diagnosis**

The differential diagnosis includes minimally differentiated AML, AML-MRC, acute panmyelosis with myelofibrosis, lymphoblastic leukaemia, pure erythroid leukaemia, blast transformation of chronic myeloid leukaemia, and the blast phase of any other myeloproliferative neoplasm. In blast transformation of chronic myeloid leukaemia and the blast phase of any other myeloproliferative neoplasm, there is usually a history of a chronic phase, and splenomegaly is an almost invariable finding. Some metastatic tumours in the bone marrow, in particular in children (e.g. alveolar rhabdomyosarcoma), may resemble acute megakaryoblastic leukaemia. In general, acute megakaryoblastic leukaemia constitutes a proliferation predominantly of megakaryoblasts, whereas acute panmyelosis is characterized by a trilineage proliferation of granulocytes, megakaryocytes, and erythroid precursors. In some cases, the distinction between acute megakaryoblastic leukaemia, acute panmyelosis with fibrosis, and AML-MRC can be problematic. In such cases, careful immunohistochemical analysis of bone marrow biopsy can be particularly helpful (2991).

**Immunophenotype**

The megakaryoblast expresses one or more of the platelet glycoproteins: CD41 (glycoprotein IIb/IIIa), CD61 (glycoprotein IIa), and CD42b (glycoprotein Ib). The myeloid-associated markers CD13 and CD33 may be positive. CD34, the panleukocyte marker CD45, and HLA-DR are often negative, especially in children; CD36 is characteristically positive but not specific. Blasts are negative with MPO antibody and with other markers of granulocytic differentiation. Lymphoid markers and TdT are not expressed, but there may be aberrant expression of CD7. Cytoplasmic expression of CD41 or CD61 is more specific and sensitive than is surface staining, due to possible adherence of platelets to blast cells, which can be misinterpreted as positive staining on flow cytometry. In cases with fibrosis, immunophenotyping on bone marrow trephine biopsy sections is particularly important for diagnosis. Megakaryocytes (and in some cases megakaryoblasts)

![Fig. 8.35 Acute megakaryoblastic leukaemia. Bone marrow biopsy section showing virtually complete replacement by a population of blasts; one micromegakaryocyte can be seen.](image_url)

![Fig. 8.36 Acute megakaryoblastic leukaemia. Bone marrow smear (A) and bone marrow section (B) from a 22-month-old child, with complete replacement by poorly differentiated blasts.](image_url)

![Fig. 8.37 Acute megakaryoblastic leukaemia. Bone marrow smear shows two megakaryoblasts, which are large cells with cytoplasmic pseudopod formation; portions of the cytoplasm are zoned, with granular basophilic areas and clear cytoplasm; nucleoli are unusually prominent. B The cytoplasm of the megakaryoblasts is intensely reactive with antibody to CD61 (platelet glycoprotein IIa).](image_url)
Acute basophilic leukaemia

**Definition**

Acute basophilic leukaemia is an acute myeloid leukaemia (AML) in which the primary differentiation is to basophils. Acute basophilic leukaemia does not fulfil the criteria for inclusion in any of the previously described groups (i.e., AML with recurrent genetic abnormalities, AML with myelodysplasia-related changes, or therapy-related AML).

**ICD-O code**

9870/3

**Synonym**

Basophilic leukaemia (no longer used)

**Epidemiology**

Acute basophilic leukaemia is a very rare disease, with a relatively small number of reported cases. It accounts for <% of cases of AML.

**Clinical features**

As with other acute leukaemias, patients present with features related to bone marrow failure and may or may not have circulating blasts. Cutaneous involvement, organomegaly, lytic lesions, and symptoms related to hyperhistaminaemia may be present.

**Microscopy**

The circulating peripheral blood and bone marrow blasts are medium-sized, with a high N:C ratio; an oval, round, or irregular nucleus characterized by dispersed chromatin, and 1–3 prominent nucleoli. The cytoplasm is moderately basophilic and contains a variable number of coarse basophilic granules that are positive with metachromatic staining; there may be vacuolation of the cytoplasm. Immature forms can be seen, but mature basophils are usually sparse. Dysplastic features in the erythroid precursors may be present. Electron microscopy shows that the granules have features characteristic of basophil precursors; they contain an electron-dense particulate substance, are internally bisected (e.g., have a theta character), or contain crystalline material arranged in a pattern of scrolls or lamellae (which is more typical of mast cells) [3148]. The most characteristic cytochemical reaction is metachromatic staining with toluidine blue. The blasts usually show a diffuse pattern of staining with acid phosphatase, and in some cases show periodic acid–Schiff (PAS) positivity in large blocks; the blasts are often negative for naphthol AS-D chloroacetate esterase (CAE), Sudan Black B staining, MPO, and non-specific esterase. The lack of CAE reactivity can be helpful in distinguishing blasts of acute basophilic leukaemia from mast cells [1360]. Bone marrow biopsy shows diffuse replacement by blast cells.

**Differential diagnosis**

The differential diagnosis includes the blast phase of a myeloproliferative neoplasm; other AML subtypes with basophilia, such as AML with t(6;9) (p23;q34.1); AML with BCR–ABL1; mast cell leukaemia; and (more rarely) a subtype of lymphoblastic leukaemia with prominent coarse granules. The clinical features and cytogenetic pattern distinguish cases presenting de novo from those resulting from transformation of chronic myeloid leukaemia and from other AML subtypes with basophilia. Immunological markers distinguish between
granular lymphoblastic leukaemia and acute basophilic leukaemia; cytochemistry and immunphenotyping distinguish acute basophilic leukaemia from other leukaemias.

**Immunophenotype**

Leukaemic blasts express myeloid markers such as CD13 and CD33; are usually positive for CD123, CD203c, and CD11b; and are negative for other monocytic markers and KIT (CD117) \(^{3761}\). Blasts may express CD34. Unlike normal basophils, they may be positive for HLA-DR but are negative for KIT. Immunophenotypic detection of abnormal mast cells expressing KIT, mast cell tryptase, and CD25 distinguishes mast cell leukaemia from acute basophilic leukaemia. Blasts usually express CD9. Some cases are positive for membrane CD22 and/or TdT. Other membrane and cytoplasmic lymphoid-associated markers are usually negative \(^{2322,3762}\).

**Genetic profile**

No consistent chromosomal abnormality has been identified, but a recurrent t(X;6)(p11.2;q23.3) resulting in MYB-GATA1 appears to occur in male infants with acute basophilic leukaemia \(^{372,3260}\). Other cytogenetic abnormalities reported in acute basophilic leukaemia include t(3;6)(q21;p21) and abnormalities involving 12p \(^{1710,1711}\). AML with t(6;9) \(^{p23};q34.1\) is specifically excluded, as are cases associated with BCR-ABL1.

**Prognosis and predictive factors**

There is little information on survival with this rare type of acute leukaemia. The cases observed have generally been associated with a poor prognosis.

### Acute panmyelosis with myelofibrosis

**Definition**

Acute panmyelosis with myelofibrosis (APMF) is an acute panmyeloid proliferation with increased blasts (≥20% of cells in the bone marrow or peripheral blood) and accompanying fibrosis of the bone marrow \(^{307,2991,3831}\). APMF does not fulfil the criteria for inclusion in any of the previously described groups; i.e. acute myeloid leukaemia (AML) with recurrent genetic abnormalities, acute myeloid leukaemia with myelodysplasia-related changes, or therapy-related acute myeloid leukaemia.

**ICD-O code**

9931/3

**Synonyms**

Acute panmyelosis, NOS; acute (malignant) myelofibrosis; acute (malignant) myelosclerosis, NOS; malignant myelosclerosis; acute myelofibrosis; acute myelosclerosis, NOS

**Epidemiology**

APMF is a very rare form of AML. It occurs *de novo* and is primarily a disease of adults, but has also been reported in children.

**Clinical features**

Patients are acutely sick at presentation, with severe constitutional symptoms including weakness and fatigue. Fever and bone pain are also common. Pancytopenia is always present. There is no or only minimal splenomegaly. The clinical evolution is usually rapidly progressive \(^{3986}\).

**Microscopy**

The peripheral blood shows pancytopenia, which is usually marked. The red blood cells show no or minimal anisopoikilocytosis and variable macrocytosis; rare erythroblasts can be seen, but teardrop-shaped cells (dacryocytes) are not observed. Occasional neutrophil precursors, including blasts, may be seen. Dysplastic changes in myeloid cells are frequent, but the criteria for AML with myelodysplasia-related changes are not met. Abnormal platelets may be noted. Bone marrow aspiration is frequently unsuccessful; either no bone marrow can be obtained or the specimen is suboptimal. Bone marrow biopsy supplemented with immunohistochemistry is required for diagnosis \(^{3837,3986}\). The bone marrow is hypercellular and shows, within a
Diffusely fibrotic stroma, increased erythroid precursors, increased granulocyte precursors, and increased megakaryocytes; i.e. there is panmyelosis, which is variable in terms of the relative proportion of each component. Characteristic findings include foci of blasts associated with conspicuously dysplastic megakaryocytes predominately of small size with eosinophilic cytoplasm showing variable degrees of cytological atypia, including the presence of hyposegmented or nonlobated nuclei with dispersed chromatin. Micromegakaryocytes may be present but should not be counted as blasts. The viability of the small megakaryocytes may be improved with periodic acid–Schiff (PAS) staining and immunohistochemistry [3986]. The overall frequency of blasts in APMF marrows is variable; based on bone marrow biopsy, a median value of 22.5% was found in one study [2991]. Most cases have a range of 20–25%. The degree of marrow fibrosis is variable. In most patients, reticulin staining shows markedly increased fibrosis with coarse fibres; diffuse collagenous fibrosis is less common.

Differential diagnosis
The major differential diagnosis of APMF includes other types of AML with associated bone marrow fibrosis, including acute megakaryoblastic leukaemia [2991]. Usually less problematic is the distinction from primary myelofibrosis, post-polycythaemia vera myelofibrosis, post–essential thrombocythaemia myelofibrosis, and other neoplasms that can be encountered in a myelofibrotic bone marrow such as metastatic malignancies with a desmoplastic stromal reaction. It can be difficult to distinguish APMF from AML (especially cases with myelodysplasia-related changes and myelofibrosis) or acute megakaryoblastic leukaemia with myelofibrosis, particularly if no specimen suitable for cytogenetic analysis can be obtained.

If the proliferative process is predominantly of one cell type (i.e. myeloblasts) and there is associated myelofibrosis, the case should be classified as AML with a specific subtype (e.g. myelodysplasia-related) and designated with the qualifying phrase 'with myelofibrosis'. Acute megakaryoblastic leukaemia is associated with the presence of ≥20% blasts, of which ≥50% are megakaryoblasts. Usually they do not express CD34. In contrast, the blasts of APMF are myeloid and poorly differentiated, express CD34, do not express megakaryocytic markers, and are associated with a panmyelotic proliferative process that involves all of the major bone marrow cell lines [2991].

Another difficult distinction is from cases of myelodysplastic syndrome (MDS) with excess blasts associated with marrow fibrosis, which can share most of the morphological findings seen in APMF. However, bone marrow biopsy supplemented by immunohistochemistry reveals more blasts in APMF than in MDS with excess blasts. Clinically, APMF can be distinguished from MDS by its more-abrupt onset with fever and bone pain. APMF is distinguished from primary myelofibrosis by its more numerous blast cells, and the megakaryocytes in primary myelofibrosis show distinctive cytological characteristics. The presence of a metastatic malignancy or (rarely) a lymphoid disorder can be excluded by studies with appropriate antibodies.

Immunophenotype
If the bone marrow specimen obtained for immunological markers is adequate or if circulating blasts are present, the cells show phenotypic heterogeneity, with varying degrees of expression of myeloid-associated antigens. The blasts usually express the progenitor/early precursor marker CD34 and one or more myeloid-associated antigens: CD13, CD33, and KIT (CD117) [2991,3837,3986]. MPO is usually negative in the blasts. In some cases, some of the immature cells express erythroid antigens. Immunohistochemistry can facilitate determination of the relative proportions of the various myeloid components in the biopsy specimen, and is generally used to confirm the multilineage nature of the proliferation. This is usually done using a panel of antibodies that includes MPO, lysozyme, megakaryocytic markers (e.g. CD61, CD42b, CD41, and von Willebrand factor), and erythroid markers (e.g. CD71, glycophorin, and haemoglobin A). These confirm the presence of panmyelosis and allow exclusion of specific unilineage-predominant proliferations, such as acute megakaryoblastic leukaemia.

Cell of origin
The postulated cell of origin is a haematopoietic stem cell. The fibroblastic proliferation is secondary.

Genetic profile
If the specimen obtained for cytogenetic analysis is adequate, the results are usually abnormal. The presence of a complex karyotype, frequently involving chromosomes 5 and/or 7, such as loss of chromosome 5 or del(5q), or loss of chromosome 7 or del(7q) [3986] should result in a diagnosis of AML with myelodysplasia-related changes, rather than acute panmyelosis, as should the presence of any other MDS-related cytogenetic abnormality.

Prognosis and predictive factors
The disease is usually associated with poor response to chemotherapy and survival times of only a few months [2991,3837].
Myeloid sarcoma

Definition
A myeloid sarcoma is a tumour mass consisting of myeloid blasts, with or without maturation, occurring at an anatomical site other than the bone marrow. Infiltration of any site of the body by myeloid blasts in a patient with leukaemia is not classified as myeloid sarcoma unless it presents with tumour masses in which the tissue architecture is effaced.

ICD-O code 9930/3

Synonyms
Granulocytic sarcoma; chloroma; extramedullary myeloid tumour

Epidemiology
There is a predilection for males and older individuals, with a male-to-female of 1.2:1 and a median patient age of 56 years (range: 1 month to 89 years) [1144,3177]. Myeloid sarcoma has been the subject of >2000 case reports indexed in PubMed, but only a few comprehensive studies have been conducted [60,238,1144,2768,2846,3177,3423,4049,4424], which reflects both the rarity of the neoplasm and the difficulties encountered in its treatment.

Etiology
The etiology of myeloid sarcoma is the same as that of acute myeloid leukaemia (AML) and other myeloid neoplasms, such as myelodysplastic syndrome (MDS) and myeloproliferative neoplasm (MPN). Although most cases of myeloid sarcoma occur as de novo neoplasms, some may be therapy-related.

Localization
Almost any site in the body can be involved; the most frequently affected are the skin, lymph nodes, gastrointestinal tract, bone, soft tissue, and testes [1144,3177]. In <10% of cases, myeloid sarcoma presents at multiple anatomical sites [1144,3177].

Clinical features
Myeloid sarcoma occurs in the absence of an underlying AML or other myeloid neoplasm in about one quarter of cases; its detection should be considered equivalent of a diagnosis of AML. It may precede or coincide with AML or constitute acute blast transformation of MDSs, myelodysplastic/myeloproliferative neoplasms (MDS/MPNs), or MPNs [1144,3177]. Isolated myeloid sarcoma occurs in 8–20% of patients who have undergone allogeneic stem cell transplantation [3177]; the reasons for this are unclear, but might be related to graft-versus-leukaemia surveillance or the biology of high-risk AML treated with transplantation.

Myeloid sarcoma can be the initial manifestation of relapse in a patient with previously diagnosed AML, regardless of blood or bone marrow findings [3177]. Myeloid sarcoma can also be associated with a simultaneous or previously treated non-Hodgkin lymphoma (e.g. follicular lymphoma; mycosis fungoides; or peripheral T-cell lymphoma, NOS), or with a previous history of non-haematopoietic tumour (e.g. germ cell tumour, endometrial carcinoma, breast cancer, or intestinal adenocarcinoma) [3177]; in the setting of such a history, myeloid sarcoma might be secondary to prior chemotherapy [3177].

Microscopy
Myeloid sarcoma most commonly consists of myeloblasts with or without features of promyelocytic or neutrophilic maturation. In a significant proportion of cases, it displays myelomonocytic or pure monoblastic morphology [1144, 3177]. Tumours predominantly composed of erythroid precursors or megakaryoblasts are rare and have been reported more often in conjunction with blast transformation of MPN [3177]. Architecturally, at extranodal sites neoplastic cells frequently mimic metastatic carcinoma by forming cohesive nests and/or single files surrounded by fibrotic septa. In the lymph node, they can either infiltrate the paracortex surrounding reactive follicles or grow diffusely, often extending into the perinodal fat.

Cytochemistry
On imprints, cytochemical stains for MPO, naphthol AS-D chloroacetate esterase (CAE), and non-specific esterase may enable differentiation of granulocytic-lineage forms (positive for MPO and CAE) and monoblastic forms (positive for non-specific esterase). In addition, CAE reaction can be applied to routine sections, although the results may depend on fixation and decalcifying agents.

Fig. 8.40 Myeloid sarcoma. The tumour consists of blasts with scant cytoplasm and round-oval nuclei with finely dispersed chromatin and minute but distinct nucleoli. Mitotic figures are numerous. Neoplastic cells strongly express MPO (inset).
Differential diagnosis
The major differential diagnosis is with malignant lymphoma. The diagnosis of myeloid sarcoma is validated by the results of cytochemical and/or immunophenotypic analyses. These allow the distinction of myeloid sarcoma from B- and T-lymphoblastic leukemia/lymphoma, Burkitt lymphoma, diffuse large B-cell lymphoma, small round cell tumors (in particular in children), and blastic plasmacytoid dendritic cell neoplasm [3177]. Myeloid sarcoma must be distinguished from non-effacing extramedullary blastic proliferations, which can occur in AML or in conjunction with acute transformation of MPN, MDS, or MDS/MPN, as well as from extramedullary haematopoiesis following the administration of growth factors that can produce pseudotumoral masses in virtually every part of the body [2915,3177]. Nodular accumulations of mature haematopoietic cells may occur in advanced-stage MPN. Although they may clinically mimic myeloid sarcoma, the often trilineage haematopoiesis and the lack of a significant blast component confirm a diagnosis of extramedullary haematopoiesis (myeloid metaplasia) and exclude myeloid sarcoma [3177, 3232].

Immunophenotype
On immunohistochemistry in paraffin sections, tumors with a more immature myeloid profile express CD33, CD34, CD68 (KP1 but not PGM1), and KIT (CD117). Staining for TdT, MPO, and CD45 is inconsistent [1144,2039,3177, 4424]. Promyelocytic cases lack CD34 and TdT but express MPO and CD15. Myelomonocytic tumors are homogeneously positive for CD68/KP1, with MPO and CD68/PGM1 (or CD163) confined to distinct subpopulations, which are CD34-negative. The monoblastic variant expresses CD68/PGM1 and CD163 but lacks MPO and CD34. CD14 and KLF4 are also useful markers [2915]. The rare erythroid cases show strong positivity for glycophorin A and C, as well as hemoglobin and CD71. Megakaryoblastic myeloid sarcoma expresses CD61, LAT, and von Willebrand factor. CD99 staining is positive in more than half of all cases, with no association with a specific subtype. About 20% of myeloid sarcomas show variable degrees of CD56 expression, irrespective of their myeloid, myelomonocytic, or monoblastic profile. Foci of plasmacytoid dendritic cell differentiation (CD123+, CD303+, but CD56−) are occasionally detected, more often in cases with inv(16) [4424]. About 16% of tumors stain for NPM1 at the nuclear and cytoplasmic level; this indicates the presence of NPM1 mutations [1144]. Exceptionally, aberrant antigenic expressions (cytokeratins, B-cell or T-cell markers, or CD30) are observed [2295,3177]. Cases that meet the criteria for mixed-phenotype acute leukemia are not classified as myeloid sarcoma. Flow cytometric analysis on cell suspensions reveals positivity for CD13, CD33, KIT, and MPO in tumors with myeloid differentiation, and for CD14, CD163, and CD11c in monoblastic tumors.

Postulated normal counterpart
A haematopoietic stem cell

Genetic profile
By FISH and/or cytogenetics, chromosomal aberrations are detected in about 55% of cases. They include monosomy 7; trisomy 8; KMT2A rearrangement; inv(16); trisomy 4; monosomy 16; loss of 16q, 5q, or 20q; and trisomy 11 [3177]. About 16% of cases carry NPM1 mutations, as evidenced by aberrant cytoplasmic NPM1 expression [1144,1149]. Such cases frequently have myelomonocytic or monoblastic morphology, CD34 negativity, normal karyotype, and mutual exclusivity with MDS or MPN [1144,1149]. Interestingly, next-generation sequencing studies show a much higher (>50%) prevalence of NPM1 mutations in AML involving the skin, and 75% of these cases have monocytic features [2419]. The 18;21(q22;q22) observed in paediatric series seems to be less frequent in adulthood [516,3177,3596]. Inv(16) or amplification of CBFB has been linked to breast, uterus, or intestinal involvement and possible foci of plasmacytoid dendritic cell differentiation [60,2475,3177]. Trisomy 8 and KMT2A-MLLT3 fusion seem to have a higher incidence in myeloid sarcoma involving the skin or breasts [60, 3177,4373]. In one series, FLT3 internal tandem duplication (FLT3-ITD) was observed in < 15% of cases [111].

Prognosis and predictive factors
Clinical behaviour and response to therapy do not seem to be influenced by age, sex, anatomical site(s) involved, type of presentation (with or without clinical history of AML, MDS, MDS/MPN, or MPN), therapy-relatedness, histological features, immunophenotype, or cytogenetic findings [3177]. Radiotherapy or surgery is sometimes used upfront in patients who need debulking or rapid symptom relief [238,4424]. Patients who undergo autologous or allogeneic bone marrow transplantation seem to have a higher probability of prolonged survival or cure [452,3177]; in one study, the 5-year overall survival rate among 51 patients with myeloid sarcoma treated with autologous bone marrow transplantation was 47% [706].
Individuals with Down syndrome have an increased risk of leukaemia [1229,4269]. This increased risk is variously estimated at 10- to 100-fold and extends into adulthood. The incidence ratio of lymphoblastic leukaemia to acute myeloid leukaemia (AML) in children aged <4 years with Down syndrome is 1.0:1.2, whereas the ratio in the same age group of children without Down syndrome is 4:1. There is an approximately 150-fold increase in AML in children aged <5 years with Down syndrome; acute megakaryoblastic leukaemia accounts for 70% of cases of AML in children aged <4 years with Down syndrome, versus only 3–6% in children without Down syndrome.

The acute megakaryoblastic leukaemia that occurs in children with Down syndrome has unique morphological, immunophenotypic, molecular genetic, and clinical characteristics that distinguish it from other forms of acute megakaryoblastic leukaemia, including GATA1 mutations [1447,2216,2438,3437]. These features serve as the rationale for the recognition of this form of leukaemia as a distinct type in the WHO classification. In addition, approximately 10% of neonates with Down syndrome manifest a haematological disorder referred to as transient abnormal myelopoiesis (or transient myeloproliferative disorder), which may be morphologically indistinguishable from the predominant form of AML in children with Down syndrome [456,2544]. This disorder resolves spontaneously over a period of several weeks to 3 months. In 20–30% of cases, non-remitting acute megakaryoblastic leukaemia subsequently develops within 1–3 years.

The aforementioned disorders, which occur in specific age groups in individuals with Down syndrome, have received the most attention, but other forms of acute leukaemia (both lymphoblastic leukaemia and AML) also affect individuals with Down syndrome. Such cases should not automatically be classified as myeloid leukaemia associated with Down syndrome; if they meet the diagnostic criteria for leukaemic disorders not associated with Down syndrome, they should be classified as such. The overall increased risk of leukaemia reported for individuals with Down syndrome applies for all types of leukaemias, not just those included in this category. To ensure that appropriate therapy is administered, the same approach for characterizing specific types of leukaemia (including careful morphological, immunophenotypic, cytogenetic, and molecular evaluation) must be used regardless of whether a patient has Down syndrome [4466].

**Transient abnormal myelopoiesis associated with Down syndrome**

**Definition**

Transient abnormal myelopoiesis (TAM) associated with Down syndrome is a unique disorder of newborns with Down syndrome that presents with clinical and morphological findings indistinguishable from those of acute myeloid leukaemia. The blasts have morphological and immunological features of megakaryocytic lineage.

**ICD-O code**

9898/1

**Epidemiology**

TAM is diagnosed in approximately 10% of newborns with Down syndrome, but the true incidence may be higher because a subset of patients are asymptomatic. It uncommonly occurs in phenotypically normal neonates with trisomy 21 mosaicism, and is extremely rare in neonates without chromosome 21 abnormalities [3441,3555].
Clinical features
TAM associated with Down syndrome is usually diagnosed at the age of 3-7 days, but some patients are asymptomatic and diagnosed later [3437,3438]. At presentation, thrombocytopenia is most common; other cytopenias are less frequently encountered. There may be a marked leukocytosis and the percentage of blasts in the peripheral blood may exceed the blast percentage in the bone marrow. Hepatosplenomegaly is often present. Less common clinical features include jaundice, ascites, respiratory distress, bleeding, and pericardial or pleural effusions. Rarely, clinical complications include skin rash, cardiopulmonary failure, hyperviscosity, splenic necrosis, renal failure, hydrops fetalis, and progressive hepatic fibrosis [1009,3438]. In most patients, the process undergoes spontaneous remission within the first 3 months of life; a few children experience life-threatening or fatal clinical complications.

Microscopy
The morphological and immunophenotypic features of TAM are similar to those seen in most cases of acute myeloid leukaemia associated with Down syndrome (2363). Peripheral blood and bone marrow blasts often have basophilic cytoplasm with coarse basophilic granules and cytoplasmic blebbing suggestive of megakaryoblasts. Some patients have peripheral blood basophilia; erythroid and megakaryocytic dysplasia is often present in the bone marrow [456].

Immunophenotype
The blasts in TAM have a characteristic megakaryoblastic immunophenotype [441,1939,2217]. In most cases, they are positive for CD34, KIT (CD117), CD13, CD33, HLA-DR, CD4 (dim), CD41, CD42, CD110 (TPOR), IL3R, CD36, CD61, and CD71, often with aberrant expression of CD7 and CD56. The blasts are negative for MPO, CD15, CD14, CD11a, and glycophorin A. Immunohistochemistry with CD41, CD42b, and CD61 may be particularly useful for identifying blasts of megakaryocytic lineage in bone marrow biopsies.

Postulated normal counterpart
A haematopoietic stem cell

Genetic profile
In addition to trisomy 21, acquired GATA1 mutations are present in blast cells of TAM [1447,1644,4222]. The mutation results in a truncated protein that appears to promote megakaryocytic proliferation [1923,4012]. Gene array studies have suggested differences in expression between myeloid leukaemia associated with Down syndrome and TAM [435, 2327,2596], and mutation studies have shown acquisition of additional mutations in cases that progress to acute leukaemia [4434].

Prognosis and predictive factors
Although the disorder is characterized by a high rate of spontaneous remission, non-transient acute myeloid leukaemia develops 1-3 years later in 20-30% of these children [4500]. Indications for chemotherapy in TAM are not firmly established, but treatment for life-threatening hepatic, renal, or cardiac failure is sometimes necessary [3438].

Myeloid leukaemia associated with Down syndrome

Definition
Among individuals with Down syndrome, the incidence rate of acute leukaemia during the first 5 years of life is 50 times the rate among individuals without Down syndrome. In Down syndrome, most cases of acute myeloid leukaemia (AML) are acute megakaryoblastic leukaemia, which accounts for ≥50% of all cases of acute leukaemia in Down syndrome beyond the neonatal period. AML often follows a prolonged myelodysplastic syndrome (MDS)-like phase [2216]. In individuals with Down syndrome, there are no biological differences between MDS and overt AML; therefore, a comparable diagnostic differentiation algorithm is not relevant and would have no prognostic or therapeutic implications. Because this type of disease is unique to children with Down syndrome, the term 'myeloid leukaemia associated with Down syndrome' encompasses both MDS and AML.

ICD-O code
9898/3

Synonym
Acute myeloid leukaemia associated with Down syndrome

Epidemiology
The vast majority of children with myeloid leukaemia associated with Down syndrome are aged <5 years. About 1-2% of children with Down syndrome develop AML during the first 5 years of life. Children with Down syndrome account for about 20% of all paediatric patients with AML/MDS [456,1009,1587]. Myeloid leukaemia associated with Down syndrome occurs in 20-30% of children with a history of transient abnormal myelopoiesis (TAM), and the leukaemia usually occurs 1-3 years after TAM.

Localization
The blood and bone marrow are the principal sites of involvement. Extramedullary involvement, mainly of the spleen and liver, is almost always present as well.

Fig. 8.45 Myeloid leukaemia associated with Down syndrome. Section of an abdominal lymph node from a child with Down syndrome and acute megakaryoblastic leukaemia; the node is completely replaced by blasts and occasional megakaryocytes, some of which are dysplastic.
Clinical features
The disorder manifests predominantly in the first 3 years of life. The clinical course in children with <20% blast cells in the bone marrow appears to be relatively indolent, initially presenting with a period of thrombocytopenia. A preleukaemic phase comparable to refractory cytopenia of childhood generally precedes MDS with excess blasts or overt leukaemia.

Microscopy
In the preleukaemic phase, which can last for several months, the disease has the features of refractory cytopenia of childhood lacking a significant increase of blasts. Erythroid cells are macrocytic. Dysplastic features may be more pronounced than in primary refractory cytopenia.

In cases of AML, blasts and occasionally erythroid precursors are usually present in the peripheral blood. Erythrocytes often show considerable anisopoikilocytosis, and dacryocytes can be observed. The platelet count is usually decreased, and giant platelets may be observed.

In the bone marrow aspirate, the morphology of the leukaemic blasts shows distinctive features, with round to slightly irregular nuclei and a moderate amount of basophilic cytoplasm; cytoplasmic blebs may be present. The cytoplasm of a variable number of blasts contains coarse granules resembling basophilic granules. The granules are generally MPO-negative. Erythroid precursors often show megaloblastic changes and dysplastic forms, including binucleated or trinucleated cells and nuclear fragments. Dysgranulopoiesis may be present.

The bone marrow core may show a dense network of reticulin fibres, making adequate bone marrow aspiration difficult or impossible. Erythropoiesis may be increased in cases with a low blast percentage, and decreases with disease progression. Maturing cells of neutrophil lineage are usually decreased. In cases with a dense blast cell infiltrate, rare dysplastic megakaryocytes may be seen. In other cases, megakaryocytes may be markedly increased, with clusters of dysplastic small forms and micromegakaryocytes.

Immunophenotype
Leukaemic blasts in acute megakaryocytic leukaemia associated with Down syndrome have an immunophenotype similar to that of blasts in TAM [1939, 2217]. In most cases, the blasts are positive for KIT (CD117), CD13, CD33, CD11b, CD7, CD4, CD42, CD110 (TPOR), IL3R, CD36, CD41, CD61, and CD71, and are negative for MPO, CD15, CD14, and glycoporphin A (4243). But unlike in TAM, CD34 is negative in 50% of cases, and approximately 30% of cases are negative for CD56 and CD41. Leukaemic blasts in other types of AML associated with Down syndrome have phenotypes corresponding to the particular AML category. Antibodies to CD41, CD42b, and CD61 may be particularly useful for identifying cells of megakaryocytic lineage in immunohistochemical preparations.

Postulated normal counterpart
A haematopoietic stem cell

Genetic profile
In addition to trisomy 21, somatic mutations of the gene encoding the transcription factor GATA1 are considered pathognomonic of TAM associated with Down syndrome or MDS/AML-associated with Down syndrome [1447,1644,2438]. Children aged >5 years with myeloid leukaemia may not have GATA1 mutations, and such cases should be considered conventional MDS or AML. Trisomy 8 is a common cytogenetic abnormality in myeloid leukaemia associated with Down syndrome, occurring in 13–44% of patients [1587,1609]. Monosomy 7 is very rare in myeloid leukaemia associated with Down syndrome. Although GATA1 mutations are present in both TAM and myeloid leukaemia associated with Down syndrome, myeloid leukaemias in this setting appear to arise from a GATA1-mutant TAM clone that has acquired additional mutations. Implicated additional mutations include mutations of CTCF, EZH2, KANSL1, JAK2, JAK3, MPL, SH2B3, and RAS pathway genes [1923,2895,3529,4434].

Prognosis and predictive factors
The clinical outcome for young children with Down syndrome and myeloid leukaemia with GATA1 mutations is unique; these cases are associated with a better response to chemotherapy and a very favourable prognosis compared with that of AML in children without Down syndrome [2216]. The children should be treated using Down syndrome–specific protocols. Myeloid leukaemia in older children with Down syndrome with GATA1 mutation has a poorer prognosis, comparable to that of AML in patients without Down syndrome [1287].
CHAPTER 9

Blastic plasmacytoid dendritic cell neoplasm
Blastic plasmacytoid dendritic cell neoplasm

Definition
Blastic plasmacytoid dendritic cell neoplasm (BPDCN) is a clinically aggressive tumour derived from the precursors of plasmacytoid dendritic cells (PDCs, also called professional type I interferon-producing cells or plasmacytoid monocytes), with a high frequency of cutaneous and bone marrow involvement and leukaemic dissemination.

ICD-O code 9727/3

Synonyms
Blastic NK-cell lymphoma (obsolete); agranular CD4+ NK leukaemia (obsolete); blastic NK leukaemia/lymphoma (obsolete); agranular CD4+ CD56+ hematodermic neoplasm/tumour

Epidemiology
This rare form of haematological neoplasm has no known racial or ethnic predilection. The male-to-female ratio is 3:3:1. Most patients are elderly, with a mean/median patient age at diagnosis of 61–67 years, but this neoplasm can occur at any age, including in children (1209,1812).

Etiology
There are currently no clues to the etiology of BPDCN, but its association with myelodysplastic syndrome (MDS) in some cases may suggest a related pathogenesis. There is no association with EBV.

Localization
The disease tends to involve multiple sites, most commonly the skin (involved in 64–100% of cases), followed by the bone marrow and peripheral blood (in 60–90%) and lymph node (in 40–50%) (2525,3151).

Clinical features
Skin manifestations are the most frequent clinical presentation of typical cases of BPDCN, and the diagnosis is made on skin biopsy. Patients usually present with asymptomatic solitary or multiple lesions. Three types of presentation are most commonly observed: an isolated (one or few) purplish nodule type (accounting for two thirds of cases), an isolated (one or few) bruise-like papule type, and a disseminated type with purplish nodules and/or papules and/or macules (820,1887). Isolated nodules are preferentially found on the head and lower limbs and can be > 10 cm in diameter. The isolated bruise-like papule type is clinically very challenging. The disseminated type is the most characteristic clinical presentation. In some patients lacking skin involvement and with leukaemic presentation, the diagnosis is made based on peripheral blood or bone marrow analyses. Regional lymphadenopathy at presentation is common (seen in 20% of cases). Peripheral blood and bone marrow involvement can be minimal at presentation, but invariably develops with progression of disease. Oral mucosal infiltration may be seen. Cytopenias (especially thrombocytopenia) can occur at diagnosis and in a minority of cases is severe, indicating bone marrow failure (1209). Following initial response to chemotherapy, relapses invariably occur, involving skin alone or skin and other sites, including soft tissue and the CNS. In most cases, a fulminant leukaemic phase ultimately develops (1209). About 10–20% of cases of BPDCN are associated with or develop into other myeloid neoplasms, most commonly chronic myelomonocytic leukaemia, but also MDS or acute myeloid leukaemia (AML) (1209,1618,2004,3151,3332,4206).

BPDCN must be distinguished from mature plasmacytoid dendritic cell proliferation (MPDCP), in which PDCs are morphologically mature and CD56-negative. This condition may be associated with cutaneous lesions (rash, macules...
or papules, and rarely nodules) together with lymph node and/or bone marrow infiltration. MPDCP is invariably associated with a myeloid disorder (most commonly chronic myelomonocytic leukaemia, MDS, or AML) [869,3155,4191,4206].

**Microscopy**
BPDCN is characterized by a diffuse, monomorphous infiltrate of mediumsized blast cells resembling either lymphoblasts or myeloblasts. Nuclei have irregular contours, fine chromatin, and one to several small nucleoli. The cytoplasm is usually scant and appears greyish-blue and agranular on Giemsa staining. Mitoses are variable in number, and the Ki-67 proliferation index is 20–80%; angioinvasion and coagulative necrosis are absent [820,1887,2004,3182]. In cutaneous infiltrates, the dermis is usually massively involved, with extension to the subcutaneous fat; the epidermis and adnexa are spared, with rare exceptions [820]. Lymph nodes are diffusely involved in the interfollicular areas and medulla, whereas B cell follicles are often spared. Bone marrow biopsy shows either a mild interstitial infiltrate (detectable only by immunophenotyping) or massive replacement; residual haematopoietic tissue may exhibit dysplastic features, especially in megakaryocytes [3151]. On peripheral blood and bone marrow smears, tumour cells may show cytoplasmic microvacuoles localized along the cell membrane and pseudopodia; granules and crystals are absent [1209].

**Cytochemistry**
The neoplastic cells in BPDCN are negative with alpha-naphthyl butyrate esterase, naphthol AS-D chloroacetate esterase (CAE), and MPO cytochemical reactions [300,1209,2004,3332].

**Immunophenotype**
The tumour cells express CD4, CD43, CD45RA, and CD56, as well as the PDC-associated antigens CD123 (IL3 alpha-chain receptor), CD303, TCL1A, CD2AP, and SPIB and the type I interferon–dependent molecule MX1 [107,406,1618,1887,2497,2702,3151,3152,3157,3182,3248,3332]. Recently, the TFCD (E2-2) transcription factor, which is essential to drive PDCs development, was found to represent a faithful diagnostic marker for BPDCN [605B]. In about 8% of cases, CD4 or CD56 is negative, which does not rule out the diagnosis if other PDC-associated antigens (in particular CD123, TCL1A, or CD303) are expressed [53,406,1887]. Tumours that have some immunophenotypic features of BPDCN may be better classified as one of the subtypes of acute leukaemia of ambiguous lineage. CD68 (an antigen typically expressed strongly on normal PDCs) is detected in 50–80% of cases, in the form of small cytoplasmic dots [3151,3157]. Of the lymphoid and myeloid-associated antigens, CD7 and CD33 are relatively commonly expressed; some cases show expression of CD2, CD5, CD36, CD38, and CD79a, whereas CD3, CD13, CD16, CD19, CD20, LAT, lysozyme, and MPO are negative. Granzyme B, which is found in normal PDCs, has also been demonstrated on flow cytometry immunophenotyping and mRNA analysis in BPDCN [663,1410], but it is typically negative on tissue sections, as are other cytotoxic molecules such as perforin and TIA1. In addition to CD56, BPDCN may also express other antigens that are usually negative in normal PDCs, such as BCL6, IRF4, and BCL2 [820] (with BCL2 potentially acting against tumour cell apoptosis [3520]). In addition, S100 protein is expressed in 25–30% of all cases [1887], and even more frequently in paediatric cases [1847,1849]; the zonal distribution of this antigen and its variable expression in tumour cells may reflect divergent subclones with maturation...
The identification of BPDCN by flow cytometry benefits from gate and intensity evaluation criteria ([663,1209,1303,1410,4058,4065]). BPDCN is characterized by high levels of CD123 and weak expression of CD45 (blast gate); in addition, CD36, CD304, and LILRB4 (also called ILT3), but not CD141, are expressed ([4058,4065]). The absence of lineage-associated antigens, together with positivity for CD4, CD45RA, CD56, and CD123, is considered a unique phenotype virtually pathognomonic of BPDCN ([4058]). CD303 positivity has the highest diagnostic score within a panel of markers used for BPDCN identification ([1303]). Subgroups of tumours may be identified on the basis of mutually exclusive expression of antigens, such as TdT, CD303 ([1846]), TdT/S100 ([1887]), and CD34/KIT/CD56 ([2525]). These subgroups may correspond to diverse levels of maturation and may be associated with different clinical presentations ([2525]), but their prognostic significance remains unclear. Among the antigens generally overexpressed by BPDCN blasts, CD123 could serve as a therapeutic target of engineered monoclonal antibodies ([108,506,1248,2877]). Because other haematological neoplasms can share morphological and immunophenotypic features with BPDCN (especially AML with monocytic differentiation, which can express CD4, CD56, and CD123 [987,3157,4206]), extensive immunohistochemical and/or genetic analysis is necessary before a definitive diagnosis of BPDCN can be made. BPDCNs must also be distinguished from MPDCPs associated with other myeloid neoplasms, which are predominantly found in lymph nodes, skin, and bone marrow. MPDCPs consist of nodules or irregular aggregates composed of cells morphologically similar to normal PDCs; these nodules can be very numerous, are sometimes confluent, and may show prominent apoptosis. The PDCs in these nodules generally have the same phenotype as their normal counterparts, although in occasional cases aberrant singe or multiple antigen expression (e.g. of CD2, CD5, CD7, CD10, CD13, CD14, CD15, and/or CD33) has been reported ([333,369,2497,4191,4206]). Most importantly, CD56 is negative in most cases, or shows only focal and weak reactivity ([1130,4206]). The PDCs in MPDCP have a low Ki-67 proliferation index (<10%) and lack TdT. Their neoplastic nature and relatedness with the associated myeloid neoplasm have been evidenced by the demonstration of identical clonal chromosomal abnormalities in the two cellular components ([684,3177,4191]).

**Cell of origin/normal counterpart**

The cell of origin is a haematopoietic stem cell; the normal counterpart is the precursor of PDCs. Data on antigen ([663,664,1410,1618,1812,1846,3152,3157]) and chemokine receptor expression ([329]), in vitro functional assays ([329,664]), gene expression profiling ([987,3520]), and the tumour-derived cell lines ([2437,2828]) all point towards derivation from the precursors of a special subset of dendritic cells: PDCs ([597,1476,3739]). In humans, these cells are distinguished by their production of large amounts of type I interferon ([598]), they have been known by many names, such as lymphoblasts, T-cell-associated plasma cells, plasmacytoid T cells, and plasmacytoid monocytes ([1129]).

Recent gene expression profiling studies have revealed that the neoplastic cells show a gene expression signature similar to that of resting normal PDCs and closer to that of myeloid than of lymphoid precursors ([3520]). The immunophenotypic heterogeneity with regard to TdT and the association with myeloid disorders suggest a multilineage potential for some cases.

Furthermore, recent data obtained by TCF4 (E2-2) ChIP-seq and gene expression changes following TCF4 knockdown revealed the similarity between normal PDCs, BPDCN cell lines, and primary BPDCN, distinguishing BPDCN from AML lines ([605B]).

**Genetic profile**

T-cell and B-cell receptor gene mutations are usually germline ([3151,3332]), except in a few cases that show T-cell receptor gamma rearrangement, possibly due to clonal bystander T cells ([53,3151]). Two thirds of patients with BPDCN have an abnormal karyotype. Specific chromosomal aberrations are lacking, but complex karyotypes are common; six major recurrent chromosomal abnormalities have been recognized, involving 5q21 or 5q34 (seen in 72% of cases), 12p13...
Genomic abnormalities mainly involve tumour suppressor genes or genes related to the G1/S transition [53,987, 1840,2278,3153,3332,4312]; the most recurrent being deletions of CDKN2A [2409,3795]. Array-based comparative genomic hybridization shows recurrent deletions of regions on chromosomes 4 (4q34), 9 (9p11-p13 and 9q12-q34), and 13 (13q12-q31), with diminished expression of tumour suppressor genes (RB1, LATS2), whereas elevated expression of the products of the oncogenes HES6, RUNX2, and FLT3 is not associated with genomic amplification [987].

Gene expression analysis of BPDCN reveals a unique signature, distinct from those of myeloid and lymphoid acute leukaemias [987,3520]; this proves that BPDCN originates from the myeloid lineage; in particular, from resting PDCs (3520). Compared with normal PDCs, BPDCN shows increased expression of genes involved in Notch signalling [987] and BCL2, as well as aberrant activation of the NF-kappaB pathway, which is a potential therapeutic target (3520).

**Prognosis and predictive factors**

The clinical course is aggressive, with a median survival of 10.0–19.8 months, irrespective of the initial pattern of disease. Most cases (80–90%) show an initial response to multiagent chemotherapy, but relapses with subsequent resistance to drugs are regularly observed [820,1812, 1888,3026,3151]. Age has an adverse impact on prognosis [315,3839], and long-term survival has been reported in 36% of paediatric patients [1847]. Among biological parameters, high marrow or peripheral blood blastosis [3839], low TdT expression [1846,3839], positivity for CD30L (also called CLEC4C or BDCA2) [1846], low Ki-67 proliferation index [1887], CDKN2A/B deletions [2409], and mutations in DNA methylation pathway genes [2629] have been associated with shorter survival. Lymphoblastic leukaemia–oriented induction treatment seemed to be more effective than AML-oriented therapy in both children [1847] and adults [3026], which might be due to the deregulation of several genes in common with lymphoblastic leukaemia, resulting in higher sensitivity to drugs included in lymphoma/lymphoblastic leukaemia–tailored regimens (3520). In patients in first complete remission, allogeneic haematopoietic stem cell transplantation has been recommended as the best way to achieve long-term survival [861,1604,3400], even in elderly patients (with reduced-intensity conditioning) [984].
CHAPTER 10

Acute leukaemias of ambiguous lineage

Acute undifferentiated leukaemia

Mixed-phenotype acute leukaemias with
gen rearrangements

Mixed-phenotype acute leukaemia

Acute leukaemias of ambiguous lineage, NOS
Acute leukaemias of ambiguous lineage

**Definition**
Acute leukaemias of ambiguous lineage are leukaemias that show no clear evidence of differentiation along a single lineage. They include acute undifferentiated leukaemias, which show no lineage-specific antigens, and mixed-phenotype acute leukaemias (MPALs), which have blasts that express antigens of more than one lineage to such a degree that it is not possible to assign the leukaemia to any one lineage with certainty. MPALs can contain distinct blast populations each of a different lineage, one population with multiple antigens of different lineages on the same cells, or a combination. The ambiguity of antigen expression most often involves myeloid antigens coexisting with either T-cell or B-cell antigens, but rare cases of ambiguity of assignment between B-cell and T-cell lineages also occur. Leukaemia with well-defined lineage engagement but expressing one or more antigens associated with a different lineage should be considered acute leukaemia with aberrant antigen expression rather than MPAL.

**Epidemiology**
Ambiguous-lineage leukaemias are rare, accounting for <4% of all acute leukaemia cases. Many cases that have been reported as undifferentiated leukaemia can in fact be demonstrated to be leukaemias of unusual lineages, and many cases that have been reported as biphenotypic acute leukaemias may in fact be acute lymphoid or myeloid leukaemias with cross-lineage antigen expression; therefore, the true frequency of ambiguous-lineage leukaemias may be even lower than reported. These leukaemias occur in both children and adults, but more frequently in adults, although some subtypes of MPAL may be more common in children [2013,3013].

**Terminology**
Historically, there has been confusion regarding the terminology and definitions of all ambiguous-lineage leukaemias and in particular the MPALs. The term ‘acute bilineage (or bilineal) leukaemia’ has been applied to MPALs containing separate populations of blasts of more than one lineage. The term ‘biphenotypic leukaemia’ has typically been reserved for MPALs containing a single population of blasts coexpressing antigens of more than one lineage [1539,2257,2578,3830], but is sometimes used more broadly to also encompass bilineage leukaemias. In this volume, the term ‘mixed-phenotype acute leukaemia’ is applied to this group in general; the more specific terms ‘B/myeloid leukaemia’ and ‘T/myeloid leukaemia’, as defined below, refer to leukaemias containing the two lineages specified, irrespective of whether one or more than one population of blasts is seen.

Some well-defined myeloid leukaemia entities have immunophenotypic features that might suggest that they be classified as B/myeloid or T/myeloid leukaemia. However, the MPAL group, as defined in this volume, excludes cases that can be classified (either by genetic or clinical features) in another category, such as cases with the recurrent genetic abnormalities t(8;21), inv(16), or PML-RARA fusion, which are associated with acute myeloid leukaemia (AML); cases with t(8;21) express multiple B-cell markers.
especially frequently [3996]. Cases of leukaemia with FGFR1 rearrangements are not considered to be T/myeloid leukaemias either. All cases of chronic myeloid leukaemia in blast crisis, AML with myelodysplasia-related changes, and therapy-related AML should be classified primarily as such, with a secondary notation that they have a mixed phenotype if applicable.

**General approach to diagnosis**

The diagnosis of ambiguous-lineage leukaemias relies on immunophenotyping. Flow cytometry is the preferred method for establishing the diagnosis, especially when a diagnosis of MPAL requires demonstrating coexpression of lymphoid and myeloid differentiation antigens on the same cell. Cases in which the diagnosis requires demonstration of two distinct leukaemic populations with different phenotypes can also be established by immunohistochemistry in tissue sections, or with cytochemical stains for MPO on smears coupled with flow cytometry to detect a leukaemic B-cell or T-cell lymphoid population. The hallmark of MPAL is that the combination of antigens expressed on the blast population or populations does not allow classification into a single lineage. There are several scenarios in which this can occur. In the most straightforward scenario, there are two (or more) distinct populations of leukaemic cells: one (or more) of which would independently fulfil the immunophenotypic criteria for AML and one (or more) the criteria for T- and/or B-lymphoblastic leukaemia (ALL); the specific antigens associated with these diagnoses are described in the respective sections devoted to these diseases. However, although abnormal blasts must constitute ≥20% of all nucleated cells overall, this need not be true of each distinct population. Cases like these would previously have been considered bilineage leukaemia.

In the second scenario, there is a dominant single population of blasts that expresses combinations of antigens that alone would be considered specific for more than one lineage. There are also hybrid cases in which this dominant population is accompanied by one or more minor populations with a different immunophenotype. In this setting, the criteria for classifying a leukaemia are more stringent (Table 10.01), and there are several caveats as to how the expression of lineage-specific markers should be interpreted to achieve a diagnosis of MPAL.

<table>
<thead>
<tr>
<th>Table 10.01</th>
<th>Requirements for assigning more than one lineage to a single blast population.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Myeloid lineage</strong></td>
<td>MPO (by flow cytometry, immunohistochemistry, or cytochemistry)</td>
</tr>
<tr>
<td>or</td>
<td>Monocytic differentiation (≥ 2 of the following: non-specific esterase, CD11c, CD14, CD64, lysozyme)</td>
</tr>
<tr>
<td><strong>T-cell lineage</strong></td>
<td>Cytoplasmic CD3 (by flow cytometry with antibodies to CD3 epsilon chain. Immunohistochemistry using polyclonal anti-CD3 antibody may detect CD3 zeta chain, which is not T-cell-specific)</td>
</tr>
<tr>
<td>or</td>
<td>Surface CD3 (rare in mixed-phenotype acute leukaemias)</td>
</tr>
<tr>
<td><strong>B-cell lineage (multiple antigens required)</strong></td>
<td>Strong CD19 with ≥ 1 of the following strongly expressed: CD79a, cytoplasmic CD22, CD10</td>
</tr>
<tr>
<td>or</td>
<td>Weak CD19 with ≥ 2 of the following strongly expressed: CD79a, cytoplasmic CD22, CD10</td>
</tr>
</tbody>
</table>

- **T-cell component of mixed-phenotype acute leukaemia**
  - The T-cell component of an MPAL is characterized by strong expression of cCD3, usually in the absence of surface CD3. For cCD3 to be T-cell-specific, it must be expressed strongly. Expression of cCD3 is best determined by flow cytometry, using relatively bright fluorophores such as phycoerythrin or allophycocyanin. Although cCD3 expression is commonly heterogeneous, the brightest cCD3-positive blasts should reach the intensity of the normal residual T cells present in the sample. Some cases of demonstrable T-ALL have cCD3 that is dimmer than this, but brighter expression is required to establish a diagnosis of MPAL. T-cell lineage can also be demonstrated by immunohistochemistry for CD3 expression in blasts on bone marrow biopsies, but the polyclonal T-cell antibodies used in immunohistochemistry can also react with CD3 zeta chains that can be present in the cytoplasm of activated NK cells, and are therefore not absolutely T-cell-specific.

- **B-cell component of mixed-phenotype acute leukaemia**
  - The B-cell component of an MPAL is characterized by surface expression of CD19 together with CD10, or in the absence of CD10, together with cCD79a, CD22, or PAX5 (with PAX5 typically detected by immunohistochemistry). As with cCD3 and T-cell lineage, bright CD19 expression at a level comparable to that of normal B cells has considerable specificity for the B-cell lineage, and is almost always expressed together with one or more of the other markers noted above; caution is required if CD19 is the only marker seen. If the expression of CD19 is dimmer, the presence of at least two other markers is required. CD19 can even be negative with three other B-cell markers present, but this is exceptionally rare.

- **Myeloid component of mixed-phenotype acute leukaemia**
  - The single most specific hallmark of the myeloid component of an MPAL is MPO in the blast cytoplasm. In cases in which the myeloid component is monocytic, MPO can be negative and unequivocal evidence of monoblastic differentiation would be considered acceptable, including diffuse positivity for non-specific esterase or expression of more than one monocytic marker, such as CD11c, CD14, CD36, CD64, or lysozyme. However, despite the generally high specificity associated with MPO, using it as the sole diagnostic criterion for MPAL has been problematic, in part due to technical factors associated with its measurement. Several anti-MPO antibodies have been shown to react with B-lymphoblastic leukaemia/lymphoma on flow cytometry [2277,2815] or immunohistochemistry [131], and MPO staining on flow cytometry can appear positive but actually...
constitute non-specific staining. Small numbers of cases of otherwise typical B-ALL have also been identified in which MPO is definitively positive by flow cytometry; the clinical significance of this is still uncertain. Therefore, a diagnosis of MPAL may not be appropriate for cases that otherwise have a typical precursor B-cell lymphoid phenotype with only a single blast population present; these should not be considered MPAL if weak MPO expression by flow cytometry is the only evidence of myeloid differentiation. Additional criteria that can be helpful for diagnosis include cytochemical positivity for MPO, expression of myeloid antigens such as KIT (CD117) [2890], and very bright CD13 and CD33. However, the most recognizable feature of MPAL is that nearly all cases display a particular pattern of heterogeneity of antigen expression: in appropriate dual-parameter displays, populations that are relatively bright for lymphoid markers show lower-level expression of myeloid antigens, and vice versa (Figure 10.01). In such cases, the subset of blasts expressing a more myeloid pattern may be larger by forward scatter than the subset of those showing a lymphoid pattern. Caution should be exercised when making a diagnosis of MPAL if such heterogeneity cannot be demonstrated.

A recent report discussed a variety of cases of MPAL submitted to a session of the Society for Hematopathology/European Association for Haematopathology Workshop that focused on acute leukaemias of ambiguous origin [3222].

Other considerations
MPAL cases with a single population of blasts at diagnosis (so-called bipheno-
typic leukaemia) can change over time to (or can relapse as) a leukaemia containing separate blast populations (so called bilineage leukaemia), and vice versa. Following therapy, persistent disease or relapse can also occur as pure ALL or AML. Some cases of what has been termed lineage switch [3047,3326] may reflect this phenomenon.

A variety of genetic lesions have been reported in ambiguous lineage leukaemias, especially in MPAL; two such lesions, t(9;22)(q34.1;q11.2) resulting in BCR-ABL1 and translocations associated with KMT2A (previously called MLL), occur frequently enough and are associated with such distinctive features that they are considered to define separate entities.

**Acute undifferentiated leukaemia**

**Definition**
Acute undifferentiated leukaemia expresses no markers considered to be specific for either lymphoid or myeloid lineage. Before categorizing a leukaemia as undifferentiated, it is necessary to perform immunophenotyping with a comprehensive panel of monoclonal antibodies in order to exclude leukaemias of unusual lineages, such as those derived from plasmacytoid dendritic cell precursors, NK-cell precursors, basophils, or even non-haematopoietic tumours.

**ICD-O code** 9801/3

**Synonyms**
Blast cell leukaemia; stem cell leukaemia; stem cell acute leukaemia; undifferentiated leukaemia.

**Epidemiology**
These leukaemias are very rare. Their precise frequency is unknown.

**Localization**
Acute undifferentiated leukaemia affects the bone marrow and blood. Due to the small number of cases reported to date, it is unknown whether there is any predilection for other sites.

**Microscopy**
The blasts have no morphological features of myeloid differentiation. They are negative for MPO and esterase.

**Immunophenotype**
These leukaemias typically express no more than one membrane marker for any given lineage. By definition, they lack the T-cell and myeloid markers CD3 and MPO and do not express B-cell markers such as cCD22, cCD79a, or strong CD19. They also lack the specific features of cells of other lineages, such as megakaryocytes or plasmacytoid dendritic cells. Blasts often express HLA-DR, CD34, and/or CD38 and may be positive for TdT, CD7, although often considered a T-cell antigen, is expressed weakly on some CD34+ haematopoietic progenitors and may be similarly expressed in these leukaemias.

**Postulated normal counterpart**
A haematopoietic stem cell.

**Genetic profile**
The limited data available suggest that genes associated with poor prognosis in acute myeloid leukaemia (such as BAALC, ERG, and MNI) are often expressed. To date, there have been no reports of cases showing the genetic mutations commonly seen in lymphoblastic leukaemia or acute myeloid leukaemia, including FLT3, WT1, rearrangements of KMT2A (previously called MLL), or BCR-ABL1 [1599].

**Prognosis and predictive factors**
The limited data suggest that these leukaemias have a very poor prognosis [1599].

**Mixed-phenotype acute leukaemia with t(9;22)(q34.1;q11.2); BCR-ABL1**

**Definition**
Mixed-phenotype acute leukaemia (MPAL) with t(9;22)(q34.1;q11.2) fulfils the criteria for MPAL and has blasts with t(9;22) and/or BCR-ABL1 rearrangement. Some patients with chronic myeloid leu-
kaemia (CML) develop or even present with a mixed blast phase that would fulfill the criteria for MPAL; however, this diagnosis should not be made in patients known to have had CML.

**ICD-O code** 9806/3

**Synonym**
Mixed-phenotype acute leukaemia with t(9;22)(q34;11.2).

**Epidemiology**
Although t(9;22)(q34.1;q11.2) is the most common recurrent genetic abnormali-
ity seen in MPAL, this leukaemia is rare, probably accounting for <1% of all acute leukaemia. It occurs in both children and adults, but is more common in adults [560,2013].

**Clinical features**
Patients present similarly to other patients with acute leukaemia. Although there are not enough data to be certain, it is likely that patients with MPAL with t(9;22) (q34.1;11.2) present with high white...
blood cell counts, similar to patients with Philadelphia (Ph) chromosome-positive lymphoblastic leukaemia.

**Microscopy**
Many cases show a dimorphic blast population, with some blasts resembling lymphoblasts and others myeloblasts, although other cases have no distinguishing features. Cases generally do not show significant myeloid maturation; caution should be exercised when making this diagnosis in a case of myeloid leukaemia with maturation that also expresses lymphoid markers, because such a pattern can also be seen in the blast phase of CML.

**Immunophenotype**
The great majority of cases have blasts that meet the criteria for B-cell and myeloid lineage, as described above, although some cases have T-cell and myeloid blasts. Triphenotypic cases have also rarely been reported.

**Postulated normal counterpart**
The postulated normal counterpart is a multipotent haematopoietic stem cell. There is no evidence that this leukaemia derives from a different cell than do other cases of Ph chromosome-positive acute leukaemia.

**Genetic profile**
All cases have either t(9;22) detected by conventional karyotyping or the BCR-ABL1 translocation detected by FISH or PCR. The p190 fusion transcript is more common than the p210 transcript (4256). If the p210 transcript is present, CML in a mixed blast crisis should be considered in the differential diagnosis, especially if there are two distinct lymphoid and myeloid blast populations. Many cases have additional cytogenetic abnormalities, and complex karyotypes are common.

**Prognosis and predictive factors**
This type of leukaemia has a poor prognosis, which appears to be worse than that of other MPALs (2013), in particular in adults (2583). It is unclear whether the prognosis is worse than that of Ph chromosome-positive lymphoblastic leukaemia. Some data suggest that treatment with tyrosine kinase inhibitors may improve outcome as is seen in lymphoblastic leukaemia with BCR-ABL1 (1973,3659). There are no known biological factors that predict whether patients with this leukaemia will do better or worse with therapy.

**Mixed-phenotype acute leukaemia with t(v;11q23.3); KMT2A-rearranged**

**Definition**
Mixed-phenotype acute leukaemia (MPAL) with t(v;11q23.3) fulfils the criteria for MPAL and has blasts with a translocation involving KMT2A (previously called MLL). Many cases of lymphoblastic leukaemia (ALL) with KMT2A translocations express myeloid-associated antigens, but such cases should not be considered MPAL unless they fulfil the specific criteria noted above.

**ICD-O code**
9807/3

**Synonyms**
Mixed-phenotype acute leukaemia, t(v;11q23), MLL rearranged; mixed-phenotype acute leukaemia with MLL rearrangement

**Epidemiology**
This rare leukaemia is more common in children than in adults. Like ALL and acute myeloid leukaemia with KMT2A rearrangements, this leukaemia is relatively more common in infancy (2013,3013).

**Clinical features**
Patients present similarly to other patients with acute leukaemia. As with other acute leukaemias with KMT2A translocations, high white blood cell counts are common.

**Microscopy**
These leukaemias typically show a dimorphic blast population, with some blasts clearly resembling monoblasts and others resembling lymphoblasts. However, some cases have no distinguishing features and appear only as undifferentiated blast cells. Cases in which the entire blast population appears monoblastic are more likely to be acute myeloid leukaemia with a KMT2A translocation, but this diagnosis requires flow cytometry to exclude the presence of a small lymphoblastic population.

**Immunophenotype**
In most cases, it is possible to recognize a lymphoblast population with a CD19+, CD10−, pro-B (B-cell precursor, B-1) immunophenotype, frequently positive for CD15. Expression of other B-cell markers, such as CD22 and CD79a, is often weak. Cases also fulfil the criteria for myeloid lineage as defined above, most commonly via demonstration of a separate population of myeloid (usually monoblastic) leukaemic cells (3013,4279). Co-expression of MPO on lymphoid blasts is rare. Because KMT2A translocations can also produce T-ALL, it is theoretically possible that T/myeloid leukaemias with t(v;11q23.3) could occur, although no such cases have been reported.

**Genetic profile**
All cases have rearrangements of KMT2A, with the most common partner gene being AFF1 (AF4) on chromosome 4 band q21.3 (560,3013). Translocations t(9;11) and t(11;19) have also been reported.
been reported. The rearrangement may be detected by standard karyotyping, by FISH with a KMT2A break-apart probe, or (less commonly) by PCR. Cases with deletions of chromosome 11q23.3 detected by karyotyping should not be considered part of this category. The KMT2A translocation may be the only lesion present or there may be secondary cytogenetic or molecular abnormalities, but no additional genetic lesions common to multiple cases have been described.

Prognosis and predictive factors
This leukaemia has a poor prognosis (2013,4279). Patients with B/myeloid leukaemia with KMT2A translocations are often treated differently than are patients diagnosed with ALL with KMT2A translocations, but there is no evidence that this is necessary or beneficial.

**Mixed-phenotype acute leukaemia, B/myeloid, not otherwise specified**

**Definition**
B/myeloid mixed-phenotype acute leukaemia (MPAL), not otherwise specified (NOS), fulfills the criteria for B/myeloid leukaemia as described above, but does not fulfill the criteria for any of the genetically defined subgroups.

**ICD-O code**
9808/3

**Epidemiology**
This is a rare leukaemia, probably accounting for about 1% of all leukaemia cases. It occurs in both children and adults, but more commonly in adults.

**Microscopy**
Most cases either have blasts with no distinguishing features - morphologically resembling lymphoblastic leukaemia (ALL) - or have a dimorphic population with some blasts resembling lymphoblasts and others myeloblasts.

**Immunophenotype**
The blasts meet the criteria for both B-cell and myeloid lineage assignment as listed above. MPO-positive myeloblasts and monoblasts commonly also express other myeloid-associated markers, including CD13, CD33, and KIT (CD117). Expression of more mature B-cell markers, such as CD20, is rare, occurring most commonly when a separate population of B-cell lineage blasts is present (4279).

**Postulated normal counterpart**
The postulated normal counterpart is a multipotent haematopoietic stem cell. There is growing evidence of a possible relationship between B-cell and myeloid development, suggesting the involvement of either a common precursor or a precursor of one lineage that has reactivated a differentiation programme of the other (1974,2193).

**Genetic profile**
Most cases have clonal cytogenetic abnormalities. Many different lesions have been demonstrated, although none commonly enough to suggest specificity for this group of leukaemias. The lesions that have been seen in more than a single case include del(6p), 12p11.2 abnormalities, del(5q), structural abnormalities of chromosome 7, and numerical abnormalities including near-tetraploidy (560, 3013). Complex karyotypes are common (2487,2583). Gene expression profile studies suggest a signature intermediate between that of ALL and that of acute myeloid leukaemia in most cases (3447). Mutations frequently found in either acute myeloid or ALLs have also been reported in MPAL, including mutations of ASXL1, TET1/2, IDH1, IDH2, DNMT3A, NOTCH1, and ETV6, and deletion of IKZF1 (4414). There are as yet insufficient data in the literature and too few reported cases to determine whether any genetic entities can be defined other than those discussed above, involving BCR-ABL1 and KMT2A.

**Prognosis and predictive factors**
B/myeloid MPAL, NOS, is generally considered a poor-prognosis leukaemia, although data on outcome of these cases versus other MPALs are limited. In children, outcome is worse than that of ALL (923,1343,2402,3447); in adults,
outcome appears to be better than that of acute myeloid leukaemia and no different than that of other ALLs [923,3646,4275]. Many cases that meet the criteria for B/myeloid MPAL, NOS, have one or more of the unfavourable genetic lesions noted above; it has been suggested that this accounts for their poor prognosis [2013]. Whether adverse cytogenetic features entirely explain the poor outcome has not been definitively established [2257, 4279]. Patients with B/myeloid MPAL, NOS, have not been treated uniformly. Various combinations and sequential administration of myeloid-directed and lymphoid-directed therapies have been tried [2583,3447,4347], and some patients may respond to one or the other.

**Mixed-phenotype acute leukaemia, T/myeloid, not otherwise specified**

**Definition**
T/myeloid mixed-phenotype acute leukaemia (MPAL), not otherwise specified (NOS), fulfills the criteria for both T-cell and myeloid lineage as described above, but its blasts lack the above-described genetic abnormalities.

**ICD-O code**
9809/3

**Epidemiology**
This is a rare leukaemia, probably accounting for <1% of all leukaemia cases. It can occur in both children and adults. It may be more frequent in children than is B/myeloid MPAL.

**Microscopy**
Most cases either have blasts with no distinguishing features (morphologically resembling lymphoblastic leukaemia) or have a dimorphic population with some blasts resembling lymphoblasts and others myeloblasts.

**Immunophenotype**
The blasts meet the criteria for both T-cell and myeloid lineage assignment as listed above. MPO-positive myeloblasts and monoblasts commonly also express other myeloid-associated markers, including CD13, CD33, and KIT (CD117). In addition to cCD3, the T-cell component frequently also expresses other T-cell markers, including CD7, CD5, and CD2. Expression of surface CD3 can occur when a separate population of T-cell lineage blasts is present [4279].

**Postulated normal counterpart**
The postulated normal counterpart is a multipotent haematopoietic stem cell. There is growing evidence of a possible relationship between T-cell and myeloid development, suggesting the involvement of either a common precursor or a lymphoid precursor that has reactivated a myeloid differentiation programme [1974,2193].

**Genetic profile**
Most cases have clonal chromosomal abnormalities, although none is frequent enough to suggest specificity for this group of leukaemias. There are insufficient data in the literature to determine whether B/myeloid and T/myeloid MPALs have different frequencies of various

---

**Fig. 10.04** T/myeloid mixed-phenotype acute leukaemia, NOS, infiltrating a lymph node. **A and B** Diffuse replacement of node by a population of cells with high nuclear/cytoplasmic ratios and fine chromatin, histologically indistinguishable from lymphoblastic leukaemia/lymphoma. **C** Immunoperoxidase staining for CD3 stains most of the blast cells. **D** Immunoperoxidase staining for MPO shows distinct staining of a subpopulation of cells with round nuclei; this indicates that they are part of the neoplasm rather than infiltrating granulocytes.
Fig. 10.05 T/myeloid mixed-phenotype acute leukaemia, NOS. There is a dimorphic population of blasts, with many small lymphoblasts. Larger blasts also have a high nuclear:cytoplasmic ratio, fine chromatin and inconspicuous nucleoli.

Prognosis and predictive factors
T/myeloid MPAL, NOS is generally considered a poor-prognosis leukaemia, although data on outcome of these cases versus other MPALs are limited (923, 1343, 2402, 3447). Patients with T/myeloid MPAL, NOS have not been treated uniformly. Various combinations and sequential administration of myeloid-directed and lymphoid-directed therapies have been tried (3447, 4347), and some patients may respond to one or the other.

Mixed-phenotype acute leukaemia, not otherwise specified, rare types

Definition
In some documented cases of leukaemia, the leukaemic blasts show clear-cut evidence of both T-cell and B-cell lineage commitment as defined above. This is a very rare phenomenon, with a frequency that is likely even lower than has typically been reported in the literature (2583). As strictly applied, the European Group for the Immunological Characterization of Leukemias (EGIL) criteria for biphenotypic leukaemia (i.e. scores > 2 in more than one lineage), which assign a 2-point value to CD79a expression (331, 332) may overestimate the incidence of B/T-leukaemia, because CD79a can be detected in T-lymphoblastic leukaemia with some antibodies (1584). For the purpose of assigning B-cell lineage to a case of T-lymphoblastic leukaemia, CD79a and CD10 should not be considered evidence of B-cell differentiation. There have also been a few cases with evidence of trilineage (B-cell, T-cell, and myeloid lineage) assignment. Overall, there are too few cases with these characteristics for any specific statements to be made about clinical features, genetic lesions, or prognosis.

To date, there have been no reports of mixed B- or T-cell and megakaryocytic or mixed B- or T-cell and erythroid leukaemias. It has been postulated that erythroid and megakaryocytic lineages are the earliest to branch off from the pluripotent haematopoietic stem cell, leaving progenitor cells with T-cell, B-cell, and myeloid potential (1803); therefore, neo-
Acute leukaemias of ambiguous lineage, not otherwise specified

Definition
Acute leukaemias of ambiguous lineage, not otherwise specified (NOS), express combinations of markers that do not allow for their classification as either acute undifferentiated leukaemia or mixed-phenotype acute leukaemia as defined above, and definitive classification along a single lineage is difficult.

Epidemiology
These are rare leukaemias, but there are no specific data on their frequency. They occur in both children and adults.

Immunophenotype
There is no unique immunophenotype that defines this class of leukaemias. Acute leukaemias of ambiguous lineage, NOS include cases that express T-cell-associated markers such as CD7 and CD5 but lack more specific markers such as cCD3, along with myeloid-associated antigens such as CD33 and CD13 without MPO. Care should be taken not to misinterpret a case as ambiguous based on the expression of antigens with limited lineage specificity, especially when the antigens are expressed only dimly. For example, a leukaemia expressing CD13, CD33 and KIT (CD117) along with CD7 and dim CD19 is more properly classified as acute myeloid leukaemia with aberrant antigen expression. With more-extended panels containing newer, less commonly used markers, such leukaemias might be able to be classified more specifically.

Cell of origin
The postulated cell of origin is a multipotent haematopoietic stem cell.

Genetic profile
Like other leukaemias in this category, most cases of acute leukaemia of ambiguous lineage, NOS, have clonal chromosomal abnormalities, although none is frequent enough to suggest specificity for this group of leukaemias.

Prognosis and predictive factors
These leukaemias are generally considered to have a poor prognosis, although data on outcome of these cases versus other ambiguous-lineage leukaemias are limited. Patients with this type of leukaemia are most often treated with myeloid-directed therapy, but definitive data justifying this approach have not been published.
CHAPTER 11

Introduction and overview of the classification of lymphoid neoplasms
Introduction and overview of the classification of lymphoid neoplasms

Definition
B-cell and T/NK-cell neoplasms are clonal tumours of mature and immature B cells, T cells, or NK cells at various stages of differentiation. Because NK cells are closely related to and share some immunophenotypic and functional properties with T cells, these two classes of neoplasms are considered together. In many respects, B-cell and T-cell neoplasms appear to recapitulate stages of normal B-cell or T-cell differentiation, so to some extent they can be classified according to the corresponding normal stage. However, some common B-cell neoplasms (e.g. hairy cell leukaemia) do not clearly correspond to a normal B-cell differentiation stage. Some neoplasms exhibit lineage heterogeneity, or even more rarely, lineage plasticity (1819, 2431). Therefore, the normal counterpart of the neoplastic cell cannot be the sole basis for classification.

Pathobiology of lymphoid neoplasms and the normal immune system
The immune system has two major subsystems, which differ in the nature of their targets and types of immune response: the innate and adaptive immune systems. The innate immune system provides a first line of defence, a primitive response. The cells of the innate immune system include NK cells, CD3+ CD56+ T cells (NK-like T cells), and gamma delta T cells. These cells play a role in barrier immunity involving mucosal and cutaneous defences. They do not need to encounter antigens in the context of major histocompatibility complex (MHC) molecules, and therefore do not require antigen-presenting cells in order to initiate an immune response. The adaptive immune system provides a more sophisticated type of immune response; two key features are antigen specificity and memory. This contrasts with the innate immune response, which is non-specific and does not require or result in immunological memory.

B-cell lymphomas: Lymphocyte differentiation and function
B-cell neoplasms tend to mimic various stages of normal B-cell differentiation, and this resemblance to normal cell stages is a major basis for their classification and nomenclature. Normal B-cell differentiation begins with B lymphoblasts, which undergo IG VDJ gene rearrangement and differentiate into mature surface immunoglobulin (sIg)-positive (IgM+ IgD+) naïve B cells via pre-B cells with cytoplasmic mu heavy chains and immature IgM+ B cells. Naïve B cells, which are often CD5-positive, are small resting lymphocytes that circulate in the peripheral blood and also occupy primary lymphoid follicles and follicle mantle zones (so-called recirculating B cells) (1758,2032). Many cases of mantle cell lymphoma are thought to correspond to CD5+ naïve B cells (1733), but somatically mutated variants also exist (1187).

Upon encountering antigen that fits their sIg receptors, naïve B cells undergo transformation, proliferate, and ultimately mature into antibody-secreting plasma cells and memory B cells. Transformed cells derived from naïve B cells that have encountered antigen may mature directly into plasma cells that produce the early IgM antibody response to antigen. T-cell-independent maturation can take place outside the germinal centre (721). Other antigen-exposed B cells migrate into the centre of a primary follicle, proliferate, and fill the follicular dendritic cell meshwork, forming a germinal centre (2374, 2427). Germinal centre centroblasts express low levels of sIg and switch off expression of BCL2; therefore, they and their progeny are susceptible to apoptosis (3329). Centroblasts express CD10 as well as BCL6, a nuclear transcription factor also expressed by centrocytes. BCL6 is not expressed in naïve B cells and is switched off in memory B cells and plasma cells (3483,3199). More recently de-
Normal B-cell differentiation and its relationship to major B-cell neoplasms. B-cell neoplasms correspond to various stages of normal B-cell maturation, although the normal cell counterparts are unknown in some instances. Precursor B cells, which mature in the bone marrow, may undergo apoptosis or develop into mature naive B cells. After exposure to antigen (AG) and blast transformation, they may develop into short-lived plasma cells or enter the germinal centre (GC), where somatic hypermutation and heavy-chain class switching occur. Centroblasts, the transformed cells of the GC, either undergo apoptosis or develop into centrocytes. Post-GC cells include both long-lived plasma cells and memory/marginal-zone B cells. Most B cells are activated within the GC, but T-cell-independent activation can take place outside the GC and probably also leads to memory-type B cells. Monocytoid B cells, many of which lack somatic hypermutation, are not illustrated. The red bars indicate IGH gene rearrangement and the blue bars IG light chain gene rearrangement; the black insertions in red and blue bars indicate somatic hypermutation.

CLL/SLL, chronic lymphocytic leukaemia/small lymphocytic lymphoma; D, surface IgD; DLBCL, diffuse large B-cell lymphoma; FDC, follicular dendritic cell; M, surface IgM; MALT, mucosa-associated lymphoid tissue.

scribed germinal centre markers include LMO2 and HGAL. LMO2 is expressed in haematopoietic precursors in the bone marrow, but appears to be specific for germinal centre B cells in normal reactive lymphoid tissues; it lacks this specificity in lymphoid neoplasms. HGAL (also called GCET2) is expressed in germinal centre B cells and germinal centre–derived malignancies (3043). In the germinal centre, somatic hypermutation occurs in the IGV genes; these mutations can result in a non-functional gene or a gene that produces antibody with lower or higher affinity for antigen. Also in the germinal centre, some cells switch from IgM production to IgG or IgA production. Through these mechanisms, the germinal centre reaction gives rise to the higher-affinity IgG or IgA antibodies of the late primary or secondary immune response (2428). BCL6 also undergoes somatic mutation in the germinal centre, but at a lower frequency than do the IGV genes (3084). Ongoing IGV gene mutation with intraclonal diversity is a hallmark of germinal centre cells, and both IGV gene and BCL6 mutations serve as markers of cells that have been through the germinal centre. Most diffuse large B-cell lymphomas (DLBCLs) are composed of cells that at least in part resemble centroblasts and have mutated IGV genes, consistent with a derivation from cells that have been exposed to the germinal centre. Burkitt lymphoma cells are BCL6-positive and have mutated IGV genes, and are therefore also thought to correspond to a germinal centre blast cell. Both Burkitt lymphoma and DLBCL correspond to proliferating cells and are clinically aggressive tumours. Centroblasts mature to centrocytes, and these cells are seen predominantly in the light zone of the germinal centre. Centrocytes express sig that has an altered antibody-combining site compared with that of their progenitors, due to both somatic mutations and heavy-chain class switching. Centrocytes with mutations that result in increased affinity are rescued from apoptosis and re-express BCL2 (2427). Through interaction with surface molecules on follicular dendritic cells and T cells (e.g. CD23 and CD40 ligand), centrocytes switch off BCL6 expression (584,3199) and differentiate into either memory B cells or plasma cells (2427). BCL6 and IRF4 (also called MUM1) are reciprocally expressed, with
IRF4 being positive in late centrocytes and plasma cells (1141,3483). IRF4 plays a critical role in downregulating BCL6 expression (3483). Recent studies indicate that MYC plays an important role in germinal centre formation (1020). MYC is upregulated upon interaction of naïve B cells with antigen and T cells by the action of BCL6, and is essential for germinal centre formation. In normal reactive lymph nodes, staining for MYC highlights a population of centrocytes in the light zone of the germinal centre, and is repressed in the dark zone. However, MYC is re-induced in a subset of light-zone B cells (centrocyes), allowing re-entry into the dark zone and maintenance of the germinal centre reaction (1020). Follicular lymphomas are tumours of germinal centre B cells (centrocytes and centroblasts) in which the germinal centre cells fail to undergo apoptosis, in most cases due to a chromosomal rearrangement, t(14;18), that prevents the normal switching off of BCL2 expression. Centrocytes usually predominate over centroblasts, and these neoplasms tend to be indolent.

Post-germinall centre memory B cells circulate in the peripheral blood and account for at least some of the cells in the follicular marginal zones of lymph nodes, spleen, and mucosa-associated lymphoid tissue (MALT). Marginal-zone B cells of this compartment typically express pan-B-cell antigens and surface IgM (with only low levels of IgD), and lack both CD5 and CD10 (3749,4124). Plasma cells produced in the germinal centre enter the peripheral blood and home to the bone marrow. They contain predominantly IgG or IgA; they lack slg and CD20 but express IRF4, CD79a, CD38, and CD138. Both memory B cells and long-lived plasma cells have mutated IGV genes, but do not continue to undergo mutation. Post-germinall centre B cells retain the ability to home to tissues in which they have undergone antigen stimulation, probably through surface integrin expression, so that B cells that arise in MALT tend to return there, whereas B cells that arise in the lymph nodes home to nodal sites and bone marrow (508). Marginal zone lymphomas of the MALT type, splenic type, and nodal type correspond to post-germinall centre memory B cells of marginal zone type that derive from and proliferate specifically in extranodal, splenic, and nodal tissues, respectively. Plasma cell myeloma corresponds to a bone marrow-homing plasma cell.

**Fig. 11.03** B-cell differentiation. B cells go through various stages of differentiation as they mature from pre-B cells to plasma cells. The antigen-dependent phase of differentiation usually begins in the germinal centre, where B cells encounter antigen. Red bar in nucleus indicates heavy chain gene rearrangement; blue bar indicates light chain rearrangement; black boxes connote somatic hypermutation. Cells coloured in yellow have not encountered antigen, as opposed to antigen-dependent stages shown in violet. Modified from Swerdlow SH et al. (3848).

**BCR**: B-cell receptor; **D**: surface IgD; **M**: surface IgM; **SHM**: somatic hypermutation.

*T-cell lymphomas: Lymphocyte differentiation and function*

T-lymphocytes arise from a bone marrow precursor that undergoes maturation and acquisition of function in the thymus gland. Antigen-specific T cells mature in the thymic cortex. T cells recognizing self peptides are eliminated via apoptosis, in a process mediated by cortical epithelial cells and thymic nurse cells. Cortical thymocytes have an immature T-cell phenotype and express TdT, CD2a, CD3, CD5, and CD7. CD3 is first expressed in the cytoplasm, prior to complete T-cell receptor (TR) gene rearrangement and export to the cell membrane. Cortical thymocytes are initially double-negative for CD4 and CD8. These antigens are coexpressed in maturing thymocytes; more mature T cells express only CD4 or CD8. These various stages of T-cell maturation are reflected in T-lymphoblastic leukemia/lymphoma.

Medullary thymocytes have a phenotype similar to that of mature T cells of the peripheral lymphoid organs. There are two classes of T cells: alpha beta T cells and gamma delta T cells (3850). This distinction is based on the structure of the T-cell receptor. The alpha beta and gamma delta chains are each composed of a variable portion and a constant portion. They are both associated with the CD3 complex, which contains gamma, delta, and epsilon chains. NK cells do not have a complete T-cell receptor complex; activated NK cells express the epsilon and zeta chains of CD3 in the cytoplasm. They express CD2, CD7, and sometimes CD8, but not surface CD3. They also typically express CD16 and CD56, variably express CD57, and contain cytoplasmic cytotoxic granule proteins. NK cells kill their targets through antibody-dependent cell-mediated cytotoxicity or a second mechanism involving killer activation receptors and inhibitory killer-cell immunoglobulin-like receptors. Because NK cells do not rearrange the TR genes, antibodies to the various killer-cell immunoglobulin-like receptors can be used for analysis of clonality in NK-cell proliferations.

---

Introduction and overview of the classification of lymphoid neoplasms
Fig. 11.04 T-cell differentiation. T cells mature in the thymus gland and then leave to occupy peripheral lymphoid tissues. T-cell receptor (TR) genes are shown schematically with a solid red bar indicating absence of rearrangement. The black boxes in the red bars reflect the rearrangements of the TR genes. The double red lines on the cell membrane represent the expressed T-cell receptor complex. Antigen dependent maturation leads to the different T-cell subsets, also illustrated in Fig. 11.05. The phenotypes of several key T-cell subsets are illustrated: T follicular helper (TFH), T regulatory (T-reg), T helper 1 (Th1), T helper 2 (Th2), and T helper 17 (Th17). Modified and updated from (3848).

Fig. 11.05 T-cell differentiation. T-cell neoplasms correspond to various stages of normal T-cell maturation. Mature T cells include alpha beta and gamma delta T cells, both of which mature in the thymus gland. Recently recognized T-cell subsets include the various types of CD4+ effector T cells, including T helper 1 (Th1), T helper 2 (Th2), T regulatory (Treg), T helper 17 (Th17), and T follicular helper (TFH) cells. Modified and updated from (3848).

Toll-like receptors play a role in cell–cell interactions and signalling. They play a critical role in the recognition of infectious agents, initiating signalling through NF-kappaB. They function most prominently in innate immune responses, but they also play a role in the adaptive immune system (2284).

The lymphomas of the innate immune system are predominantly extranodal in presentation, mirroring the distribution of the functional components of this system. It is interesting that many T-cell and NK-cell lymphomas observed commonly in the paediatric and young-adult age groups are derived from cells of the innate immune system (1816). Lymphoid neoplasms derived from innate lymphoid cells include aggressive NK-cell leukaemia, systemic EBV-positive T-cell lymphoma of childhood, hepatosplenic gamma delta T-cell lymphoma, and gamma delta T-cell lymphomas often arising in cutaneous and mucosal sites. Gamma delta T cells usually lack expression of CD4 and CD8, as well as CD5. A subpopulation expresses CD8. Gamma delta T cells account for <5% of all normal T cells and have a restricted distribution, being found mainly in the splenic red pulp, intestinal epithelium, and other epithelial sites. These sites are more commonly affected by gamma delta T-cell lymphomas, which are otherwise relatively rare (150,1299,3850). Gamma delta T cells have a restricted range of antigen recognition, and serve as a first line of defence against bacterial peptides, such as heat shock proteins (3850). They are often involved in responses to mycobacterial infections and in mucosal immunity. The T cells of the adaptive immune system, which are heterogeneous and functionally complex, include naive, effector (regulatory and cytotoxic), and memory T cells. T-cell lymphomas of the adaptive immune system present primarily in adults and are mainly nodal in origin, in contrast with the extranodal T-cell lymphomas of the innate immune system (1816). CD4+ T cells are primarily regulatory, acting via cytokine production; based on their cytokine secretion profiles, CD4+ T cells are divided into two major types: T helper 1 (Th1) cells and T helper 2 (Th2) cells. Th1 cells secrete IL2 and interferon gamma, but not IL4, IL5, or IL6. In contrast, Th2 cells secrete IL4, IL5, IL6, and IL10 (3850). Th1 cells provide help mainly to other T cells and to macrophages, whereas Th2 cells provide help mainly to B cells, in the production of antibodies (1758A). CD4+ T cells can act both to help and to suppress immune responses, and consist of multiple subpopulations only recently recognized. For example, T regulatory (Treg) cells have diverse functions, including suppressing immune responses to cancer and limiting inflammatory responses in tissues (2403,4350). Recent studies have identified transcription factors, TBX21 (also...
Inflammatory diseases and in other conditions, Th17 cells are a more recently identified subset of CD4+ effector T cells. They are characterized by expression of IL17 family of cytokines and play a role in immune-mediated inflammatory diseases and in other conditions.

Much has been learned about a unique CD4+ T-cell subset found in the normal germinal centre. These cells, called T follicular helper (TFH) cells, provide help to B cells in the context of the germinal centre reaction [1067,1469,2993]. They have a unique phenotype, expressing the germinal centre markers BCL6 and CD10, which are also normally found on germinal centre B cells. TFH cells also express CD4, CD57, and CD279/PD1 and produce the chemokine CXCL13 and its receptor CXCR5. CXCL13 causes induction and proliferation of follicular dendritic cells; it also facilitates the migration of B cells and T cells expressing CXCR5 into the germinal centre. Increased expression of CXCL13 in angioimmunoblastic T-cell lymphoma (AITL) is a finding that helps to link many of its clinical and pathological features [1067,1469,2993]. Notably, AITL is associated with polyclonal hypergammaglobulinaemia and expansion and proliferation of both B cells and CD21+ follicular dendritic cells within the lymph node. Some other nodal PTCLs display a TFH-cell phenotype and share many genetic features with AITL [178]. However, a TFH-cell phenotype is also seen in a very different disease: primary cutaneous CD4+ small/medium T-cell lymphoproliferative disorder.

A CD4+ T cell with very different properties is the Treg cell, which functions to shut off and suppress immune responses [3715]. This cell is thought to play an important role in preventing autoimmunity. Treg cells express high-density CD25 and the transcription factor FOXP3, in combination with CD4. Adult T-cell leukaemia/lymphoma (ATLL) has been linked to Treg cells on the basis of expression of both CD25 and FOXP3, and this finding helps to explain the marked immunosuppression associated with ATLL [1948,3398].

Recent studies have tried to relate the pathological or clinical manifestations of T-cell lymphomas to cytokine or chemokine expression by the neoplastic cells or by accompanying accessory cells within the lymph node. For example, the hypercalcemia associated with ATLL has been linked to secretion of factors with osteoclast-activating activity [2896A]. The haemophagocytic syndrome seen in some T-cell and NK-cell malignancies has been associated with secretion of both cytokines and chemokines, in the setting of defective cytokine function [1834,2011,2970].

Genetics

Several mature B-cell neoplasms have characteristic genetic abnormalities that are important in determining their biological features and can be useful in differential diagnosis. These genetic abnormalities include t(11;14)(q13;q32) in mantle cell lymphoma, t(14;18)(q32;q21) in follicular lymphomas, t(6;14)(q24;q32) and variants in Burkitt lymphoma, and t(11;18)(q21;q21) in MALT lymphoma (889,1933,2286). The t(11;14) translocation is seen in both mantle cell lymphoma and some cases of plasma cell myeloma, but there are minor differences in the translocation, involving different portions of the IGH gene [350]. The most common paradigm for translocations involving an IGH gene on 14q32 is that a cellular proto-oncogene comes under the influence of the IGH enhancer. For example, in follicular lymphoma, the overexpression of BCL2 blocks apoptosis in germinal centre B cells. The t(11;18) translocation, which is common in MALT lymphomas, results in a fusion gene, in which BIRC3 (also known as API2) is fused to the C-terminal sequences of MALT1 [982,2406,3341,3812]. The chimeric protein encoded by the fusion gene promotes cell survival and proliferation via activation of NF-kappaB [2406]. Other translocations found in MALT lymphomas, such as t(1;14)(p22;q32) and t(14;18)(q32;q21), act in a similar fashion by deregulating the oncogenes BCL10 and MALT1, respectively, through juxtaposition to the IGH enhancer.

The number of mature T-cell lymphomas with recurrent genetic aberrations has increased significantly in recent years with the introduction of next-generation sequencing and mutation analysis. ALK-positive anaplastic large cell lymphoma was the first T-cell lymphoma to be linked to a specific aberration: translocations involving the ALK gene on chromosome 2p23. ALK is fused to a variety of partner genes, most often NPM1, as a consequence of t(2;5)(p23;q35) [1136].
1815, 2199]. Hepatosplenic T-cell lymphoma of gamma delta origin has mutations in STAT5B in approximately 35% of cases (2869), and the same mutation is recurrent in gamma delta T-cell lymphomas involving the gastrointestinal tract and skin (2160). The JAK/STAT pathway is implicated in many forms of T-cell lymphoma, including anaplastic large cell lymphoma (both ALK-positive and ALK-negative) and monomorphic epithelioid intestinal T-cell lymphoma (836, 2807).

Other genetic tools have also been applied in the study of mature lymphoid neoplasms. These include comparative genomic hybridization and more sensitive techniques of array-based copy-number profiling, both of which can identify areas of deletion or amplification within the genome. Gene expression microarrays can interrogate the expression of thousands of genes at the RNA level, helping to elucidate pathways of activation and transformation (877, 878, 884, 1208, 1775, 2719, 3409, 3538). Most recently, studies have begun to explore changes at the epigenetic level that control the expression of multiple genes (2352, 3528).

**Principles of classification**

The classification of lymphoid neoplasms is based on all available information to define disease entities (1557). Having sufficient tissue for this multiparameter approach is critical. Great caution is advised when core needle biopsies are used for the primary diagnosis of lymphoma; fine-needle aspiration is generally inadequate for this purpose. Morphology and immunophenotype are sufficient for the diagnosis of most lymphoid neoplasms. However, no one antigenic marker is specific for any neoplasm, and a combination of morphological features and a panel of antigenic markers are necessary for correct diagnosis. Most B-cell lymphomas have characteristic immunophenotypic profiles that are very helpful in diagnosis. However, immune profiling is somewhat less helpful in the subclassification of T-cell lymphomas.

Although certain antigens are commonly associated with specific disease entities, these associations are not entirely disease-specific. For example, CD30 is a universal feature of anaplastic large cell lymphoma, but can also be expressed in other T-cell and B-cell lymphomas and in classic Hodgkin lymphoma (CHL). Similarly, CD56 is a characteristic feature of nasal NK/T-cell lymphoma, but it can also be found in other T-cell lymphomas, in plasma cell neoplasms, and in non-lymphoid cells such as blastic plasmacytoid dendritic cell neoplasms (173, 315, 746, 987, 4357). Within a given disease entity, variation in immunophenotypic features can be seen. For example, most hepatosplenic T-cell lymphomas are of the gamma delta T-cell phenotype, but some cases are of alpha beta T-cell derivation. Likewise, some follicular lymphomas are CD10-negative. An aberrant immunophenotype may suggest or help to confirm a diagnosis of malignancy (1815).

Although lineage is a defining feature of most lymphoid malignancies, in recent years there has been an increasing appreciation of lineage plasticity within the haematopoietic system. Lineage switch or demonstration of multiple lineages is most often encountered in immature haematolymphoid neoplasms, but also can be seen rarely in mature lymphomas (772, 1172, 1536).

### Table 11.01 Immunophenotypic features of common mature B-cell neoplasms; the symbols indicate the proportion of cases in which each marker listed is positive.

Modified and updated from Swerdlow SH, et al. (3848).

<table>
<thead>
<tr>
<th>Neoplasm</th>
<th>slg, clg</th>
<th>CD5</th>
<th>CD10</th>
<th>CD23</th>
<th>CD43</th>
<th>CD103</th>
<th>BCL6</th>
<th>IRF4/MUM1</th>
<th>Cyclin D1</th>
<th>ANXA1</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLL</td>
<td>+, -/+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>(PCs)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>LPL</td>
<td>+/-, +</td>
<td>+/-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>a</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>SMZL</td>
<td>+, +/-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>HCL</td>
<td>+, -</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+/-</td>
<td>+/-/</td>
<td>-</td>
</tr>
<tr>
<td>PCM</td>
<td>-</td>
<td>+</td>
<td>-/+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-/+-</td>
<td>-</td>
</tr>
<tr>
<td>MALT lymphoma</td>
<td>+, +/-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>a</td>
<td>-/+-</td>
<td>-</td>
</tr>
<tr>
<td>FL</td>
<td>+, -</td>
<td>-/c</td>
<td>+/-</td>
<td>-/+</td>
<td>-/+</td>
<td>-</td>
<td>+</td>
<td>+/-b</td>
<td>-/+-</td>
<td>-</td>
</tr>
<tr>
<td>MCL</td>
<td>+/-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>DBLCL</td>
<td>+/-, +/-</td>
<td>-c</td>
<td>+/-</td>
<td>-/+</td>
<td>n/a</td>
<td>-/+</td>
<td>n/a</td>
<td>+/-d</td>
<td>+/-e</td>
<td>-</td>
</tr>
<tr>
<td>BL</td>
<td>+/-</td>
<td>-</td>
<td>-</td>
<td>+/-</td>
<td>n/a</td>
<td>+</td>
<td>+/-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

>90% (+), >50% (+/−), <50% (−/+), or <10% (−)


- The plasma cell components of LPL and MALT lymphoma are IRF4+.
- Some grade 3A and 3B FLs are IRF4+.
- Some DLBCLs are CD5+. Other B-cell neoplasms can sometimes be CD5+, including LPL, MALT and FL.
- DLBCLs of germinal centre B-cell type express CD10 and BCL6.
- DLBCLs of activated B-cell type are typically IRF4+. 

Genetic features are playing an increasingly important role in the classification of lymphoid malignancies. However, the molecular pathogenesis of many forms of lymphoma remains unknown. Genetic studies, in particular PCR studies of IG and TR genes and FISH, are valuable diagnostic tools for the determination of clonality in B-cell and T-cell proliferations (aiding in the differential diagnosis with reactive hyperplasia) and for the identification of translocations associated with some disease entities. The identification of the MYD88 L265P mutation in most cases of lymphoplasmacytic lymphoma but only rarely in marginal zone lymphoma has provided new tools for diagnosis [1527]. Similarly, mutations in BRAF are recurrent in certain histiocytic and dendritic cell proliferations, such as Langerhans cell histiocytosis [215] and Erdheim–Chester disease [1102], but are nearly ubiquitous in hairy cell leukaemia [3997,3998].

The WHO classification emphasizes the importance of knowledge of clinical features, both for accurate diagnosis and for the definition of some diseases, such as extranodal marginal zone lymphoma of MALT (MALT lymphoma) versus nodal or splenic marginal zone lymphoma, primary mediastinal large B-cell lymphoma (PMLBCL) versus DLBCL, and most mature T-cell and NK-cell neoplasms. The diagnosis of lymphoid neoplasms should not take place in a vacuum, but rather within the context of a complete clinical history.

Lymphoid malignancies range in their clinical behaviour from indolent to aggressive. Within any given entity, a range in clinical behaviour can also be seen, and histological or clinical progression is often encountered during a patient’s clinical course. For these reasons, the WHO classification does not attempt to stratify lymphoid malignancies in terms of histologic grade or clinical aggressiveness. Both morphology and immunophenotype often change over time, as the lymphoid neoplasm undergoes clonal evolution with the acquisition of additional genetic changes. Transformation can arise by divergent evolution from a common precursor, or by more-direct linear transformation, and examples of both patterns have been shown [1434]. In addition, evolution over time does not necessarily lead to the development of a more aggressive lymphoma. For example, patients with DLBCL can relapse with a more indolent clonally related follicular lymphoma. Some of these clonal evolutions can be unexpected and not obviously connected, such as the development of a plasmacytoma in a patient with CHL [1824]. Traditionally, CHLs have been considered separately from the so-called non-Hodgkin lymphomas. However, with the recognition that CHL is of B-cell lineage, greater overlap has been recognized between CHL and many other forms of B-cell malignancies. This revised 4th edition of the WHO classification acknowledges these gray zones and provides for the identification of cases that bridge the gap between these various forms of lymphoma [4047]. The borders are further blurred by conditions such as nodular lymphocyte predominant Hodgkin lymphoma, which manifests many clinical and biological characteristics of Hodgkin and non-Hodgkin lymphomas.

### Epidemiology

Precursor lymphoid neoplasms, including B-lymphoblastic leukaemia/lymphoma and T-lymphoblastic leukaemia/lymphoma, are primarily diseases of children. About 75% of cases occur in children aged <6 years. Approximately 85% of cases presenting as lymphoblastic leukaemia are of precursor B-cell type, whereas lymphoblastic malignancies of precursor T-cell type more often present as lymphoma, with mediastinal masses. A male predominance is seen in lymphoblastic malignancies of both B-cell and T-cell lineages.

According to the *World Cancer Report 2014* [205], there were 566,000 new cases of lymphoma and about 305,000 deaths due to lymphoma in 2012. Mature B-cell neoplasms constitute >90% of lymphoid neoplasms worldwide [1,148] and account for approximately 4% of all new cancer cases each year. They are more common in developed countries, in particular in North America, Australia, New Zealand, and northern and western Europe. Globally, the incidence is greatest in Israel, followed by Lebanon, Australia, and the USA. The incidence of lymphomas, in particular B-cell lymphomas, has increased worldwide, but may be plateauing over the past decade. The International Lymphoma Epidemiology (InterLymph) Consortium is examining factors associated with increased lymphoma incidence using a case–control study methodology [2758].
The most common lymphoma types are follicular lymphoma and DLBCL, which together make up >60% of all lymphomas other than Hodgkin lymphoma and plasma cell myeloma [1,95]. The individual B-cell neoplasms vary in their relative frequency in various parts of the world. Follicular lymphoma is more common in the USA (where it accounts for 35% of non-Hodgkin lymphomas) and western Europe, and is uncommon in South America, eastern Europe, Africa, and Asia. Burkitt lymphoma is endemic in equatorial Africa, where it is the most common childhood malignancy, but it accounts for only 1–2% of lymphomas in the USA and western Europe. The median patient age for all types of mature B-cell neoplasms is in the sixth or seventh decade of life, but PMLBCL has a median patient age of about 35 years. Of the mature B-cell lymphomas, only Burkitt lymphoma and DLBCL occur with any significant frequency in children. Most types have a male predominance (with 52–55% of cases occurring in males), but mantle cell lymphoma has a striking male predominance (with 74% of the cases occurring in males), whereas females account for 58% of follicular lymphoma cases and 66% of PMLBCL cases. PMLBCL and nodular sclerosis CHL have similar clinical profiles at presentation, most commonly affecting adolescent and young-adult females. It was their shared clinical features that first prompted consideration that these lymphomas might be related [3412,3538,4047].

One known major risk factor for mature B-cell neoplasia appears to be an abnormality of the immune system, either immunodeficiency or autoimmune disease. Although evidence of immune system abnormality is absent in most patients with mature B-cell neoplasms, immunodeficient patients have a markedly increased incidence of B-cell neoplasia, in particular DLBCL and Burkitt lymphoma [345, 547,2819]. Major forms of immunodeficiency include HIV infection, iatrogenic immunosuppression to prevent allograft rejection or graft-versus-host disease, and primary immune deficiencies. Some autoimmune diseases are also associated with an increased risk of lymphoma [4252]; for example, patients with Sjögren syndrome or Hashimoto thyroiditis have a particularly high risk of developing B-cell lymphomas [1953,1959].

Mutations in genes controlling lymphocyte apoptosis have been linked to increased risk of both autoimmune diseases and lymphomas (mainly B-cell types). Patients with autoimmune lymphoproliferative syndrome (which can be caused by germline mutations in FAS) have an increased risk of B-cell lymphomas and Hodgkin lymphomas [3810]. Somatically acquired FAS mutations have also been reported in some sporadic B-cell lymphomas, most commonly marginal zone lymphomas [1483]. Genome-wide association studies have identified a substantial number of SNPs that are associated with increased risk of lymphoma [603]. Many of these polymorphisms involve immunoregulatory genes [604,2206,4251].

Mature T-cell and NK-cell neoplasms are relatively uncommon. In a large international study that evaluated lymphoma cases from the USA, Europe, Asia, and South Africa, T-cell and NK-cell neoplasms accounted for only 12% of all non-Hodgkin lymphomas [1]. The most common mature T-cell lymphomas are PTCL, NOS (accounting for 25.9% of cases) andAITL (accounting for 18.5%) [4217]. T-cell and NK-cell lymphomas show substantial variations in incidence across geographical regions and racial populations. In general, T-cell lymphomas are relatively more common in Asia [4217], due to both a higher incidence of some subtypes and a lower relative frequency of some B-cell lymphomas, such as follicular lymphoma. In Japan, one of the main risk factors for T-cell lymphoma is HTLV-1 infection. In endemic regions of south-western Japan, the seroprevalence of HTLV-1 is 8–10%. The cumulative lifetime risk for the development of ATLL is 6.9% for seropositive males and 2.9% for
seropositive females (3869). Other regions with relatively high seroprevalence of HTLV-1 include the Caribbean basin, where Black populations are primarily affected, over other racial groups (1818, 2287). Differences in viral strain may also affect the incidence of the disease (796, 3869).

Another major factor influencing the incidence of T-cell and NK-cell lymphomas is racial predisposition. EBV-associated NK-cell and T-cell neoplasms, including extranodal NK/T-cell lymphoma, nasal type (ENKTL), aggressive NK-cell leukaemia, and paediatric EBV-positive T-cell and NK-cell lymphomas, are much more common among Asians than in other populations (640,780,1814,2058). In Hong Kong SAR, China, ENKTL is one of the more common subtypes, accounting for 8% of cases. By contrast, in Europe and North America it accounts for <1% of all lymphomas. Other populations at increased risk for this disease are individuals of Native American descent in Central and South America and Mexico (275,780,3266,3269), who are genetically related to Asians (3280A). Enteropathy-associated T-cell lymphoma is most common in individuals of Welsh and Irish descent, who share HLA haplotypes that confer an increased risk of gluten allergy and susceptibility to gluten-sensitive enteropathy (837).

PTCLs with a gamma delta phenotype occur with increased frequency in the setting of immune suppression (in particular following organ transplantation), a finding that is not well understood (1301,2842). The risk appears to be increased by the combination of two factors: immunosuppression and chronic antigenic stimulation. Hepatosplenic T-cell lymphomas are most common, but primary cutaneous and mucosa-associated T-cell lymphomas have also been reported (150). Recent data from the SEER programme indicate a modest increase in the annual incidence of T-cell neoplasms in the USA: to 2.6 cases per 100 000 population, with the greatest increase occurring in the category of cutaneous T-cell lymphoma (839,2759).

**Etiology**

Infectious agents have been shown to contribute to the development of several types of mature B-cell, T-cell, and NK-cell lymphomas. EBV is present in nearly 100% of endemic Burkitt lymphoma cases and in 15–35% of sporadic and HIV-associated cases (1531,3238); it is also involved in the pathogenesis of many B-cell lymphomas arising in immunosuppressed or elderly patients, including many post-transplant lymphoproliferative disorders, plasmablastic lymphoma, and EBV-positive DLBCL, NOS. EBV is also associated with ENKTL and with two paediatric T-cell diseases: systemic EBV-positive T-cell lymphoma of childhood and hydroa vacciniforme–like lymphoproliferative disorder. The exact cause of these EBV-positive T-cell neoplasms of childhood is unclear. Risk factors may include either a high viral load at presentation or a defective immune response to the infection (3269). Chronic active EBV infection may precede the development of some EBV-positive T-cell lymphomas (1918,3269). In cases associated with chronic active EBV infection, a polyclonal process may be seen early in the course, with progression to monoclonal EBV-positive T-cell lymphoma (1918).

HHV8 is found in primary effusion lymphoma, multicentric Castleman disease, and HHV8-positive DLBCL, NOS, all of which are mainly seen in HIV-infected patients (623,1046), but the virus has also been implicated in germinotropic lymphoproliferative disease not associated with HIV. HTLV-1 is the causative agent of ATLL, and is clonally integrated into the genome of transformed T cells (796). In this condition, like in HHV8-associated disorders, a spectrum of clinical behaviours is seen, although most cases of ATLL are aggressive. Hepatitis C virus has been implicated in some cases of lymphoplasmatocytic lymphoma associated with type II cryoglobulinaemia, splenic marginal zone lymphoma, nodal marginal zone lymphoma, and DLBCL (32,168,909,912,1884, 2589,3229,4503). The role of the virus in tumour initiation is unclear; however, it does not directly infect neoplastic B cells, and appears to influence lymphoma development through activation of a B-cell immune response.

Bacteria, or at least immune responses to bacterial antigens, have also been implicated in the pathogenesis of MALT lymphoma, including Helicobacter pylori in gastric MALT lymphoma (1741,2853, 4366,4367); Borrelia burgdorferi in cutaneous MALT lymphoma in Europe (611); Chlamydia psittaci, C. pneumoniae, and C. trachomatis in ocular adnexal MALT lymphomas in some geographical areas (658,3454); Campylobacter jejuni in intestinal MALT lymphoma associated with alpha heavy chain disease (3239,3298), and Achromobacter xylosoxidans in pulmonary MALT lymphomas (20).

Environmental exposures have also been linked to a risk of developing B-cell lymphoma. Epidemiological studies have implicated herbicide and pesticide use in the development of follicular lymphoma and DLBCL (787,1566). Exposure to hair dyes was identified as a risk factor in some older studies, but potential carcinogens have been removed from newer dye formulations (4485).

**Conclusion**

The multiparameter approach to classification adopted by the WHO classification has been validated in international studies. It is highly reproducible and improves the interpretation of clinical and translational studies. Accurate and precise classification of disease entities also facilitates the determination of the molecular basis of lymphoid neoplasms in the basic science laboratory (1,1815,2759).
CHAPTER 12

Precursor lymphoid neoplasms
B-lymphoblastic leukaemia/lymphoma, NOS
B-lymphoblastic leukaemia/lymphoma with recurrent genetic abnormalities
T-lymphoblastic leukaemia/lymphoma
NK-lymphoblastic leukaemia/lymphoma
B-lymphoblastic leukaemia/lymphoma, not otherwise specified (NOS)

Definition
B-lymphoblastic leukaemia/lymphoma (B-ALL/LBL) is a neoplasm of precursor lymphoid cells committed to the B-cell lineage, typically composed of small to medium-sized blast cells with scant cytoplasm, moderately condensed to dispersed chromatin, and inconspicuous nucleoli, involving bone marrow and blood (B-ALL) and occasionally presenting with primary involvement of nodal or extranodal sites (B-LBL). By convention, the term lymphoma is used when the process is confined to a mass lesion with no or minimal evidence of blood and marrow involvement. With extensive marrow and blood involvement, the appropriate term is B-ALL. If a patient presents with a mass lesion and lymphoblasts in the marrow, the distinction between leukaemia and lymphoma is arbitrary (2792). In many treatment protocols, a value of >25% marrow blasts is used to define leukaemia. Unlike with myeloid leukaemias, there is no agreed-upon lower limit for the proportion of blasts required to establish a diagnosis of lymphoblastic leukaemia (ALL). In general, the diagnosis should be avoided when there are <20% blasts. Presentations of ALL with low blast counts are uncommon; there is no compelling evidence that failure to treat a patient when there are <20% marrow lymphoblasts has an adverse effect on outcome.

Exclusionary criteria
The term B-ALL should not be used to indicate Burkitt leukaemia/lymphoma. Furthermore, some cases of B-ALL/LBL have specific recurrent genetic abnormalities that are associated with distinctive clinical and phenotypic properties, have important prognostic implications, or demonstrate other evidence that they are mutually exclusive of other entities. These cases should not be classified as B-ALL/LBL, NOS, but rather according to their genetic abnormalities. There are currently nine genetically defined B-ALL/LBLs, which are further described in section B-lymphoblastic leukaemia/lymphoma with recurrent genetic abnormalities (p. 203).

ICD-O code 9811/3

Synonyms
Pro-B lymphoblastic leukaemia; common precursor B-lymphoblastic leukaemia; pre-B lymphoblastic leukaemia; pre-pre-B lymphoblastic leukaemia; common lymphoblastic leukaemia; precursor B-cell lymphoblastic lymphoma; precursor B-cell lymphoblastic leukaemia; precursor B-cell lymphoblastic leukaemia, NOS; B-cell acute lymphoblastic leukaemia

Epidemiology
ALL is primarily a disease of children; 75% of cases occur in children aged <6 years. The estimated annual incidence worldwide is 1–4.75 cases per 100 000 population (3327). The estimated number of new cases in the USA is approximately 6000 per year (1603), with approximately 80–85% being of precursor B-cell phenotype (1501,3358). B-LBL constitutes about 10% of lymphoblastic lymphomas (LBLs); the remainder are of T-cell lineage (419). In one literature review, approximately 64% of 98 reported cases were in patients aged <18 years (2448). One report indicated a male predominance (2345).

Etiology
The etiology of B-ALL/LBL is unknown. There is an increased risk of B-ALL in children with Down syndrome and other constitutional genetic disorders (4494). Genome-wide association studies have shown increased risk of B-ALL associated with certain single nucleotide polymorphisms (SNPs) of genes including GATA3, ARID5B, IKZF1, CEBPE, and CDKN2A/B (3051,3133). However, true familial ALL is rare, with some kindreds
described having mutations in PAX5 (3630), ETV6 (4435), and TP53 (3226). TP53 mutation, as discussed below, shows a specific association with low hypodiploid B-ALL [1929]. Some translocations associated with ALL have been detected in neonatal specimens long before the onset of leukaemia, and monozygotic twins with concordant leukaemia frequently share genetic abnormalities [1428,2731]. However, these findings are thought to reflect somatic mutations occurring in one twin and shared via in utero circulation rather than constitutional genetic lesions.

Localization
By definition, the bone marrow is involved in all cases classified as B-ALL, and the peripheral blood is usually involved. Extramedullary involvement is common, with particular predilection for the central nervous system (CNS), lymph nodes, spleen, liver, and testes. The most frequent sites of involvement in B-LBL are the skin, soft tissue, bone, and lymph nodes [354, 2345,2448]. Mediastinal masses are uncommon [354,2448,3506].

Clinical features
Most patients with B-ALL present with evidence and consequences of bone marrow failure: thrombocytopenia, anaemia, and/or neutropenia. The leukocyte count may be decreased, normal, or markedly elevated. Lymphadenopathy, hepatomegaly, and splenomegaly are frequent. Bone pain and arthralgias may be prominent symptoms. Patients presenting with B-LBL are usually asymptomatic, and most have limited-stage disease. Head and neck presentations are particularly common, especially in children. Marrow and blood involvement may be present, but by definition the proportion of lymphoblasts in the marrow is <25% [2345,2448].

Microscopy
In smear and imprint preparations, the lymphoblasts in B-ALL/LBL vary from small blasts with scant cytoplasm, condensed nuclear chromatin, and indistinct nucleoli to larger cells with moderate amounts of light-blue to bluish-grey cytoplasm (occasionally vacuolated), dispersed nuclear chromatin, and multiple variably prominent nucleoli. The nuclei are round or show convolutions. Coarse azurophilic granules are present in some lymphoblasts in approximately 10% of cases. In some cases, the lymphoblasts have cytoplasmic pseudopods (hand-mirror cells). Normal B-cell precursors (haematogones) can mimic lymphoblasts, but they typically have even higher nuclear:cytoplasmic ratios, more-homogeneous chromatin, and no discernible nucleoli.

In bone marrow biopsies, the lymphoblasts in B-ALL are relatively uniform in appearance, with round to oval, indented, or convoluted nuclei. Nucleoli range from inconspicuous to prominent. The chromatin is finely dispersed. The number of mitotic figures varies. LBL is generally characterized by a diffuse or (less commonly) paracortical pattern of involvement of lymph node. A single-file pattern of infiltration of soft tissue is common. Mitotic figures are usually numerous, and in some cases there may be a focal so-called starry-sky pattern. The morphological features of B-lymphoblastic and T-lymphoblastic proliferations are indistinguishable.

Cytochemistry
Cytochemistry seldom contributes to the diagnosis of ALL. Lymphoblasts are negative for MPO. Granules, if present, may stain light grey with Sudan Black B but are less intense than myeloblasts. Lymphoblasts may show periodic acid–Schiff (PAS) positivity, usually in the form of coarse granules. They may react with non-specific esterase, with a multifocal punctate or Golgi region pattern that shows variable inhibition with sodium fluoride.

Immunophenotype
The lymphoblasts in B-ALL/LBL are almost always positive for the B-cell markers CD19, cCD79a, and cCD22; although none of these by itself is specific, their positivity in combination or at high intensity strongly supports the diagnosis. The lymphoblasts are positive for CD10, surface CD22, CD24, PAX5, and TdT in most cases, whereas CD20 and CD34 expression is variable; CD45 may be absent and if present is nearly always more dimly expressed than on mature B cells. The myeloid-associated antigens CD13 and CD33 may be expressed and the presence of these myeloid markers...
ALL and is not specific [1584]. PAX5 is generally considered the most sensitive and specific marker for B-cell lineage in tissue sections [4028], but it is also positive in acute myeloid leukaemia with t(8;21)(q22;q22,1) resulting in RUNX1-RUNX1T1 and rarely in other acute myeloid leukemias [4102]. MPO can sometimes be detected, most commonly by immunohistochemistry but also sometimes by flow cytometry, and should not automatically exclude the diagnosis, but MPO immunoreactivity most often indicates either acute myeloid leukaemia or B/myeloid acute leukaemia [131].

The degree of differentiation of precursor B-ALL and PAX5 is most frequently used to demonstrate B-cell differentiation, but CD79a is positive in many cases of T-ALL and is not specific [1584].

Fig. 12.05 B-lymphoblastic leukaemia in lymph node. The neoplastic cells infiltrate diffusely, sparing normal follicles.

does not exclude the diagnosis of precursor B-ALL. In tissue sections, CD79a and PAX5 are most frequently used to demonstrate B-cell differentiation, but CD79a is positive in many cases of T-ALL and is not specific [1584]. PAX5 is generally considered the most sensitive and specific marker for B-cell lineage in tissue sections [4028], but it is also positive in acute myeloid leukaemia with t(8;21)(q22;q22,1) resulting in RUNX1-RUNX1T1 and rarely in other acute myeloid leukemias [4102]. MPO can sometimes be detected, most commonly by immunohistochemistry but also sometimes by flow cytometry, and should not automatically exclude the diagnosis, but MPO immunoreactivity most often indicates either acute myeloid leukaemia or B/myeloid acute leukaemia [131].

Haematogenotypes nearly always differ. Haematogenotypes show a continuum of expression of markers of B-cell maturation, including surface immunoglobulin light chain, and display a reproducible pattern of acquisition and loss of normal antigens [2600]. In contrast, B-ALL shows patterns that differ from normal, with either overexpression or underexpression of many markers, including CD10, CD45, CD38, CD58, and TdT [537,674,2408,4280]. These differences can be very useful in the evaluation of follow-up marrow specimens for minimal residual disease.

Postulated normal counterpart
Either a haematopoietic stem cell or a B-cell progenitor

Genetic profile
Antigen receptor genes
Nearly all cases of B-ALL have clonal rearrangements of IGH. In addition, T-cell receptor (TR) gene rearrangements may be seen in a substantial proportion of cases (as many as 70%) [4126]. Therefore, IGH and TR gene rearrangements are not helpful for lineage assignment.

Cytogenetic abnormalities and oncogenes
Cytogenetic abnormalities are seen in most cases of B-ALL/LBL; in many cases, they define specific entities with unique phenotypic and prognostic features (see following section). Additional cytogenetic lesions that are not associated with the entities in the category of B-ALL with recurrent genetic abnormalities include del(6q), del(9p), and del(12p), but these do not have an impact on prognosis. However, it is likely that some genetic lesions are prognostically important, although there is not yet sufficient evidence to considering these as distinct entities. For example, the very rare ALL with t(17;19)(q22;p13.3) resulting in TCF3-HLF is associated with a very poor prognosis, but there are too few cases to include this in the classification.

A very large number of recurrent genetic alterations occur in B-ALL, detected either as copy-number alterations or as specific mutations. Many of these, such as alterations of PAX5, are seen in most subtypes of B-ALL and are likely fundamental to the pathogenesis of the disease. Mutations in other genes, such as the RAS family of oncogenes and IKZF1, are seen in a more restricted distribution. Although they are not strictly part of the definition of genetic entities, they do tend to be associated with particular types.

Prognosis and predictive factors
B-ALL has a good prognosis in children, but a less favourable prognosis in adults. The overall complete remission rate is >95% in children, versus 60–85% in adults. Approximately 80% of children with B-ALL appear to be cured, versus <50% of adults. More-intensive therapy improves cure rates, and there is some evidence that, at least in younger adults, therapy with more-intensive so-called paediatric-type regimens is associated with better outcome [408,1521].

Infancy, older patient age, higher white blood cell count, slow response to initial therapy as assessed by morphological examination of blood and/or bone marrow, and the presence of minimal residual disease after therapy are all associated with adverse prognosis [380,572,829,3589,3679,4129]. The presence of CNS disease at diagnosis is associated with adverse outcome and requires specific therapy [764]. The important effect of genetic lesions is discussed in the sections below.

The prognosis of B-LBL is also relatively favourable, and like that of B-ALL, it appears to be better in children than in adults [2448].
B-lymphoblastic leukaemia/lymphoma with recurrent genetic abnormalities

Definition
B-lymphoblastic leukaemia/lymphoma (B-ALL/LBL) with recurrent genetic abnormalities is a group of diseases characterized by recurrent genetic abnormalities, including balanced translocations and abnormalities involving chromosome number. Many chromosomal abnormalities that are non-randomly associated with B-ALL are not included as separate entities in this section. Although the inclusion or exclusion of a given genetic entity is somewhat arbitrary, those included have been chosen because they are associated with distinctive clinical or phenotypic properties, have important prognostic implications, demonstrate other evidence that they are biologically distinct, and are generally mutually exclusive with other entities.

B-lymphoblastic leukaemia/lymphoma with t(9;22)(q34.1;q11.2); BCR-ABL1

Definition
B-lymphoblastic leukaemia/lymphoma (B-ALL/LBL) with t(9;22)(q34.1;q11.2) is a neoplasm of lymphoblasts committed to the B-cell lineage in which the blasts harbour a translocation between BCR on chromosome 22 and the ABL1 oncogene on chromosome 9.

ICD-O code 9812/3

Epidemiology
B-ALL with BCR-ABL1 is relatively more common in adults than in children, accounting for about 25% of adult ALL but only 2–4% of childhood cases.

Clinical features
The presenting features are generally similar to those seen in patients with other B-ALLs. Most children with B-ALL with BCR-ABL1 are considered to have high-risk on the basis of age and white blood cell count, but there are otherwise no characteristic clinical findings. Although patients with B-ALL with BCR-ABL1 may have organ involvement, lymphomatous presentations are rare.

Microscopy
There are no unique morphological or cytochemical features that distinguish this entity from other types of ALL.

Immunophenotype
B-ALL with BCR-ABL1 is typically positive for CD10, CD19, and TdT. There is frequent expression of myeloid-associated antigens CD13 and CD33 [1712]; KIT (CD117) is typically not expressed. CD25 is highly associated with B-ALL with BCR-ABL1, at least in adults [3030]. Rare cases of ALL with BCR-ABL1 have a T-cell precursor phenotype.

Cell of origin
There is some evidence that the cell of origin of B-ALL with BCR-ABL1 is more immature than that of other B-ALL cases [773].

Genetic profile
The t(9;22) translocation results from fusion of BCR at 22q11.2 and the cytoplasmic tyrosine kinase gene ABL1 at 9q34.1, with production of a BCR-ABL1 fusion protein. In most childhood cases of ALL with t(9;22), a p190 BCR-ABL1 fusion protein is produced. In adults, about half of all cases produce the p210 fusion protein that is characteristic of BCR-ABL1-positive chronic myeloid leukaemia, and the remainder produce the p190 transcript. No definite clinical differences have been attributed to these two gene products. The t(9;22) may be associated with other genetic abnormalities, including (in rare cases) abnormalities that might otherwise cause a case to be placed in one of the other categories discussed below. It is generally believed that the clinical features in such cases are governed by the presence of the t(9;22).

Prognosis and predictive factors
Historically, in both children and adults, B-ALL with BCR-ABL1 has been considered to have the worst prognosis of the major cytogenetic subtypes of ALL. Its higher frequency in adult ALL explains in part the relatively poor outcome of adults with ALL. In children, favourable clinical features including younger age, lower white blood cell count, and response to therapy are associated with somewhat better outcome [146]. Therapy with tyrosine kinase inhibitors has had a significantly favourable effect on outcome [3588].

B-lymphoblastic leukaemia/lymphoma with t(v;11q23.3); KMT2A-rearranged

Definition
B-lymphoblastic leukaemia/lymphoma (B-ALL/LBL) with t(v;11q23.3) is a neoplasm of lymphoblasts committed to the B-cell lineage in which the blasts harbour a translocation between KMT2A (also called MLL) at band 11q23.3 and any of a large number of fusion partners. Leukaemias that have deletions of 11q23.3 without KMT2A rearrangement are not included in this group.

ICD-O code 9813/3
Precursor lymphoid neoplasms recognize distinct lymphoblastic and monocytic presentations. In some cases of leukaemias with cytochemical features that distinguish this entity from other types of ALL, there are no unique morphological or cytochemical presentations. FISH studies using a break-apart probe for KMT2A (previously called MLL) or probes (MLLsp) for the normal KMT2A gene (MLL(11q23)) appear as juxtaposed red and green signals. The translocation is demonstrated by separation of the red and green probes, allowing the identification of specific fusion partners.

Clinical features
Patients with this leukaemia typically present with very high white blood cell counts, frequently > 100 x 10^9/L. There is also a high frequency of CNS involvement at diagnosis. Although organ involvement may be seen, pure lymphomatous presentations are not typical.

Microscopy
There are no unique morphological or cytochemical features that distinguish this entity from other types of ALL. In some cases of leukaemias with KMT2A rearrangement, it may be possible to recognize distinct lymphoblastic and monocytic populations, a finding that can be confirmed by immunophenotyping; such cases should be considered B/myeloid leukaemias.

Epidemiology
B-ALL with KMT2A rearrangement is the most common leukaemia in infants aged < 1 year. It is less common in older children and then becomes increasingly common with age into adulthood.

Genetic profile
The KMT2A gene on chromosome 11q23.3 is a promiscuous oncogene, with >100 fusion partners. Translocations involving this gene can be detected by standard karyotyping studies or by FISH with a break-apart probe directed against the KMT2A gene. PCR can be used to identify major translocation partners, but a negative PCR result cannot exclude an alternative fusion partner. The most common partner gene is AFF1 (AF4) on chromosome 4q21. Other common partner genes include MLLT1 (ENL) on chromosome 19p13 and MLLT3 (AF9) on chromosome 9p21.3. KMT2A-MLLT1 fusions are also common in T-ALL, whereas fusions between KMT2A and MLLT3 are more typically associated with acute myeloid leukaemia. Leukaemias with KMT2A rearrangement are frequently associated with overexpression of FLT3 [149]. In contrast to nearly all other categories of B-ALL, B-ALL with KMT2A rearrangement in infants has very few associated additional mutations, with frequencies among the lowest of all cancers [99]; when mutations occur, they typically involve RAS pathways [1039].

Prognosis and predictive factors
Leukaemias with the KMT2A-AFF1 translocation have a poor prognosis. There is some controversy as to whether leukaemias with translocations other than the KMT2A-AFF1 translocation have as poor a prognosis as those with KMT2A-AFF1. Leukaemias with KMT2A rearrangement, in particular those aged < 6 months, have a particularly poor prognosis [1039].

B-lymphoblastic leukaemia/lymphoma with t(12;21)(p13.2;q22.1); ETV6-RUNX1

Definition
B-lymphoblastic leukaemia/lymphoma (B-ALL/LBL) with t(12;21)(p13.2;q22.1) is a neoplasm of lymphoblasts committed to the B-cell lineage in which the blasts harbour a translocation between ETV6 (also called TEL) on chromosome 12 and RUNX1 (also called AML1) on chromosome 21.

ICD-O code
9814/3

Synonym
B acute lymphoblastic leukaemia/lymphoma with t(12;21)(p13.2;q221), TEL/AML1 (ETV6-RUNX1)

Epidemiology
This leukaemia is not seen in infants, but is common in children, accounting for about 25% of cases of B-ALL in that age group. It decreases in frequency with age to the point that it is rare in adulthood.

Clinical features
The presenting features are generally similar to those seen in patients with other ALLs.

Microscopy
There are no unique morphological or cytochemical features that distinguish this entity from other types of ALL.

Immunophenotype
Blasts have a CD19+, CD10+ phenotype and are most often CD34 positive; other phenotypic features, including near or
complete absence of CD9, CD20, and CD66c [421,914,1712], are relatively specific. Myeloid-associated antigens, especially CD13, are frequently expressed [283].

Cell of origin
This leukaemia appears to derive from a B-cell progenitor rather than a haematopoietic stem cell [580].

Genetic profile
The ETV6-RUNX1 translocation results in the production of a fusion protein that probably acts in a dominant negative fashion to interfere with normal function of the transcription factor RUNX1. This leukaemia appears to have a unique gene expression signature [4423]. The ETV6-RUNX1 translocation is considered to be an early lesion in leukaemogenesis, as evidenced by studies of neonatal blood spots that have shown the presence of the translocation in children who develop leukaemia many years later [4311]. There is evidence that the translocation is necessary but not sufficient for the development of leukaemia [4311].

Prognosis and predictive factors
B-ALL with the ETV6-RUNX1 translocation has a very favourable prognosis, with cure seen in >90% of children, especially if they have other favourable risk factors. Relapses often occur much later than do those of other types of ALL. Because this translocation appears to occur as an early event, it has been suggested that some late relapses in fact derive from persistent so-called preleukaemic clones that harbour the translocation and undergo additional genetic events after the first leukaemic clone has been eliminated [1234]. Children with this leukaemia who also have adverse prognostic factors, such as age > 10 years or high white blood cell count, do not have as good a prognosis, but may still fare better as a group than other ALL patients with the same adverse factors.

**B-lymphoblastic leukaemia/lymphoma with hyperdiploidy**

**Definition**
B-lymphoblastic leukaemia/lymphoma (B-ALL/LBL) with hyperdiploidy is a neoplasm of lymphoblasts committed to the B-cell lineage whose blasts contain >50 chromosomes (usually <66), typically without translocations or other structural alterations. There is controversy as to whether the specific chromosomal additions, rather than the specific number of chromosomes, should be part of the definition [1554,3284,3835].

**ICD-O code** 9815/3

**Synonyms**
Hyperdiploid acute lymphoblastic leukaemia; high hyperdiploid acute lymphoblastic leukaemia; acute lymphoblastic leukaemia with favourable trisomies

**Epidemiology**
This leukaemia is common in children, accounting for about 25% of cases of B-ALL in this age group. It is not seen in infants, and decreases in frequency among older children. It is uncommon in adulthood, accounting for about 7-8% of cases of B-ALL in adults [2771].

**Clinical features**
The presenting features are generally similar to those seen in patients with other ALLs.

**Microscopy**
There are no unique morphological or cytochemical features that distinguish this entity from other types of ALL.
**Immunophenotype**
Blasts are positive for CD19 and CD10, and express other markers typical of B-ALL. Most cases are CD34 positive, and CD45 is often absent [1712]. Patients with T-ALL with hyperdiploidy should not be considered part of this group, although most such patients have near-tetraploid karyotypes.

**Genetic profile**
Hyperdiploid B-ALL contains a numerical increase in chromosomes, usually without structural abnormalities. Extra copies of chromosomes are non-random: chromosomes 21, X, 14, and 4 are the most common, and chromosomes 1, 2, and 3 are the least often seen [1598]. Hyperdiploid B-ALL can be detected by conventional karyotyping, FISH, or flow cytometric DNA index (2523). Some cases that appear to be hyperdiploid ALL by conventional karyotyping may in fact be hypodiploid ALL that has undergone endoreduplication, doubling the number of chromosomes. The specific chromosomes that appear as trisomies may be more important to prognosis than the actual number of chromosomes, with simultaneous trisomies of chromosomes 4 and 10 carrying the best prognosis [3835].

**Prognosis and predictive factors**
Hyperdiploid B-ALL has a very favourable prognosis, with cure seen in >90% of children overall, and even more commonly among children with a favourable risk profile. Adverse factors such as advanced patient age and high white blood cell count may adversely affect the prognosis, but these patients may not fare as badly as others without this favourable genetic abnormality. Too few adult cases have been studied to determine the prognosis in adulthood.

**B-lymphoblastic leukaemia/lymphoma with hyperdiploidy**

**Definition**
B-lymphoblastic leukaemia/lymphoma (B-ALL/LBL) with hyperdiploidy is a neoplasm of lymphoblasts committed to the B-cell lineage whose blasts contain <46 chromosomes. Hyperdiploid ALL is divided into three (or sometimes four) subtypes: (1) near-haploid ALL (with 23-29 chromosomes), (2) low hypodiploid ALL (with 33-39 chromosomes), and (3) high hypodiploid ALL (with 40-43 chromosomes). A fourth category, near-diploid ALL (with 44-45 chromosomes), is often not included in definitions of hypodiploid ALL, at least for treatment purposes, because such cases do not share the poor prognostic features of the other three categories [2802].

**ICD-O code**
9816/3

**Epidemiology**
Hypodiploid ALL accounts for about 5% of ALL cases overall, but when the definition is restricted to cases with <45 chromosomes, the figure is closer to 1%. Hyperdiploid ALL occurs in both children and adults, although near-haploid ALL (23-29 chromosomes) appears to be limited to childhood.

**Clinical features**
The presenting features are generally similar to those seen in patients with other ALLs.

**Microscopy**
There are no unique morphological or cytochemical features that distinguish this entity from other types of ALL.

**Immunophenotype**
Blasts have a B-cell precursor phenotype (typically CD19+, CD10+), but there are no other distinctive phenotypic features.

**Genetic profile**
By definition, all cases show loss of one or more chromosomes. Structural abnormalities may be seen in the remaining chromosomes, although there are no specific abnormalities that are characteristically associated. Structural abnormalities are almost never seen in near-haploid ALL. The diagnosis of near-haploid or low hypodiploid ALL may be missed by standard karyotyping, because the hypodiploid clone can undergo endoreduplication, which doubles the number of chromosomes and results in a near-diploid or hyperdiploid karyotype. Flow cytometry can generally detect a clone with a DNA index of <1.0, although this may be a minor population. FISH may also identify cells hypodiploid for chromosomes, and this diagnosis should be suspected when there is a discrepancy between a karyotype and FISH results with respect to the number of chromosomes present.

The various classes of hypodiploid ALL are associated with distinctive genetic lesions [1929]. Near-haploid ALL often has RAS or receptor tyrosine kinase mutations. Low hypodiploid ALL is the most distinctive class: the great majority of cases show loss-of-function mutations in TP53 and/or RB1. Some of the TP53 mutations are germline, suggesting a form of Li-Fraumeni syndrome. These genetic lesions do not occur in high hypodiploid ALL, which is not associated with any specific gene alterations.

**Prognosis and predictive factors**
Hypodiploid ALL has a poor prognosis, with near-haploid ALL having the worst prognosis in some studies [1560,2802]. There is some evidence that patients may fare poorly even if they do not have minimal residual disease following therapy, which is in contrast to other types of ALL.

**B-lymphoblastic leukaemia/lymphoma with t(5;14)(q31.1;q32.1); IGH/IL3**

**Definition**
B-lymphoblastic leukaemia/lymphoma (B-ALL/LBL) with t(5;14)(q31.1;q32.1) is a neoplasm of lymphoblasts committed to the B-cell lineage in which the blasts harbour a translocation between IL3 and an IGH gene, resulting in variable eosinophilia. This diagnosis can be made on the basis of immunophenotypic and genetic findings even if the bone marrow blast count is low.

**ICD-O code**
9817/3

**Epidemiology**
This is a rare disease, accounting for <1% of cases of ALL. It has been reported in both children and adults.

**Clinical features**
The presenting clinical characteristics may be similar to those seen in patients with other ALLs, or patients may present with an asymptomatic eosinophilia, and blasts may be deceptively absent in the peripheral blood.
Fig. 12.10 B-lymphoblastic leukaemia with t(5;14)(q31.1;q32.1); GHIIL3. Bone marrow smear showing a population of typical lymphoblasts along with numerous mature eosinophils. The granule distribution in some of the eosinophils is unusual, but this is not a consistent factor.

Microscopy
Blasts in this neoplasm have the typical morphology of lymphoblasts, but the striking finding is an increase in circulating eosinophils. This is a reactive population and not part of the leukaemic clone.

Immunophenotype
Blasts have a CD19+, CD10+ phenotype. The finding of even small numbers of blasts with this phenotype in a patient with eosinophilia strongly suggests this diagnosis.

Genetic profile
The unique characteristics of this neoplasm derive from a functional rearrangement between the IL3 gene on chromosome 5 and an IGH gene on chromosome 14, resulting in constitutive overexpression of IL3 [1463]. Other than eosinophilia, the functional consequences of this rearrangement are not well understood. The abnormality is typically detected by conventional karyotyping; it can also be detected by FISH, although appropriate probes are not widely available.

Prognosis and predictive factors
The prognosis is not considered to be different from that of other types of ALL, although there are too few cases to be certain. Blast percentage at diagnosis is not known to be a predictive factor.

B-lymphoblastic leukaemia/lymphoma with t(1;19)(q23;p13.3); TCF3-PBX1

Definition
B-lymphoblastic leukaemia/lymphoma (B-ALL/LBL) with t(1;19)(q23;p13.3) is a neoplasm of lymphoblasts committed to the B-cell lineage in which the blasts harbour a translocation between TCF3 (also known as E2A) on chromosome 19 and PBX1 on chromosome 1.

ICD-O code 9818/3

Epidemiology
This leukaemia is relatively common in children, accounting for about 6% of cases of B-ALL. It is less common in adults.

Clinical features
The presenting features are generally similar to those seen in patients with other ALLs.

Microscopy
There are no unique morphological or cytochemical features that distinguish this entity from other types of ALL.

Immunophenotype
Blasts typically have a pre-B phenotype, with positivity for CD19, CD10, and cytoplasmic mu heavy chain, although not all cases of pre-B ALL have the t(1;19).

Genetic profile
The TCF3-PBX1 translocation results in the production of a fusion protein that has an oncogenic role as a transcriptional activator, and also likely interferes with the normal function of the transcription factors coded by TCF3 and PBX1 [2243]. The functional fusion gene resides on chromosome 19, and there may be loss of the derivative chromosome 1 in some cases, resulting in an unbalanced translocation. Gene expression profiling studies have identified a signature unique to this lesion [4423]. An alternative TCF3 translocation, t(17;19), occurs in rare cases of ALL involving the HLF gene on chromosome 17 and is associated with a dismal prognosis. Therefore, demonstration of a TCF3 rearrangement by itself is not a diagnostic criterion for this leukaemia.

A subset of B-ALL cases, most commonly hyperdiploid B-ALLs, have a karyotypically identical t(1;19) that involves neither TCF3 nor PBX1, and should not be confused with this entity.

Prognosis and predictive factors
In early studies, B-ALL with TCF3-PBX1 was associated with a poor prognosis, but this is now readily overcome with modern intensive therapy. However, there may be an increased relative risk of CNS relapse in these patients [1850]. Many treatment protocols no longer require identification of this genetic lesion; the importance of identifying it as a distinct entity is controversial, although the findings of unique immunophenotypic and genetic features support its inclusion as a distinct entity.
B-lymphoblastic leukaemia/lymphoma, BCR-ABL1–like

Definition
BCR-ABL1–like B-lymphoblastic leukaemia/lymphoma (B-ALL/LBL) is a neoplasm of lymphoblasts committed to the B-cell lineage that lack the BCR-ABL1 translocation but show a pattern of gene expression very similar to that seen in ALL with BCR-ABL1. Leukaemias with these properties frequently have translocations involving other tyrosine kinases, or alternatively have translocations involving CRLF2 or (less commonly) rearrangements leading to truncation and activation of EPOR. From a practical standpoint, it is difficult to identify these leukaemias without complex laboratory analysis, and it is also difficult to screen cases of B-ALL to determine which cases would need such analysis; however, diagnostic technology in this area is progressing rapidly. Because of the clinical importance of identifying these cases, BCR-ABL1–like B-ALL/LBL has been included as a provisional entity.

ICD-O code 9819/3

Epidemiology
This is relatively common, occurring in 10–25% of patients with ALL; the frequency is lowest in children with United States National Cancer Institute (NCI) standard-risk ALL and progressively higher in children with high-risk ALL, adolescents, and adults. Children with Down syndrome have a very high frequency of B-ALL with CRLF2 translocations. The frequency of certain genomic lesions varies with ethnicity; IGH/CRLF2 translocations are more common in Hispanics and in individuals with Native American genetic ancestry [1577].

Etiology
No details are known about the etiology of this leukaemia, but it has been shown that certain inherited GATA3 variants confer an increased risk of this entity [3133].

Clinical features
The presenting clinical features are generally similar to those seen in patients with other ALLs, although patients tend to have high white blood cell counts at presentation.

Microscopy
There are no unique morphological or cytochemical features that distinguish this entity from other types of ALL.

Immunophenotype
Blasts typically have a CD19+, CD10+ phenotype. The subset of cases with CRLF2 translocations show very high levels of surface expression of the protein product by flow cytometry; this can be a useful screen for translocations, because cases without elevated expression essentially never show a CRLF2 translocation. There are no specific immunophenotypic features associated with translocations involving EPOR or tyrosine kinases.

Genetic profile
BCR-ABL1–like B-ALLs were originally identified by their distinctive gene expression profile, although different groups’ algorithms do not always identify the same patients [398]. BCR-ABL1–like leukaemias show various types of chromosomal rearrangements, involving many different genes and various partners [3370]. Cases with CRLF2 rearrangements, which account for about half of cases overall, often show an interstitial deletion of the PARI gene family on Xp22.3 and Yp11.3, which juxtaposes CRLF2 to the promoter of the P2RY8 gene. An alternative translocation involves IGH. IGH translocation can also involve EPOR. The tyrosine kinase–type translocations have been reported to involve ABL1 with partners other than BCR, as well as other kinases, including ABL2, PDGFRB, NTRK3, TYK2, CSF1R, and JAK2. More than 30 partner genes have been described. Kinase translocations only rarely coexist with CRLF2 rearrangements. Some of these translocations can be detected by standard cytotype analysis, but many are cryptic, in particular those involving an interstitial deletion of CRLF2. Many cases of BCR-ABL1–like ALL also show deletions or mutations in other genes known to be important to leukaemogenesis, including IKZF1 and CDKN2A/B, although IKZF1 in particular is not specific enough for this group of diseases to be part of the definition [398]. About half of the cases of CRLF2-rearranged ALL have mutations in JAK2 or JAK1.

Prognosis and predictive factors
Overall, patients with BCR-ABL1–like ALL have a poor prognosis [397,398]; however, these patients also have a higher risk of being positive for minimal residual disease, and determining the extent to which the outcome is impacted by minimal residual disease status is difficult [3371]. CRLF2 translocations have specifically been associated with poor prognosis in several studies, but there is some controversy as to whether this is true only in certain subgroups of patients [1111,1577,3038,4125]. Many of the small number of children who are primarily resistant to induction therapy have a translocation targeting PDGFRB, most often with EBF1 as the partner. These patients have shown dramatic responses to ABL-class tyrosine kinase inhibitors such as imatinib and dasatinib [4306]. Clinical trials are being developed to test the hypothesis that treatment of all patients with ABL-class fusions with tyrosine kinase inhibitors will greatly improve their poor outcome. Patients with JAK mutations or translocations may be candidates for treatment with JAK inhibitors, although the effectiveness of such treatment has not yet been proven.

B-lymphoblastic leukaemia/lymphoma with iAMP21

Definition
B-lymphoblastic leukaemia/lymphoma (B-ALL/LBL) with iAMP21 is a neoplasm of lymphoblasts committed to the B-cell lineage characterized by amplification of a portion of chromosome 21, typically detected by FISH with a probe for RUNX1 that reveals ≥5 copies of the gene (or ≥3 extra copies on a single abnormal chromosome 21) [1561,1597].

ICD-O code 9811/3

Epidemiology
This disease is most often identified in children with ALL, and is more common in older children who present with low white blood cell counts. It accounts for about 2% of cases of B-ALL. The incidence in adult ALL has not been established, but appears to be lower than in children.

Etiology
Although the etiology of this entity is not fully understood, mechanistic clues have been derived from the observation that
individuals with the rare constitutional Robertsonian translocation rob(15:21) (q10;q10)c have a nearly 3000-fold increased risk of developing this leukaemia, which appears to involve chromothripsis; this mechanism is likely to be contributory in sporadic cases as well [2314].

Microscopy
There are no unique morphological or cytochemical features that distinguish this entity from other types of ALL.

Immunophenotype
Other than the observation that these cases occur exclusively in B-ALL, no detailed immunophenotypic information is known.

Genetic profile
This leukaemia is recognized by FISH with the probes used to identify ETV6-RUNX1 translocation. However, the pathogenesis of the disease does not involve RUNX1, although it is part of the critical region consistently amplified. In about 20% of cases, this is the only cytogenetic abnormality; in the remainder, many other chromosomal abnormalities are seen, the most common of which include gains of the X chromosome and abnormalities of chromosome 7. This entity is associated with deletions of RB1 and ETV6, and with rearrangements of CRLF2 at a frequency greater than is seen in other ALLs; but the role of these additional alterations in this leukaemia is uncertain. Given the unique nature of the iAMP21 alteration, cases with genetic lesions that might suggest another category of classification, such as CRLF2 translocations, should still be included as B-ALL/LBL with iAMP21.

Prognosis and predictive factors
ALL with iAMP21 has a relatively poor prognosis among children with cases that would otherwise be classified and treated as standard-risk ALL, although it appears that treatment of these children with more intensive therapy overcomes this adverse risk [1561]. Children with high-risk ALL with iAMP21 probably do not require any special therapy.

T-lymphoblastic leukaemia/lymphoma

Definition
T-lymphoblastic leukaemia/lymphoma (T-ALL/LBL) is a neoplasm of lymphoblasts committed to the T-cell lineage, typically composed of small to medium-sized blast cells with scant cytoplasm, moderately condensed to dispersed chromatin, and inconspicuous nucleoli, involving bone marrow and blood (T-ALL) or presenting with primary involvement of the thymus or nodal or extranodal sites (T-LBL). By convention, the term lymphoma is used when the process is confined to a mass lesion with no or minimal evidence of peripheral blood and bone marrow involvement. With extensive bone marrow and peripheral blood involvement, the appropriate term is T-ALL. If a patient presents with a mass lesion and lymphoblasts in the bone marrow, the distinction between leukaemia and lymphoma is arbitrary. In many treatment protocols, a value of >25% bone marrow blasts is used to define leukaemia. Unlike with myeloid leukaemias, there is no agreed-upon lower limit for the proportion of blasts required to establish a diagnosis of ALL. In general, the diagnosis should be avoided when there are <20% blasts.

ICD-O code 9837/3

Synonyms
Precursor T-lymphoblastic leukaemia/lymphoma; T acute lymphoblastic leukaemia

Epidemiology
T-ALL accounts for about 15% of childhood ALL cases; it is more common in adolescents than in younger children, and more common in males than in females. T-ALL accounts for approximately 25% of cases of adult ALL. T-LBL accounts for approximately 85–90% of all LBLs; like its leukaemic counterpart, it is most frequent in adolescent males, but can occur in any age group.

Fig. 12.11 T-lymphoblastic leukaemia. A Blood smear. The lymphoblasts vary in size from large cells to small cells with a very high N:C ratio. B Bone marrow biopsy section showing mitotic activity in the lymphoblasts.
dispersed chromatin and distinct but not unusually prominent nucleoli. Several mitotic figures are present.

Etiology
One study reported a set of T-ALL cases in monozygotic twins that shared the same TR gene rearrangement (1235), suggesting an in utero origin of the earliest genetic lesions.

Localization
The bone marrow is involved in all cases of T-ALL. Unlike in B-ALL, aleukaemic presentations in the setting of bone marrow replacement are uncommon. T-LBL frequently shows mediastinal (thymic) involvement, although it may involve any lymph node or extranodal site. The skin, tonsils, liver, spleen, CNS, and testes may be involved, although presentation at these sites without nodal or mediastinal involvement is uncommon.

Clinical features
T-ALL typically presents with a high leukocyte count, and often with a large mediastinal mass or other tissue mass. Lymphadenopathy and hepatosplenomegaly are common. For a given leukocyte count and tumour burden, T-ALL often shows relative sparing of normal bone marrow haematopoiesis compared to B-ALL. T-LBL frequently presents with a mass in the anterior mediastinum, often exhibiting rapid growth and sometimes presenting as a respiratory emergency. Pleural effusions are common.

Microscopy
The lymphoblasts in T-ALL/LBL are morphologically indistinguishable from those of B-ALL/LBL. In smears, the cells are of medium size with a high N:C ratio; there may be a considerable size range, from small lymphoblasts with very condensed nuclear chromatin and no evident nucleoli to larger blasts with finely dispersed chromatin and relatively prominent nucleoli. Nuclei range from round to irregular to convoluted. Cytoplasmic vacuoles may be present. Occasionally, blasts of T-ALL may resemble more mature lymphocytes; in such cases, immunophenotypic studies may be required to distinguish this disease from a mature (peripheral) T-cell leukaemia.

In bone marrow sections, the lymphoblasts have a high N:C ratio, a thin nuclear membrane, finely stippled chromatin, and inconspicuous nucleoli. The number of mitotic figures is reported to be higher in T-ALL than in B-ALL. In T-LBL, the lymph node generally shows complete effacement of architecture and involvement of the capsule. Partial involvement in a paracortical location with sparing of germinal centres may occur. Sometimes, a multinodular pattern is produced due to stretching of the fibrous framework, mimicking follicular lymphoma. A starry-sky effect may be present, sometimes mimicking Burkitt lymphoma, although the nucleoli and cytoplasm are typically less prominent in T-LBL. The blasts can have round or convoluted nuclei. Mitotic figures are often numerous. In the thymus, there is extensive replacement of the thymic parenchyma and permissive infiltration of the surrounding fibroadipose tissue.

Cases with histological findings of T-LBL may be TdT-positive and variably express CD1a, CD2, CD3, CD4, CD5, CD7, and CD8. Of these markers, CD7 and CD3 (cytoplasmic) are most often positive, but only CD3 is considered lineage specific. CD4 and CD8 are frequently coexpressed on the blasts, and CD10 may be positive; however, these immunophenotypes are not specific for T-ALL, because CD4 and CD8 double positivity can also be seen in T-cell prolymphocytic leukaemia and CD10 positivity in peripheral T-cell lymphomas (most commonly angioimmunoblastic T-cell lymphoma). In addition to TdT and CD34, CD1a and CD99 may help to indicate the precursor nature of T-lymphoblasts (3373). In 29–48% of cases, there is nuclear staining for TAL1, but this does not necessarily correlate with presence of TAL1 gene alteration (695,931).

CD79a positivity has been observed in approximately 10% of cases (3188). One or both of the myeloid-associated antigens CD13 and CD33 are expressed in 19–32% of cases (1998,4088). KIT (CD117) is positive in occasional cases; such cases have been associated with activating mutations of FLT3 (3027). The

abnormality involving the FGFR1 gene (see Myeloid/lymphoid neoplasms with FGFR1 rearrangement, p. 77) [15,1760].

Cytochemistry
T-lymphoblasts frequently show focal acid phosphatase activity in smear and imprint preparations, although this is not specific.

Immunophenotype
The lymphoblasts in T-ALL/LBL are usually TdT-positive and variably express CD1a, CD2, CD3, CD4, CD5, CD7, and CD8. Of these markers, CD7 and CD3 (cytoplasmic) are most often positive, but only CD3 is considered lineage specific. CD4 and CD8 are frequently coexpressed on the blasts, and CD10 may be positive; however, these immunophenotypes are not specific for T-ALL, because CD4 and CD8 double positivity can also be seen in T-cell prolymphocytic leukaemia and CD10 positivity in peripheral T-cell lymphomas (most commonly angioimmunoblastic T-cell lymphoma). In addition to TdT and CD34, CD1a and CD99 may help to indicate the precursor nature of T-lymphoblasts (3373). In 29–48% of cases, there is nuclear staining for TAL1, but this does not necessarily correlate with presence of TAL1 gene alteration (695,931).

CD79a positivity has been observed in approximately 10% of cases (3188). One or both of the myeloid-associated antigens CD13 and CD33 are expressed in 19–32% of cases (1998,4088). KIT (CD117) is positive in occasional cases; such cases have been associated with activating mutations of FLT3 (3027).
T-lymphoblastic leukaemia/lymphoma

The presence of myeloid markers does not exclude the diagnosis of T-ALL/LBL, nor does it indicate T/myeloid mixed-phenotype acute leukaemia.

Many markers characteristic of immature T cells, such as CD7, CD2, and even CD5 and CD3-epsilon, may also be seen in NK-cell precursors. Therefore, it can be very difficult to distinguish the rare true NK-cell ALL/LBL from T-ALL that expresses only immature markers. CD56 expression, although characteristic of NK cells, does not exclude T-cell leukaemia. T-ALL/LBL has previously been stratified into four stages of intrathymic differentiation according to the antigens expressed (332): (1) pro-T/T-I, (2) pre-T/T-II, (3) cortical T/T-III, and (4) medullary T/T-IV. Many cases previously classified as pro-T or pre-T would now meet the criteria for early T-cell precursor ALL (see next section). Like normal thymocytes, T-ALL of the cortical T stage often has a double-positive (CD4+, CD8+) immunophenotype together with CD1a positivity, whereas the medullary T stage expresses either CD4 or CD8. Some studies have shown a correlation between the stages of T-cell differentiation and survival. T-ALL tends to have a more immature immunophenotype than does T-LBL, but the groups overlap (4287).

Postulated normal counterpart

A T-cell progenitor (T-ALL) or a thymic lymphocyte (T-LBL)

Genetic profile

Antigen receptor genes

T-ALL/LBL almost always shows clonal rearrangements of the T-cell receptor (TR) genes, and there is simultaneous presence of IGH gene rearrangements in approximately 20% of cases (3187,3860).

Cytogenetic abnormalities and oncogenes

An abnormal karyotype is found in 50–70% of cases of T-ALL/LBL (1426, 1534). The most common recurrent cytogenetic abnormality involves the alpha and delta TR loci at 14q11.2, the beta locus at 7q35, and the gamma locus at 7p14-15, with a variety of partner genes (1426,1534). In most cases, these translocations lead to a dysregulation of transcription of the partner gene by juxtaposition with the regulatory region of one of the TR loci. The most commonly involved genes include the transcription factors TLX1 (also called HOX11) at 10q24, which is involved in 7% of childhood and 30% of adult cases, and TLX3 (also called HOX11L2) at 5q35, which is involved in 20% of childhood and 10–15% of adult cases (1426). Other transcription factors that may be involved in translocations include MYC at 8q24.1, TAL1 at 1p32, LMO1 (also called RBTN1) at 11p15, LMO2 (also called RBTN2) at 11p13, and LYL1 at 19p13 (897,1426). The cytoplasmic tyrosine kinase LCK at 1p34.3-35 can also be involved in a translocation. In many cases, translocations are not detected by karyotyping but only by molecular genetic studies. For example, the TAL1 locus is altered by translocation in about 20–30% of cases of T-ALL, but a t(1;14)(p32;q11) translocation can be detected in only about 3% of cases. Much more often, TAL1 is fused to STIL (also called S1L) as a result of a cryptic interstitial deletion at chromosome 1p32 (467,1596,1837). Aberrant TAL1 expression interferes with differentiation and proliferation by inhibiting the transcriptional activity of TCF3 (also called E47) and TCF12 (also called HEB) (2919). Other important translocations in T-ALL include t(10;11)(p13;q14), which results in PICALM-MLLT10 (also called CALM-AF10) and is found in 10% of cases, and translocations involving KMT2A (also called MLL), which occur in 8% of cases, most often with the partner MLLT1 (also called ENL) at 19p13 (1426); both result in activation of HOXA genes (2614). It has been proposed that T-ALL be divided into four distinct, non-overlapping genetic subgroups based on specific translocations that lead to aberrant expression of (1) TAL or LMO genes, (2) TLX1, (3) TLX3, and (4) HOXA genes, resulting in arrest of T-cell maturation at distinct stages of thymocyte development (2614,4143). The TLX1 group appears to have a relatively favourable prognosis (1189). Another group, characterized by overexpression of LYL1, may correspond more closely to early T-cell precursor ALL (2614).

Deletions also occur in T-ALL. The most important is del(9p), resulting in loss of the tumour suppressor gene CDKN2A (an inhibitor of the cyclin-dependent kinase CDK4), which occurs at a frequency of about 30% by cytogenetics, and a greater frequency by molecular testing. This leads to loss of G1 control of the cell cycle. About 50% of cases have activating mutations involving the extracellular heterodimerization domain and/or the C-terminal domain.
PEST domain of NOTCH1, which encodes a protein critical for early T-cell development (4294). The direct downstream target of NOTCH1 appears to be MYC, which contributes to the growth of the neoplastic cells (4295). According to one study, NOTCH1 mutation is associated with shorter survival in adults but not in paediatric patients (4492). In about 30% of cases, there are mutations in FBXW7, a negative regulator of NOTCH1. These missense mutations result in an increased half-life of the NOTCH1 protein (2478).

Prognosis and predictive factors
T-ALL in childhood is generally considered a higher-risk disease than B-ALL, although this is in part due to the frequent presence of high-risk clinical features (i.e. older age and higher white blood cell count). However, patients with T-ALL lacking high-risk features do not fare as well as those with standard-risk B-ALL unless intensive therapy is given. Compared to B-ALL, T-ALL is associated with a higher risk of induction failure, early relapse, and isolated CNS relapse (1393). Unlike in B-ALL, white blood cell count does not appear to be a prognostic factor. Minimal residual disease following therapy is a strong adverse prognostic factor (4319), although there is evidence that patients who are positive for minimal residual disease at the end of induction therapy still do very well if the minimal residual disease is cleared by day 78 (3584). In adult protocols, T-ALL is treated similarly to other types of ALL. The prognosis of T-ALL may be better than that of B-ALL in adults, although this may reflect the lower incidence of adverse cytogenetic abnormalities. The prognosis of T-LBL, like that of other lymphomas, depends on patient age, disease stage, and lactate dehydrogenase levels (2727).

Several cases of an entity referred to as indolent T-lymphoblastic proliferation have been described. These typically involve the upper aerodigestive tract and are characterized by multiple local recurrences without systemic dissemination (2941). These cases are morphologically and immunophenotypically similar to T-LBL, but are cytologically less atypical, show a developmentally normal (rather than aberrant) thymic phenotype, and lack clonal rearrangements of the TR genes (2941).

Early T-cell precursor lymphoblastic leukaemia

**Definition**
Early T-cell precursor (ETP) lymphoblastic leukaemia (ETP-ALL) is a neoplasm composed of cells committed to the T-cell lineage but with a unique immunophenotype indicating only limited early T-cell differentiation.

**Epidemiology**
This is an uncommon neoplasm found in both children and adults, accounting for approximately 10–13% of cases of T-ALL in childhood, and for 5–10% of cases of adult ALL.

**Microscopy**
Blasts from patients with ETP-ALL are similar to those from patients with other ALLs: small to medium-sized with scant cytoplasm and inconspicuous nucleoli.

**Immunophenotype**
ETP-ALL expresses CD7 but by definition lacks CD8 and CD1a and is positive for one or more of the myeloid / stem cell markers CD34, KIT (CD117), HLA-DR, CD13, CD33, CD11b, and CD65. Blasts also express cytoplasmic, or in rare cases, surface CD3, and may express CD2 and/or CD45. CD5 is often negative; when positive, it is present on <75% of the blast population (828). It has been suggested that leukaemias that express brighter or more uniform CD5 but otherwise meet the criteria for ETP-ALL be called near-ETP-ALL (4362). By definition, MPO is negative, because a leukaemia with an otherwise ETP immunophenotype that also expresses MPO would most likely meet the criteria for T/myeloid mixed-phenotype acute leukaemia. A unique case of an MPO-negative leukaemia with an ETP phenotype with blasts containing Auer rods has been described (1275). This finding, coupled with the frequent expression of myeloid markers in ETP-ALL, as well as the genetic findings outlined below, underscores the promiscuity between immature precursor T cells and the myeloid lineage, supporting the notion of lymphocyte-primed multipotent progenitors (3342).

Cell of origin
ETP-ALL is postulated to derive from a subset of cells that have migrated to the thymus from the bone marrow but are not yet irreversibly committed to the T-cell lineage and retain the potential for myeloid/dendritic-cell differentiation.

**Genetic profile**
Gene expression profiling studies of ETP-ALL have identified an expression profile similar to that of normal early thymocyte precursors and different from that of cases of T-ALL corresponding to later maturation stages. The overexpressed genes included many that are more typically associated with myeloid or stem cell profiles, such as CD44, CD34, KIT, GATA2, and CEL1A (828, 4475). In addition, previously described cases of the immature T-ALL characterized by LYL1 overexpression (1189) likely constitute ETP-ALL. The mutation profile is also more similar to that of myeloid leukaemias than to those of other T-cell leukaemias (2855, 2856, 4142, 4475), with mutations reported at high frequencies in FLT3, the RAS family of genes, DNMT3A, IDH1, and IDH2; more-typical ALL lesions, such as NOTCH1 activating mutations and mutations in CDKN1A genes, are reported at low frequencies (4142).

Prognosis and predictive factors
Initial descriptions of this entity suggested that the outcome of the small numbers of children with ETP-ALL was very poor compared with that of other patients with T-ALL (828), and other small series showed similar results (7165, 2420). However, more recent, larger series with more effective therapy showed either a small but statistically non-significant difference in outcome (3106) or (in the largest series to date) no effect whatsoever (4362), despite the fact that rates of minimal residual disease in ETP-ALL at the end of induction therapy are higher than among other patients with T-ALL. There are fewer data in adults, but one small study suggested no prognostic effect of ETP-ALL (1497). Therefore, although the kinetics of response to therapy appears to be very different in ETP-ALL compared with other T-ALL, the ultimate outcome with appropriate therapy appears to be the same.
NK-lymphoblastic leukaemia/lymphoma

Definition
NK-lymphoblastic leukaemia/lymphoma has been very difficult to define, and there is considerable confusion in the literature. Contributing to this confusion is the fact that many cases reported as NK-leukaemia due to expression of CD56 (NCAM) are now recognized to in fact be blastic plasmacytoid dendritic cell neoplasms [3151,3152]. Similarly, the entity known as myeloid/NK acute leukaemia [3599,3841], which has been suggested to be of precursor NK-cell origin [3000], has a primitive immunophenotype indistinguishable from that of acute myeloid leukaemia with minimal differentiation. Until further evidence emerges, these should be considered as cases of acute myeloid leukaemia.

Early in their development, NK-cell progenitors express no specific markers [1255], or express markers that overlap with those seen in T-ALL, including CD7, CD2, and even CD5 and cCD3-epsilon [3757]; therefore, distinguishing between T-ALL and NK-cell tumours can be difficult. More-mature but more-specific markers such as CD16 are rarely expressed in any acute leukaemia; some markers that might be considered relatively specific but that are still expressed on NK-cell progenitors (e.g. CD94 and CD161 [1255]) are not commonly tested. Some well-characterized cases of NK precursor tumours with lymphomatous presentations that expressed NK-specific CD94 1A transcripts have been described [2338]. It is hoped that wider availability of more specific NK-cell markers, including panels of antibodies against killer-cell immunoglobulin-like receptors, will help clarify this disease, but until then, NK-ALL/LBL is best considered a provisional entity. The diagnosis of precursor NK-ALL/LBL may be considered in a case that expresses CD56 along with immature T-associated markers such as CD7 and CD2, and even including cCD3, provided that the case lacks B-cell and myeloid markers, TCR and IG genes are in the germline configuration [1975,2070,3000], and blastic plasmacytoid dendritic cell neoplasm has been excluded.
CHAPTER 13

Mature B-cell neoplasms
Chronic lymphocytic leukaemia / small lymphocytic lymphoma

Definition
Chronic lymphocytic leukaemia / small lymphocytic lymphoma (CLL/SLL) is a neoplasm composed of monomorphic small mature B cells that coexpress CD5 and CD23. There must be a monoclonal B-cell count > 5 x 10⁹/L, with the characteristic morphology and phenotype of CLL in the peripheral blood. Individuals with a clonal CLL-like cell count < 5 x 10⁹/L and without lymphadenopathy, organomegaly, or other extramedullary disease are considered to have monoclonal B-cell lymphocytosis. Although CLL and SLL are the same disease, the term SLL is used for cases with a circulating CLL cell count < 5 x 10⁹/L and documented nodal, splenic, or other extramedullary involvement [1523].

ICD-O code 9823/3

Synonyms
Chronic lymphocytic leukaemia, B-cell type; chronic lymphoid leukaemia; chronic lymphatic leukaemia

Epidemiology
CLL is the most common leukaemia of adults in western countries. The annual incidence rate is about 5 cases per 100 000 population, and dramatically increases with age, to as many as > 20 cases per 100 000 individuals aged > 70 years. The median patient age at diagnosis of CLL is approximately 70 years, but CLL can also present in younger adults [3060]. There is a male preponderance, with a male-to-female ratio of 1.5 - 2:1. CLL/SLL accounts for 7% of non-Hodgkin lymphomas [2759, 3515]. The disease is rare in Asian countries, with the low incidence maintained in emigrant populations [2451]. This finding, together with the reported familial cases, indicates a genetic basis and predisposition for the disease.

Etiology
B-cell receptors of CLL cells demonstrate highly selected IGHV gene usage or even very similar antigen-binding sites, coded by both heavy and light chain genes (so-called stereotypes), and thus differ from the B-cell receptors of much broader diversity found in normal B lymphocytes. These findings support the concept of a limited set of (auto-)antigenic elements promoting division of precursor cells and clonal evolution [28].

Localization
CLL/SLL involves the blood, bone marrow, and secondary lymphoid tissues such as the spleen, lymph nodes, and Waldeyer ring. Extranodal involvement (e.g. of the skin, gastrointestinal tract, kidneys, or CNS) occurs in a small subset of cases [3315]. SLL is diagnosed in 10-20% of cases, and as many as 20% evolve into frank CLL [3516].

Clinical features
Most cases of CLL in western countries are diagnosed on the basis of routine blood analysis in asymptomatic subjects. Less often, lymphadenopathy, splenomegaly, anaemia, or thrombocytopenia can lead to the diagnosis. In a few cases, the diagnosis is reached after work-up for other manifestations of CLL/SLL, such as an autoimmune cytopenia (i.e. autoimmune haemolytic anaemia, immune thrombocytopenia, or erythroblastopenia) [1652] or an infection, most frequently pulmonary. A small paraprotein, usually of IgM type, can be observed in approximately 10% of the patients [87]. The frequency of hypogammaglobulinaemia is about 30% at diagnosis and increases over time, to as much as 60% among patients with advanced disease [3059]. Extramedullary involvement (e.g. of the skin, gastrointestinal tract, kidneys, or CNS) occurs in a small proportion of patients [3315]. Patients with CLL can experience severe allergic reactions to insect bites [235].

Microscopy
Lymph nodes and spleen
Enlarged lymph nodes show diffuse architectural effacement by a proliferation of small lymphocytes with variably prominent scattered paler proliferation centres (so-called pseudofollicles) [2267]. In some cases, there is a vaguely nodular appearance. Only partial nodal involvement with an interfollicular or perifollicular infiltration pattern may be seen [224, 3894]. The predominant cell in the diffuse areas is a small lymphocyte with scant cytoplasm, usually a round nucleus with clumped chromatin, and occasionally a

Fig. 13.01 Chronic lymphocytic leukaemia / small lymphocytic lymphoma (CLL/SLL). A Bone marrow trephine section illustrating a nodular pattern of infiltration. B Bone marrow trephine section illustrating an interstitial pattern of lymphocytic infiltration. C Peripheral blood. The CLL lymphocytes are small and round, with distinct clumped chromatin. Smudge cells are commonly seen.
small nucleolus. Mitotic activity is usually very low. In some cases, the small lymphoid cells show moderate nuclear irregularity, which can lead to a differential diagnosis of mantle cell lymphoma [411]. Some cases show plasmacytoid differentiation. The proliferation centres are composed of a continuum of small lymphocytes, prolymphocytes, and paraimmunoblasts. Prolymphocytes are small to medium-sized cells with relatively clumped chromatin and small nucleoli; paraimmunoblasts are larger cells with round to oval nuclei, dispersed chromatin, central eosinophilic nucleoli, and slightly basophilic cytoplasm [411,2267].

In some cases, the proliferation centres are very large (broader than a 20x field) and confluent [760,1373]. Such cases are usually associated with increased proliferation, deletion in 17p13, trisomy 12, and a more aggressive course compared to cases with smaller proliferation centres [760,1134,1373]. In the spleen, white pulp involvement is usually prominent, but the red pulp is also involved; proliferation centres may be seen but are less conspicuous than in lymph nodes.

Bone marrow and blood

CLL cells are small lymphocytes with clumped chromatin and scant cytoplasm. Smudge or basket cells are typically seen in peripheral blood smears. In most cases, besides typical CLL cells, other lymphoid cells (e.g. prolymphocytes, cells with irregular nuclear contours, and larger cells with more dispersed chromatin and more abundant cytoplasm) are also observed, but they usually constitute <15% of the lymphoid cells. Cases with a higher proportion of these cells but <55% prolymphocytes have been called atypical CLL. In such cases, trisomy 12 and strong positivity for surface immunoglobulin, CD20, and FMC7 are frequently found [341]. The finding of >55% prolymphocytes defines B-cell prolymphocytic leukaemia [341,1523]. Bone marrow biopsy may show interstitial, nodular, mixed (nodular and interstitial), or diffuse involvement; diffuse involvement is usually associated with more advanced disease [2720]. Paratrabecular aggregates are not typical. Proliferation centres can be observed, although they are not as prominent as in lymph nodes, and follicular dendritic cells may be present [716]. Most cases have >30% CLL cells in the bone marrow aspirate [1523].

Immunophenotype

Circulating leukaemic B cells express CD19 and dim surface IgM/IgD, CD20, CD22, and CD79b. They are also positive for CD5 and CD43 and strongly positive for CD23 and CD200 [1027]. CD10 is negative and FMC7 is usually negative or only weakly expressed. The immunophenotype of CLL cells has been integrated into a scoring system that helps in the differential diagnosis of CLL and other B-cell leukaemias [2582,2722]. Some cases have an atypical immunophenotype (e.g. CD5- or CD23-, FMC7+, strong surface immunoglobulin, or CD79b+) [838, 2580]. However, in these cases it is imperative that the possibility of some other type of B-cell neoplasm, such as splenic marginal zone lymphoma in CD5- cases, be excluded.

In tissue sections, cytoplasmic immunoglobulin may be detectable. CD20 and CD23 expression is usually stronger in cells of the proliferation centres than in the diffuse areas [2202]. Follicular dendritic cell meshworks are present in some cases, and may be associated with the proliferation centres. LEF1 is useful to identify CLL/SSL infiltration in tissues, because it is aberrantly expressed in almost all CLL/SSL, whereas normal mature B lymphocytes and virtually all smaller B-cell lymphomas are negative [3893]. Cyclin D1 is not expressed, but some positive cells can be seen in proliferation centres in about 30% of cases. These cells are SOX11-negative and do not carry chromosomal translocations affecting the CCND1 gene [1419]. MYC and NOTCH1 proteins may also be expressed.
in proliferation centres independently of gene alterations [2047, 2123].

Postulated normal counterpart
An antigen-experienced mature CD5+ B cell with mutated or unmutated IGHV genes [721]

Genetic profile
Antigen receptor genes
IGHV gene usage in CLL is highly skewed; IGHV genes are mutated (i.e. <98% identity with the germline) in 50–70% of cases and unmutated (i.e. ≥98% identity) in 30–50% [863, 1528]. Patients can have very similar, if not identical, immunoglobulin sequences, a phenomenon that is present in 30% of all CLL cases and termed ‘BCR stereotypy’ [28]. BCR signalling is more active in CLL with unmutated IGHV genes [4483], whereas other cases, in particular those with mutated IGHV genes, have a response resembling anergy. CLL B-cell receptors have been shown to recognize both foreign and self antigens, including the possibility of the monoclonal immunoglobulin recognizing itself on the same or an adjacent cell (so-called autonomous signalling) [1072].

Cytogenetic abnormalities and oncogenes
CLL has no specific genetic markers. About 80–90% of the cases have cytogenetic abnormalities detected by FISH or copy-number arrays [1073, 2469, 3244]. The most common alterations are deletions in 13q14.3 (miR-16-1 and miR-15a; present in ~50% of cases) and trisomy 12 or partial trisomy 12q13 (present in ~20%); less commonly, there is deletion in 11q22-23 (ATM and BIRC3), 17p13 (TP53), or 6q21 [1073, 1510, 4469]. The distribution of these abnormalities varies depending on the IGHV mutation status (see Table 13.01). High-resolution genomic arrays have helped to refine and expand the known DNA copy-number alterations, identifying gains in 2p (present in 7% of cases) and 8q24 (MYC; in 3%), as well as losses of 14q (in 4%), among others [1079, 2469, 3244]. Chromosomal translocations in CLL are uncommon, but t(14;18)(q32;q21), resulting in IGH/BCL2, can be found, most likely as a secondary change, in 2% of cases, usually with mutated IGHV. Translocations involving the 13q14 region, present in 2% of cases, are associated with miR-16-1 and miR-15a deletions [3244, 3247]. The t(14;19) (q32;q13) translocation, resulting in IGH/BCL3, is present in occasional cases of CLL with unmutated IGHV genes [1730]. Complex rearrangements within single chromosomes (chromothripsis) and among chromosomes have been identified in 2% of cases, mainly with unmutated IGHV genes and frequently in association with TP53 mutations [1079, 3244]. The most commonly mutated genes, affected in 3–15% of cases, are NOTCH1.
 Whole-genome DNA methylation studies have identified three epigenetic subgroups of CLL with methylation signatures closely related to different stages of B-cell differentiation: one resembling naïve B cells, one resembling memory B cells, and one with a signature intermediate between those of naïve and memory B cells [2130]. These three epigenetic CLL subgroups (naïve-like, memory-like, and intermediate) have different biological characteristics and only partially overlap with IGHV mutation status [2921,3259]. Naïve-like CLLs have mainly unmutated IGHV genes, whereas most epigenetically intermediate and memory-like CLLs have mutated IGHV genes. However, the clinical behaviour of the intermediate subgroup is more aggressive than that of memory-like CLL [3259]. Although these epigenetic subgroups have been identified using genomics-wide methylation arrays, they can also be reproducibly identified in clinical practice using pyrosequencing of only five epigenetic biomarkers [3259].

**Genetic susceptibility**

CLL is a multifactorial disease with considerable heritability. A familial predisposition can be documented in 5–10% of patients with CLL [1396,3697]. The overall risk of developing CLL is 2–7 times higher in first-degree relatives of CLL patients. These patients also have an increased risk for other lymphoid neoplasms [603]. Family members of patients with CLL also show an increased incidence of CLL-like monoclonal B-cell lymphocytosis. Genetic studies have identified as many as 30 genomic loci related to inherited susceptibility to CLL [355,3747].

**Prognosis and predictive factors**

The Rai and Binet clinical staging systems are used to define disease extent and prognosis [382,3263]. Patients with mutated IGHV genes have a better prognosis than those with unmutated genes [4469]. Expression of ZAP70, CD38, or CD49d is associated with an adverse prognosis [833]. The three genetic subtypes are also of prognostic significance; naïve-like cases have the worst prognosis and memory-like cases the best [2921,3259]. Deletion in 11q and in particular deletion in 17p confers a worse clinical outcome, whereas isolated deletion in 13q14 is associated with a more favourable clinical course [833]. CLL with a high proportion of cells with isolated 13q deletion, however, do not do as well [1722A,3246A,4132A]. TP53 abnormalities (i.e. deletion in 17p13 and TP53 mutations) are predictive of lack of response to fludarabine-containing regimes; therefore, these aberrations should be checked for in all patients before starting any line of therapy. Prognostic and predictive factors need to be more firmly established for newer therapeutic strategies such as B-cell receptor or BCL2 inhibitors. Complex karyotype also correlates with poor outcome [940,3012]. The presence of a stereotyped B-cell receptor utilizing the IGHV3-21 gene (so-called subset #2) is an adverse prognostic marker independent of IGHV mutations [240]. Additional adverse predictive factors include a rapid lymphocyte doubling time in the blood (<12 months) and serum markers of rapid cell turnover, including elevated thymidine kinase and beta-2 microglobulin [383]. Mutations in TP53, ATM, NOTCH1, SF3B1, and BIRC3, among others, are associated with a poor outcome [2208,3244]. The integration of these results with other prognostic parameters requires further study [241].

**Progression and transformation of chronic lymphocytic leukaemia into high-grade lymphoma**

Clinical progression of CLL/SLL is often associated with an increase in size and proliferative activity of the CLL cells. Proliferation centres in lymph nodes may expand with a higher proliferation rate and become confluent [760,1134,1373]. Histologically aggressive CLLs are recognized by proliferation centres that are

<table>
<thead>
<tr>
<th>Aberration(s)</th>
<th>Mutated IGHV n = 132 (44% of cases)</th>
<th>Unmutated IGHV n = 168 (56% of cases)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clonal aberrations</td>
<td>80%</td>
<td>84%</td>
</tr>
<tr>
<td>13q deletion*</td>
<td>65%</td>
<td>48%</td>
</tr>
<tr>
<td>Isolated 13q deletion*</td>
<td>50%</td>
<td>26%</td>
</tr>
<tr>
<td>Trisomy 12</td>
<td>15%</td>
<td>19%</td>
</tr>
<tr>
<td>11q deletion*</td>
<td>4%</td>
<td>27%</td>
</tr>
<tr>
<td>17p deletion*</td>
<td>3%</td>
<td>10%</td>
</tr>
<tr>
<td>17p or 11c deletion*</td>
<td>7%</td>
<td>35%</td>
</tr>
</tbody>
</table>

*Significant difference between cases with and without IGHV mutation.

**Table 13.01 Relation of IGHV mutation status and genomic aberrations in 300 cases of chronic lymphocytic leukaemia. From: Kröber A, et al. [2120A]**

---

**Fig. 13.06** Diffuse large B-cell lymphoma (DLBCL) transformed from chronic lymphocytic leukaemia. The area with DLBCL is composed of a monotonous population of large cells with immunoblastic features.

**Fig. 13.07** Diffuse large B-cell lymphoma (DLBCL) transformed from chronic lymphocytic leukaemia. The transformed DLBCL cells are intermingled with residual small lymphocytes.
broader than a 20x field or becoming confluent. Although data are limited, cases may also belong in this category when the Ki-67 proliferation index is >40% or there are >2.4 mitoses in the proliferation centres (760,1373). These cases are reported to have an outcome intermediate between those of typical CLL and classic Richter syndrome (diffuse large B-cell lymphoma; DLBCL) (760,1373). An increasing proportion of prolymphocytes in the blood may also be seen (prolymphocytoid transformation). However, progression of CLL into B-cell prolymphocytic leukaemia does not occur, by definition. Approximately 2–8% of patients with CLL develop DLBCL, and <1% develop classic Hodgkin lymphoma (453,2492,4006). Most cases of DLBCL-type Richter syndrome are clonally related to the previous CLL, i.e. they express the same immunoglobulin gene rearrangement, and are IGHV-unmutated, whereas clonally unrelated cases usually occur in IGHV-mutated CLL (2492, 4006). The former are associated with a median survival time of <1 year, whereas the prognosis of the latter is identical to that of a de novo DLBCL (3058). DLBCL transformation is associated with TP53 and NOTCH1 mutations, CDKN2A deletions, and MYC translocations (712, 1125,3245). The vast majority of Hodgkin lymphoma cases occur in mutated CLL, are EBV-positive, and are unrelated to the CLL clone [2492]. The diagnosis of Hodgkin lymphoma in the setting of CLL requires classic Reed–Sternberg cells in an appropriate background. The presence of scattered EBV-positive or sometimes EBV-negative Reed–Sternberg cells in the background of CLL does not fulfill the criteria for the diagnosis of Hodgkin lymphoma. EBV-associated lymphoproliferative disorders, including Hodgkin lymphoma–type proliferations, may occur in patients with CLL following immunosuppressive therapy (16,3993).

Monoclonal B-cell lymphocytosis

Definition
Monoclonal B-cell lymphocytosis (MBL) is defined by a monoclonal B-cell count <5×10^9/L in the peripheral blood in subjects who have no associated lymphadenopathy, organomegaly, other extramedullary involvement, or any other feature of a B-cell lymphoproliferative disorder (2516). MBL is classified into three categories on the basis of phenotype: (1) chronic lymphocytic leukaemia (CLL)-type, (2) atypical CLL-type, and (3) non-CLL-type. Caution is advised, because many small B-cell lymphomas/leukaemias have low-level peripheral blood involvement.

MBL with a CLL-type phenotype is the most common, accounting for as many as 75% of all cases. It is characterized by coexpression of CD19, CD5, CD23, and CD20 (dim). The B cells show light chain class restriction or ≥25% lack surface...
Fig. 13.09 Low-count and high-count chronic lymphocytic leukaemia (CLL)-type monoclonal B-cell lymphocytosis (MBL). The cumulative percentage of cases according to the absolute number of clonal B cells in studies of individuals from the general population with a normal blood count (dotted line) and in series of individuals referred for clinical haematology investigations, usually with current or prior lymphocytosis (solid line). There is a marked difference in the clonal B-cell count in CLL-type MBL in cases from population studies versus clinical haematology series. In population studies, the median clonal B-cell count is 1/μL, with 95% of cases having <56/μL (white background). In clinical haematology series, the median is 2939/μL, with 95% of cases having >447/μL (dark-grey background). Very few cases from either series have a clonal B-cell count within the same range as polyclonal B-cell levels in individuals with no detectable abnormal B cells (light-grey background). From Rawstron AC et al. {3321}.

immunoglobulin. More than one clone may coexist. The reported frequency of CLL-type MBL in the general population depends on the sensitivity of the method used for detection, ranging from 3.5% to 12% among healthy individuals [1167, 2876,3320]. The frequency increases with age; it is negligible among individuals aged <40 years and 50–75% among 90-year-old individuals [1353,2876,3320]. It has been reported that virtually all CLls are preceded by MBL, although not all MBL progresses to CLL [1167]. Some patients have counts that oscillate between MBL and CLL for some time. CLL-type MBL must be further classified on the basis of the size of the monoclonal population, as low-count (<0.5 x 10^9/L) or high-count (≥0.5 x 10^9/L) MBL. Low-count MBL has some biological differences from high-count MBL and CLL, and does not seem to progress, whereas high-count MBL has biological features identical to those of low-stage (Rai stage 0) CLL, and progresses to frank leukaemia requiring therapy at an annual rate of 1–2% [1167,3319,3666]. The higher the MBL count, the more likely there will be progression. The adverse prognostic indicators identified in patients with CLL are also associated with progression from MBL to CLL and shorter time to treatment [1949,1992]. MBL cases usually have mutated IGHV genes (present in 75–90% of cases) and may carry the same chromosomal abnormalities and somatic mutations as CLL, including NOTCH1, SF3B1, ATM, and TP53 aberrations, although at lower frequencies [1992,3244]. Nodal infiltration by CLL-type cells without apparent proliferation centres in individuals without lymphadenopathy >1.5 cm on CT who otherwise have MBL may constitute a nodal equivalent of MBL rather than small lymphocytic lymphoma [1369]. MBL with an atypical CLL phenotype expresses CD19, CD5, CD20 (bright), and moderate to bright surface immunoglobulin. CD23 may be negative. It is critical to exclude the possibility of mantle cell lymphoma or other B-cell lymphoma in these cases. MBL with a non-CLL phenotype is characterized by CD5+ (or CD5(dim) in 20% of cases), CD19+, CD20+ B cells with moderate to bright surface immunoglobulin expression [1353,3631]. Some of these clonal expansions may be transient and self-limited. Additional phenotypic and cytogenetic studies are mandatory to rule out a specific lymphoid neoplasm [1949]. Some cases have aberrant karyotypes involving chromosome 7q, and as many as 17% may eventually develop splenomegaly, suggesting a relationship to splenic marginal zone lymphoma [4390]. Some very similar cases with a low rate of progression may have a non-CLL B-cell count >5 x 10^9/L, therefore not fulfilling the criteria for MBL [4390]. Some cases of diffuse large B-cell lymphoma with simultaneous MBL with a CLL or non-CLL phenotype are clonally related [2468].

ICD-O codes
- MBL, CLL-type 9823/1
- MBL, non-CLL-type 9591/1
B-cell prolymphocytic leukaemia

Definition
B-cell prolymphocytic leukaemia (B-PLL) is a neoplasm of B-cell prolymphocytes affecting the peripheral blood, bone marrow, and spleen. Prolymphocytes must constitute >55% of lymphoid cells in peripheral blood. Cases of chronic lymphocytic leukaemia (CLL) with increased prolymphocytes and lymphoid proliferations with relatively similar morphology but with a t(11;14)(q13;q32) (IGH/CCND1) translocation or SOX11 expression are excluded; they instead constitute mantle cell lymphoma with leukaemic expression.

ICD-O code 9833/3

Synonym
Prolymphocytic leukaemia, B-cell type

Epidemiology
B-PLL is an extremely rare disease, accounting for approximately 1% of lymphocytic leukaemias. Most patients are aged >60 years, with a median age of 65–69 years. The frequencies in males and females are similar [2621].

Localization
The leukaemic cells are found in the peripheral blood, bone marrow, and spleen.

Clinical features
Most patients present with B symptoms, massive splenomegaly with absent or minimal peripheral lymphadenopathy, and a rapidly increasing lymphocyte count, usually >100×10^9/L. Anaemia and thrombocytopenia are seen in 50% of cases [2117].

Microscopy
Peripheral blood and bone marrow
The majority (>55% and usually >90%) of the circulating cells are prolymphocytes. These are medium-sized lymphoid cells (twice the size of a normal lymphocyte) with a round nucleus, moderately condensed nuclear chromatin, a prominent central nucleolus, and a relatively small amount of faintly basophilic cytoplasm [1284,2621]. Although the nucleus is typically round, in some cases it can be indented. The bone marrow shows an interstitial or nodular inter trabecular infiltration of lymphoid cells similar to those found in blood.

Other tissues
The morphology of B-PLL in tissues is not well characterized, because initial descriptions of the disease included cases with the t(11;14)(q13;q32) (IGH/CCND1) translocation characteristic of mantle cell lymphoma [3449,3563]. The spleen shows expanded white pulp nodules and red pulp infiltration by intermediate to large cells with abundant cytoplasm and irregular or round nuclei with a central eosinophilic nucleolus [3449]. Lymph nodes display diffuse or vaguely nodular infiltration by similar-looking cells. Proliferation centres (pseudofollicles) are not seen.

Distinguishing B-PLL from pleomorphic mantle cell lymphoma, splenic marginal zone lymphoma, and CLL with an increased number of prolymphocytes can be difficult on morphological grounds. The diagnosis of B-PLL cannot be made without excluding other conditions, because there are no specific markers for B-PLL. The diagnosis of mantle cell lymphoma, for example, is based on immunophenotyping and genetic studies to detect cyclin D1 overexpression and t(11;14)(q13;q32). Evaluation of SOX11 expression may be useful to exclude leukaemic cyclin D1-negative mantle cell lymphoma [3492]. In pure leukaemic cases, the evaluation of SOX11 and cyclin D1 expression may require mRNA analysis by quantitative PCR [3439].

Immunophenotype
B-PLL cells strongly express surface IgM/IgD, as well as B-cell antigens (CD19, CD20, CD22, CD79a, CD79b, and FMC7). CD5 and CD23 are only positive in 20–30% and 10–20% of cases, respectively, and CD200 is weakly positive or negative [927,2117,3449]. ZAP70 and CD38 are expressed in approximately 50% of cases. ZAP70 expression does not correlate with IGHV mutation status [927].

Postulated normal counterpart
A mature B cell of unknown type

Genetic profile
Antigen receptor genes
IGH genes are clonally rearranged, with an unmutated IGH gene in about half of the cases. B-PLL has been reported to use members of the IGHV3 and IGHV4 gene families in 68% and 32% of cases, respectively [927].
Cytogenetic abnormalities and oncogenes

Initial studies demonstrated t(11;14) (q13;q32) (IGH/CCND1) in as many as 20% of B-PLLs (459). However, these cases are now considered to be leukemic variants of mantle cell lymphoma (3449, 3563, 4358). Complex karyotypes are common (3563). Deletion in 17p13 is detected in 50% of the cases (927) and is associated with TP53 mutations (2270). This probably underlies the progressive course and relative treatment resistance of B-PLL. FISH analysis detects deletions at 13q14 in 27% of the cases (927). Trisomy 12 is uncommon (927). Aberrations of MYC, including gains, amplifications, and translocations with the IGH, IGK, or IGL loci, have been reported (1224). This is consistent with the documented increased expression of MYC mRNA and protein (929, 1224). B-PLL has a transcriptional profile different from those of CLL and CLL with increased prolymphocytes, but has features that overlap those of some other lymphomas, such as mantle cell lymphoma (929, 4127). However, the number of investigated cases is limited, and the relationship of B-PLL without CCND1 rearrangement to mantle cell lymphoma is still uncertain (4127).

Prognosis and predictive factors

B-PLL responds poorly to therapies for CLL, with a median survival of 30–50 months (927, 3449). There is no correlation between survival and ZAP70 expression, CD38 positivity, deletion in 17p, or IGHV mutation status (927). Splenectomy may improve symptoms. Responses have been recorded with the CHOP chemotherapy regimen, fludarabine, and cladribine. A combination of chemotherapy and rituximab may be a reasonable treatment approach (2117). In selected cases, allogeneic bone marrow transplantation should be considered.

Splenic marginal zone lymphoma

Definition

Splenic marginal zone lymphoma (SMZL) is a B-cell neoplasm composed of small lymphocytes that surround and replace the splenic white pulp germinal centres, efface the follicle mantle, and merge with a peripheral (marginal) zone of larger cells, including scattered transformed blasts; both small and larger cells infiltrate the red pulp. Splenic hilar lymph nodes and bone marrow are often involved; lymphoma cells are frequently found in the peripheral blood as villous lymphocytes.

ICD-O code 9689/3

Synonyms

Splenic B-cell marginal zone lymphoma; splenic lymphoma with villous lymphocytes; splenic lymphoma with circulating villous lymphocytes (no longer used)

Epidemiology

SMZL is a rare disorder, accounting for <2% of lymphoid neoplasms (148), but it may account for most cases of otherwise unclassifiable chronic lymphocytic leukemias that are CD5-negative. Most patients are aged >50 years, with a median age of 67–68 years. The incidence rates among males and females are equal (347, 1999, 4388).

Localization

The tumour involves the spleen and splenic hilar lymph nodes, the bone marrow, and often the peripheral blood. The liver may also be involved. Peripheral lymph nodes are not typically involved (347, 2691).

Clinical features

Patients present with splenomegaly, sometimes accompanied by autoimmune thrombocytopenia or anaemia and a variable presence of peripheral blood villous lymphocytes. The bone marrow is regularly involved, but peripheral lymphadenopathy and extranodal infiltration are extremely uncommon. About one third of patients have a small para-

Macroscopy

Gross examination of the spleen reveals marked expansion of the white pulp and infiltration of the red pulp.

Microscopy

In the splenic white pulp, a central zone of small round lymphocytes surrounds or, more commonly, replaces reactive germinal centres, with effacement of the normal follicle mantle (1783, 2691). This zone...
Fig. 13.14 Splenic marginal zone lymphoma, spleen. A Infiltration of white and red pulp. The white pulp nodules show a central dark zone of small lymphocytes (sometimes surrounding a residual germinal centre) giving way to a paler marginal zone. B High-magnification view of white pulp nodule showing the small lymphocytes merging with a marginal zone consisting of larger cells with pale cytoplasm and occasional transformed blasts.

merges with a peripheral zone of small to medium-sized cells with more dispersed chromatin and abundant pale cytoplasm, which resemble marginal zone cells, and interspersed transformed blasts. The red pulp is always infiltrated, with small nodules of the larger cells and sheets of the small lymphocytes, which often invade sinuses. Epitheloid histiocytes may be present in the lymphoid aggregates. Some cases have a markedly predominant population of the larger marginal zone-like cells [1532,1793]. Plasmacytic differentiation may occur, and in rare cases, clusters of plasma cells are present in the centres of the white pulp nodules. In splenic hilar lymph nodes, the sinuses are dilated and lymphoma surrounds and replaces germinal centres, but the two cell types, clusters of plasma cells are present in the centres of the white pulp nodules. In the bone marrow, there is a nodular interstitial infiltrate cytologically similar to that in the lymph nodes. Occasionally, neoplastic cells surround reactive follicles. Intrasinusoidal lymphoma cells, which are more apparent after CD20 immunostaining, are a helpful feature, although they are sometimes observed in other lymphomas [1242]. When lymphoma cells are present in the peripheral blood, they are usually characterized by short polar villi. Some may appear plasmacytoid [2622].

Differential diagnosis
The differential diagnosis includes other small B-cell lymphomas/leukaemias, including chronic lymphocytic leukaemia (CLL), hairy cell leukaemia (HCL), mantle cell lymphoma, follicular lymphoma, and lymphoplasmaicytic lymphoma. It is also important to recognize that many small B-cell lymphomas (other than HCL, which diffusely involves the red pulp) can have slightly larger cells with pale cytoplasm when they involve the splenic marginal zone, mimicking SMZL [3197]. The nodular pattern on bone marrow biopsy excludes HCL, but the morphological features on bone marrow examination may not be sufficient to distinguish between the other small B-cell neoplasms. Immunophenotypic and molecular/cytogenetic findings may also be very helpful, but the diagnosis can be rendered most confidently from a splenectomy specimen; however, such a specimen is often not available.

Immunophenotype
Tumour cells express surface IgM and usually IgD. They are positive for CD20 and CD79a and negative for CD5, CD10, CD23, CD43, and annexin A1 [1783, 2579]. CD103 is usually negative, and cyclin D1 is absent [3540]. Ki-67 staining shows a distinctive targetoid pattern due to an increased growth fraction in both the germinal centre, if present, and the marginal zone. The absence of cyclin D1 and LEF1 is useful in excluding mantle cell lymphoma and CLL, respectively. The absence of annexin A1 excludes HCL, and lack of CD10 and BCL6 helps to exclude follicular lymphoma [3540]. A group of CD5+ SMZL cases has been described, distinguished by a higher lymphocytosis and diffuse bone marrow infiltration [285].

Postulated normal counterpart
A marginal-zone B cell that may or may not demonstrate evidence of antigen exposure

Genetic profile
Antigen receptor genes
IG heavy and light chain genes have clonal rearrangements, and approximately half of the cases have somatic hypermutation. Bias in IGHV1-2*04 usage has been found in 30% of SMZL cases, suggesting that this tumour derives from a highly selected B-cell population [62, 378]. Stereotyped HCDR3 sequences, specific for SMZL, support a potential role of antigen selection in the pathogenesis of these lymphomas [4493].
Cytogenetic abnormalities and oncogenes

SMZL lacks recurrent chromosomal translocations, including translocations that are typical of other lymphoma types, such as the t(14;18)(q32;q21) translocation affecting BCL2 in follicular lymphoma; the t(11;14)(q13;q32) translocation affecting CCND1 in mantle cell lymphoma; and the t(11;18)(q21;q1), t(14;18)(q32;21) and t(1;21)(p22;q32) translocations resulting in BIRC3/MALT1, IGH/MALT1, and IGH/BCL10 juxtaposition, respectively, in extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma). The absence of these abnormalities helps distinguish SMZL from some of the lymphomas that can mimic it. A small number of SMZLs carry a recurrent t(2;7) (p12;q21) translocation, which activates the CDK6 gene through juxtaposition with the IGK locus [810]. Approximately 30% of SMZLs show a homozygous deletion in 7q, which is rarely found in other lymphoma subtypes [3362,3497]. The gene(s) targeted by the 7q deletion remain unknown, despite the combined investigation of genomic and transcriptomic profiles and mutation analysis of a number of candidate genes [1254,3376, 4268]. Gain of 3q is present in a considerable subset of cases.

NOTCH2 is one of the most frequently mutated genes in SMZL, mutated in approximately 10–25% of cases [771,2006, 2532,3070,3203,3419]. Although diagnostically useful, NOTCH2 mutations are also seen in infrequent other small B-cell lymphomas [305,1945,2006, 3419]. KLF2 is somatically mutated in approximately 10–40% of SMZLs [771, 3070,3203], but these mutations are also found in some other small B-cell neoplasms [3203]. Mutations in both of these genes have been associated with SMZLs that have deletion in 7q. MYD88 mutations are rare in SMZL, and may therefore be a useful biomarker for the differentiation of SMZL from lymphoplasmacytic lymphoma in pathologically challenging cases with evidence of plasmacytic differentiation [2532,3070]. The fact that the most frequently mutated genes in SMZL (i.e. NOTCH2 and KLF2) are physiologically involved in proliferation and commitment of mature B cells to the marginal zone, points to homing to the spleen compartment and marginal zone differentiation as the major programmes deregulated in this lymphoma. Congruently, SMZL has an expression signature characterized by the upregulation of genes belonging to the marginal zone differentiation programme [3455, 4064].

Prognosis and predictive factors

The clinical course is indolent, with a 10-year survival probability from 67% to 95% [135,347,2266,2781,3956,3957,3768A]. Response to chemotherapy of the type that is typically effective in other small B-cell neoplasms is often poor, but patients generally have haematological responses to splenectomy and/or rituximab, with long-term survival [2266,3139A,3768A]. Transformation to large B-cell lymphoma occurs in 10–15% of cases [4388], and is usually associated with a shorter time to progression [2266,2781]. Hepatitis C virus–positive cases have been reported to respond to antiviral treatment using interferon gamma, with or without ribavirin [1620,3955]. Adverse clinical prognostic factors include a large tumour mass and poor general health status [627]. A clinical scoring system has been proposed that incorporates haemoglobin concentration, platelet count, lactate dehydrogenase level, and presence of extrahilar lymphadenopathy [2696]. Although data are limited, NOTCH2, KLF2, and in particular TP53 mutations have been reported to be adverse prognostic indicators [3070].
Hairy cell leukaemia

Definition
Hairy cell leukaemia (HCL) is a cytologically and immunophenotypically distinct, indolent neoplasm of small mature lymphoid cells with oval nuclei and abundant cytoplasm with so-called hairy projections involving peripheral blood and diffusely infiltrating the bone marrow and splenic red pulp.

ICD-O code 9940/3

Synonym
Leukaemic reticuloendotheliosis (obsolete)

Epidemiology
HCL is a rare disease, accounting for 2% of lymphoid leukaemias. The annual incidence rate in the USA is 0.32 cases per 100,000 population (1025). Patients are predominantly middle-aged to elderly adults, with a median age of 58 years; HCL has been diagnosed rarely in patients in their 20s, but it is exceptionally uncommon in children. The male-to-female ratio is 4:1, and the incidence is substantially higher in White versus Black populations (1025).

Etiology
The presence of the BRAF V600E mutation in virtually 100% of cases of HCL is strong evidence of a disease-defining genetic event (3998). This leads to constitutive activation of MAPK (3997).

Localization
Tumour cells are found predominantly in the bone marrow and spleen. Typically, a small number of circulating cells are noted. Tumour infiltrates may occur in the liver and lymph nodes, and occasionally also in the skin. Rare patients demonstrate prominent abdominal lymphadenopathy (3638).

Clinical features
The most common presenting symptoms include weakness and fatigue, left upper quadrant pain, fever, and bleeding. Most patients present with splenomegaly and pancytopenia, with few circulating neoplastic cells. Monocytopenia is characteristic. Other common distinctive manifestations include hepatomegaly and recurrent opportunistic infections; less common unique findings include vasculitis, bleeding disorders, neurological disorders, skeletal involvement, and other immune dysfunction (364).

Macroscopy
Diffuse expansion of the red pulp with variably sized blood lakes in markedly enlarged spleen

Microscopy
Peripheral blood and bone marrow
Hairy cells are small to medium-sized lymphoid cells with an oval or indented (kidney-shaped) nucleus with homogeneous, spongy, ground-glass chromatin that is slightly less clumped than that of a normal lymphocyte. Nucleoli are typically absent or inconspicuous. The cytoplasm is abundant and pale blue, with circumferen-

Table 13.02 Pathogenesis of hairy cell leukaemia (HCL) (287,586,1360,3994,3998)

<table>
<thead>
<tr>
<th>Property</th>
<th>Proposed pathogenetic mechanism(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clonal B cell</td>
<td>Derived from a BRAF V600E-mutant mature memory B cell</td>
</tr>
<tr>
<td>Hairy cell morphology</td>
<td>Shaped by BRAF V600E mutation and influenced by overexpression</td>
</tr>
<tr>
<td>Reticulin fibrosis</td>
<td>HCL cells synthesize and bind to fibronectin in bone marrow microenvironment</td>
</tr>
<tr>
<td>Homing to bone marrow, splenic</td>
<td>Hairy cells home to blood-related compartments via constitutively activated integrin receptors</td>
</tr>
<tr>
<td>red pulp, and hepatic sinusoids</td>
<td>and overexpression of matrix metalloproteinase inhibitors</td>
</tr>
<tr>
<td>Phagocytosis</td>
<td>Overexpression of annexin A1 and actin possible mediators</td>
</tr>
<tr>
<td>Pseudosinus formation in spleen</td>
<td>Interaction of hairy cells with endothelial cells resulting in replacement of endothelial cells</td>
</tr>
<tr>
<td>CD25 and tartrate-resistant acid</td>
<td>Induced by BRAF V600E</td>
</tr>
<tr>
<td>phosphatase expression</td>
<td>Prolonged cell survival</td>
</tr>
<tr>
<td>Inhibition of normal haematopoiesis (hypocellular HCL)</td>
<td>Constitutive production of TGF-beta by hairy cells</td>
</tr>
</tbody>
</table>

Fig. 13.18 Hairy cell leukaemia (HCL). The spleen is markedly enlarged, with diffuse expansion of the red pulp. White pulp is not discernible. Numerous blood lakes of varying size are visible.

Foucar K.
Falini B.
Stein H.
Hairy cell leukaemia. A, B Note the typical morphological features of circulating hairy cells highlighting the range in nuclear morphology between these two peripheral blood smear photomicrographs. C There is strong tartrate-resistant acid phosphatase positivity, characteristic of hairy cell leukaemia.

Spleen and other tissues
In the spleen, HCL infiltrates are found in the red pulp. The white pulp is typically atrophic. The cells characteristically fill the red pulp cords. Red blood cell lakes, collections of pooled erythrocytes surrounded by elongated hairy cells, are the presumed consequence of disruption of normal blood flow in the red pulp (364, 3638). The liver may show infiltrates of hairy cells, predominantly in the sinusoids. Lymph node infiltration may occur, especially with advanced disease, and is variable interfollicular/paracortical, with sparing of follicles and intact sinuses.

Cytochemistry
The only cytochemical stain used in the diagnosis is tartrate-resistant acid phosphatase, but the use of this technically challenging stain has been largely supplanted by immunophenotypic/immunohistochemical techniques. When appropriate air-dried unfixed slide preparations are available, virtually all cases of HCL are found to contain at least some cells with strong, granular cytoplasmic tartrate-resistant acid phosphatase positivity; weak staining is of no diagnostic utility.

Immunophenotype
The classic immunophenotypic profile of HCL consists of bright monoclonal surface immunoglobulin; bright coexpression of CD20, CD22, and CD11c; and expression of CD103, CD10, CD123, TBX21 (also called TBET), annexin A1, FMC7, CD200, and cyclin D1 (1153,1302,1896, 3643). Most cases of HCL lack both CD5 and CD10, but CD10 expression is reported in about 10–20% of cases and CD5 expression in about 0–2% (685,
1024,1844,3632). Other immunophenotypic variants are also recognized [685]. Annexin A1 is the most specific marker; it is not expressed in any B-cell lymphoma other than HCL [1153]. Expression of annexin A1 can be used to distinguish HCL from splenic marginal zone lymphoma and HCL variant, which are both annexin A1-negative. Immunostaining for annexin A1 must always be compared with staining for a B-cell antigen (e.g. CD20), because annexin A1 is also expressed by myeloid cells and a proportion of T cells. For this reason, annexin A1 is not a suitable marker for monitoring minimal residual disease. A more suitable approach for assessment for residual disease after therapy is multicolour flow cytometry targeting the distinctive HCL profile or immunostaining for TBX21 [1896,2730,3185,3643]. Immunohistochemical staining for V600E-mutant BRAF protein can also be helpful and may be useful for recognizing residual disease [4076].

Postulated normal counterpart
Although the BRAF V600E mutation has been detected in the haematopoietic stem cell compartment of patients with HCL, the postulated normal counterpart is a late, activated memory B cell, as suggested by gene expression profiling studies [287,755].

Genetic profile
Antigen receptor genes
Although exceptions have been reported, the majority (>85%) of cases of HCL demonstrate IGHV genes with somatic hypermutation indicative of a post-germinal centre stage of maturation [152,586,3994]. A unique feature of HCL is the common coexpression of multiple clonally related immunoglobulin isotypes, suggesting arrest at some point during isotype switching [3994].

Cytogenetic abnormalities and oncogenes
No cytogenetic abnormality is specific for HCL; numerical abnormalities of chromosomes 5 and 7 have been rarely described, but translocations are distinctly uncommon [586]. The high frequency of BRAF V600E mutation, confirmed by multiple investigators, suggests a key role in the pathogenesis of HCL [3160]. Sensitive methods for the detection of this HCL-defining mutation have been published [138,3997,3998]. It remains to be established whether cases that lack BRAF V600E mutation, use the IGHV4-34 family, and have MAP2K1 mutations are more closely related to classic HCL or HCL variant [4267,4382].

Prognosis and predictive factors
HCL is uniquely sensitive to either interferon alfa or nucleosides (purine analogues) such as pentostatin and cladribine. Patients receiving purine analogues often achieve complete and durable remission [1458]. However, as many as 50% of patients with HCL relapse. In refractory or relapsed cases, salvage therapeutic options include chemotherapy combined with rituximab, anti-CD22 immunotoxin therapy, and, more recently, BRAF inhibitors [3160,3995].
Splenic B-cell lymphoma/leukaemia, unclassifiable

Definition
There are a number of variably well-defined entities that constitute small B-cell clonal lymphoproliferations involving the spleen but that do not fit into any of the other categories of B-cell lymphoid neoplasms in the WHO classification. The best-defined of these relatively rare provisional entities are splenic diffuse red pulp small B-cell lymphoma and hairy cell leukaemia variant.

The relationship of splenic diffuse red pulp small B-cell lymphoma to hairy cell leukaemia variant and other primary splenic B-cell lymphomas remains uncertain; the precise diagnostic criteria and most-appropriate terminology for these provisional entities have not yet been fully established.

Other splenic small B-cell lymphomas that do not fulfil the criteria for either of these provisional entities should be diagnosed as splenic B-cell lymphoma/leukaemia, unclassifiable, until more is known [3848].

ICD-O code 9591/3

Synonyms
Splenic marginal zone lymphoma, diffuse variant; splenic red pulp lymphoma with numerous basophilic villous lymphocytes; splenic lymphoma with villous lymphocytes; prolymphocytic variant of hairy cell leukaemia

Splenic diffuse red pulp small B-cell lymphoma

Definition
Splenic diffuse red pulp small B-cell lymphoma (SDRPL) is an uncommon lymphoma with a diffuse pattern of involvement of the splenic red pulp by small monomorphous B lymphocytes. The neoplasm also involves bone marrow sinusoids and peripheral blood, commonly with a villous cytology [1921,4043,4044]. This is a provisional entity that requires additional molecular studies for defining its main features and diagnostic markers. This diagnosis should be restricted to characteristic cases fulfilling the major features described here, and should not be applied to any lymphoma growing diffusely in the spleen. Chronic lymphocytic leukaemia, hairy cell leukaemia, lymphoplasmacytic lymphoma, and B-cell prolymphocytic leukaemia should be excluded through appropriate studies. A diagnosis of SDRPL may be suggested for cases showing purely intrasinusoidal bone marrow involvement and villous lymphocytes in the peripheral blood, but the differential with splenic marginal zone lymphoma (SMZL) may require examination of the spleen (3216). In case of doubt, the use of the term splenic B-cell lymphoma/leukaemia, unclassifiable, is warranted.

There is some degree of overlap with cases that fulfil the criteria for hairy cell leukaemia variant; however, additional studies are required to further evaluate the extent of overlap between these entities, particularly given that not all studies report the same phenotypic, cytogenetic, or molecular findings [2584,3481]. Although the rare large B-cell lymphomas that involve the splenic and bone marrow sinusoids may be related to SDRPL, they should not be included in this category, which is restricted to indolent lymphomas composed of small lymphocytes [2688,2743].

ICD-O code 9591/3

Epidemiology
SDRPL is a rare disorder, accounting for <1% of non-Hodgkin lymphomas. It accounts for about 10% of the B-cell lymphomas diagnosed in splenectomy specimens. Most patients are aged >40 years, and there is no sex predilection.

Localization
All cases are diagnosed at clinical stage IV, with spleen, bone marrow, and peripheral blood involvement. Peripheral lymph node involvement is only rarely reported.

Clinical features
SDRPL is a leukaemic neoplasm, usually with a relatively low lymphocytosis. Almost all patients have splenomegaly (frequently massive). Although not consistent among all studies, thrombocytopenia and leukopenia are frequently present, whereas anaemia has been reported more rarely. B symptoms are infrequent. The presence of a paraprotein has not been reported.

Microscopy

Peripheral blood
Villous lymphocytes similar to those

Fig. 13.23 Splenic diffuse red pulp small B-cell lymphoma. A Peripheral blood cytology with villous cell. B Reticular staining in spleen outlines the infiltration of red pulp cords and sinusoids. C Bone marrow intrasinusoidal infiltration highlighted by CD20 staining.
reported in SMZL are present. Clonal B-cell expansions with an immunophenotype consistent with marginal zone lymphoma presenting with isolated lymphocytosis may constitute an early stage of splenic B-cell lymphoma/leukaemia, unclassifiable, or of SMZL, although most of these cases remain stable over time (4390). Some of these cases fulfil the criteria for non-chronic lymphocytic leukaemia–type monoclonal B-cell lymphocytosis.

Bone marrow
Intrasinusoidal infiltration is the rule, occasionally as a sole finding. This can be accompanied by interstitial and nodular infiltration. Lymphoid follicles, as seen in SMZL, have not been reported.

Spleen
There is a diffuse pattern of involvement of the red pulp, with both cord and sinu¬soid infiltration. Characteristic blood lakes lined by tumoural cells may be seen. Unlike in SMZL, white pulp involvement is absent, although there may be residual lymphoid nodules composed of T cells or, much less often, residual white pulp nodules (2530). The neoplastic infiltrate is composed of a monomorphic population of small to medium-sized lymphocytes, with round and regular nuclei, compact chromatin, and occasional distinct small nucleoli, with scattered nucleolated blast cells. The tumoural cells have pale cytoplasm and plasmacytoid features but lack phenotypic features of plasmacytic differentiation such as cytoplasmic immunoglobulin and CD38 expression. Some cases show focal plasmacytic differentiation. Rare cases have clusters of large cells (2530).

Immunophenotype
SDRPL is characteristically positive for CD20, DBA.44 (CD72), and IgG and negative for IgD, annexin A1, CD25, CD5, CD103, CD123, CD11c, CD10, and CD23 (2581,2689,3499). IgD+ cases can be seen with similar features. There have also been reports of cases that are positive for IgM (with or without IgG), CD103, and CD11c, with infrequent CD5 and CD123 expression (4043).

Postulated normal counterpart
A mature B cell of unknown type

Genetic profile
Somatic hypermutation in IGHV genes is present in most cases, but about 20–30% of cases are unmutated (2530,4043). Over-representation of IGHV3-23 and IGHV4-34, as is seen in hairy cell leukaemia, has been reported (4043). The reported proportion of cases that use the IGHV1-2 gene, which is overrepresented in SMZL, has varied; in one series, its use was reported in 3 of 13 cases; the same number of cases used IGHV4-34 (2530,4043).

Complex cytogenetic alterations, including t(9;14)(p13;q32) involving PAX5 and IGH genes, have been found in some cases, but the neoplasms lack CCND1 rearrangements, and usually do not demonstrate deletions in 7q or trisomies of chromosomes 3 or 18 (262,2530,4043). Copy-number arrays have identified abnormalities in almost 70% of cases (2530). TP53 mutations have been reported in a small number of cases, and some cases show increased p53 expression (2530,2689). Sequencing studies have shown that SDRPL has a distinct pattern of somatic mutations amongst B-cell malignancies (4047A), with an increased expression of cyclin D3 and recurrent mutations in the CCND3 PEST domain in a high proportion of SDRPL cases (848A). Infrequent mutations in NOTCH1, MAP2K1, BRAF, and SF3B1 have also been reported (2530).

Prognosis and predictive factors
This is an indolent but incurable disease, with good responses after splenectomy in most patients; however, some patients do develop progressive disease and have an adverse outcome. The small number of patients with mutations in NOTCH1, MAP2K1, and TP53 have been reported to have shorter progression-free survival (2530).

Hairy cell leukaemia variant

Definition
The designation of hairy cell leukaemia variant (HCL-v) encompasses cases of B-cell chronic lymphoproliferative disorders that resemble classic HCL but exhibit variant cytological and haematological features such as leukocytosis, presence of monocytes, cells with prominent nucleoli, cells with blastic or convoluted nuclei, and/or absence of circumferential shaggy contours. They also have a variant immunophenotype (including absence of CD25, CD123, annexin A1, and tartrate-resistant acid phosphatase), have wildtype BRAF, and are resistant to conventional HCL therapy (i.e. show lack of response to cladribine). These cases are not considered to be biologically related to HCL.
with villous projections, but unlike the cells of typical hairy cell leukaemia, they have visible nucleoli, resembling HCL, although several other morphological subtypes (e.g. blastic and convoluted) have also been described (3367). Nuclear features range from condensed chromatin with the prominent central nucleoli of a prolymphocytic cell to dispersed chromatin with highly irregular nuclear contours. Cytoplasmic features are similarly variable, although some degree of hairy projections is typically noted. Transformation to large cells with convoluted nuclei has been described, and cases of so-called convoluted HCL may be explained by this phenomenon (2584,4461). Unlike in classic HCL, the bone marrow is aspirable, without significant reticulin fibrosis (1920). The infiltrates of HCL-v may be subtle and very inconspicuous, often requiring immunohistochemical staining to highlight the pattern and extent of infiltration (624). A distinct predilection for sinusoidal infiltration has been described (624).

Like in HCL and splenic diffuse red pulp small B-cell lymphoma, the red pulp of the spleen is diffusely involved and expanded in HCL-v, with atretic or absent white pulp follicles (1920). The leukaemic cells fill dilated sinusoids, and red blood cell lakes may be noted (2584). Liver involvement is characterized by both portal tract and sinusoidal infiltrates.

Cytochemistry
Unlike in classic HCL, cytochemical staining for tartrate-resistant acid phosphatase is weak to negative in HCL-v (1920,2584).

Immunophenotype
Cases of HCL-v share many phenotypic features with HCL, although HCL-v cells characteristically lack several key HCL antigens, usually including CD25, annexin A1, tartrate-resistant acid phosphatase, CD200, and CD123 (52,1024,1153,3367). Positive markers in HCL-v include DBA-44 (CD72), pan-B-cell antigens, CD11c, bright monotypic surface immunoglobulin (most frequently IgG), CD103, and FMC7 (2574).

Postulated normal counterpart
An activated B cell at a late stage of maturation

Genetic profile
Studies are limited, but about one third of cases of HCL-v demonstrate no somatic mutations of IGHV; these unmutated cases have a high frequency of TP53 mutation (1647). There is preferential usage of the IGHV4-34 gene family, although this is not a feature exclusive to HCL-v (1648). High-resolution genomic profiling has shown a large number of DNA copy-number alterations, the most frequent being gains on chromosome 5 and losses on 7q and 17p (1649). BRAF V600E mutations have not been documented in HCL-v (3278,3997,3998,4382). Recurrent MAP2K1 mutations have been found in HCL-v and in cases described as classic HCL with IGHV4-34 gene family usage (4267).

Prognosis and predictive factors
The 5-year survival rate is reported to be 57% (1647). Most patients with HCL-v require therapy, which can range from splenectomy to combination chemotherapy with rituximab (1647). Agents that are effective in classic HCL (i.e. cladribine and pentostatin) are not effective in HCL-v (1024,3367). However, patients seem to achieve a long-lasting response to the combination of cladribine and rituximab (2106). Significant adverse prognostic factors include older patient age, greater severity of anaemia, and TP53 mutations (1647).
**Lymphoplasmacytic lymphoma**

**Definition**
Lymphoplasmacytic lymphoma (LPL) is a neoplasm of small B lymphocytes, plasmacytoid lymphocytes, and plasma cells, usually involving bone marrow and sometimes lymph nodes and spleen, which does not fulfil the criteria for any of the other small B-cell lymphoid neoplasms that can also have plasmacytic differentiation. Because the distinction between LPL and some of the other small B-cell lymphoid neoplasms, especially some marginal zone lymphomas (MZLs), is not always clear-cut, some cases may need to be diagnosed as a small B-cell lymphoid neoplasm with plasmacytic differentiation and a differential diagnosis provided. The great majority (>90%) of LPLs have MYD88 L265P mutation, which can make the diagnosis either more or less likely; however, this abnormality is neither specific nor required. Although LPL is often associated with a paraprotein, usually of IgM type, this is not required for the diagnosis. Waldenström macroglobulinaemia (WM) is found in a substantial subset of patients with LPL, but is not synonymous with it; it is defined as LPL with bone marrow involvement and an IgM monoclonal gammopathy of any concentration [3017]. Cases of gamma heavy chain disease are no longer considered a variant of LPL [1526].

**ICD-O codes**
- Lymphoplasmacytic lymphoma 9671/3
- Waldenström macroglobulinaemia 9761/3

**Synonym**
Malignant lymphoma, lymphoplasmacytoid

**Epidemiology**
LPL occurs in adults, with a median age in the seventh decade of life, and shows a slight male predominance [991,4195].

**Etiology**
Hepatitis C virus is associated with type II cryoglobulinaemia and with LPL in some series, perhaps related to geographical differences [908,2263,2616,2875,3037,3263,3514,3913]. Some of the hepatitis C virus-associated lymphoplasmacytic proliferations, even if monotypic, are non-progressive and may be similar to monoclonal B-cell lymphocytosis [2690,2717]. Treatment of these patients with antiviral agents may lead to regression of the lymphoplasmacytic proliferations [2589,3913]. So-called immune-stimulating conditions, such as autoimmune disorders, are associated with an increased risk [2483].

**Localization**
Most cases involve the bone marrow, and some cases involve the lymph nodes and other extranodal sites. About 15–30% of patients with WM also have splenomegaly, hepatomegaly, and/or adenopathy, with a higher proportion with disease progression [991,4051]. The peripheral blood may also be involved. Rare involvement of the CNS, associated with WM, is known as Bing–Neel syndrome [3225]. LPL can occur at sites typically involved by extranodal M2L of mucosa-associated lymphoid tissue (MALT lymphoma), such as the ocular adnexa [2347,3851].

**Clinical features**
Most patients present with weakness and fatigue, usually related to anaemia. Most patients have an IgM serum paraprotein, and would therefore also fulfil the criteria for WM. Some, however, have a different paraprotein or no paraprotein at all. A minority have both IgM and IgG or other paraproteins. Hyperviscosity occurs in as many as 30% of cases. The paraprotein may also have autoantibody or cryoglobulin activity, resulting in autoimmune phenomena or cryoglobulinaemia (seen in as many as ~20% of patients with WM). Cold agglutinins may also be present; however, primary cold agglutinin disease may be distinct from LPL [3301]. Neuropathies occur in a minority of patients and may result from reactivity of the IgM paraprotein with myelin sheath antigens.

---

**Fig. 13.26** Lymphoplasmacytic lymphoma. A Bone marrow biopsy shows a lymphoplasmacytic infiltrate with a Dutcher body (arrow), which gives a positive periodic acid–Schiff (PAS) reaction. B The lymphoplasmacytic infiltrate is also seen in the aspirate smear. C Giemsa staining highlights the characteristic increased mast cells and haemosiderin (arrow).
cryoglobulinaemia, or paraprotein deposition. Deposits of IgM may occur in the skin or the gastrointestinal tract, where they may cause diarrhoea. Coagulopathies may be caused by IgM binding to clotting factors, platelets, and fibrin. IgM paraproteins are not diagnostic of either LPL or WM, because they can also occur in patients with other lymphoid neoplasms or without an overt neoplasm. A minority of patients initially present with an IgM-related disorder such as cryoglobulinaemia or IgM monoclonal gammopathy of undetermined significance (see below) and only later develop an overt LPL (621,2173,2752).

**Microscopy**

**Bone marrow and peripheral blood**

Bone marrow involvement is characterized by a nodular, diffuse, and/or interstitial infiltrate, sometimes even with paratrabeicular aggregates. The infiltrate is usually composed predominantly of small lymphocytes admixed with variable numbers of plasma cells, plasmacytoid lymphocytes, and often increased mast cells [2347,3016,3017]. The plasma cells may also form distinct clusters separate from the lymphoid component [2347, 2736]. Residual disease after treatment may demonstrate virtually all plasma cells [2347,4157]. A similar spectrum of cells as are present in the bone marrow may be present in the peripheral blood, but the white blood cell count is typically lower than in chronic lymphocytic leukaemia.

**Lymph nodes and other tissues**

In the most classic cases, which are usually associated with WM, the lymph nodes show retention of normal architectural features with dilated sinuses with periodic acid-Schiff (PAS) positive material and sometimes small portions of residual germinal centres. There is a relatively monotonous proliferation of small lymphocytes, plasma cells, and plasmacytoid lymphocytes, with relatively few transformed cells. Dutcher bodies (PAS-positive intranuclear pseudoinclusions), increased mast cells, and haemosiderin are also typical features. Other cases show greater architectural destruction and may have a vaguely follicular growth pattern, more prominent residual germinal centres (even with follicular colonization), epithelioid histioyte clusters, sometimes a much greater proportion of plasma cells, and sometimes a paucity of frank plasma cells [3521,3851]. The presence of prominent large transformed cells should raise the possibility of either disease progression or a diagnosis other than LPL. Proliferation centres like those seen in chronic lymphocytic leukaemia / small lymphocytic lymphoma must be absent, and the presence of paler...
appearing marginal zone–type differentiation should suggest a diagnosis of one of the MZLs. There may be associated amyloid, other immunoglobulin deposition, or crystal-storing histiocytes. The growth pattern in spleen is not well established, but there should be a lymphoplasmacytic infiltrate with diffuse and/or nodular red pulp involvement and sometimes white pulp nodules [1527,2343].

**Immunophenotype**

Most cells express surface immunoglobulin, and the light chain–restricted plasmacytic cells express cytoplasmic immunoglobulin (usually IgM, sometimes IgG, and rarely IgA). LPLs are typically IgD-negative; express B-cell–associated antigens (CD19, CD20, CD22, and CD79a); and are most typically negative for CD5, CD10, CD103, and CD23, with frequent CD25 and CD38 expression. However, a minority of cases are positive for CD5 or CD10 (but BCL6-negative), and CD23 expression is not at all uncommon in some studies [2347,2736,3851]. The precise phenotype may also change over time [2347]. The plasma cells are CD138-positive; unlike in plasma cell myeloma, they are usually also positive for CD19 and often for CD45 [2736,3401]. The CD138+ plasma cells in LPL, although usually positive for IRF4/MUM1, are more likely to be IRF4/MUM1-negative and PAX5-positive compared with normal plasma cells or those in MZL [1502,2736,3372]. These differences are not easily assessed in daily practice. In addition to the neoplastic plasma cells, polytypic plasma cells may also be present.

**Postulated normal counterpart**

A post-follicular B cell that differentiates into plasma cells

**Genetic profile**

*Antigen receptor genes*

IG genes are rearranged, usually with variable regions that show somatic hypermutation but lack ongoing mutations [4226]. There may be biased IGHV gene usage [1801,2114]. Clonal cytotoxic T-cell populations may be present, at least in the peripheral blood [2299].

*Cytogenetic abnormalities and oncogenes*

No specific chromosomal abnormalities are recognized in LPL; however, >90% of cases have MYD88 L265P mutation, and there is a small proportion of other small B-cell lymphomas in which it is present [3851]. MYD88 L265P mutation is also seen in some non-germinal centre subtype DLBCL, NOS, primary cutaneous DLBCL, leg type, and primary CNS and testicular DLBCL cases. Similarly, CXCR4 mutations are also present in a very small proportion of other small B-cell lymphomas. These mutations are important in the pathogenesis of LPL, at least in part by leading to NF-kappaB signalling, and for developing improved therapeutic strategies [548,549,3379]. The previously reported t(1;14)(q13;q32) translocation leading to IGH/PAX5 juxtaposition is rarely, if ever, found in LPL [792,1328,2489]. Deletion in 6q is reported in somewhat more than half of bone marrow–based cases, but it is not a specific finding and may be less frequent in tissue-based LPL [793,2489,2925,3582]. Small copy-number abnormalities leading to varied B-cell regulatory gene losses are also commonly found [1735]. Trisomies 3 and 18 are infrequent. Trisomy 4, present in about 20% of WM, is another finding that can be used to support the diagnosis [444,3941]. LPLs do not demonstrate the translocations associated with other B-cell lymphomas (e.g. those involving CCND1, MALT1, or BCL10), with the possible rare exception of 6CL2 gene rearrangements. One study found that WM had a homogeneous gene expression profile, independent of 6q deletion, which is more similar to chronic lymphocytic leukaemia and normal B cells than to myeloma [727]. The study also suggested the importance of upregulated IL6 and its downstream MAPK signalling pathway.

**Fig. 13.29** Lymphoplasmacytic lymphoma with MYD88 L265P mutation involving cerebrospinal fluid (Bing–Neel syndrome). Cytology preparation of the cerebrospinal fluid shows a population of small lymphocytes, as well as small and larger plasmacytoid forms (Diff-Quik stain). The patient had an IgM monoclonal gammopathy with bone marrow involvement (Waldenström macroglobulinaemia).

**Fig. 13.30** Lymphoplasmacytic lymphoma (LPL). Real-time PCR with MYD88 L265P–specific primers shows positive amplification in a case of LPL (blue) and no amplification in a negative control cell line (red).
Genetic susceptibility
A familial predisposition may exist in as many as 20% of patients with WM [80, 4053, 4054]. These patients are diagnosed at a younger age and with greater bone marrow involvement. There may also be prognostic and therapeutic implications [4054].

Prognosis and predictive factors
The clinical course is typically indolent, with median survival times of 5–10 years, and with improved survival in more recent years [579, 991, 4195]. Advanced patient age, peripheral blood cytopenias (especially anaemia), poor performance status, and high beta-2 microglobulin levels have been reported to be associated with a worse prognosis [991, 4195]. An international prognostic scoring system for WM has been proposed that also includes a high (>7.0 g/dL) serum paraprotein level but not performance status [2726]. Cases with increased numbers of transformed cells/immunoblasts may also be associated with an adverse prognosis; however, a validated grading system does not exist [103, 346]. Cases with del(6q) have been associated with adverse prognostic features [2925]. Cases lacking MYD88 L265P mutation are reported to have an adverse prognosis and a lower response to ibrutinib; however, the diagnosis in these cases may be less certain, and data are limited [4052, 4055]. Although there are no documented survival differences, CXCR4-mutated LPL (in particular cases with nonsense mutations), has been associated with more-symptomatic/active disease, other clinical and laboratory findings, and greater resistance to ibrutinib and possibly other therapeutic agents [4051, 4052, 4055, 4056]. Transformation to diffuse large B-cell lymphoma occurs in a small proportion of cases and is associated with poor survival [2346].
IgM monoclonal gammopathy of undetermined significance

**Definition**
IgM monoclonal gammopathy of undetermined significance (MGUS) is defined by a serum IgM paraprotein concentration <30 g/L; bone marrow lymphoplasmacytic infiltration of <10%; and no evidence of anaemia, constitutional symptoms, hyperviscosity, lymphadenopathy, hepatosplenomegaly, or other end-organ damage that can be attributed to the underlying lymphoproliferative disorder [239]. IgM MGUS is a precursor condition that may progress to overt lymphoma or primary amyloidosis.

**Epidemiology**
MGUS of any isotype is reported in approximately 3% of individuals aged ≥50 years; of these cases, approximately 15% are of IgM type, yielding a prevalence of approximately 0.5% [1004,1085, 2175]. The proportion of MGUS due to IgM is higher among White populations than among Black or Asian populations [2215]. The median patient age at diagnosis is 74 years, and there is a male predominance, with a male-to-female ratio of 1.4:1 [2177].

**Localization**
There may be as much as 10% bone marrow infiltration by an IgM+ clonal lymphoplasmacytic population. Cases with any degree of bone marrow infiltration are considered by some pathologists to constitute asymptomatic Waldenström macroglobulinaemia (WM), lymphoplasmacytic lymphoma (LPL).

**Clinical features**
Patients with IgM MGUS lack the signs and symptoms of an overt lymphoproliferative disorder or plasma cell neoplasm, and the paraprotein is typically discovered incidentally on serum protein electrophoresis. Reduced polyclonal immunoglobulins are reported in 35% of cases, and Bence Jones proteinuria has been reported in approximately 20% [2162,2751]. The term IgM-related disorder has been proposed for cases in patients who have no evidence of an overt lymphoma or plasma cell neoplasm but have symptoms related to the IgM paraprotein, such as anti-myelin-associated glycoprotein-mediated peripheral neuropathy, cold agglutinin disease, or cryoglobulinaemia [3017].

**Microscopy**
By definition, IgM MGUS lacks findings diagnostic of LPL, another lymphoproliferative disorder, or plasma cell neoplasm. Distinguishing IgM MGUS from LPL and other lymphomas requires examination of a bone marrow biopsy [3017]. The marrow contains clonal lymphoplasmacytic cells; however, they must not represent an infiltrate of ≥10%. In addition, sometimes the clonal cells are not easily identified in the background of normal polyclonal plasma cells.

**Immunophenotype**
On sensitive multiparameter flow cytometry, clonal B cells are reported in as many as 75% of IgM MGUS bone marrows, although complex gating strategies may be required to identify the clonal population within the background of benign polytypic B cells [2924,3032,3034]. When present, the clonal B-cell population shows a non-specific phenotype similar to that of LPL (CD19+, CD20+, CD5−, CD10−, and CD103−). The plasma cells in IgM MGUS lack expression of CD56 [3034].

**Postulated normal counterpart**
B cells with somatic hypermutation of the IGHV genes but without class switching [2177]

**Genetic profile**
The gene expression profile of IgM MGUS is similar to that of LPL [3032]. Approximately half of IgM MGUS cases are positive for the MYD88 L265P mutation [1861,2214,4155,4393], and 20% have been reported to have CXCR4 S338X mutations [3379], but further genetic data are limited. Deletions of chromosome 6q have been reported, but are non-specific and appear to be less frequent than in LPL and WM [3032,3582].

**Prognosis and predictive factors**
IgM MGUS progresses to LPL/WM, other B-cell neoplasms, or primary amyloidosis at a rate of 1.5% per year. Progression to plasma cell myeloma occurs rarely, if at all. The rate of progression in patients with symptomatic IgM-related disorders appears similar to that in patients without monoclonal protein-related symptoms [2751]. Patients remain at risk for progression even after having stable disease for 20 years [2177]. A detectable MYD88 L265P mutation and higher levels of serum monoclonal protein are independent risk factors for progression [239,4116,4155,4156]. In one study, the risk of progression doubled for every increase of 7 g/L in serum monoclonal protein [239].
Heavy chain diseases

**Definition**
The heavy chain diseases (HCDs) are three rare B-cell neoplasms characterized by the production of monoclonal immunoglobulin heavy chains (IgG in gamma HCD, IgA in alpha HCD, and IgM in mu HCD) and typically no light chains \( \{370,4230\} \). The heavy chain is usually truncated, preventing normal assembly with light chains. Variably sized proteins are produced, which may not yield a characteristic monoclonal peak by routine serum protein electrophoresis, and require immunoelectrophoresis or immunofixation to detect.

In some cases, HCD can show morphological features consistent with another well-defined histological entity. Some cases of gamma HCD resemble typical examples of splenic marginal zone lymphoma or extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma) \( \{371\} \), whereas mu HCD typically resembles chronic lymphocytic leukaemia, and alpha HCD is considered a variant of MALT lymphoma \( \{370,4230\} \). However, each of these HCDs is sufficiently distinct to be considered a separate entity. Establishing the diagnosis of an HCD requires demonstration of free heavy chains by protein electrophoresis/immunofixation.

---

**Mu heavy chain disease**

**Definition**
Mu heavy chain disease (HCD) is a B-cell neoplasm resembling chronic lymphocytic leukaemia (CLL), in which a defective mu heavy chain lacking a variable region is produced. The bone marrow contains an infiltrate of characteristic vacuolated plasma cells, admixed with small, round lymphocytes.

**ICD-O code**
9762/3

**Epidemiology**
This is an extremely rare disease of adults, with only 30–40 cases reported, a median patient age of 60 years, and an approximately equal frequency in males and females \( \{370,4230\} \).

**Localization**
The spleen, liver, bone marrow, and peripheral blood may be involved; peripheral lymphadenopathy is usually absent.

**Clinical features**
Most patients present with a slowly progressive disease resembling CLL. Mu HCD differs from most cases of CLL in the high frequency of hepatosplenomegaly and the absence of peripheral lymphadenopathy. Routine serum protein electrophoresis is frequently normal. Immunoelectrophoresis reveals reactivity to anti-mu in polymers of diverse sizes. Although mu chain is not found in the urine, Bence Jones light chains (particularly kappa chains) are common (found in the urine in 50% of cases). Light chains, although still produced in mu HCD, are not assembled into a complete immunoglobulin protein, due to IGH gene aberrations leading to truncated forms \( \{242,4229,4230\} \).

**Microscopy**
The bone marrow contains vacuolated plasma cells, which are typically admixed with small, round lymphocytes similar to CLL cells.

---

Fig. 13.31 Structure of the immunoglobulin molecule in heavy chain disease. An immunoglobulin molecule is composed of two heavy chains (H) and two light chains (L), which are joined by disulfide bonds (S–S). The normal heavy chain constant region has three constant domains: CH1 is responsible for binding to the light chain, CH2 for binding to complement, and CH3 for binding to Fc receptors. In the absence of an associated light chain, the CH1 domain binds to HSP78 and undergoes proteasomal degradation; thus, normal free heavy chains are not secreted. In heavy chain diseases, non-contiguous deletions in the CH1 domain prevent both binding of the heavy chain to the light chain and degradation in the proteasome, and free heavy chains are secreted. Variably sized deletions also occur in the heavy chain diversity region (D\(_{\mu}\)), the heavy chain joining region (J\(_{\mu}\)), and the heavy chain variable region (V\(_{\mu}\)). Reprinted with permission from Munshi NC et al. (2785).
Immunophenotype
The cells contain monoclonal cytoplasmic mu heavy chain (with or without monotypic light chain), express B-cell antigens, and are usually negative for CD5 and CD10.

Postulated normal counterpart
A post-germinal centre B cell that can differentiate into a plasma cell, with an abnormal IGHM gene

Genetic profile
Immunoglobulin genes are clonally rearranged and contain high levels of somatic hypermutation [370,4230]. Deletions in the IGHM gene are present that result in expression of a defective heavy chain protein that cannot bind light chain to form a complete immunoglobulin molecule. These deletions involve IGHV and variable proportions of the CH1 domain, and there may be insertions of large amounts of DNA of unknown origin [1244, 4230].

Prognosis and predictive factors
The clinical course is slowly progressive in most cases [242,1244,4229,4230].

Gamma heavy chain disease

Definition
Gamma heavy chain disease (HCD) is a small B-cell neoplasm with plasmacytic differentiation that produces a truncated immunoglobulin gamma heavy chain that lacks light chain-binding sites and therefore cannot form a complete immunoglobulin molecule.

ICD-O code
9762/3

Synonym
Franklin disease

Epidemiology
This is a rare disease of adults, with a median patient age of 60 years; approximately 150 cases have been described. There is no particular geographical distribution, and recent series report a female predominance [371,4231].

Localization
The tumour may involve the lymph nodes, Waldeyer ring, gastrointestinal tract and other extranodal sites, bone marrow, liver, spleen, and peripheral blood.

Clinical features
Most patients have systemic symptoms such as anorexia, weakness, fever, weight loss, and recurrent bacterial infections. Autoimmune manifestations are reported in 25–70% of cases, most frequently rheumatoid arthritis and systemic lupus erythematosus, but also autoimmune haemolytic anaemia, thrombocytopenia, or both; vasculitis; Sjögren syndrome; myasthenia gravis; and thyroiditis [371, 1739,4231]. Autoimmune disease may precede the diagnosis of lymphoma by several years. Most patients have generalized disease, including lymphadenopathy, splenomegaly, and hepatomegaly. Involvement of the Waldeyer ring, skin and subcutaneous tissues, thyroid, salivary glands, or gastrointestinal tract may occur. Circulating plasma cells or lymphocytes may occasionally be present. Patients generally do not have lytic bone lesions or amyloid deposition. The bone marrow is involved in 30–60% of cases [371,1739,4231]. Clinical and laboratory distinction from an infection or inflammatory process may be difficult given the constellation of symptoms and the sometimes broad band or near-normal serum protein electrophoresis results. Approximately 10–20% of patients have no overt lymphadenopathy or other mass lesions; most of these patients have autoimmune disorders. The diagnosis is made by demonstration of IgG without light chains by immunofixation in the peripheral blood, the urine, or both.

Microscopy
The morphological findings in gamma HCD are heterogeneous [371,2785,4231]. Some cases resemble typical examples of splenic marginal zone lymphoma or MALT lymphoma. Most frequently, lymph nodes show a polymorphous proliferation of admixed lymphocytes, plasmacytoid lymphocytes, plasma cells, immunoblasts, histiocytes, and eosinophils. The presence of eosinophils, histiocytes, and immunoblasts may cause a resemblance to angioimmunoblastic T-cell lymphoma or classic Hodgkin lymphoma. In some cases, plasma cells predominate; these cases may resemble plasmacytoma. The peripheral blood may show lymphocytosis with or without plasmacytoid lymphocytes, resembling chronic lymphocytic leukaemia or lymphoplasmacytic lymphoma. Transformation to diffuse large B-cell lymphoma is rare [370,4230].

Fig. 13.32 Mu heavy chain disease. Bone marrow aspirate shows predominantly plasma cells with prominent cytoplasmic vacuolation.

Fig. 13.34 Gamma heavy chain disease. This case displays a diffuse proliferation of small lymphocytes, plasmacytoid cells, plasma cells, and scattered large transformed cells.

Fig. 13.33 Gamma heavy chain disease. This case presented with splenomegaly. The white pulp contains a nodular proliferation (A) of small lymphocytes with abundant pale cytoplasm (B), morphologically suggestive of a splenic marginal zone lymphoma. The diagnosis of gamma heavy chain disease was established by serum immunofixation studies and the finding of gamma heavy chain–expressing plasma cells that lacked kappa and lambda staining.
bone marrow may show lymphoplasmacytic aggregates or only a subtle increase in plasma cells with monotypic gamma heavy chains without light chains.

**Immunophenotype**
The cells express CD79a and cytoplasmic gamma chain and are negative for CD5 and CD10. CD20 is found on the lymphocytic component and CD138 on the plasma cell component. Kappa and lambda light chains are typically not expressed, but a minority of cases show staining for monotypic light chains by immunohistochemistry or in situ hybridization, despite the absence of light chains on immunofixation studies [370,371,4230].

**Postulated normal counterpart**
A post-germinal centre B cell that can differentiate into a plasma cell, with a defective IGHG gene

**Genetic profile**
IG genes are clonally rearranged and contain high levels of somatic hypermutation. Deletions in the IGHG gene are present that result in expression of an abnormally truncated heavy chain protein that cannot bind light chain to form a complete immunoglobulin molecule. These deletions involve IGHV and variable proportions of the CH1 domain, and there may be insertions of large amounts of DNA of unknown origin [57,370,1132,1243,1249,4230].

Abnormal karyotypes have been found in about half of the reported cases, but no specific or recurrent genetic abnormality has been reported [371,4231]. The MYD88 L265P mutation that is characteristic of lymphoplasmacytic lymphoma is absent in gamma HCD [1526].

**Prognosis and predictive factors**
The clinical course is highly variable, ranging from indolent to rapidly progressive. One study of 23 cases reported a median survival time of 7.4 years, with more than half of the deaths unrelated to the lymphoproliferative disorder [4231]. There is no standardized therapy. Most patients with low-grade—appearing lymphoplasmacytic infiltrates appear to respond to non-anthracycline-containing chemotherapy, and responses to rituximab and other single agents have also been reported [370,4230].

**Fig. 13.36 Gamma heavy chain disease.** A A distinct band was identified by serum protein electrophoresis (SPE) in the IG region anodal to the point of origin (arrow). The M protein (also called M component) typed as IgG (denoted by the band seen in the IgG lane), but without a corresponding light chain (only faint polyclonal patterns were seen in the kappa and lambda lanes). B Urine protein electrophoresis (UPE) revealed similar results, with a broad monoclonal band corresponding to IgG without a corresponding light chain. Reprinted from Munshi NC et al. [2785].
**Alpha heavy chain disease**

**Definition**
Alpha heavy chain disease (HCD), also known as immunoproliferative small intestinal disease, is a variant of extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma) in which defective immunoglobulin alpha heavy chains are secreted [48, 4230].

**ICD-O code**
9762/3

**Synonyms**
Mediterranean lymphoma; immunoproliferative small intestinal disease

**Epidemiology**
Alpha HCD is the most common of the HCDs. Unlike the other HCDs, alpha HCD involves a young age group, with a peak incidence rate in the second and third decades; it is rare in young children and older adults, and there is an equal incidence in males and females. It is most common in areas bordering the Mediterranean Sea, including northern Africa, Israel and Saudi Arabia. It is associated with factors linked to low socioeconomic status, including poor hygiene, malnutrition, and frequent intestinal infections [48, 3298, 3617, 4230].

**Etiology**
Chronic intestinal infection, in some cases with Campylobacter jejuni, is believed to result in chronic inflammation, a setting in which neoplastic transformation of a clone of abnormal B cells develops [2248, 3072].

**Localization**
Alpha HCD involves the gastrointestinal tract (mainly the small intestine) and mesenteric lymph nodes. The gastric and colonic mucosa may also be involved. The bone marrow and other organs are usually not involved, although respiratory tract and thyroid involvement has been described in rare cases [3617, 4040].

**Clinical features**
Patients typically present with malabsorption, diarrhoea, hypocalcaemia, abdominal pain, wasting, fever, and steatorrhoea. Typically, serum protein electrophoresis is normal, and identification of the abnormal alpha heavy chain requires immunofixation, immunoelectrophoresis, or immunoselection techniques [370, 4230].

**Microscopy**
The lamina propria of the bowel is heavily infiltrated with plasma cells and admixed small lymphocytes; marginal zone B cells may be present, with formation of lymphoepithelial lesions. The lymphoplasmacytic infiltrate separates the crypts, and villous atrophy may be present [1781, 3239, 3298]. Sheets of large plasmacytoid cells and immunoblasts that form solid, destructive aggregates with ulceration characterize progression to diffuse large B-cell lymphoma [370, 4230].

**Immunophenotype**
The plasma cells and marginal zone cells express monoclonal cytoplasmic alpha chain without light chains. Marginal zone cells express CD20 and are negative for CD5 and CD10; plasma cells are typically CD20-negative and CD138-positive [1781].

**Postulated normal counterpart**
A post-germinal centre B cell that can differentiate into a plasma cell, with an abnormal IGHA gene

**Genetic profile**
Immunoglobulin heavy and light chain genes are clonally rearranged [3710]. Deletions in the IGHA gene are present that result in expression of a defective heavy chain protein that cannot bind light chain to form a complete immunoglobulin molecule. These deletions involve IGHV and the CH1 domain, and there may be insertions of DNA of unknown origin [48, 4230]. Cytogenetic abnormalities have been reported in rare single cases. The t(11;18)(q21;q21) (BIRC3/MALT1) translocation associated with gastric and pulmonary MALT lymphomas has not been described [4421].

**Prognosis and predictive factors**
In the early phase, alpha HCD may completely remit with antibiotic therapy. In patients with more advanced disease, multiagent chemotherapy is typically required. Treatment with anthracycline-containing regimens has been reported to result in remission and long-term survival in 67% of patients [48, 3295, 4230].
Plasma cell neoplasms

Plasma cell neoplasms result from expansion of a clone of immunoglobulin-secreting, heavy chain class-switched, terminally differentiated B cells that typically secrete a single homogeneous monoclonal immunoglobulin called an M protein; the presence of such a protein is called monoclonal gammopathy. The plasma cell neoplasms discussed in this section include plasma cell myeloma, plasmacytoma, disorders defined by tissue immunoglobulin deposition (primary amyloidosis and light and heavy chain deposition diseases), and clonal plasma cell proliferations with an associated paraneoplastic syndrome (POEMS syndrome and TEMPI syndrome) (see Table 13.04). Non-IgM monoclonal gammapathy of undetermined significance, a precursor lesion with the potential to evolve to a malignant plasma cell neoplasm, is also included. Other immunoglobulin-secreting disorders that consist of both clonal lymphocytes and plasma cells, including lymphoplasmacytic lymphoma, the heavy chain diseases, and IgM monoclonal gammapathy of undetermined significance, are discussed in other sections.

### Non-IgM monoclonal gammapathy of undetermined significance

#### Definition
There are two major types of monoclonal gammapathy of undetermined significance (MGUS): plasma cell and lymphoid/lymphoplasmacytic, which have different genetic bases and different outcomes in terms of malignant progression. Plasma cell MGUS and lymphoid/lymphoplasmacytic MGUS can usually be distinguished by morphology, but this analysis is not always precise. Instead, MGUS is classified as IgM MGUS, which is mostly lymphoid/lymphoplasmacytic, or non-IgM MGUS, which is mostly plasma cell, although about 1% of plasma cell MGUS cases actually produce an IgM M protein. Features of IgM MGUS are detailed in the section on lymphoplasmacytic lymphoma and will not be discussed further in this section. Non-IgM (plasma cell) MGUS is defined as the presence in the serum of an IgG, IgA, or (rarely) IgD M protein at a concentration <30 g/L; clonal bone marrow plasma cells <10%; and absence of end-organ damage such as hypercalcaemia, renal insufficiency, anaemia, and bone lesions (CRAB) and amyloidosis attributable to the plasma cell proliferative disorder (Table 13.05) [3290]. The risk of progression to plasma cell myeloma, light-chain amyloidosis, or a related disorder is 1% per year. Light-chain MGUS consists only of monoclonal light chains. It is defined by an abnormal free light chain ratio and an increase of involved light chain with complete loss of heavy chain expression. The urinary light chain excretion must be <0.5 g/24 hour. The plasma cell content is <10%, and there is no end-organ damage attributable to the plasma cell disorder [1004,2167]. The rate of progression of light-chain MGUS is approximately 0.3% per year (Table 13.06). Although the M protein reflects the presence of an expanded clone of immunoglobulin-secreting plasma cells, non-IgM MGUS is considered a premalignant neoplasm, which in most cases does not progress to overt malignancy.

#### ICD-O code
9765/1

### Table 13.04 Plasma cell neoplasms

<table>
<thead>
<tr>
<th>Clinical variants</th>
<th>Non-IgM (plasma cell) monoclonal gammapathy of undetermined significance (precursor lesion)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smouldering (asymptomatic) plasma cell myeloma</td>
<td></td>
</tr>
<tr>
<td>Non-secretory myeloma</td>
<td></td>
</tr>
<tr>
<td>Plasma cell leukaemia</td>
<td></td>
</tr>
<tr>
<td>Solitary plasmacytoma of bone</td>
<td></td>
</tr>
<tr>
<td>Extraosseous (extramedullary) plasmacytoma</td>
<td></td>
</tr>
<tr>
<td>Primary amyloidosis</td>
<td></td>
</tr>
<tr>
<td>Systemic light and heavy chain deposition diseases</td>
<td></td>
</tr>
<tr>
<td>POEMS syndrome</td>
<td></td>
</tr>
<tr>
<td>TEMPI syndrome (provisional)</td>
<td></td>
</tr>
</tbody>
</table>

#### Synonym
Monoclonal gammapathy, NOS

#### Epidemiology
MGUS is uncommon in patients aged <40 years but is found in approximately 3-4% of individuals aged >50 years and in >5% of individuals aged >70 years; 80-85% of cases are non-IgM MGUS [2175, 2176]. Approximately 60% of patients with non-IgM MGUS are men, and it is nearly twice as frequent in Black populations as in White populations [2171, 2210,2211,3685].

#### Etiology
No cause of MGUS has been identified, but there is an increased prevalence in families with members with a lymphoid or plasma cell proliferative disorder [1441, 2212]. Transient oligoclonal and monoclonal gammopathies may occur in solid organ and bone marrow / stem cell transplant recipients [2171,2679,3330].

#### Localization
The clonal plasma cells producing non-IgM MGUS are in the bone marrow.

#### Clinical features
Patients exhibit no symptoms or physical findings related to non-IgM MGUS, and the typical laboratory and radiographical abnormalities associated with plasma cell myeloma are lacking. The M protein is often identified in the course...
Table 13.05 Diagnostic criteria for non-IgM monoclonal gammopathy of undetermined significance (MGUS) and light-chain MGUS. Adapted from the International Myeloma Working Group (IMWG) updated criteria for the diagnosis of multiple myeloma. From: Rajkumar SV et al. (3290).

<table>
<thead>
<tr>
<th>Non-IgM MGUS:</th>
<th></th>
<th>Light-chain MGUS:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum M protein (non-IgM) concentration &lt; 30 g/L</td>
<td>Abnormal free light chain ratio (&lt; 0.26 or &gt; 1.65)</td>
<td></td>
</tr>
<tr>
<td>Clonal bone marrow plasma cells &lt; 10%</td>
<td>Increased level of the involved free light chain</td>
<td></td>
</tr>
<tr>
<td>Absence of end-organ damage; e.g. hypercalcaemia, renal insufficiency, anaemia, and bone lesions (CRAB) and amyloidosis attributable to the plasma cell proliferative disorder</td>
<td>No immunoglobulin heavy chain expression on immunofluorescence micrography</td>
<td></td>
</tr>
<tr>
<td>Microscopy</td>
<td>Urinary M protein &lt; 500 mg/24 hours</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Clonal plasma cells &lt; 10%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Absence of end-organ damage (CRAB) and amyloidosis</td>
<td></td>
</tr>
</tbody>
</table>

Table 13.06 Diagnostic criteria for monoclonal plasma cell proliferative disorders with complete loss of immunoglobulin heavy chain (HC) expression, i.e. with light chain (LC) expression only. Adapted from Kyle RA et al. (2167)

<table>
<thead>
<tr>
<th>Feature</th>
<th>LC-MGUS</th>
<th>LC-SPCM</th>
<th>LC-PCM</th>
</tr>
</thead>
<tbody>
<tr>
<td>M protein concentration</td>
<td>Abnormal FLC ratio and increase of involved LC with complete loss of HC expression in serum; urinary LC M protein &lt; 0.5 g/24 hours</td>
<td>Urinary LC M protein ≥ 0.5 g/24 hours</td>
<td>Presence of LC-only M protein (usually in urine but can sometimes be seen in serum)</td>
</tr>
<tr>
<td>Percentage of plasma cells in bone marrow</td>
<td>&lt; 10%</td>
<td>≥ 10%</td>
<td>≥ 10% or biopsy-proven plasmacytoma</td>
</tr>
<tr>
<td>End-organ damage attributable to the plasma cell disorder</td>
<td>Absent</td>
<td>Absent</td>
<td>Present</td>
</tr>
<tr>
<td>Annual rate of progression</td>
<td>0.3%</td>
<td>First 5 years: 5%; Next 5 years: 3%; Following 5 years: 2%</td>
<td>n/a</td>
</tr>
</tbody>
</table>

FLC, free light chain; LC-MGUS, light-chain monoclonal gammopathy of undetermined significance; LC-PCM, light-chain plasma cell myeloma; LC-SPCM, light-chain smouldering plasma cell myeloma.

For the diagnosis of LC-MGUS, both M protein and bone marrow plasma cell percentage criteria must be fulfilled; in contrast, the diagnosis of LC-SPCM requires only that at least one of these two criteria is fulfilled.

of evaluation for another disorder, but there is no specific association with any particular disease [2171]. The M proteins are usually discovered unexpectedly on serum protein electrophoresis. Approximately 60% are IgG, 15% IgA, 1% IgD, 1% IgE, and 3% biclonal (2171). About 20% of non-IgM MGUS consists only of an immunoglobulin light chain, which can be detected with the serum free light chain assay [1004,1969,2476,3290]. Reduction of uninvolved immunoglobulin is found in 30–40% of patients with non-IgM MGUS, and monoclonal light chain in urine in nearly one third [2171,2176,3330].

Microscopy
Marrow aspirates contain a median of 3% plasma cells, and trephine biopsies show no or only a minimal increase in plasma cells, which are interstitial and evenly scattered throughout the bone marrow or occasionally in small clusters [1762,3330]. They are usually mature-appearing, but mild changes, including cytoplasmic inclusions and nucleioli, are occasionally observed.

Immunophenotype
Immunohistochemical staining for CD138 facilitates enumeration of plasma cells on bone marrow biopsy sections. The detection of plasma cells that express monoclonal cytoplasmic light chain of the same isotype as the M protein is often problematic, because the clone may be small and may occur in a background of normal polyclonal plasma cells. In some non-IgM MGUSs, the kappa/lamba ratio is within the normal reference range; in others, it is skewed (but less so than in myeloma) [2450,3147]. Flow cytometry frequently shows two populations of plasma cells: a polyclonal population with a normal immunophenotype (CD38bright, CD19+, CD56–) and a monoclonal population with an aberrant phenotype (most often either CD19–/CD56+ or CD19–/CD56–) [2974,3138]. The monoclonal population may exhibit weaker expression of CD38 than normal, along with other aberrant antigen expression [2926,2974,3138]. Residual normal polyclonal bone marrow plasma cells are a consistent finding by flow cytometry in non-IgM MGUS, whereas they are absent or present in only very low numbers in myeloma [2926,2974].

Cell of origin
Non-IgM (plasma cell) MGUS is produced by post-germinal centre plasma cells with IG genes that have somatic hypermutation of the variable regions and are class-switched.

Genetic profile
Abnormal karyotypes are rarely found in non-IgM MGUS, but FISH identifies numerical and/or structural abnormalities in most cases [202,728,1230,1231]. The abnormalities are the same as those found in myeloma, although the prevalence may differ. Translocations involving the IGH locus (14q32) are found in nearly half of non-IgM MGUs, with various studies showing t(11;14)(q13;q32) (IGH/CCND1) in 15–25%, t(4;14)(p16.3;q32) (IGH/NSD2, also called IGH/MMSET) in 2–9%, and t(14;16)(q32;q23) (IGH/MAF) in 1–5% [202,1230]. Hyperdiploidy is observed in about 40% of non-IgM MGUs, with chromosomal trisomies similar to those in myeloma [728]. Deletions of 13q are present in about 35–40% of non-IgM MGUSs, versus about 50% of myelomas [1230,1231,1970,2179]. Activating NRAS mutations are much less frequent in MGUS (present in ~7%) compared with myeloma (~15–20%), whereas activating KRAS mutations have not been detected in MGUS but are present in about 15–20% of myelomas [2128,3312]. No obvious clinical correlations are associated with chromosome abnormalities, but this may reflect a lack of sufficient data [1230]. Although genetic alterations and gene expression patterns can probably distinguish advanced myeloma from
MGUS, there are no unequivocal intrinsic differences that distinguish the two.

Prognosis and predictive factors
In most cases, the clinical course of MGUS is stable, with no increase in M protein or other evidence of progression, but in a substantial minority, there is evolution to an active plasma cell myeloma, solitary plasmacytoma, or amyloidosis [1762, 2171]. The risk of progression is about 1% per year (0.3% for light-chain MGUS) and indefinite, persisting even after 30 years [2171,2176]. The size and type of M protein and serum free light chain ratio are significant prognostic indicators [1762, 2172,2176,3080,4078]. The risk of progression for patients with an M protein concentration of 25 g/L is >4 times that of patients with a concentration <5 g/L. Patients with IgA MGUS are at greater risk of progression (≈1.5% per year) to a malignant disorder than are patients with IgG or light-chain MGUS [2171]. Patients with IgG or IgA MGUS with an abnormal serum free light chain ratio at diagnosis are at higher risk of progression than are patients with a normal ratio [2171,3292]. Individuals with marked predominance of aberrant plasma cells (>90%) on flow cytometry have a significantly higher risk of progression to myeloma [2974,3138]. DNA aneuploidy and subnormal levels of polyclonal immunoglobulin appear to be additional clinical risk factors [3138]. An evolving clinical phenotype also predicts an increased probability of progression to myeloma [3137]. Several risk stratification models identify subgroups of cases of non-IgM MGUS that progress to myeloma at rates ranging from approximately 0.3% to 12% per year [2167,3137,3138, 3292,4496].

Plasma cell myeloma

Definition
Plasma cell myeloma (PCM) is a bone marrow-based, multifocal neoplastic proliferation of plasma cells, usually associated with an M protein in serum and/or urine and evidence of organ damage related to the plasma cell neoplasm. Bone marrow is the site of origin of nearly all PCMs, and in most cases there is disseminated bone marrow involvement. Other organs may be secondarily involved. The disease spans a clinical spectrum from asymptomatic to highly aggressive. Diagnosis is based on a combination of clinical, morphological, immunological, and radiological features. The diagnostic criteria for PCM are listed in Table 13.07.

| Synonyms |
| Plasma cell myeloma; medullary plasmacytoma; myelomatosis; Kahler disease (no longer used); myeloma, NOS |

Epidemiology
PCM accounts for about 1% of malignant tumours, 10–15% of haematopoietic neoplasms, and 20% of deaths from haematological malignancies [1851, 2164,3357]. In the USA in 2015, an estimated 26 000 cases were diagnosed and >11 000 patients died of PCM [3673]. PCM is more common in men than in women, with a male-to-female ratio of 1.1:1. It is nearly twice as frequent in Black populations as in White populations [2164,3357,3673]. PCM is almost never found in children and very infrequently in adults aged <30 years [840, 390]; the incidence increases progressively with patient age thereafter, with about 90% of cases occurring in patients aged >50 years (median patient age at diagnosis: ~70 years).

Etiology
Chronic antigenic stimulation from infection or other chronic disease and exposure to specific toxic substances or radiation has been associated with an increased incidence of PCM [2292,2353]. An antigenic stimulus giving rise to multiple benign clones could be followed by a mutagenic event initiating malignant transformation [1522]. Most patients have

Table 13.07 Diagnostic criteria for plasma cell myeloma (PCM) and smouldering (asymptomatic) PCM. Adapted from the International Myeloma Working Group (IMWG) updated criteria for the diagnosis of multiple myeloma [3290]

| Criteria |
| Clonal bone marrow plasma cell percentage ≥10% or biopsy-proven plasmacytoma and ≥1 of the following myeloma-defining events: |
| End-organ damage attributable to the plasma cell proliferative disorder: |
| - Hypercalcaemia: serum calcium >0.25 mmol/L (>1 mg/dL) higher than the upper limit of normal or >2.75 mmol/L (>11 mg/dL) |
| - Renal insufficiency: creatinine clearance <40 mL/minute or serum creatinine >177 µmol/L (>2 mg/dL) |
| - Anaemia: a haemoglobin value of >20 g/L below the lower limit of normal or a haemoglobin value <100 g/L |
| - Bone lesions: ≥1 osteolytic lesion on skeletal radiography, CT, or PET/CT |
| Smouldering (asymptomatic) PCM |
| Both criteria must be met: |
| - Serum M protein (IgG or IgA) ≥30 g/L or urinary M protein ≥500 mg/24 hours and/or clonal bone marrow plasma cell percentage of 10–60% |
| - Absence of myeloma-defining events or amyloidosis |

A image of a skull and femoral head (B) demonstrate multiple lytic bone lesions.
no identifiable toxic exposure or known chronic antigenic stimulation [2353]. Almost all PCMs arise in patients with a precursor monoclonal gammopathy of undetermined significance (MGUS) [2213,4284].

**Localization**
Generalized or multifocal bone marrow involvement is typically present. Lytic bone lesions and focal tumoural masses of plasma cells also occur, most commonly in sites of active haematopoiesis. Extramedullary involvement is usually a manifestation of advanced disease.

**Clinical features**
In most patients, there is clinical evidence of PCM-related end-organ damage in the form of one or more of the following: hypercalcaemia, renal insufficiency, anaemia, and bone lesions (CRAB). Renal failure occurs due to tubular damage resulting from monoclonal light chain proteinuria, and anaemia results from bone marrow replacement and renal damage. Bone pain and hypercalcaemia result from PCM-induced lytic lesions and osteoporosis [2164]. Other presenting findings may include infections (partly a consequence of depressed normal immunoglobulin [Ig] production), bleeding, and occasionally neurological manifestations due to spinal cord compression or peripheral neuropathy [1762,2164]. The serum M protein is usually >30 g/L of IgG and >20 g/L of IgA. In 90% of patients, there is a decrease in polyclonal Ig (<50% of normal). Other laboratory findings include hypercalcaemia (found in up to 10% of cases), elevated creatinine (in 20–30%), hyperuricaemia (in >50%), and hypoalbuminaemia (in ~15%) [1456,2164].

**Imaging**
Bone lesions are found on radiographical skeletal survey in about 70% of cases of PCM, and even more frequently by MRI and PET/CT [988,2164,3348,4455]. Lytic lesions are most common (accounting for ~70% of the bone lesions found), but abnormalities also include osteoporosis (accounting for 10–15% of bone lesions), pathological fractures, and vertebral compression fractures. The most frequent sites of lesions, in decreasing order, are the vertebrae, ribs, skull, shoulders, pelvis, and long bones [4455].

**Macroscopy**
The bone defects apparent on gross examination are filled with soft, gelatinous, fish-flesh haemorrhagic tissue.

**Microscopy**

**Bone marrow biopsy**
Monoclonal plasma cells may be scattered interstitially, in small clusters, in focal nodules, or in diffuse sheets [281,480,3330]. There is often bone marrow sparing and preservation of normal haematopoiesis, with interstitial and focal patterns of involvement. With diffuse involvement, expansive areas of the bone marrow are replaced and haematopoiesis may be markedly decreased. There is typically progression from interstitial and focal disease in early PCM to diffuse involvement in advanced stages of disease [281]. Generally, when 30% of the bone marrow volume is composed of plasma cells, a diagnosis of myeloma is likely, although rare cases of reactive plasma cytosis can reach that level. A tumoural

---

![Fig. 13.39 Plasma cell myeloma. A Gross photograph of the vertebral column, showing multiple lytic lesions filled with grey, fleshy tumour. B Vertebral column after maceration, showing multiple lytic lesions.](image1)

![Fig. 13.40 Plasma cell myeloma. Low-magnification (A) and high-magnification (B) views of a bone marrow biopsy. There is extensive marrow replacement with neoplastic plasma cells. The pattern of involvement is mixed, interstitial, and focal. The plasma cells exhibit mature features, with abundant cytoplasm and eccentric nuclei with coarse chromatin; most lack visible nucleoli.](image2)
mass of plasma cells displacing normal bone marrow elements strongly favours a diagnosis of PCM, even if the volume of bone marrow replaced is <30%. Prominent osteoclastic activity is observed in some cases.

Immunohistochemistry is useful in quantifying plasma cells on biopsies, in confirming a monoclonal proliferation, and in distinguishing PCM from other neoplasms. CD138 staining is useful for quantifying plasma cells, and clonality can usually be established with staining for Ig kappa and lambda light chains (1762,3147). Staining for commonly expressed aberrant antigens such as CD56 and KIT (CD117) may be used to detect populations of neoplastic plasma cells. The small-cell or lymphoplasmacytic variant of PCM may be confused with small B-cell lymphoma or mantle cell lymphoma, especially given that these cases frequently show strong CD20 expression and/or strong cyclin D1 expression, due to the common presence of a t(11;14)(q13;q32) (IGH/CCND1) translocation (3242,3744).

Bone marrow aspiration

The proportion of plasma cells on aspirate smears varies from barely increased to >90% (2164). Myeloma plasma cells vary from mature forms indistinguishable from normal cells to immature, plasmablastic, and pleomorphic cells (281,480,1454). Mature plasma cells are usually oval, with a round eccentric nucleus and so-called spoke-wheel or clock-face chromatin without nucleoli. There is generally abundant basophilic cytoplasm and a perinuclear hof. The small-cell variant shows a lymphoplasmacytic appearance, with a narrow rim of basophilic cytoplasm and the occasional perinuclear hof. In contrast, immature forms have more-dispersed nuclear chromatin, a higher N:C ratio, and (often) prominent nucleoli. In almost 10% of cases, there is plasmablastic morphology (1454). Multinucleated, multilobed, pleomorphic plasma cells are prominent in some cases (281,480). Because nuclear immaturity and pleomorphism rarely occur in reactive plasma cells, they are reliable indicators of neoplastic plasma cells. The cytoplasm of myeloma cells has abundant endoplasmic reticulum, which may contain condensed or crystallized cytoplasmic Ig producing a variety of morphological findings, including multiple pale bluish-white, grape-like accumulations (Mott cells and morula cells); cherry-red refractive round bodies (Russell bodies); vermilion-staining glycoprotein-rich IgA (flame cells); overstuffed fibrils (pseudo-Gaucher cells and thesaurocytes); and crystalline rods (480). These changes are not pathognomonic of PCM; they can also be found in reactive plasma cells.

In about 5% of cases of PCM, there are <10% plasma cells in the bone marrow aspirate smears (1762). This may be due to a suboptimal bone marrow aspirate or the frequent focal distribution of PCM in the bone marrow. In such instances, larger numbers of plasma cells and focal clusters are sometimes observed in the trephine biopsy sections. Biopsies directed at radiographical lesions may be necessary to establish the diagnosis in some patients.

Peripheral blood

Rouleaux formation is the most striking feature on blood smears and is related to the quantity and type of M protein. A leukoerythroblastic reaction is observed in some cases. Plasma cells are found...
on blood smears in about 15% of cases, usually in small numbers. Marked plasmacytosis accompanies plasma cell leukaemia.

Kidney
Bence Jones protein accumulates as aggregates of eosinophilic material in the lumina of the renal tubules. Renal tubular reabsorption of Bence Jones protein is largely responsible for renal damage in PCM.

Immunophenotype
Flow cytometry shows that the neoplastic plasma cells have monotypic cytoplasmic Ig and usually lack surface Ig; the cells typically express CD38 and CD138, but the CD38 expression signal tends to be dimmer and the CD138 signal brighter than in normal plasma cells (291,2926). Unlike in normal plasma cells, CD45 is negative or expressed at low levels; CD19 is negative in 95% of cases, and CD27 and CD61 are frequently negative or underexpressed (291,2348,2557,2725,2826,3033,3474,3937). Aberrant expression of antigens not found on normal plasma cells (or present only in small subsets of normal cells) is identified in nearly 90% of cases (2348). These antigens include CD56 (found in 75–80% of cases), CD200 (60–75%), CD28 (~40%), KIT (CD117; 20–40%), CD20 (10–20%), CD52 (8–14%), CD10 and myeloid and monocytic antigens (found occasionally), and stem cell–associated antigens (found rarely) (73,291,292,2348,2973,3123,3227,3268,3375,3474). Increased expression of MYC may be detected on immunohistochemistry, and cyclin D1 is expressed in cases with t(11;14)(q13;q32) (IGH/CCND1) and some cases with hyperdiploidy (795,3744,4384). There is conflicting evidence regarding the value of immunophenotype as an indicator of prognosis in PCM (2724,3124). Expression of CD19, CD28, and CD200; lack of expression of CD45 or KIT (CD117); and underexpression of CD27 have all been associated with more-aggressive disease; however, none of these markers has been proven by multivariate analysis to have independent prognostic significance (52,2557,2725,2973).

Postulated normal counterpart
The postulated normal counterparts are post-germinal centre long-lived plasma cells in which the IG genes have undergone class switch and somatic hypermutation. The cell of origin has not been established.

Genetic profile
Antigen receptor genes
IG heavy and light chain genes are clonally rearranged. There is a high load of IGHV gene somatic hypermutation without ongoing mutations, consistent with derivation from a post-germinal centre, antigen-driven B cell (237).

Cytogenetic abnormalities and oncogenes
Abnormalities are detected by karyotype in about one third of PCMs, and by FISH in >90% (200,728,1231,2180,2186,3541). Abnormalities are both numerical and structural and include...
trisomies and whole or partial chromosome deletions and translocations; complex cytogenetic abnormalities are common. A molecular cytogenetic classification of PCM proposed by the International Myeloma Working Group (IMWG) is shown in Table 13.08 [1232]. The genenic categories are major indicators of prognosis and form the basis for risk stratification of PCM (see Prognosis and predictive factors). The IMWG consensus recommendations on genetic testing are listed in Table 13.09 [1232].

The most frequent chromosome translocations involve IGH on chromosome 14q32 and are present in 55–70% of PCMs [202,1231]. Seven recurrent oncogenes are involved in 14q32 translocations: CCND1 on 11q13 (involved in 16% of cases), MAF on 16q23 (in 5%), FGFR3/NSD2 (also called FGFR3/MMSET) on 4p16.3 (in 15%), CCND3 on 6p21 (in 2%), MAFA on 20q11 (in 2%), MAFA on 8q24 (in <1%), and CDND2 on 12p13 (in <1%) (see Table 13.08 and Table 13.10 [200,1232,2128]). Together these seven translocations are found in about 40% of cases of PCM, most of which are non-hyperdiploid (i.e. with <48 or >75 chromosomes). The remaining PCMs are mostly hyperdiploid (usually with gains in three or more of the odd-numbered chromosomes 3, 5, 7, 9, 11, 15, 19, and 21) and only infrequently have one of the seven recurrent IGH translocations listed above (728,729,1231).

IGH translocations and hyperdiploidy appear to be early events in the genesis of plasma cell neoplasms, unified by associated upregulation of one of the cyclin D genes (CCND1, CCND2, or CCND3) [351,2128]. Gene expression profiling can determine the expression levels of CCND1, CCND2, and CCND3 RNA and identify tumours that overexpress oncoproteins dysregulated by the seven recurrent IGH translocations. On the basis of patterns of translocations and cyclin D expression (TC groups), non-IgM MGUS and PCM can be classified into groups that are based mostly on early pathogenic events (Table 13.10). Some or all of these groups may represent distinct disease entities that require different therapeutic strategies [2128,3798]. Two other molecular classifications based on unsupervised clustering of tumours by gene expression profiles are similar to the TC groups [473,4474]. However, they are not generally applicable for non-IgM MGUS, because some of the groups are based on progression events not found in MGUS (e.g. proliferation) [2128].

Monosomy or partial deletion of chromosome 13 (13q14) is found by FISH in nearly half of PCMs. It is sometimes an early event (present in about 35% of non-IgM MGUSs) but can also be a progression event, particularly in PCM with t(11;14) [711,1231]. MYC (and rarely MYCN) locus rearrangements are present in nearly half of PCM tumours. These repositioning MYC near a promiscuous array of plasma cell–specific super-enhancers (including IGH, IGK, and IGL super-enhancers). The MYC rearrangements may sometimes contribute to the progression from non-IgM MGUS to PCM, but can also occur at later stages of PCM progression [26]. Mutually exclusive activating mutations of KRAS, NRAS, or BRAF are present in about 40% of PCMs and are candidates for mediating the transition of non-IgM MGUS to myeloma in some patients [2363,3312,4496].

Other recurrent genetic changes associated with disease progression include secondary IGH or IGL translocations; deletion and/or mutation of TP53 (17p13); gains of chromosome 1q and loss of 1p; mutations of genes that result in activation of the NF-kappaB pathway; mutations of FGFR3 in tumours with t(4;14); and inactivation of CDKN2C, RB1, FAM46C, and DIS3 [109,1232,2128,2383]. Epigenetic changes manifested by DNA methylation are also associated with tumour progression.

Myeloma tumour cells from individual patients often have heterogeneous somatic genetic abnormalities reflecting the presence of multiple subclones. Tumour subclones can have activating mutations of NRAS, KRAS, or BRAF; although each subclone can have only one of these mutations. This has important therapeutic implications, because a specific therapeutic regimen may target one or more specific subclones but have little or no effect on other subclones [179,2383]. Although genetic events appear to play the key role in initiation and progression.

<table>
<thead>
<tr>
<th>Genetic category</th>
<th>Proportion of cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hyperdiploid</td>
<td>45%</td>
</tr>
<tr>
<td>Cyclin D translocation</td>
<td>18%</td>
</tr>
<tr>
<td>t(11;14)(q13;q32)</td>
<td>16%</td>
</tr>
<tr>
<td>t(8;14)(p25;q32)</td>
<td>2%</td>
</tr>
<tr>
<td>t(12;14)(p13;q32)</td>
<td>&lt;1%</td>
</tr>
<tr>
<td>NSD2 (also called MMSET) translocation</td>
<td>15%</td>
</tr>
<tr>
<td>t(4;14)(p16;q32)</td>
<td>15%</td>
</tr>
<tr>
<td>MAF translocation</td>
<td>8%</td>
</tr>
<tr>
<td>t(14;16)(q32p23)</td>
<td>5%</td>
</tr>
<tr>
<td>t(14;20)(q32q11)</td>
<td>2%</td>
</tr>
<tr>
<td>t(6;14)(q24;q32)</td>
<td>1%</td>
</tr>
<tr>
<td>Unclassified (other)</td>
<td>15%</td>
</tr>
</tbody>
</table>

Table 13.08 The International Myeloma Working Group (IMWG) molecular cytogenetic classification of plasma cell myeloma. Adapted from Fonseca R, et al. (1232)

<table>
<thead>
<tr>
<th>Minimal panel:</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>t(4;14)(p16;q32)</td>
<td></td>
</tr>
<tr>
<td>t(14;16)(q32;p23)</td>
<td></td>
</tr>
<tr>
<td>del(17p13)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>More comprehensive panel:</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>t(11;14)(q13;q32)</td>
<td></td>
</tr>
<tr>
<td>del 13, ploidy category, chromosome 1 abnormalities</td>
<td></td>
</tr>
</tbody>
</table>

Table 13.09 The International Myeloma Working Group (IMWG) consensus recommendations on genetic testing. Adapted from Fonseca R, et al. (1232)

Fig. 13.46 Plasma cell myeloma. Morphological variants based on cytoplasmic features. A So-called Mott cell with abundant grape-like cytoplasmic inclusions of immunoglobulin. B Numerous Russell bodies.
of PCM, the bone marrow microenvironment is also important in pathogenesis and progression [97]. Extracellular matrix proteins, secreted cytokines and growth factors (including IL6), and/or the functional consequences of direct interaction of the bone marrow stromal cells with neoplastic plasma cells are major constituents that influence the pathophysiology of PCM [2677].

Table 13.10 Molecular classifications of plasma cell myeloma, modified from Kuehl WM and Bergsagel PL (2128)

<table>
<thead>
<tr>
<th>Group</th>
<th>TC</th>
<th>Gene</th>
<th>Ploidy</th>
<th>%</th>
<th>UAMS</th>
<th>HOVON-GMMMG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyclin D TLC</td>
<td>1q13</td>
<td>CCND1</td>
<td>N</td>
<td>15</td>
<td>CD1, CD2</td>
<td>CD1, CD2</td>
</tr>
<tr>
<td></td>
<td>6p21</td>
<td>CCND3</td>
<td>N</td>
<td>2</td>
<td>CD1, CD2</td>
<td>CD1, CD2</td>
</tr>
<tr>
<td></td>
<td>12p13</td>
<td>CCND2</td>
<td>N</td>
<td>&lt;1</td>
<td>CD1, CD2</td>
<td>CD1, CD2</td>
</tr>
<tr>
<td>NSD2 TLC</td>
<td>4p16</td>
<td>NSD2, FGFR3 (CCND2)</td>
<td>N &gt; H</td>
<td>15</td>
<td>MS</td>
<td>MS</td>
</tr>
<tr>
<td>MAF TLC</td>
<td>16q23</td>
<td>MAF (CCND2)</td>
<td>N</td>
<td>5</td>
<td>MF</td>
<td>MF</td>
</tr>
<tr>
<td></td>
<td>20q12</td>
<td>MAFB (CCND2)</td>
<td>N</td>
<td>2</td>
<td>MF</td>
<td>MF</td>
</tr>
<tr>
<td></td>
<td>8q24</td>
<td>MAF (CCND2)</td>
<td>N</td>
<td>&lt;1</td>
<td>MF</td>
<td>MF</td>
</tr>
<tr>
<td>No primary TLC</td>
<td>D1</td>
<td>CCND1</td>
<td>H</td>
<td>33</td>
<td>HY</td>
<td>Y, CD1, NFKB, CTA, PRL3</td>
</tr>
<tr>
<td>D1 + D2</td>
<td>CCND1, CCND2</td>
<td>H</td>
<td>7</td>
<td>PR</td>
<td>PR, CTA</td>
<td></td>
</tr>
<tr>
<td>D2</td>
<td>CCND2</td>
<td>H, NH</td>
<td>18</td>
<td>PR, LB</td>
<td>LB, CTA, PRL3</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>No cyclin D genes</td>
<td>N</td>
<td>2</td>
<td>PR</td>
<td>PR, CTA</td>
<td></td>
</tr>
</tbody>
</table>

H, mostly hyperdiploid; HOVON-GMMMG, Dutch-Belgian Cooperative Trial Group for Hematology-Oncology and German Multiple Myeloma Group classification; N, mostly non-hyperdiploid; PR, proliferation; TC, translocations and cyclin D classification; TLC, IGH translocation; UAMS, University of Arkansas for Medical Science classification.

Table 13.11 International Staging System (ISS) for plasma cell myeloma. From Greipp PR, et al. (1456)

<table>
<thead>
<tr>
<th>Stage</th>
<th>Criteria</th>
<th>Median survival (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Serum beta-2 microglobulin &lt; 3.5 g/dL</td>
<td>62</td>
</tr>
<tr>
<td></td>
<td>Serum albumin ≥ 3.5 g/dL</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>Not stage I or II^a</td>
<td>44</td>
</tr>
<tr>
<td>III</td>
<td>Serum beta-2 microglobulin ≥ 5.5 mg/L</td>
<td>29</td>
</tr>
</tbody>
</table>

^a There are two categories for stage II:
(1) serum beta-2 microglobulin < 3.5 mg/dL but serum albumin < 3.5 g/dL and
(2) serum beta-2 microglobulin of 3.5 to < 5.5 mg/L, irrespective of the serum albumin level.

Table 13.12 Mayo Stratification of Myeloma and Risk-Adapted Therapy. Adapted from Chesi M and Bergsagel PL (690)

<table>
<thead>
<tr>
<th>Standard risk (60%)</th>
<th>Intermediate risk (20%)</th>
<th>High risk (20%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>t(11;14)</td>
<td>t(4;14)</td>
<td>Del 1p</td>
</tr>
<tr>
<td>t(6;14)</td>
<td>Del 13</td>
<td>t(14;16)</td>
</tr>
<tr>
<td>Hyperdiploid</td>
<td>Hypodiploid</td>
<td>t(14;20)</td>
</tr>
<tr>
<td>All others</td>
<td>GEP high-risk signature</td>
<td></td>
</tr>
<tr>
<td>OS: 8–10 years</td>
<td>OS: 4–5 years</td>
<td>OS: 3 years</td>
</tr>
</tbody>
</table>

GEP, gene expression profiling; OS, overall survival.

Genetic susceptibility
The risk of PCM in individuals with a first-degree relative with PCM or MGUS is 3.7 times that in the general population (468,1441).

Prognosis and predictive factors
For most patients, PCM is an incurable progressive disease, but newer therapeutic approaches have significantly improved quality of life and survival [2723]. Survival ranges from <6 months to >10 years (median: ~5.5 years) [666]. Patients aged >70 years and those with significant comorbidities and poor performance status have a less favourable prognosis. The International Staging System (ISS) for PCM, based on pretreatment serum beta-2 microglobulin and albumin levels, provides a strong predictor of survival (Table 13.11) [1456]. Treatment response as measured by minimal residual disease detection by flow cytometry is a significant predictor of both progressions-free and overall survival, especially following autologous stem cell transplantation [3035,3036]. Genetics is a powerful predictor of prognosis and has been combined with the ISS (R-ISS) to provide improved risk stratification [3041]. Genetic risk stratification based primarily on FISH analysis stratifies cases into standard-risk, intermediate-risk, and high-risk categories [690,1232,2661]. The most important genetic indicators of high-risk myeloma are deletion of 17p/TP53 sequences and the MAF translocations t(14;16) and t(14;20) (Table 13.12). Several high-risk molecular signatures, based on gene expression profiling using various panels of genes, serve as prognostic indicators for PCM. Increased expression of genes associated with proliferation is an important component of these prognostic signatures. The UAMS-70 and related UAMS-17 prognostic scores [3639] and the EMC-92-gene signature [2129] appear to be the most robust prognostic gene signature models when applied to cohorts of PCM from various institutions. Other reported indicators of less favourable risk include reduced polyclonal (uninvolved) serum Igs, elevated lactate dehydrogenase, high C-reactive protein, increased plasma cell proliferative activity, a high degree of bone marrow replacement, plasmablastic morphology, and elevated serum soluble receptor for IL6 [281,289, 290,1454,1455,2164,3291].
**Plasma cell myeloma variants**

**Smouldering (asymptomatic) plasma cell myeloma**

Patients with smouldering plasma cell myeloma (SPCM) have 10–60% clonal plasma cells in their bone marrow and/or an M protein at myeloma levels but absence of myeloma-defining events (i.e., hypercalcaemia, renal insufficiency, anaemia, and bone lesions; CRAB) and amyloidosis (see Table 13.06, p. 242, and Table 13.07, p. 243) [2165,2174,3290]. Approximately 8–14% of patients with plasma cell myeloma (PCM) are initially diagnosed with SPCM [2119,2174]. The disorder is similar to monoclonal gammopathy of undetermined significance in its lack of clinical manifestations, but is much more likely to progress to symptomatic PCM [992,2165,2174,2176]. Most patients have 10–20% bone marrow plasma cells, and the median level of serum M protein is nearly 30 g/L. Normal polyclonal immunoglobulins (Igs) are reduced in >83% of patients, and 53% of patients have monoclonal light chains in the urine [2164,2165]. Light chain SPCM is characterized by 10–60% bone marrow clonal plasma cells and urinary light chain M protein excretion of ≥0.5 mg/24 hours.

Some patients with SPCM have stable disease for long periods, but the cumulative probability of progression to symptomatic PCM or amyloidosis is approximately 10% per year for the first 5 years, 3% per year for the next 5 years, and 1% per year thereafter; the median time to progression is about 5 years [2174]. For light chain SPCM, the rate of progression is 5% per year for the first 5 years, 3% per year for the next 5 years, and then 2% per year for the following 5 years. Risk factors for earlier progression to symptomatic PCM include the presence of both >10% bone marrow plasma cells and >30 g/L M protein, detection of bone lesions by MRI, a high percentage of bone marrow plasma cells with an aberrant immunophenotype, an abnormal serum free light chain ratio, a high-risk gene expression profile, a high plasma cell proliferation rate, and circulating plasma cells [969,1005,1007,2174,2558]. Therapeutic intervention for the highest-risk patients has been shown to delay progression to symptomatic PCM and to improve overall survival [1007,2558,3293].

---

**Fig. 13.47** Plasma cell myeloma. Flow cytometry histograms of bone marrow. The neoplastic plasma cells are indicated in red and normal B lymphocytes in blue. The myeloma cells express bright CD38 and are negative for CD20, CD19, and CD10. They express CD56 and partial CD45, are negative for surface light chains, and express cytoplasmic kappa.

**Fig. 13.48** Plasma cell myeloma. Interphase FISH analyses of recurrent abnormalities. A Fusion signals for t(4;14) (p16;q32). Probes for IGH are green and probes for FGFR3/NSD2 (also called FGFR3/MMSET) are red. Two fusion signals (indicated by arrowheads) most likely identify der(4) and der(14), but could represent two copies of der(4). B Extra copies of three chromosomes in a hyperdiploid tumour. Three copies of chromosome 5 (LSI D5S23/D5S721, green) and chromosome 9 (CEP 9, aqua, circled) and four copies of chromosome 15 (CEP 15, red). C Two copies of chromosome 17 (CEP 17, green) and deletion of one copy of TP53 (red). D Loss of one copy of chromosome 13/13q. LSI 13 containing RB1 (green), D13S19 (red). In all four panels, the cytoplasm is blue due to immunostaining of Ig-kappa or Ig-lambda expressed by the tumour plasma cells, and the probes are from Vysis. Courtesy of Dr R. Fonseca.
with 53 chromosomes, including 4 rearranged chromosomes involving >2 different chromosomes; trisomies of chromosomes 3, 9, 11, and 19; and tetrasomies of chromosomes 15 and 21. From FISH analyses (not shown) confirm the presence of a t(2;14)(p23;q32) translocation involving NMYC (also called NMyc). A higher incidence of high-risk genetic findings (201,1185,1862,4000). The t(11;14) translocation, typically associated with a more favourable prognosis in PCM, is also overrepresented in primary PCL (201,4000). Patients with PCL have aggressive disease, poor response to therapy, and a relatively short survival (201,993,1185,1300,1400,2169,2886,4000).

**Plasmacytoma**

**Definition**

Solitary plasmacytomas are single localized tumours consisting of monoclonal plasma cells with no clinical features of plasma cell myeloma (PCM) and no physical or radiographical evidence of additional plasma cell tumours. There are two types of plasmacytoma: solitary plasmacytoma of bone and extramedullary (extramedullary) plasmacytoma.

**Solitary plasmacytoma of bone**

**Definition**

Solitary plasmacytoma of bone (SPB) is a localized tumour consisting of monoclonal plasma cells with no clinical features of PCM. Radiographical studies, including MRI and CT, show no other bone lesions (988,2324,3290,3742). Approximately 30% of patients with a solitary plasmacytoma defined only by radiographical skeletal survey have additional lesions identified on MRI or CT (988,2765). These patients are consid-
Table 13.13 Diagnostic criteria for solitary plasmacytoma. Adapted from the International Myeloma Working Group (IMWG) updated criteria for the diagnosis of multiple myeloma. From: Rajkumar SV, et al. (3290).

<table>
<thead>
<tr>
<th>Condition</th>
<th>Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solitary plasmacytoma</td>
<td>Biopsy-proven solitary lesion of bone or soft tissue consisting of clonal plasma cells. Normal random bone marrow biopsy with no evidence of clonal plasma cells. Normal skeletal survey and MRI or CT (except for the primary solitary lesion). Absence of end-organ damage, such as hypercaelema, renal insufficiency, anaemia, and bone lesions (CRAB) attributable to a plasma cell proliferative disorder.</td>
</tr>
<tr>
<td>Solitary plasmacytoma with minimal bone marrow involvement</td>
<td>Same as above plus clonal plasma cells &lt;10% in random bone marrow biopsy (usually identified by flow cytometry).</td>
</tr>
</tbody>
</table>

An M protein is found in the serum or urine in 24–72% of patients; the serum free light chain ratio is abnormal in about half (992,997,1762,3742,4315). In most cases, polyclonal immunoglobulins are at normal levels (992,3742). There is no anaemia, hypercaelema, or renal failure related to the plasmacytoma (1762).

An indolent variant of IgA-expressing, monoclonal plasma cell proliferative disorder is observed in about 10% of patients. It is characterized by an M protein for >1 year following local treatment. With masses in the upper respiratory tract, symptoms may include rhinorrhea, epistaxis, and nasal obstruction. Radiographical and morphological assessments show no evidence of bone marrow involvement. Approximately 20% of patients have a small M protein, most commonly IgA (1279,1762,3742).

**Extraosseous plasmacytoma**

Definition

Extraosseous (extramedullary) plasmacytomas are localized plasma cell neoplasms that arise in tissues other than bone (Table 13.13). Lymphomas with prominent plasmacytic differentiation, especially extranodal marginal zone lymphoma (MZL) of mucosa-associated lymphoid tissue (MALT lymphoma), must be excluded (Table 13.14).

**ICD-O code**

9734/3

**Epidemiology**

Extraosseous plasmacytomas occur most commonly in the mucous membranes of the upper air passages, but they can also occur in numerous other sites, including the gastrointestinal tract, lymph nodes, bladder, breasts, thyroid, testes, parotid glands, skin, and CNS (61,1279). Plasmacytomas of the upper respiratory tract spread to cervical lymph nodes in about 15% of cases (2631). An indolent variant of IgA-expressing, predominantly nodal plasmacytoma has been described in younger adults, with frequent signs of immune dysfunction (3634).

**Clinical features**

Symptoms are generally related to the tumor mass. With masses in the upper airway, symptoms may include rhinorrhea, epistaxis, and nasal obstruction. Radiographical and morphological assessments show no evidence of bone marrow involvement. Approximately 20% of patients have a small M protein, most commonly IgA (1279,1762,3742). In extraosseous plasmacytoma there are no clinical features of plasma cell myeloma.
Table 13.14  Differential diagnosis of neoplasms with plasmacytic (PC) or plasmablastic (PB) differentiation in extraosseous locations

<table>
<thead>
<tr>
<th>Clinical features and predisposing factors</th>
<th>Extraosseous infiltrates of plasma cell myeloma (PCM)</th>
<th>PB lymphoma</th>
<th>Primary extraosseous plasmacytoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical features</td>
<td>Usually in advanced PCM, sometimes pure extraosseous relapse after treatment</td>
<td>HIV infection and iatrogenic immunosuppression, elderly immunocompetent patients</td>
<td>No known predisposing factors, broad patient age range, rare cases in post-transplantation setting (3352)</td>
</tr>
<tr>
<td>Location</td>
<td>Any site, with or without leukaemic peripheral blood involvement</td>
<td>Predominantly extranodal, oral cavity, gastrointestinal tract, skin, and lymph nodes; 50% in immunocompetent patients</td>
<td>80% in head and neck region, mostly extranodal</td>
</tr>
<tr>
<td>Osteolytic lesions</td>
<td>Common, disseminated</td>
<td>Rare</td>
<td>Rare local infiltration (skull)</td>
</tr>
<tr>
<td>M protein</td>
<td>&gt;95%</td>
<td>Rare</td>
<td>20%, low level</td>
</tr>
<tr>
<td>Bone marrow involvement</td>
<td>Yes</td>
<td>Rare</td>
<td>No manifest involvement (by definition), 15% during disease evolution</td>
</tr>
<tr>
<td>Disease stage</td>
<td>Usually in advanced-stage PCM</td>
<td>&gt;90% either stage I or IV</td>
<td>Mostly stage IE-IIE</td>
</tr>
<tr>
<td>Morphology</td>
<td>PB/PC</td>
<td>Immunoblastic/PB, occasionally PC component</td>
<td>Usually mature PC</td>
</tr>
<tr>
<td>Immunophenotype</td>
<td>PC markers and cytoplasmic immunoglobulin light chains positive CD56+ in 70–80% (PC leukaemia usually CD56−)</td>
<td>PC markers positive Immunoglobulin light chains positive in 50% B-cell markers negative CD56+ in 10–30%</td>
<td>PC markers and cytoplasmic immunoglobulin light chains positive CD56 less common, weak Cyclin D1 negative</td>
</tr>
<tr>
<td>Molecular alterations</td>
<td>PCM cyogenetics, with 50–70% IG translocations MYC rearrangement frequent with PB morphology</td>
<td>PCM-type translocations absent 50% MYC rearrangement</td>
<td>t(11;14) translocation and MYC rearrangement absent</td>
</tr>
<tr>
<td>EBV infection</td>
<td>Absent</td>
<td>50–75%, depending on patient background</td>
<td>Rare, 50–70% in extramedullary plasmacytoma–like post-transplant lymphoproliferative disorder</td>
</tr>
<tr>
<td>Outcome</td>
<td>Poor</td>
<td>Poor</td>
<td>Good, progression to PCM in 15%</td>
</tr>
</tbody>
</table>

Fig. 13.51  A The so-called mass effect of plasma cells simulates a neoplasm.  
B,C Immunoperoxidase staining reveals polyclonal cytoplasmic immunoglobulin, with some plasma cells expressing kappa light chains (B) and some expressing lambda light chains (C).

Microscopy
The morphological features are similar to those of SPB. However, in extraosseous sites, the distinction between lymphomas that exhibit extreme plasma cell differentiation and plasmacytoma can be difficult [990]. MZL of the mucosa-associated lymphoid tissue type, lymphoplasmacytic lymphoma, and (possibly) plasmablastic lymphoma may be misdiagnosed as plasmacytoma [990,1742]. Distinction from an MZL with marked plasma cell differentiation is especially problematic, particularly in the skin, upper airway, and gastrointestinal tract, and may not be possible by morphological assessment. A search for areas of a biopsy with lymphocyte proliferation typical of MZL is fruitful in some cases. In others, a clonally related lymphocyte population may be identified by flow cytometry [1742,3609]. Distinguishing plasmacytoma from extraosseous

infiltrates of PCM is impossible by morphology, although extraosseous PCM more frequently shows cellular atypia or blastic features [2107]. The differential diagnosis of extraosseous tumours with plasmacytic or plasmablastic features is detailed in Table 13.14. Rarely, extraosseous plasmacytoma is accompanied by a local, occasionally tumour-forming amyloid deposit.

Immunophenotype
The immunophenotype appears to be similar to that of PCM, although certain differences may be helpful for diagnosis. Extraosseous plasmacytoma usually lacks cyclin D1 expression and shows less-frequent and weaker positivity for CD56 [2107]. Immunohistochemistry or in situ hybridization for immunoglobulin light chains can be useful in distinguishing a monotypic plasmacytoma from polyclonal reactive plasma cell infiltrates. Expression of CD20 by lymphocytes within the lesion or by the plasmacytoid cells, or expression of mu rather than alpha or gamma heavy chain, favours a diagnosis of lymphoma over plasmacytoma. On flow cytometry, the clonal plasma cells of lymphomas are more likely than those of PCM or plasmacytoma to express CD19 (positive in 95% vs 10%) and CD45 (in 91% vs 41%) and to lack CD56 (positive in 33% vs 71%) [3609]. In some cases, extraosseous plasmacytoma and lymphoma with extreme plasma cell differentiation cannot be immunophenotypically distinguished with certainty.

Genetic profile
The genetic features have not been extensively studied, but appear to be similar to those of PCM, with the exception of a lack of t(11;14)(q13;q32) (IGH/CCND1) translocations and aberrations of MYC [379,384,410].

Prognosis and predictive factors
In most cases, the lesions are eradicated with local radiation therapy. Regional recurrences develop in as many as 25% of patients, and occasionally there is metastasis to distant extraosseous sites. Progression to PCM is infrequent, occurring in about 15% of cases [61,662,4335]. Cases with minimal bone marrow involvement have a higher rate of progression to PCM [410,3290]. About 70% of patients remain disease free at 10 years [989].
Primary amyloidosis

Definition
Primary amyloidosis is caused by a plasma cell or (rarely) a lymphoplasmacytic neoplasm in which the monoclonal plasma cells secrete intact or fragments of abnormal immunoglobulin light chains that deposit in various tissues and form a beta-pleated sheet structure (amyloid light chain). The abnormal light chains include the N-terminal (variable) region and part of the constant region of the light chain [509]. Most light chain variable (V) region subgroups are potentially amyloidogenic, but V lambda VI is always associated with amyloidosis [509]. The amyloid tissue deposits accumulate and lead to organ dysfunction [1487]. In most cases, the diagnostic criteria for plasma cell myeloma (PCM) are lacking, but there is a moderate increase in monoclonal plasma cells in the bone marrow.

ICD-O code 9769/1

Synonyms
Immunoglobulin deposition disease; systemic light chain disease

Epidemiology
The reported annual incidence of primary amyloidosis is approximately 1 case per 100,000 population, and appears to have been stable [2163,2168]. The median patient age at diagnosis is 64 years, and >95% of patients are aged >40 years; 65-70% are male [2163,2166,2168]. Approximately 20% of patients with primary amyloidosis have PCM [1762,2163,2164,2168,3626].

Localization
Amyloid light chain accumulates in many tissues and organs, including the subcutaneous fat, kidneys, heart, liver, gastrointestinal tract, peripheral nerves, and bone marrow. The diagnostic biopsy site is typically the abdominal subcutaneous fat pad or bone marrow [2163,3626]. In most cases, the monoclonal plasma cell proliferation is in the bone marrow.

Clinical features
An M protein is detected in the serum or urine by the combination of immunofixation and serum free light chain ratio in 99% of patients [1487,1968,2163]. The median concentration of the serum M protein is

Fig. 13.54 Primary amyloidosis. Pulmonary blood vessel with amyloid deposition, showing Congo red staining (A) and apple-green birefringence in polarized light (B).
1.4 g/dL. IgG is most frequent, followed by light chain only, IgA, IgM, and IgD; the light chain is lambda in 70% of cases [1345,2163,4272]. About 20% of patients present with hypogammaglobulinaemia [2163]. Proteinuria is present in >80% of patients and nephrotic syndrome or renal failure in approximately 30%, with serum creatinine >2.0 mg/dL in 20–25% of cases [2163]. Hypercalcaemia is occasionally found, most often in patients with myeloma.

Macroscopy
On gross inspection, amyloid has a dense so-called porcelain-like or waxy appearance.

Microscopy
Bone marrow specimens vary from revealing no pathological findings to showing extensive replacement with amyloid, overt PCM, or (rarely) involvement with lymphoplasmacytic lymphoma. The most common finding is a mild increase in plasma cells, which may appear normal or may exhibit any of the changes found in myeloma. Amyloid deposits are found in the bone marrow in about 60% of cases [2163,3159,3843]. Amyloid is also present in many other tissues and organs. On H&E-stained sections, amyloid is a pink, amorphous, waxy-looking substance with a characteristic cracking artefact. Typically, it is found focally in thickened blood vessel walls, on basement membranes, and in the interstitium of tissues such as fat and bone marrow [3843]. Macrophages and foreign-body giant cells may be found around deposits. Rarely, organ parenchyma may be massively replaced by amyloid. Plasma cells may be increased in the adjacent tissues. Congo red stains amyloid pink to red by standard light microscopy, and under polarized light produces a characteristic apple-green birefringence. Congo red fluorescence microscopy may be a more sensitive method for amyloid detection [2503]. Electron microscopic studies can differentiate light-chain amyloidosis from non-amyloid immunoglobulin deposition diseases.

Immunophenotype
The immunophenotypic features of the monotypic plasma cells in primary amyloidosis are similar to those of PCM. Immunohistochemical staining for immunoglobulin kappa and lambda light chains on bone marrow sections usually shows a monoclonal plasma cell staining pattern, but if the clone is small, it may be masked by normal polyclonal plasma cells [4349,4376].

Genetic profile
The genetic abnormalities reported in primary amyloidosis are similar to those in non-IgM monoclonal gammopathy of undetermined significance and PCM. One exception is the unexplained observation that t(11;14) is present in >40% of individuals with amyloidosis but only 15–20% of those with non-IgM monoclonal gammopathy of undetermined significance or myeloma [484,1231,1594]. Other frequent chromosomal abnormalities include 13q14 deletion and gain of 1q21 [396].

Prognosis and predictive factors
In recent years, the survival of patients with primary amyloidosis (especially low-stage disease) has greatly improved, but prognosis remains poor for those with high-stage disease [2131,3502]. The major determinant of outcome is extent of cardiac involvement. Other indicators of higher risk are bone marrow monoclonal plasma cells >10%, a high serum free light chain level, and elevated beta-2 microglobulin [2131,2640]. Multiple organ involvement and an elevated serum uric acid level also have negative prognostic significance [1345]. A recently revised staging system for primary amyloidosis uses two cardiac biomarkers and the free light chain level [2131]. Four stages are defined by the elevation of 0, 1, 2, or all 3 of these parameters. Stages 1 and 2 are associated with median overall survivals of 94 and 40 months, and stages 3 and 4 with median overall survivals of only 14 and 6 months, respectively [2131]. The single most frequent cause of death (responsible for ~40% of deaths) is amyloid-related cardiac disease [2166].

Light chain and heavy chain deposition diseases
Definition
Monoclonal light chain and heavy chain deposition diseases are plasma cell or (rarely) lymphoplasmacytic neoplasms that secrete an abnormal light or (less often) heavy chain, or both, which deposit in tissues, causing organ dysfunction, but do not form amyloid beta-pleated sheets, bind Congo red stain, or contain an amyloid P component. These disorders comprise light chain deposition disease (LCDD), heavy chain deposition disease (HCDD), and light and heavy chain deposition disease (LHCDD) [190,509,510,1283,1627,1906,2537,3230,3234,3300].

Fig. 13.55 Light chain deposition disease. A Bone marrow biopsy showing patches of pale amorphous material. B Bone marrow aspirate showing numerous plasma cells. C Joint fluid aspirate showing clumps of amorphous material and plasma cells, both staining for kappa light chain by immunoperoxidase.
Fig. 13.56 Light chain deposition disease in kidney showing (A) pale amorphous patches within glomeruli (nodular glomerulosclerosis and (B) immunofluorescence stain showing renal tubular and extratubular deposition of kappa light chain in a smooth linear pattern.

ICD-O code 9769/1

Synonym
Randall disease

Epidemiology
These are rare diseases occurring at a median patient age of 58 years (range: 33–79 years); 60–65% of patients are men [509,1906,3230,3234,3542]. There is no evidence of an ethnicity effect. LCDD is the most common, and frequently occurs in association with plasma cell myeloma (PCM; in 40–65% of cases) or in patients with M protein and marrow plasma cells at monoclonal gammopathy of undetermined significance levels. Some cases are idiopathic or occur with a lymphoproliferative disorder [510,3230].

Localization
The plasma cell proliferative disorder is in the bone marrow. Deposition of aberrant immunoglobulin (Ig) may involve many organs, most commonly the kidneys [3230]. The liver, heart, peripheral nerves, blood vessels, and occasionally joints may also be involved [366,2641,3230]. Diffuse or nodular pulmonary involvement has also been reported [366,3420]. There is prominent deposition of the aberrant Ig on basement membranes, elastic fibres, and collagen fibres.

Clinical features
Patients have symptoms of organ dysfunction as a result of diffuse, systemic Ig deposits. As many as 96% of patients with LCDD present with renal manifestations [3230]. Nephrotic syndrome and renal failure are the most common features [968,3230,3542]. Symptomatic extrarenal deposition, which is uncommon in LCDD, involves the heart (in 21% of cases), liver (in 19%), and peripheral nervous system (in 8%) [366,2641,3230]. HCDD of the IgG3 or IgG1 isotype results in hypocomplementaemia, because the IgG3 and IgG1 subclasses most readily fix complement [1627,1906,2340]. There is a detectable M protein in about 85% of cases. Kappa deposition is found in at least two thirds of cases of LCDD. Gamma deposits are most common in HCDD, but alpha deposition has also been reported [2340].

Microscopy
In most cases, bone marrow is involved with a plasma cell proliferative disorder, most frequently PCM [3230]. Rarely, lymphoplasmacytic lymphoma, marginal zone lymphoma, or chronic lymphocytic leukaemia is the associated neoplasm [4299]. Deposition of light or heavy chains is most frequently found in renal biopsies but can be observed in bone marrow and other tissues in some cases. The aberrant Ig deposits consist of amorphous eosinophilic material that is non-amyloid and non-fibrillary, and does not stain with Congo red. In LCDD, renal biopsies typically show nodular sclerosing glomerulonephritis. The deposits consist of refractile eosinophilic material in the glomerular and tubular basement membranes. Immunofluorescence microscopy most often identifies kappa chains. A hallmark of LCDD is prominent, smooth, ribbon-like linear peritubular deposits of monotypic Ig along the outer edge of the tubular basement membrane. By electron microscopy, these deposits are typically non-fibrillary, powdery, and electron-dense, with an absence of the beta-pleated sheet structure by X-ray diffraction [2340,2537,3230]. In some cases, plasma cells are found in the vicinity of Ig deposits in visceral organs, but most commonly, few if any are present [366,510].

Immunophenotype
LCDD has a high prevalence of kappa light chains (80%), with overrepresentation of the V kappa IV variable region [509,3230]. Immunohistochemistry on bone marrow sections may reveal an aberrant kappa/lambda ratio [4349].

Genetic profile and pathophysiology
The genetic profile of cases associated with PCM is similar to that of other PCMs. The M protein in non-amyloid Ig deposition diseases has undergone structural change due to deletion and mutation events [509,510,1906,3234]. In LCDD, the primary defect involves multiple mutations of the Ig light chain variable region, with kappa light chain V kappa IV type notably overrepresented [509,510,3234]. In HCDD, the critical event is deletion of the CH1 constant domain, which causes failure to associate with heavy chain-binding protein, resulting in premature secretion [190,1627,1906,2340,3234]. In HCDD, the variable regions also contain amino acid substitutions that cause an increased propensity for tissue deposition and for binding blood elements [190,509,1906].

Prognosis and predictive factors
Older patient age, associated PCM, and extrarenal light chain deposition are predictors of higher risk and unfavourable survival [2641,3230]. The median overall survival of patients with LCDD varies from 4 years to 14 years in one recent series [3230,3542].

Plasma cell neoplasms with associated paraneoplastic syndrome

POEMS syndrome

Definition
POEMS syndrome is a paraneoplastic syndrome associated with a plasma cell neoplasm, usually characterized by fibrosis and osteosclerotic changes in bone trabeculae, and often with lymph node changes resembling the plasma cell variant of Castleman disease. The POEMS acronym stands for polyneuropathy, organomegaly, endocrinopathy, mono-
clonal gammopathy, and skin changes (1983), but these components are not all required for diagnosis; in many cases, not all are present. The diagnostic criteria for POEMS syndrome are shown in Table 13.15 (1001).

**Synonyms**
Osteosclerotic myeloma; Crow–Fukase syndrome

**Epidemiology**
POEMS syndrome is a rare disease, estimated to account for < 1% of plasma cell neoplasms. Many cases have been reported from Asia. Men are affected more often than women, with a male-to-female ratio of 1.4:1, and the median patient age is about 50 years (1006).

**Etiology**
The etiology and pathogenesis of POEMS syndrome are not well understood, but markedly elevated levels of VEGF are present and appear to be an important pathogenic factor and to be responsible for some of the symptoms (1070, 3737, 4266). The pathophysiological connection between POEMS syndrome, osteosclerotic myeloma, and Castleman disease is not clearly defined. A few reported patients, typically with cases associated with Castleman disease, have been infected with HHV8 (318, 1002, 2671).

**Clinical features**
The mandatory criteria for diagnosis of POEMS syndrome are a chronic progressive polyneuropathy and a monoclonal plasma cell proliferative disorder. There is usually an associated M protein of either IgG or IgA type, with lambda light chain restriction in almost all cases. The quantity of M protein is typically below myeloma levels (median: 1.1 g/dL) (1006). In addition, patients must have one or more major and one or more minor criteria (Table 13.15) (1001, 1003, 2300). Two thirds of patients with lymphadenopathy have changes consistent with the plasma cell variant of Castleman disease (1006). Osteosclerotic bone lesions are present in > 95% of cases (1003, 1006). These vary from single sclerotic lesions (seen in about half of patients) to > 3 lesions (in one third of patients) (1006). Plasma and serum VEGF is markedly elevated in nearly all cases, and the levels correlate with disease activity (1003, 2300). Organomegaly, primarily hepatomegaly or splenomegaly, is present in at least half of patients, and an endocrinopathy, most frequently hypogonadism or thyroid abnormality, is found in more than two thirds of patients. Skin changes occur in more than two thirds of cases, most commonly hyperpigmentation and hypertrichosis (1003, 1006, 2300). Other relatively common clinical findings include papilloedema, thrombocytosis, oedema and serous cavity effusions, weight loss, fatigue, fingernail clubbing, bone pain, and arthralgias. Hypercalcaemia, renal insufficiency, and pathological fractures are rare.

**Microscopy**
The characteristic lesion in bone marrow is a single or multiple osteosclerotic plasmacytoma. The lesion is composed of focally thickened trabecular bone with associated paratrabecular fibrosis containing entrapped plasma cells. The plasma cells may appear elongated due to distortion by small bands of connective tissue. In the bone marrow away from the osteosclerotic lesion, plasma cells are usually < 5%, but can be > 50% in patients with disseminated disease (867, 3738). The plasma cells are distributed interstitially or in small or large clusters, depending on their abundance. Lymphoid aggregates rimmed by monotypic or polytypic plasma cells are found in half of patients. Megakaryocyte hyperplasia in clusters and often with atypical morphological features similar to those seen in myeloproliferative neoplasms is frequently observed (867). Lymph node biopsies commonly reveal features of the plasma cell variant of Castleman disease (1006).

**Immunophenotype**
In most patients with POEMS syndrome, a bone marrow monoclonal plasma cell population is detectable by flow cytometry or immunohistochemistry, frequently in a background of polyclonal plasma cells. The neoplastic plasma cells are of IgG or IgA type, and are lambda-restricted in almost all cases. The common phenotypic aberrancies found in other plasma cell neoplasms are also seen in POEMS syndrome (867, 1006, 3738).

**Genetic profile**
The few published studies on the genetics of POEMS syndrome report abnormalities similar to those in plasma cell myeloma but with different prevalence rates (483, 1924). No significant correlations between genetic abnormalities and clinical features have been established (1924).

**Prognosis and predictive factors**
In most cases, POEMS syndrome is a chronic and progressive disease but with a median overall survival as long as 165 months and a 5-year survival rate of 60–94% (856, 1006, 1983, 2300). Patients with localized plasma cell tumours treated with radiation therapy fare best, with improvement of the paraneoplastic symptoms and in some instances apparent cure (1003). Several clinical factors are associated with shorter survival, including extrascular fluid overload, fingernail clubbing, respiratory symptoms, and pulmonary hypertension (1003, 2300). There are no known genetic findings that are predictors of prognosis (1003, 1006).

**TEMPI syndrome**

**Definition**
TEMPI syndrome is a paraneoplastic syndrome associated with a plasma cell neoplasm. The acronym stands for telangiectasias, elevated erythropoietin and erythrocytosis, monoclonal gammopathy, perinephric fluid collection, and intrapulmonary shunting. TEMPI syndrome is similar to POEMS syndrome in that its manifestations appear to result from the monoclonal plasma cell proliferation and associated M protein. However, the clinical and laboratory findings are mostly distinct from those of POEMS syndrome. Because TEMPI syndrome is a rare and only recently described disease, it is

---

**Table 13.15 Diagnostic criteria for POEMS syndrome; adapted from Dispensieri A (1001)**

<table>
<thead>
<tr>
<th>Mandatory criteria</th>
<th>Minor criteria (≥ 1 required)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polyneuropathy (typically demyelinating)</td>
<td>Organomegaly</td>
</tr>
<tr>
<td>Monoclonal plasma cell proliferative disorder</td>
<td>Endocrinopathy</td>
</tr>
<tr>
<td></td>
<td>Skin changes</td>
</tr>
<tr>
<td></td>
<td>Papilloedema</td>
</tr>
<tr>
<td></td>
<td>Thrombocytosis</td>
</tr>
<tr>
<td></td>
<td>Extravascular volume overload</td>
</tr>
</tbody>
</table>

**POEMS syndrome**
Polyneuropathy, organomegaly, endocrinopathy, monoclonal gammopathy, and skin changes.
included in the WHO classification as a provisional category of plasma cell neoplasm (17,2153,2684,3402,3585,3858).

Epidemiology
TEMPI syndrome is a rare disease, with only 11 cases reported in the medical literature as of mid-2015 (3402). The lack of familiarity with this disease until very recently and its propensity to mimic other disorders suggest that TEMPI syndrome may be underrecognized. The reported patient age range is 35–58 years, and it occurs in both men and women.

Etiology
There is no published information on the etiology of TEMPI syndrome. The successful results of treatment aimed at ablation of the monoclonal plasma cells suggest that the monoclonal plasma cells and their M protein product play a major role in the pathophysiology of the disease and its paraneoplastic manifestations (2153,3585).

Localization
The clonal plasma cells producing TEMPI syndrome are in the bone marrow.

Clinical features
TEMPI syndrome has an insidious onset with slowly progressive symptoms, which may cause delay in diagnosis. Erythrocytosis seems to be a uniform feature, associated with a steadily progressive increase in erythropoietin to very high levels exceeding those produced by most other causes of erythrocytosis. Telangiectasia is reported in most cases, prominent on the face, trunk, arms, and hands. These findings appear to precede development of intrapulmonary shunting and hypoxia. The perinephric fluid, which collects between the kidney and its capsule, is clear, serous, and of low protein content. Spontaneous intracranial haemorrhage and venous thrombosis have been reported in some patients (3858). M protein has been present in all reported cases. IgG kappa predominates; both IgG and IgA lambda have been reported in single cases (2153,2684,3402,3858). In at least one patient, the serum free light chain ratio was skewed (2153). Unlike in POEMS syndrome, VEGF levels are reportedly normal (3402).

Microscopy
There are no reported blood or bone marrow morphological findings that are specific for TEMPI syndrome, but erythrocytosis and a hypercellular marrow due to erythroid hyperplasia are recurrent findings (3402). Mild erythroid and megakaryocytic atypia has been described in one patient, and reactive lymphoid aggregates were present in another (3402). Most patients have a percentage of bone marrow clonal plasma cells in the range of monoclonal gammopathy of undetermined significance (<10%). Two patients have been reported to have had >10% plasma cells (one diagnosed with smouldering plasma cell myeloma), but no case reported to date has fulfilled the criteria for the diagnosis of symptomatic plasma cell myeloma. Slight plasma cell atypia is generally present, with prominent cytoplasmic vacuolization reported in one case (2153,3402).

Immunophenotype
The monoclonal plasma cell proliferation is most commonly IgG kappa, but both IgG and IgA lambda have also been reported. There are no detailed descriptions of the immunophenotype of the monoclonal plasma cells.

Prognosis and predictive factors
There is insufficient experience with TEMPI syndrome to predict its overall prognosis or risk factors, but it seems to be an indolent plasma cell neoplasm of low tumour burden, with symptomatology related to the constellation of paraneoplastic manifestations. Recognition of the disease and initiation of treatment before advanced symptoms develop seems key to successful management. Complete or partial resolution of symptoms has been achieved through treatment with the proteasome inhibitor bortezomib (2153,3585). A significant decrease in erythropoietin level following treatment is one indicator of good therapeutic response (3402). In at least one case, a bortezomib regimen was followed by autologous stem cell transplantation, with complete remission and resolution of symptoms (3402).
Extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma)

Definition
Extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma) is an extranodal lymphoma composed of morphologically heterogeneous small B cells including marginal zone (centrocyte-like) cells, cells resembling monocytoid cells, small lymphocytes, and scattered immunoblasts and centroblast-like cells. There is plasmacytic differentiation in some cases. The neoplastic cells reside in the marginal zones of reactive B-cell follicles and extend into the interfollicular region as well as into the follicles (follicular colonization). In epithelial tissues, the neoplastic cells typically infiltrate the epithelium, forming lymphoepithelial lesions [1044,1782]. Thus, MALT lymphomas variably recapitulate Peyer’s patch-type lymphoid tissue, the prototypical normal mucosa-associated lymphoid tissue (MALT). MALT lymphomas arising at any anatomical site share many characteristics, but there are also site-specific differences with respect to etiology, morphological features, molecular cytogenetic abnormalities, and clinical course [502, 1044,2140].

ICD-O code  9699/3

Epidemiology
MALT lymphoma accounts for 7-8% of all B-cell lymphomas [1] and for as many as 50% of primary gastric lymphomas [1016,3275]. Most cases occur in adults, with a median patient age in the seventh decade of life. Men and women are about equally affected, although there are site-specific sex differences, with a female predominance reported for cases in the thyroid and salivary glands [1, 1999]. There is geographical variability, with a higher incidence of gastric MALT lymphoma reported in north-eastern Italy [1016], and a special subtype called alpha heavy chain disease (also known as immunoproliferative small intestinal disease) occurs in the Middle East [48,4230], the Cape region of South Africa [3239], and a variety of other tropical and subtropical locations (see Alpha heavy chain disease, p. 240).

Etiology
In many MALT lymphoma cases, there is a history of a chronic inflammatory disorder that results in accumulation of extranodal lymphoid tissue (called acquired MALT). The chronic inflammation may be the result of infection, autoimmunity, or unknown other stimuli. The link between infection and MALT lymphoma is most clearly established for Helicobacter pylori and gastric MALT lymphoma [1199,3954]. The continued proliferation of gastric MALT lymphoma cells from patients infected with H. pylori depends on the presence of T cells specifically activated by H. pylori antigens and/or direct oncogenic effects of H. pylori proteins on B cells [1741,2137]. The importance of this stimulation in vivo has been clearly demonstrated by the...
induction of remissions in gastric MALT lymphomas with antibiotic therapy to eradicate *H. pylori* [4366]. In the first study in which the association of gastric MALT lymphoma with *H. pylori* infection was examined, the organism was present in >90% of cases [4367]. More recent studies, in the era of antibiotic eradication therapy for *H. pylori* gastritis, suggest that the overall incidence of gastric MALT lymphoma is decreasing, and that a much smaller proportion of cases (32%) are now associated with *H. pylori* at diagnosis [2414, 3621].

A role for antigenic stimulation by *Chlamydia psittaci* and *Borrelia burgdorferi* has been proposed for some cases of ocular adnexal MALT lymphoma and cutaneous MALT lymphoma, respectively (611,1199,1201). There is great variation in the strength of these associations, which might relate in part to geographical diversity [658,2296,3454]. A similar role has been proposed for *Campylobacter* infection in patients with alpha heavy chain disease. In other cases, acquired MALT secondary to autoimmune disease may serve as the substrate for lymphoma development [1779]. Autoimmune-based chronic inflammation in the form of Sjögren syndrome and Hashimoto thyroiditis is known to precede salivary gland and thyroid MALT lymphomas, respectively. Patients with primary Sjögren syndrome have an estimated risk of lymphoma 14–19 times that of the general population [2319, 4497]; most lymphomas in patients with Sjögren syndrome are MALT lymphomas. In patients with Hashimoto thyroiditis, the risk of developing lymphoma is 3 times that in the general population, and the risk of thyroid lymphoma 70 times that in the general population, for an overall lymphoma risk of 0.5–1.5% [117,1671, 1959]. Approximately 90% of thyroid lymphomas have evidence of lymphocytic thyroiditis [957,4265].

**Localization**

The stomach is the most common site of MALT lymphoma, affected in 35% of all cases. Other common sites include the eyes and ocular adnexa (affected in 13% of cases), skin (9%), lungs (9%), salivary glands (8%), breasts (3%), and thyroid (2%) [1999].

**Clinical features**

Most patients present with stage I or II disease, but 23–40% have involvement of multiple extranodal sites [1905, 2140]. Staging in patients with multiple extranodal lesions may be challenging, because at least some cases constitute multiple clonally unrelated proliferations rather than truly disseminated disease [2081]. Making this distinction may not be possible in routine practice. A minority of patients (2–20%) have bone marrow involvement [148,3276,3953]. The frequency of bone marrow involvement and involvement of multiple extranodal sites is higher in non-gastric MALT lymphoma than in gastric cases. Generalized nodal involvement is rare (reported in < 10% of cases) [1905,2140,3952]. Plasmacytic
differentiation is a feature of many of the cases, and a serum paraprotein can be detected in one third of patients with MALT lymphoma (4381).

Microscopy
The characteristic marginal zone B cells have small to medium-sized, slightly irregular nuclei with moderately dispersed chromatin and inconspicuous nucleoli, resembling those of centrocytes, and relatively abundant, pale cytoplasm. The accumulation of even more pale-staining cytoplasm may lead to a monocytoid appearance, which is especially common in salivary gland MALT lymphomas. Alternatively, the marginal zone cells may more closely resemble small lymphocytes. Plasmacytic differentiation is present in approximately one third of gastric MALT lymphomas, is frequently found in cutaneous MALT lymphomas, and is a constant and often striking feature in thyroid MALT lymphomas. In some MALT lymphomas, there is a marked predominance of plasma cells, resulting in resemblance to an extramedullary plasmacytoma. Cutaneous plasmacytomas are diagnosed as MALT lymphoma. Amyloid deposition is seen in some cases. Large cells resembling centroblasts or immunoblasts are usually present, but are in the minority. The lymphoma cells infiltrate around reactive B-cell follicles external to a preserved mantle in a marginal zone distribution, and spread out to form larger confluent areas that eventually replace some or most of the follicles, often leaving small remnants of germinal centres, which can be highlighted by negativity for BCL2 (1784,1785). The lymphoma cells sometimes specifically colonize reactive germinal centres; in extreme examples, this can lead to a close resemblance to follicular lymphoma. Lymphoepithelial lesions, defined as aggregates of ≥3 marginal zone cells with distortion or destruction of the epithelium, may be seen in glandular tissues, often together with eosinophilic degeneration (oxyphilic change) of epithelial cells. In lymph nodes, MALT lymphoma invades the marginal zone, with subsequent interfollicular expansion. Discrete aggregates of monocytoid-like B cells may be present in a parafollicular and perisinusoidal distribution. Cytological heterogeneity is still present, and both plasmacytic differentiation and follicular colonization may be seen. MALT lymphoma, by definition, is a lymphoma composed predominantly of small cells. Transformed centroblast-like or immunoblast-like cells may be present in variable numbers, but when solid or sheet-like proliferations of transformed cells are present, the tumour should be diagnosed as diffuse large B-cell lymphoma (DLBCL) and the presence of accompanying MALT lymphoma noted. The term ‘high-grade MALT lymphoma’ should not be used, and the term ‘MALT lymphoma’ should not be applied to a DLBCL even if it has arisen in a MALT site or is associated with lymphoepithelial lesions.

Immunophenotype
The neoplastic cells of MALT lymphoma are CD20+, CD79a+, CD5-, CD10-, CD23-, CD43+, and CD11c+/- (weak). Infrequent cases are CD5+, and very rare cases are CD5+ but BCL6- (2140,3954). Staining for CD21, CD23, and CD35 typically reveals expanded meshworks of follicular dendritic cells, corresponding to colonized follicles. The demonstration of light chain restriction is helpful in the differential diagnosis with reactive hyperplasia. Recent reports have highlighted IRTA1 as a possible specific marker for marginal zone lymphomas, including MALT lymphoma, although IRTA1 antibodies are not yet widely available (1137, 1154). MNDA staining may facilitate the differential diagnosis of MALT lymphoma versus follicular lymphoma, because this nuclear antigen is expressed in 61–75% of MALT lymphomas but < 10% of follicular lymphomas (1922,2645). The tumour cells of MALT lymphoma typically express IgM heavy chains, and less
often IgA or IgG. A notable exception is cutaneous marginal zone lymphoma, of which two subsets have been described: a more common class-switched subset (accounting for 75–85% of cases) with IgG (including many IgG4-i-) cases or IgA expression and usually a T-cell–predominant background, and a less common (15–25% of cases) IgM-i- subset that tends to be B-cell-predominant [455, 1080, 4137].

**Postulated normal counterpart**
A post-germinal centre marginal-zone B cell

**Genetic profile**
IG heavy and light chain genes are rearranged and show somatic hypermutation of variable regions [1043,3254]. There is biased usage of certain IGHV gene families at different anatomical sites, suggesting antigen-induced clonal expansion during the process of lymphomagenesis [1905,3954]. Chromosomal translocations associated with MALT lymphomas include t(11;18) (q21;q21), t(14;18) (q32;q21), and t(3;14)(p14.1;q32), resulting in the production of a chimeric protein (BIRC3-MALT1) and in transcriptional deregulation of BCL10, MALT1, and FOXP1, respectively [1044,3813]. Trisomy of chromosome 3 or 18 (or less commonly of other chromosomes) is a non-specific but also not infrequent finding in MALT lymphomas. The frequencies at which the translocations or trisomies occur vary markedly with the primary site of disease. The t(11;18)(q21;q21) translocation is mainly detected in pulmonary and gastric tumours; t(14;18)(q32;q21) in ocular adnexa, orbit, and salivary gland lesions; and t(3;14)(p14.1;q32) in MALT lymphomas arising in the thyroid, ocular adnexa, orbit, and skin (Table 13.16). Similarly, geographical variability in incidence and anatomical site specificity of the translocations has been noted, suggesting different environmental influences, such as infectious and other etiological factors [3340,3813].

Abnormalities of TNFAIP3 on chromosome 6q23, which may include deletions, mutations, and promoter methylation, occur in 15–30% of cases, most frequently cases lacking specific translocations [657,1045,2898]. However, TNFAIP3 abnormalities are not specific for MALT lymphoma, and can be found in many types of non-Hodgkin lymphoma [1681]. MYD88 L265P mutation has been reported in 6–9% of MALT lymphomas [1267, 2315, 2860].

**Prognosis and predictive factors**
MALT lymphomas have an indolent natural course and are slow to disseminate. Recurrences, which can occur after many years, may involve other extranodal sites and occur more often in patients with extragastric MALT lymphomas than in patients with primary gastric disease [3276]. Cutaneous marginal zone lymphomas have a particularly indolent course, with 5-year survival rates approaching 100% [4320]. MALT lymphomas are sensitive to radiation therapy, and local treatment may be followed by prolonged disease-free intervals. Involvement of multiple extranodal sites and even bone marrow involvement do not appear to confer a worse prognosis [3953,3954]. Protracted remissions may be induced in H. pylori–associated gastric MALT lymphoma by antibiotic therapy for H. pylori [2853,4366]. The presence or absence of H. pylori should be investigated in both gastric MALT lymphoma and gastric DLBCL, because some primary gastric DLBCLs may also respond to antibiotic eradication therapy alone [676,1200]. Cases with t(11;18)(q21;q21) appear to be resistant to H. pylori eradication therapy [2366]. Antibiotics have also been used to successfully treat selected other MALT lymphomas. Transformation to DLBCL may occur but is uncommon (reported in < 10% of cases) [3953,3954].

**Table 13.16** Anatomical site distribution and frequency of chromosomal translocations and trisomies 3 and 18 in MALT lymphomas.
Data summarized according to Streubel B et al. [3812] and Remstein ED et al. [3340]

<table>
<thead>
<tr>
<th>Site of disease</th>
<th>t(11;18)(q21;q21)</th>
<th>t(14;18)(q32;q21)</th>
<th>t(3;14)(p14.1;q32)</th>
<th>t(1;14)(p22;q32)</th>
<th>+3</th>
<th>+18</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stomach</td>
<td>6–26</td>
<td>1–5</td>
<td>0</td>
<td>0</td>
<td>11</td>
<td>6</td>
</tr>
<tr>
<td>Intestine</td>
<td>12–56</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>75</td>
<td>25</td>
</tr>
<tr>
<td>Ocular adnexa/orbit</td>
<td>0–10</td>
<td>0–25</td>
<td>0–20</td>
<td>0</td>
<td>36</td>
<td>13</td>
</tr>
<tr>
<td>Salivary gland</td>
<td>0–5</td>
<td>0–16</td>
<td>0</td>
<td>0</td>
<td>55</td>
<td>19</td>
</tr>
<tr>
<td>Lung</td>
<td>31–53</td>
<td>6–10</td>
<td>0</td>
<td>2–7</td>
<td>20</td>
<td>7</td>
</tr>
<tr>
<td>Skin</td>
<td>0–8</td>
<td>0–14</td>
<td>0–10</td>
<td>0</td>
<td>20</td>
<td>4</td>
</tr>
<tr>
<td>Thyroid</td>
<td>0–17</td>
<td>0</td>
<td>0–50</td>
<td>0</td>
<td>17</td>
<td>0</td>
</tr>
</tbody>
</table>
Nodal marginal zone lymphoma

Definition
Nodal marginal zone lymphoma (NMZL) is a primary nodal B-cell neoplasm that morphologically resembles lymph nodes involved by marginal zone lymphoma (MZL) of the extranodal or splenic types, but without evidence of extranodal or splenic disease.

ICD-O code 9699/3

Synonyms
Monocytoid B-cell lymphoma; parafollicular B-cell lymphoma (obsolete)

Epidemiology
NMZL accounts for only 1.5–1.8% of all lymphoid neoplasms, and has an annual incidence of 0.8 cases per 100,000 adults [106,347,2834]. Most cases occur in adults, with a median age of ~60 years, and the proportion of males and females affected is similar [106,4123]. This lymphoma can also occur in children, and is then separately designated as paediatric NMZL [3866]. A significantly increased incidence has been observed among females with autoimmune disorders [442]. A relationship to hepatitis C virus infection has been detected in some studies [137,4503], but not in others [442,4046].

Localization
NMZL involves peripheral lymph nodes, but can also involve the bone marrow and occasionally the peripheral blood [106,347,2834,4123].

Clinical features
Most patients present with asymptomatic, localized, or generalized peripheral lymphadenopathy [137,347]. The head and neck lymph nodes are more frequently involved [4123]. B symptoms are present in 10–20% of patients. Bone marrow infiltration is seen in one third of patients [4123]. The presence of a primary extranodal MZL should be ruled out, because approximately one third of cases presenting as NMZL in fact constitute nodal dissemination of a MALT lymphoma, which is particularly common in patients with Hashimoto thyroiditis or Sjögren syndrome [542].

Microscopy
Lymph nodes demonstrate a small-cell lymphoid proliferation that surrounds reactive follicles and expands into the interfollicular areas. Follicular colonization may be present. In cases with a diffuse pattern, follicle remnants may be detected with immunohistochemical stains for follicular dendritic cells and germinal centre markers. The neoplastic cells are composed of variable numbers of marginal zone (centrocyte-like and monocytoid) B cells, plasma cells in some cases, and scattered transformed B cells [533, 2834,2884,4046]. Cases with a predominant monocytoid B-cell population are uncommon. Plasma cell differentiation may be prominent, and the differential diagnosis with lymphoplasmacytic lymphoma or even nodal plasmacytoma may be difficult. The presence of remnants of follicular dendritic cell meshworks suggestive of colonized follicles favours the diagnosis of NMZL. Prominent eosinophilia may be present. Some cases have more-numerous large transformed cells (sometimes >20%). However, these cells are usually mixed with small cells and may be more common in the colonized germinal centres [2834,4046]. Some cases mimic splenic MZL, with the neoplastic cells being small to medium-sized lymphocytes with pale cytoplasm and occasional transformed cells growing inside an attenuated mantle zone and often around a residual germinal centre [542]. Composite NMZL and Hodgkin lymphoma have been reported [4473]. Bone marrow involvement is usually interstitial or nodular, with an intertrabecular or paratrabecular distribution. An intrasinusoidal infiltration may be seen but is less common [437,1757].

Immunophenotype
Most NMZLs express pan-B-cell markers, with CD43 coexpression in 20–75% of cases [3486]. CD23 is usually negative, but may be expressed in as many as 29% of cases [4123]. CD5 expression
Genetic profile

The IG genes are clonally rearranged, with a predominance of mutated IGHV3 and IGHV4 family members, in particular IGHV4-34 (533,4045). Cases associated with hepatitis C virus preferentially use IGHV1-69 (2499,4123).

NMZL shares gains of chromosomes 3 and 18 and loss of 6q23-24 with extranodal MZL of mucosa-associated lymphoid tissue (MALT lymphoma) and splenic MZL. However, deletions in 7q31 and the recurrent translocations associated with extranodal MZL are not detected (443,983,3362,4046).

Gene expression profiling analysis has demonstrated an increased expression of NF-κB-related genes (154). MYD88 L265P mutation is usually absent but has been detected in occasional cases not specifically associated with plasmacytic differentiation (1527,2534,3851).

Prognosis and predictive factors

The 5-year overall survival rate is about 60–70% (137). Advanced patient age, B symptoms, and advanced disease stage are associated with a worse prognosis (106). However, on a multivariate analysis, only the Follicular Lymphoma International Prognostic Index (FLIPI) applied to these patients predicted overall survival (137). The proportion of scattered or clustered large cells does not appear to be of prognostic significance (4046). However, transformation to diffuse large B-cell lymphoma may occur. This diagnosis requires the presence of sheets of large cells (2687).

Paediatric nodal marginal zone lymphoma

Definition

Paediatric nodal marginal zone lymphoma (NMZL) has distinctive clinical and morphological characteristics (3866). It presents predominantly in males (with a male-to-female ratio of 20:1) with asymptomatic and localized disease (stage I in 90% of cases), mainly in the head and neck lymph nodes. Histologically, it is similar to adult NMZL, except that there are often large follicles with extension of mantle zone B cells into the germinal centres, resembling progressively transformed germinal centres. The immunophenotype is similar to that of adult NMZL, with expansion of the interfollicular...
Fig. 13.67 Paediatric nodal marginal zone lymphoma. A. These lymphomas commonly exhibit progressive transformation of germinal centres. The atypical cells are found primarily in the interfollicular areas and may disrupt the follicles. B. IgD stain highlights the disrupted and expanded mantle zone; the tumour cells are IgD-negative.

lar areas by CD20+ B cells that commonly coexpress CD43 [3866]. Light chain restriction can often be demonstrated by immunohistochemistry or flow cytometry [3366]. IgD staining may help to delineate an irregular and expanded mantle zone. BCL2 is positive in half of the cases. CD10 is usually negative [3272]. Staining for CD279/PD1 shows numerous positive cells in the reactive germinal centres, a feature that may help in the differential diagnosis with paediatric-type follicular lymphoma, in which these cells are less numerous and pushed to the periphery of the germinal centre [2369,3272].

Clonal rearrangements of the IGHV region are detected in almost all cases [3366]. Trisomy 18 may be present in approximately one fifth of cases, and occasionally trisomy 3 [3366]. The prognosis of paediatric NMZL is excellent, with a very low relapse rate and long survival following conservative treatment [3272,3866].

The differential diagnosis with atypical marginal zone hyperplasia with mono-typic immunoglobulin expression may be difficult, because the large cells in this condition also express CD43 [183]; although this type of hyperplasia has been reported in extranodal sites, some caution is advised because a similar process might also occur in the lymph nodes. Particularly for these reasons, genetic studies in paediatric marginal zone lymphomas are strongly recommended [3866]. A marginal zone hyperplasia mimicking NMZL in head and neck lymph nodes of children has been associated with Haemophilus influenzae. The marginal zone cells in these cases are IgD-positive [2046].

ICD-O code 9699/3
Follicular lymphoma

Definition
Follicular lymphoma (FL) is a neoplasm composed of follicle centre (germinal centre) B cells (typically both centrocytes and centroblasts/large transformed cells), which usually has at least a partially follicular pattern. Lymphomas composed of centrocytes and centroblasts with an entirely diffuse pattern in the sampled tissue may be included in this category, but are relatively rare at presentation. Progression in cytological grade is common during the natural history of the disease. A diffuse lymphoma composed of centroblasts is considered evidence of progression to diffuse large B-cell lymphoma (DLBCL). Four variants of FL are recognized: (1) in situ follicular neoplasia, formerly called FL in situ; (2) duodenal-type FL; (3) testicular FL; and (4) the diffuse variant of FL.

Primary cutaneous follicle centre lymphomas are separately classified [2242, 3848]. FL is nearly exclusively a disease of adults, and very rarely occurs in patients aged <18 years. Paediatric-type FL, which is a nodal lymphoma that occurs in children and young adults, is considered a separate entity.

ICD-O codes
Follicular lymphoma 9690/3
Grade 1 9695/3
Grade 2 9691/3
Grade 3A 9698/3
Grade 3B 9698/3

Epidemiology
FL accounts for about 20% of all lymphomas. The highest incidence rates are reported in the USA and western Europe. In eastern Europe, Asia, and developing countries, the incidence is much lower [95]. It affects predominantly adults, with a median age in the sixth decade of life and a male-to-female ratio of 1:1.7 [1]. FL is 2–3 times as common in White populations as in Black populations [1477]. Unlike paediatric-type FL, usual FL rarely occurs in individuals aged <18 years. Agricultural exposure to pesticides and herbicides has been associated with an increased risk [33,1257,3678].

Etiology
Individuals with a high environmental exposure to pesticides and herbicides have increased numbers of cells carrying t(14;18)(q32;q21) (IGH/BCL2) in the peripheral blood [33]. This may help explain the reported increased risk of FL among such individuals.

Localization
FL predominantly involves the lymph nodes, but also involves the spleen, bone marrow, peripheral blood, and less commonly Waldeyer ring. Any nodal group can be involved, but most patients present with peripheral lymphadenopathy. Pure extranodal presentations are uncommon. The most commonly affected extranodal sites include the gastrointestinal tract (often in association with mesenteric lymph node involvement), soft tissue, breast, and ocular adnexa. FL arising in the small intestine, in particular the duodenum, has distinctive features (duodenal-type FL). FLs can occur in almost any extranodal site [1203]. In some cases, the morphology, phenotype, and genetics are similar to those of nodal FL. However, many FLs in extranodal sites tend to be of higher grade (grade 3), and may lack BCL2 protein and the BCL2 translocation [3021, 4148].

Clinical features
Most patients have widespread disease at diagnosis, including peripheral and central (abdominal and thoracic) lymphadenopathy and splenomegaly. The bone marrow is involved in 40–70% of cases. Only 15–25% of cases are stage I or II at the time of diagnosis [1632]. Despite widespread disease, patients are usually otherwise asymptomatic. B symptoms such as fever and weight loss are uncommon. Waxing and waning of the disease without therapy is common. The disease follows a chronic relapsing clinical course.

Imaging
Conventional imaging tools such as CT and MRI are useful in assessing the degree of lymphadenopathy and extent of disease. FDG-PET is less useful in assessing disease than in more aggressive lymphomas. However, the detection of PET-avid disease may be useful in identifying patients with higher risk for progression [112].

Staging
The stage of the disease is now determined using the Lugano classification, a modification of the Ann Arbor staging system [691]. Assessment of bone marrow involvement should be accomplished with bone marrow biopsy. Bone marrow aspiration has a lower yield, due to the difficulty is aspirating cells from the parabreptacular lymphoid aggregates. The designation of a case as A or B (asymptomatic or symptomatic) is no longer required for non-Hodgkin lymphoma subtypes, according to the Lugano system.

Macroscopy
The cut surface of lymph nodes involved...
by FL displays a vaguely nodular pattern that can be seen macroscopically. The neoplastic follicles often have a bulging appearance. However, reactive follicular hyperplasia can display the same pattern. Spleens involved by FL show uniform expansion of the white pulp, usually with no evidence of involvement of the red pulp.

Microscopy

Pattern
Most cases of FL have a predominantly follicular pattern, with closely packed follicles that efface the nodal architecture. Neoplastic follicles are often poorly defined and usually have attenuated or absent mantle zones. Unlike in reactive germinal centres, where the proportion of centroblasts and centrocytes varies in different zones (polarization), in FL the two types of cells are randomly distributed. Similarly, tingible body macrophages, characteristic of reactive germinal centres, are usually absent in FL. In some cases, follicles may be irregular and serpiginous, but this growth pattern does not constitute progression to a diffuse growth pattern. Staining for follicular dendritic cell (FDC) markers (CD21 and/or CD23) can be helpful in highlighting the follicular pattern. Interfollicular infiltration by neoplastic cells is common and does not constitute a diffuse pattern. The interfollicular neoplastic cells are often centrocytes that are smaller than those in the germinal centres, with a less irregular nuclear contour, and they may show immunophenotypic differences from the cells in the germinal centres (1015). Infrequent cases have a so-called floral growth pattern that resembles progressively transformed germinal centres (4004).

Spread beyond the lymph node capsule is often associated with sclerosis, particularly in mesenteric and retroperitoneal locations. With limited sampling in small biopsies, it may be difficult to appreciate a follicular pattern. A diffuse area is defined as an area of the tissue completely lacking follicles as evidenced by the absence of CD21+/CD23+ FDCs. The distinction between an extensive interfollicular component and a diffuse component is sometimes arbitrary. Diffuse areas composed predominantly of centrocytes are not thought to be clinically significant. Nevertheless, it is recommended that the relative proportions of follicular and diffuse areas be noted in the pathology report as follicular (>75% follicular), follicular and diffuse (25-75% follicular), or focally follicular / predominantly diffuse (<25% follicular) (1556). However, the presence of diffuse areas composed entirely or predominantly of large centroblasts (that would fulfil the criteria for grade 3 FL) in FL of any grade is equivalent to DLBCL, and a separate diagnosis of DLBCL should be made (1556) (see Grading).

Some cases have the morphology and immunophenotype of FL but with no evidence of a follicular growth pattern. This phenomenon is usually seen in small biopsy specimens, and likely constitutes a diffuse area in an FL that was not adequately sampled. A repeat biopsy in the same or another site may reveal a follicu-
Fig. 13.71 Follicular lymphoma, grade 3B. A The follicle is composed of a uniform population of centroblasts, with no evident centrocytes. B This case was negative for CD10 but positive for IRF4/MUM1. Such cases are often negative for the BCL2 translocation (1944). The border of the neoplastic follicle is shown at lower left. C The neoplastic cells show nuclear staining for IRF4/MUM1, but are negative for CD10.

Cytology

FL is typically composed of the two types of B cells normally found in germinal centres. Small to medium-sized cells with an-gulated, elongated, twisted, or cleaved nuclei; inconspicuous nucleoli; and scant pale cytoplasm are called centrocytes. Large centrocytes with dispersed chromatin and inconspicuous nucleoli may also be present. Large cells with usually round or oval nuclei, vesicular chromatin, 1–3 peripheral nucleoli, and a rim of cyto-

plasm are called centroblasts. Typically, they are ≥3 times the size of lymphocytes, but they may be smaller in some cases. Centrocytes predominate in most cases; centroblasts are always present, but are usually in the minority. The number of centroblasts varies from case to case and is the basis of grading. In some cases, neoplastic centroblasts have irregular or multilobed nuclei. Rare cases of FL are composed of blastoid-appearing cells with dispersed chromatin resembling lymphoblasts (4250). This variant appears to have an aggressive clinical course, equivalent to grade 3. Unlike in reactive germinal centres, polarization is usually absent, and starry-skyclistic hystocytes are absent or few in number.

In about 10% of FLs, there are discrete foci of marginal zone or monocytoid-appearing B cells, typically at the periphery of the neoplastic follicles (1407, 2833,4029). These cells are part of the neoplastic clone (3374). Plasmacytic differentiation can be seen uncommonly. In cases with plasmacytic differentiation, the plasmacytoid cells have an interfollicular distribution and carry the BCL2 translocation, indicating they are part of the neoplastic clone (1418). Other cases resembling FL have intrafollicular plasmacytoid cells and lack the translocation. Such t(14;18)-negative cases might constitute an unusual variant of marginal zone lymphoma with follicular colonization.

Bone marrow and blood

In bone marrow, FL characteristically localizes to the paratrabecular region and may spread into the interstitial areas. A follicular growth pattern with a meshwork of FDCs can be rarely seen. The morphology of the tumour cells most commonly resembles that of the neoplastic interfollicular cells in lymph nodes. The same cells may be seen in the peripheral blood.

Diffuse follicular lymphoma variant

A novel diffuse FL variant is characterized by a predominantly diffuse growth pattern and consistent absence of the t(14;18)(q32;q21) (IGH/BCL2) chromosomal translocation (1962,3670A). In all cases, small follicles or so-called microfollicles are seen, with weak to absent BCL2 staining. This particular FL variant mainly occurs in the inguinal region, forming larger tumours, but with little tendency to disseminate. The neoplastic cells are usually CD10-positive, and in almost all cases express the CD23 antigen as well. These cases cluster with typical FL by gene expression profiling. A recurrent genetic aberration, deletion in 1p36, is seen in most cases. These alterations are not specific to this variant; the region at 1p36, which contains TNFRSF14, is also commonly affected in t(14;18)-positive FL.

Testicular follicular lymphoma

Testicular FLs (1219) are a distinctive variant of FL. They are reported with higher frequency in children, but are also seen rarely in adults (214). They differ biologically from nodal FL in that they lack evidence of the BCL2 translocation. Cytologically they are of high cytological grade, usually grade 3A, but have a good prognosis, even without additional therapy beyond surgical excision (1608, 2369,2386).
**Immunophenotype**

The tumour cells are usually positive for surface immunoglobulin (IgM with or without IgD, IgG, or rarely IgA). They express B-cell-associated antigens (CD19, CD20, CD22, and CD79a) and are usually positive for BCL2, BCL6, and CD10 and negative for CD5 and CD43. Some cases, that are most commonly grade 3B, lack CD10 but retain BCL6 expression (424,584,1944,2190,3008,3199). CD10 expression is often stronger in the follicles than in interfollicular neoplastic cells, and may be absent in the interfollicular component as well as in areas of marginal zone differentiation, peripheral blood, and bone marrow (1015,1558). BCL6 is frequently downregulated in the interfollicular areas and is more variably expressed than in normal germinal centres. Other germinal centre markers, such as LMO2, GCET1, and HGAL (also called GCET2), are positive, but are generally not required for routine diagnosis (2634, 2840,3043). However, they may be useful in the differential diagnosis of FL and marginal zone lymphoma with follicular colonization. CD5 is expressed in rare cases of FL, possibly more frequently in those with a floral growth pattern (2313, 4004). Meshworks of FDCs are present in follicular areas (4505) but are usually sparser and more irregularly distributed than in normal follicles. They may variably express CD21 and CD23, so antibodies to both antigens may be needed to detect FDC meshworks.

BCL2 overexpression is the hallmark of FL, and BCL2 protein is expressed by a variable proportion of the neoplastic cells in 85–90% of cases of grade 1–2 FL, but in <50% of grade 3 FLs (2189). In some cases, the apparent absence of BCL2 protein is due to mutations in the BCL2 gene that eliminate the epitopes recognized by the most commonly used antibody; however, BCL2 can be detected in those cases using antibodies to other BCL2 epitopes (1990,2540, 3583). BCL2 protein can be useful in distinguishing neoplastic from reactive follicles, although absence of BCL2 protein does not exclude the diagnosis of a FL. BCL2 protein is not useful in distinguishing FL from other types of low-grade B-cell lymphomas, most of which also express BCL2. The interpretation of BCL2

Fig. 13.73 Follicular lymphoma, grade 1–2, with marginal zone differentiation. A At the periphery of the follicles, there is a pale rim corresponding to marginal zone differentiation. B The centres of the follicles contain the typical mixture of centrocytes and centroblasts. C The cells at the periphery of the follicles are medium-sized cells with slightly irregular nuclei and abundant lightly eosinophlic to pale-staining cytoplasm, consistent with marginal zone or monocytoid B cells.

Fig. 13.74 Bone marrow involvement by follicular lymphoma. A At low magnification, paratrabecular lymphoid aggregates are visible. B The cells are small centrocytes.
immunostaining in germinal centre cells
requires caution, because T cells, primary follicles, and mantle zones normally express this protein.
In addition to FDCs, neoplastic follicles contain numerous other non-neoplastic cells normally found in germinal centres, including follicular T cells (CD3+, CD4+, CD57+, PD1/CD279+, CXCL13+) and varying numbers of histiocytes. Consistent with the germinal centre phenotype of the neoplastic cells, most cases are negative for IRF4/MUM1. However, a subset of FLs negative for CD10 are positive for IRF4/MUM1 [1944]. Such tumours typically lack the BCL2 translocation but show amplification of BCL6 [1950]. They tend to occur in elderly patients and cytologically are of higher grade (3A or 3B). These cases must be distinguished from large B-cell lymphomas with IRF4/MUM1 rearrangement, which often has at least a partially follicular growth pattern [2369, 3491]. The Ki-67 proliferation index in FL generally correlates with histological grade; most grade 1–2 cases have a proliferation index <20%, whereas most grade 3 cases have a proliferation index >20%, although there is considerable variation among studies, probably due to technical differences in immunostaining [2088, 2520, 3008, 4250]. A subgroup of morphologically low-grade FLs with a high proliferation index has been described [2088, 4250]; these cases behaved more aggressively than did those with a low proliferation index, and similarly to grade 3 FL [4250]. Therefore, Ki-67 staining should be considered as an adjunct to histological grading, and its use is clinically justified, although not formally required at this time.

**Postulated normal counterpart**
The postulated normal counterpart is a germinal centre B cell. In cases with t(14;18)(q32;q21), the IGH/BCL2 translocation occurs in bone marrow pre-B cells; fully malignant transformation of these t(14;18)-positive primed cells occurs during (re)entry into the germinal centres in secondary lymphoid organs [3425, 3426].

**Grading**
FL is graded by counting or estimating the absolute number of centroblasts (large or small) in 10 neoplastic follicles, expressed per high-power (40x magnification, 0.159 mm²) microscopic field (HPF) [1820, 2485, 2835]. At least 10 HPFs within different follicles must be evaluated; these should be representative follicles, not those with the most numerous large cells. Grade 1–2 cases have a marked predominance of centrocytes, with few centroblasts (grade 1: 0–5 centroblasts per HPF; grade 2: 6–15 centroblasts per HPF). As recommended in the 2008 WHO classification, the combined designation of grade 1–2 is preferred, due to the lack of clinically significant differences between grades 1 and 2, the considerable interobserver variation in grading, and variations in grade within a given biopsy. Grade 3 cases have >15 centroblasts per HPF. Grade 3 is further subdivided on the basis of the presence or absence of centrocytes. In grade 3A, centrocytes are still present, whereas grade 3B
Follicles are composed entirely of large blastic cells (centroblasts or immunoblasts). Recent data indicate that grade 3B FL differs from other forms of FL both biologically and clinically, as discussed below. If distinct areas of grade 3 FL are present in a biopsy of an otherwise grade 1–2 FL, a separate diagnosis of grade 3 FL should also be made and the approximate percentages of each grade reported. Because both pattern and cytology vary among follicles, lymph nodes must be adequately sampled. Accurate grading cannot be performed on fine-needle aspirations and may be difficult on core needle biopsies. Therefore, an excisional biopsy is recommended for primary diagnosis. The presence of a diffuse component with grade 3 cytology always warrants an additional diagnosis of DLBCL.

The vast majority of FLs (80–90% in most unselected series) are of grade 1–2 (4228). Only a few studies have compared the frequency of grade 3A versus 3B cases. However, pure grade 3B FL is rare, with most cases containing at least focal diffuse areas composed mainly of centroblasts, constituting DLBCL (1963,3008).

Grade 3B FL is biologically more closely related to DLBCL than to other FLs (1684). Translocations involving BCL2 are relatively rare in such cases (3493). In addition, biopsies frequently contain diffuse areas composed mainly of centroblasts, constituting DLBCL. The natural history appears to differ from that of other forms of FL. There may be higher short-term mortality, but patients in remission after anthracycline-based therapy at 5 years, are likely cured of disease (4228). Thus, the clinical course resembles that of DLBCL. However, some studies have not found significant differences between grade 3A and 3B FL (3668). These data underscore the often subjective nature of grading in FL, with considerable interobserver variation.

Genetic profile

Antigen receptor genes

IG heavy and light chain genes are rearranged; IGV genes show extensive and ongoing somatic hypermutation (765,3010). As a result of mutations in the complementarity-determining regions, a high false-negative rate on IGH PCR was observed with older primer sets. Multiplex PCR reactions using BIOMED-2 expanded primer sets detect closer to 90% of IGH VDJ gene rearrangements, and clonality detection approximates 100% when primers detecting IGH DJ and light chain gene rearrangements are included (1117). FL undergoes genetic evolution over time, which can be linear, but in most cases follows a pattern of divergent evolution rather than direct evolution. FL is associated with the development of multiple subclones (1434). This observation has relevance for histological Table 13.17 Follicular lymphoma grading, based on the absolute number of centroblasts per high-power (40 x objective, 0.159 mm^2) microscopic field (HPF)^a

<table>
<thead>
<tr>
<th>Grading</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade 1–2 (low grade)</td>
<td>0–15 centroblasts per HPF</td>
</tr>
<tr>
<td>1</td>
<td>0–5 centroblasts per HPF</td>
</tr>
<tr>
<td>2</td>
<td>6–15 centroblasts per HPF</td>
</tr>
<tr>
<td>Grade 3</td>
<td>&gt;15 centroblasts per HPF</td>
</tr>
<tr>
<td>3A</td>
<td>Centrocytes present</td>
</tr>
<tr>
<td>3B</td>
<td>Solid sheets of centroblasts</td>
</tr>
</tbody>
</table>

Reporting of pattern

- Follicular: >75%
- Follicular and diffuse: 25–75%
- Focally follicular/predominantly diffuse: <25%
- Diffuse: 0%

Diffuse areas containing >15 centroblasts per HPF are reported as diffuse large B-cell lymphoma with follicular lymphoma (grade 1–2, grade 3A, or grade 3B)^b.

To determine the number of centroblasts per 0.159 mm^2 HPF: if using an 18 mm field of view ocular, count the centroblasts in 10 fields and divide by 10; if using a 20 mm field of view ocular, count in 8 fields and divide by 10 or count in 10 fields and divide by 15.

Mention the approximate percentage of each component in the report.

If the biopsy specimen is small, a note should be added that the absence of follicles may reflect sampling error.

Table 13.18 Frequency of genetic alterations in follicular lymphoma at diagnosis

<table>
<thead>
<tr>
<th>Gene</th>
<th>Frequency of alterations (%)^a</th>
<th>Predominant type of alteration</th>
</tr>
</thead>
<tbody>
<tr>
<td>BCL2</td>
<td>85–90</td>
<td>Translocation, mutation</td>
</tr>
<tr>
<td>KMT2D (MLL2)</td>
<td>85</td>
<td>Mutation</td>
</tr>
<tr>
<td>TNFRSF14</td>
<td>45–65</td>
<td>Deletion, mutation</td>
</tr>
<tr>
<td>EZH2</td>
<td>60</td>
<td>Mutation (Y641)</td>
</tr>
<tr>
<td>EPHA7</td>
<td>70</td>
<td>Mutation</td>
</tr>
<tr>
<td>CREBBP</td>
<td>33</td>
<td>Deletion, mutation</td>
</tr>
<tr>
<td>BCL6</td>
<td>45</td>
<td>Translocation, mutation</td>
</tr>
<tr>
<td>MEF2B</td>
<td>15</td>
<td>Mutation</td>
</tr>
<tr>
<td>EP300</td>
<td>10</td>
<td>Deletion, mutation</td>
</tr>
<tr>
<td>TNFAIP3 (also called A20)</td>
<td>20</td>
<td>Deletion, mutation</td>
</tr>
<tr>
<td>FAS</td>
<td>5</td>
<td>Mutation</td>
</tr>
<tr>
<td>TP53</td>
<td>&lt;5</td>
<td>Deletion, mutation</td>
</tr>
<tr>
<td>MYC</td>
<td>&lt;5</td>
<td>Translocation, gain</td>
</tr>
</tbody>
</table>

^a Approximate frequency of alterations; some alterations may be subclonal.
progression in FL, in which transformation develops in an earlier common progenitor rather than one of the later subclones [1432].

**Cytogenetic abnormalities and oncogenes**

FL is genetically characterized by the t(14;18)(q32;q21) translocation between the IGH and BCL2 genes. Alternative BCL2 translocations to Ig light chain genes have been reported. The t(14;18) translocation is present in as many as 90% of grade 1–2 FLs [1700,3434]. Due to the variation in breakpoint regions, FISH is more sensitive than PCR-based approaches for detecting the translocation [4094]. Caution is advised because classic cytogenetics cannot be used to distinguish IGH/BCL2 from IGH/MALT1 translocations. FLs negative for BCL2 rearrangement are more likely to have a late germinal centre gene expression profile [2259], but the absence of the translocation has no apparent impact on prognosis [2258]. However, BCL2 rearrangements are much less frequent in grade 3B FL [3008]. Similarly, testicular FL, seen mainly in young boys, is negative for the BCL2 rearrangement. Abnormalities of 3q27 and/or BCL6 rearrangement are found in 5–15% of FLs, and have also been reported in testicular FL [1219].

In addition to t(14;18), other genetic alterations are found in 90% of FLs: most commonly, loss of 1p, 6q, 10q, and 17p and gains of chromosomes 1, 6p, 7, 8, 12q, X, and 18q [1750,2931,4005]. One of the most commonly affected regions is 1p36, which contains TNFRSF14 [1434]. Copy-number alterations, acquired copy-neutral LOH, and mutations in TNFRSF14 are among the most common findings in all forms of FL, including the diffuse variant of FL and paediatric-type FL [2228]. TNFRSF14 mutations were initially suggested to be associated with an adverse prognosis [702], but other studies have not confirmed this association [2228].

The number of additional alterations increases with histological grade and transformation [3508]. Rare cases of FL carry t(8;14)(q24;q32) or variants together with t(14;18) [185,4214], and progression to a high-grade so-called double-hit lymphoma may occur, with translocations affecting both BCL2 and MYC [3082].

Gain-of-function mutations in the H3K27 methyltransferase EZH2 are relatively common in FL, and appear to be an early event in the evolution of the disease [513]. Additionally, driver mutations in the chromatin regulator genes CREBBP and KMT2D (MLL2) play a key role [1435, 2966]. EZH2, KMT2D, and CREBBP have all been proposed as possible therapeutic targets. More recently, activating somatic mutations in RRAGC have been found in approximately 17% of cases [2967].

Gene expression studies have shown the importance of the microenvironment in the pathogenesis, evolution, and prognosis of FL [878,1382]. Transformation to DLBCL may occur via various genetic pathways, including inactivation of TP53 and CDKN2A and activation of MYC [884,1089,2395,3195,3508].

**Genetic susceptibility**

Genome-wide association studies have identified five susceptibility loci for FL outside the HLA region [3690]. Consistent with these data, FL has an increased incidence in patients with a history of lymphoma in first-degree family members [2354].

**Prognosis and predictive factors**

The prognosis of FL is closely related to the extent of the disease at diagnosis. The FLIP, a modification of the InternationaL Prognostic Index (IPI), is useful in predicting outcome [1,2,3720]. The FLIP uses five independent predictors of inferior survival: age >60 years, haemoglobin concentration <12 g/dL, elevated serum lactate dehydrogenase, Ann Arbor stage III/IV (disseminated disease in the Lugano system), and >4 involved nodal areas. The presence of 0–1, 2, 3–5 of these adverse factors, respectively, defines low-risk, intermediate-risk, and high-risk disease. Genetic profiling may provide additional information [3094].
Most published studies show a more aggressive clinical course for FLs classified as grade 3, but the use of regimens containing doxorubicin and/or rituximab may obviate these differences, and requires further study [1252,4228,4293]. With current therapies, the median survival for patients with FL of grades 1–3A is > 12 years [4228]. There are continued relapses over time, with no plateau in the survival curves.

Transformation or so-called progression usually to DLBCL occurs in 25–35% of patients with FL. Rare patients with initial DLBCL develop a late relapse as FL [2249]. This sequence is analogous to chronic myeloid leukaemia presenting in blast-phase crisis. The clinical outcome for patients with histological progression is better than in past years [4227]. FL may also progress to a lymphoma resembling Burkitt lymphoma or with features intermediate between those of DLBCL and Burkitt lymphoma [77,1260,1689,3279]. Transformation typically involves additional genetic abnormalities, in particular MYC translocations; the combination of a BCL2 and a MYC rearrangement is associated with a particularly aggressive course [1212,2252,4394,4420]; cases with this combination should be diagnosed as high-grade B-cell lymphoma with MYC and BCL2 rearrangements, transformed from FL, so-called double-hit lymphoma.

Rarely, patients develop B-lymphoblastic leukaemia/lymphoma, which in the cases studied is clonally related to the original B-cell tumour [896,1311,2061,2121]. This form of transformation also involves the acquisition of a MYC rearrangement in addition to the underlying BCL2 rearrangement. However, the term ‘high-grade B-cell lymphoma with MYC and BCL2 rearrangements’ should not be used in the setting of lymphoblastic transformation.

FL can relapse as classic Hodgkin lymphoma. The association of FL and other B-cell lymphomas with classic Hodgkin lymphoma provided early evidence for the B-cell origin of the Hodgkin/Reed-Sternberg cell [1825]. Genetic studies identified the BCL2 translocation in cases of classic Hodgkin lymphoma associated with FL [2244] and established a common clonal identity for the two morphologically different neoplasms [486]. Rarely, the occurrence of histiocytic or dendritic cell sarcoma has been described in patients with FL, in which the sarcomas also had the IGH and BCL2 rearrangements. However, the histiocytic cells show loss of PAX5 activity and loss of all B-cell markers [1172]. Genetic studies show that the histiocytic sarcoma and the FL both arise from a common precursor, rather than constituting differentiation of the mature FL B cell [479]. However, this precursor is beyond the haematopoietic stem cell level, and has already undergone IG rearrangement.
In situ follicular neoplasia

Definition
In situ follicular neoplasia (ISFN), formerly referred to as follicular lymphoma in situ [789], is defined as partial or total colonization of germinal centres by clonal B cells carrying the BCL2 translocation characteristic of FL in an otherwise reactive lymph node. It can also be seen in reactive follicles in lymphoid tissue in other sites, including the spleen. ISFN is biologically similar to the presence of cells carrying the BCL2 rearrangement found in the peripheral blood of otherwise healthy individuals by PCR amplification for BCL2 rearrangement [2479], referred to as FL-like B cells. Both FL-like B cells and ISFN have been described in the same patient [703]. ISFN may be seen uncommonly in patients with overt FL at another site or subsequently. In the few such cases studied, there have been differences in the genetic profiles of the two lesions, suggesting that in at least some cases the ISFN lesion does not simply constitute secondary spread of FL from another site [3568]. ISFN should be distinguished from lymph nodes showing only partial involvement by FL. Although patients with partial involvement tend to have lower-stage disease, partial involvement is considered a form of FL for clinical purposes [21].

ICD-O code 9695/1

Synonyms
Intrafollicular neoplasia; follicular lymphoma in situ

Epidemiology
ISFN can be detected in approximately 2% of randomly selected reactive lymph node biopsies [353,1610]. Epidemiological studies have shown that FL-like B cells are more commonly found in patients with increased environmental risk, such as prolonged exposure to herbicides and pesticides [33]. FL-like B cells can be detected in as many as 70% of adults aged >50 years. The incidence of this finding increases with age, and is uncommon in individuals aged <18 years, in whom FL is rare [1076,3424]. Some cases of ISFN are discovered incidentally in lymph nodes involved by other forms of lymphoma, most often other B-cell lymphomas [353,1848,2698,3184]. ISFN has also been observed in lymph nodes from nodal dissections performed in connection with surgery for other cancers [353,2747]. In patients with subsequent FL, the in situ lesion preceded the lymphoma diagnosis by 23 months to 10 years.

Microscopy
ISFN is generally not apparent in routine H&E-stained sections [789]. Lymph nodes contain reactive follicles with well-formed germinal centres. The affected follicles are similar in size and shape to adjacent uninvolved follicles, although on close inspection closely packed centrocytes may be appreciated. The lesion can also be seen in reactive follicles in extranodal sites, i.e. any location where reactive follicles are identified. ISFN is distinct from partial involvement by FL.
in which only selected follicles within a lymph node may be involved (1848). Partial involvement can usually be suspected based on H&E-stained sections, whereas ISFN can only be observed with subsequent immunohistochemical stains. Partial involvement is associated with lower-stage disease (21), but progression can occur.

ISFN may be detected in lymph nodes involved by other forms of lymphoma, most often other lymphomas of B-cell lineage, which include chronic lymphocytic leukaemia, mantle cell lymphoma, marginal zone lymphoma, diffuse large B-cell lymphoma, and classic Hodgkin lymphoma (1848,2698,3184). ISFN has also been reported to coexist in the same anatomical site with in situ mantle cell neoplasia (3428). Activation-induced cytidine deaminase is involved in initiating chromosomal breaks that lead to both the t(14;18) and the t(11;14) translocations, which is suggestive of common molecular mechanisms (1457).

**Immunophenotype**

ISFN can only be detected by immunohistochemistry for BCL2, and is generally not suspected on H&E staining. The BCL2-positive cells are exclusively centrocytes, and BCL2 is very strongly expressed, with a higher intensity than in adjacent T cells or mantle zone cells. The BCL2-positive centrocytes also show increased expression of CD10. Flow cytometry may demonstrate CD10-positive, BCL2-positive, light chain restricted B cells, often in the presence of more numerous other CD10-positive or CD10-negative B cells (3184).

**Genetic profile**

ISFN cells are positive for t(14;18), but are found to have very few other genetic aberrations when examined by array comparative genomic hybridization (2480,3568), suggesting that this is a very early step in lymphomagenesis. In addition to the BCL2 translocation, mutations in EZH2 (Y641) were also reported (1848). Deletions at 1p36 encompassing the TNFRSF14 gene have also been seen (2480). This aberration is common to many B-cell lymphomas. Lymph nodes showing partial involvement by FL have also been found to have a relatively low level of genomic alterations (2480, 3568). In cases in which ISFN and overt FL are seen in the same patient, there are additional genetic alterations in the FL that are not present in the ISFN (414, 3568). Some of these secondary events lead to mutations in BCL2, resulting in negative staining of the FL with the commonly used clone 124 antibody to BCL2 (414,1848).

**Prognosis and predictive factors**

For patients with incidentally diagnosed ISFN and no other evidence of FL on clinical evaluation, the risk of subsequent FL is very low (< 5%) (353,1848). Most studies have not found that the number of follicles involved within a single lymph node showing ISFN predicts the subsequent risk of lymphoma (1848, 3184). High levels of FL-like B cells in the peripheral blood are associated with an increased risk of subsequent FL (3425), but levels so low that the cells can only be identified with multiple rounds of PCR amplification carry no increased risk. For patients with other sites of lymphadenopathy at presentation with ISFN, additional biopsy is recommended, because other forms of lymphoma (usually B-cell lymphomas) may exist with ISFN (353,1288, 1848,3184).
Duodenal-type follicular lymphoma

Definition
Most cases of primary follicular lymphoma (FL) in the gastrointestinal tract occur in the small intestine, usually with involvement of the duodenum [2675, 3530, 3648, 4439]. Duodenal-type FL is a specific variant of FL defined by distinctive clinical and biological features. The lesions are predominantly found in the second portion of the duodenum, presenting as multiple small polyps, often as an incidental finding on endoscopy performed for other reasons [3564]. The immunophenotype is similar to that of nodal FLs. Most patients have localized disease (stage IE or IIE), and survival appears to be excellent even without treatment. Surveillance of the small bowel in patients with duodenal-type FL reveals involvement of the more distal small intestine in approximately 80–85% of cases [3564, 3876]. Cases not exhibiting the typical features of duodenal-type FL should be evaluated for evidence of FL at other sites. Classic FL can involve the intestine, usually in association with involvement of mesenteric lymph nodes. Such cases lack the indolent clinical features of the duodenal-type variant [2676]. They usually show more-extensive involvement of the intestinal wall, with infiltration into the muscularis propria. They show variation in cytological grade (similar to that seen in nodal FL) and are distinct from duodenal-type FL [3648].

ICD-O code 9695/3

Synonym
Primary intestinal follicular lymphoma

Epidemiology
Most patients are middle-aged, with an equal male-to-female ratio in most large series [2732, 3564]. Several large series have been reported from Japan, but it is not clear that this reflects an increased incidence; it could instead be a result of increased surveillance by endoscopy in this population [1802, 2732].

Microscopy
Duodenal-type FL demonstrates neoplastic follicles in the mucosa/submucosa. The atypical follicles are composed almost uniformly of centrocytes (with only infrequent centroblasts), constituting grade 1–2 disease in the grading system for nodal FL. The neoplastic cells also infiltrate the lamina propria outside of the follicles, a feature best illustrated with immunohistochemical staining.

CCL20
MADCAM1

{ BCL family

Fig. 13.81 Gene expression profiling of duodenal-type follicular lymphoma (DFL), extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma), and nodal follicular lymphoma (nodal FL). The gene expression pattern of DFL is closer to that of Helicobacter-associated gastric MALT lymphoma than to that of nodal FL. DFL and MALT lymphoma both show high expression of CCL20 and MADCAM1, which are expressed at low levels in nodal FL. In contrast, for the BCL gene family, DFL and nodal FL show similar patterns, which are distinct from that of MALT lymphoma. These data were compared with data from 17 normal control samples: 5 normal duodenal mucosa (yellow), 8 nodal reactive lymphoid hyperplasia (green), and 4 normal gastric mucosa (vermilion) samples [3879].

Fig. 13.82 Duodenal-type follicular lymphoma. Large nodules of lymphoid cells are present in the mucosa (A). The follicles are uniformly positive for BCL2 (B) and positive for CD10 (C).
Fig. 13.83 Duodenal-type follicular lymphoma. A This duodenal polyp was an incidental finding in a patient undergoing screening endoscopy. Another similar polypoid lesion was observed. B CD20 stain. Note the extension of the infiltrate into the lamina propria. C The cells are strongly positive for CD10. D The cells are intensely BCL2-positive. E CD3 stain. Few T cells are present surrounding the abnormal follicle. F CD21 stain shows that the follicular dendritic cells are few in number and mainly located in the periphery of the lymphoid infiltrate.

**Immunophenotype**

The immunophenotype of duodenal-type FL is similar to that of nodal FL. The cells are positive for CD20, BCL2, and CD10, with variable expression of BCL6 (3564). The proliferation rate is generally low. The cells are negative for activation-induced cytidine deaminase (3878), which plays a key role in class switching and in somatic hypermutation in normal and neoplastic B cells. Follicular dendritic cells (as identified by CD21) are usually restricted to the periphery of the follicle (3878). The cells express the intestinal homing receptor alpha4beta7 (328). Consistent with the intestinal origin of the neoplastic cells, they usually express IgA heavy chain.

**Postulated normal counterpart**

A B cell that expresses germinal centre markers and has features of a memory B cell (3878)

**Genetic profile**

The cells carry the t(14;18)(q32;q21) (IGH/BCL2) translocation. They are thought to be memory B cells and show evidence of somatic hypermutation of the IGH gene (3877). Restricted IGHV usage suggests similarities to MALT lymphomas (3879). Gene expression studies have also suggested an overlap with MALT lymphoma, showing overexpression of CCL20 and MADCAM1, genes that were not found to be upregulated in nodal FL (3879). A study using array comparative genomic hybridization showed that although duodenal-type FL is positive for the IGH/BCL2 translocation, it has a lower frequency of other genetic aberrations than does nodal FL, consistent with its indolent clinical course and low incidence of progression (2480). However, in common with nodal FL, recurrent deletion of chromosome 1p was observed, encompassing the TNFRSF14 gene, as well as mutations in TNFRSF14 exons (2480). The same study identified a limited set of amplified oncogenes (BCL2, BCL6, FGFR1, ELF4A2, and TFRC) and deleted tumour suppressor genes (PTEN, FAS, and TP73) in some cases of duodenal-type FL (2480). These aberrations are also found in nodal FL.

**Prognosis and predictive factors**

Long-term survival is excellent, even with local recurrences in the intestine (2732,3564). There is a low (<10%) risk of progression to nodal disease. Various therapies have been used, including local radiation therapy, chemotherapy, and rituximab. Given the indolent clinical course, a watch-and-wait approach is reasonable for most patients (1288,3564, 3903).
Paediatric-type follicular lymphoma

Definition
Paediatric-type follicular lymphoma (PTFL) is an uncommon nodal follicular lymphoma (FL) that occurs primarily in children and young adults, but also occurs sporadically in older individuals [2369,2400]. This term should not be used for cases with areas of diffuse large B-cell lymphoma (DLBCL) or other lymphomas of follicle centre derivation that occur in the paediatric age group. In particular, this category does not include testicular FLs [1219,2386] or large B-cell lymphomas with IRF4 rearrangement, which often have a follicular or partially follicular growth pattern [2369,3491]. PTFL most often involves lymph nodes of the head and neck, and usually presents with stage I disease. The median age at onset is 15–18 years, with only rare cases presenting in patients aged >40 years. The male-to-female ratio is ≥10:1. Cytologically, the lesions appear to be of high grade, most often with a high proliferation rate, but the prognosis is excellent, and many patients achieve continuous complete remission following only complete surgical excision of the affected lymph node. The usual translocations found in other B-cell lymphomas of germinal centre origin (i.e. of BCL2 and BCL6) are absent.

ICD-O code 9690/3

Epidemiology
There are no known risk factors and no known associations with immunodeficiency or autoimmune disease. Most patients are aged 5–25 years. There is a marked male predominance, with a male-to-female ratio of ≥10:1.

Localization
Most patients present with enlarged lymph nodes in the head and neck region (i.e. the cervical, submental, submandibular, postauricular, and periparotid nodes) [2369,2400]. The inguinal and femoral lymph nodes are less often the presenting site. Virtually all patients present with isolated peripheral lymphadenopathy, without involvement of the paraaortic or mesenteric lymph nodes.

Clinical features
Most patients present with isolated, asymptomatic lymph node enlargement. Occasional cases of FL in children have been reported with more extensive disease or focal progression to DLBCL [2393,2524]. However, these reports did not use the current WHO definition of PTFL, and at least some of the reported cases had significant genetic or immunophenotypic differences from PTFL as it is currently defined [1817]. In clinical practice, cases with areas of DLBCL or disseminated disease are excluded from this category.

Imaging
Imaging studies confirm the localized nature of the disease, with absence of radiological evidence of mediastinal or intrabdominal lymph node involvement.

Staging
The vast majority of patients present with a single site of lymph node enlargement. Bone marrow involvement has not been reported. B symptoms such as fever and weight loss are absent.

Table 13.20 Primary diagnostic criteria for paediatric-type follicular lymphoma (PTFL)

<table>
<thead>
<tr>
<th>Morphology</th>
<th>At least partial effacement of nodal architecture (required)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pure follicular proliferation (required)®</td>
</tr>
<tr>
<td></td>
<td>Expansile follicles®</td>
</tr>
<tr>
<td></td>
<td>Intermediate-sized so-called blastoid cells (not centrocytes)®</td>
</tr>
<tr>
<td>Immunohistochemistry</td>
<td>BCL6 positivity</td>
</tr>
<tr>
<td>(required)</td>
<td>BCL2 negativity or weak positivity</td>
</tr>
<tr>
<td></td>
<td>High proliferative fraction (&gt;30%)</td>
</tr>
<tr>
<td>Genomics</td>
<td>No BCL2, BCL6, IRF4, or aberrant IG rearrangement</td>
</tr>
<tr>
<td>(required)</td>
<td>No BCL2 amplification</td>
</tr>
<tr>
<td>Clinical features</td>
<td>Nodal disease (required)</td>
</tr>
<tr>
<td></td>
<td>Stage I–II disease (required)</td>
</tr>
<tr>
<td></td>
<td>Patient age &lt;40 years®</td>
</tr>
<tr>
<td></td>
<td>Marked male predominance</td>
</tr>
</tbody>
</table>

® The presence of any component of diffuse large B-cell lymphoma or advanced-stage disease excludes PTFL

The cells have a mature B-cell phenotype and are positive for CD20, CD79a, and PAX5. CD10 is usually strongly expressed, and BCL6 is positive. Most cases are negative for BCL2 expression, but weak staining is seen in a minority of cases. Ki-67 staining usually reveals a high proliferative fraction (>30%). Mitotic figures are readily apparent. Some cases contain more typical centroblasts. Areas of DLBCL preclude the diagnosis of PTFL. Historically, most cases have been reported as grade 3A or 3B [2393,2997], but grading is not typically used, unlike for usual FL.

Immunophenotype

Microscopy
Lymph node architecture is totally or subtotally effaced by large expansile follicles, often with a serpiginous growth pattern. Partial involvement can be seen, with a rim of normal node at the edge of the biopsy. On low-power magnification, the follicles show a starry-sky pattern and thin or absent mantle zones. In some cases, evidence of marginal zone differentiation may be seen peripheral to the neoplastic follicles. The cellular composition is typically monotonous; the atypical cells are intermediate in size, often have a blastoid appearance, and lack prominent nucleoli [2369]. Mitotic figures are readily apparent. Some cases contain more typical centroblasts. Areas of DLBCL preclude the diagnosis of PTFL. Historically, most cases have been reported as grade 3A or 3B [2393,2997], but grading is not typically used, unlike for usual FL.

Table 13.20 Primary diagnostic criteria for paediatric-type follicular lymphoma (PTFL)

<table>
<thead>
<tr>
<th>Morphology</th>
<th>At least partial effacement of nodal architecture (required)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pure follicular proliferation (required)®</td>
</tr>
<tr>
<td></td>
<td>Expansile follicles®</td>
</tr>
<tr>
<td></td>
<td>Intermediate-sized so-called blastoid cells (not centrocytes)®</td>
</tr>
<tr>
<td>Immunohistochemistry</td>
<td>BCL6 positivity</td>
</tr>
<tr>
<td>(required)</td>
<td>BCL2 negativity or weak positivity</td>
</tr>
<tr>
<td></td>
<td>High proliferative fraction (&gt;30%)</td>
</tr>
<tr>
<td>Genomics</td>
<td>No BCL2, BCL6, IRF4, or aberrant IG rearrangement</td>
</tr>
<tr>
<td>(required)</td>
<td>No BCL2 amplification</td>
</tr>
<tr>
<td>Clinical features</td>
<td>Nodal disease (required)</td>
</tr>
<tr>
<td></td>
<td>Stage I–II disease (required)</td>
</tr>
<tr>
<td></td>
<td>Patient age &lt;40 years®</td>
</tr>
<tr>
<td></td>
<td>Marked male predominance</td>
</tr>
</tbody>
</table>

® The presence of any component of diffuse large B-cell lymphoma or advanced-stage disease excludes PTFL

These are common features of PTFL, but not required for diagnosis.
Fig. 13.34 Paediatric-type follicular lymphoma. A The follicles are large and lack mantle zones. No polarization is evident, and the cellular composition is monotonous. B High-magnification view shows that the follicles are composed of monotonous, medium-sized lymphoid cells. Abundant starry-sky histiocytes are present. C CD20 stain. The follicles are closely packed. D IgD stain. The mantle zones are attenuated. E Serpiginous follicles are strongly CD10-positive. F BCL2 protein is negative in the neoplastic follicles.

Moderate to high proliferation rate (> 30% of follicular cells), usually without evidence of polarization in the follicles. The follicular dendritic cell markers (CD21 and CD23) outline meshworks within the follicles. IgD is negative and shows either absent or attenuated mantle cuffs. IRF4/MUM1 is negative; strong positivity should raise the possibility of large B-cell lymphoma with IRF4 rearrangement.

Plasma cells are sparse; an abundance of plasma cells raises the possibility of reactive hyperplasia. Flow cytometry identifies a monotypic population of B cells positive for CD10 and negative for CD5. However, rare cases of florid follicular hyperplasia, most commonly in young boys, can have clonal populations of CD10+ B cells detected by flow cytometry that are usually small, with monoclonality in IG rearrangement studies [2145]. Architectural effacement is a key feature that distinguishes PTFL from reactive follicular hyperplasia with clonal B cells.

Postulated normal counterpart
A germinal centre B cell

Grading
Although most cases would meet the criteria for grade 3 FL, grading is not used if the criteria for the diagnosis of PTFL are met.

Genetic profile
PCR techniques for the detection of IG gene rearrangements are positive, which is helpful in ruling out many, but not all, cases of florid follicular hyperplasia [2145]. Aberrations affecting the BCL2, BCL6, or IRF4 loci are absent. PTFLs generally lack mutations in KMT2D (MLL2), CREBBP, and EZH2, genes frequently mutated in usual FL [1435,2966], including cases negative for BCL2 rearrangements [2400A,3567A]. The most common genetic aberrations are deletion at 1p36 and deletions or mutations affecting TNFRSF14 [2400A,2524, 3567A]. MAP2K1 mutations are identified in approximately 40–50% of cases [2400A,2567B].

Prognosis and predictive factors
The prognosis is excellent. Most data indicate that patients with localized disease amenable to surgical excision do not require radiation or chemotherapy [176,2400]. In one study, there was no difference in clinical outcome between patients with and without genetic aberrations [2524]; however, most of the patients received multiagent chemotherapy. Areas of DLBCL exclude the diagnosis of PTFL as it is currently defined [2997]. The diagnosis should be made with caution in patients aged > 25 years, because the differential diagnosis with usual FL of grade 3A or 3B can be challenging. Correlation with clinical features is essential in older patients.
Large B-cell lymphoma with IRF4 rearrangement

Definition
Large B-cell lymphoma (LBCL) with IRF4 rearrangement is an uncommon subtype of LBCL that can be entirely diffuse, follicular and diffuse, or entirely follicular. It is characterized by strong expression of IRF4/MUM1, usually with IRF4 rearrangement. It occurs primarily in children and young adults, with predominantly Waldeyer ring or head and neck lymph node involvement (2369,3491). Despite its common occurrence in the paediatric age group, it is distinct from paediatric-type follicular lymphomas, even when purely follicular (2369,2400).

ICD-O code 9698/3

Epidemiology
LBCL with IRF4 rearrangement is rare, accounting for just 0.05% of diffuse LBCLs. The patient age range at presentation is 4–79 years, with a median age of 12 years and an equal sex distribution. This entity is significantly more frequent in children (individuals aged <18 years) than in adults (P<0.001) (3491).

Localization
Most patients present with enlarged lymph nodes of the head and neck region. The Waldeyer ring is also a frequent site of disease. Another reported site of involvement is the gastrointestinal tract (898,2369,3491).

Clinical features
Most patients present with isolated lymph node or tonsillar enlargement (stage I–II) (3491).

Microscopy
The neoplastic cells are medium-sized to large, with chromatin which is more open than typically seen in centrocytes and small, basophilic nucleoli. Mitotic figures are infrequent, and a starry-sky pattern is absent. When a follicular pattern is present, the neoplastic follicles are large, with a back-to-back growth pattern and absent or attenuated mantle zones. Many cases have an entirely diffuse growth pattern. Unlike in paediatric-type follicular lymphoma, the follicles generally lack a serpiginous configuration and starry-sky pattern.

Immunophenotype
The atypical cells have a mature B-cell phenotype and are positive for CD20, CD79a, and PAX5. IRF4/MUM1 is typically strongly expressed, and BCL6 is positive, whereas PRDM1 (also known as BLIMP1) is usually negative. CD10 and BCL2 expression is observed in 66% of cases (3491). The proliferation rate is usually high, with no evidence of polarization in the neoplastic follicles. In the appropriate clinical context, cases with coexpression of CD10, BCL6, and IRF4/MUM1 should be screened for IRF4 rearrangements.

Postulated normal counterpart
Germinal centre B cell with IRF4 rearrangement resulting in IRF4/MUM1 expression

Genetic profile
Immunoglobulin genes are clonally re-
Large B-cell lymphoma with IRF4 rearrangement. A There is uniform expression of IRF4/MUM1, (B) CD10 and (C) BCL6. D MIB-1/Ki-67 stain shows numerous positive cells in the reactive germinal centres (upper right) and somewhat fewer positive cells in the neoplastic follicles (lower left).

arranged. A cytogenetically cryptic rearrangement of IRF4 with an IGH locus is detected in most cases, whereas light chains are rarely involved in the translocation [3491]. In rare cases with similar clinical and pathological features, the IRF4 translocation may not be demonstrated with current techniques. However, an IGH rearrangement may be detected in these cases. BCL6 locus breakpoints may be seen in some cases, but virtually all cases lack MYC and BCL2 rearrangements [3272, 3491]. These cases display a complex pattern of genetic changes, including loss of TP53 in a subset of patients, independent of age, but these changes are most likely not predictors of clinical behaviour [3489]. Although most cases are found to have a germinal centre B-cell origin (either by gene expression profiling or by immunohistochemistry), the cases have a unique gene expression signature that is distinct from those of both germinal centre B cells and activated B cells [3491].

Prognosis and predictive factors
Patients have favourable outcome after treatment (combination immunotherapy with or without radiation) [3491]. This clinical picture is in contrast with that of paediatric-type follicular lymphoma, which tends to have a good prognosis with local management.
Primary cutaneous follicle centre lymphoma

Definition
Primary cutaneous follicle centre lymphoma (PCFCL) is a tumour of neoplastic follicle centre cells, including centrocytes and variable numbers of centroblasts, with a follicular, follicular and diffuse, or diffuse growth pattern. It generally presents on the head or trunk (4320). Lymphomas with a diffuse growth pattern and a monotonous proliferation of centroblasts and immunoblasts are classified, irrespective of site, as primary cutaneous diffuse large B-cell lymphoma, leg type (4320).

ICD-O code 9597/3

Synonyms
Reticulohistiocytoma of the dorsum; Crosti lymphoma

Epidemiology
PCFCL accounts for approximately 50% of primary cutaneous B-cell lymphomas. It mainly affects middle-aged adults, with a male-to-female ratio of approximately 1.5:1 (1530,3623,4499).

Localization
PCFCL characteristically presents with solitary or localized skin lesions on the scalp, forehead, or trunk. Approximately 5% of cases present with skin lesions on the legs, and 15% with multifocal skin lesions (2062,3623,4499).

Clinical features
The clinical presentation consists of firm erythematous to violaceous plaques, nodules, or tumours of variable size. Particularly on the trunk, tumours may be surrounded by erythematous papules and slightly infiltrated, sometimes figurate plaques, which may precede the development of tumorous lesions by months or years (358,3517,4322). PCFCL with this typical presentation on the back was formerly referred to as reticulohistiocytoma of the dorsum or Crosti lym-
phoma [358]. The skin surface is smooth and ulceration is rarely observed. Presentation with multifocal skin lesions is observed in a minority of patients, but is not associated with a more unfavourable prognosis [1421,3623]. If left untreated, the skin lesions gradually increase in size over the course of several years, but dissemination to extracutaneous sites is uncommon (occurring in ~10% of cases) [3623,4499]. Recurrences tend to be proximate to the initial site of cutaneous presentation.

Microscopy
PCFCL shows perivascular and periadnexal to diffuse infiltrates, with almost invariable sparing of the epidermis. The infiltrates show a spectrum of growth patterns, with a morphological continuum from follicular to follicular and diffuse to diffuse [3517,4320,4322]. Cases with a follicular growth pattern show nodular infiltrates throughout the entire dermis, often extending into the subcutis. Unlike in cutaneous follicular hyperplasias, the follicles in PCFCL may be poorly defined, show a monotonous proliferation of BCL6+ follicle centre cells enmeshed in a meshwork of CD21+/CD35+, follicular dendritic cells, lack tingible body macrophages, generally have an attenuated or absent mantle zone, and show a low proliferation rate [606,1408]. Reactive T cells may be numerous, and a prominent stromal component is usually present.

Cases with a diffuse growth pattern usually show a monotonous population of large centrocytes, some of which may have a multilobated appearance, and variable numbers of admixed centroblasts [358,1421,3517,4322]. In rare cases, the large centrocytes may be spindle-shaped [607,1406]. In some cases, foci of CD21+/CD35+ follicular dendritic cells may still be present; in other cases, they may be totally absent [1494]. The proliferative fraction in these diffuse PCFCLs is generally high.

Immunophenotype
The neoplastic cells express CD20 and CD79a, but are usually immunoglobulin-negative by immunohistochemistry. Flow cytometric studies are reported to demonstrate restricted light chain expression in almost 3/4 of cases [3549A]. PCFCLs consistently express BCL6. CD10 may be positive in cases with a follicular growth pattern, but is generally negative in cases with a diffuse growth pattern [903,1409,1654,2016,2062,2674,3623]. Most cases either do not express BCL2 or only show faint BCL2 staining (weaker than in admixed T cells) in a minority of neoplastic B cells [606,610,715,1318,1654,2062,3623]. However, several studies have reported BCL2 expression in a substantial proportion of PCFCLs with at least a partially follicular growth pattern [37,1409,2016,2674,3164]. Strong expression of both BCL2 and CD10 by the neoplastic B cells should always raise suspicion of a nodal follicular lymphoma involving the skin secondarily. Staining for IRF4/MUM1 and FOXP1 is negative in most cases; staining for CD5 and CD43 is always negative [2062,3623].

Postulated normal counterpart
A mature germinal centre B cell

Genetic profile
Antigen receptor genes
Clonally rearranged IG genes with somatic hypermutation are present, but may not be detectable by PCR [6,1325].

Cytogenetic abnormalities and oncogenes
In many studies, PCFCLs, including cases with a follicular growth pattern, do not show (or rarely show) BCL2 rearrangements [10,610,715,1318,1408,1409,1494,1524,1525,3164,4183]. However, other studies using PCR and/or FISH report BCL2 rearrangements in about 10–40% of PCFCLs with a follicular growth pattern, as well as in some totally diffuse cases [37,2016,2674,3859]. PCFCLs have the same gene expression profile as germinal centre B-cell– subtype diffuse large B-cell lymphomas, and often show amplification of REL [6,986,1653]. Deletion of chromosome 14q32.33 has been reported [986]. Unlike in primary cutaneous diffuse large B-cell lymphoma, leg type, inactivation of CDKN2A and CDKN2B gene loci on chromosome 9p21.3 by deletion or pro-
motor hypermethylation is only rarely found in PCFCL [986].

**Prognosis and predictive factors**
Irrespective of the growth pattern (follicular or diffuse), the number of blast cells, the presence of t(14;18) and/or BCL2 expression, or the presence of either localized or multifocal skin disease, PCFCLs have an excellent prognosis, with a 5-year survival rate of >95% [1408,1421, 2674,3517,3623,4499]. PCFCLs presenting on the leg are reported to have a less favourable prognosis [2062,3623]. In patients with localized or few scattered lesions, local radiotherapy is the preferred treatment [1530,3624]. Cutaneous relapses, observed in about 30% of cases, do not indicate progressive disease. Systemic therapy is only required for patients with very extensive cutaneous disease, extremely thick skin tumours, or extracutaneous disease.

![Fig. 13.92 Primary cutaneous follicle centre lymphoma, spindle-shaped morphology.](image)
- A Diffuse proliferation of centrocytes with a spindled morphology.
- B Detail of spindle-shaped cells.
- C Ki-67 stain shows high proliferation rate.
- D The cells are strongly positive for CD79a but (E) negative for BCL2.
Mantle cell lymphoma

Definition
Mantle cell lymphoma is a mature B-cell neoplasm usually composed of monomorphic small to medium-sized lymphoid cells with irregular nuclear contours; in >95% of cases, there is a \text{CCND1} translocation \{245,543,2219,2269,3849,4018\}. Neoplastic transformed cells (centroblasts), paraimmunoblasts, and proliferation centres are absent. Mantle cell lymphoma has traditionally been considered a very aggressive and incurable lymphoma, but more indolent variants, including leukaemic non-nodal mantle cell lymphoma and in situ mantle cell neoplasia, are now also well recognized.

Table 13.21  Morphological variants of mantle cell lymphoma

| Aggressive variants | Blastoid:  
|--------------------|-----------------  
| Cells resemble lymphoblasts with dispersed chromatin and a high mitotic rate (usually \(\geq 20-30\) mitoses per 10 high-power fields).  
| Pleomorph:  
| Cells are pleomorphic, but many are large with oval to irregular nuclear contours, generally pale cytoplasm, and often prominent nucleoli in at least some of the cells.  
| Other variants | Small-cell:  
| Cells are small round lymphocytes with more clumped chromatin, either admixed or predominant, mimicking a small lymphocytic lymphoma.  
| Marginal zone-like:  
| There are prominent foci of cells with abundant pale cytoplasm resembling marginal zone or monocytoid B cells, mimicking a marginal zone lymphoma; sometimes these paler foci also resemble proliferation centres of chronic lymphocytic leukaemia / small lymphocytic lymphoma.

Fig. 13.93  Mantle cell lymphoma involving the colon (multiple lymphomatous polyposis). Gross photographs.  
A Overview showing one large and multiple small polypoid mucosal lesions.  
B Closer view showing tiny polypoid mucosal lesions.

ICD-O codes
Mantle cell lymphoma 9673/3  
in situ mantle cell neoplasia 9673/1

Synonyms
Mantle zone lymphoma (obsolete); malignant lymphoma, lymphocytic, intermediate differentiation, diffuse (obsolete); malignant lymphoma, centrocytic (obsolete); malignant lymphomatous polyposis; in situ mantle cell lymphoma (for in situ mantle cell neoplasia)

Epidemiology
Mantle cell lymphoma accounts for approximately 3–10% of non-Hodgkin lymphomas [1]. It occurs in middle-aged to older individuals, with a median age of about 60 years. There is a variably marked male predominance, with a male-to-female ratio of \(\geq 2:1\) \{139,423,543,2219,3849,3854\}. Other extranodal sites are also important sites of disease \{139,423,2896,3849\}. Other extranodal sites are also frequently involved, including the gastrointestinal tract (where infiltration may be subclinical), Waldeyer ring, lungs, and pleura [1324,3487]. An uncommon but distinctive presentation is

Fig. 13.94  Mantle cell lymphoma, peripheral blood, cytological variation.  
A This typical mantle cell lymphoma demonstrates relatively small lymphoid cells with clumped chromatin and prominent nuclear clefts.  
B In contrast, the cells in this blastoid mantle cell lymphoma are larger and have prominent nucleoli.  
C This mantle cell lymphoma could easily be confused morphologically with chronic lymphocytic leukaemia.
with multiple intestinal polyps (so-called multiple lymphomatous polyposis), although these findings are not specific for mantle cell lymphoma [2134,2910,3459]. CNS involvement may occur, most commonly at the time of relapse [667].

**Clinical features**

Most patients present with stage III or, usually, stage IV disease with lymphadenopathy, hepatosplenomegaly, and bone marrow involvement [423,543,2896,3854]. Extravascular involvement, usually in the presence of extensive lymphadenopathy, is common. Peripheral blood involvement is also common, and can be identified by flow cytometry in almost all patients [1192]. Some patients have a marked lymphocytosis, which can closely mimic prolymphocytic leukaemia [139,423,2896], an acute leukaemia [4204], or chronic lymphocytic leukaemia. Some patients present with leukaemic non-nodular disease, sometimes with splenomegaly. These cases constitute a different variant of the disease (see below).

**Macroscopy**

Most cases of multiple lymphomatous polyposis constitute mantle cell lymphoma.

**Microscopy**

Classic mantle cell lymphoma is a monomorphic lymphoid proliferation with a vaguely nodular, diffuse, mantle zone, or rarely follicular growth pattern [245,2219,2269,3849,4018]. Mantle cell lymphoma with a mantle zone growth pattern should be distinguished from in situ mantle cell neoplasia (see discussion below). Most cases are composed of small to medium-sized lymphoid cells with slightly to markedly irregular nuclear contours, most closely resembling centrocytes. The nuclei have at least somewhat dispersed chromatin but inconspicuous nucleoli. Neoplastic transformed cells resembling centroblasts, immunoblasts, or paraimmunoblasts and proliferation centres are absent; however, foci mimicking proliferation centres may be present [3855A], and a spectrum of morphological variants is recognized, which can also cause diagnostic confusion (Table 13.21, p.285). The blastoid and pleomorphic variants are considered to be of important clinical significance. The small-cell variant is overrepresented among cases of leukaemic, non-nodular mantle cell lymphoma [1187], and the marginal zone–like variant is of greatest interest because of its potential confusion with marginal zone lymphomas. Mantle cell lymphoma in the peripheral blood or in bone marrow aspirates shows the same cytological spectrum that is seen in tissue sections; however, nucleoli are sometimes more prominent, even in cases of classic type. Although mantle cell lymphoma is not graded, evaluation of the proliferative fraction (either by counting mitotic figures or estimating the proportion of Ki-67-positive nuclei) is critical because of its prognostic impact.

Hyalinized small vessels are commonly seen. Many cases have scattered single epithelioid histiocytes, which in occasional blastoid or pleomorphic cases can create a so-called starry-sky appearance. Non-neoplastic plasma cells may be present, but true plasmacytic differentiation, which can be very marked, is seen only rarely [2645,3347,3851,4444,4445]. Splenic involvement, characterized by white pulp and variable red pulp infiltr-
Mantle cell lymphomas, with smaller cells with little cytoplasm centrally in the white pulp nodules and a peripheral zone where the neoplastic cells more closely resemble marginal zone cells, being somewhat larger and with more abundant cytoplasm.

Histological transformation to a typical diffuse large B-cell lymphoma does not occur; however, loss of a mantle zone growth pattern, increase in nuclear size, pleomorphism and chromatin dispersal, and increase in mitotic activity and Ki-67 expression can be seen in some cases at relapse [139,2219,2896,3849,4206]. Some such cases fulfil the criteria for a blastoid or pleomorphic mantle cell lymphoma (see below). Cases that are blastoid at diagnosis may relapse with a blastoid or pleomorphic variant [2769,2816,3721]. Caution is advised, because the specificity and sensitivity of SOX11 antibodies vary widely. Aberrant phenotypes have been described (sometimes in association with blastoid or pleomorphic variants), including absence of CD5 and expression of CD10 and BCL6 [41,535,1294,2769,2816,3721]. Rare cases express other antigens more typically associated with chronic lymphocytic leukaemia, such as LEF1 or CD200, with LEF1 more likely to be seen in blastoid or pleomorphic mantle cell lymphoma and CD200 in the leukaemic non-nodal variant [636,1115,1157,2633,3505]. Immunohistochemical staining often reveals loose meshworks of follicular dendritic cells.

**Postulated normal counterpart**

The postulated normal counterpart is a peripheral B cell of the inner mantle zone; this postulate is based in part on the growth pattern, with early involvement in lymphoid organs. The possibility that mantle cell lymphoma may derive from more than one B-cell compartment has also been suggested [2075]. Most cases are of pre-germinal centre origin, but some are of post-germinal centre origin.

**Genetic profile**

**Antigen receptor genes**

IG genes are clonally rearranged. IGV genes are unmutated or minimally mutated in most cases, but in 15–40% of cases, IG genes show somatic hypermutation, although the load of mutations is usually lower than in mutated chronic lymphocytic leukaemia [534,2008,2992,3950]. A biased use of the IGHV genes has been reported, suggesting that mantle cell lymphoma may originate from specific subsets of B cells [534,2008,2992,3950]. Together with other observations, this finding suggests that at least a substantial proportion of mantle cell lymphomas show evidence of antigenic drive [1509,4391].

**Cytogenetic abnormalities and oncogenes**

The t(11;14)(q13;q32) translocation between an IGH gene and CCND1 (encoding cyclin D1) is present in >95% of cases and is considered to be the primary genetic event [2301,3408,4095,4146,4323,4324]. Variant CCND1 translocations with the IG light chains have also been reported but are very uncommon. The translocation results in deregulated overexpression of CCND1 mRNA and protein [422,889,3627]. Some mantle cell lymphomas express aberrant transcripts, resulting in an increased half-life of cyclin D1. Tumours with these truncated transcripts have very high levels of cyclin D1 expression [422,889,3627], high proliferation rates, and more-aggressive clinical behaviour [3413]. Deregulated expression of cyclin D1 is assumed to overcome the cell cycle suppressive effect of RB and p27 in addition to other effects, leading to the development of mantle cell lymphoma [1841,3264]. Nevertheless, it is not sufficient by itself to lead to mantle cell lymphoma, as demonstrated both with animal models and by observations related to CCND1-rearranged clonal populations in the peripheral blood of healthy individuals [2247]. In addition to the molecular cytogenetic abnormalities described below,
tetraploid clones, which are more common. Mature B-cell neoplasms, such as mantle cell lymphoma, may also demonstrate abnormalities, leading to clinical and sometimes morphological progression. Modified from TP53 ties, in particular genetically stable for long periods of time and that preferentially involve the peripheral blood (PB), bone marrow (BM), and sometimes the spleen. However, even these MCLs can acquire additional molecular and cytogenetic abnormalities, including gains of 3q26 (p14ARF, in 18-31%), 11q22-23 (IGH/CCND1), and 8q24 (MYC, in 16-36%), as well as losses of 1p13-31 (in 29-52% of cases), 6q23-27 (TNFAIP3, in 23-38%), 9p21 (CDKN2A which codes for p16INK4a and p14ARF, in 18-31%), 11q22-23 (ATM, in 21-59%), 13q11-13 (in 22-55%), 13q14-34 (in 43-51%), and 17p13 (TP53, in 21-45%) [302,3444,3627]. SNP studies also demonstrate copy-neutral LOH in as many as approximately 60% of cases, often involving the same regions where copy losses are found, such as in the region of TP53 [303,3440]. High-level amplifications, such as of 18q21 (the site of the BCL2 locus) or of the translocated CCND1 region, are also found in a minority of cases [303]. Trisomy 12 has been reported in 25% of cases, but usually in the context of other alterations [847]. In addition to chromosomal imbalances, mantle cell lymphoma may also demonstrate tetraploid clones, which are more common in the pleomorphic variant (present in 80% of these cases) and the blastoid variant (in 36%) than in cases with typical morphology (present in 8%) [3007]. The t(8;14)(q24;q32) translocation and variant MYC translocations are rarely present, and are associated with an aggressive clinical course [4098]. BCL6 (3q27) translocations also occur uncommonly and are reportedly associated with BCL6 expression [535]. Some cytogenetic abnormalities may also have associations with various clinical parameters, including a leukemic presentation [1192,3071]. Oncogenic alterations have been found in genes targeting cell-cycle regulatory elements, the DNA damage response pathway, cell survival, and other pathways [305,1186,2115,2615,3280,4477]. Inactivating mutations of ATM at 11q22-23 have been detected in 40-75% of mantle cell lymphomas, as well as in the germline of some patients with mantle cell lymphoma [305,532,3550]. CCND1 is reported to be mutated in 35% of cases (usually IGH-soft mutated cases), KMT2D (MLL2) in 14%, NOTCH1 in 5-12%, and many other genes in < 10% of cases; some mutations, such as those of NOTCH1/2, are of prognostic and potential therapeutic importance [305,2115,2615,4477]. Mutations in some genes (ATM, KMT2D, and NOTCH1/2) have been reported only in SOX11-positive mantle cell lymphomas. Highly proliferative variants of mantle cell lymphoma have frequent TP53 mutations, homozygous deletions of CDKN2A and the cyclin-dependent kinase inhibitor CDKN2C, amplifications and overexpression of the BMLI polycomb and CDK4 genes, and occasional microdeletions of the RB1 gene [302,304,543,1453,1622,2399,3194,3196,4325,4326].

**Cyclin D1-negative mantle cell lymphoma**

Rare cases with the morphology and phenotype of mantle cell lymphoma are negative for cyclin D1 and t(11;14)(q13;q32) (IGH/CCND1) but have gene expression and global genomic profiles as well as other features, including clinical presentation and evolution, indistinguishable from those of cyclin D1-positive mantle cell lymphoma [1262,3413,3492,3494]. Cyclin D2 or cyclin D3 is highly expressed. Approximately half of these cases have CCND2 translocations, usually with an IG partner (often either IGK or IGL), and are associated with high cyclin D2 expression levels [3492]. Immunostaining for cyclin D2 or D3 is not useful in recognizing these cases, because such staining is also positive in other B-cell lymphomas; however, SOX11 staining is very useful [3492]. Diminished p27 staining (less intense than in the T-cell population) may also be helpful [3264]. In the absence of SOX11 staining, this diagnosis must be made with extreme caution, if at all, given the many lymphomas that can mimic mantle cell lymphoma. Very rare cases in which cyclin D1 expression cannot be demonstrated despite the presence of the CCND1 rearrangement do occur [3505]. Conversely, rare cases express cyclin D1 but lack a demonstrable CCND1 rearrangement [3851].

**Genetic susceptibility**

Familial aggregation of mantle cell lymphoma and of mantle cell lymphoma with other B-cell neoplasms has been reported [4032].

**Prognosis and predictive factors**

Mantle cell lymphoma is associated with a median survival of 3-5 years, with the vast majority of patients not being cured even with the newer therapeutic modalities being used today [46,543,544,651A,1037,1115,1187,2992,3854,4218].
In recent years, overall survival appears to have improved among at least some subsets of patients. A subset of asymptomatic patients with indolent disease who can be followed, at least initially, without any therapy has also been increasingly recognized and includes many of the patients with one of the clinicopathological mantle cell lymphoma variants described below [571,1037,1118,1187,2522,2992]. Assessment of proliferation rate is critical; high mitotic rate (>10–37.5 mitoses per 15 high-power fields or >50 mitoses/mm²) [139,423,3849,4001] and high Ki-67 proliferation index (variably defined, but with >30% as a currently accepted cut-off point) are associated with an adverse prognosis [962,1038,1416,1964,4001]. Cases with <10% Ki-67-positive cells have a more indolent course. Ki-67 expression has also been incorporated into the biological, prognostically important Mantle Cell Lymphoma International Prognostic Index (MIPI) score, which also includes patient age, Eastern Cooperative Oncology Group (ECOG) performance score, lactate dehydrogenase level, and white blood cell count [1319,1704]. In a major gene expression profiling study, the most significant prognostic indicator in mantle cell lymphoma was also the proliferation signature score, which further highlights the clinical importance of proliferation rate in this entity [3413]. The following features have been reported in at least some studies to be adverse prognostic factors (although they are not necessarily all independent of the proliferative fraction): blastoid or pleomorphic morphology, karyotypic complexity, TP53 mutation/overexpression/loss, CDKN2A deletion, and a variety of individual clinical parameters including overt peripheral blood involvement (at least in patients with adenopathy) [302,423,543,847,939,1453,2399,2892,3200,3494,3522,3849]. Lack of SOX11 expression has been associated with more-indolent mantle cell lymphoma in some studies; however, other studies have found these cases to be more aggressive, perhaps related to acquisition of TP53 abnormalities, which are present in a substantial subset of the cases [1187,2841,2892,2908]. The small-cell variant also appears to have a more indolent course; the impact of a mantle zone or nodular pattern is less certain, but at least some nodular cases are probably associated with a more indolent course [423,571,2219,2449,2896,3413,3849,4001]. The literature is not uniform with regard to the impact of individual chromosomal gains or deletions, limiting their utility. The prognostic value of 9p (CDKN2A) and 17p (TP53) deletions has been confirmed in clinical trials with new therapies, and seems to be independent of proliferation [939]. Other chromosomal alterations have been associated with a poor prognosis independent of the proliferative fraction, but these associations need to
be confirmed in patients treated with current therapeutic approaches [3494,3522].

**Leukaemic non-nodal mantle cell lymphoma**

Leukaemic non-nodal mantle cell lymphoma is defined as mantle cell lymphoma in which the patient presents with peripheral blood, bone marrow, and sometimes splenic involvement but without significant adenopathy (typically defined as peripheral lymph nodes < 1–2 cm and without adenopathy on CT, if performed) [1115,1187,2992]. Like in chronic lymphocytic leukaemia, it appears that these circulating cells may reversibly infiltrate extranodal inflammatory sites (e.g. with *Helicobacter pylori*-associated gastritis) and may remain localized to the mantle zone, overlapping with in situ mantle cell neoplasia. The neoplastic cells in leukaemic non-nodal mantle cell lymphoma are more likely to be small, resembling chronic lymphocytic leukaemia-type cells, SOX11-negative, and to have somatic IG hypermutation [928,1115,1187,2992]. CD5 expression may be less common than in other mantle cell lymphomas. Leukaemic non-nodal mantle cell lymphomas are more likely than classic mantle cell lymphomas to have <30–40% CD38 positivity, and at least a subset is more likely to have ≥2% CD200 positivity, a phenotype that may overlap with that of chronic lymphocytic leukaemia [1115,2992]. Cytogenetic studies show few abnormalities other than the *CCND1* translocation, whereas classic mantle cell lymphoma typically has greater genomic instability and a more complex karyotype [1187,3439]. Although not considered a prognostic indicator, mutated IG genes have been identified in patients with longer-term survival. Expression profiling and experimental studies suggest that these cases have a lack of tumour invasion properties and angiogenic potential, perhaps accounting for the absence of significant adenopathy [928,3039]. Patients with this variant have been reported to have a better prognosis than those with classic mantle cell lymphoma (median survival: 79 months) and are overrepresented in studies of patients with mantle cell lymphomas that have followed an indolent course, often not requiring therapy for long periods [1115,1187,2992]. However, some cases of this subtype may progress to an aggressive disease, with or without the development of lymphadenopathy, sometimes with rapidly progressive splenomegaly and sometimes with transformation to a blastoid or pleomorphic variant. Acquisition of *TP53* mutations or other oncogenic alterations may be associated with this aggressive evolution [3439]. Although monoclonal B-cell lymphocytosis of mantle cell lymphoma type is not recognized, there is a small proportion of healthy individuals (at least 7%) with circulating cells that have *IGH/CCND1* translocations detected with highly sensitive techniques, which can persist for years and may increase in number over time [2247]. A report of simultaneous development of mantle cell lymphoma in a recipient and donor 12 years after allogeneic bone marrow transplantation is also consistent with a very long latent period for mantle cell lymphoma after an initial event [750].

**In situ mantle cell neoplasia**

In situ mantle cell neoplasia, formerly referred to as in situ mantle cell lymphoma or mantle cell lymphoma-like B cells of uncertain/undetermined significance, is defined as the presence of cyclin D1-positive lymphoid cells with *CCND1* rearrangements restricted to the mantle zones of otherwise hyperplastic-appearing lymphoid tissue [571,2885]. Peripheral blood involvement or involvement at more than one site does not exclude the diagnosis, with some cases probably constituting the leukaemic non-nodal type of mantle cell lymphoma but with involvement of the mantle zone (i.e. in situ) in lymph nodes enlarged for some other reason. Extranodal involvement may also be present. The cyclin D1-positive cells are typically in the inner mantle zone, but may rarely be scattered throughout the mantle zone, in the outer mantle zone, or very rarely intrafollicular. When the mantle zones are expanded and completely replaced by cyclin D1-positive lymphoid cells, the diagnosis of overt mantle cell lymphoma with a mantle zone growth pattern is more appropriate. Compared with classic mantle cell lymphoma, these cases are more likely to be CD5-negative, and they include both SOX11-positive and a moderate number of SOX11-negative cases. These cases are very rare (none were found in two series of at least 100 hyperplastic lymph nodes) and are usually identified as an incidental finding, sometimes associated with other malignant lymphomas, when cyclin D1 immunohistochemical staining is performed [571,22A]. In situ mantle cell neoplasia often has an indolent course with long-term survival, frequently with stable disease even without therapy. However, caution is advised, because rare cases may progress to overt mantle cell lymphoma and occasional patients have been reported who have not done well [571,1949,3351]. The proportion of overt mantle cell lymphoma preceded by in situ mantle cell neoplasia is controversial.
Diffuse large B-cell lymphoma, NOS

Definition
Diffuse large B-cell lymphoma (DLBCL) is a neoplasm of medium or large B lymphoid cells whose nuclei are the same size as, or larger than, those of normal macrophages, or more than twice the size of those of normal lymphocytes, with a diffuse growth pattern. Morphological, biological, and clinical studies have subdivided DLBCLs into morphological variants, molecular subtypes, and distinct disease entities (Table 13.22). However, there remain many cases that may be biologically heterogeneous, but for which there are no clear and accepted criteria for subdivision. These cases are classified as DLBCL, NOS, which encompasses all cases that do not belong to a specific diagnostic category listed in Table 13.22. DLBCL, NOS can be subdivided into germinal centre B-cell (GCB) subtype and activated B-cell (ABC) subtype. Focal involvement of follicular structures in DLBCL, which can be highlighted by immunohistochemical staining for follicular dendritic cells, does not require the additional diagnosis of a grade 3B follicular lymphoma.

ICD-O codes
Diffuse large B-cell lymphoma, NOS 9680/3
Germinal centre B-cell subtype 9680/3
Activated B-cell subtype 9680/3

Epidemiology
DLBCL, NOS, constitutes 25–35% of adult non-Hodgkin lymphomas in developed countries, and a higher percentage in developing countries. It is more common in elderly individuals. The median patient age is in the seventh decade of life, but it can also occur in children and young adults. It is slightly more common in males than in females (1,95).

Etiology
The etiology of DLBCL, NOS, remains unknown. These tumours usually arise de novo (referred to as secondary), such as chronic lymphocytic leukaemia/small lymphocytic lymphoma, follicular lymphoma, marginal zone lymphoma, or nodular lymphocyte predominant Hodgkin lymphoma. Underlying immunodeficiency is a significant risk factor. DLBCLs, NOS, occurring in the setting of immunodeficiency are more often EBV-positive than sporadic cases. In DLBCL cases without an overt immunodeficiency, the EBV infection rate varies from 3% in western populations to approximately 10% in Asian and Latin American populations, and the lymphoma is typically of the ABC subtype (325, 1017,1367,1655,2701,2956,3696). Importantly, EBV positivity in most tumour cells should lead to a diagnosis of either EBV-positive DLBCL, NOS, or another specific type of EBV-positive lymphoma (e.g. DLBCL associated with chronic inflammation or lymphomatoid granulomatosis).

Localization
Patients may present with nodal or extranodal disease; as many as 40% of cases are confined to extranodal sites at least initially (1,95). The most common

![Image of involved spleen contains large tumour nodules.](Image)
extranodal site is the gastrointestinal tract (stomach and ileocecal region), but virtually any extranodal location can be primarily involved. Other common sites of extranodal presentation include the bone, testes, spleen, Waldeyer ring, salivary glands, thyroid, liver, kidneys, and adrenal glands. Primary CNS lymphoma and primary testicular lymphoma are both lymphomas of immune-privileged sites and therefore share some overlapping biology. DLBCLs involving the kidneys and adrenal glands are associated with an increased risk of spread to the CNS. Cutaneous lymphomas composed mostly of large B lymphocytes (i.e., primary cutaneous follicle centre lymphoma and primary cutaneous DLBCL, leg type) are considered distinct entities and discussed separately in this volume. Bone marrow involvement in DLBCL can be discordant (low-grade B-cell lymphoma in the marrow, seen in 10–25% of cases) or concordant (large cell lymphoma in the marrow, seen in a similar proportion of cases) [128, 474, 538, 3061, 3612]. The detection rate for minimal involvement may be increased with the use of ancillary techniques such as flow cytometry, immunohistochemistry, and molecular genetics [3883, 3884]. Recent studies have suggested that FDG-PET is a sensitive technique for detecting concordant bone marrow involvement, but is not reliable for discordant disease [24, 273, 2000]. The most recent consensus criteria for lymphoma staging indicate that a routine staging bone marrow biopsy is no longer required if FDG-PET is negative [691]. Morphologic involvement of the peripheral blood by DLBCL is rare.

Clinical features
Patients usually present with a rapidly enlarging tumour mass at single or multiple nodal or extranodal sites. Almost half of the patients have stage I or II disease, but the inclusion of PET/CT in the initial staging of DLBCL has resulted in stage migration, reducing the percentage of patients with limited-stage disease. Many patients are asymptomatic, but B symptoms may be present. Specific localizing symptoms may be present and are highly dependent on the site of extranodal involvement [1, 148].

Microscopy
Lymph nodes demonstrate partial or more commonly total architectural effacement by a diffuse proliferation of medium or large lymphoid cells. Partial nodal involvement may be interfollicular and/or less commonly sinusoidal. The perinodal tissue is often infiltrated. Broad or fine bands of sclerosis may be present. The morphology of DLBCL, NOS, is diverse and the disease can be divided into common and rare morphological variants. For this reason, ancillary studies are critical before making the diagnosis of DLBCL, NOS. Cases with medium-sized cells are particularly prone to misclassification. Special studies are required to exclude extramedullary leukaemias, Burkitt lymphoma, high-grade B-cell lymphoma with MYC and BCL2 and/or BCL6 rearrangements, and blastoid mantle cell lymphoma.

Common morphological variants
Three common and several rare morphological variants have been recognized. All variants may be admixed with a high number of T cells and/or histiocytes. These cases should not be categorized as T-cell/histiocyte-rich large B-cell lymphoma as long they do not fulfil all the criteria for that entity.

Centroblastic variant: This is the most common variant. Centroblasts are medium-sized to large lymphoid cells with usually oval to round, vesicular nuclei containing fine chromatin. There are 2–4 nuclear membrane-bound nucleoli. The cytoplasm is usually scant and amphophilic or basophilic. In some cases, the tumour is monomorphic, i.e. it is composed almost entirely (>90%) of centroblasts. Centroblastic cases are more frequently of the GCB subtype [3411]. However, in most cases the tumour is polymorphic with an admixture of
centroblasts and immunoblasts (<90%) [1105,3009]. The tumour cells may have multilobated nuclei, which predominate in rare instances, especially in tumours localized to the bone and other extranodal sites.

Immunoblastic variant: In this variant, >90% of the cells are immunoblasts, with a single centrally located nucleolus and an appreciable amount of basophilic cytoplasm. Immunoblasts with plasmacytoid differentiation may also be present. Clinical and/or immunophenotypic findings may be essential in for differentiating this variant from extramedullary involvement by a plasmablastic lymphoma or an immature plasma cell myeloma. The distinction of the immunoblastic variant from the common centroblastic variant has generally shown poor intraobserver and interobserver reproducibility [1105,3009].

Anaplastic variant: This variant is characterized by large to very large cells with bizarre pleomorphic nuclei that may resemble, at least in part, Hodgkin/Reed-Sternberg cells, and may resemble the neoplastic cells of anaplastic large cell lymphoma. The cells may show a sinusoidal and/or a cohesive growth pattern and may mimic undifferentiated carcinoma (1548). The anaplastic variant is biologically and clinically unrelated to anaplastic large cell lymphoma, which is often of cytotoxic T-cell derivation, and unrelated to ALK-positive large B-cell lymphoma.

Rare morphological variants
Rare cases of DLBCL, NOS, have a myxoid stroma or a fibrillary matrix. Pseudorosette formation is rarely seen. Occasionally, the neoplastic cells are spindle-shaped or display features of signet ring cells. Cytoplasmic granules, microvillous projections, and intercellular junctions can also be seen ultrastructurally.

Immunophenotype
The neoplastic cells typically express pan-B-cell markers such as CD19, CD20, CD22, CD79a, and PAX5, but may lack one or more of these. Surface and cytoplasmic immunoglobulin (most commonly IgM, followed by IgG and IgA) can be demonstrated in 50–75% of the cases. The presence of cytoplasmic immunoglobulin does not correlate with the expression of plasma cell–associated markers such as CD138. CD138 is rarely coexpressed in CD20-positive cells. CD30 may be expressed in 10–20% of cases, especially in the anaplastic variant [1717,3696]. The presence of EBV in most of the cells should lead to a diagnosis of EBV-positive DLBCL, NOS; most of these cases are CD30-positive.

The neoplastic cells express CD5 in 5–10% of the cases [4399,4406]. CD5+ DLBCLs usually constitute de novo DLBCL, only rarely arising from chronic lymphocytic leukaemia / small lymphocytic lymphoma. CD5+ DLBCL can be distinguished from the blastoid or pleomorphic variant of mantle cell lymphoma by the absence of cyclin D1 and/or SOX11 expression [4467]. Rare cases express cyclin D1 in the absence of CCND1 translocation and SOX11 expression. However, it is usually not as strong and uniform as in MCL [1714,2959,4170].

The expression of MYC and BCL2 varies considerably, in part depending on the threshold used to define positivity [1440, 1686,1718,1866,2048,2049,2446,3141, 3601,4113,4415]. In most studies, BCL2 is considered positive if ≥50% of the tumour cells are positive, and MYC is considered positive if ≥40% of the tumour cell nuclei are positive. Coexpression of these two proteins (so-called double expressers) is more frequent in the ABC subtype (see below) [1866,3601].

The reported incidence of CD10, BCL6, IRF4/MUM1, FOXP1, GCET1, and LMO2 expression varies. The Hans algorithm uses three markers to distinguish the GCB from the non-GCB subtype: CD10, BCL6, and IRF4/MUM1 are each considered positive if ≥30% of the tumour cell stain positively [1537]. CD10 is positive in 30–50% of cases, BCL6 in 60–90%, and IRF4/MUM1 in 35–65% [348,766,902,2790]. Unlike in normal GCBs, in which expression of IRF4/MUM1 and BCL6 is mutually exclusive, coexpression of these markers is found in 50% of DLBCLs [1141]. FOXP1 expression has been reported in about 20% of DLBCL cases that lack the germinal centre phenotype and express IRF4/MUM1 and BCL2 in the absence of t(14;18) (q32,q21.3) [271]. GCT1, a germinal centre marker, is expressed in 40–50% of cases and is highly correlated with the GCB type [2703]. LMO2 expression is found in approximately 45% of DLBCL and is highly correlated with the germinal centre markers CD10, BCL6, and HGal, but not with IRF4/MUM1 or BCL2 [2840]. Expression of BCL2 varies between reports, with a range of 47–84% [902,1305,1619,1770]. The observed frequency may also vary depending on the BCL2 antibody used [1990]. In the GCB subtype, BCL2 expression is closely linked to the presence of t(14;18)(q32;q21.3), whereas expression is more common in the ABC subtype, but is the result of copy number gains and transcriptional upregulation [2761,3601].

The Ki-67 proliferation index is high; it is usually much more than 40% and may be >90% in some cases [1468,1589,2069,2664,4430]. Expression of p53 is seen in 20–60% of cases, but is more common than the presence of mutations, suggesting upregulation of the wildtype TP53 in some cases [701,2279,2312,4400,4445].

Cell of origin/postulated normal counterpart
The postulated normal counterparts are peripheral mature B cells of either germinal centre origin (GCB subtype) or germinal centre exit / early plasmablastic or post-germinal centre origin (ABC subtype). Cell of origin distinctions underlie fundamentally different biology based on gene expression, chromosomal aberrations, and recurrent mutations; they are also associated with reproducible survival differences in patients treated with the CHOP chemotherapy regimen.
plus rituximab (R-CHOP). The accurate distinction of the GCB from the ABC subtype is an important predictive factor in DLBCL, NOS.

**Cell of origin subtyping**

On the basis of gene expression profiling, DLBCL can be divided into two main molecular subgroups: the GCB subtype and the ABC subtype. Approximately 10–15% of cases cannot be included in either of these subtypes and remain unclassified [67,2272,2273,3411,4372]. The relative frequencies of the GCB subtype and the ABC subtype vary based on geographical location, median age of the patient population, and methodology used, but are typically about 60% and 40%, respectively [3601]. The frequency of the GCB subtype is lower in Asian countries {1745,2308,3061,3615,3663,4383}. Low density gene expression analysis platforms can recapitulate these same groups, and many are applicable to formalin-fixed, paraffin-embedded material {2505,3601,3603}. Numerous immunohistochemical algorithms exist, but most are binary classifiers (i.e. two-class predictors) [738,830,1537,2652,2790,2838,2909,4199]. For example, the Hans algorithm classifies DLBCL into the two subgroups of GCB subtype and non-GCB subtype, and thus does not recognize the unclassified cases. Although routinely available, all immunohistochemical algorithms suffer from a lack of reproducibility and accuracy, but the determination of cell of origin has begun to penetrate clinical practice and is therefore required [830,3325]. Enrolment in current clinical trials requires the determination of cell of origin status, because preliminary data from phase I/II trials suggest that the benefit from the addition of bortezomib, lenalidomide, and ibrutinib to R-CHOP is preferentially seen in the ABC subtype {1057,2562,2902,2903,2932,4334,4419}. Therefore, the distinction between the GCB subtype and the ABC subtype should be made for all cases of DLBCL, NOS, at diagnosis. If gene expression technologies are not available, then immunohistochemistry technologies are considered an acceptable alternative. The algorithm used should be specified.

**Genetic profile**

**Antigen receptor genes**

Clonally rearranged IG heavy and light chain genes are detectable. The IG genes show ongoing somatic hypermutation in the GCB subtype or evidence of prior somatic hypermutation in the ABC subtype.

**Mutation landscape**

Several studies have examined the mutation landscape of DLBCL. (Table 13.23), implicating many novel mutations in the pathogenesis of this disease {2384,2745,3085,4476}. For many genes, the frequency of specific mutations varies depending on the cell of origin subtype; others are found in both the GCB subtype and the ABC subtype. For example, mutations in EZH2 and GNA13 are seen almost exclusively in the GCB subtype, whereas CARD11, MYD88, and CD79B mutations are characteristic of the ABC subtype {886,2744,2745,2860}. Recurrent copy number gains have also been studied. Gains and deletions of chromosomal material are common and are differentially seen across the cell of origin subtypes (Table 13.23) [2274,2718]. For example, GCB DLBCLs often harbour gains or amplification of 2p16 and 8q24 and deletions of 1p36 and 10q23; ABC cases show gains of 3q27, 11q23-4, and 18q21 and deletions of 6q21 and 9p21 {306,1433,1839,2274,2718,3081,3085,3545,3578,3607}. Copy number gains and amplifications of the MYC locus vary in frequency; they occur in both the GCB subtype and the ABC subtype, but are slightly more common in GCB DLBCL (2404,3601,4432). Candidate gene studies of DLBCL arising at specific extranodal sites have, in some cases, shown both overlapping and unique molecular features. Primary breast DLBCL arising in women is uncommon, but studies reveal that most cases are of the ABC subtype and show recurrent MYD88 L265P and CD79B mutations, similar to nodal ABC DLBCL, NOS {3900}. Rearrangements of either BCL2 or BCL6 are rare. Primary gastric DLBCLs are mostly of the ABC subtype. Recurrent mutations of MYD88 and CD79B are uncommon. Translocations involving BCL6 occur in a subset of cases, as do MYC rearrangements, but BCL2 translocations are uncommon {687}. Primary DLBCLs are common testicular tumours in elderly men, and like primary CNS lymphomas, they are considered lymphomas of immune-privileged sites. Testicular DLBCL, NOS, can spread to the CNS and the contralateral testis {954}. Most cases are of the ABC subtype. Molecular studies reveal a high frequency of MYD88 mutations, accompanied in a subset of cases by CD79B mutations {2098}. Occasional
cases show translocations of MYC or BCL6, but BCL2 translocations are rare. Recent data reveal copy-number gains of the 9p21 locus, the site of the ligands of PD1 [665]. In addition, rare translocations of the ligands are also seen, characterized by promotor substitution with promiscuous genes actively transcribed in B cells, leading to overexpression of PDL1, PDL2, or both proteins in primary testicular DLBCL [665,4082]. Further evidence of an immune escape phenotype is loss of major histocompatibility complex (MHC) class I and II expression, which is also a common genetic alteration in primary testicular DLBCL resulting from deletion of HLA loci on chromosome 6p21.3 [415,1883,3355,3356].

Chromosomal translocations
As many as 30% of cases show rearrangement of the 3q27 region involving BCL2, which is the most common translocation in DLBCL [40,272,288,1769,2396,2930,3667]. These rearrangements tend to occur more commonly in the ABC subtype [1768,3601,4422]. Translocation of the BCL2 gene, i.e. t(14;18)(q32;q21.3), a hallmark of follicular lymphoma, occurs in 20–30% of DLBCL cases, more commonly in the GCB subtype, where it is present in about 40% of cases and is closely associated with BCL2 and CD10 protein expression [270,1719,1769,1771,3601,4200]. MYC rearrangement is observed in 8–14% of cases, evenly distributed between the GCB subtype and the ABC subtype; unlike in Burkitt lymphoma, it is typically associated with a complex karyotype [44,269,802,848,1440,1686,1718,1866,2074,2100,3537,4085,4200]. Approximately half of the DLBCL cases that harbour a MYC translocation also show a BCL2 and/or BCL6 translocation, and therefore belong in the newly created category of high-grade B-cell lymphoma with MYC and BCL2 and/or BCL6 rearrangements (so-called double-hit lymphoma) [194,802,1865,1943,2878,3117,3601]. Cases with otherwise typical DLBCL morphology and an isolated MYC translocation belong in the category of DLBCL, NOS [802,1865,4398]. A variable proportion of the MYC translocations involve IG loci as partners: IGH, IGK, or IGL. Others partners include non-IG loci such as PAX5, BCL6, BCL11A, IKZF1 (IKAROS), and BTG1 [193,362,1865,3118]. The ability to detect MYC translocations with non-IG partner

Table 13.23 Genetic, molecular, and clinical characteristics of the diffuse large B-cell lymphoma (DLBCL) subtypes and primary mediastinal large B-cell lymphoma (PMBL)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>ABC DLBCL</th>
<th>GCB DLBCL</th>
<th>PMBL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rearrangements</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BCL2</td>
<td>&lt;5%</td>
<td>40%</td>
<td>0%</td>
</tr>
<tr>
<td>BCL6</td>
<td>25–30%</td>
<td>15%</td>
<td>0%</td>
</tr>
<tr>
<td>MYC, single hit</td>
<td>5–6%</td>
<td>5–8%</td>
<td>0%</td>
</tr>
<tr>
<td>CD74/PDCD1LG2 (also called PDL1/2)</td>
<td>Rare</td>
<td>Rare</td>
<td>20%</td>
</tr>
<tr>
<td>CIITA</td>
<td>Rare</td>
<td>Rare</td>
<td>38%</td>
</tr>
<tr>
<td>TBL1XR1</td>
<td>0%</td>
<td>5%</td>
<td>0%</td>
</tr>
<tr>
<td>Copy-number aberrations</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1p36.32 deletion (TNFRSF14)</td>
<td>Rare</td>
<td>30%</td>
<td>Rare</td>
</tr>
<tr>
<td>2p16 gain/amplification (REL)</td>
<td>Rare</td>
<td>30%</td>
<td>60–75%</td>
</tr>
<tr>
<td>3q27 gain/amplification</td>
<td>45%</td>
<td>15–20%</td>
<td>Rare</td>
</tr>
<tr>
<td>6q21 deletion (PRDM1)</td>
<td>45%</td>
<td>25%</td>
<td>n/a</td>
</tr>
<tr>
<td>9p21 deletion (CDKN2A)</td>
<td>40%</td>
<td>20%</td>
<td>Rare</td>
</tr>
<tr>
<td>9p24.1 gains/amplification (CD74/PDCD1LG2)</td>
<td>Uncommon</td>
<td>Uncommon</td>
<td>60–75%</td>
</tr>
<tr>
<td>18q21.3 gain/amplification (BCL2)</td>
<td>55%</td>
<td>15%</td>
<td>Rare</td>
</tr>
<tr>
<td>Recurrent mutations</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EZH2</td>
<td>Rare</td>
<td>20–25%</td>
<td>n/a</td>
</tr>
<tr>
<td>GNA13</td>
<td>Rare</td>
<td>25%</td>
<td>n/a</td>
</tr>
<tr>
<td>KMT2D (also called MLL2)</td>
<td>Rare</td>
<td>35%</td>
<td>40%</td>
</tr>
<tr>
<td>TP53</td>
<td>25%</td>
<td>20%</td>
<td>n/a</td>
</tr>
<tr>
<td>MEF2B</td>
<td>5%</td>
<td>15–20%</td>
<td>n/a</td>
</tr>
<tr>
<td>SGK1</td>
<td>5–10%</td>
<td>15–20%</td>
<td>n/a</td>
</tr>
<tr>
<td>CREBBP</td>
<td>10%</td>
<td>30%</td>
<td>n/a</td>
</tr>
<tr>
<td>TNFRSF14</td>
<td>Rare</td>
<td>30%</td>
<td>n/a</td>
</tr>
<tr>
<td>SOCS1</td>
<td>Uncommon</td>
<td>10–15%</td>
<td>40%</td>
</tr>
<tr>
<td>PTPN1</td>
<td>n/a</td>
<td>n/a</td>
<td>20%</td>
</tr>
<tr>
<td>STAT6</td>
<td>Rare</td>
<td>5%</td>
<td>35%</td>
</tr>
<tr>
<td>CARD11</td>
<td>10–15%</td>
<td>10–15%</td>
<td>n/a</td>
</tr>
<tr>
<td>CD79B</td>
<td>20–25%</td>
<td>Uncommon</td>
<td>n/a</td>
</tr>
<tr>
<td>MYD88</td>
<td>35%</td>
<td>Uncommon</td>
<td>n/a</td>
</tr>
<tr>
<td>PRDM1</td>
<td>15%</td>
<td>Rare</td>
<td>n/a</td>
</tr>
<tr>
<td>B2M</td>
<td>15–20%</td>
<td>20–25%</td>
<td>n/a</td>
</tr>
<tr>
<td>CD58</td>
<td>10%</td>
<td>10%</td>
<td>n/a</td>
</tr>
<tr>
<td>Pathway perturbations</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NF-kappaB activation</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>PI3K/AKT</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>JAK/STAT signalling</td>
<td>Rare</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Immune escape</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Clinical features</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-year PFS (R-CHOP)</td>
<td>40–50%</td>
<td>70–80%</td>
<td>85–90%</td>
</tr>
</tbody>
</table>

ABC, activated B-cell subtype; GBC, germinal centre B-cell subtype; n/a, not available; PFS, progression-free survival; R-CHOP, CHOP chemotherapy regimen plus rituximab.

a Some cases with recurrent mutations also harbour copy number alterations of the same locus, in particular alterations associated with loss of function.
Mature B-cell neoplasms

Genetic susceptibility
Recent case control studies have identified genetic loci that may predispose individuals to the development of DLBCL (602,2135,3691,3704). Some of the findings in European studies have been replicated in eastern Asian populations, suggesting a common risk [286,3868]. The identities of the candidate genes in these loci suggest that immune recognition and immune function may underlie the pathogenesis of DLBCL [602].

Prognosis and predictive factors

Clinical features
In the R-CHOP era, the 5-year progression-free and overall survival rates are approximately 60% and 65%, respectively [3611]. Disease stage and patient age are significant factors affecting survival. The International Prognostic Index (IP), which incorporates five clinical variables, remains a valuable prognostic tool, although newer variations have been described that are better able to identify patients with the highest-risk clinical features [3610,4491]. Other clinical prognostic factors associated with inferior outcome include tumour bulk (masses ≥10 cm), male sex, vitamin D deficiency, low body mass index, elevated serum free light chains, monoclonal serum IgM proteins, low absolute lymphocyte/monocyte count, and discordant (but not discordant) bone marrow involvement [568,831,1035,1673,2586,2797,3161,3220,3612,4314]. Discordant bone marrow involvement also predicts an increased risk of CNS relapse and in some centres dictates CNS prophylaxis.

Morphology
There are many conflicting reports on the prognostic impact of immunoblastic features [1105,3009]. Some studies have found an adverse prognostic impact of immunoblastic morphology, whereas others have not. Reproducibility and variable criteria remain significant obstacles in these studies. The immunoblastic variant is associated with MYC translocations, typically involving IG loci. These cases frequently express CD10 [1685].

Immunophenotype
Many immunohistochemical markers have been reported to have prognostic impact, but most have not been validated and are therefore not accepted as robust or routine biomarkers [212,2397,2917]. All biomarkers require reassessment using standard of care therapy. De novo CD5+ DLBCL is variably reported to have prognostic importance [754,2681,2879,4399]. It is often associated with high-risk clinical features, especially in Asian countries, and is usually of ABC subtype [1110,2681,4406]. BCL2 and BCL6 are examples of biomarkers of which the reported prognostic effect was altered by the addition of rituximab to the CHOP chemotherapy regimen [2766,4337]. Many of these immunohistochemistry-based biomarkers reflect biology, but are not predictive. Moreover, the results obtained in these studies are often conflicting, and their interpretation is therefore controversial. The current emphasis is on developing predictive biomarkers in DLBCLs where knowledge of a biomarker impacts an initial or subsequent therapeutic decision. Predictive markers currently include markers for the determination of cell of origin (i.e. GCB subtype vs ABC subtype) being tested now in the context of phase III clinical trials and markers of the presence of relevant oncogene translocations (see below), in particular involving the MYC gene [3601]. A recent meta-analysis clearly established the prognostic significance of cell of origin as determined by gene expression profiling, but not based on most immunohistochemical algorithms [3325]. More controversial is the assessment of
the double-expression status of MYC and BCL2 proteins, found in approximately 30% of all cases of DLBCL, NOS, and associated with inferior survival in most studies [2686,3523]. Double-expression status also predicts an increased risk of CNS relapse in DLBCL, NOS, and is independent of the CNS International Prognostic Index (CNS-IPI) [3539]. Cases with MYC and BCL2 double-expression that have rearrangements of these genes or of MYC and BCL6 belong in the high-grade B-cell lymphoma, with MYC and BCL2 and/or BCL6 rearrangements category. CD30 expression in EBV-negative DLBCL (occurring in ~10–20% of cases), excluding primary mediastinal large B-cell lymphomas, is associated with a favourable outcome in some studies [1542,1717,3696]. CD30 expression might have therapeutic implications with the existence of anti-CD30 therapy.

**Proliferation**

The prognostic importance of a high proliferative fraction, as assessed by the Ki-67 proliferation index, is controversial. The findings of studies from both the CHOP and the R-CHOP eras are often conflicting and are typically confounded by the lack of consideration of patient age, other clinical variables, and the cell of origin status [1866,2069,2922,4085].

**Genetics**

In some studies, the presence of a BCL2 translocation is associated with inferior outcome in GCB DLBCL in patients treated with R-CHOP [270,1769,4200]. BCL2 copy-number gain predicts inferior survival in the ABC subtype [2404]. Translocation of BCL6 is more frequent in ABC DLBCL, and in some studies it has been associated with improved survival [272,1768,3667]. MYC translocations occur in about 8–14% of DLBCL, NOS, cases and are associated with inferior survival [269,802,3537,4085]. Published data are confounded by whether or not FISH is also performed for additional oncogenes, including BCL2 and BCL6. Most studies have confirmed that MYC and BCL2 double-hit lymphomas are much more common among GCB cases and are associated with inferior survival [194,269,1665,3601]. The prognostic relevance of MYC and BCL6 translocations is more controversial, because studies are contradictory [2304,3183,4422]; these translocations are more common within the ABC subtype [3183,3601,4422]. These double-hit lymphomas are now excluded from DLBCL, NOS and diagnosed as high-grade B-cell lymphoma with MYC and BCL2 and/or BCL6 rearrangements (p.335). Cases of DLBCL with MYC translocations only are also associated with decreased survival in some series [551,778,802,848,4085,4398]. The results for MYC copy-number gains or amplification suggest inferior outcomes, but are inconsistent, in part due to the varying definitions of gain versus amplification [2404,3770,3947,4109,4432]. TP53 loss and/or mutations are associated with inferior survival [4400,4445]. Deletions of the CDKN2A locus on chromosome 9p21 and trisomy 3 are also associated with diminished survival, in particular with the ABC subtype [1839,2271]. Definitive data regarding the prognostic role of other recurrently mutated genes in DLBCL, with the exception of FOXO1, are lacking [4060]. There are increasing expectations that at least some of the mutations found in DLBCL will become important in the development of future targeted therapies [1763,3405].

**Microenvironment**

Gene expression profiling studies indicate a prognostic role for both non-neoplastic cells and extracellular matrix components in the tumour microenvironment in DLBCL [3600]. Stromal-1 (extracellular matrix deposition and histiocytic infiltration) and stromal-2 (tumour blood vessel density/angiogenesis) signatures have been shown to be prognostic in the current R-CHOP treatment era [2273]. A two-gene expression signature including one gene representing the microenvironment (TNFRSF9) has also been shown to predict prognosis [68]. Mutation landscape studies in DLBCL highlight a number of recurrently mutated genes targeting the cross-talk between malignant B cells and non-neoplastic cells, including mutations and aberrant protein expression of beta-2 microglobulin and CD58 [635,2745]. Congruent data have also shown that loss of MHC class II is associated with decreased tumour-infiltrating CD8+ T cells and inferior outcome [3360,3361]. Immune escape mechanisms are also important oncogenic drivers in DLBCL. Overexpression of PDL1 in DLBCL, NOS, has been shown to be associated with inferior survival [2036]. The prognostic role of other immune cells and the assessment of these cells in the peripheral blood remain less well studied.

**MicroRNA**

Several studies have linked specific microRNA expression patterns with outcome in DLBCL [56,1772]. More recently, somatic mutations involving microRNAs have been shown to be prognostic in DLBCL and to be independent of both cell of origin and the IPI [2328].

**Host genetics**

Analysis of host genetics has recently been shown to be prognostic in DLBCL, NOS. SNPs involving loci at 5q23.2 and 6q21 have been associated with event-free survival in patients with DLBCL treated with R-CHOP [1351].

**Therapy**

The standard of care for the treatment of advanced-stage DLBCL, NOS, is R-CHOP. Other regimens exist, but it is unclear whether these provide an overall survival benefit [3611]. Attempts to improve the survival of ABC DLBCLs are currently being made with the addition of novel agents to an R-CHOP backbone [1057,2562,2902,2903,2932,4334,4419].
T-cell/histiocyte-rich large B-cell lymphoma

Definition
T-cell/histiocyte-rich large B-cell lymphoma (THRLBCL) is characterized by a limited number of scattered, large B cells embedded in a background of abundant T cells and histiocytes. THRLBCL may arise de novo; however, more recent data suggest the possibility of a closer relationship with progression forms of nodular lymphocyte predominant Hodgkin lymphoma (NLPHL) than previously thought, indicating that NLPHL may proceed to or contain areas indistinguishable from THRLBCL. In small biopsies in particular, differentiating between a progression form of NLPHL (i.e. NLPHL with THRLBCL-like transformation) and de novo THRLBCL may be difficult, if not impossible.

ICD-O code
9688/3

Synonyms
T-cell-rich B-cell lymphoma; B-cell lymphoma rich in T cells and simulating Hodgkin disease; histiocyte-rich/T-cell-rich large B-cell lymphoma; T-cell-rich large B-cell lymphoma; T-cell-rich/histiocyte-rich large B-cell lymphoma; histiocyte-rich large B-cell lymphoma

Epidemiology
THRLBCL mainly affects middle-aged men. It accounts for < 10% of all DLBCLs.

Localization
THRLBCL mainly affects the lymph nodes, but bone marrow, liver, and spleen involvement is frequently found at diagnosis.

Clinical features
Patients present with fever, malaise, splenomegaly, and/or hepatomegaly. At diagnosis, almost half of cases are at an advanced Ann Arbor stage, with an intermediate-risk to high-risk International Prognostic Index (IPI) score (Table 13.24). The disease is often refractory to the chemotherapy regimens currently in use.

Imaging
Because of the important differential diagnosis with NLPHL, clinical staging procedures (including imaging analyses) are important. NLPHL usually affects one or two regions, whereas THRLBCL frequently manifests as systemic disease. Because THRLBCL is more PET-avid than is NLPHL, staging procedures such as FDG-PET and CT may facilitate the differential diagnosis [246].

Microscopy
THRLBCL has a diffuse or less commonly vaguely nodular growth pattern replacing most of the normal lymph node parenchyma. It is composed of scattered, single large B cells embedded in a background of small T cells and variable numbers of histiocytes. The tumour cells are always dispersed and do not form aggregates or sheets. These cells may mimic the neoplastic lymphocyte predominant (LP) cells of NLPHL, but usually show greater variation in size and, in some cases, may resemble centroblasts or more pleomorphic cells, mimicking Reed-Sternberg or Hodgkin cells [19,3299]. They are typically found within clusters of bland-looking non-epithelioid histiocytes that may not be obvious on conventional examination. These histiocytes are a main and distinctive component of THRLBCL and are useful for the diagnosis [1448].

Nearly all of the background lymphocytes are of T-cell lineage, with typically only very few scattered B cells. Meshworks of follicular dendritic cells are absent. Eosinophils and plasma cells are not found. De novo THRLBCLs are usually diffuse and do not show the typical small B-cell background of NLPHL. However, there are cases of NLPHL in which the small B cells are diminished in number, and in
which nodular very T-cell rich areas can be seen. Follow-up data suggest that these variant histologies may negatively affect prognosis [1572], and there are some cases of histological progression in NLPHL, in which the process is entirely diffuse and the histological appearance is virtually indistinguishable from that of de novo THRLBCL, constituting THRLBCL-like transformation. More recent genetic and gene expression data suggest that the relationship between de novo THRLBCL and secondary THRLBCL may be closer than previously thought.

In cases predominantly affecting the spleen, there is a multifocal or micronodular involvement of the white pulp; in the liver, the lymphomatous foci are localized in the portal tracts [1014]. In these extranodal locations as well as in the bone marrow, the lymphoma is characterized by the same composition as in the lymph node.

On recurrence, the number of atypical cells may increase, resulting in a picture of DLBCL, which portends an inferior outcome [19].

Several studies have recognized cases with similar morphology but without histiocytes. Whether these cases constitute the same entity as typical THRLBCL is not yet clear. Studies including cases rich in T cells with and without histiocytes have defined a more heterogeneous group of large B-cell lymphomas, which probably include more than one entity [13,1448, 2118, 2330, 3299]. Further studies should clarify the relationship between these lymphomas. Although cases lacking significant numbers of histiocytes may currently be included in the THRLBCL category, the paucity or absence of histiocytes should be noted. Lymphomas containing B cells with a spectrum of cell size, morphology, and distribution (clusters or sheets of medium-sized to large B cells) should not be included within the category of THRLBCL, and may be considered a subtype of DLBCL, NOS.

**Immunophenotype**

The large atypical cells express pan-B-cell markers such as CD19, CD20, and CD79a. BCL6 is also positive. A variable number stain for BCL2 and EMA, and no expression of CD15, CD30, or CD138 is found. The background is composed of variable numbers of CD68+ and CD163+ histiocytes and CD3- and CD5+ T cells. T-cell rosettes around the tumour cells and remnants of B-cell follicles or clusters of small B lymphocytes are absent in de novo THRLBCL [2822]. CD279/PD1 rosettes are a feature of NLPHL, and may be seen in cases progressing to a diffuse pattern resembling THRLBCL. However, the presence or absence of CD279/PD1+ T cells is not specific for NLPHL. Lack of residual IgD+ mantle cells and lack of follicular dendritic cell meshworks are of further diagnostic help in differentiating de novo THRLBCL from NLPHL [18,1240].

There are aggressive B-cell lymphomas, rich in reactive T cells, in which the neoplastic cells are sparse and EBV-positive. In some cases, the neoplastic cells exhibit a Hodgkin-like morphology. Such cases should not be classified as THRLBCL, and should be considered within the spectrum of EBV-positive DLBCL [2330, 2867].

**Postulated normal counterpart**

A germinal centre B cell

**Genetic profile**

The tumour B cells harbour clonally rearranged IG genes carrying high numbers of somatic mutations and intraclonal diversity indicating derivation from germinal centre cells [487].

Limited karyotypic studies failed to show recurrent abnormalities. Comparative genomic hybridization on microdissected tumour cells demonstrated more imbalances in NLPHL than in THRLBCL [1246]. However, more recent array comparative genomic hybridization studies showed that the number of genomic aberrations was higher in THRLBCL than in typical and THRLBCL-like variants of NLPHL [1571]. Gains of 2p16.1 and losses of 2p11.2 and 9p11.2 were recurrent aberrations in both typical and THRLBCL-like variants of NLPHL, as well as in THRLBCL. Expression of the REL protein was observed at similar frequencies in NLPHL and THRLBCL. Gene expression profiling has identified a subgroup of DLBCL characterized by a host immune response and a very bad prognosis [2719], which includes most of the cases diagnosed as THRLBCL. Microdissected histiocytes from NLPHL and THRLBCL showed similar gene expression profiles, expressing genes related to proinflammatory and regulatory macrophage activity. Unlike histiocytes of NLPHL, those from THRLBCL strongly expressed metal-binding proteins [1576]. Overall, more recent expression profiling and genetic studies have revealed similarities between NLPHL and THRLBCL that suggest that these entities may constitute a pathobiological continuum with various clinical presentations [1570, 1576].

**Prognosis and predictive factors**

THRLBCL is considered an aggressive lymphoma, although clinical heterogeneity is described. Cases with histiocytes are reported to define a more homogeneous group of patients with a very aggressive lymphoma and frequent failure of current therapies. The IPI score is the only known parameter of prognostic significance [19,428].

---

**Table 13.24** T-cell/histiocyte-rich large B-cell lymphoma

<table>
<thead>
<tr>
<th>Median patient age</th>
<th>12–61 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>75%</td>
</tr>
<tr>
<td>Stage III–IV</td>
<td>64%</td>
</tr>
<tr>
<td>Liver involvement</td>
<td>13–70%</td>
</tr>
<tr>
<td>Spleen involvement</td>
<td>33–67%</td>
</tr>
<tr>
<td>Bone marrow involvement</td>
<td>17–60%</td>
</tr>
</tbody>
</table>


**Fig. 13.109** T-cell/histiocyte-rich large B-cell lymphoma. A Small lymphocytes correspond to CD3-positive T cells. B A large proportion of histiocytes stained for CD68.
Primary diffuse large B-cell lymphoma of the CNS

Definition
Primary diffuse large B-cell lymphoma (DLBCL) of the CNS is defined as DLBCL arising within the brain, spinal cord, leptomeninges or eye. Excluded are lymphomas of the dura, intravascular large B-cell lymphomas, lymphomas with evidence of systemic disease or secondary lymphomas, and all immunodeficiency-associated lymphomas.

ICD-O code
9680/3

Synonyms
Primary CNS lymphoma; primary intraocular lymphoma; lymphomatosis cerebri (no longer recommended)

Epidemiology
CNS DLBCL accounts for <1% of all non-Hodgkin lymphomas and 2.4–3% of all brain tumours [3556]. The overall annual incidence rate of CNS DLBCL is 0.47 cases per 100 000 population [4196]. This lymphoma can affect patients of any age, with a peak incidence in the fifth to seventh decade of life, a median patient age of 56 years, and a male-to-female ratio of 3:2. In the past two decades, an increased incidence has been reported among patients aged >60 years [821,4196].

Etiology
In immunocompetent individuals, the etiological factors are unknown. Viruses, including EBV, HHV6 [3114], HHV8 [2708], and the polyomaviruses SV40 and BK virus [2705,2795], do not play a role. Pathogenically, expression or absence of chemokines and chemokine receptors or cytokines may contribute to the specific localization [3706]. Tumour cells and endothelial cells may interact via activation of IL4 to create a favourable microenvironment for tumour growth [3443]. Tumour cells of CNS DLBCL recognize proteins present in the CNS via their poly-reactive B-cell receptor, and thus have the capacity to stimulate B-cell receptor signalling. This interaction between CNS antigens and the lymphoma cells may also contribute to organ restriction [2710]. Upon relapse, CNS DLBCLs show a restricted homing to the main immune sanctuaries (i.e. the brain, eyes, and testes) [416,1552,1828,3289], and primary testicular and intraocular lymphomas frequently spread to the CNS [4205], suggesting that the tumour cells need to hide in an immune sanctuary. Many DLBCLs of the CNS and testis show decreased or absent expression of HLA class I and II proteins, allowing the tumour cells to further escape from immune attack [415,3355].

Localization
About 60% of CNS DLBCLs involve the supratentorial space, including the frontal lobe (affected in 15% of cases), temporal lobe (in 8%), parietal lobe (in 7%), occipital lobe (in 3%), basal ganglia and periventricular brain parenchyma (in 10%), and corpus callosum (in 5%). Less frequently affected sites include the posterior fossa (affected in 13% of cases) and spinal cord (in 1%) [919]. A single tumour is present in 60–70% of cases, with the remainder presenting as multifocal disease [919]. The leptomeninges may be involved, but exclusive meningeal manifestation is unusual. Approximately 20% of patients present with or develop intraocular lesions, and 80–90% of patients with intraocular DLBCL develop contralateral tumours.
and parenchymal CNS lesions. Dissemination to extraneural sites, including the bone marrow, is very rare; in these cases, preferential spread to the testis has been noted (416,1552,1828,3289).

Clinical features
Patients more frequently present with cognitive dysfunction, psychomotor slowing, and focal neurological symptoms than with headache, seizures, and cranial nerve palsies. Blurred vision and eye floaters are symptoms of ocular involvement (293,2084,3128).

Imaging
MRI is the most sensitive technique for detecting CNS DLBCL, which is hypointense on T1-weighted and isointense to hyperintense on T2-weighted images, typically appearing densely enhancing on postcontrast images. Peritumoral oedema is relatively limited, and less extensive than in malignant gliomas and metastases (2084). Meningeal involvement may present as foci of abnormal contrast enhancement (2161). With steroid therapy, lesions may vanish within hours (919).

Macroscopy
As observed in postmortem examination, CNS DLBCL occurs as single or multiple masses in the brain parenchyma, most frequently in the cerebral hemispheres. The masses are often deep-seated and adjacent to the ventricular system. The tumours can be firm, friable, granular, haemorrhagic, and greyish-tan or yellow, with central necrosis or virtually indistinguishable from the adjacent neuropil. Demarcation from surrounding parenchyma is variable. Some tumours appear well delineated, like metastases. When diffuse borders and architectural effacement are present, the lesions resemble gliomas. Like malignant gliomas, the tumours may diffusely infiltrate large areas of the hemispheres without forming a distinct mass. Meningeal involvement may resemble meningitis or meningioma, but can also be grossly inconspicuous.

Microscopy
CNS DLBCLs are usually highly cellular, diffusely growing tumours. Centrally, large areas of geographical necrosis are common, which may harbour viable perivascular lymphoma islands. At the periphery, this perivascular infiltration pattern is frequent. Infiltration of cerebral blood vessels causes fragmentation of the argyrophilic fibre network. From these perivascular cuffs, tumour cells invade the neural parenchyma, either with a well-delineated invasion front with small clusters or with single tumour cells diffusely infiltrating the tissue; this is accompanied by a prominent astrocytic and microglial activation and a reactive inflammatory infiltrate consisting of mature T cells and B cells and sometimes also many foamy histiocytes. In some cases, a distinct tumour mass is difficult to identify on imaging and the biopsy may have been taken from the periphery, resulting in the finding of an entirely interstitial pattern with isolated tumour cells intermingled among astrocytes. In such cases, immunohistology with CD20 or other B-cell markers is necessary to identify the lymphoma cells. Cytomorphologically, CNS DLBCL consists of atypical cells with medium-sized to large round, oval, irregular, or pleomorphic nuclei and distinct nucleoli, corresponding to centroblasts or immunoblasts. Some cases show a relatively monomorphic cell population with intermingled macrophages, mimicking Burkitt lymphoma.

Postulated normal counterpart
A late germinal centre exit B cell arrested in terminal B-cell differentiation that shares genetic characteristics with both activated B cells and germinal centre B cells (2706).
Genetic profile
Because the tumour cells correspond to late germinal centre exit B cells with blocked terminal B-cell differentiation, they carry rearranged and somatically mutated IG genes with evidence of ongoing somatic hypermutation [2709, 3127,3990]. Consistent with the ongoing germinal centre programming, they show persistent BCL6 activity [478]. The process of somatic hypermutation is not confined to its physiological targets (IG and BCL6 genes), but extends to other genes that have been implicated in tumorigenesis, including BCL2, MYC, PIM1, PAX5, RHOH (also called TTF), KLHL14, OSBPPL10, and SUSD2 [482,2715,4163]. The fixed IgM/IgD phenotype of the tumour cells is in part due to miscarried IG class-switch rearrangements during which the S-mu region is deleted (2713). The L265P mutation is highly recurrent in patients with CNS DLBCL [2084]. The inclusion of whole-genome sequencing has revealed recurrent gains of genetic material most frequently affecting 18q21.33-23 (in 43% of cases), including the BCL2 and MALT1 genes; chromosome 12 (in 26%); and 10q23.21 (in 21%) [3595]. Loss of genetic material most frequently involves 6q21 (in 52% of cases), 6p21 (in 37%), 8q12.1-12.2 (in 32%), and 10q23.21 (3595). Heterozygous deletions, homozygous loss, or copy-neutral LOH of chromosomal region 6p21.32 affects 73% of CNS DLBCLs; the 6p21 region harbours the MHC class II encoding genes HLA-DRB, HLA-DQA, and HLA-DQB [1883, 3355,3595]. Approximately 50% of CNS DLBCLs have lost expression of HLA class I and II gene products [415,3356]. The MYD88 L265P mutation is highly recurrent: present in more than half of the cases [2707]. Other pathways involving the B-cell receptor, the toll-like receptor, and the NF-kappaB pathway are frequently activated due to genetic alterations affecting the genes CD79B (in 20% of cases), INPP5D (also called SHIP; in 25%), CBL (in 4%), BLNK (in 4%), CARD11 (in 16%), MALT1 (in 43%), and BCL2 (in 43%), which may foster proliferation and prevent apoptosis [665,1403,2097,2707, 2712,2714,3595].

Epigenetic changes may also contribute to CNS DLBCL pathogenesis, including gene silencing by DNA methylation. Hypermethylation of DAPK1 (seen in 84% of cases), CDKN2A (in 75%), MGMT (in 52%), and RFC (in 30%) may be of potential therapeutic relevance [753,774,1194, 3595].

Prognosis and predictive factors
Patients with CNS DLBCL have a remarkably worse outcome than do patients with systemic DLBCL. Older patient age (> 65 years) is a major negative prognostic factor and is associated with reduced survival as well as an increased risk of neurotoxicity related to therapy [14,2084]. High-dose methotrexate-based polychemotherapy is currently the treatment of choice [2084]. The inclusion of whole-brain irradiation may improve outcome, but carries the risk of neurotoxicity resulting in severe cognitive, motor, and autonomic dysfunction, particularly in elderly patients [14]. Most protocols report a median progression-free survival of about 12 months and an overall survival of approximately 3 years. In a subgroup of elderly patients with methylated MGMT within the lymphoma cells, temozolomide monotherapy appeared to be therapeutically effective [2144]. On biopsy, the presence of reactive perivascular CD3+ T-cell infiltrates has been associated with improved survival [3213]. LM02 protein expression by the tumour cells has been associated with prolonged overall survival [2394]. BCL6 expression has been suggested as a prognostic marker, although conflicting favourable versus unfavourable conclusions have been reported [2694,3237, 3308,3727]. Del(6)(q22) has been associated with inferior overall survival [519, 1403]. With improvement of outcome, some sporadic systemic relapses have been observed; these can involve any organ, but relatively frequently involve the testis and breast [1828].
Primary cutaneous diffuse large B-cell lymphoma, leg type

**Definition**
Primary cutaneous diffuse large B-cell lymphoma (PCLBCL), leg type, is a PCLBCL composed exclusively of centroblasts and immunoblasts, most commonly arising in the leg.

**ICD-O code**
9680/3

**Epidemiology**
PCLBCL, leg type, accounts for 4% of all primary cutaneous lymphomas and 20% of all primary cutaneous B-cell lymphomas [1530,4320]. It typically occurs in elderly patients, in particular women, with a male-to-female ratio of 1:3-4. The median patient age is in the seventh decade of life [4189].

**Localization**
These lymphomas preferentially affect the lower legs, but 10–15% of cases arise at other sites [2062,3623,4499].

**Clinical features**
PCLBCL, leg type, presents with red or bluish-red, often rapidly growing tumours on one or both of the lower legs [1421,2062,3623,4499]. These lymphomas frequently disseminate to extracutaneous sites.

**Microscopy**
These lymphomas are composed of a monotonous, diffuse, non-epidermotropic infiltrate of confluent sheets of centroblasts and immunoblasts [1421,4189]. Mitotic figures are frequently observed. Small B cells and CD21+/CD35+ follicular dendritic cell meshworks are absent. Reactive T cells are relatively few and are often confined to perivascular areas.

**Immunophenotype**
The neoplastic B cells express monotypic immunoglobulin, CD20, and CD79a. Unlike primary cutaneous follicle centre lymphomas, PCLBCLs, leg type, usually strongly express BCL2, IRF4/MUM1, FOXP1, MYC, and clgM, with coexpression of IgD in 50% of cases [953,1318,1409,1424,1653,1654,2062,2065,2066]. However, BCL2 and IRF4/MUM1 expression are absent in approximately 10% of the cases. [2062,3623]. The proliferation rate is high. BCL6 is expressed by most cases, but may be dim, whereas CD10 staining is usually negative [1654].

**Postulated normal counterpart**
A peripheral B cell of post-germinal centre origin

**Genetic profile**
PCLBCL, leg type, has many genetic similarities with diffuse large B-cell lymphomas arising at other sites, but shows marked differences from primary cutaneous follicle centre lymphoma. PCLBCL, leg type, has the gene expression profile of the ABC subtype of DLBCL [1653]. Interphase FISH analysis frequently shows translocations involving MYC or BCL6, and IGH genes in PCLBCL, leg type [1524]. High-level DNA amplifications of 18q21.31-21.33, including the BCL2 and MALT1 genes, are detected in 67% of cases by array comparative genomic hybridization and FISH analyses [986]. Amplification of BCL2 may well explain the strong BCL2 expression in these cases, particularly given that t(14;18) is not found [986,1525]. Loss of the CDKN2A and CDKN2B gene loci on chromosome 9p21.3, due to either gene deletion or promoter methylation, has been reported in as many as 67% of PCLBCLs, leg type, and correlates with an adverse prognosis [986,3625]. MYD88 L265P mutation, found in 60% of cases, and mutations in various components of the B-cell receptor signalling pathway, including CARD11 (in 10% of
cases), CD79B (in 20%), and TNFAIP3 (encoding TNFAIP3, also called A20; in 40%), strongly suggest constitutive NF-kappaB activation in PCLBCL, leg type [2067,3163,3165]. The similarities in gene expression profile and cytogenetic alterations, including translocations and NF-kappaB activating mutations, underscore that PCLBCL, leg type, may be considered a cutaneous counterpart of activated B-cell subtype diffuse large B-cell lymphoma [3165].

Prognosis and predictive factors
Earlier studies reported a 5-year survival rate of approximately 50% [1421,1422,4189]. However, recent studies have reported a significantly better clinical outcome for patients when rituximab is added to a multiagent chemotherapy (CHOP or CHOP-like) regimen [1423,1530]. Multiple skin lesions at diagnosis, inactivation of CDKN2A, and MYD88 L265P mutation have been reported to be associated with an inferior prognosis [986,1421,1422,3162,3623,3625].

EBV-positive diffuse large B-cell lymphoma, not otherwise specified (NOS)

Definition
EBV-positive diffuse large B-cell lymphoma (DLBCL), NOS, is an EBV-positive clonal B-cell lymphoid proliferation [780,1017,2701,2867,2957,3018,3019,3063,3661]. Excluded from this category are cases of lymphomatoid granulomatosis, cases with evidence of acute or recent EBV infection, other well-defined lymphomas that may be EBV-positive (such as plasmablastic lymphoma and DLBCL associated with chronic inflammation), and EBV-positive mucocutaneous ulcer (localized EBV-driven proliferations affecting cutaneous or mucosal sites). This disease was formerly designated as EBV-positive DLBCL of the elderly, but the restriction to elderly patients has been removed; although the disease usually occurs in individuals aged >50 years, it can present over a wide age range [326,781,1675,2867,3661]. The NOS designation has been added to emphasize the exclusion of the more specific types of EBV-positive lymphoma. Although many cases have a distinctive histological appearance, routine EBV testing is required for all cases to be identified. The clinical outcome is variable [165,1017,1018,2867,3018,3019].

ICD-O code 9680/3

Synonyms
EBV-positive diffuse large B-cell lymphoma of the elderly; senile EBV-associated B-cell lymphoproliferative disorder; age-related EBV-positive lymphoproliferative disorder

Epidemiology
EBV-positive DLBCL accounts for <5–15% of DLBCLs among Asian and Latin American patients and <5% among western patients, with no documented predisposing immunodeficiency [325,1017,1367,1674,2405,2701,2956,2957,3018,3019,3063]. Most cases occur in patients over age 50, with a peak in the eighth decade [3661]. However, cases also occur in younger patients sporadically, with a second smaller peak in the third decade [2867]. EBV-positive DLBCL is more common in males, with a male-to-female ratio of 1.2–3.6:1 [1017,1675,2867,3018,3019,3661].

Etiology
The increased incidence of EBV-positive DLBCL in older patients is believed to be related to immunosenescence [1017,2746,3018,3019]. Alterations in the immune microenvironment may play a role at any patient age [668,2867].

Localization
Nodal or extranodal sites can be involved. The most common extranodal sites are the lungs and gastrointestinal tract [1017,
In young patients (aged <45 years), the disease is predominantly nodal, with only about 10% of cases showing extranodal involvement [2867,4087]. Approximately 5–10% of patients have both nodal and extranodal involvement.

Clinical features
The clinical features at presentation are variable [1017,1663,2867,3018,3019]. More than half of the patients have a high or high-intermediate International Prognostic Index (IPI) score. Most patients have detectable EBV DNA in serum or whole blood, but this can also be seen in patients with EBV-negative DLBCL [2316,2961].

Microscopy
The histological features overlap with those of other EBV-related lymphoid proliferations, including EBV-positive classic Hodgkin lymphoma. The neoplastic component most often consists of a variable number of large transformed cells/immunoblasts and Hodgkin/Reed–Sternberg–like cells. There is a variable component of reactive elements, including small lymphocytes, plasma cells, histiocytes, and epithelioid cells. The rich background of small lymphocytes and histiocytes may resemble T-cell/histiocyte-rich large B-cell lymphoma, also referred to as the polymorphic pattern in young patients. Other cases are more monomorphic, and may be difficult to distinguish from EBV-negative DLBCL without ancillary studies [165,780,1017,1018,2701,3018,3019]. Large areas of geographical necrosis and angioinvasion are other characteristic findings, but they are not always present.

Immunophenotype
The neoplastic cells are usually positive for the pan–B-cell antigens CD19, CD20, CD22, CD79a, and PAX5, and have an activated–B-cell immunophenotype, being positive for IRF4/MUM1 and negative for CD10 and commonly BCL6 [1017,2867,3018,3019]. CD30 is frequently positive and CD15 is sometimes coexpressed, but other phenotypic features typical of classic Hodgkin lymphoma are usually lacking [668,1017,2867]. Light chain restriction may be difficult to demonstrate. EBNA2 and LMP1 are expressed in 7–36% and >90% of cases, respectively, indicating type III and (more often) type II EBV latency [2867]. The tumour cells often express PDL1 and PDL2, providing a mechanism for immune escape [668,2867,4431]. In situ hybridization for EBV-encoded small RNA (EBER) is mandatory for the diagnosis of EBV-positive DLBCL, NOS. With EBER in situ hybridization, more than 80% of the atypical cells are positive. Small numbers of EBER-positive cells may be present as bystander B-cells in EBV-negative B-cell or T-cell lymphomas [2867,2960,3821].

Postulated normal counterpart
A mature B cell, transformed by EBV...
Genetic profile
Clonality of the IG genes and EBV can usually be detected by molecular techniques, and is helpful for distinguishing polymorphous cases from reactive hyperplasia and infectious mononucleosis [2867,3018,3019]. Restricted/clonal T-cell receptor responses can be seen in some cases [1018,2867], but can also be present in other EBV-associated lymphoproliferations such as infectious mononucleosis [2447]. IG translocations are uncommon (seen in ~15% of cases). The presence of an IGH/MYC translocation or variants should suggest a diagnosis of plasmablastic lymphoma [2365, 4110]. Mutations in CD79B, CARD11, and MYD88, which are often found in the activated B-cell type of DLBCL, are absent [1316]. Chromosomal gains at 9p24.1 may contribute to increased expression of PDL1 and PDL2 [4431]. Gene expression profiling shows activation of the JAK/STAT and NF-kappaB pathways [1958,2956].

Prognosis and predictive factors
With an age cut-off point of 45 years, the prognosis of EBV-positive DLBCL differs significantly between elderly and young patients (P <0.0001) [1017,2867,3019]. The disease is aggressive, with a median survival of about 2 years in elderly patients, even when treated with rituximab immunochemo therapy [1017,3019,3527, 3820], but younger patients appear to have an excellent prognosis, with long-term complete remission in >80% [2867, 4087]. Cases with the T-cell/histiocyte-rich large B-cell lymphoma-like or polymorphic pattern appears to have a better prognosis than do monomorphic EBV-positive DLBCL in young patients [1017, 1018,2867]. Positivity for CD30 [2956] and EBNA2 [3820] may have an adverse prognostic impact. In elderly patients, B symptoms and age > 70 years appear to be adverse prognostic factors [3019]; patients with neither, one, or both of these factors have median overall survival times of 56, 25, and 9 months, respectively.
Definition

EBV-positive mucocutaneous ulcer (EBVMCU) is a newly recognized clinico-pathological entity occurring in patients with age-related or iatrogenic immunosuppression, often with Hodgkin-like features and a typically indolent course, with spontaneous regression in some cases (1018). It presents in cutaneous or mucosal sites. The most common site of involvement is the oral cavity, including gingiva. The outgrowth of the EBV-positive cells may be related to local trauma or inflammation.

ICD-O code

9680/1

Epidemiology

The incidence of EBVMCU has not been established (498,971,1017,1018,2010,3852,4408). EBVMCU occurs in a variety of clinical settings associated with defective surveillance for EBV, including advanced age in a high proportion of cases, but also in patients with iatrogenic immunosuppression, such as those receiving methotrexate, azathioprine, cyclosporine, or tumour necrosis factor inhibitors for autoimmune diseases, and in solid organ transplant recipients (1565). Similar cases have been reported in allogeneic transplant recipients and HIV-infected patients (498,2847). The disease has a mild male predominance and a median patient age >70 years (1018). As would be expected, iatrogenically immunosuppressed patients with EBVMCU are younger on average than those with age-related EBVMCU.

Etiology

The disease is uniformly associated with EBV and occurs in patients with various forms of immunosuppression (1018). At least in elderly patients, alterations in T-cell responses, with the accumulation of clonal or oligoclonal restricted CD8+ T cells with diminished functionality, likely play a role in the pathogenesis of this EBV-associated lymphoproliferative disorder (1017). The lesions often arise in locations subjected to local tissue damage or inflammation, such as in the intestine in patients with inflammatory bowel disease (2132).

Localization

EBVMCU presents with ulcerated lesions, usually in the oral mucosa (tonsils, tongue, buccal mucosa, and palate), skin, and gastrointestinal tract (oesophagus, large bowel, rectum, and perianal region) (498,971,1017,1018,2010,3852). Regional isolated lymphadenopathy is rarely seen, but there is no evidence of systemic lymphadenopathy, hepatosplenomegaly, or bone marrow involvement. Regional lymph nodes may show reactive hyperplasia.

Clinical features

The symptoms are related to the ulcerated lesion, whether in the oral cavity, skin, or intestine. Systemic symptoms are rare.

Macroscopy

Patients with EBVMCU present with sharply circumscribed, isolated, indurated mucosal or cutaneous ulcers.

Microscopy

The mucosal or cutaneous surface is ulcerated, sometimes with pseudoepitheliomatous hyperplasia of the adjacent intact epithelium. Beneath the ulcer, there is a dense polymorphic infiltrate with a variable number of plasma cells, histiocytes, and eosinophils, as well as a substantial number of large transformed cells, resembling either atypical immunoblasts or Hodgkin/Reed-Sternberg-like cells. Scattered apoptotic cells are often seen. Angioinvasion and necrosis can be present in addition to surface ulceration (1017,1018). The lymphocytes in the background are abundant, many with an-gulated and medium-sized nuclei.
Some cases resemble diffuse large B-cell lymphoma or a polymorphic post-transplant lymphoproliferative disorder; others show Hodgkin-like morphology, with the distinction from classic Hodgkin lymphoma sometimes very difficult. However, the diagnosis of classic Hodgkin lymphoma in the skin or in mucosa should be rendered only with extreme caution. The deepest margin of the lesion usually contains a band-like infiltrate of mature lymphocytes. These cells are mainly T cells negative for EBV.

Immunophenotype

The large transformed immunoblasts and Hodgkin/Reed–Sternberg–like cells are B cells that in most cases have CD20 expression ranging from strong to weak and heterogeneous. These cells are positive for PAX5 and OCT2, with variable expression of BOB1. They have an activated-B-cell phenotype, being negative for CD10 and BCL6 and positive for IRF4/MUM1, and are CD30-positive. CD15 is expressed in about half of the cases. CD79a is often positive. EBV is consistently positive, with transformed cells commonly positive for LMP1. Positivity for EBV-encoded small RNA (EBER) parallels expression of most B-cell antigens, and is found in a range of cell sizes, from small lymphocytes to immunoblasts and cells with Hodgkin/Reed–Sternberg cell morphology. The background consists mainly of T cells, with numerous CD8+ T cells. A dense rim of CD3+ lymphocytes is present between the lesion and adjacent soft tissue.

Postulated normal counterpart

An EBV-transformed post-germinal centre B cell

Genetic profile

Fewer than half of all EBVMCUs show clonal IG gene rearrangements. Studies of TR gene rearrangement often reveal an oligoclonal or restricted pattern by PCR (971,1017,1018).

Prognosis and predictive factors

Case reports and series suggest a benign natural history, with nearly all reported cases responding to reduction of immunosuppressive therapy. In patients in whom immunosuppression cannot be reversed, responses to rituximab, local radiation, and chemotherapy have been observed. Spread to distant sites is rare, but local progression may be seen. The outcome is superior to that to other immunodeficiency-associated EBV-driven lymphoproliferative disorders (1017, 1566,2597,3852,4408). However, rare cases of relapses or progression to more widespread disease (2721) have been reported.
Diffuse large B-cell lymphoma associated with chronic inflammation

Definition
Diffuse large B-cell lymphoma (DLBCL) associated with chronic inflammation is a lymphoid neoplasm occurring in the setting of longstanding chronic inflammation and showing association with EBV. Most cases involve body cavities or narrow spaces. Pyothorax-associated lymphoma (PAL) is the prototypical form, developing in the pleural cavity of patients with longstanding pyothorax.

ICD-O code
9680/3

Synonym
Pyothorax-associated lymphoma

Epidemiology
PAL develops in patients with a 20 to 64-year (median: 37-year) history of pyothorax resulting from artificial pneumothorax for treatment of pulmonary or pleural tuberculosis [121,1797,2818,2827,3150]. Patient age at diagnosis ranges from the fifth to eighth decade of life (median: 65–70 years) [2818,2827]. The male-to-female ratio is 12:1 versus nearly equal in chronic pyothorax, suggesting that males are more susceptible to this type of lymphoma than are females [1797]. Although most cases of PAL have been reported in Japan, this lymphoma has also been described in the west [166,2518,3150]. For DLBCLs arising in other settings of chronic suppuration or inflammation, such as chronic osteomyelitis, metallic implant insertion, surgical mesh implantation, and chronic skin venous ulcer, the interval between the predisposing event and malignant lymphoma is usually >10 years (range: 1.2–57 years) [696, 804,1263].

Etiology
Artificial pneumothorax, used in the past as a form of surgical therapy for pulmonary tuberculosis, is the only significant risk factor for development of PAL among patients with chronic pyothorax [120, 1679]. PAL is strongly associated with EBV, with expression of EBNA2 and/or LMP1 together with EBNA1 (i.e. usually type III EBV latency) [1264,2949,3150,3525,3873,3874]. Chronic inflammation at the local site probably plays a role in the proliferation of EBV-transformed B cells by enabling them to escape from the host immune surveillance through production of IL10 (an immunosuppressive cytokine) and by providing autocrine to paracrine growth via IL6 and IL6R [1925,1928]. DLBCLs associated with chronic inflammation that occur in other settings similarly harbour EBV, likely facilitated by so-called local immunodeficiency resulting from longstanding chronic suppuration or inflammation in a confined space [696, 804].

Localization
The most common sites of involvement are the pleural cavity (PAL), bone (especially femur), joints, and periarticular soft tissue [696]. In more than half of all PAL cases, the tumour mass is >10 cm [121]. There is direct invasion of adjacent structures, but the tumour is often confined to the thoracic cavity at the time of diagnosis, with about 70% of patients presenting with clinical stage I or II disease [2818]. PAL differs from primary effusion lymphoma, which is characterized by lymphomatous serous effusions in the absence of tumour mass formation and is HHV8+.

Clinical features
Patients with PAL present with chest pain, back pain, fever, or tumorous swelling in the chest wall, or with respiratory symptoms such as productive cough, haemoptysis, and dyspnoea. Radiologi-
cal examination reveals a tumour mass in the pleura (in 80% of cases), pleura and lung (in 10%), or lung near the pleura (in 7%). The serum lactate dehydrogenase level is commonly elevated (2818,3150). Patients who develop lymphoma in the bone, joint, periarticular soft tissue, or skin usually present with pain or mass lesion. The involved bone typically shows lytic lesions on radiological examination.

Microscopy
The morphological features are the same as those of DLBCL, NOS. Most cases show centroblastic or immunoblastic morphology, with round nuclei and large single or multiple nucleoli. Massive necrosis and angiocentric growth may be present.

Immunophenotype
Most cases express CD20 and CD79a. However, a proportion of cases may show plasmacytic differentiation, with loss of CD20 and/or CD79a, and expression of IRF4/MUM1 and CD138. The lymphoma has an activated B-cell phenotype. CD30 can be expressed. Occasional cases also express one or more T-cell markers (CD2, CD3, CD4, and/or CD7), causing problems in lineage assignment (2734, 2818,3150,4022).

In situ hybridization for EBV-encoded small RNA (EBER) shows positive labelling of the lymphoma cells. Type III EBV latency (i.e. positivity for LMP1 and EBNA2) is characteristic (1263,3150).

Postulated normal counterpart
An EBV-transformed post-germinal centre B cell

Genetic profile
IG genes are clonally rearranged and hypermutated, but lack ongoing mutations (2680,3875). TP53 mutations are found in about 70% of cases, usually involving dipyrimidine sites, which are known to be susceptible to mutagenesis induced by ionizing radiation (1679). MYC gene amplification is common (4412), and TNFAIP3 (also called A20) is deleted in a proportion of cases (100). Cytogenetic studies show complex karyotypes with numerous numerical and structural abnormalities (3874). The gene expression profile of PAL is distinct from that of nodal DLBCL, which may be attributable to the presence of EBV (2882). One of the most differentially expressed genes is IFI27, which is known to be induced in B lymphocytes by stimulation of interferon alpha, consistent with the role of chronic inflammation in this condition. Downregulation of HLA class I expression, which is essential for efficient induction of host cytotoxic T lymphocytes, and mutations of cytotoxic T-lymphocyte epitopes in EBNA3B, an immunodominant antigen for cytotoxic T-lymphocyte responses, might also contribute to escape of PAL cells from host cytotoxic T lymphocytes (1926,1927).

Fig. 13.125 The patterns of gene expression in pyothorax-associated lymphoma (PAL) and nodal diffuse large B-cell lymphoma (DL) are significantly different. Modified from Nishiu M et al. (2882).

310 Mature B-cell neoplasms
Prognosis and predictive factors

DLBCL associated with chronic inflammation is an aggressive lymphoma. For PAL, the 5-year overall survival rate is 20–35% [2818,2827]. For patients achieving complete remission with chemotherapy and/or radiotherapy, the 5-year survival rate is 50% [2818]. Complete tumour resection (pleuropneumonectomy with or without resection of adjacent involved tissues) has also been reported to give good results [2809]. Poor performance status; high serum levels of lactate dehydrogenase, alanine transaminase (also called glutamic-pyruvic transaminase), or urea; and high clinical stage are unfavourable prognostic factors [119,2827].

Fibrin-associated diffuse large B-cell lymphoma

An unusual form of diffuse large B-cell lymphoma associated with chronic inflammation is not mass-forming and does not directly produce symptoms, but is discovered incidentally on histological examination of surgical pathology specimens excised for various pathologies other than lymphoma. The specimens typically contain fibrinous materials, such as in the walls of pseudocysts (having been reported in splenic false cyst, renal pseudocyst, adrenal pseudocyst, para-testicular pseudocyst, and pseudocyst in ovarian teratoma), hydrocoele, lesions or materials located in the cardiovascular system (having been reported in cardiac myxoma, cardiac prosthesis, cardiac fibrin thrombus, and synthetic tube graft), wear debris (associated with metallic implants), and chronic subdural haematoma [36,418,1479,1907,2388,2663,4114,4115]. Suggestions have been made to rename this group of lymphomas fibrin-associated EBV+ large B-cell lymphoma [440A].

Histologically, single and small aggregates of large lymphoma cells are found in only small foci within the fibrinous or amorphous material. The lymphoma cells show irregular nuclear foldings, coarse chromatin, distinct nucleoli, and amphophilic cytoplasm. Mitotic figures are easily found, and admixed apoptotic bodies are prominent. Chronic inflammatory cell infiltration in the background or vicinity is usually not prominent. The immunophenotypic features are similar to those of pyothorax-associated lymphoma, with expression of B-cell lineage markers and an activated B-cell phenotype. EBV is positive, with type III latency (typically EBNA2-positive).

Unlike in pyothorax-associated lymphoma, the clinical outcome is highly favourable, even with surgical excision alone. However, one report raised the possibility of progression to an infiltrative tumour; an incidental diffuse large B-cell lymphoma associated with chronic inflammation arising in a chronic subdural haematoma was accompanied by brain parenchymal infiltration [1907].
Lymphomatoid granulomatosis

Definition
Lymphomatoid granulomatosis (LYG) is an angiocentric and angiodestructive lymphoproliferative disease involving extranodal sites, composed of EBV-positive B cells admixed with reactive T cells, which usually predominate. The lesion has a spectrum of histological grade and clinical aggressiveness, which is related to the proportion of large B cells.

ICD-O codes
Lymphomatoid granulomatosis
  - Grade 1 or 2: 9766/1
  - Grade 3: 9766/3

Synonym
Angiocentric immunoproliferative lesion (obsolete)

Epidemiology
LYG is a rare condition. It usually presents in adulthood, but may be seen in children with immunodeficiency disorders. It affects males more often than females, with a male-to-female ratio of ≥2:1 [1965, 3726]. It appears to be more common in western countries than in Asia.

Etiology
LYG is an EBV-driven lymphoproliferative disorder. Individuals with underlying immunodeficiency are at increased risk [1490, 1544]. Predisposing conditions include allogeneic organ transplantation, Wiskott–Aldrich syndrome (eczema-thrombocytopenia-immunodeficiency syndrome), HIV infection, and X-linked lymphoproliferative syndrome. Patients presenting without evidence of underlying immunodeficiency usually manifest reduced immune function on careful clinical or laboratory analysis [3733, 4332].

Localization
Pulmonary involvement occurs in > 90% of patients and is usually present at initial diagnosis. Other common sites of involvement include the brain, kidneys, liver, and skin. Involvement of the upper respiratory tract or gastrointestinal tract is relatively uncommon [841, 1966, 3726].

Clinical features
Patients frequently present with signs and symptoms related to the respiratory tract, such as cough, dyspnoea, and chest pain. Constitutional symptoms are also common, including fever, malaise, weight loss, neurological symptoms, arthralgias, myalgias, and gastrointestinal symptoms. Patients with CNS disease may be asymptomatic or have varied presentations depending on the site of involvement, such as hearing loss, diplopia, dysthria, ataxia, and/or altered mental status [1966, 3107, 3726]. Few patients present with asymptomatic disease [1823].

Macroscopy
LYG most commonly presents as pulmonary nodules that vary in size. The lesions are most often bilateral in distribution, involving the mid- and lower lung fields. Larger nodules frequently exhibit central necrosis and may cavitate. Nodular lesions are found in the kidneys and brain, usually associated with central necrosis [1966, 3726]. Skin lesions are extremely diverse in appearance. Nodular lesions are found in the subcutaneous tissue. Dermal involvement may also be seen, sometimes with necrosis and ulceration. Cutaneous plaques or a maculopapular rash are less common cutaneous manifestations [309, 1823, 1965, 2604].

Microscopy
LYG is characterized by an angiocentric and angiodestructive polymorphous lymphoid infiltrate [1965, 1966, 2087, 3726]. Lymphocytes predominate and are admixed with plasma cells, immunoblasts, and histiocytes. Neutrophils and eosinophils are usually inconspicuous. The background small lymphocytes may show some atypia or irregularity, but do not appear overtly neoplastic. LYG is composed of a variable but usually small number of EBV-positive B cells admixed with a prominent inflammatory background [1490, 1966, 1967]. The EBV-positive cells usually show some atypia. They may resemble immunoblasts or less commonly have a more pleomorphic appearance reminiscent of Hodgkin cells. Multinucleated forms may be seen. Classic Reed–Sternberg cells are generally not present; if seen, they should raise the
possibility of Hodgkin lymphoma. Well-formed granulomas are typically absent in the lungs and most other extranodal sites [2357]. However, skin lesions often exhibit a prominent granulomatous reaction in subcutaneous tissue [309]. Vascular changes are prominent in LYG. Lymphocytic vasculitis, with infiltration of the vascular wall, is seen in most cases. The vascular infiltration may compromise vascular integrity, leading to infarct-like tissue necrosis. More direct vascular damage, in the form of fibrinoid necrosis, is also common, and is mediated by chemokines induced by EBV [3943]. LYG must be distinguished from nasal-type extranodal NK/T-cell lymphoma, which often has an angiodestructive growth pattern and is also associated with EBV [1819,3726].

**Immunophenotype**
The EBV-positive B cells usually express CD20 [1491,1966,3726,3899,4332]. The cells are variably positive for CD30, but negative for CD15. LMP1 may be positive in the larger atypical and more pleomorphic cells. EBNA2 is frequently positive, consistent with latency type III [3726]. Stains for cytoplasmic immunoglobulin are frequently non-informative, although in rare cases monotypic cytoplasmic immunoglobulin expression may be seen, particularly in cells showing plasmacytoid differentiation [3726,4332]. The background lymphocytes are CD3+ T cells, with CD4+ cells more frequent than CD8+ cells [3726].

**Postulated normal counterpart**
A mature B cell, transformed by EBV

**Grading**
The grading of LYG relates to the proportion of EBV-positive B cells relative to the reactive lymphocyte background [1491, 1966,2357,3726]. It is most important to distinguish grade 3 from grade 1 or 2. A uniform population of large atypical EBV-positive B cells without a polymorphous background should be classified as EBV-positive, diffuse large B-cell lymphoma, NOS and is beyond the spectrum of LYG as currently defined.

Grade 1 lesions contain a polymorphous lymphoid infiltrate without cytological atypia. Large transformed lymphoid cells are absent or rare, and are better appreciated by immunohistochemistry. When present, necrosis is usually focal. By in situ hybridization for EBV-encoded small RNA (EBER), only infrequent EBV-positive cells are identified (<5 per high-power field) [4332]. In some cases, EBV-positive cells may be absent; in this setting, the diagnosis should be made with caution, with studies to rule out other inflammatory or neoplastic conditions.

Grade 2 lesions contain occasional large lymphoid cells or immunoblasts in a polymorphous background. Small clusters can be seen, in particular with CD20 staining. Necrosis is more commonly seen. In situ hybridization for EBER readily identifies EBV-positive cells, which usually number 5–20 per high-power field. Variation in the number and distribution of EBV-positive cells can be seen within a nodule or among nodules, and occasionally as many as 50 EBV-positive cells per high-power field can be observed.

Grade 3 lesions still show an inflammatory background, but contain large atypical B cells that are readily identified by CD20 and can form larger aggregates. Markedly pleomorphic and Hodgkin-like cells are often present, and necrosis is usually extensive. By in situ hybridization, EBV-positive cells are extremely numerous (>50 per high-power field), and focally may form small confluent sheets. It is important to take into consideration that in situ hybridization for EBV can be unreliable when large areas of necrosis are present, due to poor RNA preservation; additional molecular studies for EBV may be helpful [3726].

**Genetic profile**
In most cases of grade 2 or 3 disease, clonality of the IG genes can be demonstrated by molecular genetic techniques [1490,2604]. In some cases, different

---

**Fig. 13.130** Lymphomatoid granulomatosis. Cutaneous manifestation showing subcutaneous infiltration, with fat necrosis and a granulomatous response.

**Fig. 13.131** Lymphomatoid granulomatosis. A Grade 1 lesion of the lung shows a polymorphous infiltrate in the vascular wall. B Grade 3 lesion of the brain contains numerous large transformed lymphoid cells. C Grade 3 lesion. The cells are positive for EBER by in situ hybridization for EBV-encoded small RNA (EBER). D Large pleomorphic cells may be seen, most commonly in grade 3 lesions and rarely in grade 2 lesions.
clonal populations may be identified in different anatomical sites \( (2678, 4332) \). Southern blot analysis may also show clonality of EBV \( (2607) \). Demonstration of clonality in grade 1 cases is less consistent, an observation that may be related to the relative rarity of the EBV-positive cells in these cases. Alternatively, some cases of LYG may be polyclonal \( (3726) \). TR gene analysis shows no evidence of monoclonality \( (2604, 2607, 3726) \). Alterations of oncogenes have not been identified.

**Genetic susceptibility**
Genetic susceptibility includes Wiskott-Aldrich syndrome (eczema–thrombocytopenia–immunodeficiency syndrome), X-linked lymphoproliferative syndrome, and conditions linked to immunodeficiency.

**Prognosis and predictive factors**
The clinical behaviour of LYG varies widely; the disease ranges from an indolent process to an aggressive large B-cell lymphoma. In its most indolent form, LYG presents with pulmonary nodules in an otherwise asymptomatic patient, which can wax and wane and rarely resolve. A more typical course is characterized by symptoms and multiorgan involvement. In the largest retrospective series reported, from 1979, 63.5% of patients died, most within the first year of diagnosis, and the median overall survival was 14 months \( (1965) \). Historically, corticosteroids and chemotherapy were the most common treatments, with most patients eventually succumbing to disease-related complications, including EBV-positive diffuse large B-cell lymphomas. More recently, the use of chemoimmunotherapy with dose-adjusted EPOCH-R and/or interferon has resulted in a 5-year overall survival rate of 70% \( (3406, 4332) \).

---

**Primary mediastinal (thymic) large B-cell lymphoma**

**Definition**
Primary mediastinal (thymic) large B-cell lymphoma (PMBL) is a mature aggressive large B-cell lymphoma (LBCL) of putative thymic B-cell origin arising in the mediastinum, with distinctive clinical, immunophenotypic, genotypic, and molecular features. Cases that arise outside the mediastinum are probably very uncommon, and cannot be confidently recognized without gene expression profiling studies.

**ICD-O code**
9679/3

**Synonyms**
Primary mediastinal clear cell lymphoma of B-cell type (obsolete); mediastinal diffuse large cell lymphoma with sclerosis (no longer recommended)

**Epidemiology**
PMBL accounts for about 2–3% of non-Hodgkin lymphomas. Unlike most other mature aggressive B-cell lymphomas, it occurs predominantly in young adults (median patient age: ~35 years) and has a female predominance, with a female-to-male ratio of about 2:1 \( (1,589,3535, 4498) \).

**Localization**
The vast majority of patients with PMBL present with a localized anterosuperior mediastinal mass in the thymic area. The mass is often bulky (> 10 cm in 60–70% of patients) and frequently invades adjacent structures, such as the lungs, pleura, and pericardium. Regional involvement of supraclavicular and cervical lymph nodes can occur. At progression, dissemination to distant extranodal sites, such as the kidneys, adrenal glands, liver, and CNS, is relatively common; however, bone marrow involvement is usually absent \( (279, 589, 2234) \). Leukaemia is not observed. Uncommon cases of PMBL at non-mediastinal sites, and without evident mediastinal disease, have been identified, in particular by gene expression profiling \( (672, 3412, 3538, 4452) \). Most of these cases are missed in routine practice, because gene expression profiling is not a routine clinical test.

**Clinical features**
Symptoms are related to the mediastinal mass, frequently with superior vena cava syndrome. B symptoms may be present. Pleural or pericardial effusion is present in one third of cases \( (4498) \). The absence of distant lymph node and bone marrow involvement is important to help exclude a systemic diffuse LBCL (DLBCL) with secondary mediastinal involvement. About 80% of cases are stage I–II at the time of diagnosis \( (4498) \).

**Microscopy**
PMBL shows wide morphological/cytological variation from case to case \( (3112) \). The growth pattern is diffuse, and it is commonly associated with compartmentalizing, alveolar fibrosis \( (279, 589, 2628, 2799, 3112, 3179) \). The stromal component is frequently absent in involved lymph nodes. The neoplastic cells are usually medium-sized to large, with abundant pale cyto-
plasm and relatively round or ovoid nuclei. In some cases, the lymphoma cells have pleomorphic and/or multilobated nuclei and may resemble Reed–Sternberg cells, raising suspicion of Hodgkin lymphoma (3112,4047). Rarely, there are so-called grey-zone lymphomas combining features of PMBL and classic Hodgkin lymphoma (CHL); these are separately designated as B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and CHL. Examples of composite PMBL and nodular sclerosis CHL have also been described, and PMBL can occur either before or at relapse of nodular sclerosis CHL (4047,4333).

**Immunophenotype**

PMBL expresses B-cell–lineage antigens such as CD19, CD20, CD22, and CD79a, but commonly lacks immunoglobulin despite a functional IG gene rearrangement and expression of the transcription factors PAX5, BOB1, OCT2, and PU1 [1915, 2379,3179,4274]. Flow cytometric studies may demonstrate surface IG in a subset of cases [4274]. CD30 is present in >80% of cases; however, staining is usually weak and heterogeneous compared to that in CHL [1633,3179]. CD15 may be expressed in a minority of cases [2800]. EBV is almost always absent [589]. The neoplastic cells are frequently positive for IRF4/MUM1; present in 75% of cases, and have variable expression of BCL2, present in 55–80% of cases, and BCL6, in 45–100%; CD10 positivity is less common, seen in 8–32% of cases [899,3179]. CD11c is positive in about 30% of cases [3364]. Unlike most cases of DLBCL, approximately 70% of PMBL express CD23, MAL antigen, and programmed cell death ligands PDL1 and PDL2 [524, 803,805,1327A,3179]. MYC can be expressed, sometimes on >30% of cells, independently of MYC gene alteration (2303). Aberrant expression of TNFAIP2 is a frequent feature, in common with CHL but rarely found in other types of DLBCL (2080). PMBL is also positive for CD54 and FAS (also known as CD95), and coexpresses TRAF1 and nuclear REL [3384,4460]. It often lacks HLA class I and/or class II molecules [2799,2800].

**Postulated normal counterpart**

A thymic medullary, asteroid, activation-induced cytidine deaminase–positive B cell

**Genetic profile**

*Antigen receptor genes*  
Immunoglobulin genes are rearranged and may be class switched, with a high load of somatic hypermutations without ongoing mutation activity [2262].

*Gene expression profiling*  
PMBL has a unique transcriptional signature that is distinct from those of other LBCLs, but shares similarities with CHL [3412,3538].

**Fig. 13.133** Primary mediastinal large B-cell lymphoma.  
A Nuclei are round (centroblast-like) or sometimes multilobated.  
B Sheets of large cells with abundant pale cytoplasm, separated by collagenous fibrosis.  
C Medium-sized cells with abundant pale cytoplasm.  
D Classic clear-cell appearance of the tumour cells, with associated delicate interstitial fibrosis.

**Genetic profile**

*Antigen receptor genes*  
Immunoglobulin genes are rearranged and may be class switched, with a high load of somatic hypermutations without ongoing mutation activity [2262].

*Gene expression profiling*  
PMBL has a unique transcriptional signature that is distinct from those of other LBCLs, but shares similarities with CHL [3412,3538].

**Fig. 13.134** Primary mediastinal large B-cell lymphoma.  
A All large cells express CD20 on the membrane.  
B Nests of CD3+ lymphocytes are present, with a perivascular distribution.

**Fig. 13.135** Primary mediastinal large B-cell lymphoma.  
A More than 60% of the large cells express nuclear Ki-67.  
B Thymic remnants infiltrated by tumour cells; the epithelial component is positive for cytokeratin.
Cytogenetic abnormalities and oncogenes
Rearrangements of BCL2, BCL6, and MYC are absent or rare (3548, 4067). Rearrangements or mutations in the class II major histocompatibility complex (MHC) transactivator CIITA at 16p13.13 have been reported in as many as 53% of PMBLs, resulting in downregulated MHC class II molecules, creating an immune-privileged phenotype in PMBL. Translocations involving CIITA occur with CD274 (also called PDCD1LG1 or PDL1) and PDCD1LG2 (also called PDL2). CD274 and PDCD1LG2 are also reported to fuse with partners other than CIITA in some cases (2763, 3779, 4081). Together with gains and amplifications at chromosome 9p24.1, including the JAK2/PDCD1LG2/PDCD1LG1 locus (in as many as 75% of cases) (344, 1881, 2624, 4300), these aberrations in the PDL locus likely explain the common overexpression of PDL1 and PDL2 in PMBL (668, 3645). This profile, with CIITA alterations and also copy-number gains and high-level amplification of the PDL locus (in 29–70% of cases), occurs almost exclusively in PMBL; it is unique among the LBCLs but shows similarities to what is found in CHL (1436, 3779, 4081).

The genomic profile also typically contains gains in chromosome 2p16.1 (seen in ~50% of cases), where candidate genes REL and BCL11A are amplified in a proportion of cases, leading to a frequent, albeit inconsistent, nuclear accumulation of their proteins (3384, 4296, 4297). Gains involving chromosomes Xp11.4-21, Qx24-26, 7q22, 12q31, and 9q34 (344) are also seen in approximately one third of PMBLs. PMBL has a constitutively activated NF-kappaB pathway (1208), which might be due to deleterious mutations in the TNFAIP3 gene, present in as many as 60% of PMBLs (1049, 3572A). These tumours also have a constitutively activated JAK/STAT signalling pathway, also found in CHL, that seems to be frequently related to inactivating mutations of SOCS1 (2624, 3777, 4073, 4297). Mutations affecting the STAT6 DNA-binding domain and in PTPN1, a negative regulator of JAK/STAT signalling, are also common, found in as many as 72% and 25% of PMBLs, respectively, but are almost absent in DLBCL (1049, 1495, 2280, 2504, 3363). The genetic landscape of PMBL suggests activation-induced cytidine deaminase–mediated aberrant somatic hypermutation as the mutational mechanism (2763), with BCL6 mutations detected in about half of the cases (2477). The landscape also includes truncating immunity pathway, ITPKB, MFHAS1, and XPO1 mutations (1049).

Genetic susceptibility
Exome sequencing of a family with three siblings with PMBL suggested KMT2A (previously called MLL) as a candidate predisposition gene, but these findings warrant replication (3465).

Prognosis and predictive factors
Variations in microscopic appearance do not predict differences in survival (3112). PMBL should be distinguished from B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and CHL, which is more aggressive (4047). Response to intensive chemotherapy, with or without radiotherapy, is usually good. PMBL is associated with a more favourable survival than are the germinal centre B-cell and activated B-cell subtypes of DLBCL, and recently developed chemotherapy protocols have shown high cure rates in adults and children (1058, 2667, 3412, 4346). Extension into adjacent thoracic viscera, pleural or pericardial effusion, and poor performance status are associated with inferior outcome (115, 589, 2233, 2234, 3535, 3538). FDG-PET predicts survival after chemotherapy (605, 2514).
Intravascular large B-cell lymphoma

Definition

Intravascular large B-cell lymphoma is a rare type of extranodal large B-cell lymphoma characterized by the selective growth of lymphoma cells within the lumina of vessels, in particular capillaries, and with the exception of larger arteries and veins.

ICD-O code

9712/3

Synonyms

Malignant angioendotheliomatosis; angioendotheliomatosis proliferans syndrome; intravascular lymphomatosis; angioendotheliotropic lymphoma (all obsolete)

Epidemiology

This tumour occurs in adults, with a median age of 67 years (range: 13–85 years) and a male-to-female ratio of 1:1:1. The frequency and clinical presentation differ according to patients’ geographical origin (West vs Far East) (1196,2789,3214,3215,3655).

Localization

This lymphoma is usually widely disseminated in extranodal sites, including the bone marrow, and may present in virtually any organ. However, the lymph nodes are usually spared.

Clinical features

Two major patterns of clinical presentation have been recognized: a so-called classic form (mostly present in western countries) characterized by symptoms related to the main organ involved, predominantly neurological or cutaneous, and a haemophagocytic syndrome–associated form. In the CNS, recurrences may be associated with extravascular brain masses (1642,3657). Sinusoidal involvement occurs in the liver, spleen, and bone marrow. Malignant cells are occasionally detected in peripheral blood (3215).

Microscopy

The neoplastic lymphoid cells are mainly lodged in the lumina of small or intermediate-sized vessels in many organs. Fibrin thrombi, haemorrhage, and necrosis may be observed in some cases. The tumour cells are large, with prominent nucleoli and frequent mitotic figures. Rare cases have cells with anaplastic features or smaller size (3215). Minimal extravascular location of neoplastic cells may be seen. In the CNS, recurrences may be associated with extravascular brain masses (1642,3657). Sinusoidal involvement occurs in the liver, spleen, and bone marrow. Malignant cells are occasionally detected in peripheral blood (3215).

Immunophenotype

Tumour cells express mature B-cell–associated antigens. CD5 and CD10 coexpression is seen in 38% and 13% of the cases, respectively. Almost all CD10-negative cases are positive for IRF4/MUM1. An increasing number of intravascular NK/T-cell lymphomas with EBV-positive tumour cells (1317,4241) and rarely intralymphatic anaplastic large cell lymphomas, ALK-negative (2644,3871), have been reported, but they should be considered different entities.
The intravascular growth pattern has been hypothesized to be secondary to a defect in homing receptors on the neoplastic cells (1204), such as the lack of CD29 (integrin beta-1) and CD54 (ICAM1) adhesion beta-molecules (3212).

**Postulated normal counterpart**
A transformed peripheral B cell

**Genetic profile**
Immunoglobulin genes are clonally rearranged. Karyotypic abnormalities have been described, but too few cases have been studied to demonstrate recurrent abnormalities (3655,3656).

**Prognosis and predictive factors**
Intravascular large B-cell lymphoma is generally aggressive, except for the cases with disease limited to the skin (3655). The poor prognosis is due in part to the delay of timely and accurate diagnosis related to the protean presentation of this lymphoma. Chemotherapeutic regimens with rituximab have significantly improved the clinical outcomes of these patients, with a 3-year overall survival rate of 60–81% (1195,1197,3655,3656). However, CNS relapse, which occurs in ~25% of cases (3657) and neurolymphomatosis (2570) are serious complications in the rituximab era. Neither the clinical type of presentation nor clinical parameters predict CNS relapse.
ALK-positive large B-cell lymphoma

**Definition**
ALK-positive large B-cell lymphoma (LBCL) is an aggressive neoplasm of ALK-positive monomorphic large immunoblast-like B cells, which usually have a plasma cell phenotype.

**ICD-O code** 9737/3

**Synonyms**
Large B-cell lymphoma expressing the ALK kinase and lacking the t(2;5) translocation; ALK-positive plasmablastic B-cell lymphoma (both obsolete)

**Epidemiology**
This lymphoma is very rare, accounting for <1% of diffuse LBCLs. It seems to occur more frequently in young men, with a male-to-female ratio of 5:1, but spans all age groups, with a patient age range of 9–85 years (median: 43 years). One third of cases occur in the paediatric age group [2230, 3333]. There is no association with immunosupression.

**Localization**
The tumour mainly involves lymph nodes [22, 251, 1307, 1790, 3333, 3763] or presents as a mediastinal mass [907, 1348]. Extranodal involvement has also been reported, at sites such as the nasopharynx [2976], tongue [907], stomach [2601], bone [2976], soft tissue [713], liver, spleen, and skin [2230].

**Clinical features**
Most patients present with generalized lymphadenopathy, and 60% present in advanced stage III/IV. Bone marrow is infiltrated in 25% of cases [2230].

**Microscopy**
The lymph nodes show a marked diffuse infiltrate, frequently with a sinusoidal growth pattern. The infiltrate is composed of monomorphic large immunoblast-like cells with round pale nuclei containing a large central nucleolus and abundant amphophilic cytoplasm. Some cases show plasmablastic differentiation [951, 1307]. Atypical multinucleated neoplastic giant cells may be seen.

**Immunophenotype**
Lymphoma cells are strongly positive for the ALK protein, with most demonstrating a restricted granular cytoplasmic staining pattern highly indicative of the expression of the CLTC-ALK fusion protein. Few cases show cytoplasmic, nuclear, and nucleolar ALK staining associated with the NPM1-ALK fusion protein. ALK translocations with other partners may be associated with a cytoplasmic staining pattern. The tumours also characteristically strongly express EMA and plasma cell markers such as CD138, VS38, PRDM1 (also known as BLIMP1), and XBP1, and are negative or only positive in occasional cells for B-cell lineage–associated antigens (CD20, CD79a, and PAX5). IRF4/MUM1 is also positive [951, 3763, 4111]. CD45 is weak or negative [951, 1307, 2230]. CD30 is negative [951], although focal and weak staining has been reported in a few cases [3763]. Most tumours express cytoplasmic immunoglobulin (usually IgA, more rarely IgG) with light chain restriction [951]. As described in some plasma cell tumours,

Fig. 13.141 ALK-positive large B-cell lymphoma. A, B Neoplastic cells show immunoblastic and plasmablastic features.
occasional cases are positive for cytokeratin, which may lead (in addition to EMA positivity, weak or negative staining for CD45, and the morphological features of cohesiveness and sinusoidal infiltration of the cells) to the mistaken diagnosis of carcinoma [2230]. The tumours are negative for T-cell markers but may be positive for CD4, CD57, CD43, and perforin [2230,3763]. All cases are EBV-negative and HHV8-negative [2230,4111]. These tumours should be distinguished from ALK-positive anaplastic large cell lymphoma, which is of T-cell origin; from other LBCLs with a sinusoidal growth pattern that are ALK-negative, may be CD30-positive, and express pan-B-cell antigens; and from other immunoblastic-appearing or plasmablastic lymphomas that are ALK-negative [785].

Postulated normal counterpart
A post-germinal centre B cell with plasmablastic differentiation

Genetic profile
The IG genes are clonally rearranged [713,1307]. The key oncogenic factor of this tumour is ALK overexpression due to the fusion protein generated by the translocation of the ALK locus on chromosome 2. The most frequent abnormality is t(2;17)(p23;q23), responsible for a CLTC-ALK fusion protein. Few cases are associated with the t(2;5)(p23;q35) translocation, as described in ALK-positive anaplastic large cell lymphoma [22, 2976]. A cryptic insertion of three ALK gene sequences into chromosome 4q22-24 has also been reported. ALK may also be fused to SQSTM1, SEC31A, or other uncommon fusion partners. These translocations are typically detected in the context of complex karyotypes [713,907,1307, 1348,1790,2230,2601,3333,3763,4111]. ALK protein oncogenic mechanisms include activation of the STAT3 pathway; concordantly, ALK-positive LBCLs express high phospho-STAT3 [4111]. MYC is also expressed in the absence of MYC translocations or amplifications, probably due to transcriptional activation downstream of STAT3 [4111]. Oncogenic AILK activation in murine B cells generates plasmablastic B-cell tumours [709].

Prognosis and predictive factors
In one study, the reported median overall survival of patients with stage III/IV disease was 11 months [3333]. Longer survival (> 156 months) has been reported in children [951,2976]. These tumours are usually negative for CD20 antigen, and are thus unlikely to be sensitive to rituximab. Patients presenting with localized disease (stage I–II) have been found to have significantly longer survival [2230].
Plasmablastic lymphoma

Definition
Plasmablastic lymphoma (PBL) is a very aggressive lymphoma with a diffuse proliferation of large neoplastic cells, most of which resemble B immunoblasts or plasmablasts, that have a CD20-negative plasmacytic phenotype. It was originally described in the oral cavity and frequently occurs in association with HIV infection, but it may also occur in other sites, predominantly extranodal, and in association with other causes of immunodeficiency (785,936). Some cases, particularly in the oral cavity (i.e. the oral mucosa type), look most like a diffuse large B-cell lymphoma (LBCL); other cases have morphologically recognizable plasmacytic differentiation. Other subtypes of LBCLs with a plasmablastic immunophenotype (e.g. ALK-positive LBCL and HHV8-associated lymphoproliferative disorders) are not included in this category.

ICD-O code
9735/3

Epidemiology
This lymphoma occurs predominantly in adults with immunodeficiency, most commonly due to HIV infection but also in the setting of iatrogenic immunosuppression (transplantation and autoimmune diseases) and in elderly patients with presumptive immunosenescence. PBL also occurs in children with immunodeficiency, mainly due to HIV infection [417,758,785,936,2365].

Etiology
Immunodeficiency, due to various causes, predisposes individuals to the development of PBL. The tumour cells are EBV-infected in most patients [417,785,936,1023].

Localization
PBL most frequently presents as a mass in extranodal regions of the head and neck, in particular the oral cavity, with the gastrointestinal tract being the next most common site. Other extranodal localizations reported in >1% of cases include the skin, bone, genitourinary tract, nasal cavity and paranasal sinuses, CNS, liver, lungs, and orbits. Nodal involvement is found in <10% of cases overall, but in 30% of post-transplant cases (578,785,936,1023).

Clinical features
Disseminated stage III/IV disease, including bone marrow involvement, is found at presentation in 75% of HIV-positive patients and 50% of post-transplant patients, but in only 25% of patients without apparent immunodeficiency [578]. Most patients have an intermediate-risk or high-risk International Prognostic Index (IPI) score. CT and PET show disseminated bone involvement in 30% of patients [3942]. A paraprotein may be detected in some cases [3665]. Tumours with features of PBL may occur in patients with prior plasma cell neoplasms, including plasma cell myeloma. Such cases should be considered plasmablastic transformation of myeloma and distinguished from primary PBL.

Microscopy
PBLs show a morphological spectrum varying from a diffuse and cohesive proliferation of cells resembling immunoblasts to cells with more obvious plasmacytic differentiation, which may resemble cases of plasmablastic plasma cell myeloma. Mitotic figures are frequent. Apoptotic cells and tingible body macrophages may be present. Cases with monomorphic plasmablastic cytology are most commonly seen in the setting of HIV infection and in the oral, nasal, and paranasal sinus areas (i.e. the oral mucosal type). Conversely, cases with plasmacytic differentiation tend to occur more commonly in other extranodal sites, as well as in lymph nodes (785,936). The differential diagnosis of cases with plasmacytic differentiation may include anaplastic or plasmablastic plasma cell myeloma. A history of immune deficiency and the presence of EBV by in

Fig 13.143 Plasmablastic lymphoma (PBL). A PBL of the oral mucosa with a monomorphic proliferation of large, immunoblastic cells with prominent nucleoli. B PBL with plasmacytic differentiation. Many of the tumour cells are large, with round nuclei and variably prominent nucleoli and showing coarse chromatin. Smaller cells with plasmacytic differentiation are also present.
In situ hybridization for EBV-encoded small RNA (EBER) are useful in establishing the diagnosis of PBL. However, some cases occurring in HIV-positive patients have overlapping features with plasma cell myeloma, such as lytic bone lesions and monoclonal serum immunoglobulins (3865,3942). In some cases, a definite distinction cannot be made, and a descriptive diagnosis, such as ‘plasmablastic neoplasm, consistent with PBL or anaplastic plasmacytoma’, may be acceptable. LBCLs with plasmablastic features may occur as transformation of small B-cell lymphoid neoplasms. These cases have a morphology and phenotype similar to those of PBL, but immunodeficiency does not seem to play a role and EBV infection and MYC translocation are only rarely seen (2531).

Immunophenotype
The neoplastic cells express a plasma cell phenotype, including positivity for CD138, CD38, VS38c, IRF4/MUM1, PRDM1 (also called BLIMP1), and XBP1. CD45, CD20, and PAX5 are either negative or sometimes weakly positive in a minority of cells. CD79a is positive in approximately 40% of cases (578,2699, 4111). Cytoplasmic immunoglobulin is commonly expressed, most frequently IgG and either kappa or lambda light chain. CD56 is detected in 25% of cases. It is usually negative in the oral mucosal type, but may be seen in cases with plasmacytic differentiation. EMA and CD30 are frequently expressed. The Ki-67 proliferation index is usually very high (>90%). BCL2 and BCL6 expression is usually absent, whereas CD10 is expressed in 20% of cases. Cyclin D1 is negative. Some cases express the T-cell associated markers CD43 and CD45RO (578,785,936,1023,4164). Reactive infiltrating T cells are usually very scarce. Some LBCLs may have marked morphological plasmablastic features but strongly express CD20, CD79a, and PAX5 (3682). These cases should not be considered PBL and are better classified as diffuse LBCL, NOS.

In situ hybridization for EBER is positive in 60–75% of cases, but LMP1 is rarely expressed. PBL is more frequently EBV-positive in HIV-positive and post-transplant patients than in HIV-negative patients (578,2755). HHV8 is consistently absent.

Postulated normal counterpart
A plasmablast (i.e. a blastic proliferating B cell that has switched its phenotype to the plasma cell gene expression programme)

Genetic profile
Clonal IGH rearrangement is demonstrable, even when immunoglobulin expression is not detectable, and IGHV may have somatic hypermutation or be unmutated with a germline configuration (1273).

Genetic studies have revealed frequent complex karyotypes. MYC translocation has been identified in approximately 50% of cases, more frequently in EBV-positive tumours (74%) than in EBV-negative tumours (43%), and it is associated with MYC protein expression (400,3865,4110, 4111). The rearrangement usually occurs with IG genes (4110).

Prognosis and predictive factors
The prognosis is generally poor; more than three quarters of patients die of the disease, with a median survival of 6–11 months (578,2755). Newer therapies and better treatment of HIV infection may be associated with a better prognosis, but the results are not consistent across studies (578). Evaluation of prognostic parameters has not yielded consistent results. However, MYC translocation has been associated with a worse outcome in two studies (578,2755).
Primary effusion lymphoma

**Definition**
Primary effusion lymphoma (PEL) is a large B-cell neoplasm usually presenting as serous effusions without detectable tumour masses. It is universally associated with the human herpesvirus 8 (HHV8), also called Kaposi sarcoma–associated herpesvirus. It most often occurs in the setting of immunodeficiency. Some patients with PEL secondarily develop solid tumours in adjacent structures such as the pleura. Rare HHV8-positive lymphomas indistinguishable from PEL, present as solid tumour masses, and have been termed extracavitary PEL.

**ICD-O code**
9678/3

**Synonym**
Body cavity–based lymphoma (obsolete)

**Epidemiology**
Most cases arise in young or middle-aged men who have sex with men and who have HIV infection and severe immunodeficiency [2803,3479]. There is frequent coinfection with monoclonal EBV [623,2803,3479]. PEL has also been reported in recipients of solid organ transplants [1030,1875,2417]. The disease also occurs in the absence of immunodeficiency, usually in elderly patients, both men and women [3946]. In these patients, the lymphoma cells contain HHV8 and may lack EBV [775,3478].

**Etiology**
The neoplastic cells are positive for HHV8 (Kaposi sarcoma–associated herpesvirus or KSHV) in all cases. Most cases are co-infected with EBV [110,157,1683,3479], but EBV has restricted gene expression and is not required for the pathogenesis. HHV8 encodes > 1 homologue of cellular genes that provide proliferative and anti-apoptotic signals [116,156,172,1895]. HHV8-encoded proteins and microRNAs include LANA1, vIRF3, vFLIP, and miR-K1 [116,156,172,1895,4090]. HHV8 IL6 prevents apoptosis by suppressing proapoptotic caspase D [670]. HHV8-encoded vFLIP activates NF-kappaB by binding IKBKG/NEMO and prevents death-receptor induced apoptosis. PELs also express vIRF3, which inhibits HLA transactivators, resulting in inefficient recognition and killing by T cells [3468]. Secretome analysis has revealed proteins involved in inflammation, immune response, and cell cycle and growth; structural proteins; and other proteins [1385].

**Localization**
The most common sites are the pleural, pericardial, and peritoneal cavities. Typically only a single body cavity is involved (308,955,3006). PEL has also been reported in unusual cavities, such as an artificial cavity related to the capsule of a breast implant [3478]. Most cases of PEL remain restricted to the body cavity of origin, but subsequent dissemination can occur. Extracavitary tumours with morphological and phenotypic characteristics similar to those of PEL can occur in extranodal sites including the gastrointestinal tract, skin, lungs, and CNS, or can involve the lymph nodes [631,824,955].

**Clinical features**
PELs occur mainly in males. The median patient age at presentation is 42 years in HIV-infected individuals and 73 years in the general population. Male homosexual contact is the most common risk factor, followed by injection drug use. PELs also occur in HIV-seronegative men and women who have been exposed to HHV8. Patients typically present with effusions in the absence of lymphadenopathy or organomegaly. Approximately one third to half of the patients have pre-existing or develop Kaposi sarcoma [110], and the CD4+ cell count is generally low. Occasional cases are associated with multicentric Castleman disease [3946]. PEL should be distinguished from the rare HHV8-negative effusion-based lymphoma morphologically similar to PEL that has been described in patients with fluid overload states [59,1753,2891,3386,4378], as well as from the EBV-associated HHV8-negative large B-cell lymphomas also occurring with chronic suppurative inflammation (diffuse large B-cell lymphoma associated with chronic inflammation), such as pyothorax-associated lymphoma [121,804]. Cases of extracavitary PEL occur in lymph nodes or extranodal sites without lymphomatous effusions during the course of the disease. These cases are similar to PEL in their clinical presentation occurring in HIV-positive men and morphology [3044]. Other lymphomas, including Burkitt lymphoma, can present with a malignant effusion and are unrelated to PEL.

**Microscopy**
In cytocentrifuge preparations, the cells exhibit a variety of appearances, ranging from large immunoblastic or plasma-
blastic cells to cells with more-anaplastic morphology. Nuclei are large and round to more irregular in shape, with prominent nucleoli. The cytoplasm can be abundant and is deeply basophilic, with vacuoles in occasional cells. A perinuclear hof consistent with plasmacytid differentiation may be seen. Some cells resemble Hodgkin or Reed–Sternberg cells. Mitotic figures are numerous. The cells often appear more uniform in histological sections than in cytospin preparations [110, 955, 2803].

The histological features of extracavitary PELs include an immunoblastic to anaplastic morphology similar to that seen in effusions. There may be lymph node sinus involvement, and staining for HHV8 may be helpful in differentiating these cases from anaplastic large cell lymphoma [826]. Pleomorphic large cells may have a Hodgkin-like appearance, necessitating differentiation from classic Hodgkin lymphoma. There may be involvement of endothelial-lined lymphatic or vascular channels, and cases resembling intravascular lymphoma have been reported [826].

**Immunophenotype**

The lymphoma cells usually express CD45 but lack pan-B-cell markers such as CD19, CD20, and CD79a [2054, 2803]. Surface and cytoplasmic immunoglobulin is absent. BCL6 is usually absent. Activation and plasma cell–related markers and a variety of non–lineage associated antigens such as HLA-DR, CD30, CD38, VS38c, CD138, and EMA are often demonstrable. Levels of immunoglobulin expression are usually undetectable or low. The cells usually lack T/NK-cell antigens, although aberrant expression of T-cell markers may occur, and may be more frequent in cases of extracavitary PEL [308, 431, 826, 3044, 3477]. The nuclei of the neoplastic cells are positive for the HHV8-associated latent protein LANA1 (also called ORF73) [1065]. This is very useful in establishing a diagnosis. Despite the usual positivity for EBV by in situ hybridization for EBV-encoded small RNA (EBER), EBV LMP1 is absent [110, 775, 1683, 3946]. EBV-negative PELs usually occur in elderly HIV-negative patients from HHV8-endemic areas such as the Mediterranean [3725]. Solid tumours constituting the extracavitary variant of PEL have a phenotype similar to that of PEL but express B-cell associated antigens and immunoglobulins slightly more frequently [631]. They may have lower expression of CD45 but higher expression of CD20 and CD79a, as well as aberrant expression of T-cell markers [3044].

**Postulated normal counterpart**

Post-germinal centre B cell with plasmablastic differentiation

**Genetic profile**

Immunoglobulin genes are clonally rearranged and hypermutated, indicating a B-cell derivation [2565, 4234]. Some cases also have rearrangement of TR genes in addition to IG genes (so-called genotypic infidelity) [1667, 3477]. Rare cases diagnosed as T-cell PEL have been reported as well as a case with monoclonal TR and IGH pseudomonoclonality due to extremely low numbers of B cells [824, 2245]. Such cases should not be diagnosed as PEL. Nearly all cases of PEL contain clonal EBV; the exceptions are in the non–HIV infected population, which may be EBV-negative. No recurrent chromosomal abnormalities have been identified. HHV8 viral genomes are present in all cases. Gene expression profiling of AIDS-related PEL shows a distinct profile, with features of both plasma cells and EBV-transformed lymphoblastoid cell lines [2040]. PELs lack structural alterations in the MYC gene but have deregulated MYC protein due to the activity of HHV8 encoded latent proteins [4017]. They also lack mutations in the RAS family of genes and TP53, as well as rearrangements of CCND1, BCL2, and BCL6. A subset have mutations involving BCL6 [1271]. They have complex karyotypes with numerous abnormalities, including trisomy 12, trisomy 7, and abnormalities of 1q21-25 [1271]. Comparative genomic analysis has revealed gains in chromosomes 12 and X [2780].

**Prognosis and predictive factors**

The clinical outlook is extremely unfavourable, and median survival is <6 months. Rare cases have responded to chemotherapy and/or immune modulation [1357].
HHV8-associated lymphoproliferative disorders

Definition
In addition to causing Kaposi sarcoma, which may involve the lymph nodes, the human herpesvirus HHV8 (also called Kaposi sarcoma–associated herpesvirus) is responsible for a spectrum of lymphoproliferative disorders. These include HHV8-positive multicentric Castleman disease (MCD); HHV8-positive diffuse large B-cell lymphoma (DLBCL), NOS, which frequently arises in the background of MCD; and germinotropic lymphoproliferative disorder (GLPD). Except for GLPD, these disorders are most commonly seen in the setting of HIV infection and in HHV8-endemic areas, but they can also occur in other immunosuppressed states, including following transplantation (2238). GLPD is most commonly seen in immunocompetent individuals. Primary effusion lymphoma (PEL) and extracavitary PEL are also caused by HHV8, but are discussed elsewhere (see Primary effusion lymphoma, p. 323).

Associated conditions
Kaposi sarcoma is frequently present in patients with MCD and HHV8-positive DLBCL, NOS, arising in MCD. PEL and its extracavitary counterpart may complicate HHV8-positive MCD.

Other HHV8-positive lymphoproliferative disorders
Although most cases of HHV8-positive lymphoproliferative disorders fall within the spectrum defined above, individual cases have been reported in which there are overlapping features. For example, cases arising in the background of MCD intermediate between HHV8-positive DLBCL and GLPD have been reported in HIV-positive and HIV-negative patients (1404A). They may resemble HHV8-positive DLBCL, NOS, but are also positive for EBV-encoded small RNA (EBER) (3052,3616). Although GLPD tends not to progress, one reported case evolved to a high-grade HHV8-positive, EBV-positive lymphoma (826). The differential between HHV8-positive DLBCL, NOS, and extracavitary PEL may be problematic, but most cases of PEL are EBV-positive, lack cytoplasmic immunoglobulins, express activation and plasma cell-associated antigens, including CD30, CD38, CD138, and EMA, and arise from a terminally differentiated rather than a naive B cell.

Multicentric Castleman disease
Definition
Multicentric Castleman disease (MCD) is a clinicopathological entity that encompasses a group of systemic polyclonal lymphoproliferative disorders in which there is a proliferation of morphologically benign lymphocytes, plasma cells, and vessels, due to excessive production of cytokines, in particular IL6 (556, 1133). Activation of the IL6R signalling pathway by the virus plays a key role in the development of HHV8-infected B-cell lymphoproliferative lesions, including MCD (1048). In patients with HIV, MCD is almost always HHV8-related. In the absence of HIV, MCD is HHV8-related in as many as 50% of cases, and usually occurs in HHV8-endemic areas (1029).

<table>
<thead>
<tr>
<th>Feature</th>
<th>HHV8-positive MCD</th>
<th>HHV8-positive DLBCL, NOS</th>
<th>HHV8-positive GLPD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical presentation</td>
<td>Generalized lymphadenopathy</td>
<td>Large lymph node</td>
<td>Localized or sometimes multifocal lymph node involvement</td>
</tr>
<tr>
<td></td>
<td>Splenomegaly</td>
<td>Splenic mass</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Constitutional symptoms</td>
<td>Extranodal sites</td>
<td></td>
</tr>
<tr>
<td>Microscopy</td>
<td>Abnormal follicles</td>
<td>Sheets of large plasmablastic cells</td>
<td>Retention of architecture with germinal centres containing variable numbers of plasmablasts sometimes replacing follicles</td>
</tr>
<tr>
<td></td>
<td>Plasmablasts predominantly in mantle zones</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Interfollicular plasma cell hyperplasia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phenotype</td>
<td>B-cell antigens +/-</td>
<td>B-cell antigens +/-</td>
<td>B-cell antigens –</td>
</tr>
<tr>
<td></td>
<td>clgM lambda +</td>
<td>clgM lambda +</td>
<td>Monotypic kappa or lambda</td>
</tr>
<tr>
<td></td>
<td>IRF4a +</td>
<td>IRF4a +</td>
<td>Any heavy chain</td>
</tr>
<tr>
<td></td>
<td>CD138 –</td>
<td>CD138 –</td>
<td>CD138 –</td>
</tr>
<tr>
<td>Clonality</td>
<td>Polyclonal</td>
<td>Monoclonal</td>
<td>Polyclonal or oligoclonal</td>
</tr>
<tr>
<td>HHV8 LANAI</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>EBER</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>HIV status</td>
<td>+/-</td>
<td>+/-</td>
<td>–</td>
</tr>
<tr>
<td>Prognosis</td>
<td>Poor but improved with new therapies</td>
<td>Poor</td>
<td>Usually responds to treatment</td>
</tr>
</tbody>
</table>

EBER, EBV-encoded small RNA.

a Also known as MUM1.

**Fig. 13.148** HHV8-positive multicentric Castleman disease. A Immunostaining for HHV8 LANA1 shows localization in the plasmablasts present in the mantle zones. B Staining for IgM shows localization within the cytoplasm of the plasmablastic cells. C Plasmablasts are negative for kappa light chains. The interfollicular reactive plasma cells stain positively. D Plasmablasts stained for lambda light chains show lambda light chain restriction.

**Fig. 13.149** HHV8-positive multicentric Castleman disease. A Immunostaining for HHV8 LANA1 shows localization in the plasmablasts present in the mantle zones. B Staining for IgM shows localization within the cytoplasm of the plasmablastic cells. C Plasmablasts are negative for kappa light chains. The interfollicular reactive plasma cells stain positively. D Plasmablasts stained for lambda light chains show lambda light chain restriction.

MCD is idiopathic in HHV8-negative and HIV-negative patients. Idiopathic HIV- and HHV8-negative multicentric Castleman disease (iMCD) is a systemic disease with constitutional symptoms, laboratory abnormalities, and multicentric lymphadenopathy characterized by polytypic plasmacytosis and variably prominent hypervascular or regressed germinal centres [1132A,3475A,4450A]. The diagnosis requires exclusion of infectious, neoplastic, and autoimmune diseases that may have similar clinical presentations. In this syndrome, the hypercytokinaemia may be driven by inflammatory disease or inflammatory gene mutations, autoantibodies, ectopic cytokine secretion, as seen in paraneoplastic syndromes, or viral signalling by a non-HHV8 virus [1133].

**Epidemiology**

HHV8-positive MCD occurs worldwide in immunosuppressed patients, particularly in association with HIV/AIDS. It may also affect immunocompetent individuals in HHV8-endemic areas (e.g. sub-Saharan Africa and Mediterranean countries) [425]. In HIV-infected patients, there is a strong association with sexual transmission, and men are predominantly affected.

**Etiology**

MCD is a heterogeneous group of disorders thought to arise from excessive hypercytokinaemia, most notably of IL6 [1133]. In HHV8-positive MCD, the plasmablastic cells are infected with HHV8, which produces viral IL6. In addition, HHV8-encoded proteins and microRNAs provide proliferative and anti-apoptotic signals contributing to the pathogenesis. These include LANA1, LANA2, IL10, vFLIP, and miR-K1 [116,156,172,1895,4090]. HHV8-encoded vGPCR induces expression of proinflammatory and angiogenic factors contributing to the inflammatory and hyperproliferative nature of the lesions, and also constitutively activates the nuclear factor of activated T cells [3097].

**Localization**

HHV8-positive MCD usually presents with generalized lymphadenopathy and splenomegaly.

**Clinical features**

Patients with HHV8-positive MCD present with constitutional symptoms, enlarging lymph nodes, and splenomegaly. Constitutional symptoms include fever, night sweats, fatigue, weight loss, and respiratory symptoms [440]. Kaposi sarcoma is commonly also present [1064, 2968]. In addition to lymphadenopathy, patients may have hepatosplenomegaly, and a skin rash [556]. Laboratory findings include anaemia, thrombocytopenia, hypoalbuminaemia, hypergammaglobulinaemia, and elevated C-reactive protein [556].

**Microscopy**

The B-cell follicles of lymph nodes and spleen show varied degrees of involution and hyalinization of their germinal cen-
tres, with prominent mantle zones that may intrude into the germinal centres and completely efface them. Follicles may show onion skinning or widened concentric rings of mantle zone lymphocytes, and prominent penetrating venules typical of Castleman disease. Among these mantle zone cells and adjacent interfollicular regions, there are variable numbers of medium-sized to large plasmablastic cells with amorphophilic cytoplasm and vesicular, often eccentrically placed nuclei containing one or two prominent nucleoli. The blasts may be single in the intrafollicular and perifollicular areas, or may form small clusters or aggregates. Sheets of mature plasma cells expand the interfollicular region, including cells with cytoplasmic inclusions (Russell bodies) and crystalline forms. As the disease progresses, the plasmablasts may coalesce to form clusters (1064,1404A). There may be clonal expansion of these clusters to form sheets of lymphoma cells effacing the architecture, with progression to HHV8-positive diffuse large B-cell lymphoma (see below).

**Immunophenotype**
The plasmablasts in MCD show stippled nuclear staining for HHV8 LANA1 and strong clgM expression, with lambda light chain restriction. A proportion of the plasmablasts are positive for viral IL6. Plasmablasts have a CD20/-, CD79a-/+ , CD138-, PAX5-, CD38/-/+ , CD27- phenotype and are negative for EBV-encoded small RNA (EBER) (2968). The interfollicular plasma cells are typically clgM-negative and clgA-positive, express polytypic light chains, and do not show nuclear expression of LANA1 antigen.

**Postulated normal counterpart**
A naïve B cell

**Genetic profile**
Despite the constant expression of monotypic IgM lambda by the plasmablasts in HHV8-positive MCD, careful molecular studies have shown that they constitute a polyclonal population (1048). The plasmablastic aggregates that can develop during the progression of MCD may be monoclonal or oligoclonal.

**Prognosis and predictive factors**
Prognosis has been poor, related to the lymphoid proliferation and underlying immune disorder. However, multitarget treatment strategies including rituximab, antiherpesvirus therapy, and targeted therapy against IL6 have improved outcome (556,1133,2064,4090).

### HHV8-positive diffuse large B-cell lymphoma, NOS

**Definition**
HHV8-positive diffuse large B-cell lymphoma (DLBCL), NOS, usually arises in association with HHV8-positive multicentric Castleman disease (MCD). However, similar lymphomas (HHV8-positive, EBV-positive, with lambda light chain restriction) have been reported in the absence of MCD (1108). The lymphoma is characterized by a monoclonal proliferation of HHV8-infected lymphoid cells resembling plasmablasts expressing IgM lambda. It is usually associated with HIV infection. The cells may morphologically resemble plasmablasts and have abundant cytoplasmic immunoglobulin; however, they correspond to a naïve, IgM-producing B cell without IG somatic hypermutations. This lymphoma must be distinguished from plasmablastic lymphomas presenting in the oral cavity or other extranodal sites that frequently show class-switched and hypermutated IG genes. HHV8-positive DLBCLs, NOS, differ from primary effusion lymphoma (PEL) in that they are EBV-negative, do not have IG gene mutations, and are thought to arise from naïve IgM lambda-positive B cells rather than terminally differentiated B cells.

**ICD-O code**
9738/3

**Epidemiology**
Among patients with HIV and MCD, the risk of developing non-Hodgkin lymphoma is 15 times that within the general HIV-positive population (2700,2968). In a series of 60 patients with HIV-positive MCD, 6 patients developed HHV8-positive DLBCL with a plasmablastic appearance (2968).

---

**Fig. 13.150** Leukaemic HHV8-positive diffuse large B-cell lymphoma, NOS, arising in multicentric Castleman disease. Wright-Giemsa-stained tumour cells in the peripheral blood.

**Fig. 13.151** HHV8-positive diffuse large B-cell lymphoma, NOS, arising in multicentric Castleman disease. A Sheets of plasmablasts efface normal lymph node architecture. B High magnification showing sheets of neoplastic plasmablasts. C Tumour cells are negative for immunoglobulin kappa light chain. D Tumour cells are positive for lambda light chain indicating lambda light chain restriction.
Etiology
By definition, the large lymphoid/plasma-blastic cells in all cases are positive for HHV8. The molecular mechanisms involved in this lymphoma seem similar to those of the other HHV8-positive entities (116,156,172,1895).

Localization
HHV8-positive DLBCL, NOS, characteristically involves the lymph nodes and/or spleen, but can disseminate to other viscer, including the liver, lungs, and gastrointestinal tract, and can also manifest as a leukaemia, with involvement of the peripheral blood (1064,2968).

Clinical features
HHV8-positive DLBCL, NOS, usually arises in patients with clinical features of HHV8-positive MCD. HHV8-positive DLBCL, NOS, usually manifests with profound immunodeficiency, enlarging lymph nodes, and massive splenomegaly. There may also be manifestations of Kaposi sarcoma (1064,2968). More rarely, HHV8-positive DLBCL, NOS, may arise in the absence of MCD (826,1108,1404A).

Microscopy
The emergence of frank lymphoma is heralded by expansion of the small confluent sheets of HHV8 LANA1-positive plasmablasts to efface the lymph node and splenic architecture, often with massive splenomegaly. The large plasmablastic cells have vesicular, often eccentrically placed nuclei containing one or two prominent nucleoli and amphophilic cytoplasm. Infiltrates can also be present in the liver, lungs, and gastrointestinal tract, and in some cases there is involvement of the bone marrow and peripheral blood by HHV8-positive IgM lambda plasmablasts (1064,2968,2969). Distinction from extracavitary PEL may be difficult, but EBV is usually positive in extracavitary PEL and there may be kappa or lambda light chain restriction.

Immunophenotype
The malignant large plasmablastic lymphoid cells show stippled nuclear staining for LANA1, and like the plasmablasts in HHV8-positive MCD, strongly express cIgM with lambda light chain restriction (2968). They have a CD20+/−, CD79a−, CD138−, CD38+/−, CD27− phenotype, and are negative for EBV-encoded small RNA (EBER).

Postulated normal counterpart
A naïve B cell

Genetic profile
Frank HHV8-positive DLBCLs, NOS, are monoclonal. The IG genes are unmutated, unlike in PEL and extracavitary PEL, in which IG genes are clonally rearranged and hypermutated.

Prognosis and predictive factors
HHV8-positive DLBCL, NOS, is an extremely aggressive disorder.

HHV8-positive germinotropic lymphoproliferative disorder

Definition
HHV8-positive germinotropic lymphoproliferative disorder (GLPD) is a monotypic HHV8-positive lymphoproliferative lesion that usually occurs in HIV-negative individuals (1047,1404A). HHV8-positive

Fig. 13.152 HHV8-positive germinotropic lymphoproliferative disorder. A Germinal centre replaced by confluent sheets of plasmablasts. B Germinal centre largely replaced by plasmablasts. C Plasmablasts are seen within a hyperplastic germinal centre. D Clusters of plasmablasts within hyperplastic germinal centres.
Fig. 13.153 HHV8-positive germinotropic lymphoproliferative disorder. A Plasmablasts stained for HHV8 LANA1. B Plasmablasts stain for lambda light chains. C Plasmablasts are positive for EBV-encoded small RNA (EBER).

plasmablasts partially or completely replace germinal centres [1047,2938, 3904]. The plasmablasts show either kappa or lambda light chain restriction but are polyclonal or oligoclonal. Coinfection with EBV is characteristic.

ICD-O code 9738/1

Epidemiology GLPD mainly affects immunocompetent individuals, but occasional cases have been described in HIV-positive patients [1404A]. There is no known epidemiological association.

Etiology The large plasmablastic cells are positive for HHV8 in all cases. Unlike in MCD and HHV8-positive DLBCL, NOS, in GLPD, the large cells are also positive for EBV-encoded small RNA (EBER). The contribution of EBV to the pathogenesis is uncertain. Occasional EBV-negative cases have been described [1404A].

Localization GLPD involves the lymph nodes.

Clinical features The disorder presents with localized and sometimes multifocal lymph node involvement in otherwise healthy individuals.

Microscopy There is overall retention of nodal architecture. The lymphoid proliferation is characterized by medium-sized to large lymphoid cells resembling plasmablasts that involve or replace germinal centres. In some nodes, there may be atrophic follicles resembling those seen in multicentric Castleman disease.

Immunophenotype Plasmablastic cells are negative for CD20, CD79a, CD138, BCL6, and CD10, and negative or positive for CD30. Occasional cases may co-express CD3 in the absence of other T-cell markers [1404A]. They may be positive for IRF4/MUM1 and may show monotypic kappa or lambda light chain, unlike the cells in multicentric Castleman disease, which are always lambda-positive. In some cases, immunoglobulin expression cannot be demonstrated. They are positive for HHV8 LANA1 and EBER. Cases are negative for the EBV latency proteins LMP-1, EBNA2, and BZLF-1 indicating latency 1 [366A].

Postulated normal counterpart A germinal centre B cell [1047]

Genetic profile Despite the constant expression of monoclonal immunoglobulin, HHV8-positive GLPD has a polyclonal or oligoclonal pattern of IG gene rearrangements. There may be somatic mutation and intraclonal variation in the rearranged IG genes [1047].

Prognosis and predictive factors In most cases, there is a favourable response to chemotherapy or radiation [1047]. However, there are rare cases with features of both GLPD and HHV8-positive diffuse large B-cell lymphoma, NOS; one reported case in an HIV-negative patient progressed from GLPD to HHV8-positive EBV-positive diffuse large B-cell lymphoma, NOS, suggesting that there can be overlap between these conditions [826,3616,1404A].
Burkitt lymphoma

Definition
Burkitt lymphoma (BL) is a highly aggressive but curable lymphoma that often presents in extranodal sites or as an acute leukaemia. It is composed of monomorphic medium-sized B cells with basophilic cytoplasm and numerous mitotic figures, usually with a demonstrable MYC gene translocation to an IG locus. The frequency of EBV infection varies according to the epidemiological subtype of BL. No single parameter, such as morphology, genetic analysis, or immunophenotyping, can be used as the gold standard for diagnosis of BL; a combination of several diagnostic techniques is necessary.

ICD-O code 9687/3

Synonyms
Burkitt tumour (obsolete); malignant lymphoma, undifferentiated, Burkitt type (obsolete); malignant lymphoma, small noncleaved, Burkitt type (obsolete); Burkitt cell leukaemia (9826/3)

Epidemiology
Three epidemiological variants of BL are recognized, which mainly differ in their geographical distribution, clinical presentation, subtle morphological aspects, molecular genetics, and biological features. Endemic BL occurs in equatorial Africa and in Papua New Guinea, with a distribution that overlaps with regions endemic for malaria. In these areas, BL is the most common childhood malignancy, with an incidence peak among children aged 4–7 years and a male-to-female ratio of 2:1 [503, 4369]. Sporadic BL is seen throughout the world, mainly in children and young adults. The incidence is low, accounting for only 1–2% of all lymphomas in western Europe and in the USA. In these countries, BL accounts for approximately 30–50% of all childhood lymphomas. The median age of the adult patients is 30 years, but there is also an incidence peak in elderly patients [2590]. The male-to-female ratio is 2–3:1. In some parts of the world (e.g. South America and northern Africa), the incidence of BL is intermediate between that of sporadic BL in developed countries and endemic BL [2441, 3258]. Immunodeficiency-associated BL is more common in the setting of HIV infection than in other forms of immunosuppression. In HIV-infected patients, BL appears early in the progression of the disease, when CD4+ T-cell counts are still high [2332, 3309]. The increased risk of developing BL seems to have persisted among HIV-infected patients over time, across the pre- and post-HAART eras [1370].

Etiology
In all patients with endemic BL, the EBV genome is present in >95% of the neoplastic cells. There is also a strong epidemiological link with holoendemic malaria. Therefore, EBV and Plasmodium falciparum are thought to be responsible for endemic BL [915, 1870]. Recent data have provided new insight into how these two human pathogens interact to cause the disease, supporting the emerging concepts of polymicrobial disease pathogenesis [688, 2692, 2937, 3285, 3368]. Malaria and EBV are ubiquitous within the lymphoma belt of Africa, suggesting that other etiological agents may also be involved, including arboviruses, schistosomiasis, and plant tumour promoters [4121, 4122, 2486]. A recent study using RNA sequencing found herpesviruses in 12 of 20 cases (60%) of endemic BL, in particular HHV5 and HHV8, and confirmed their presence by immunohistochemistry in the adjacent non-neoplastic tissue [8]. The polymicrobial nature of endemic BL is further supported by the status of B-cell receptor, which carries the signs of antigen selection due to chronic antigen stimulation [84, 3171]. In sporadic BL, EBV can be detected in...
approximately 20–30% of cases; however, low socioeconomic status and early EBV infection are associated with a higher prevalence of EBV-positive cases [2441]. The proportion of EBV-positive sporadic BL appears to be much higher in adults than in children [3531]. In immunodeficiency-associated cases, EBV is identified in only 25–40% of cases [1531,3396]. The variation in EBV association among the different forms of BL and among different countries makes it difficult to determine the role of the virus in BL pathogenesis. EBV may impact host cell homeostasis in various ways by encoding its own genes and microRNAs and by interfering with cellular microRNA expression [85,1982, 2283,3173,4181]. However, recent studies have shown that the mutation landscape and viral landscape of BL is more complex than previously reported. In fact, a distinct latency pattern of EBV involving the expression of LMP2 along with that of lytic genes has been demonstrated [8, 158,4003]. These results confirm recent evidence that LMP2A cooperates in re-programming normal B-lymphocyte function and increases MYC-driven lymphomagenesis through activation of the PI3K pathway, crucial cooperating mechanisms of MYC transformation [1008,1221, 3509]. However, expression of the latency pattern in BL is heterogeneous, not only from case to case, but also within a given case from cell to cell, suggesting that the tumour is under selective pressure and needs alternative mechanisms to survive and proliferate.

Localization
Extranodal sites are most often involved, with some variation among the epidemiological variants. However, in all three variants, patients are at risk for CNS involvement. In endemic BL, the jaws and other facial bones (e.g. the orbit bones) are the site of presentation in about 50–70% of cases. The distal ileum, caecum, omentum, gonads, kidneys, long bones, thyroid, salivary glands, and breasts are frequently involved. Bone marrow involvement may be present, but may not be associated with leukaemic expression [503,2912]. In sporadic BL, tumours in facial structures, in particular the jaws, are very rare. Most cases present with abdominal masses. The ileocaecal region
is the most frequent site of involvement. Like in endemic BL, the ovaries, kidneys, and breasts may also be involved [4369]. Breast involvement, often bilateral and massive, has been associated with onset during puberty, pregnancy, or lactation. Retroperitoneal masses may result in spinal cord compression with paraplegia. Lymph node presentation is unusual, but more common in adults than in children. Waldeyer ring or mediastinal involvement is rare. In immunodeficiency-associated BL, nodal localization and bone marrow involvement are frequent [3396].

Clinical features
Patients often present with bulky disease and high tumour burden due to the short doubling time of the tumour. In the typical paediatric cases, the parents of affected children usually report symptoms of only a few weeks' duration. Specific clinical manifestations at presentation may vary according to the epidemiological subtype and the site of involvement.

Paediatric BL cases are staged according to the system proposed by Murphy and Hustu [2793]. A revised international paediatric non-Hodgkin lymphoma staging system has been recently proposed [3417]. Localized-stage (I or II) disease and advanced-stage (III or IV) disease are found in approximately 30% and 70% of patients, respectively, at presentation. Upon initiation of therapy, a tumour lysis syndrome can occur due to rapid tumour cell death.

Burkitt leukaemia variant
A leukaemic phase can be observed in patients with bulky disease, but only rare cases, typically in males, present purely as leukaemia with peripheral blood and bone marrow involvement [2443, 2444, 3741]. Burkitt leukaemia tends to involve the CNS at diagnosis or early in the disease course. Its high and immediate chemosensitivity easily leads to an acute tumour lysis syndrome. Involvement of the bone marrow or presentation as acute leukaemia is uncommon in endemic BL [2442].

Macrosopy
Involved organs are replaced by masses with a fish-flesh appearance, often associated with haemorrhage and necrosis. Adjacent organs or tissues are compressed and/or infiltrated. Nodal involvement is rare in endemic and sporadic BL, but more frequent in immunodeficiency-associated BL. Even when nodal involvement is not present, uninvolved lymph nodes may be surrounded by tumour.

Microscopy
The prototype of BL is observed in endemic BL, in a high proportion of sporadic BL cases, in particular in children, and in many cases of immunodeficiency-related BL. The tumour cells are medium-sized and show a diffuse monotonous pattern of growth. The cells appear to be cohesive but often exhibit squared-off borders of retracted cytoplasm in formalin-fixed material. The nuclei are round, with finely clumped chromatin, and contain multiple basophilic medium-sized, paracentrally located nucleoli. The cytoplasm is deeply basophilic and usually contains lipid vacuoles, which are better seen in imprint preparations or fine-needle aspiration cytology. The tumour has an extremely high proliferation rate, with many mitotic figures, as well as a high rate of spontaneous cell death (apoptosis). A so-called starry sky pattern is usually present, which is due to the presence of numerous tingible body macrophages. Some cases have a florid granulomatous reaction that may cause difficulties in the recognition of the tumour. These cases typically present with limited stage disease and have an especially good prognosis [1549, 1666].

Some cases of BL may show greater nuclear pleomorphism despite clinical, immunophenotypic, and molecular features characteristics of typical BL. In such cases, the nucleoli may be more prominent and fewer in number. In other cases, particularly in adults with immunodeficiency, the tumour cells exhibit plasmacytoid differentiation, with eccentric basophilic cytoplasm and often a single central nucleolus [3396]. These morphological features are consistent with gene expression profile studies suggesting that the morphological spectrum of BL is broader than previously thought [1732].

Immunophenotype
The tumour cells typically express moderate to strong membrane IgM with light chain restriction, B-cell antigens (CD19, CD20, CD22, CD79a, and PAX5), and germinal centre markers (CD10 and BCL6). CD38, CD77, and CD43 are also frequently positive [280, 2190, 2826]. Almost all BLs have strong expression of MYC protein in most cells [3902]. The proliferation rate is very high, with nearly 100% of the cells positive for Ki-67. The characteristic cytoplasmic lipid vesicles can also be demonstrated by immunohistochemistry on paraffin-embedded tissue.
Genetic profile

Antigen receptor genes

The tumour cells show clonal IG rearrangements with somatic hypermutation and intraclonal diversity [84].

Cytogenetic abnormalities

The molecular hallmark of BL is the translocation of MYC at band 8q24 to the IGH region on chromosome 14q32, t(8;14) (q24;q32), or less commonly to the IGK locus on 2p12 [t(2;8)] or the IGL locus on 22q11 [t(8;22)]. Most breakpoints originate from aberrant somatic hypermutation mediated by the activity of activation-induced cytidine deaminase, in contrast to the previous assumption that they derived from aberrant VDJ gene recombination, and the translocation primarily involves the switch regions of the IGH locus. In sporadic and immunodeficiency-associated BL, most breakpoints are nearby or within MYC, whereas in endemic cases most breakpoints are dispersed over several hundred kilobases further upstream of the gene [2093]. MYC translocations are not specific for BL, and may occur in other types of lymphoma. Additional chromosomal abnormalities may also occur in BL, including gains of 1q, 7, and 12 and losses of 6q, 13q32-34, and 17p, that may play a role in the progression of the disease [195, 2507, 3579, 4036]. Approximately, 10% of classic BL cases lack an identifiable MYC rearrangement [1550, 1732, 1827, 2282]. However, none of the techniques currently used to diagnose genetic changes can unambiguously rule out all MYC translocations [1827], in particular due to very distant breakpoints or insertions of the MYC gene in an IG locus or vice versa. The expression of MYC mRNA and protein in these cases suggests that there are also alternative mechanisms deregulating MYC [2282, 2979, 3579]. In these cases, strict clinical, morphological, and phenotypic criteria should be used to exclude lymphomas that can mimic BL. At least some of these cases constitute the new provisional entity Burkitt-like lymphoma with 11q aberration.

Gene expression profile

Gene and microRNA expression profiling can identify molecular signatures that are characteristic of BL and different from those of other lymphomas (e.g. diffuse large B-cell lymphoma) [877, 1732, 2275, 3171]. Slight differences in the expression profiles have been identified between the endemic and sporadic BL subtypes [2275, 3171]. In addition, molecularly defined BLs do include some cases that are best not diagnosed as BL, and some cases of BL may have a gene expression profile intermediate between those of BL and diffuse large B-cell lymphoma.

Next-generation sequencing

Next-generation sequencing analysis has revealed the importance of the B-cell receptor signalling pathway in the pathogenesis of BL. Mutations of the transcription factor TCF3 (also known as E2A) or its negative regulator ID3 have been reported in about 70% of sporadic BL cases. These mutations activate B-cell receptor signalling, which sustains BL cell survival by engaging the PI3K pathway [2401, 3354, 3509, 3573]. Other recurrent mutations, in CCND3, TP53, RHOA, SMARCA4, and ARID1A, occur in 5–40% of BLs [1381, 2112, 2401, 3354, 4225]. Both the number of mutations overall and the proportion of cases with mutations in TCF3 or ID3 are lower in endemic than sporadic BL [8, 3573]. An inverse correlation between EBV infection and the number of mutations has been observed, suggesting that these mutations may serve in place of the virus for the activation of the B-cell receptor signalling [8, 1381].
Genetic susceptibility
Individuals with X-linked lymphoproliferative syndrome (also known as 'Duncan disease') associated with SH2D1A mutations are at greatly increased risk of developing BL.

Prognosis and predictive factors
BL is a highly aggressive but potentially curable tumour; intensive chemotherapy leads to long-term overall survival in 70–90% of cases, with children doing better than adults. However, there are several adverse prognostic factors: advanced-stage disease, bone marrow and CNS involvement, unresected tumour >10 cm in diameter, and high serum lactate dehydrogenase levels. Relapse, if it occurs, is usually seen within the first year after diagnosis. The overall survival rate in endemic BL has improved from <10–20% to almost 70% due to the introduction of the International Network for Cancer Treatment and Research (INCTR) protocol INCTR 03-06 in African institutions (2861).

Burkitt-like lymphoma with 11q aberration

Definition
Burkitt-like lymphoma with 11q aberration is a subset of lymphomas identified by several recent studies that resemble Burkitt lymphoma (BL) morphologically, to a large extent phenotypically, and in terms of microRNAs and gene expression profile, but that lack MYC rearrangements. Instead, they have a chromosome 11q alteration characterized by proximal gains and telomeric losses: specifically, interstitial gains including a minimal region of gain in 11q23.2-23.3 and losses of 11q24.1-ter (195,3175,3490). These lymphomas lack the 1q gain frequently seen in BL and have more-complex karyotypes than BL. They also have a certain degree of cytological pleomorphism, occasionally a follicular pattern, and frequently a nodal presentation (3490,4454). The clinical course seems to be similar to that of BL, but only a limited number of cases have been reported. Very similar cases have also been reported in the post-transplant setting (1191).

ICD-O code 9687/3

Fig. 13.161 Burkitt-like lymphoma with 11q aberration. The tumour cells are medium-sized to large, with a high mitotic index. A starry-sky pattern is usually seen.

Fig. 13.162 Burkitt-like lymphoma with 11q aberration. Chromosomal view of chromosome 11 analysed by OncoScan array, depicting gains of 11q21-23.3 in blue and terminal losses of 11q23.3-25 in red.
High-grade B-cell lymphoma

Definition
High-grade B-cell lymphoma (HGBL) is a group of aggressive, mature B-cell lymphomas that, for biological and clinical reasons should not be classified as diffuse large B-cell lymphoma (DLBCL), NOS, or as Burkitt lymphoma (BL). There are two categories of HGBL (1547,3846). The first category, HGBL with MYC and BCL2 and/or BCL6 rearrangements, encompasses all B-cell lymphomas (except some rare follicular lymphomas and B-lymphoblastic leukaemia/lymphomas) that have a MYC (8q24) rearrangement in combination with a BCL2 (18q21) and/or a BCL6 (3q27) rearrangement, i.e. the so-called double-hit and triple-hit lymphomas. Morphologically, these cases typically either resemble DLBCL, NOS, or can have features of both BL and DLBCL (referred to in the 2008 WHO classification as B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and BL). Less commonly, they have a blastoid appearance, morphologically mimicking lymphoblastic lymphoma or the blastoid variant of mantle cell lymphoma.

The second category, HGBL, NOS, encompasses cases that either have features intermediate between DLBCL and BL or appear blastoid, but by definition do not harbour a genetic double hit as defined above. This category excludes cases with the morphology of DLBCL, which should be diagnosed as such, even if they have a high proliferative fraction.

Synonym
B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma and Burkitt lymphoma (no longer recommended)

High-grade B-cell lymphoma with MYC and BCL2 and/or BCL6 rearrangements

Definition
High-grade B-cell lymphoma (HGBL) with MYC and BCL2 and/or BCL6 rearrangements is an aggressive mature B-cell lymphoma that harbours a MYC rearrangement at chromosome 8q24 and a rearrangement in BCL2 (at chromosome 18q21) and/or in BCL6 (at chromosome 3q27). These lymphomas are often called double-hit lymphomas, or triple-hit lymphomas if there are both BCL2 and BCL6 rearrangements in addition to the MYC rearrangement. The term "double-hit+ as defined for this category refers only to the co-occurrence of MYC and BCL2 and/or BCL6 translocations. Lymphomas with two oncogenic translocations other than MYC (e.g. concomitant BCL2 and BCL6 translocations) without a MYC breakpoint) or other gene translocations associated with MYC translocations (e.g. CCND1 translocations) are not included in this category.
Except for proven follicular lymphoma and rare cases of B-lymphoblastic leukaemia/lymphoma, NOS, all other lymphomas and leukaemias with these molecular features should be included in this category. The category therefore includes (1) double-hit cases previously classified as B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma (DLBCL) and Burkitt lymphoma (BL); (2) blastoid cases with a double hit; and (3) cases with a DLBCL, NOS, morphology that upon evaluation have rearrangements of MYC and BCL2 and/or BCL6. Grade 3B follicular lymphomas with a double hit that are completely follicular should still be diagnosed as follicular lymphoma, with a comment indicating the cytogenetic findings; however, if there is an associated DLBCL with a double hit, the diagnosis of a follicular lymphoma and HGBL with MYC and BCL2 and/or BCL6 rearrangements should be rendered. Given the possibility of prognostic implications, the morphological appearance of the HGBL with MYC and BCL2 and/or BCL6 rearrangements should be noted in a diagnostic comment.

Rare cases of B-lymphoblastic leukaemia/lymphoma with MYC and BCL2 translocations, sometimes transformed from an antecedent or synchronous follicular lymphoma, are not included in this category, and should be classified as B-lymphoblastic leukaemia/lymphoma with the translocations specified in a comment. Patients with such cases are usually treated like patients with lymphoblastic leukaemia [896,1349,2061,2121,4446]. Rearrangements of MYC, BCL2, and BCL6 should be detected by a cytogenetic/molecular method such as FISH. The presence of only copy-number increase/amplification or somatic mutations, without an underlying rearrangement, is insufficient to qualify a case for this category. There are indications that such cases are also aggressive, similar to the double-hit lymphomas, but there are insufficient data to justify the inclusion of such cases in this category. Although so-called double-expressor DLBCLs that show immunohistochemical overexpression of MYC and BCL2 protein also have a relatively poor prognosis, overexpression cannot be used as a surrogate marker for double-hit cytogenetic status. Most double-hit lymphomas are also double-expressers, but most double-expressers are not double-hit lymphomas; the majority are the activated B-cell subtype of DLBCL, and do not harbour translocations [3846]. Specifically, it is important to distinguish DLBCL with MYC and BCL2 co-expression, which is not a diagnostic category, from high grade B-cell lymphomas with MYC and BCL2 and/or BCL6 rearrangements that also often show this double-expression. This classification is primarily applicable to de novo cases; lymphomas with a proven history of a pre-existing or co-existent indolent lymphoma (of follicular or other type) should be diagnosed as such (e.g. HGBL with MYC and BCL2 rearrangements, transformed from follicular lymphoma).

**ICD-O code** 9680/3

**Synonyms**
(Subset of) B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma and Burkitt lymphoma (no longer recommended); (subset of) diffuse large B-cell lymphoma; double or triple-hit lymphoma

**Epidemiology**
These lymphomas mostly present in elderly patients, with a median age at diagnosis in the sixth to seventh decade, with the youngest reported patients aged approximately 30 years. Both men and women are affected, with a slight male predominance [194,2350,3143,3183,3846]. In one study of triple-hit lymphomas (i.e. with involvement of MYC, BCL2, and BCL6), all patients were men [4254].

**Etiology**
By definition, these mature B-cell lymphomas harbour two or more recurrent cytogenetic events, and in almost all cases, classic cytogenetic analysis also shows many additional abnormalities (complex karyotype). It is likely that the rearrangement of MYC is a secondary event, but this has only been proven for the combination of BCL2 and MYC rearrangements in patients who first had a follicular lymphoma and later developed a MYC rearrangement, and in patients with de novo disease in which two subclones with and without the MYC rearrangement are present. Although it might be envisaged that all lymphomas with
both MYC and BCL2 rearrangements are the result of transformation from an antecedent indolent lymphoma, this is found in only half of the cases.

**Localization**
More than half of all patients present with widespread disease, including involvement of the lymph nodes. There can also be involvement of more than one extranodal site (occurring in 30–88% of cases), the bone marrow (in 59–94%), and even the CNS (in as many as 45%) [2350, 3158, 3523].

**Clinical features**
Most patients (70–100%) present with advanced disease (stage IV according to the Ann Arbor classification), more than one extranodal localization, a high International Prognostic Index (IPI), and elevated lactate dehydrogenase levels [2350, 3158, 3523, 3846]. Extranodal localizations include the bone marrow and CNS. Double-hit HGBLs are particularly enriched in patients diagnosed with DLBCL who do not respond well to induction therapy with the CHOP chemotherapy regimen plus rituximab (R-CHOP) or who have early relapses after complete remission [843].

**Microscopy**
Double-hit HGBLs have variable morphology. The specific morphological appearance should be stated in a diagnostic comment. Although the incidence differs between studies, most authors conclude that approximately half of the cases have the morphology of a DLBCL, NOS [193, 194, 1820, 1865, 2304, 3158, 3523, 3712]. This is due to the fact that DLBCL is by far the most frequent lymphoma subtype, and approximately 4–8% of all DLBCLs are double-hit lymphomas. In a recent study, 69% of the MYC and BCL2 double-hit lymphomas and 85% of the MYC and BCL6 double-hit lymphomas had DLBCL morphology [2304]. The differences between individual studies may be due to referral bias, inclusion bias, and the variable morphological criteria [2720A, 3712]. The growth pattern is completely diffuse, often with relatively few small lymphocytes. Some fibrosis may be present. Starry-sky macrophages may be present, sometimes only focally. The numbers of mitotic figures and apoptotic figures are highly variable, with some cases having a low number of mitotic figures and also a low Ki-67 proliferation index (see below). Therefore, a low proliferation rate does not exclude this type of lymphoma. The nuclei have a variable size and contour, with some cases showing nuclei that are 3–4 times the size of normal lymphocytes (much larger than BL cells). The cytoplasm is usually more abundant and less basophilic than in BL.

Because these lymphomas can be indistinguishable from other DLBCL, a double-hit status should be investigated in all DLBCLs, NOS, using cytogenetic or molecular cytogenetic studies. Some pathologists may prefer to look for evidence of a double-hit only after immunohistochemical or other pre-selection (see below).
Double-hit high-grade B-cell lymphoma with MYC and BCL2 rearrangements, blastoid morphology. As is typical in blastoid cases, this patient had a history of follicular lymphoma. The lymphoma cells are monotonous and mimic lymphoblasts, with overlapping nuclei, finely dispersed chromatin, and inconspicuous nucleoli. However, in this case they are mature B cells with expression of CD20, CD10, and BCL2 but negative for BCL6. TdT was negative. The Ki-67 proliferation index was 70%.

Another subset (also accounting for ~50% of cases) shows a morphology that mimics that of BL, or has features intermediate between DLBCL and BL (193,194,3143,3712,3846). Approximately 50% of cases with this morphologic appearance have a double-hit status. They show a diffuse proliferation of medium-sized to large cells with very few admixed small lymphocytes and no stromal reaction or fibrosis. Starry-sky macrophages are generally present, along with many mitotic figures and prominent apoptosis. The cellular morphology varies. Some cases are relatively monomorphic, very closely resembling BL. Others exhibit more variation in nuclear size and nucleolar features than is generally seen in BL. The cytoplasm is usually less basophilic than in BL, a feature best appreciated on Giemsa-stained imprints. In most cases, cytoplasmic vacuoles are absent. In cases that closely mimic BL, the diagnosis of BL can be excluded on the basis of an aberrant clinical presentation, immunophenotype (typically strong BCL2 expression), and molecular genetic findings (see below).

Other cases may have a blastoid cytomorphology, with medium-sized cells often resembling small centroblasts. Nucleoli are inconspicuous. The chromatin has a finely granular texture. The cells have a small rim of cytoplasm. Thus, they are considered true lymphoblasts, and staining for TdT should be performed in all cases (1914,4097). Because the blastoid variant of mantle cell lymphoma shares many of these features, cyclin D1 staining should also be performed. These tumour cells are CD10+ and BCL6+ mature B cells. In many of these cases, an antecedent or synchronous follicular lymphoma is present; such cases should be diagnosed as double-hit HGBL transformed from a follicular lymphoma.

Immunophenotype

These lymphomas are mature B-cell lymphomas with expression of CD19, CD20, CD79a, and PAX5 and lack of TdT. Some double-hit HGBL cases lack surface immunoglobulin expression as detected by flow cytometry, which may be related to the involvement of multiple IG loci by translocations (2101). This absence should not be interpreted as proof of a precursor B-cell phenotype. CD10 and BCL6 expression in most of these lymphomas (75–90%), and IRF4/MUM1 is expressed in approximately 20% of the cases (193,3846). Almost all cases with a BCL2 (18q21) breakpoint have strong cytoplasmic BCL2 positivity, in contrast to the absent or weak expression of BCL2 in BLs.

It has been suggested that immunostaining for CD10, BCL6, IRF4/MUM1, and BCL2 or gene expression analysis of paraffin-embedded materials could be used to select cases of DLBCL to be tested by MYC FISH (3117,3601,3846). However, the less-frequent double-hit lymphomas with MYC (8q24) and BCL6 (3q27) rearrangements without a concomitant BCL2 (18q21) breakpoint variably express BCL2 and CD10 and express IRF4/MUM1 more commonly than do the other double-hit lymphomas (2304,3183,338,3712,3846).
Fig. 13.172 Double-hit (DH) high-grade B-cell lymphoma with MYC and BCL2 rearrangements and subsequent lymphoblastic lymphoma in a patient with a prior follicular lymphoma. A 66-year-old man presented with grade 2 follicular lymphoma (FL2) in 2005 and a histologically documented relapse in 2006. In 2009, he presented with an abdominal mass and extensive bone marrow involvement. The 2005 biopsies showed a classic phenotype: CD20+, CD10+, BCL6+, and BCL2+. The bone marrow biopsy in 2009 (2009A) showed a diffuse infiltration of blastoid cells. Immunohistochemistry revealed a persistent mature phenotype, with expression of CD20 and BCL6 and absence of TdT. The Ki-67 proliferation index was ~60%. Flow cytometry confirmed this phenotype and showed expression of surface IgG without detectable light chains. The simultaneously obtained needle biopsy of the para-iliac abdominal mass (2009B) showed a blastoid/lymphoblastic morphology, with loss of both CD20 and BCL6 expression, strong CD10 and BCL2 expression, a Ki-67 proliferation index of ~90%, and expression of TdT in ~30% of the cells. By FISH analysis, rearrangements of both MYC and BCL2 were identified, the MYC rearrangement without colocalization of the IGH locus (IGK and IGL were not tested).

4077) and therefore could be missed by such a selection procedure. Ki-67 immunohistochemistry shows variable results. In cases that mimic BL, the Ki-67 proliferation index is 80–95%. However, in cases with DLBCL morphology, the index may be deceptively low (<30%). Therefore, the Ki-67 proliferation index cannot be used to select cases for MYC FISH [2564,4113]. Similarly, MYC protein expression cannot be used to select cases for FISH either. Although there is consensus that high expression (in >80% of nuclei) is present in most cases of BL with an IG/MYC translocation, there is much more variability in the double-hit lymphomas; most authors have concluded that MYC staining is not reliable enough to be used for the selection of cases that should have cytogenetic or molecular/cytogenetic testing [722, 1439,1440,1866,3143,4085]. Nevertheless, some authors suggest performing FISH studies only in cases with >30% or >40% MYC-positive tumour cells.

Postulated normal counterpart
The limited gene expression data available and the applied immunohistochemical algorithms suggest that almost all cases with MYC and BCL2 rearrangements originate from mature germinal centre B cells, whereas the cell of origin for cases with MYC and BCL6 rearrangements is more variable [802,3601].

Genetic profile
By definition, these lymphomas have a MYC (8q24) rearrangement as detected by classic cytogenetics, FISH, or other molecular genetic tests. In approximately 65% of cases, MYC is juxtaposed to one of the IG genes (usually IGH, less frequently IGK or IGL); in the other cases, MYC has a non-IG partner, such as at 9p13 (gene unknown), 3q27 (BCL6), or other loci [193,3118]. Some reports suggest that an IG/MYC translocation confers a poor outcome compared with cases in which MYC has a non-IG partner [193,802,1865,3118], but the clinical impact of the individual non-IG partners is not fully established. Identification of an IGK-MYC or IGL fusion requires the use of dual fusion probes, because the identification of MYC and IGK or IGL rearrangements using only break-apart probes does not exclude the possibility of two separate unrelated translocations [802]. In addition to MYC rearrangement, all cases contain a BCL2 rearrangement at 18q21 and/or a BCL6 rearrangement at...
3q27. Other infrequent recurrent combinations with MYC rearrangement, such as rearrangement of BCL3 at 19q13 and of an unknown gene at 9p13, are also recognized, but there have been no systematic studies of these lymphomas; therefore, such cases should not be included in this category \[194\]. Cases with a combination of MYC and CCND1 (at 11q13) breakpoints constitute aggressive mantle cell lymphoma with the acquisition of a secondary MYC breakpoint and should not be included in this category either \[734,933,1069,3628\].

Lymphomas can show a combination of a chromosomal rearrangement of one gene and copy-number increase or amplification of other genes, for example, a MYC (8q24) rearrangement with gain or amplification of BCL2 (18q21) or vice versa. In the current classification, such a combination is not sufficient to classify a case as a double-hit HGBL. Notably, the definitions of amplification and copy-number increase differ across publications, and in some clinically oriented papers, these phenomena are lumped with rearrangements \[2307,2404,4113\]. High-level amplification at 8q24 may occur together with a rearrangement \[2527\], and in combination with a BCL2 rearrangement, it likely has a similar clinical impact as the classic double-hit configuration \[4113\]. In contrast, the biological and clinical impact of a small increase in copy number (mostly caused by aneusomy) in DLBCL is controversial \[2307,2404,4113\]. Therefore, until more data are available, cases with only gains or amplification without a proven rearrangement should not be included in this category, but rather in the category of DLBCL or HGBL, NOS.

Double-hit HGBLs often have complex karyotypes, with many other structural and numerical abnormalities \[193\]. Sequencing studies reveal frequent TP53 mutations (especially frequent in the MYC and BCL2 double-hit cases \[1314\]) and few MYD88 mutations \[1315\]. Whereas TCF3 mutations and in particular homozygous mutations or deletion of its inhibitor, ID3, are frequent in BL, hemizygous mutations of ID3 may be present in double-hit HGBL as well \[193,1313,2401,2693,3573\].

**Prognosis and predictive factors**

With R-CHOP or comparable therapies, the complete response rate is relatively low, and overall survival is short, with median survivals of 4.5–18.5 months \[269,1055,1865,2237,2304,2306,3712,4019,4020\]. Clinical trials are under way to test other polychemotherapy modalities and new drugs that may improve the outcome of these patients \[778,1055,1709\]. Several clinical and biological factors, including the tumour morphology, the MYC partner, and extent of the disease, may influence survival and warrant further studies \[541\]. A small subset of patients with no risk factors may have a favourable outcome \[1865,3158\].

---

**High-grade B-cell lymphoma, NOS**

**Definition**

High-grade B-cell lymphoma (HGBL), NOS, is a heterogeneous category of clinically aggressive mature B-cell lymphomas that lack MYC plus BCL2 and/or BCL6 rearrangements and do not fall into the category of diffuse large B-cell lymphoma (DLBCL), NOS, or Burkitt lymphoma (BL). However, they do share some morphological, immunophenotypic, and genetic features with these lymphomas. These cases are rare; the diagnosis should be made sparingly, and only when the pathologist is truly unable to confidently classify a case as DLBCL or BL.

In the 2008 edition of the WHO classification, these cases were included in the category of B-cell lymphoma, un-classifiable, with features intermediate between DLBCL and BL \[3848\], which also included cases now classified as HGBL with MYC and BCL2 and/or BCL6 rearrangements. Because the double-hit and triple-hit HGBLs have now been classified as a distinct category of their own, the category of HGBL, NOS, represents the remaining cases of the previous classification system. This category also includes cases of blastoid-appearing mature B-cell lymphomas (not of mantle cell type), which in the past might have
High-grade B-cell lymphoma

Fig. 13.175 High-grade B-cell lymphoma, NOS, with some features of Burkitt lymphoma. A The tumour cells show a squared-off cytoplasm, but no distinct starry-sky pattern. Immunohistochemistry showed expression of CD20, CD10, BCL6, and BCL2. The Ki-67 proliferation index showed a heterogeneous staining of ~50%. Strong and homogeneous p53 staining suggested TP53 mutation. FISH analysis revealed a BCL2 rearrangement but no rearrangements of MYC or BCL6. B Same case at higher magnification. Despite the impression at low magnification, the cytological appearance is blastoid: the nuclei show a finely granular chromatin pattern and absence of nucleoli.

be included among the DLBCLs. Cases of otherwise typical DLBCL, NOS, harbouring an isolated MYC translocation should still be classified as DLBCL, NOS. Some paediatric lymphomas also share features of both DLBCL and BL, and more than half of such cases harbour a MYC rearrangement in combination with a relatively simple karyotype. They often have a molecular Burkitt or intermediate gene expression profile and show an excellent prognosis. It is therefore recommended that these cases be classified as BL or DLBCL, and not as HGBL, NOS [2038].

ICD-O code 9680/3

Epidemiology

Few epidemiological data are available for this category, because in most reports these lymphomas and the double-hit HGBLs with a similar morphology are described together. In general, elderly patients are affected, with incidence increasing with age. Men and women are affected almost equally.

Microscopy

Most cases have a morphology that mimics that of BL more closely than that of DLBCL. They show a diffuse proliferation of medium-sized to large cells with very few admixed small lymphocytes and no stromal reaction or fibrosis. Starry-sky macrophages may be present, along with many mitotic figures and prominent apoptosis. The cellular morphology varies. Some cases are relatively monomorphic, resembling BL; others exhibit more variation in nuclear size and nucleolar features than is generally seen in BL. The cytoplasm is usually less basophilic than in BL, a feature best appreciated on Giemsa-stained sections or imprints. In most cases, cytoplasmic vacuoles are absent. In cases that closely mimic BL, the diagnosis of BL can be excluded on the basis of an aberrant clinical presentation, immunophenotype, and/or molecular genetic findings (see below).

Rare mature (CD20+ and TdT-) B-cell lymphomas with a blastoid appearance that do not constitute a blastoid variant of mantle cell lymphoma and that lack a double hit (MYC rearrangement in combination with BCL2 and/or BCL6 rearrangement) are also included in this category.

Immunophenotype

The immunophenotype is not well described, due to the heterogeneous nature of these lymphomas and the fact that most cases were previously included in reports that also included cases with double or triple translocations, or were classified as DLBCL. All cases are CD20-positive, mature B-cell lymphomas. Most show expression of BCL6, but CD10 expression is variable. In most cases, expression of IRF4/MUM1 is absent. Ki-67 positivity is also variable. MYC expression is variable, partially dependent on the presence of a MYC rearrangement.

Genetic profile

Molecular/cytogenetic data have been systematically analysed in few studies. By definition, the presence of a BCL2 and/or a BCL6 rearrangement in combination with a MYC rearrangement should be excluded. Approximately 20–35% of cases have a MYC rearrangement, with or without increased copy numbers or, rarely, amplification of 18q21 involving BCL2. Cases with a BCL2 rearrangement and increased copy number or high-level amplification of MYC have also been identified [2307,2344,3143]. Among the so-called blastoid cases, 40% of 24 cases studied lacked rearrangements in both MYC and BCL2 [1914,4097]; in another study, none of the 8 cases studied contained such rearrangements [725].

Prognosis and predictive factors

Patients with HGBL, NOS, have a poor outcome, although it may be slightly better than that of patients with double-hit HGBL [794,2344,3143]. A relatively poor outcome in HGBL, NOS, with amplification of MYC, with or without rearrangement of BCL2 (or vice versa) has been reported. However, in most studies, this aspect was analysed mainly or exclusively in patients with DLBCL [2307,2344,2404,3143,4113]. In general, clinical correlates from studies on HGBL, NOS, are hampered by their retrospective nature, by the lumping together of these cases with other lymphoma types, and by small cohort sizes.
B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma and classic Hodgkin lymphoma

Definition
B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma (DLBCL) and classic Hodgkin lymphoma (CHL) is a B-cell–lineage lymphoma that demonstrates overlapping clinical, morphological, and/or immunophenotypic features between CHL and DLBCL, especially primary mediastinal (thymic) large B-cell lymphoma (PMBL). These lymphomas are most commonly associated with mediastinal disease, but similar cases have been reported in peripheral lymph node groups as the primary site. Mediastinal presentations are infrequent among elderly patients \{1077,1121\}. Most cases have been reported from western countries. Like CHL, these tumours are less common in Black and Asian populations.

ICD-O code
9596/3

Synonyms
Mediastinal grey-zone lymphoma; Hodgkin-like anaplastic large cell lymphoma (obsolete)

Epidemiology
MGZL is most common in young men, usually presenting in patients aged 20–40 years \{1297,4047\}. It has been reported rarely in children \{2995\}. Mediastinal presentations are infrequent among elderly patients \{1077,1121\}. Most cases have been reported from western countries. Like CHL, these tumours are less common in Black and Asian populations.

Clinical features
Most patients have bulky mediastinal masses, sometimes leading to superior vena cava syndrome or respiratory distress. Supraclavicular lymph nodes may be involved. Non-mediastinal GZL more often presents in older patients, and shows less of a male predominance \{1077,1121\}. Cases of composite CHL and PMBL and cases of sequential development of CHL and PMBL in the same patient are not strictly accepted as examples of MGZL, but are thought to be biologically related phenomena \{1402, 3140\}. When seen sequentially, CHL is more often the initial presentation, followed by PMBL \{4463\}.

Microscopy
A characteristic feature is the broad spectrum of cytological appearances within a given tumour; some areas more closely resemble CHL and others resemble PMBL. There is also variation across different cases, with some examples being more Hodgkin-like and others more closely resembling either PMBL or diffuse large cell lymphoma. As discussed below (see Immunophenotype), discordance between the cytological appearance and the immunophenotype is common. Tumour cell density is high, often with sheet-like growth of pleomorphic tumour cells in a diffusely fibrotic stroma \{1297,4047\}. Focal fibrous bands may be seen in some cases. The cells are larger and more pleomorphic than is typical in PMBL, although some centroblast-like cells may be present. There is usually a
Fig. 13.177 B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and CHL. Mediastinal mass. A The lymphoma is composed of sheets of cells with clear cytoplasm and fine sclerosis. The appearance resembles that of primary mediastinal large B-cell lymphoma. However, CD20 and CD79a are both negative. B The tumour cells are strongly CD15-positive and also CD30-positive (not shown).

sparse inflammatory infiltrate, although eosinophils, lymphocytes, and histiocytes may be present focally. Necrosis is frequent, but unlike in CHL, the necrotic areas do not contain neutrophilic infiltrates. Due to the variations in histological pattern in different portions of the tumour, diagnosing these lymphomas on a core needle biopsy is challenging and not recommended.

Immunophenotype
The lymphoma cells exhibit an aberrant immunophenotype, making the distinction between CHL and PMBL difficult (1297,4047). Neoplastic cells typically express CD45. Cases in which the cytological appearance might suggest CHL show preservation of the B-cell programme, with strong and uniform positivity for CD20 and CD79a. CD30 is usually positive, and CD15 may be expressed. Cases in which the histological appearance on H&E staining might suggest PMBL show loss of B-cell antigens but positivity for CD30 and CD15. Surface or cytoplasmic immunoglobulin is absent. The transcription factors PAX5, OCT2, and BOB1 are usually expressed. BCL6 is variably positive, but is not useful in differential diagnosis, because it is positive in most cases of CHL and PMBL. In one series, p53 was expressed in most cases (1300). Expression of cyclin E and p63 in most cases has also been reported (1077). Most cases of MGZL are negative for EBV; positivity for EBV-encoded small RNA (EBER) or LMP1 should prompt suspicion for EBV-positive DLBCL, especially in elderly patients. However, rare cases of MGZL have been EBV-positive (2867).

In instances of composite or metachronous lymphomas, the various components exhibit a phenotype characteristic of that entity, either CHL or PMBL.

Fig. 13.178 B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and CHL. There is a sheet-like growth of pleomorphic lymphoid cells. Some binucleated cells are present, but there is marked variation in cell size and shape. The biopsy was taken from a 28-year-old man with a mediastinal mass and supraclavicular lymph node involvement.
Postulated normal counterpart
The postulated cell of origin for cases arising in the mediastinum is a thymic B cell [4047]; cases arising in the peripheral lymph nodes are thought to arise from a non-thymic B cell.

Genetic profile
Clonal rearrangement of the IG genes is positive by PCR in most cases, presumably due to the high content of tumour cells in comparison with CHL. Many of the genetic aberrations identified by FISH are similar to those observed in PMBL [1077]. Gains and amplification of the JAK2 and PDCD1LG2 (also called PDL2) loci at 9p24.1 are common, seen in > 50% of cases. Increased expression of CD274 (PD-L1) may occur as a result. Also frequent are gains/amplification at 2p16.1 involving REL. Breaks in the CIITA locus at 16p13.13 have been reported in approximately one third of cases [1077]. Gains in MYC have been observed in 20–30% of cases. The aberrations above are seen in both mediastinal and non-mediastinal cases, although gains/amplification at 9p24.1 are more common in patients with mediastinal than non-mediastinal presentations, occurring in 61% and 38% of such cases, respectively [1077].

Prognosis and predictive factors
MGZLs generally have a more aggressive clinical course and poorer outcome than do either CHLs or PMBLs [1060]. Combined modality treatment appears to be required in most cases, and systemic multiagent chemotherapy followed by radiation to the mediastinal mass produces event-free survival in a majority of patients [4333]. Given the strong expression of CD20, the addition of rituximab appears to be of benefit. Regimens effective in the treatment of CHL, such as ABVD chemotherapy (i.e. doxorubicin, bleomycin, vinblastine, and dacarbazine), have been reported to be less effective than regimens used for treating DLBCLs [1121]. Like in CHL, a decrease in the absolute lymphocyte count has been associated with a worse outcome [4333]. In one study, a high content of DC-SIGN-positive dendritic cells was associated with an adverse prognosis [4333]. This association may be comparable to the association between a high content of tumour-associated macrophages and adverse outcome in CHL [3778].
CHAPTER 14

Mature T- and NK-cell neoplasms
T-cell prolymphocytic leukaemia

**Definition**
T-cell prolymphocytic leukaemia (T-PLL) is an aggressive T-cell leukaemia characterized by the proliferation of small to medium-sized prolymphocytes with a mature post-thymic T-cell phenotype, involving the peripheral blood, bone marrow, lymph nodes, liver, spleen, and skin.

**ICD-O code**
9834/3

**Synonym**
Prolymphocytic leukaemia, T-cell type

**Epidemiology**
T-PLL is rare, accounting for approximately 2% of cases of mature lymphocytic leukaemias in adults aged >30 years; the median patient age is 65 years (range: 30–94 years). It is very infrequent among individuals aged <30 years.

**Localization**
Leukaemic T cells are found in the peripheral blood, bone marrow, lymph nodes, spleen, liver, and sometimes skin.

**Clinical features**
Most patients present with hepatosplenomegaly and generalized lymphadenopathy. Skin infiltration is seen in 20% of cases, and serous effusions in a minority (2575). Anaemia and thrombocytopenia are common, and the lymphocyte count is usually >100 x 10^9/L. Serum immunoglobulins are normal. Serology for HTLV-1 is negative.

**Microscopy**

*Peripheral blood and bone marrow*
The diagnosis is made on peripheral blood films, which show a predominance of small to medium-sized lymphoid cells with non-granular basophilic cytoplasm; round, oval, or markedly irregular nuclei, and visible nucleoli. In 25% of cases, the cell size is small and the nucleolus may not be visible by light microscopy (small-cell variant) (2577). In 5% of cases, the nuclear outline is very irregular and can even be cerebriform (cerebriform variant) (3115). Irrespective of the nuclear features, a common morphological feature is cytoplasmic protrusions or blebs. The bone marrow is diffusely infiltrated, but the diagnosis is difficult to make on the basis of bone marrow histology alone.

*Other tissues*
Cutaneous involvement consists of perivascular and periadnexal or more diffuse dermal infiltrates, without epidermotropism (2472,2575). The spleen contains a dense red pulp infiltrate, which invades the spleen capsule, blood vessels, and atrophic white pulp (3004). In lymph nodes, the involvement is diffuse and tends to predominate in the paracortical areas, sometimes with sparing of follicles. Prominent high endothelial venules may be numerous and are often infiltrated by neoplastic cells.

**Cytochemistry**
T-cell prolymphocytes stain strongly with alpha-naphthyl acetate esterase and acid phosphatase, with a dot-like pattern (2576). However, cytochemistry is rarely used for routine diagnosis.

**Immunophenotype**
T-cell prolymphocytes are peripheral T cells that are negative for TdT and CD1a, and positive for CD2, CD5, CD3, and CD7; the membrane expression of CD3 may be weak. CD52 is usually expressed at high density, and can be used as a target of therapy (916).
TR genes (TRB and TRG) are clonally rearranged. Antigen receptor genes
Genetic profile
T cell at an intermediate stage of differentiation between a cortical thymocyte and a mature T lymphocyte.

The postulated normal counterpart is an unknown T cell with a mature (post-thymic) immunophenotype. Strong expression of CD7, coexpression of CD4 and CD8, and weak membrane expression of CD3 may suggest that T-PLL arises from a T cell at an intermediate stage of differentiation between a cortical thymocyte and a mature T lymphocyte.

Genetic profile
Antigen receptor genes
TR genes (TRB and TRG) are clonally rearranged.

Cytogenetic abnormalities and oncogenes
The most frequent chromosome abnormality in T-PLL involves inversion of chromosome 14 with breakpoints in the long arm at q11 and q32, seen in 80% of patients and described as inv(14)(q11q32). In 10%, there is t(14;14)(q11;q32) [457, 2470]. These translocations juxtapose the TRA locus with the oncogenes TCL1A and TCL1B at 14q32.1, which are activated through the translocation [3121]. The t(X:14)(q28;q11) translocation is less common, but it also involves the TRA locus, at 14q11 with the MTCP1 gene, which is homologous to TCL1A/B at Xq28 [3797]. Both TCL1A/B and MTCP1 have oncogenic properties; both can induce a T-cell leukaemia (CD4−, CD8+) in transgenic mice [1467, 4198]. The oncprotein TCL1 inhibits activation-induced death in the neoplastic T cells, further contributing to the neoplastic process [961]. Rearrangements of TCL1A/B or MTCP1 are initiating events but are probably not sufficient to drive leukaemogenesis.

Abnormalities of chromosome 8, idic(8)(p11), t(8:8)(p11-12;q12), and trisomy 8q are seen in 70–80% of cases [3121], and gains in the MYC gene have been documented by FISH in some cases [1713]. Deletions at 12p13 and 22q and amplification of 5p are also a feature of T-PLL on FISH and/or SNP array [493, 1631, 2901]. Molecular and FISH studies also show deletions at 11q23 (the locus for ATM), and mutation analysis has shown missense mutations at the ATM locus in T-PLL [3803, 4215]. T-PLL is not an uncommon neoplasm in patients with ataxia-telangiectasia [457]. Abnormalities of chromosomes 6 (present in 33% of cases) and 17 (in 26%) have also been identified in T-PLL by conventional karyotyping and comparative genomic hybridization [457, 818]. The TP53 gene (at 17p13.1) is deleted, with overexpression of p53, in some cases [458]. Whole-exome and targeted sequencing studies have shown recurrent alterations in genes of the JAK/STAT signalling pathway. Mutations of JAK3 have been documented in 30–42% of cases, of JAK1 in about 8%, and of STAT5B in 21–36% [322, 349, 2007, 3794]. These mutations, which are largely mutually exclusive, lead to constitutive activation of the STAT signalling pathway. In addition, although more rarely, genes encoding epigenetic modifiers, such as EZH2 and BCO1, have been described to be recurrently mutated in T-PLL [2007, 3794].

Genetic susceptibility
Patients with ataxia-telangiectasia may be at increased risk for the development of T-PLL.

Prognosis and predictive factors
The disease course is aggressive, with a median survival of 1–2 years. Cases with a more chronic course have also been reported [1296], but such cases may progress after 2–3 years. The best responses have been reported with the monoclonal antibody alemtuzumab (anti-CD52) [917, 1978]. Autologous or allogeneic stem cell transplantation should be considered for eligible patients who achieve remission following immunotherapy [1488, 2116]. The findings of mutation activation of the JAK/STAT signalling pathway may provide opportunities to develop novel therapies with inhibitors targeting this pathway. High levels of expression of both TCL1 and AKT1 have been identified as poor prognostic markers [1617], and more recently, STAT5B mutations have been documented to have a negative prognostic impact [3794].
T-cell large granular lymphocytic leukaemia

Definition
T-cell large granular lymphocytic leukaemia (T-LGLL) is a heterogeneous disorder characterized by a persistent (>6 months) increase in the number of peripheral blood large granular lymphocytes (LGLs), usually to 2–20 × 10^9/L, without a clearly identified cause.

ICD-O code
9831/3

Synonyms
T-cell large granular lymphocytosis; CD8+ T-cell chronic lymphocytic leukaemia (obsolete); T-cell lymphoproliferative disease of granular lymphocytes; T-gamma lymphoproliferative disease (obsolete)

Epidemiology
T-LGLL accounts for 2–3% of mature small lymphocytic leukemias. There is an approximately equal male-to-female ratio, with no clearly defined age peak. The disease is rare in individuals aged <25 years, with <3% of cases occurring in this age group; most cases (73%) occur in individuals aged 45–75 years.

Etiology
The underlying pathophysiological mechanisms of T-LGLL are not well understood, and this disorder is fairly unique in that the clonal T-cell LGLs (T-LGLs) retain many phenotypic and functional properties of normal cytotoxic T lymphocyte effector memory cells (377). One theory is that T-LGLL arises in a setting of sustained immune stimulation. The frequent association of T-LGLL with autoimmune disorders supports this hypothesis (377,4343). The absence of homeostatic apoptosis is also a feature of the T-LGLs; these cells express high levels of FAS and FASL, leading investigators to propose activation of prosurvival pathways in T-LGLs, which prevents activation-induced cell death (1112,3549). FASL levels are elevated in the sera of many patients, and this elevation is postulated to be important in the pathogenesis of neutropenia (2368). Other pathways reported to be dysregulated include the MAPK, PI3K/AKT, NF-kappaB, and JAK/STAT signalling pathways (3791). About one third of the cases carry STAT3 mutations, which affect the SH2 domain of STAT3 (1854,2086). Rarely, mutations affecting the SH2 domain of STAT5B are observed (3288). There is evidence that these are activating mutations that may contribute to the pathogenesis of the disease by providing prosurvival and growth signals (1854,2086). It is possible that T-LGLL starts as an immune response to a chronic persistent stimulus, with clonal selection and eventually the acquisition of an oncogenic mutation that allows the further expansion and establishment of a monoclonal population.

Localization
T-LGLL involves the peripheral blood, bone marrow, liver, spleen, and rarely skin. Lymphadenopathy is very rare.

Clinical features
Most cases have an indolent clinical course. Severe neutropenia (with or without anaemia) is frequent, whereas thrombocytopenia is not; 60% of patients are symptomatic at presentation (650,967,2203,3046). The lymphocyte count is usually 2–20 × 10^9/L. There is disagreement about the level of lymphocytosis required for the diagnosis of T-LGLL (3620), but a T-LGL count of >2 × 10^9/L is frequently associated with a large clonal proliferation. However, cases that have LGL counts of <2 × 10^9/L but meet
all other criteria are consistent with this diagnosis. Cases with low counts of LGLs in the blood and low bone marrow infiltration generally have small clonal populations; their designation as T-LGLL is questionable, and other names have been proposed [3687]. A pitfall in diagnosis is the frequent development of oligoclonal T-cell populations following allogeneic bone marrow transplantation as lymphocyte reconstitution occurs [1253, 2552, 3687]. Severe anaemia due to red blood cell hypoplasia has been reported in association with T-LGLL [2203]. Moderate splenomegaly is the main physical finding. Rheumatoid arthritis, the presence of autoantibodies, circulating immune complexes, and hypergammaglobulinaemia are also common [650, 2203, 3001]. Oligoclonal or clonal expansions of T-LGLs can be observed in a variety of situations. For example, rare cases of T-LGLL have been observed as a form of post-transplant lymphoproliferative disorder [151, 186, 2829]. Clonal populations of T-LGLs are also seen in association with low-grade B-cell malignancies, including hairy cell leukaemia and chronic lymphocytic leukaemia, and in particular following immunochemotherapy [151, 2529]. Cases that are CD4-positive have been reported to be frequently (i.e. in 30% of cases) associated with an underlying haematological or (less often) non-haematological malignancy [2335]. Morbidity and mortality are mostly due to the accompanying cytopenias or other accompanying diseases. There is no difference in survival between cases with and without STAT3 mutation, although mutation seems to be associated with more-symptomatic disease and shorter time to treatment failure [1854, 2086]. The rare finding of STAT5B mutations appears to be associated with more-aggressive disease [3288].

Microscopy
The predominant lymphocytes in blood and bone marrow films are LGLs with moderate to abundant cytoplasm and fine or coarse azurophilic granules [464, 650, 2599]. The granules in the LGLs often exhibit a characteristic ultrastructural appearance described as parallel tubular arrays [2599] and contain a number of proteins that play a role in cytology, such as perforin and granzyme B. Despite the cytopenias, the bone marrow is normocellular or hypocellular in about 50% of cases; in the other 50%, the marrow is slightly hypercellular [2741, 3003]. The granulocytic series often shows left-shifted maturation, and mild to moderate reticulin fibrosis is present [650]. The extent of bone marrow involvement is variable, and usually constitutes <50% of the cellular elements, with interstitial/intrasinusoidal infiltrates that are difficult to identify by morphological review [2741]. Non-neoplastic nodular lymphoid aggregates containing many B cells surrounded by a rim of CD4+ T cells are also frequently present [3003]. Splenic involvement is characterized by infiltration and expansion of the red pulp cords and sinusoids by T-LGLs, with sparing of the often hyperplastic white pulp [3004].
Mature T- and NK-cell neoplasms
serve as a surrogate indicator of clonality
of a KIR family member, and this finding can
cases usually show uniform expression of
CD56 is infrequent. KIR-positive
cells, which are major histocompatibility
complex (MHC) class I receptors, can be
detected in >50% of T-LGLs, but expres¬
sion of CD56 is infrequent. KIR-positive
cases are clonal as documented by TR gene
rearrangement studies (2203). The TRG
gene is rearranged in all cases, regardless
of the type of TCR expressed. TRB is
rearranged in cases expressing the al¬
pha beta TCR, but the TRB gene may be
in germline configuration in gamma delta
TCR cases (4194). Deep sequencing of
the TRB CDR3 has demonstrated that the
dominant clonotypes in the CD8+ LGLs
tend to be private and not commonly
shared in the CDR3 repertoire seen in
normal individuals (768).

Immunophenotype
T-LGLL is typically a disorder of mature
CD2+, CD3+, CD8+, CD57+, and al¬
pha beta TCR-positive cytotoxic T cells
(650,3046). Uncommon variants include
CD4+ / alpha beta TCR-positive cases and
gamma delta TCR-positive cases; ap¬
proximately 60% of the latter express CD8,
and the remainder are CD4/CD8-negative
(650,2335,3046,3504,3620). Abnormally
diminished or lost expression of CD5
and/or CD7 is common in T-LGLL (2416,
2740). CD57 and CD16 are expressed in
>80% of cases (2203,2740). Expression of
CD94/NKG2 family and killer-cell immu¬
noglobulin-like receptor (KIR) family mem¬
bers, which are major histocompatibility
complex (MHC) class I receptors, can be
detected in ≥50% of T-LGLLs, but expres¬
sion of CD56 is infrequent. KIR-positive
cases usually show uniform expression of
a KIR-family member, and this finding can
serve as a surrogate indicator of clonality
similar to the restricted expression of TCR-
alpha beta family members (2416,2740). T-LGLs
express the cytotoxic effector proteins
TIA1, granzyme B, and granzyme M. Bone
marrow core biopsy immunohistochem¬
istry can confirm the diagnosis by high¬
lighting the interstitial and intrasinusoidal
T-LGL infiltrates and revealing the non-ne¬
oplastic nature of the nodular aggregates
(2738,2741,3003).

Variant Cases that morphologically resemble
T-LGLL but have an NK-cell immunophe¬
notype, i.e., that are negative for surface
CD3 (sCD3) and T-cell receptor (TCR)
expression, are classified with the NK-
cell disorders.

Cytochemistry The granules are positive for acid
phosphatase and beta-glucuronidase. En¬
zyme cytochemical studies are rarely
performed for routine diagnosis.

Prognosis and predictive factors The lymphoproliferation is typically in¬
olent and non-progressive, and some
investigators feel that this entity is better
regarded as a clonal disorder of uncer¬
tain significance than as a leukaemia.
Morbidity is associated with the cytope¬
nias (especially neutropenia), but mortal¬
ity due to this cause is uncommon. In a
series of 68 cases, the actuarial median
survival was 161 months (967). There
appears to be no difference in survival
between cases with and without STAT3
mutation, although mutation seems to
be associated with more-symptomatic
disease and shorter time to treatment
failure (1854,2086). The rare finding of
STAT5B mutations appears to be asso¬
ciated with more aggressive disease
(3288,1327,4025). Rare cases have un¬
dergone transformation to a peripheral
T-cell lymphoma composed of large cells
(2585). Some of the aggressive cases
may represent peripheralization of a pe¬
ripheral T-cell lymphoma. Conversely,
rare cases with spontaneous remission
have also been reported (4338). Patients
who require treatment may benefit from
cyclosporine, cyclophosphamide, and
corticosteroids or low-dose methotrex¬
ate, which has been reported to induce
clinical remission in as many as 50% of
patients (650,2203,3005). Some patients
have benefited from pentostatin (3005).
Splenectomy has been performed in
patients with a large spleen, but does
not correct the cytopenia. In light of the
discovery of activation of the STAT3 or
STAT5B pathway in patients with (and
possibly also patients without) activating
mutations (1854), inhibiting this pathway
could be an option for the treatment of
T-cell and NK-cell LGL proliferations.

Fig. 14.08 STAT3 mutations in large granular lymphocytic leukaemia (LGL). From: Jerez A et al. (1854)
Chronic lymphoproliferative disorder of NK cells

Definition
Chronic lymphoproliferative disorders of NK cells (CLPD-NKs) are rare and heterogeneous. They are characterized by a persistent (>6 months) increase in the peripheral blood NK-cell count (usually to \( \geq 2 \times 10^9/L \)) without a clearly identified cause. It is difficult to distinguish between reactive and neoplastic conditions without highly specialized techniques. CLPD-NK is a proliferation of NK cells associated with a chronic clinical course, and is considered a provisional entity.

ICD-O code 9831/3

Synonyms
Chronic NK-cell lymphocytosis; chronic NK large granular lymphocyte lymphoproliferative disorder; NK-cell large granular lymphocyte lymphocytosis; indolent large granular NK-cell lymphoproliferative disorder; indolent leukaemia of NK cells

Epidemiology
CLPD-NK occurs predominantly in adults, with a median patient age of 60 years and no sex predominance [2204,2336,3274]. Unlike in EBV-associated aggressive NK-cell leukaemia, there is no racial or genetic predisposition.

Etiology
A transient increase in circulating NK cells can be encountered in many conditions, such as autoimmune disorders and viral infections [2204,3274]. NK-cell activation due to an unknown stimulus, presumably viral, is postulated to play a role in the early pathogenesis of CLPD-NKs by selecting and expanding NK-cell clones, although no evidence of direct NK-cell infection has been observed [1113,2336,2398,3274,3605,4456,4457]. The tyrosine kinase inhibitor dasatinib produces a sustained increase in NK cells that can be monoclonal [2113,2796]. One third of cases have activating mutations in the STAT3 SH2 domain [1163,1854].

Localization
The peripheral blood and bone marrow are the predominant sites.

Clinical features
Most patients are asymptomatic, but some present with systemic symptoms and/or cytopenia (mainly neutropenia and anaemia). Lymphadenopathy, hepatomegaly, and cutaneous lesions are infrequent [260,2336,3001,3274]. CLPD-NKs may occur in association with other medical conditions, such as solid and haematological tumours, vasculitis, spleenectomy, neuropathy, and autoimmune disorders [2336,3001,3075,3274]. CLPD-NK is distinguished from NK-cell lymphoproliferative disorders involving the gastrointestinal tract, designated as NK-cell enteropathy or lymphomatoid gastroscopy of NK-cell type [2489A; 3882A].

Microscopy
The circulating NK cells are typically intermediate in size, with round nuclei with condensed chromatin and moderate amounts of slightly basophilic cytoplasm containing fine or coarse azurophilic granules. These large granular lymphocytes are monotonous, and features of lymphocyte activation are not apparent. Lymphocytosis may be apparent on bone marrow aspirate smears, but large granular lymphocyte morphology is often subtle. The bone marrow biopsy is characterized by intrasinusoidal and interstitial infiltration by cells with small, minimally irregular nuclei and modest amounts of pale cytoplasm. These infiltrates are difficult to detect without immunohistochemistry.

Immunophenotype
CLPD-NK shows a distinctive profile by flow cytometric immunophenotyping. Surface CD3 is negative, whereas cCD3-epsilon is often positive. CD16 is positive, and weak CD56 expression is frequently observed [2336,2738,2739,2740,3075,4259]. Cytotoxic markers (including TIA1, NK-cell activation markers, and CD103) are usually negative. The restricted pattern of killer-cell immunoglobulin-like receptor expression by NK cells is abnormal and supports the diagnosis of CLPD-NK.
granzyyme B, and granzyme M) are positive. There may be diminished or lost expression of CD2, CD7, and CD57, and abnormal uniform expression of CD8 (2735,2740). Expression of the killer-cell immunoglobulin-like receptor (KIR) family of NK-cell receptors is abnormal in CLPD-NK: either restricted KIR isofrom expression or a complete lack of detectable KIRs may be seen (1113,1661,2740,3075,4456,4459). KIR-positive cases preferentially express activating receptor isoforms (1113,4456). Other abnormalities of NK-cell receptors include uniform, bright CD94/NKG2A heterodimer expression and diminished CD161 expression (2336,2740,3605,4259).

Postulated normal counterpart
A mature NK cell

Genetic profile
The karyotype is normal in most cases (3001,3274,3925). Activating mutations in the STAT3 SH2 domain are present in 30% of cases, and the finding of this mutation excludes non-neoplastic NK-cell proliferations (1163). There are no rearrangements of the IG and TR genes, as expected for NK cells. In female patients, it is possible to use X-chromosome inactivation as an indirect marker of clonality. A skewed ratio of X-chromosome inactivation restricted to NK cells is indicative of a clonal population.

Fig. 14.10 Chronic lymphoproliferative disorder of NK cells. A–C Peripheral blood films show a lymphocyte with coarse azurophilic granulation (A), a lymphocyte with numerous fine granulations (B), and a lymphocyte with scarce granulation at the limit of visibility (C). D Intrasinusoidal marrow infiltration by cells positive for granzyme B. Note the bland nuclear cytology of the antigen-positive cells.

With such methodologies, clonality is found in some patients (1981,2336,2831). Unlike in aggressive NK-cell leukaemia, EBV is negative (2204,2398,4457).

Genetic susceptibility
A genetic susceptibility may be linked to haplotypes containing higher numbers of activating KIR genes (1113,3605,4456).

Prognosis and predictive factors
In most patients, the clinical course is indolent over a prolonged period, and no therapy is needed. In general, the management of CLPD-NKs is similar to that of T-cell large granular lymphocytic leukaemia (2205). Disease progression with increasing lymphocytosis and worsening of cytopenias is observed in some cases. Cytopenias, recurrent infections, and comorbidity may be harbingers of a worse prognosis. Rare cases with either spontaneous complete remission (2336,3001,3274,4458) or transformation to an aggressive NK-cell disorder have been described (1722,2948,3427). Cytogenetic abnormalities may imply a worse prognosis and could be associated with the rare transformations reported in the literature (2948).
Aggressive NK-cell leukaemia

Definition
Aggressive NK-cell leukaemia is a systemic neoplastic proliferation of NK cells frequently associated with EBV and an aggressive clinical course.

ICD-O code 9948/3

Synonym
Aggressive NK-cell leukaemia/lymphoma

Epidemiology
This rare form of leukaemia is much more prevalent among Asians than in other ethnic populations [3460]. Patients are most commonly young to middle-aged adults, with a median age of 40 years and two incidence peaks, in the third and fifth decades of life [1786,2297,3062,3728,3840]. There is no definite sex predilection [640,647,1756,2155,2157,2297,2999,3460,3462,3728,3840].

Etiology
Little is known about the etiology of aggressive NK-cell leukaemia, but the strong association with EBV suggests a pathogenetic role of the virus. In younger patients, the leukaemia may evolve from chronic active EBV infection [1788,1789,3062].

Localization
The most commonly involved sites are the peripheral blood, bone marrow, liver, and spleen, but any organ can be involved.

There can be overlap with extranodal NK/T-cell lymphoma showing multiorgan involvement; it is unclear whether aggressive NK-cell leukaemia is the leukaemic counterpart of extranodal NK/T-cell lymphoma [640].

Clinical features
Patients usually present with fever, constitutional symptoms, and a leukaemic blood picture. The number of circulating leukaemic cells may be low or high (a few per cent to >80% of all leukocytes); anaemia, neutropenia, and thrombocytopenia are common. Serum lactate dehydrogenase levels are often markedly elevated. Hepatosplenomegaly is common, sometimes accompanied by lymphade-
nopathy, but skin lesions are uncommon. Effusions are common. The disease may be complicated by coagulopathy, haemophagocytic syndrome, or multiorgan failure (647,1756,2157,2297,2733,2970,3728,3840). Rare cases may evolve from extranodal NK/T-cell lymphoma or chronic lymphoproliferative disorder of NK cells (1591,2948,3001,3722,4482).

Microscopy
Circulating leukaemic cells can show a range of appearances, from cells indistinguishable from normal large granular lymphocytes to cells with atypical nuclei featuring enlargement, irregular foldings, open chromatin, or distinct nucleoli. There is ample pale or lightly basophilic cytoplasm containing fine or coarse azurophilic granules. The bone marrow shows massive, focal, or subtle infiltration by the neoplastic cells, and there can be intermingled reactive histiocytes with haemophagocytosis. In tissue sections, the leukaemic cells show diffuse or patchy destructive infiltrates. They often appear monotonous, with round or irregular nuclei, condensed chromatin, and small nucleoli, but they can sometimes show substantial nuclear pleomorphism. There are frequently admixed apoptotic bodies. Necrosis is common, and there may or may not be angioinvasion.

Immunophenotype
The neoplastic cells typically have a CD2-, surface CD3-, CD3-epsilon-, CD5-, CD56-, phenotype and are positive for cytotoxic molecules. Thus, the immunophenotype is identical to that of extranodal NK/T-cell lymphoma, except that CD16 is frequently (in 75% of cases) positive (3840). Aberrant immunophenotypes can also occur, such as loss of expression of CD2, CD7, or CD45 (2297). CD11b may be expressed, whereas CD57 is usually negative (640,2999). The neoplastic cells express FASL, and high levels can be found in the serum of affected patients (1960,2452,3891).

Cell of origin
An activated NK cell

Genetic profile
TR genes are in germline configuration. EBV is reported to be positive in 85–100% of cases, and EBV is present in a clonal episomal form (647,1564,1971). The EBV-negative subset of cases occur de novo or evolve from chronic lymphoproliferative disorder of NK cells, and have clinicopathological features similar to those of EBV-positive cases; however, it is unclear whether the clinical outcome is similar (1773,2057,2866A,3062,3139,3840). Various clonal cytogenetic abnormalities have been reported, such as del(6)(q21q25) and 11q deletion (3462). An array comparative genomic hybridization study identified significant differences in genetic changes between aggressive NK-cell leukaemia and extranodal NK/T-cell lymphoma: losses in 7p and 17p as well as gains in 1q are frequent in the former but not the latter; deletions in 6q are common in the latter but rare in the former (2817).

Prognosis and predictive factors
Most cases have a fulminant clinical course, frequently complicated by multiorgan failure, coagulopathy, and haemophagocytic syndrome. The median survival is <2 months (640,2297,3462,3728,3840,4482). Response to chemotherapy is usually poor, and relapse is common in patients who achieve remission with or without bone marrow transplantation (2297,3840).
EBV-positive T-cell and NK-cell lymphoproliferative diseases of childhood

EBV-associated T-cell and NK-cell lymphoproliferative disorders in the paediatric age group can be categorized into two major groups: systemic EBV-positive T-cell lymphoma of childhood and chronic active EBV infection. Both occur with increased frequency in Asians and in Native Americans from Central and South America and Mexico. Systemic EBV-positive T-cell lymphoma of childhood has a very fulminant clinical course, usually associated with a haemophagocytic syndrome. Chronic active EBV infection of T- and NK-cell type shows a broad range of clinical manifestations, from indolent, localized forms such as hydroa vacciniforme-like lymphoproliferative disorder and severe mosquito bite allergy to more systemic disease characterized by fever, hepatosplenomegaly, and lymphadenopathy, with or without cutaneous manifestations. Additionally, significant overlap in the morphological features of the following conditions is present. Therefore, correlation with clinical features is critical for accurate diagnosis.

**Systemic EBV-positive T-cell lymphoma of childhood**

**Definition**

Systemic EBV-positive T-cell lymphoma of childhood is a life-threatening illness of children and young adults, characterized by a clonal proliferation of EBV-infected T cells with an activated cytotoxic phenotype. It can occur shortly after primary acute EBV infection or in the setting of chronic active EBV infection (CAEBV). It has rapid progression, with multiorgan failure, sepsis, and death, usually within a timeframe of days to weeks. A haemophagocytic syndrome is nearly always present. This entity has some clinicopathological features overlapping with those of aggressive NK-cell leukaemia.

**ICD-O code** 9724/3

**Synonyms and historical terminology**

Historically, this process has been described using a variety of terms, including fulminant EBV-positive T-cell lymphoproliferative disorder of childhood (3269), sporadic fatal infectious mononucleosis, fulminant haemophagocytic syndrome in children (in Taiwan, China) (3822); fatal EBV-associated haemophagocytic syndrome (in Japan) (2011); and severe CAEBV (2025,2963,3838). The term fulminant or fatal haemophagocytic syndrome was used to describe a systemic disease secondary to acute primary EBV infection affecting previously healthy children, but the disease has since been shown to be a monoclonal CD8+ T-cell EBV-associated lymphoproliferative disorder, and is therefore now considered equivalent to systemic EBV-positive T-cell lymphoma of childhood (3269). The term CAEBV was coined to describe an infectious mononucleosis-like syndrome persisting for at least 6 months and associated with high titres of IgG antibodies against EBV viral capsid antigen and early antigen, with no association with malignancy, autoimmune diseases, or immunodeficiency (3809). Because the earliest described cases, which were found in western populations, showed EBV predominantly in B cells (779,3809), CAEBV was originally thought to be a disorder affecting B cells, but has since been shown to affect primarily T cells and NK cells (780,2025,2026). Progression to EBV-positive T-cell lymphoma is not unusual (1877,1918,2026,3269). A more severe form of CAEBV, characterized by high fever, hepatosplenomegaly, extensive lymphadenopathy, haemophagocytic syndrome, and pancytopenia, has been described in patients in Japan (2025,2028,3838). These patients had higher viral copy numbers in peripheral blood, as well as monoclonal expansion of EBV-infected T cells or NK cells. Severe CAEBV with monoclonal EBV-positive T-cell proliferation is part of the

**Table 14.01 Classification of EBV-positive T-cell and NK-cell proliferations**

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Usual patient age group(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EBV-positive haemophagocytic lymphohistiocytosis (benign, may be self-limited)</td>
<td>Paediatric, adolescent</td>
</tr>
<tr>
<td>Systemic CAEBV</td>
<td>Paediatric, adolescent</td>
</tr>
<tr>
<td>Cutaneous CAEBV, hydroa vacciniforme-like lymphoproliferative disorder</td>
<td>Paediatric, adolescent</td>
</tr>
<tr>
<td>Cutaneous CAEBV, severe mosquito bite allergy</td>
<td>Paediatric, adolescent</td>
</tr>
<tr>
<td>Systemic EBV-positive T-cell lymphoma</td>
<td>Paediatric, adolescent</td>
</tr>
<tr>
<td>Aggressive NK-cell leukaemia</td>
<td>Adult</td>
</tr>
<tr>
<td>Extracodal NK/T-cell lymphoma, nasal type</td>
<td>Adult</td>
</tr>
<tr>
<td>Nodal peripheral T-cell lymphoma, EBV-positive</td>
<td>Adult</td>
</tr>
</tbody>
</table>

*CAEBV, chronic active EBV infection.*

* Included within the category of peripheral T-cell lymphoma, NOS.

**Fig. 14.17** Systemic EBV-positive T-cell lymphoma of childhood. PCR for TR gamma rearrangement demonstrates an identical T-cell clone in liver, spleen, and lymph nodes.
Fig. 14.18 Systemic EBV-positive T-cell lymphoma of childhood. A The bone marrow shows histiocytic hyperplasia with a lymphoid infiltrate composed of relatively large cells with bland nuclei and inconspicuous nucleoli. B CD8 is positive in the large lymphoid cells. C Double staining demonstrates that many of the CD8+ lymphocytes are also positive for EBV encoded small RNA (EBER).

spectrum of systemic EBV-positive T-cell lymphoma of childhood; to avoid confusion, it should not be referred to as CAEBV.

Epidemiology
Systemic EBV-positive T-cell lymphoma of childhood is most prevalent in Asia, primarily in Japan and Taiwan, China. It has been reported in Mexico and in Central and South America, and is reported rarely in non-indigenous populations in western countries. It occurs most often in children and young adults. There is no sex predominance.

Etiology
Although the etiology of systemic EBV-positive T-cell lymphoma of childhood is unknown, its association with primary EBV infection and its racial predisposition strongly suggest a genetic defect in the host immune response to EBV. It is usually complicated by haemophagocytic syndrome, coagulopathy, multiorgan failure, and sepsis. Some cases occur in patients with a well-documented history of CAEBV. A disorder that is probably related but presents mainly with lymphadenopathy and high lactate dehydrogenase levels has recently been reported in children from Peru.

Localization
This is a systemic disease. The most commonly involved sites are the liver and spleen, followed by the lymph nodes, bone marrow, skin, and lungs. Laboratory tests show pancytopenia, abnormal liver function, and often abnormal EBV serology, with low or absent IgM antibodies against viral capsid antigen. The disease is usually complicated by haemophagocytic syndrome, coagulopathy, multiorgan failure, and sepsis. Some cases occur in patients with a well-documented history of CAEBV. A disorder that is probably related but presents mainly with lymphadenopathy and high lactate dehydrogenase levels has recently been reported in children from Peru.

Clinical features
Previously healthy patients present with acute onset of fever and general malaise suggestive of an acute viral respiratory illness. Within a period of weeks to months, patients develop hepatosplenomegaly and liver failure, sometimes accompanied by lymphadenopathy. Laboratory tests show pancytopenia, abnormal liver function, and often abnormal EBV serology, with low or absent IgM antibodies against viral capsid antigen. The disease is usually complicated by haemophagocytic syndrome, coagulopathy, multiorgan failure, and sepsis. Some cases occur in patients with a well-documented history of CAEBV. A disorder that is probably related but presents mainly with lymphadenopathy and high lactate dehydrogenase levels has recently been reported in children from Peru.

Spread
The disease is systemic, with the potential to involve all organ systems. However, involvement of the CNS is less often seen.

Fig. 14.19 Systemic EBV-positive T-cell lymphoma of childhood. Lymph node. A The lymph node shows partial preservation of the architecture with residual regressive follicles and expanded interfollicular area. Inset: The interfollicular infiltrate is polymorphic with some relatively large cells with irregular nuclei and prominent nucleoli. B The infiltrating lymphocytes are CD8-positive. Inset: The atypical cells are CD8-positive. C Many cells are positive for EBV-encoded small RNA (EBER). Inset: Double staining demonstrates that the EBER positive cells (black) are positive for CD8 (brown).
**Microscopy**

The infiltrating T cells are usually small and lack substantial cytological atypia (3269). However, cases with pleomorphic medium-sized to large lymphoid cells, irregular nuclei, and frequent mitoses have been described (3838). The liver and spleen show mild to marked sinusoidal infiltration, with striking hemophagocytosis. The splenic white pulp is depleted. The liver has prominent portal and sinusoidal infiltration, cholestasis, steatosis, and necrosis. The lymph nodes usually show preserved architecture with open sinuses. The B-cell areas are depleted, whereas the paracortical areas may be expanded and show a subtle to dense infiltration and a broad cytological spectrum ranging from small or medium-sized lymphocytes to large atypical lymphocytes with hyperchromatic and irregular nuclei. The more advanced the disease, the more depleted the lymph nodes look. A variable degree of sinus histiocytosis with erythrophagocytosis is present. Bone marrow biopsies show histiocytic hyperplasia with prominent erythrophagocytosis.

**Immunophenotype**

The neoplastic cells most typically have a CD2+, CD3+, CD56−, TIA1+ phenotype. Most cases secondary to acute primary EBV infection are CD8-positive (1952, 3269, 3822), whereas cases occurring in the setting of severe CAEBV are CD4-positive (1877,1918,3269). Rare cases show both CD4+ and CD8+ EBV-infected T cells (3269). EBV-encoded small RNA (EBER) is positive.

**Postulated normal counterpart**

A cytotoxic CD8+ T cell or activated CD4+ T cell.

**Genetic profile**

The cells have monoclonally rearranged TR genes. All cases harbour EBV in a clonal episomal form (1877,2011,2025, 3838). All cases analysed carry type A EBV, with either wildtype or the 30 bp-deleted product of the LMP1 gene (1952, 3269,3838). In situ hybridization for EBER shows that most of the infiltrating lymphoid cells are positive. No consistent chromosomal aberrations have been identified (675,2025,3707).

**Prognosis and predictive factors**

Most cases have a fulminant clinical course resulting in death, usually within days to weeks of diagnosis. The disease is usually complicated by haemophagocytic syndrome. Few cases have been reported to respond to an etoposide- and dexamethasone-based regimen followed by allogeneic haematopoietic stem cell transplantation (the HLH-2004 protocol) (178,1611,1882,2026,3707). The rapidly progressive clinical course is similar to that of aggressive NK-cell leukaemia.
**Chronic active EBV infection of T- and NK-cell type, systemic form**

**Definition**
Chronic active EBV infection (CAEBV) of T-cell or NK-cell type is a systemic EBV-positive polyclonal, oligoclonal, or (often) monoclonal lymphoproliferative disorder characterized by fever, persistent hepatitis, hepatosplenomegaly, and lymphadenopathy, which shows varying degrees of clinical severity depending on the host immune response and the EBV viral load. The revised diagnostic criteria for CAEBV include infectious mononucleosis-like symptoms persisting for >3 months, increased EBV DNA (>10^5 copies/mg) in peripheral blood, histological evidence of organ disease, and demonstration of EBV RNA or viral protein in affected tissues in patients without known immunodeficiency, malignancy, or autoimmune disorders.

**Synonym**
Systemic chronic active EBV infection of T-cell and NK-cell type

**Epidemiology**
Systemic CAEBV of T- and NK-cell type has a strong racial predisposition, with most cases reported from Asia, primarily in Japan [1954,2025,2026,2951,3838]; the Republic of Korea [1676]; and Taiwan, China [4244]. It has also been reported in Latin America and rarely in western [3422,3592,3730] and African populations [3353]. It occurs most often in children and adolescents. Adult-onset disease is rare and appears to be rapidly progressive and more aggressive [124,1792]. There is no sex predilection.

**Etiology**
Although the etiology is unknown, the strong racial predisposition for the development of CAEBV of T- and NK-cell type in immunocompetent individuals strongly suggests that genetic polymorphisms in genes related to the EBV immune response are responsible for the development of this disease [780,2023]. EBV-specific cytotoxic T lymphocyte activity is impaired in patients with CAEBV [780,3829,4071].

**Localization**
This is a systemic disease. The most commonly involved sites are the liver, spleen, lymph nodes, bone marrow, and skin. The lungs, kidneys, heart, CNS, and gastrointestinal tract can also be involved [2025,2026,3062].

**Clinical features**
Approximately 50% of patients present with infectious mononucleosis-like illness, including fever, hepatosplenomegaly, and lymphadenopathy. Accompanying symptoms include skin rash (occurring in 26% of cases), severe mosquito bite allergy (in 33%), hydroa vacciniforme-like eruptions (in 10%), diarrhoea (in 6%), and uveitis (in 5%). Laboratory tests reveal pancytopenia and abnormal
liver function. In most patients, EBV serology reveals high titres of IgG antibodies against EBV viral capsid antigen and early antigen. All patients have increased levels of EBV DNA (>10^2.5 copies/mg) in the peripheral blood. The clinical course varies but is usually protracted, with some patients surviving for many years without disease progression. The severity of CAEBV is probably related to the immunological response of the individual and to the EBV viral load. The clinical course also varies depending on the predominant infected cell type in the peripheral blood [2024,2025]. Patients with T-cell CAEBV have a shorter survival time than patients with NK-cell disease. Patients with T-cell CAEBV often present with prominent systemic symptoms and high titres of EBV-specific antibodies and have rapid disease progression. In contrast, patients with NK-cell disease, in addition to mild systemic symptoms, often have severe mosquito bite allergy, rash, and high levels of IgE, and do not always have elevated EBV-specific antibody titres. Life-threatening complications include haemophagocytic syndrome (which occurs in 24% of cases), coronary artery aneurysm (in 9%), hepatic failure (in 15%), interstitial pneumonia (in 5%), CNS involvement (in 7%), gastrointestinal perforation (in 11%), and myocarditis (in 4%). Due to the variety of the clinical presentations, diagnosis is often delayed. Progression to NK/T-cell lymphoma or aggressive NK-cell leukaemia occurs in 16% of cases [1676,2026,2028,2951].

Microscopy
The infiltrating cells do not show changes suggestive of a neoplastic lymphoproliferation. The liver shows sinusoidal and portal infiltration suggestive of viral hepatitis. The spleen shows atrophy of the white pulp with congestion of the red pulp. The lymph nodes exhibit variable morphology, including paracortical and follicular hyperplasia, focal necrosis, and small epithelioid granulomas. Bone marrow biopsies usually appear normal. In cases complicated by haemophagocytic syndrome, sinus histiocytosis with erythrophagocytosis is present [2026].

Immunophenotype
The immunophenotype of the EBV-infected cells varies; it includes T cells in 59% of cases, NK cells in 41%, and both T and NK cells in 4%. CAEBV of B-cell phenotype is seen in only 2% of cases. Unlike the T cells in systemic EBV-positive T-cell lymphoma of childhood, the T cells in CAEBV are predominantly CD4-positive, and less often show a cytotoxic CD8+ phenotype [780,2026]. EBV-encoded small RNA (EBER) is positive.

Cell of origin
The postulated cells of origin are CD4+ T cells, NK cells, cytotoxic CD8+ lymphocytes, and (rarely) gamma delta T cells.

Genetic profile
Chromosomal aberrations are detected in a minority of cases [2026]. One series reported monoclonally rearranged TR genes in 84% of cases, oligoclonally rearranged TR genes in 11%, and polyclonal TR genes in only 5% of cases [2026]. However, this report includes cases of 'severe CAEBV', which might be
reclassified as systemic EBV+ T-cell lymphoma using the current WHO system. Somatic mutation of the perforin gene has been reported in one case [1956].

Prognosis and predictive factors
The prognosis is variable, with some cases following an indolent clinical course and others constituting rapidly progressive disease. Patient age >8 years at onset of disease and liver dysfunction are risk factors for mortality. Adult patients with CD4+ T-cell infection may have more-aggressive disease. The 5-year survival rates associated with cases of T-cell type and NK-cell type, respectively, are 59% and 87%. Monoclonality of the proliferating cells does not correlate with increased mortality and does not warrant a diagnosis of lymphoma. Patients who undergo bone marrow transplantation have a better prognosis [2024,2025,2026]. A specific classification of CAEBV based on cytology and clonality of the proliferating cells has been proposed [2951]. A1 cases are polymorphic and polyclonal; A2 cases are polymorphic and monoclonal; A3 cases are monomorphic and monoclonal; and B cases are monomorphic and monoclonal but with a fulminant course. The A1–A3 categories are thought to represent a continuous spectrum of CAEBV from lymphoproliferative disorder (A1–A2) to overt lymphoma (A3), whereas the B category is equivalent to systemic EBV-positive T-cell lymphoma of childhood.

Hydroa vacciniforme–like lymphoproliferative disorder

Definition
Hydroa vacciniforme (HV)-like lymphoproliferative disorder is a chronic EBV-positive lymphoproliferative disorder of childhood, associated with a risk of developing systemic lymphoma. HV-like lymphoproliferative disorder is a primarily cutaneous disorder of polyclonal or (most often) monoclonal T cells or NK cells, with a broad spectrum of clinical aggressiveness and usually a long clinical course. As the disease progresses, patients develop severe and extensive skin lesions and systemic symptoms including fever, hepatosplenomegaly, and lymphadenopathy. Classic HV, severe HV, and HV-like T-cell lymphoma constitute a continuous spectrum of EBV-associated HV-like lymphoproliferative disorder.

ICD-O code
9725/1

Synonyms and historical terminology
In Western countries, classic HV was originally described as a benign photodermatosis characterized by light-induced vesicles that evolve to crusts and leave varicelliform scars after healing. It was noted that systemic symptoms were not observed and that the disease usually remitted spontaneously in adolescence [1395,1498,3731]. Because these cases were rarely biopsied, their clonality and EBV status were not thoroughly investigated. Subsequent studies in Asian populations showed that classic HV was an EBV-associated disorder [1806,1807]. A condition that clinically mimics classic HV was recognized in children and young adults who were mainly from Asia [1805] and Latin America [3456]. Patients with the condition present with marked facial oedema, vesicles, crusts, and large ulcers, sometimes with severe scarring and disfigurement. Unlike in classic HV, the skin lesions are not limited to sun-exposed areas and are not associated with light hypersensitivity; sun protection does not prevent the development of HV-like eruption. Because later studies demonstrated that these lesions are also associated with EBV infection [1807,2440] and often show monoclonal rearrangement of the TR genes [274], the term HV-like lymphoma was suggested and was included in the 2008 WHO classification. However, given the broad clinical spectrum of the disease and the lack of reliable morphological or molecular criteria to predict its clinical behaviour (classic HV vs HV-like lymphoma), the term HV-like lymphoproliferative disorder (A1–A2) to overt lymphoma (A3), whereas the B category is equivalent to systemic EBV-positive T-cell lymphoma of childhood.

Fig. 14.27 Hydroa vacciniforme–like lymphoproliferative disorder. Sun-exposed areas of the skin exhibit a papulovesicular eruption. Many of the lesions are ulcerated, with a haemorrhagic crust.
Fig. 14.28 Hydroa vacciniforme-like lymphoproliferative disorder. A The infiltrate is concentrated in the superficial dermis, but often extends to the subcutaneous tissue. B Neoplastic cells, which can be of T-cell or NK-cell lineage, are predominantly small, without marked atypia. C The lymphoid cells are positive for EBV, as demonstrated by in situ hybridization for EBV-encoded small RNA (EBER).

Lymphoproliferative disorder has been proposed, to encompass the various manifestations of the EBV-associated HV-like skin lesions [3271]. In the past, this disease has also been referred to as oedematous, scarring vasculitic panniculitis [3456]; angiocentric cutaneous T-cell lymphoma of childhood [2440]; hydroa-like cutaneous T-cell lymphoma [274]; and severe HV [1806].

Epidemiology
This condition is seen mainly in children and adolescents from Asia [731,1805, 1806,2883,4397], and in Native Americans from Central [1012] and South [274, 3389,3512] America and Mexico [2439, 3271]. The median patient age at diagnosis is 8 years (range: 1–15 years). The male-to-female ratio is slightly elevated (2:3:1). It is rare in adults [731,3512].

Etiology
The etiology is unknown. The geographical and ethnic distribution indicate that, like in other EBV-positive T-cell and NK-cell lymphomas, genetic predisposition plays a major role.

Localization
This is a cutaneous condition that affects sun-exposed and non-exposed skin areas. In the early phases, it affects mainly the face, dorsal surface of the hands, and earlobes; in advanced stages, it can be generalized [3271].

Clinical features
It is characterized by a papulovesicular eruption that generally proceeds to ulceration and scarring. The severity of the skin lesions and the clinical presentation varies between patients, with a broad spectrum. Some cases present with a very indolent course, with localized skin lesions in sun-exposed areas and no systemic symptoms (classic HV). Spontaneous remission and clearing after photoprotection can occur, but most cases show a long clinical course, with remissions and recurrences that may finally progress to more-severe disease [732]. There is seasonal variation, with increased recurrences in spring and summer. In more-severe cases, in addition to extensive skin lesions, systemic symptoms (including fever, wasting, lymphadenopathy, and hepatosplenomegaly) may be present, in particular late in the course of the disease [1806,2026, 3271,3512]. Some patients develop severe mosquito bite allergy [1639,3271]. A rare clinical presentation with primarily periorbital swelling has been reported in children from Bolivia [3206].

Macroscopy
In addition to prominent swelling of the face, lips, and eyelids, multiple vesiculo-papules with umbilication and crust are characteristic.

Microscopy
The characteristic histological feature of HV is epidermal reticular degeneration leading to intraepidermal spongiotic vesiculation. The lymphoid infiltrate predominates in the dermis but may extend deep into the subcutaneous tissue. The infiltrate is mainly located around adnexa and blood vessels, often with angiodestructive features. The intensity of the infiltrate and atypia of the lymphocytes varies. The neoplastic cells are generally small to medium-sized, without significant atypia. In severe cases, the overlying epidermis is frequently ulcerated [3271].

Immunophenotype
The cells have a cytotoxic T-cell phenotype, mostly CD8-positive, with few cases being CD4-positive. One third of the cases show an NK-cell phenotype, with expression of CD56 [1919,4484,2026, 3271,3389]. Clonal expansion of gamma delta T cells has been documented in the peripheral blood in most cases [1639, 2026,4224], but only in rare cases in the infiltrating lymphocytes in the skin [2439, 3271,4224]. CCR4 is expressed in the gamma delta T cells [1917]. CD30 is often expressed in the infiltrating EBV-positive T cells. LMP1 is usually negative [3271].

Postulated normal counterpart
The postulated normal counterpart is a skin-homing cytotoxic T cell or NK cell. Gamma delta T cells have been hypothesized to play a central role in the formation of HV-like eruptions [1972].

Genetic profile
Most cases have clonal rearrangements of the TR genes. Some cases of NK-cell derivation do not show TR gene rearrangement [2026,3271]. In situ hybridization for EBV-encoded small RNA (EBER) is positive, but the number of positive cells varies from case to case. EBV is monoclonal by terminal repeat analysis. Although LMP1 is negative by immunohistochemistry, it can be detected in most cases by PCR in peripheral blood, indicating type II EBV latency [1804].
Prognosis and predictive factors
The clinical course is variable, and patients may have recurrent skin lesions for as long as 10–15 years before progression to systemic involvement. With systemic spread, the clinical course is much more aggressive (1805). T-cell clonality, the amount of EBV-positive cells, and/or the density of the infiltrate do not predict which patients will eventually progress to systemic disease or develop a systemic lymphoma. No standard treatment has been established. The disease is resistant to conventional chemotherapy, and treated patients often die of infectious complications (274,3512). In indolent cases, a conservative approach is recommended, whereas haematopoietic stem cell transplantation has been introduced as a curative therapy in more advanced cases (2026,3271).

Severe mosquito bite allergy

Definition
Severe mosquito bite allergy is an EBV-positive NK-cell lymphoproliferative disorder characterized by high fever and intense local skin symptoms, including erythema, bullae, ulcers, skin necrosis, and deep scarring following mosquito bites. Patients have NK-cell lymphocytosis in the peripheral blood and an increased risk of developing haemophagocytic syndrome and progressing into overt NK/T-cell lymphoma or aggressive NK-cell leukaemia in the longstanding clinical course.

Synonym
Hypersensitivity to mosquito bites

Epidemiology
Severe mosquito bite allergy is very uncommon. Most cases have been reported in Japan (1788,2025,2026,4016), with a few cases from Taiwan, China (1158); the Republic of Korea (730); and Mexico (3024,3456). The patient age at onset ranges from birth to 18 years (mean: 6.7 years) (4015). There is no sex predilection.

Etiology
The etiology is unknown. Genetic background and environmental factors may play a role. Severe mosquito bite allergy is due to CD4+ T-cell proliferation in response to mosquito salivary gland secretions and plays a key role in the reactivation of EBV in NK cells inducing the expression of LMP1 (161). LMP1 expression induces NK-cell proliferation and may be responsible for the development of aggressive NK-cell leukaemia (162,4072).

Localization
This is primarily a cutaneous condition.

Clinical features
Severe mosquito bite allergy is characterized by local skin symptoms including erythema, bullae, ulcers, necrosis, and scarring. High fever and general malaise are common symptoms. Patients have a high level of serum IgE, a high EBV DNA load in the peripheral blood, and NK-cell lymphocytosis. Lymphadenopathy, hepatosplenomegaly, hepatic dysfunction, haematuria, and proteinuria are occasionally seen in the clinical course. After recovering, patients are asymptomatic until the next mosquito bite. Common complications are progression to systemic chronic active EBV infection of NK-cell type, haemophagocytic syndrome, aggressive NK-cell leukaemia, and nasal-type extranodal NK/T-cell lymphoma (160,2025,2026).

Microscopy
The skin biopsy at the bite site shows epidermal necrosis and ulceration or intraepidermal bullae. The dermis shows oedema and a dense infiltrate extending into the subcutaneous tissue. There is angiinvasion and angiodestruction. The infiltrate is polymorphic, with small lymphocytes, large atypical cells, histiocytes, and abundant eosinophils. The morphological characteristics are similar to those of hydroa vacciniforme-like lymphoproliferative disorder.

Immunophenotype
The infiltrating cells have an NK-cell phenotype, including positivity for CD3-epsilon and CD56, with expression of the cytotoxic molecules TIA1 and granzyme B. Reactive T cells, both CD4-positive and CD8-positive, are found at various intensities. LMP1 is rarely positive. CD30 is often positive in the EBV-infected cells.

Fig. 14.29 Severe mosquito bite allergy. The upper arm shows extreme oedema and erythema with necrosis and haemorrhagic crust after a mosquito bite.

Fig. 14.30 Severe mosquito bite allergy. A Skin biopsy with a subepidermal bulla with a dense infiltrate in the dermis, mainly surrounding blood vessels. B Higher magnification. The infiltrate is polymorphic but mainly composed of relatively large cells with bland nuclei, inconspicuous nucleoli, and abundant cytoplasm.
Postulated normal counterpart
Mature activated NK cell

Genetic profile
NK cells are infected with monoclonal EBV as demonstrated by terminal repeat analysis, indicating clonal expansion of NK cells. Rarely, monoclonal TR gene rearrangement has been documented (2025). Chromosomal alterations are rarely identified (2026). In situ hybridization for EBV-encoded small RNA (EBER) is positive in a fraction of the NK cells. LMP1 is detected by PCR in peripheral blood, indicating type II EBV latency (1804).

Prognosis and predictive factors
Patients usually have a long clinical course, with an increased risk of developing haemophagocytic syndrome and aggressive NK-cell leukaemia after 2–17 years (median: 12 years). Patients with chromosomal aberrations appear to have a higher risk of developing lymphoma/leukaemia (2026,4015).

Adult T-cell leukaemia/lymphoma

Definition
Adult T-cell leukaemia/lymphoma (ATLL) is a mature T-cell neoplasm most often composed of highly pleomorphic lymphoid cells. The disease is usually widely disseminated and is caused by the human retrovirus HTLV-1. Most ATLL patients present with widespread lymph node involvement as well as involvement of peripheral blood. The histology shows remarkable pleomorphism, with several morphological variants having been described. The leukaemic cells often show a multilobed appearance of so-called flower cells. Neoplastic cells show monoclonal integration of HTLV-1 and express T-cell–associated antigens (CD2, CD3, CD5), but usually lack CD7. Most cases are CD4-positive and CD8-negative. ATLL most often occurs in regions endemic for HTLV-1, and the frequency is estimated to be 2.5% among HTLV-1 carriers. ATLL occurs only in adults, with an average patient age of 58 years. The male-to-female ratio is 1.5:1. ATLL is a systemic disease, and the prognosis is poor (Fig. 14.45, p. 367).

ICD-O code 9827/3

Synonyms
Adult T-cell lymphoma/leukaemia; adult T-cell leukaemia

Epidemiology
ATLL is endemic in several regions of the world, in particular south-western Japan, the Caribbean basin, and parts of central Africa. The distribution of the disease is closely linked to the prevalence of HTLV-1 in the population. The disease has a long latency, and affected individuals are usually exposed to the virus very early in life. The virus may be transmitted in breast milk, as well as through exposure to peripheral blood and blood products. The cumulative incidence of ATLL is estimated to be 2.5% among HTLV-1 carriers in Japan (3869). Sporadic cases have been described.

Table 14.02 Clinical spectrum of HTLV-1-associated diseases

<table>
<thead>
<tr>
<th>Neoplastic disorders</th>
<th>Neonaplastic disorders (2521)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adult T-cell leukaemia/lymphoma</td>
<td>HTLV-1-associated myelopathy (tropical spastic paraparesis)</td>
</tr>
<tr>
<td>Smouldering</td>
<td>HTLV-1-associated infective dermatitis</td>
</tr>
<tr>
<td>Chronic</td>
<td>- Other HTLV-1 inflammatory disorders</td>
</tr>
<tr>
<td>Acute</td>
<td>Uveitis</td>
</tr>
<tr>
<td>Lymphomatous</td>
<td>Thyroiditis</td>
</tr>
<tr>
<td></td>
<td>Pneumonitis</td>
</tr>
<tr>
<td></td>
<td>Myositis</td>
</tr>
</tbody>
</table>

Ohshima K.
Jaffe E.S.
Yoshino T.
Siebert R.
but the affected patients often derive from an endemic region of the world. ATLL occurs only in adults, and the patient age at onset ranges from the third to the ninth decade of life, with an average patient age of 58 years. The male-to-female ratio is 1.5:1 [4405].

**Etiology**

HTLV-1 is causally linked to ATLL, but HTLV-1 infection alone is not sufficient to result in neoplastic transformation of infected cells. HTLV-1 enters cells mainly through cell-to-cell contact via three cellular molecules: heparan sulfate proteoglycan, neuropilin 1, and the glucose transporter GLUT1 [1352]. Neuropilin 1 appears to function as the viral receptor. The p40 tax viral protein leads to transcriptional activation of many genes in HTLV-1-infected lymphocytes [1241]. In addition, the HTLV-1 basic leucine zipper factor (HBZ) is thought to be important for T-cell proliferation and oncogenesis [3532]. However, additional genetic alternations acquired over time may result in the development of a malignancy. Hypermethylation is associated with disease progression [3528]. HTLV-1 can also indirectly cause other diseases (Table 14.02), such as HTLV-1-associated myelopathy / tropical spastic paraparesis [3880].

**Localization**

Most patients present with widespread lymph node involvement and involvement of the peripheral blood. The number of circulating neoplastic cells does not correlate with the degree of bone marrow involvement. This suggests that circulating cells are recruited from other organs, such as the skin, which is the most common extralymphatic site of involvement (involved in >50% of cases). The distribution of the disease is usually systemic, involving the spleen and extranodal sites including the skin, lungs, liver, gastrointestinal tract, and CNS [499]. There are epidemiological differences in the patterns of presentation. For example, a leukemic clinical presentation is much less common in patients from the Caribbean than in patients from Japan [2287].

**Clinical features**

Several clinical variants have been identified: acute, lymphomatosus, chronic, and smouldering (see Table 14.03) [3660]. The acute variant is most common and is characterized by a leukemic phase, often with a markedly elevated white blood cell (WBC) count, skin rash, and generalized lymphadenopathy. Hypercalcemia, with or without lytic bone lesions, is a common feature. Patients with acute ATLL have systemic disease accompanied by hepatosplenomegaly, constitutional symptoms, and elevated lactate dehydrogenase. Leukocytosis and eosinophilia are common. Many patients have an associated T-cell immunodeficiency, with frequent opportunistic infections such as Pneumocystis jirovecii pneumonia and strongyloidiasis. The lymphomatos variant is characterized by prominent lymphadenopathy but without peripheral blood involvement. Most patients present with advanced-stage disease similar to the acute form, although hypercalcemia is seen less often. Cutaneous lesions are common in both the acute and the lymphomatos forms.
of ATLL. They are clinically diverse and include erythematous rashes, papules, and nodules. Larger nodules may show ulceration. The chronic variant is frequently associated with an exfoliative skin rash. An absolute lymphocytosis may be present, but atypical lymphocytes are not numerous in the peripheral blood. Hypercalcaemia is absent. In the smouldering variant, the WBC count is normal with >5% circulating neoplastic cells. ATLL cells are generally small, with a normal appearance. Patients frequently have skin or pulmonary lesions, but there is no hypercalcaemia. Progression from the chronic or smouldering variant to the acute variant occurs in 25% of cases, usually after a long duration (3660).

**Imaging**

In patients with hypercalcaemia, imaging studies may show lytic bone lesions. FDG-PET/CT is usually positive in sites of disease activity (582).

**Macroscopy**

Macroscopic findings of the skin have been classified as erythema, papules, and nodules. Rare cases show tumour-like lesions or erythroderma as seen in Sézary syndrome.

**Microscopy**

ATLL is characterized by a broad spectrum of cytological features (2950). Several morphological variants have been described, reflecting this varied cytology and referred to as pleomorphic small, medium, and large cell types, anaplastic, and a rare form resembling angioimmunoblastic T-cell lymphoma (2950). The use of these variants is optional but they call attention to the spectrum of morphological appearances.

Some cases exhibit a leukaemic pattern of infiltration, with preservation or dilation of lymph node sinuses that contain malignant cells. The inflammatory background is usually sparse, although eosinophilia may be present. The neoplastic lymphoid cells are typically medium-sized to large, often with pronounced nuclear pleomorphism. The nuclear chromatin is coarsely clumped with distinct, sometimes prominent, nucleoli. Blast-like cells with transformed nuclei and dispersed chromatin are present in variable proportions (3660). Giant cells with convoluted or multilobed nuclear contours may be present. Rare cases may be composed predominantly of small lymphocytes, with irregular nuclear contours. Cell size does not correlate with the clinical course (1818), with the exception of the chronic and smouldering forms, in which the lymphocytes have a more normal appearance.

Lymph nodes in some patients in an early phase of adult T-cell lymphoma/leukaemia may exhibit a Hodgkin lymphoma-like histology (2953). The lymph nodes have expanded paracortical areas containing a diffuse infiltrate of small to medium-sized lymphocytes with mild nuclear irregularities, indistinct nucleoli, and scant cytoplasm. EBV-positive B lymphocytes with Hodgkin-like features are interspersed in this background. The expansion of EBV-positive B cells is thought to be secondary to the underlying immunodeficiency seen in patients with ATLL.

In the peripheral blood, the multilobed appearance of the neoplastic cells has led to the term ‘flower cells’. These cells have deeply basophilic cytoplasm, most readily observed with Giemsa staining of air-dried smears. Marrow infiltrates are usually patchy, ranging from sparse to moderate. Osteoclastic activity may be prominent, even in the absence of bone marrow infiltration by neoplastic cells. Skin lesions are seen in >50% of patients with ATLL. Epidermal infiltration with Pautrier-like microabscesses is common (2950). Dermal infiltration is mainly perivascular, but larger tumour nodules with extension to subcutaneous fat may be observed.

**Table 14.03 Diagnostic criteria for clinical subtypes of adult T-cell leukaemia/lymphoma. Modified, from Shimoyama M (3660)**

<table>
<thead>
<tr>
<th>Clinical manifestation</th>
<th>Smouldering</th>
<th>Chronic</th>
<th>Acute</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymphocytosis</td>
<td>No</td>
<td>Increased</td>
<td>Increased</td>
</tr>
<tr>
<td>Blood abnormal lymphocytes</td>
<td>&gt;5%</td>
<td>Increased</td>
<td>Increased</td>
</tr>
<tr>
<td>Lactate dehydrogenase</td>
<td>Normal</td>
<td>Slightly increased</td>
<td>Increased</td>
</tr>
<tr>
<td>Calcium</td>
<td>Normal</td>
<td>Normal</td>
<td>Variable</td>
</tr>
<tr>
<td>Skin rash</td>
<td>Erythema, papules</td>
<td>Variable</td>
<td>Variable</td>
</tr>
<tr>
<td>Lymphadenopathy</td>
<td>No</td>
<td>Mild</td>
<td>Variable</td>
</tr>
<tr>
<td>Hepatosplenomegaly</td>
<td>No</td>
<td>Mild</td>
<td>Variable</td>
</tr>
<tr>
<td>Bone marrow infiltration</td>
<td>No</td>
<td>No</td>
<td>Variable</td>
</tr>
</tbody>
</table>

**Fig. 14.36** Adult T-cell leukaemia/lymphoma (ATLL). Peripheral blood films. A In the acute variant, the leukaemic cells are medium-sized to large lymphoid cells with irregular nuclei and basophilic cytoplasm. The characteristic ATLL cells have been described as ‘flower cells’, with many nuclear convolutions and lobes. B ATLL cells in the chronic variant are generally small, with slight nuclear abnormalities such as notching and indentations.

**Fig. 14.37** Adult T-cell leukaemia/lymphoma cells frequently express FOXP3. The coexpression of FOXP3 and CD25 is characteristic of T regulatory (Treg) cells.
Diffuse infiltration of many organs is indicative of the systemic nature of the disease and of the presence of circulating malignant cells.

**Immunophenotype**

Tumour cells express T-cell–associated antigens (CD2, CD3, CD5), but usually lack CD7. Most cases are CD4-positive and CD8-negative, but a few are CD4-negative and CD8-positive or double-positive for CD4 and CD8. CD25 is strongly expressed in nearly all cases. The large transformed cells may be positive for CD30, but are negative for ALK (3882) and cytotoxic molecules. Tumour cells frequently express the chemokine receptor CCR4. FOXP3, a feature of T regulatory (Treg) cells, is expressed in a subset of cases (1948,3398), but often only in a subset of the neoplastic cells.

**Postulated normal counterpart**

The postulated normal counterpart is a peripheral CD4+ T cell. It has been hypothesized that the CD4-, CD25-, FOXP3+ Treg cells are the closest normal counterparts (1948), which would be consistent with the disease's characteristic association with immunodeficiency.

**Grading**

There is no formal grading system for ATLL. However, the four clinical variants – acute (leukaemic), lymphomatous, chronic, and smouldering – vary in their clinical course and cytological atypia (3660). In the chronic and smouldering forms the neoplastic cells are small, and can have minimal cytological atypia. Pronounced cytological atypia is seen in the acute and lymphomatous forms.

**Genetic profile**

Antigen receptor genes
TR genes are clonally rearranged (2952).
Oncogenes and other molecular changes

Neoplastic cells show monoclonal integration of HTLV-1. No clonal integration is present in healthy carriers (4075). Tax, encoded by the HTLV-1 pX region, is a critical non-structural protein that plays a key role in leukaemogenesis and activates a variety of cellular genes (3126). Enhancement of cAMP response element-binding transcription factor (CREB) phosphorylation by Tax appears to play a role in leukaemogenesis (2022). CREB is highly phosphorylated in a panel of HTLV-1-infected human T-cell lines, and Tax is responsible for promoting elevated levels of CREB phosphorylation. However, Tax is not critical to sustained tumour cell growth and is inactivated in a high proportion of cases of ATLL (1957). HBZ also appears to play a critical role in tumorigenesis (4487). HBZ is the only gene that is consistently expressed in all ATLL cases; it modulates a variety of cellular signalling pathways that are related to cell growth, immune response, and T-cell differentiation. In whole-transcriptome sequencing, CCR4 mutations have been detected in one quarter of cases, and are associated with gain of function and increased PI3K signalling (2808).

A recent study (1957), reviewed in (4193) provided an integrated genomic and transcriptomic analysis of >400 ATLL cases. The authors identified a single viral integration site in most cases of ATLL, confirming the clonal nature of the disease. Transcriptome analysis confirmed the critical role of HBZ, which is expressed at high levels in all cases. ATLL showed considerable genomic instability, with a high number of structural variations per case. A total of 50 genes were recurrently mutated. Among the most frequently mutated genes were PLCG1, PKRCCB, VAV1, IRF4, FYN, CARD11, and STAT3, some of which are involved in T-cell receptor signalling. This study also confirmed a high incidence of CCR4 mutations and identified mutations in CCR7 in some other cases. The additional mutations identified affect the NF-kappaB pathway and genes involved in T-cell signalling. Whole-genome sequencing identified intragenic deletions involving TP73, a homologue of TP53. These deletions resulted in mutant p73, lacking the transactivation domain (TAD). Recurrent splice-site mutations were found in GATA3, HNRNP2B1, and FAS.

ATLL genomes demonstrated prominent CpG island DNA hypermethylation, leading to transcriptome silencing of many genes, including genes encoding major histocompatibility complex (MHC) class I, death receptors, and immune checkpoints, providing a mechanism for immune escape of the tumour cells (1957).

Prognosis and predictive factors

Clinical subtype, patient age, performance status, and serum calcium and lactate dehydrogenase levels are major prognostic factors (4410). The survival time for the acute and lymphomatous variants ranges from 2 weeks to >1 year. Death is often caused by infectious complications, including P. jiroveci pneumonia, cryptococcal meningitis, disseminated herpes zoster, and hypercalcaemia (3660). The chronic and smouldering forms have a more protracted clinical course and better survival, but can progress to an acute phase with an aggressive course in approximately 25% of patients (1961, 2954).

Fig. 14.45  Adult T-cell leukaemia/lymphoma. Overall survival (OS) of 1665 patients diagnosed between 2000 and 2009 in Japan. A total of 227 patients underwent allogeneic bone marrow transplantation; 25% of those patients had long survival. MST, median survival time. Data from Katsuya H et al. (1961).
Extranodal NK/T-cell lymphoma, nasal type

Definition
Extranodal NK/T-cell lymphoma, nasal type, is a predominantly extranodal lymphoma of NK-cell or T-cell lineage, characterized by vascular damage and destruction, prominent necrosis, cytotoxic phenotype, and association with EBV. It is designated an NK/T-cell lymphoma because although most cases appear to be genuine NK-cell neoplasms, some cases are of cytotoxic T-cell lineage.

ICD-O code
9719/3

Synonyms
Angiocentric T-cell lymphoma (obsolete); malignant reticulosis, NOS (obsolete); malignant midline reticulosis (obsolete); polymorphic reticulosis (obsolete); lethal midline granuloma (obsolete); T/NK-cell lymphoma

Epidemiology
Extranodal NK/T-cell lymphoma is more prevalent in Asians and the indigenous populations of Mexico, Central and South America [187,640,2232,3266,3949]. It occurs most often in adults, with a reported median patient age of 44–54 years [189,647,704,1484,1858,2305,3062,3210]. It is more common in males than in females.

Etiology
The very strong association with EBV, irrespective of the ethnic origin of the patients, suggests a pathogenic role of the virus [133,649,1091,1916,2607,3266,3842,4133] with type II latency (EBNA1+, EBNA2−, LMP1+), and commonly shows a 30 bp deletion in the LMP1 gene [708,1000,1091,2139,3868]. Most studies show that the EBV is almost always of subtype A [275,1091,1484,3120,3842,4023]. The disease activity can be monitored by measuring circulating EBV DNA; a high titre is correlated with extensive disease, unfavourable response to therapy, and poor survival [188,4257]. Extranodal NK/T-cell lymphomas can occur in the setting of immunosuppression, including following transplantation [1702,2156].

Localization
Extranodal NK/T-cell lymphoma almost always has an extranodal presentation. The upper aerodigestive tract (nasal cavity, nasopharynx, paranasal sinuses, and palate) is most commonly involved, with the nasal cavity being the prototypical site of involvement. Preferential sites of extranasal involvement include the skin, soft tissue, gastrointestinal tract, and testes. Some cases may be accompanied by secondary lymph node involvement [640,647,1996,2154,3154,4023]. Rare cases with features of intravascular lymphoma have been reported, involving sites such as the skin and CNS [2138,2372,2810,4385]. Primary EBV-positive nodal T-cell or NK-cell lymphomas have been reported [178,718,1901]. These usually have a monoclonal pattern of infiltration and lack the angiodestruction and necrosis seen in extranodal NK/T-cell lymphoma. They are more common in elderly patients, or
in the setting of immune deficiency. They are considered a variant of peripheral T-cell lymphoma, NOS, and seem distinct from cases with primary extranodal presentations.

### Clinical features

Patients with nasal involvement present with symptoms of nasal obstruction or epistaxis due to the presence of a mass lesion, or with extensive destructive midfacial lesions (lethal midline granuloma). The lymphoma can extend to adjacent tissues, such as the nasopharynx, paranasal sinuses, orbits, oral cavity, palate, and oropharynx. The disease is often localized to the upper aerodigestive tract at presentation, and bone marrow involvement is uncommon [4356]. The disease may disseminate to various sites (e.g. the skin, gastrointestinal tract, testes, and cervical lymph nodes) during the clinical course. Some cases may be complicated by haemophagocytic syndrome [704, 2154].

Extranodal NK/T-cell lymphomas occurring outside of the upper aerodigestive tract (often referred to as extranasal NK/T-cell lymphomas) have variable presentations, depending on the major site of involvement. Skin lesions are commonly nodular, often with ulceration. Intestinal lesions often manifest as perforation or gastrointestinal bleeding. Other involved sites present as mass lesions. The patients commonly have high stage disease at presentation, with involvement of multiple extranodal sites. Systemic symptoms such as fever, malaise, and weight loss can be present [647,1996,4357]. Lymph nodes can be involved as part of disseminated disease. Marrow and peripheral blood involvement can occur, and such cases may overlap with aggressive NK-cell leukaemia.

### Microscopy

The histological features of extranodal NK/T-cell lymphoma are similar irrespective of the site of involvement. Mucosal sites often show extensive ulceration. The lymphomatous infiltrate is diffuse and permeative. Mucosal glands often

---

**Fig. 14.49** Extranodal NK/T-cell lymphoma, nasal type. A Prominent ulceration and necrosis in the nasal mucosa. B The nasal mucosa is diffusely infiltrated and expanded by an abnormal lymphoid infiltrate. The mucosal glands commonly show a peculiar clear-cell change. C,D Nasal-type NK/T-cell lymphoma in the testis. There is a diffuse dense lymphoid infiltrate, with prominent coagulative necrosis (C). The neoplastic cells appear monotonous and are medium-sized (D).

**Fig. 14.50** Extranodal NK/T-cell lymphoma, nasal type. A The lymphomatous infiltrate shows infiltration and destruction of an artery. B In this case involving the skin, the lymphomatous infiltrate has an angiocentric angiodestructive quality.
become widely spaced or are lost. An angiocentric and angiodestructive growth pattern is frequently present, and fibrinoid changes can be seen in the blood vessels even in the absence of angioinvasion. Coagulative necrosis and admixed apoptotic bodies are common findings. These have been attributed to vascular occlusion by lymphoma cells, but other factors (e.g. chemokines and cytokines) have also been implicated (3943).

The cytological spectrum is very broad. Cells may be small, medium-sized, large, or anaplastic. In most cases, the lymphoma is composed of medium-sized cells or a mixture of small and large cells. The cells often have irregularly folded nuclei, which can be elongated. The chromatin is granular, except in the very large cells, which may have vesicular nuclei. Nucleoli are generally inconspicuous or small. The cytoplasm is moderate in amount and often pale to clear. Mitotic figures are easily found, even in small cell predominant lesions. On Giemsa-stained touch preparations, azurophilic granules are commonly detected. Ultrastructurally, electron-dense membrane-bound granules are present.

Extranodal NK/T-cell lymphomas, particularly those in which small-cell or mixed-cell populations predominate, and those accompanied by a heavy admixture of inflammatory cells (small lymphocytes, plasma cells, histiocytes, and eosinophils), may mimic an inflammatory process (640,1591). The lymphoma can sometimes be accompanied by florid pseudoepitheliomatous hyperplasia of the overlying epithelium (2355).

Immunophenotype

The most typical immunophenotype of extranodal NK/T-cell lymphoma is: CD2+, CD5−, CD56−, surface CD3− (as demonstrated on fresh or frozen tissue), and cCD3-epsilon+ (as demonstrated on fresh, frozen, or fixed tissues) (640,648, 1814,1819,3266,4069). CD56, although a highly useful marker for NK cells, is not specific for extranodal NK/T-cell lymphoma, and can be expressed in some peripheral T-cell lymphomas. CD43 and CD45RO are often positive, and CD7 is variably expressed. Other T-cell-associated and NK-cell-associated antigens, including CD4, CD8, CD16, and CD57, are usually negative. The subset of cases of cytotoxic T-cell lineage may express CD5, CD8, and T-cell receptor (gamma delta or alpha beta) (1858,3210,4407).

Cytotoxic molecules (e.g. granzyme B, TIA1, and perforin) are positive (1091). HLA-DR, CD25, FAS (also known as CD95), and FASL are commonly expressed (2858,2955). CD30 is positive in about 30% of cases (189,1484,2021, 2139,2305). Nuclear expression of megakaryocyte-associated tyrosine kinase (MATK) is common (3210,3890).

Lymphomas that demonstrate a CD3-epsilon+, CD56− immunophenotype are also classified as extranodal NK/T-cell lymphomas if both cytotoxic molecules are positive.
and EBV are positive, because these cases show a similar clinical disease as cases with CD56 expression [2859]. On the other hand, nasal or other extranodal lymphomas that are CD3+ and CD56- but negative for EBV and cytotoxic molecules should be diagnosed as peripheral T-cell lymphoma, NOS. The diagnosis of extranodal NK/T-cell lymphoma should be considered with scepticism if EBV is negative [518,649,1091,2139,2859,3850,3868]. On the other hand, EBV can occasionally occur in other T-cell lymphoma types, so EBV positivity does not always equate with a diagnosis of extranodal NK/T-cell lymphoma [3850]. In situ hybridization for EBV-encoded small RNA (EBER) is the most reliable way to demonstrate the presence of EBV, with virtually all lymphoma cells being labelled. Immunostaining for EBV LMP1 is often negative, consistency with EBV latency type 1.

Postulated normal counterpart
Activated NK cells and (less commonly) cytotoxic T cells

Grading
The prognostic importance of cytological grade is controversial; some studies suggest that tumours composed predominantly of small cells are less aggressive, but other studies have not shown this feature to be of significance on multivariate analysis [275,704,1645,2139,2305,3210]. The International Peripheral T-cell Lymphoma Project reports that the presence of >40% transformed cells predicts worse overall survival in nasal (but not extranasal) cases [189].

Genetic profile
Antigen receptor genes
TR and IG genes are in germline configuration in most cases. Clonal TR gene rearrangements are reported in 10–40% of cases, presumably the cases of cytotoxic T lymphocyte derivation [189,1858,2058,2305,2859,3210,3868].

Gene expression profiling
The gene expression profiles of all extranodal NK/T-cell lymphomas cluster together, irrespective of NK-cell or gamma delta T-cell lineage [1728,1773]. Non-hepatosplenic gamma delta T-cell lymphomas show very similar gene expression profiles.

Cytogenetic abnormalities and oncogenes
A variety of cytogenetic aberrations have been reported, but so far no specific chromosomal translocations have been identified. The commonest cytogenetic abnormality is del(6)(q21q25) or i(6)(p10), but it is unclear whether this is a primary or progression-associated event [3688,3689,4002,4359]. Comparative genomic hybridization studies show that the commonest aberrations are gain of 2q and loss of 1p36.23-36.33, 6q16.1-27, 4q12, 5q34-35.3, 7q21.3-22.1, 11q22.3-23.3, and 15q11.2-14 [2817]. Recurrent mutations, deletions, and hypermethylation have been found in the genes encoding RNA helicase DDX3X, members of the JAK/STAT signalling pathway (STAT3, STAT5B, JAK3, and PTPRK) and other signalling pathways (KIT and CTNNB1), tumour suppressors (TP53, MCA, PRDM1, ATG5, AIM1, FOXO3, and HACE1), oncogenes (the RAS family of genes and MYC), epigenetic modifiers (KMT2D/MLL2, ARID1A, EP300, and ASXL3), cell-cycle regulators (CDKN2A, CDKN2B, and CDKN1A), and apoptosis regulators (FAS) [429,686,1678,1727,1859,1946,2027,2083,2158,2159,2160,3062,3268].

Prognosis and predictive factors
The prognosis of nasal NK/T-cell lymphoma is variable, with some patients responding well to therapy and others dying of disseminated disease despite aggressive therapy. Historically, the survival rate has been poor (30–40%), but the survival has improved in recent years with more intensive therapy including upfront radiotherapy [275,704,705,719,841,2058,2154,2317]. Significant unfavourable prognostic factors include advanced-stage disease (stage III or IV), unfavourable International Prognostic Index (IPI), invasion of bone or skin, high circulating EBV DNA levels, presence of EBV-positive cells in bone marrow, and a high Ki-67 proliferation index (>40–65%) [189,705,719,1724,1860,2018,2251,2859,3210]. Extranasal NK/T-cell lymphoma is highly aggressive, with short survival and poor response to therapy [189,640,647,1858,3210]. However, rare cases of primary cutaneous NK/T-cell lymphoma may pursue a protracted clinical course [655,740,1791].

Extranodal NK/T-cell lymphoma, nasal type

Fig. 14.54 Extramedullary lymphoma, nasal type. The neoplastic cells show strong staining for cCD3-epsilon (A) and CD56 (B). C The neoplastic cells show strong granular staining for granzyme B. D In situ hybridization for EBV-encoded small RNA (EBER). In this nasal tumour, virtually all the neoplastic cells show nuclear labelling.
Intestinal T-cell lymphoma

Introduction

Recent data have led to changes in the categorization of intestinal T-cell lymphomas. It has become apparent that the two subtypes formerly designated as variants of enteropathy-associated T-cell lymphoma (EATL) are distinct [178,3850]. Type I EATL, now simply designated as EATL, is closely linked to coeliac disease, and is primarily a disease of individuals of northern European origin. Type II EATL, now formally designated as monomorphic epitheliotropic intestinal T-cell lymphoma (MEITL), shows no association with coeliac disease, and appears relatively increased in incidence in Asian and Hispanic populations. EATL generally has a polymorphic cellular composition and wide range in cytology, whereas MEITL is monomorphic, is usually positive for CD8 and CD56, and expresses megakaryocyte-associated tyrosine kinase (MATK). Gains in chromosome 8q24 involving MYC are seen in a high proportion of cases of MEITL but not EATL. Many cases of MEITL are derived from gamma delta T cells, but exceptions exist; some cases are T-cell receptor-silent and some cases express the alpha beta T-cell receptor. Likewise, most cases of EATL express the alpha beta T-cell receptor, but gamma delta variants exist. Mutations in STAT5B and SETD2 have been associated with gamma delta MEITL, but investigations of classic EATL or alpha beta cases are limited [2160,2869A]. Both forms of intestinal T-cell lymphoma are clinically aggressive and almost always occur in adults. They are negative for EBV, which is strongly associated with nasal-type extranodal NK/T-cell lymphoma; this disease can present with intestinal disease [3210]. There remains a small group of intestinal T-cell lymphomas that do not meet the criteria for EATL or MEITL as currently defined [178]. These should be designated as intestinal T-cell lymphoma, NOS.

Table 14.04 Comparison of enteropathy-associated T-cell lymphoma (EATL) and monomorphic epitheliotropic intestinal T-cell lymphoma (MEITL)

<table>
<thead>
<tr>
<th>Feature</th>
<th>EATL</th>
<th>MEITL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethnicity (excess incidence)</td>
<td>Northern European</td>
<td>Asian, Hispanic</td>
</tr>
<tr>
<td>Risk factors</td>
<td>Coeliac disease, HLA-DQ2/DQ6</td>
<td>None recognized</td>
</tr>
<tr>
<td>Morphology</td>
<td>Polymorphic</td>
<td>Monomorphic</td>
</tr>
<tr>
<td>Usual immunophenotype</td>
<td>CD3+, CD5-, CD4-, CD8-, CD56-, CD103+, CD30+/-, cytotoxic +</td>
<td>CD3+, CD5-, CD4-, CD8+, CD56+, CD103+/-, CD30-, cytotoxic +</td>
</tr>
<tr>
<td>T-cell receptor expression</td>
<td>Alpha beta &gt; gamma delta</td>
<td>Gamma delta &gt; alpha beta</td>
</tr>
<tr>
<td>Localization</td>
<td>Small intestine</td>
<td>Small intestine</td>
</tr>
</tbody>
</table>

Enteropathy-associated T-cell lymphoma

Bhagat G.                      Chan J.K.C.
Jaffe E.S.                      Tan S.Y.
Chott A.                        Stein H.
Ott G.                         Isaacson P.G.

Definition

Enteropathy-associated T-cell lymphoma (EATL), previously designated type I EATL, is a neoplasm of intraepithelial T cells that occurs in individuals with coeliac disease and exhibits varying degrees of cellular pleomorphism. It commonly presents as a tumour composed of medium-sized to large lymphocytes, often accompanied by a component of chronic inflammatory cells. The adjacent small intestinal mucosa shows villous atrophy, crypt hyperplasia, and increased intraepithelial lymphocytes. Lymphomas composed of monomorphic medium-sized cells (formerly called type II EATL) are now considered to constitute a distinct entity (monomorphic epitheliotropic intestinal T-cell lymphoma).

ICD-O code 9717/3

Synonyms

Enteropathy-type intestinal T-cell lymphoma; classic enteropathy-associated T-cell lymphoma; malignant histiocytosis of the intestine (obsolete)

Epidemiology

EATL is the most common subtype of primary intestinal T-cell lymphoma in western countries, accounting for almost two thirds of all cases [932]. It is uncommon in many Asian countries due to the low population frequency of coeliac HLA risk alleles. EATL characteristically occurs in the sixth and seventh decades of life and shows a slight male predominance, with a male-to-female ratio of 1.04–2.8:1 [932,1276,2456,3675,4177]. It is seen with greater frequency in areas with a high prevalence of coeliac disease, such as Europe (0.05–0.14 cases per 100 000 population) [583,3675,
The incidence of EATL in the coeliac population is 0.22–1.9 cases per 100,000 population [171,814,823,1295].

**Etiology**

EATL is a recognized complication of coeliac disease or gluten-sensitive enteropathy [1437,2913,3857]. The association of EATL with coeliac disease is borne out by the detection of anti-endomysial (or anti-tissue transglutaminase 2) antibodies, the presence of HLA-DQ2 or HLA-DQ8 alleles, clinical findings such as dermatitis herpetiformis, and demonstration of gluten sensitivity in EATL patients, and a protective effect of gluten-free diet on EATL development [171,814,1438,1672,1708,2913,3676]. Coeliac disease may be diagnosed prior to (20–73%) or concomitant with (10–58%) EATL, and occasionally only at autopsy, because some individuals might have lifelong so-called 'silent' gluten sensitivity [876,1276,2456,2899,3675]. Risk factors for EATL include homozygosity for the HLA-DQ2 allele [49] and advanced age [2456].

**Localization**

The small intestine is involved in >90% of EATLs, most commonly the jejunum and ileum [932,1276,2456,3675], and multifocal lesions are observed in 32–54% of cases [1276,2456]. Other common gastrointestinal sites include the large intestine and stomach [932]. EATL might occasionally present at extragastrointestinal sites (e.g. the skin, lymph nodes, spleen, or CNS) [932,1389,2456,4080], usually in cases evolving from type 2 refractory coeliac disease [2454,2456,4186].

**Clinical features**

EATL most commonly presents with abdominal pain (65–100%) and gluten-insensitive malabsorption or diarrhoea (40–70%) in individuals without prior symptoms, or recurrence of symptoms in those with a history of adult-onset (or occasionally childhood-onset) coeliac disease and prior response to a gluten-free diet [599,876,932,1081,1276,2456,2899,3675]. Other presentations include weight loss (50–80%), anorexia, fatigue or early satiety and nausea or vomiting due to intestinal obstruction, and not infrequently intestinal perforation (25–50%) or haemorrhage [599,876,932,1081,1276,2456,2899,3675]. B symptoms, besides weight loss, are present in less than one third of patients [876,1276,2899]. The duration of symptoms prior to diagnosis varies widely [1276], but is <3 months in most cases [3675]. Elevated lactate dehydrogenase levels are observed in 25–62%, low serum albumin in 76–88%, and low haemoglobin in 54–91% of patients [932,1276,2456,3675]. In a proportion, there is a prodromal period of refractory coeliac disease, which is sometimes accompanied by intestinal ulceration (ulcerative jejunitis) [169,223]. A haemophagocytic syndrome occurs in 16–40% of patients [90,2456].

**Imaging**

Endoscopy is used to visualize sites of EATL involvement within the gastrointestinal tract. Double balloon enteroscopy and wireless capsule endoscopy are useful when ulceration, strictures, or large masses are present. CT is the standard imaging modality for staging EATL. FDG-PET and MRI enteroclysis are useful in screening for the development of EATL in patients with refractory coeliac disease and for assessment of treatment response [1507,1660,4144].

**Spread**

Common sites of disease dissemination include the intra-abdominal lymph nodes (affected in 35% of cases), bone marrow (in 3–18%), lungs or mediastinal lymph nodes (in 5–16%), liver (in 2–8%), and skin (in 5%) [932,2456,3675]. The CNS may be involved in occasional cases [352].
Fig. 14.58 Enteropathy-associated T-cell lymphoma. A Tumour cells are characterized by moderate amounts of eosinophilic cytoplasm and round or angulated nuclei with prominent nucleoli. B Anaplastic variant. C There is a heavy infiltrate of eosinophils between the tumour cells.

Staging
The Ann Arbor staging system, with or without the modification proposed by Rohatiner et al. [3394], is frequently used for staging EATL. High stage disease is detected in 43-90% of patients at diagnosis [932,1276,2456,3675].

Macroscopy
The tumour may form ulcerating nodules, plaques, strictures, or (less commonly) a large exophytic mass. The uninvolved mucosa can be thin and can show loss of mucosal folds. The mesentery and mesenteric lymph nodes are commonly involved. Occasionally, lymph node infiltration by EATL occurs in the absence of macroscopic evidence of intestinal disease.

Microscopy
The neoplastic lymphocytes exhibit a wide range of cytological appearances [2456,4370]. Most lymphomas show pleomorphic medium-sized to large cells with round or angulated vesicular nuclei, prominent nucleoli, and moderate to abundant pale-staining cytoplasm. As many as 40% of cases exhibit predominant large cell or anaplastic cytomyxology [2456]. Angiocentricity and angioinvasion, as well as extensive areas of necrosis, are frequently observed. Most tumours have an admixture of inflammatory cells, including large numbers of histiocytes and eosinophils, which may obscure the relatively small number of neoplastic cells in some cases. Intraepithelial spread of tumour cells may be striking, but sometimes only single scattered atypical lymphocytes are observed in the epithelium. The intestinal mucosa

Fig. 14.59 Adjacent uninvolved mucosa in enteropathy-associated T-cell lymphoma. Increased numbers of intraepithelial lymphocytes (A) are CD3-positive (B), CD8-negative (C), and CD56-negative (D).
adjacent to EATL, especially in the jejunum, usually shows features of coeliac disease, i.e. villous atrophy, crypt hyperplasia, intraepithelial lymphocytosis, and increased lymphocytes and plasma cells within the lamina propria [746,747]. However, these alterations are highly variable. Sometimes only an increase in intraepithelial lymphocytes is noted, and occasionally the jejunum appears near-normal [223]. The mesenteric lymph nodes can show intrasinusoidal and/or paracortical infiltration by EATL. However, some cases display various degrees of necrosis in the absence of morphologically recognizable lymphoma. Abdominal (and extra-abdominal) lymph nodes may also show a spectrum of degenerative changes, including dissolution of the node and replacement with lymph fluid (so-called lymph node cavitation) [1706,2573].

**Immunophenotype**

The neoplastic lymphocytes are usually CD3+, CD5−, CD7+, CD4+, CD8−, and CD103+, and they express cytotoxic granule-associated proteins (e.g. TIA1, granzyme B, and perforin). However, variability in the immunophenotype is observed. CD8 may be expressed by 19−30% of cases, overall [746,2456,2794], with a higher frequency (50%) reported in patients without a history of refractory coeliac disease, type II [2456]. In a minority of cases, the lymphoma cells express cytoplasmic T-cell receptor (TCR) beta [746,2794] or TCR gamma [178,644] chains, or rarely both. The frequency of CD30 expression varies in the different cytomorphological variants, but almost all EATLs manifesting large cell morphology are CD30+ [2456].

The immunophenotype of the intraepithelial lymphocytes in uninvolved areas may be normal in de novo EATLs, but in most cases preceded by type 2 refractory coeliac disease, the intraepithelial lymphocytes exhibit an aberrant phenotype similar to that of the EATL.

**Postulated normal counterpart**

Small intestinal intraepithelial T cells have been postulated to be the normal counterparts of EATL cells, on the basis of shared immunophenotypic features [2456,3748]. Most EATL cells correspond to conventional intraepithelial T cells (type A), expressing the CD8 alpha beta heterodimer.

**Grading**

**Precursor lesions**

EATL may be preceded by refractory coeliac disease, also referred to as refractory sprue, which is defined as persistent gastrointestinal symptoms and abnormal small intestinal mucosal architecture with increased intraepithelial lymphocytes despite a strict gluten-free diet for ≥6−12 months [3446]. The diagnosis of refractory coeliac disease requires exclusion of coeliac disease–related conditions (e.g. bacterial overgrowth, microscopic colitis, and lymphoma) and other small intestinal disorders (e.g. common variable immunodeficiency and autoimmune enteropathy) [2914]. Refractory coeliac disease can be primary (lack of response to gluten-free diet at diagnosis) or secondary (symptom onset after a variable duration of a gluten-free diet).

The range of reported prevalence rates of refractory coeliac disease is wide (1.5−10%) [140,2256,2914,3416,3445]. However, a recent epidemiological survey suggests a much lower prevalence in the community (0.3% in coeliacs) [1755]. Similar to in EATL, the duration and dose of gluten exposure are considered risk factors, based on the high frequency of HLA-DQ2 homozygosity [49,2454] and older patient age (majority >50 years) [1755,2454,3445].

Refractory coeliac disease is considered to be a biologically heterogeneous entity [50,2454,3416,3445], and it is currently categorized into two types based on immunophenotypic and molecular criteria [874].

**Type 1 refractory coeliac disease**

The small intestinal intraepithelial lymphocytes have a normal phenotype, i.e. they express CD8 and surface CD3 and TCR. Polyclonal products are detected on TR gene rearrangement analysis. Small intestinal histology is similar to that observed in uncomplicated coeliac disease. Type 1 refractory coeliac disease accounts for 68−80% of all refractory coeliac disease cases [50,875,1755,2454,3416,3445]. Surreptitious gluten ingestion is thought to sustain intestinal inflammation in many cases [1669], and transition to an autoimmune state is suspected in a minority [2458]. No genetic or molecular alterations have been identified in this disease subtype. The symptoms are milder than those of type 2 refractory coeliac disease [2454]. The 5-year survival rate is high (80−96%), and the risk for developing EATL is low (3−14% in 4−6 years) [50,1755,2454,3445].

**Type 2 refractory coeliac disease**

The small intestinal intraepithelial lymphocyte immunophenotype is aberrant, i.e. CD8, surface CD3, and TCR expression is absent. However, CD8 expression may be detected on a subset of intraepithelial lymphocytes by flow cytometry in as many as one third of cases [601]. The intraepithelial lymphocytes usually do not express CD5, and variable downregulation or loss of other T-cell antigens can be seen. CD30 expression is considered to indicate transformation to EATL [1162]. Clonal products are detected on TR gene rearrangement analysis. The degree of
villous atrophy is usually severe (subtotal or total). The intraepithelial lymphocytes lack significant cytological atypia, but they can be widely distributed throughout the gastrointestinal tract (599,2454,4186). Small aggregates of lymphocytes are seen in the lamina propria in approximately half of the cases (4178). Some cases exhibit ulcerated mucosa associated with variable degrees of chronic inflammation and a relative paucity of intraepithelial lymphocytes (ulcerative jejunitis) (169,223,562,599,4371). The identification of TCR gene rearrangements of similar size in biopsies exhibiting features of type 2 refractory coeliac disease and concurrent or subsequent EATL helped establish that the aberrant intraepithelial lymphocytes are precursors of EATL in a proportion of cases and that they constitute a neoplastic population (low-grade lymphoma of intraepithelial T lymphocytes, EATL in situ, or cryptic EATL) (169,223,562,599,4371).

In most cases, the aberrant intraepithelial lymphocytes are considered to be of alpha beta TCR lineage. However, recent studies have suggested that some cases might derive from gamma delta TCR T cells or possibly immature T/NK-cell precursors (innate immune cells), and that the maturational state of the cell of origin could impact the risk of extraintestinal dissemination and transformation to EATL (3571,3864). Disruption of intestinal immune homeostasis by deregulated expression of IL15 contributes to disease pathogenesis (7,1747,2635,2639). IL15 also increases survival of the aberrant intraepithelial lymphocytes (2457), which can facilitate genomic instability and acquisition of genetic abnormalities. Recurrent gains of chromosome 1q22-44 are detected in type 2 refractory coeliac disease in common with EATL (297,937,2454,4167,4471). This finding suggests early acquisition of chromosome 1q abnormalities in the evolution of EATL. Loss of p16 protein, in the absence of LOH at chromosome 9p21, is observed in 40% of type 2 refractory coeliac disease cases exhibiting features of ulcerative jejunitis, and aberrant nuclear p53 expression can be detected in 57% of cases in the absence of molecular lesions of TP53 (2923).

Most patients have severe symptoms and they usually have profound malnutrition (body mass index <18) due to protein-losing enteropathy (1755,2454,3445). Large ulcers or stenotic areas are frequently observed on endoscopy (2454). As systemic dissemination of aberrant intraepithelial lymphocytes occurs in a high proportion of cases (44–60%), patients may present with extraintestinal symptoms or disorders (e.g. skin lesions) (2454,4178,4186). The 5-year survival rate is low (44–58%), with 30–52% of patients developing EATL over the course of 4–6 years (50,1755,2454,3445). The current chemotherapy and bone marrow transplantation regimens used for EATL are ineffective in eradicating the aberrant clonal intraepithelial lymphocytes (445,2455,3446).

Genetic profile
TRB or TRG genes are clonally rearranged in virtually all cases (169,2456,2794). Unlike primary nodal peripheral T-cell lymphoma, most EATLs (~80%) either display gains of the 9q34 region, which harbours known proto-oncoproteins (e.g. NOTCH1, ABL1, and VAV2) or, alternatively, show deletions of 16q12.1 (297,596,937,4471). Similar changes are also observed in monomorphic epimisiotropic intestinal T-cell lymphoma. However, EATLs frequently display chromosomal gains of chromosomes 1q and 5q, which are less common in monomorphic epimisiotropic intestinal T-cell lymphoma (937,4471).

Losses at 9p are detected in 18% of EATLs; however, LOH at 9p21, targeting the cell-cycle inhibitor CDKN2A/B is observed in 56% of cases, accompanied by loss of p16 protein expression (2923,4471). Loss of the 17p12-13.2 region, containing the TP53 tumour suppressor gene, is noted in 23% of EATLs, but aberrant nuclear p53 expression can be seen in 75% of cases (937,2923). Recent studies have reported recurrent mutations in constituents of the JAK-STAT signalling pathway in EATL (2869A). Additionally, the detection of JAK1 and STAT3 mutations in type 2 refractory coeliac disease implicates deregulation of JAK-STAT signalling to be an early event in disease pathogenesis (1116A).

Genetic susceptibility
Coeliac disease, or gluten-sensitive enteropathy, predisposes to EATL. Coeliac disease is a polygenic disorder with various risk loci associated, including the HLA locus. EATL is associated with the HLA-DQA1*0501 and HLA-DQB1*0201 genotypes (49). More than 90% of EATL patients carry HLA-DQ2.5 heterodimers encoded by HLA-DQA1*05 and HLA-DQB1*02 alleles, either in cis or trans configuration (1708).
Prognosis and predictive factors
The prognosis of EATL patients is poor, due to the usually multifocal nature of the disease and a high rate of intestinal recurrence (743). The median survival is 7 months, and 1-year and 5-year overall survival rates are 31–39% and 0–59%, respectively (50,876,1081,1276,2456,2899). Better outcomes have been reported for patients receiving intensive chemotherapy and autologous stem cell transplantation (with 5-year overall and progression-free survival rates of 60% and 52%, respectively) (3675). However, an Eastern Cooperative Oncology Group (ECOG) score > 1 is noted in 88% of patients, and many have a poor performance status, making them poor candidates for chemotherapy (1276,3675). Moreover, response to most current chemotherapy regimens is suboptimal.

Prognostic factors are not well established for EATL. Disease stage and the international Prognostic Index (IPI) are not useful in predicting survival. Chemotherapy and surgical resection are associated with prolonged survival, and low serum albumin with an adverse outcome (2456). Malnutrition, which is common in EATL patients with prior type 2 refractory coeliac disease, is considered responsible for their markedly lower 5-year survival rate (0–8%) (50,2456). The Prognostic Index for T-cell Lymphoma (PIT) has been shown to be useful in predicting survival of EATL patients (932). Recently, an EATL prognostic index (EPI) has been developed that can distinguish three risk groups and reportedly performs better than the IPI and PIT (888).

Monomorphic epitheliotropic intestinal T-cell lymphoma


Definition
Monomorphic epitheliotropic intestinal T-cell lymphoma (MEITL) is a primary intestinal T-cell lymphoma derived from intraepithelial lymphocytes. Unlike in the classic form of enteropathy-associated T-cell lymphoma (EATL), there is no clear association with coeliac disease (3850). The neoplastic cells have medium-sized round nuclei with a rim of pale cytoplasm. There is usually florid infiltration of intestinal epithelium. An inflammatory background is absent, and necrosis is usually less evident than in classic EATL. Based on distinctive pathological and epidemiological features, and to facilitate distinction from EATL, this disease is no longer referred to as type II EATL.

ICD-O code 9717/3

Synonym Type II enteropathy-associated T-cell lymphoma

Epidemiology
MEITL has a worldwide distribution. There is no clear association with coeliac disease. It accounts for the vast majority of cases of primary intestinal T-cell lymphoma occurring in Asia, and also appears to occur with increased frequency in individuals of Hispanic/indigenous origin (644,1299,3832). Males are affected more often than females; the male-to-female ratio is approximately 2:1.

Localization
The disease most often presents in the small intestine, with the jejunum affected more often than the ileum. Tumour masses, with or without ulceration, are common. Diffuse spread within the intestinal mucosa is often seen. Involvement of mesenteric lymph nodes is common. There can also be involvement of the stomach (occurring in 5% of cases) or the large bowel (in 16%) (4070). With dissemination, multiple extranodal sites may be affected.

Clinical features
The tumour presents with symptoms referable to the intestinal lesions, such as abdominal pain, obstruction or perforation, weight loss, diarrhoea, and gastrointestinal bleeding (644,4070). A history of malabsorption is generally absent.

Spread
Diffuse spread of tumour cells in the adjacent mucosa is common. There is risk of dissemination to many extranodal sites, as well as regional lymph nodes.

Microscopy
The neoplastic lymphocytes are generally medium in size (747). The nuclei are round and regular in appearance, with finely dispersed chromatin and inconspicuous nucleoli. There is a generous rim of pale cytoplasm. Within a given tumour the nuclear appearance is...
generally uniform, although the disease shows variation in cell size between patients. The tumour cells show prominent epitheliotropism. The villous architecture is distorted, and broadly expanded villi may be present. Unlike in classic EATL (formerly type I EATL), an inflammatory background is absent. Areas of necrosis are uncommon.

Immunophenotype
MEITL has a distinctive immunophenotype, being positive for CD3, CD8, and CD56 in the vast majority of cases [746]. Most tumours lack CD5, a feature suggesting a gamma delta T-cell derivation. Expression of T-cell receptor (TCR) gamma is often positive, although some cases express TCR beta [178,3889]. In a minority of cases, the tumour cells are TCR-silent, or rarely both TCR gamma and TCR beta are expressed [644]. One study reported a high incidence of CD8 alpha homodimers (CD8 alpha alpha) [3889]. The cytotoxic granule–associated protein TIA1 is usually positive, but expression of other cytotoxic molecules (including granzyme B and perforin) is less consistent [4021]. Approximately 20% of cases show aberrant expression of CD20 [3889]. Most cases express MATK, a marker helpful in the distinction from EATL [3890] if present in >80% of tumour cells.

Cell of origin
MEITL arises from an intraepithelial T cell, which can be of either gamma delta or alpha beta derivation. These intestinal intraepithelial cells have a distinct ontogeny and function [1593,3255].

Grading
All cases are clinically aggressive, irrespective of cell size.

Genetic profile
Clonal rearrangement of the TR genes is seen in >90% of cases. Extra signals for MYC at 8q24 are commonly seen [937]. Gains at 9q34.3 may be identified by FISH and copy-number analysis, and are the most common genetic change, seen in >75% of cases [937,2056,4021]. Other aberrations include gains at 1q32.3, 4p15.1, 5q34, 7q34, 8p11.23, 9q22.31, 9q33.2, 8q24 (MYC locus), and 12p13.31 and losses of 7p14.1 and 16q12.1 [2807,4021]. Compared with classic EATL, gains at 1q32.2-41 and 5q34-35.5 are seen less often [2807]. Activating mutations in STAT5B have been identified in a high proportion of cases [2160]; in one study, 63% of cases had mutations in STAT5B when examined by whole-exome sequencing [2807]. Moreover, mutations in STAT5B were seen in cases of both gamma delta and alpha beta derivation. JAK3 and GNAI2 are also mutated in some cases. These findings implicate activation of the JAK/STAT and G protein-coupled receptor signalling pathways [2807]. The most commonly mutated gene is SETD2 in one study mutated in >90% of cases [3368A]. EBV is negative; if positive, it suggests a diagnosis of extranodal NK/T-cell lymphoma. As in other forms of T-cell lymphoma, EBV may sometimes be identified in background reactive B cells.

Genetic susceptibility
Unlike in EATL, there is no association with the HLA-DQA1*0501 and HLA-DQB1*0201 genotypes.

Prognosis and predictive factors
The clinical outcome of patients with MEITL is poor, with a median survival of 7 months. The five year overall and complete response rates are poor: 46% and 48%, respectively. In one study, good performance status was associated with better overall survival (P = 0.03), and response to initial treatment led to better overall survival and progression-free survival (P < 0.001) [4070].

Intestinal T-cell lymphoma, NOS

Definition
This category is used for T-cell lymphomas arising in the intestines, or sometimes other sites in the gastrointestinal tract, that do not conform to either classic enteropathy-associated T-cell lymphoma or monomorphic epitheliotropic intestinal T-cell lymphoma. Sometimes this diagnosis is made based on an inadequate biopsy in which the mucosal surface cannot be evaluated or immunophenotypic data are incomplete. It is not considered a specific disease entity. At a recent
workshop of the European Association for Haematopathology, most cases assigned to this category involved the colon (178). The cases were heterogeneous in their morphology and immunophenotype; 4 of the 5 cases with evaluable data were T-cell receptor–silent, but most had a cytotoxic phenotype. Several of the cases had widespread disease, so the intestines may not have been the primary site. All cases were clinically aggressive.

ICD-O code 9717/3

Indolent T-cell lymphoproliferative disorder of the gastrointestinal tract

Jaffe E.S. Bhagat G.
Chott A. Tan S.Y.
Ott G. Stein H.
Chan J.K.C. Isaacson P.G.

Definition
Indolent T-cell lymphoproliferative disorder of the gastrointestinal tract is a clonal T-cell lymphoproliferative disorder that can involve the mucosa in all sites of the gastrointestinal tract, but is most common in the small intestine and colon. The lymphoid cells infiltrate the lamina propria but usually do not show invasion of the epithelium. The clinical course is indolent, but most patients do not respond to conventional chemotherapy. A subset of cases progress to a higher-grade T-cell lymphoma with spread beyond the gastrointestinal tract.

ICD-O code 9702/1

Epidemiology
The disease presents in adulthood, more frequently in men than in women. Rare cases have been reported in children. No ethnic or genetic factors have been identified for increased risk. However, some patients have a history of Crohn disease (3145).

Localization
Most patients present with disease affecting the small bowel or colon (3145, 3305). However, all sites in the gastrointestinal tract can be involved, including the oral cavity and oesophagus (1082). The bone marrow and peripheral blood are usually not involved.

Clinical features
Presenting symptoms include abdominal pain, diarrhoea, vomiting, dyspepsia, and weight loss (561,3145). The clinical course is chronic, but progression to disseminated disease has been reported infrequently. Peripheral lymphadenopathy is not present, but a subset of patients exhibit mesenteric lymphadenopathy (2506).

Spread
Multiple sites in the gastrointestinal tract are involved, with a chronic relapsing clinical course. A subset of patients are at risk for disease progression and more widespread disease, usually after many years (561,2506).

Macroscopy
The mucosa of affected sites in the gastrointestinal tract is thickened, with prominent folds or nodularity. In some cases, the infiltrate produces intestinal polyps resembling lymphomatous polyposis (1640,1794). The mucosal surface can be hyperaemic, with superficial erosions.

Microscopy
The lamina propria is expanded by a dense, non-destructive lymphoid infiltrate (3145). Infiltration of the muscularis mucosae and submucosa may be seen focally. The mucosal glands are displaced by the infiltrate but not destroyed. The infiltrate is monotonous, composed of small, round, mature-appearing lymphocytes. Admixed inflammatory cells are rare, but epithelioid granulomas may be focally present (563,2506). Some of the histological changes may resemble those of Crohn disease; whether some of these patients truly have preceding inflammatory bowel disease remains uncertain.

Immunophenotype
The cells have a mature T-cell phenotype, positive for CD3. Most reported cases have been positive for CD8 (3145), but CD4 has been expressed in a significant number (561,2506,3305). The CD8+ cases express TIA1, but granzyme B is generally negative. Other

Fig. 14.68 Indolent T-cell lymphoproliferative disorder of the gastrointestinal tract, duodenum. Infiltrate fills the lamina propria and focally extends beyond the muscularis mucosae. However, glands are largely intact.

Fig. 14.69 Indolent T-cell lymphoproliferative disorder of the gastrointestinal tract. Small polypoid lesions are hyperaemic. Reprinted from Perry AM et al. (3145).

Fig. 14.70 Indolent T-cell lymphoproliferative disorder of the gastrointestinal tract. The mucosa displays multiple small polyps (arrows). From Perry AM et al. (3145).
mature T-cell markers are also positive, including CD2 and CD5, with variable expression of CD7. All reported cases have expressed the alpha beta T-cell receptor, with no cases positive for T-cell receptor gamma. CD56 is negative but expression of CD103 has been reported in some cases. The proliferation rate is extremely low, with a Ki-67 proliferation index <10% in all cases studied. Rare cases have phenotypic aberrancy, such as double-negativity for CD4 and CD8; this phenotype is associated with a more aggressive clinical course, and may constitute a different entity.

Cell of origin
The postulated cell of origin is a mature peripheral T cell

Genetic profile
All reported cases have had clonal rearrangements of TR genes, either TRG or TRB (2506,3145). Recurrent mutations or translocations have not been identified. In limited studies, activating mutations of STAT3 were absent. In situ hybridization for EBV-encoded small RNA (EBER) was negative in all cases studied.

Prognosis and predictive factors
Most patients have a chronic relapsing clinical course. Response to conventional chemotherapy is poor, but patients have prolonged survival with persistent disease. The presence of an aberrant T-cell phenotype in a small subset may indicate potential for more-aggressive clinical behaviour. Additionally, cases expressing CD4 rather than CD8 appear to be at higher risk for progression, although data are limited (561,2506).

Fig. 14.71 Indolent T-cell lymphoproliferative disorder of the gastrointestinal tract, ileum. The lamina propria is diffusely infiltrated by small lymphoid cells.

Fig. 14.72 Indolent T-cell lymphoproliferative disorder of the gastrointestinal tract, ileum. Lymphocytes are positive for CD3.

Fig. 14.73 Indolent T-cell lymphoproliferative disorder of the gastrointestinal tract. The Ki-67 proliferation index is extremely low.

Fig. 14.74 Indolent T-cell lymphoproliferative disorder of the gastrointestinal tract, CD8, duodenal biopsy. Glands are largely intact, but some epitheliotropism is seen.
Hepatosplenic T-cell lymphoma

Definition
Hepatosplenic T-cell lymphoma (HSTL) is an aggressive subtype of extranodal lymphoma characterized by a hepatosplenic presentation without lymphadenopathy and a poor outcome. The neoplasm results from a proliferation of cytotoxic T cells, usually of gamma delta T-cell receptor type. It is usually composed of medium-sized lymphoid cells, demonstrating marked sinusoidal infiltration of spleen, liver, and bone marrow.

ICD-O code 9716/3

Epidemiology
HSTL is a rare form of lymphoma, reported in both western and Asian countries. It accounts for 1–2% of all peripheral T-cell lymphomas [904,4217]. Peak incidence occurs in adolescents and young adults (median patient age at diagnosis: ~35 years), with a male predominance [178,319,797].

Etiology
As many as 20% of HSTLs arise in the setting of chronic immune suppression, most commonly during long-term immunosuppressive therapy for solid organ transplantation or prolonged antigenic stimulation [319,4168,4375]. In this setting, HSTL is regarded as a late-onset post-transplant lymphoproliferative disorder of host origin. A number of HSTL cases have been reported in patients (especially children) treated with azathioprine and infliximab for Crohn disease [922,3414]. Rare cases have been reported in patients with psoriasis or rheumatoid arthritis receiving tumour necrosis factor inhibitors and immunomodulators [3825].

Localization
Patients present with marked splenomegaly and usually hepatomegaly, but without lymphadenopathy. The bone marrow is almost always involved [178,319,797,4166].

Clinical features
HSTL typically presents with hepatosplenomegaly and systemic symptoms. Patients usually manifest marked thrombocytopenia, often with anaemia and leukopenia. Peripheral blood involvement is uncommon at presentation but may

Fig. 14.75 Hepatosplenic T-cell lymphoma. A Cords and sinusoids of the spleen are infiltrated by a monotonous population of neoplastic lymphoid cells with medium-sized nuclei and a moderate rim of pale cytoplasm. B The neoplastic cells diffusely infiltrate the hepatic sinusoids. C The bone marrow is usually hypercellular, with neoplastic cells infiltrating sinusoids. D Neoplastic cells in the bone marrow are highlighted with immunohistochemistry for CD3.

Gauiard P.
Jaffe E.S.
Krenacs L.
Macon W.R.
occur late in the clinical course [178,319,4166]. Given the almost constant bone marrow involvement, virtually all patients have Ann Arbor stage IV disease. HSTL is easily distinguishable from other gamma delta T-cell lymphomas that involve extranodal localizations (i.e. the skin and subcutaneous tissue, intestines, or nasal region) [150,1299], but may be more difficult to differentiate clinically from some cases of T-cell large granular lymphocytic leukaemia with a gamma delta phenotype [178].

Macroscopy
The spleen is enlarged, with diffuse involvement of the red pulp but no gross lesions. Diffuse hepatic enlargement is also present.

Microscopy
Histopathologically, the cells of HSTL are monotonous, with medium-sized nuclei and a rim of pale cytoplasm. The nuclear chromatin is loosely condensed, with small inconspicuous nucleoli. Some pleomorphism may occasionally be seen. The neoplastic cells involve the cords and sinuses of the splenic red pulp, with atrophy of the white pulp. The liver shows a predominant sinusoidal infiltration. Neoplastic cells are almost constantly present in the bone marrow, with a predominantly intrasinusoidal distribution. This may be difficult to identify without the aid of immunohistochemistry or flow cytometry. Cytological atypia, with large cell or blastic changes may be seen, especially with disease progression [178,319,4166].

Immunophenotype
The neoplastic cells are CD3+ and usually gamma delta T-cell receptor+; alpha beta T-cell receptor-, CD56+/-, CD4-, CD8-/+, and CD5-. Most gamma delta cases express the V delta 1 chain [319,3243]. A minority of cases appear to be of alpha beta type, which is considered a variant of the more common gamma delta form of the disease [2431,4168]. The determination of the alpha beta or gamma delta phenotype is now possible in formalin-fixed, paraffin-embedded tissues [1299,3850]. The cells express the cytotoxic granule-associated proteins TIA1 and granzyme M, but are usually negative for granzyme B and perforin (797,2109,3850). They often show aberrant coincident expression of multiple killer-cell immunoglobulin-like receptor isoforms along with dim or absent CD94 (2742). Therefore, the cells appear to be mature, non-activated cytotoxic T cells with phenotypic aberrancy. This contrasts with T-cell large granular lymphocytic leukaemia, in which the cells have a more mature lymphocytic appearance; have a CD8+, often CD57+ phenotype with granzyme B expression; and disclose a subtle, diffuse interstitial infiltrate in the marrow, with minimal (not prominent) infiltration of sinuses [178,4168].

Postulated normal counterpart
Peripheral gamma delta (or less commonly alpha beta) cytotoxic T cells of the innate immune system with a memory phenotype, recirculating between spleen, bone marrow, and liver [1816,2109].

Genetic profile
Cases of gamma delta origin have rearranged TRG genes and show a biallelic rearrangement of TRD genes. TRB genes have been reported in some gamma delta cases. Isochromosome 7q is present in most cases, and with disease progression, a variety of FISH patterns equivalent to 2-5 copies of i(7)(q10) or numerical and structural aberrations of the second chromosome 7 have been detected [4340]. Ring chromosomes leading to 7q amplification have also been reported. The common gained region, which has been mapped at 7q22, is associated with increased expression of several genes, including the gene encoding the multidrug resistance glycoprotein ABCB1 [1214]. Trisomy 8 may also be present. In situ hybridization for EBV is generally negative. Recent gene expression profiling studies have shown that HSTL discloses a distinct molecular signature unifying alpha beta and gamma delta cases [4048]. Missense mutations in STAT5B and more rarely STAT3 have been found in about 40% of HSTLs [2160,2869], consistent with the significant enrichment in genes of the JAK/STAT pathway in the gene expression profile [4048]. Chromatin-modifying genes, including SETD2, INO80, and ARID1B, are commonly mutated in HSTL, affecting 62% of cases [2600A].

Prognosis and predictive factors
The course is aggressive. Patients may respond initially to chemotherapy, but relapses are seen in the vast majority of cases. The median survival is <2 years [319,1135]. Platinum-cytarabine [319] and pentostatin have been shown to be active agents [807]. Early use of high-dose therapy followed by haematopoietic stem cell transplantation (especially allogeneic transplantation) may improve survival [4220].
Subcutaneous panniculitis-like T-cell lymphoma

Definition
Subcutaneous panniculitis-like T-cell lymphoma (SPTCL) is a cytotoxic T-cell lymphoma that preferentially infiltrates subcutaneous tissue. It is composed of atypical lymphoid cells of varying size, typically with prominent apoptotic activity of tumour cells and associated fat necrosis. Cases expressing the gamma delta T-cell receptor are excluded, and are instead classified as primary cutaneous gamma delta T-cell lymphoma. SPTCL has a very wide patient age distribution, with cases seen in both children and adults. The prognosis is generally good, especially compared with other forms of peripheral T-cell lymphoma.

ICD-O code 9708/3

Epidemiology
SPTCL is a rare form of lymphoma, accounting for <1% of all non-Hodgkin lymphomas. It is slightly more common in females than in males and has a broad patient age range [2133]. Approximately 20% of patients are aged <20 years (median: 35 years) [4321], and the disease can also present in infancy [1737]. As many as 20% of patients may have associated autoimmune disease, most commonly systemic lupus erythematosus [4321]. The differential diagnosis with lupus panniculitis may be challenging.

Etiology
Autoimmune disease may play a role in some cases. The lesions may show features overlapping with those of lupus panniculitis, and a diagnosis of systemic lupus erythematosus has been documented in some cases [2445,2550]. In some patients, the early lesions may closely mimic lobular panniculitis. Whether a benign lobular panniculitis precedes the development of SPTCL in patients without systemic lupus erythematosus is unclear. EBV is absent [2133].

Localization
Patients present with multiple subcutaneous nodules or plaques, usually in the absence of other extracutaneous sites of disease. The most common sites of localization are the extremities and trunk. The nodules range in size from 0.5 cm to several centimetres in diameter. Larger nodules may become necrotic, but ulceration is rare [2133,4321]. It is rare for patients to present with only a single skin lesion.

Clinical features
Clinical symptoms are primarily related to the subcutaneous nodules. Systemic symptoms may be seen in as many as 50% of patients. Laboratory abnormalities, including cytopenias and elevated liver function tests are common, and a frank haemophagocytic syndrome is seen in 15–20% of cases [1401]. In such cases, hepatosplenomegaly may be present. Lymph node involvement is usually absent. Bone marrow involvement has been reported rarely, with involvement of fat cells within the marrow space [2133].
### Table 14.05: Differential diagnosis of neoplasms expressing T-cell and NK-cell markers with frequent cutaneous involvement

<table>
<thead>
<tr>
<th>Disease</th>
<th>Clinical features</th>
<th>CD3</th>
<th>CD4</th>
<th>CD8</th>
<th>Cytotoxic molecules*</th>
<th>CD56</th>
<th>EBV</th>
<th>TR genes</th>
<th>Lineage</th>
</tr>
</thead>
<tbody>
<tr>
<td>SPTCL</td>
<td>Tumours (extremities and trunk)</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>R</td>
<td>T-cell</td>
</tr>
<tr>
<td>PCGD-TCL</td>
<td>Tumours, plaques, ulcerated nodules</td>
<td>+</td>
<td>-</td>
<td>-/+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>R</td>
<td>T-cell</td>
</tr>
<tr>
<td>Extracutaneous NK/TCL</td>
<td>Nodules, tumours</td>
<td>+</td>
<td>-</td>
<td>-/+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>G</td>
<td>NK-cell, sometimes T-cell</td>
</tr>
<tr>
<td>Primary C-ALCL</td>
<td>Superficial nodules with epidermal involvement</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>R</td>
<td>T-cell</td>
</tr>
<tr>
<td>Mycosis fungoides</td>
<td>Patched plaques, tumours late in course</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>R</td>
<td>T-cell</td>
</tr>
<tr>
<td>Blastic PDC neoplasm</td>
<td>Nodules, tumours</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>G</td>
<td>Precursor of PDC</td>
</tr>
</tbody>
</table>

C-ALCL, cutaneous anaplastic large cell lymphoma; G, in germline configuration; PCGD-TCL, primary cutaneous gamma delta T-cell lymphoma; PDC, plasmacytoid dendritic cell; R, rearranged; SPTCL, subcutaneous panniculitis-like T-cell lymphoma; TCL, T-cell lymphoma; TR, T-cell receptor.

* Cytotoxic granule-associated protein(s) TIA1, granzyme B, and/or perforin.

** Marked variation in T-cell antigens, including frequent antigen loss of CD3, CD4, CD8

---

**Microscopy**

The infiltrate involves the fat lobules, usually with sparing of septa. The overlying dermis and epidermis are typically uninvolved. The neoplastic cells range in size, but in any given case, cell size is relatively consistent. The neoplastic cells have irregular and hyperchromatic nuclei. The lymphoid cells have a rim of pale-staining cytoplasm. A helpful diagnostic feature is the rimming of the neoplastic cells surrounding individual fat cells. Admixed reactive histiocytes are frequently present, in particular in areas of fat infiltration and destruction. The histiocytes are frequently vacuolated, due to ingested lipid material. Other inflammatory cells are typically absent, notably plasma cells and plasmacytoid dendritic cells, which are both common in lupus panniculitis (2320,3189). Vascular invasion may be seen in some cases, and necrosis and karyorrhexis are common (2551). Karyorrhexis is helpful in the differential diagnosis from other lymphomas involving skin and subcutaneous tissue (2133). Cutaneous gamma delta T-cell lymphomas may show panniculitis-like features, but commonly involve the dermis and epidermis, and may show epidermal ulceration.

**Immunophenotype**

The cells have a mature alpha beta T-cell phenotype, usually CD8-positive, with expression of cytotoxic molecules including granzyme B, perforin, and TIA1 (Table 14.05) [2133,3495]. The cells express beta F1 and are negative for CD56.
facilitating the distinction from primary cutaneous gamma delta T-cell lymphoma (150,4030). CD123 is generally negative, whereas this marker frequently identifies increased plasmacytoid dendritic cells in lupus panniculitis [426].

Postulated normal counterpart
A mature cytotoxic alpha beta T cell

Genetic profile
The neoplastic cells show rearrangement of TR genes and are negative for EBV sequences. No specific cytogenetic features or mutation patterns have been reported.

Prognosis and predictive factors
Dissemination to lymph nodes and other organs is rare (1401,2071,3495). The median 5-year overall survival rate is 80%; however, if a haemophagocytic syndrome is present, the prognosis is poor (2551,2998,4321). Combination chemotherapy has traditionally been used, but some studies suggest that more conservative immunosuppressive regimens (cyclosporine A, prednisone) may be effective (1485,1869,2805,4074). The distinction from cutaneous gamma delta T-cell lymphomas is important, because SPTCL has a much better prognosis (4031,4321).

Mycosis fungoides

Definition
Mycosis fungoides is an epidermotropic, primary cutaneous T-cell lymphoma characterized by infiltrates of small to medium-sized T lymphocytes with cerebriform nuclei. The term mycosis fungoides should be used only for classic cases, characterized by the evolution of patches, plaques, and tumours, or for variants with a similar clinical course.

ICD-O code 9700/3

Epidemiology
Mycosis fungoides is the most common type of cutaneous T-cell lymphoma and accounts for almost 50% of all primary cutaneous lymphomas (4320). Most patients are adults/elderly, but the disease can also be observed in children and adolescents (395,1218). The male-to-female ratio is 2:1 (4320). Worldwide, the incidence is about 10 cases per one million population, with marked regional variation (2085) and a higher incidence in Black populations (1636). An elevated risk of mycosis fungoides has been associated with several professions, including crop and vegetable farming, painting, woodworking, and carpentry. These findings suggest possible environmental cofactors in the pathogenesis of the disease (167). Studies have shown increased risk for individuals in the petrochemical, textile, and metal industries (776). Certain lifestyle factors have also been associated with increased risk: obesity and a smoking history of ≥40 years (167). It has been speculated that mycosis fungoides might be associated with certain retroviruses, but no such association has been identified through high-throughput sequencing (956).

Localization
The disease is generally limited to the skin, with variable distribution, for a protracted period. Extracutaneous dissemination may occur in advanced stages, mainly to the lymph nodes, liver, spleen, lungs, and blood (892). Involvement of the bone marrow is rare (4320).

Clinical features
Mycosis fungoides has an indolent clinical course, with slow progression over years or sometimes decades from patches to more-infiltrated plaques and eventually tumours. In early stages, the
Lesions are often confined to sun-protected areas. Patients with tumour-stage mycosis fungoides characteristically show a combination of patches, plaques, and tumours, which often show ulceration. Uncommonly, patients present with or develop an erythrodermic stage of disease that lacks the haematological criteria for Sézary syndrome. Besides the clinicopathological variants discussed in detail later in this chapter, several other clinical (and histopathological) variants of the disease have been described, such as hypopigmented and hyperpigmented variants.

**Staging**

*See Table 14.06.*

**Microscopy**

The histology of the skin lesions varies with the stage of the disease. Early patch lesions show superficial band-like or lichenoid infiltrates, mainly consisting of lymphocytes and histiocytes. Atypical cells with small to medium-sized, highly indented (cerebriform) nuclei are few and mostly confined to the epidermis, where they characteristically colonize the epidermal basal layer, often as haloed cells, either singly or in a linear distribution. In typical plaques, epidermotropism is more pronounced. Intraepidermal collections of atypical cells (Pautrier microabscesses) are a highly characteristic feature, but are observed in only a minority of cases. With progression to tumour stage, the dermal infiltrates become more diffuse, and epidermotropism may be lost. The tumour cells increase in number and size, showing variable proportions of small, medium-sized, and large cerebriform cells with pleomorphic or blastoid nuclei. Histological transformation, defined by the presence of >25% large lymphoid cells in the dermal infiltrates, may occur, mainly in the tumour stage. These large cells may be CD30-negative or CD30-positive. Enlarged lymph nodes from patients with mycosis fungoides frequently show dermatopathic lymphadenopathy with paracortical expansion due to the large number of histiocytes and interdigitating cells with abundant, pale cytoplasm. The International Society for Cutaneous Lymphomas (ISCL) / European Organisation for Research and Treatment of Cancer (EORTC) staging system for clinically abnormal lymph nodes (> 1.5 cm) in mycosis fungoides and Sézary syndrome recognizes three categories: N1, reflecting no involvement; N2, early involvement (with no architectural effacement); and N3, overt involvement (with partial or complete architectural effacement).
Table 14.06 Staging of mycosis fungoides and Sézary syndrome according to the International Society for Cutaneous Lymphomas (ISCL) and the European Organisation for Research and Treatment of Cancer (EORTC). From: Olsen et al. Blood 110:1713–22 (2007) [2972]

<table>
<thead>
<tr>
<th>Stage</th>
<th>Skin</th>
<th>Lymph nodes</th>
<th>Viscera</th>
<th>Blood</th>
</tr>
</thead>
<tbody>
<tr>
<td>I A</td>
<td>T1, N0, M0, B0-1</td>
<td>I1 A: T1, N0, M0, B0-1</td>
<td>IVA1: T1-4, N0-2, M0, B2</td>
<td>B0</td>
</tr>
<tr>
<td>I I</td>
<td>T1-2, N1-2, M0, B0-1</td>
<td>I1 B: T2, N0-2, M0, B0-1</td>
<td>IVA2: T1-4, N3, M0, B0-2</td>
<td>B1</td>
</tr>
<tr>
<td>I I I</td>
<td>T4, N0-2, M0, B0-1</td>
<td>IIIA: T4, N0-2, M0, B0</td>
<td>IIV A: T4, N0-2, M0, B1</td>
<td>B2</td>
</tr>
<tr>
<td>I I V A</td>
<td>T1-4, N0-2, M0, B2</td>
<td>IIV B: T1-4, N0-3, M1, B0-2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Skin</th>
<th>T1</th>
<th>Limited patches, papules, and plaques covering &lt;10% of the skin surface. May further stratify into T1a (patch only) vs T1b (plaque ± patch)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T2</td>
<td>Patches, papules, or plaques covering &gt;10% of the skin surface. May further stratify into T2a (patch only) vs T2b (plaque ± patch)</td>
<td></td>
</tr>
<tr>
<td>T3</td>
<td>≥ 1 tumour (&gt; 1 cm in diameter)</td>
<td></td>
</tr>
<tr>
<td>T4</td>
<td>Confluence of erythema covering ≥80% of the body surface area</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Lymph nodes</th>
<th>NO</th>
<th>Clinically abnormal peripheral lymph nodes; histopathology Dutch grade 1 or NCI LN0-2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N1</td>
<td>N1a: Clone-negative, N1b: Clone-positive</td>
</tr>
<tr>
<td></td>
<td>N2</td>
<td>N2a: Clone-negative, N2b: Clone-positive</td>
</tr>
<tr>
<td></td>
<td>N3</td>
<td>Clinically abnormal peripheral lymph nodes; histopathology Dutch grade 3-4 or NCI LN4; clone-positive or clone-negative</td>
</tr>
<tr>
<td></td>
<td>Nx</td>
<td>Clinically abnormal peripheral lymph nodes; no histological confirmation</td>
</tr>
</tbody>
</table>

- For skin, the term *patch+ means a skin lesion of any size without significant elevation or induration; the presence or absence of hypo- or hyperpigmentation, scale, crusting, and/or poikiloderma should be noted.
- For skin, the term *plaque+ means a skin lesion of any size that is elevated or indurated; the presence or absence of scale, crusting, and/or poikiloderma should be noted.
- For skin, the term *tumour+ means a solid or nodular lesion >1 cm in diameter with evidence of depth and/or vertical growth; note the total number of lesions, total volume of lesions, largest size of lesion, and region of body involved; also note whether there is histological evidence of large-cell transformation; phenotyping for CD30 is recommended.
- For nodes, the term ‘abnormal peripheral lymph node+ means any palpable peripheral node that on physical examination is firm, irregular, clustered, fixed, or ≥1.5 cm in diameter; node groups examined on physical examination include cervical, supraclavicular, epitrochlear, axillary, and inguinal; central nodes, which are not generally amenable to pathological assessment, are not considered in the nodal classification unless used to establish N3 histopathologically.
- A T-cell clone is defined by PCR or Southern blot analysis of the TR gene.
- Spleen and liver involvement can be diagnosed on the basis of imaging criteria.
- Sézary cells are defined as lymphocytes with hyperconvoluted cerebriform nuclei; if Sézary cells cannot be used to determine tumour burden for B2, then one of the following modified ISCL criteria along with a positive clonal rearrangement of the TR gene can be used instead:
  1. expanded CD4+ T cells with a CD4:CD8 ratio of ≥10
  2. expanded CD4+ T cells with abnormal immunophenotype including loss of CD7 or CD26.
Recognition of the early infiltrates can be difficult and can be facilitated by PCR for clonal TR rearrangement analysis [2972]. N3 lymph nodes may simulate peripheral T-cell lymphoma, NOS, or Hodgkin lymphoma.

Variants

Folliculotropic mycosis fungoides

Folliculotropic mycosis fungoides is characterized by infiltrates of atypical (cerebriform) CD4+ T lymphocytes involving hair follicles, often with sparing of the epidermis [608]. Many cases show mucinous degeneration of the hair follicles (follicular mucinosis), but mucin deposition can be absent [1222,4131]. The lesions preferentially involve the head and neck area and often present with grouped follicular papules and plaques associated with alopecia [4131]. Due to the deep localization of the neoplastic infiltrate, folliculotropic mycosis fungoides is less accessible to skin-targeted therapies. The 5-year disease-specific survival rate is approximately 70–80%, which is significantly worse than that seen in classic plaque-stage mycosis fungoides [4131].

Pagetoid reticulosis

Pagetoid reticulosis is characterized by patches or plaques with an intraepidermal proliferation of neoplastic T cells [1516]. The term should only be used for the localized type (Woringer–Kolopp type) and not for the disseminated type (Ketron–Goodman type), because most cases corresponding to the latter category would instead be classified as aggressive epidermotropic CD8+ cytotoxic T-cell lymphoma or cutaneous gamma delta T-cell lymphoma [4320]. The atypical cells have medium-sized or large cerebriform nuclei and either a CD4-/CD8+ phenotype or (less commonly) a CD4+/CD8+ phenotype. CD30 is often expressed. Unlike with classic mycosis fungoides, neither extracutaneous dissemination nor disease-related deaths have ever been reported [4320].

Granulomatous slack skin

Granulomatous slack skin is an extremely rare subtype of cutaneous T-cell lymphoma characterized clinically by the development of bulky, pendulous skin folds in the flexural areas (axillae, groin), and histologically by a granulomatous infiltrate within the dermis and subcutaneous tissues, with clonal CD4+ T cells, abundant macrophages with many multinucleated giant cells, and loss of elastic fibres [1989,2241]. Most reported cases
have been associated with mycosis fungoides [2241], but in some cases the disease was concomitant with Hodgkin lymphoma. Granulomatous slack skin is characterized by an indolent clinical course [2241].

**Immunophenotype**
The typical phenotype is CD2+, CD3+, TCR beta+, CD5+, CD4+, CD8−, TCR gamma−. Cases with a cytotoxic phenotype (CD8+ and/or TCR gamma+) are well recognized [2547,3390]. Such cases have the same clinical behaviour and prognosis as CD4+ cases, and should not be considered a separate entity [2547]. A CD8+ phenotype has been reported more commonly in paediatric mycosis fungoides. Expression of CD56 has been observed in otherwise conventional mycosis fungoides. A lack of CD7 is frequent in all stages of the disease. Cutaneous lymphocyte antigen (CLA), associated with lymphocyte homing to the skin, is expressed in most cases. Other alterations in the expression of T-cell antigens may be seen, but mainly occur in the advanced (tumour) stages. Partial expression of CD30 by neoplastic cells may be found in all stages, in particular in plaques and tumours of the disease. Cytotoxic granule-associated proteins are uncommonly expressed in the early patch/plaque lesions, but may be positive in neoplastic cells in more-advanced lesions [4188].

**Cell of origin**
A mature skin-homing CD4+ T cell

**Genetic profile**
TR genes are found to be clonally rearranged in most cases when sensitive techniques (e.g. BIOMED-2) are used [3211]. Complex karyotypes are present in many patients, in particular in the advanced stages. Somatic copy-number variants constitute the vast majority of all driver mutations, including mutations in multiple components of the TCR and IL2 signalling pathways, in genes that drive T helper 2 (Th2) cell differentiation, in genes that facilitate escape from TGF-beta-mediated growth suppression, and in genes that confer resistance to tumour necrosis factor receptor superfamily (TNFRSF)-mediated apoptosis [736,2598,4039,4091,4134]. Constitutive activation of STAT3 and inactivation of CDKN2A (also called p16INK4a) and PTEN have been identified and may be associated with disease progression [3547].

**Prognosis and predictive factors**
The single most important prognostic factor in mycosis fungoides is the extent of cutaneous and extracutaneous disease, as reflected by the clinical stage. Patients with limited disease generally have an excellent prognosis, with a similar survival as the general population [27,958,3256,4132]. Large Pautrier microabscesses and dermal atypical lymphocytes in early lesions have been associated with progression to advanced stage [4212]. In advanced stages, the prognosis is poor, in particular in patients with skin tumours and/or extracutaneous dissemination [337,4132]. Failure to achieve complete remission after the first treatment, patient age >60 years, and elevated lactate dehydrogenase are adverse prognostic parameters [27,973], as is histological transformation with increase in blast cells (>25%) [155,609].
Sezary syndrome

Definition
Sezary syndrome (SS) is defined by the triad of erythroderma, generalized lymphadenopathy, and the presence of clonally related neoplastic T cells with cerebriform nuclei (Sezary cells) in skin, lymph nodes, and peripheral blood. In addition, one or more of the following criteria are required: an absolute Sézary cell count ≥ 1000/μL, an expanded CD4+ T-cell population resulting in a CD4:CD8 ratio of ≥ 10, and loss of one or more T-cell antigens. SS and mycosis fungoides are closely related neoplasms, but are considered separate entities on the basis of differences in clinical behaviour and cell of origin (4211).

ICD-O code 9701/3

Synonym
Sézary disease

Epidemiology
This is a rare disease, accounting for < 5% of all cutaneous T-cell lymphomas (4320). It occurs in adults, characteristically presents in patients aged > 60 years, and has a male predominance.

Localization
As a leukaemia, SS is by definition a generalized disease. Any visceral organ can be involved in advanced stages, but the most common sites are the oropharynx, lungs, and CNS. Bone marrow involvement is variable.

Clinical features
Patients present with erythroderma and generalized lymphadenopathy. Other features are pruritus, alopecia, ectropion, palmar or plantar hyperkeratosis, and onychodystrophy. An increased prevalence of secondary cutaneous and systemic malignancies has been reported in SS and attributed to the immunoparesis associated with skewing of the normal T-cell repertoire and loss of normal circulating CD4+ cells (1720).

<table>
<thead>
<tr>
<th>Table 14.07 Histopathological staging for clinically abnormal lymph nodes (&gt;1.5 cm) in mycosis fungoides and Sézary syndrome</th>
</tr>
</thead>
<tbody>
<tr>
<td>ISCL/EORTC (2972)</td>
</tr>
<tr>
<td>N1</td>
</tr>
<tr>
<td>N2</td>
</tr>
<tr>
<td>N3</td>
</tr>
<tr>
<td>N2a</td>
</tr>
<tr>
<td>N2b</td>
</tr>
</tbody>
</table>

N2 is divided into two categories: N2a (without clonally rearranged T cells) and N2b (with clonally rearranged T cells).

Staging
Staging of mycosis fungoides and SS is performed according to the International Society for Cutaneous Lymphomas / European Organisation for Research and Treatment of Cancer (EORTC) system (Table 14.06, p. 387) (2972). SS patients are erythrodermic (T4) and have peripheral blood involvement, which requires demonstration of clonal TR gene rearrangement (preferably the same clone in skin and peripheral blood) in combination with (1) a total Sézary cell count ≥ 1000/μL, (2) an expanded CD4+ T-cell population with a CD4:CD8 ratio of ≥ 10, or (3) an expanded CD4+ T-cell population with abnormal immunophenotype including loss of CD7 or CD26 (2972). For determination of the Sézary cell count, Sézary cells are defined as lymphocytes with hyperconvoluted cerebriform nuclei. SS cases are stage IVA1, IVA2, or IVB according to the ISCL/EORTC system.

Microscopy
The histological features in SS may be similar to those in mycosis fungoides. However, the cellular infiltrates in SS are more often monotonous, and epidermoporesis may sometimes be absent. In as many as one third of biopsies from patients with otherwise classic SS, the histological picture may be non-specific (4062). Involved lymph nodes characteristically show a dense, monotonous infiltrate of Sézary cells with effacement

Fig. 14.98 Sézary syndrome. Generalized skin disease with erythroderma.
of the normal lymph node architecture [3553]. Bone marrow may be involved, but the infiltrates are often sparse and mainly interstitial [3669].

**Immunophenotype**
The neoplastic T cells have a CD3+, CD4+, CD8− phenotype; characteristically lack CD7 and CD26, and express PD1 (also known as CD279) in almost all cases [625]. Sézary cells express cutaneous lymphocyte antigen (CLA) and the skin-homing receptor CCR4 [1213], as well as CCR7 [2825]. Flow cytometry analysis of peripheral blood lymphocytes shows a CD4+/CD7− (>30%) or CD4+/CD26− (>40%) T-cell population [356, 3718].

**Postulated normal counterpart**
The normal counterparts of Sézary cells are circulating central memory T cells (CD27+, CD45RA−, CD45RO+); this is in contrast to the tumour cells of mycosis fungoides, which derive from skin-resident memory T cells [539].

**Genetic profile**
TR genes are clonally rearranged [3211, 4308, 4309]. A characteristic gene expression signature consists of overexpression of PLS3, DNM3, TWIST1, and EPHA4 and underexpression of STAT4 [1519, 1941, 4130]. Recurrent balanced chromosomal translocations have not been detected in SS, but complex numerical and structural alterations are common and similar to those in mycosis fungoides [294, 552, 2491], including losses of 1p, 6q, and 10q and gains of 8q, with isochromosome 17q as a recurrent feature of SS [4190]. High-throughput sequencing techniques have revealed a markedly heterogeneous pattern of novel gene mutations and focal copy-number variants affecting genes encoding members of the JAK/STAT pathway may also explain the constitutive activation of STAT3 in tumour cells. Mutations affecting chromatin-modifying genes such as DNMT3A are also present in SS, as are frequent inactivating mutations of TP53 [736] and deletions of CDKN2A (also called p16INK4a) [2843, 3546]. Hypermethylation and inactivation of genes involved in the FAS-dependent apoptotic pathway is common [1873].

**Prognosis and predictive factors**
SS is an aggressive disease, with a median survival of 32 months and a 5-year overall survival rate of 10−30%, depending on stage [4320]. Most patients die of opportunistic infections. Lymph node involvement (stage IVA2) and visceral involvement (stage IVB) indicate a worse prognosis [343]. The degree of peripheral blood involvement at diagnosis may have an impact on prognosis [2041, 4213], but the prognostic relevance of bone marrow involvement is unknown.
Primary cutaneous CD30-positive T-cell lymphoproliferative disorders

Definition
Primary cutaneous CD30+ lymphoproliferative disorders are the second most common group of cutaneous T-cell lymphomas, accounting for approximately 30% of cases. This group includes lymphomatoid papulosis, primary cutaneous anaplastic large cell lymphoma (C-ALCL), and borderline cases. These diseases form a spectrum and may show overlapping histopathological, phenotypic, and genetic features. The clinical appearance and course are therefore critical for the definite diagnosis. The term 'borderline' refers to cases in which, despite careful clinicopathological correlation, a definite distinction between lymphomatoid papulosis and C-ALCL cannot be made. However, clinical examination during follow-up will generally disclose whether such patients have lymphomatoid papulosis or C-ALCL. Clinico-pathological correlation is not only required to differentiate between lymphomatoid papulosis and C-ALCL, but is also essential for differentiating these primary cutaneous CD30+ lymphoproliferative disorders from a wide variety of infectious and inflammatory skin diseases and other types of cutaneous T-cell lymphomas (in particular, mycosis fungoides) that contain significant numbers of CD30+ cells.

Lymphomatoid papulosis

Definition
Lymphomatoid papulosis (LyP) is a chronic, recurrent, self-healing skin disease that combines a usually benign clinical course with histological features suggestive of a cutaneous T-cell lymphoma.

ICD-O code 9718/1

Synonym
Primary cutaneous CD30+ T-cell lymphoproliferative disorder

Epidemiology
LyP most often occurs in adults (median patient age: 45 years), but children may also be affected. The male-to-female ratio is 2–3:1. LyP is a skin-limited disease that most frequently affects the trunk and extremities. In rare cases, concurrent oral mucosal lesions can be present.

Clinical features
LyP is characterized by papular, papulonecrotic, and/or nodular skin lesions at various stages of development. The number of lesions may vary from a few to more than a hundred. Individual skin...
lesions disappear within 3–12 weeks and may leave behind superficial scars. The duration of the disease may vary from several months to >40 years. In as many as 20% of patients, LyP may be preceded by, associated with, or followed by another type of malignant lymphoma, generally mycosis fungoides, cutaneous anaplastic large cell lymphoma, or Hodgkin lymphoma [314,910].

Microscopy
The histological picture of LyP is extremely variable, and in part correlates with the age of the biopsied skin lesion. Several histological subtypes of LyP have been described.

Type A LyP is the most common type (>80%) and is characterized by scattered or small clusters of large atypical (sometimes multinucleated or Reed-Sternberg-like) CD30+ cells intermingled with numerous inflammatory cells, including small lymphocytes, neutrophils, and/or eosinophils [314].

LyP, Type B is uncommon (<5%) and is characterized by a predominantly epidermotropic infiltrate of small atypical CD30+ cells with cerebriform nuclei, histologically simulating early-stage mycosis fungoides [314].

LyP, Type C lesions (~10%) demonstrate a monotonous population or cohesive sheets of large CD30+ T cells with relatively few admixed inflammatory cells, very similar to cutaneous anaplastic large cell lymphoma [314].

LyP, Type D lesions (<5%) are characterized by a strongly epidermotropic, sometimes pagetoid infiltrate of atypical small to medium-sized CD8+, CD30+ pleomorphic T cells, mimicking primary cutaneous aggressive epidermotropic CD8+ cytotoxic T-cell lymphoma [3473].

Primary cutaneous CD30-positive T-cell lymphoproliferative disorders
Some of these cases may have a gamma delta T-cell phenotype [3390].

Type E LyP (<5%) is characterized by angiocentric and angiodestructive infiltrates of small to medium-sized CD8+, CD30+ pleomorphic T cells [1988]. Vascular occlusion, haemorrhages, extensive necrosis, and ulceration may be present. Clinically, patients present with a few papulonodular lesions that rapidly ulcerate and evolve into large necrotic eschar-like lesions. LyP with 6p25.3 rearrangement (<5%) is characterized by chromosomal rearrangements involving the DUSP22-IRF4 locus on 6p25.3 [1938]. Patients are older adults and often present with localized skin lesions. Histologically, skin lesions show extensive epidermotropism by weakly CD30+ small to medium-sized T cells with cerebriform nuclei and strongly CD30+ medium-sized to large blast cells in the dermis, simulating transformed mycosis fungoides. Other rare histological variants have also been described, including folliculotropic, syringotropic, and granulomatous LyP [1986, 1987]. Different subtypes of LyP may occur in separate but concurrent lesions, and a single LyP lesion may show histological features of multiple subtypes. Recognition of these different subtypes of LyP is important, to avoid misdiagnosis of other, often more aggressive, types of cutaneous T-cell lymphoma (see Table 14.08). However, the subtypes have no therapeutic or prognostic implications.

Immunophenotype

The large atypical cells in the lesions of type A and type C LyP have the same phenotype as the tumour cells in cutaneous anaplastic large cell lymphoma.
Primary cutaneous anaplastic large cell lymphoma

Definition
Cutaneous anaplastic large cell lymphoma (C-ALCL) is composed of large cells with an anaplastic, pleomorphic, or immunoblastic cytomorphology, the majority (>75%) of which express the CD30 antigen (4320). Patients with C-ALCL should not have clinical evidence or history of mycosis fungoides; in this setting a diagnosis of mycosis fungoides with large cell transformation, which may be CD30-negative, is more likely (337). The disease must also be distinguished from systemic anaplastic large cell lymphoma with cutaneous involvement, which is a separate disease with different cytogenetics, clinical features, and outcome (4389).

ICD-O code 9718/3

Synonyms
Primary cutaneous CD30+ large T-cell lymphoma (obsolete); primary cutaneous CD30+ T-cell lymphoproliferative disorder; regressing atypical histiocytosis (obsolete)

Epidemiology
C-ALCL is the second most common type of cutaneous T-cell lymphoma (4320). The median patient age is 60 years. Children are sporadically affected (314). The male-to-female ratio is 2–3:1 (314).

Localization
This is a skin-limited disease that most frequently affects the trunk, face, and extremities (338,4361).

Clinical features
Most patients present with solitary or localized nodules or tumours, and sometimes papules, and often show ulceration (314,2367). Multifocal lesions are seen in about 20% of patients. The skin lesions may show partial or complete spontaneous regression, as is seen in lymphomatoid papulosis. These lymphomas frequently relapse in the skin. Extracutaneous dissemination occurs in about 10% of patients, and mainly involves the regional lymph nodes (314,4320).

Microscopy
Histology shows diffuse infiltrates with cohesive sheets of large CD30+ tumour cells. Epidermotropism may be present, and is particularly marked in cases carrying a DUSP22-IRF4 rearrangement (2975). In most cases, the tumour cells have the characteristic morphology of anaplastic cells, with round, oval, or irregularly shaped nuclei; prominent eosinophilic nucleoli; and abundant cytoplasm (4320). Less commonly (20–25%), they have a non-anaplastic (pleomorphic or immunoblastic) appearance (314,2548, 3111). Reactive lymphocytes are often present at the periphery of the lesions. Ulcerating lesions may show a lymphomatoid papulosis-like histology, with an abundant inflammatory infiltrate of reactive T cells, histiocytes, eosinophils, neutrophils, and relatively few CD30+ cells. In such cases, epidermal hyperplasia may be prominent. The inflammatory background is especially prominent in the rare neutrophil-rich (pyogenic) variant (500). Rare cases of intralymphatic C-ALCL have been reported (3500).

Table 14.08 Lymphomatoid papulosis: histological subtypes and differential diagnosis

<table>
<thead>
<tr>
<th>Subtype</th>
<th>Relative frequency</th>
<th>Predominant phenotype</th>
<th>Main differential diagnoses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type A</td>
<td>&gt;80%</td>
<td>CD4+, CD8-</td>
<td>C-ALCL Tumour-stage MF</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Hodgkin lymphoma</td>
</tr>
<tr>
<td>Type B</td>
<td>&lt;5%</td>
<td>CD4+, CD8-</td>
<td>Plaque-stage MF</td>
</tr>
<tr>
<td>Type C</td>
<td>~10%</td>
<td>CD4+, CD8-</td>
<td>C-ALCL Transformed MF</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(CD30+)</td>
</tr>
<tr>
<td>Type D</td>
<td>&lt;5%</td>
<td>CD4-, CD8+</td>
<td>CD8+ aggressive epidermotropic TCL</td>
</tr>
<tr>
<td>Type E</td>
<td>&lt;5%</td>
<td>CD4-, CD8+</td>
<td>Extranodal NK/TCL</td>
</tr>
<tr>
<td>With DUSP22-IRF4 rearrangement</td>
<td>&lt;5%</td>
<td>CD4-, CD8+ or CD4-,CD8-</td>
<td>Tumour-stage MF</td>
</tr>
</tbody>
</table>

C-ALCL, cutaneous anaplastic large cell lymphoma; MF, mycosis fungoides; TCL, T-cell lymphoma.

The atypical cells are predominantly CD4-positive in LyP types A–C, CD8-positive in types D and E, and either CD8-positive or double negative for CD4 and CD8 in DUSP22-IRF4–translocated cases. The expression of CD56 has also been sporadically reported (316,1223).

Postulated normal counterpart
An activated skin-homing T cell

Genetic profile

Antigen receptor genes
Clonally rearranged TRG genes have been detected in most cases of LyP (748,887,3789). Identical rearrangements have been demonstrated in LyP lesions and associated lymphomas (748,887).

Cytogenetic abnormalities
No consistent abnormalities have been described. The t(2;5)(p23;q35) translocation or its variants, leading to activation of the ALK kinase, are not detected in LyP (920). Rearrangements of the DUSP22-IRF4 locus on chromosome 6p25.3 are found in a small subset of LyP cases, which show distinctive clinicopathological features (1938,4223).

Prognosis and predictive factors
LyP has an excellent prognosis. In two large studies, together including 242 patients, only 2% of LyP patients died of associated lymphomas (314,910). However, because the risk factors for the development of a systemic lymphoma are unknown, long-term follow-up is recommended.
Immunophenotype

The neoplastic cells show an activated CD4+ T-cell phenotype with variable loss of CD2, CD5, and/or CD3 and frequent expression of cytotoxic proteins (e.g., granzyme B, TIA1, and perforin) (432, 2136, 4320). Some cases may have a CD4−/CD8+ or CD4+/CD8+ T-cell phenotype or a null-cell phenotype (2545). CD30 is by definition expressed by the majority (>75%) of the neoplastic cells (4320). Unlike systemic anaplastic large cell lymphomas, most C-ALCLs express the cutaneous lymphocyte antigen (CLA), but do not express EMA or ALK (891, 920, 3940). CD15 is expressed in approximately 40% of cases, whereas staining for IRF4/MUM1 is positive in almost all cases (336, 4262). Unlike Hodgkin lymphomas, C-ALCLs do not express PAX5 and are negative for EBV. Coexpression of CD56 is observed in rare cases, but does not appear to be associated with an unfavourable prognosis (2639).

Postulated normal counterpart

A transformed/activated skin-homing T cell

Genetic profile

Antigen receptor genes

Most cases show clonal rearrangement of the TR genes (2425). However, T-cell receptor proteins are often not expressed (413).

Cytogenetic abnormalities and oncogenes

Unlike systemic anaplastic large cell lymphomas, the vast majority of C-ALCLs do not carry translocations involving the ALK gene at chromosome 2 (920). However, unusual cases of ALK-positive C-ALCL, including cases showing strong nuclear and cytoplasmic staining characteristic of the t(2;5) chromosomal translocation and cases expressing cytoplasmic ALK protein (indicative of a variant ALK translocation), have been reported (178, 1900, 2996, 3267). Many of these cases had an excellent prognosis. Rearrangements of the DUSP22-IRF4 locus on chromosome 6p25.3 are found in approximately 25% of C-ALCLs and in a small subset of lymphomatoid papulosis (3166, 4223). Frequent chromosomal aberrations affecting almost half of the patients are gains of 7q31 and losses on 6q16-21 and 13q34 (2185, 4135). Unlike in tumour-stage mycosis fungoides and peripheral T-cell lymphoma, NOS, loss of 9p21.3 harbouring the CDKN2A tumour suppressor gene is rarely observed in C-ALCL (2870). A recurrent NPM1-TYK2 gene fusion resulting in constitutive STAT signalling has been described in both C-ALCL and lymphomatoid papulosis (4171). TYK2 breaks were found in 15% of primary cutaneous CD30+ lymphoproliferative disorder cases. TP63 rearrangements have been observed in rare C-ALCL cases with an unusually aggressive clinical behaviour (4161). Gene expression profiling showed high expression of the skin-homing chemokine receptor genes CCR10 and CCR8 in C-ALCLs, which may explain their affinity for the skin and their low tendency to disseminate to extracutaneous sites (4135).

Prognosis and predictive factors

The prognosis is usually favourable, with a 10-year disease-specific survival rate of approximately 90% (314, 2367). Patients presenting with multifocal skin lesions and patients with involvement of regional lymph nodes have a prognosis similar to that of patients with only skin lesions (314). No differences in clinical presentation, clinical behaviour, or prognosis have been found between cases with an anaplastic morphology and cases with a non-anaplastic (pleomorphic or immunoblastic) morphology (338, 2367, 4361).
Primary cutaneous peripheral T-cell lymphomas, rare subtypes

Introduction

Peripheral T-cell lymphomas commonly involve the skin, either as primary or secondary manifestations of disease. Within this group, three rare provisional entities were delineated in the WHO-EORTC classification for cutaneous lymphomas: primary cutaneous gamma delta T-cell lymphoma, primary cutaneous aggressive epidermotropic CD8+ cytotoxic T-cell lymphoma, and primary cutaneous CD4+ small/medium T-cell lymphoma. The last two entities are still considered provisional, and altered terminology is proposed for the CD4- small/medium proliferations. A diagnosis of mycosis fungoides must be ruled out by complete clinical examination and an accurate clinical history. A new provisional entity has been added: primary cutaneous acral CD8- T-cell lymphoma.

Primary cutaneous gamma delta T-cell lymphoma

Gaulard P.
Berti E.
Willemze R.
Petrella T.
Jaffe E.S.

Definition

Primary cutaneous gamma delta T-cell lymphoma (PCGD-TCL) is a lymphoma, involving primarily the skin, composed of a clonal proliferation of mature, activated gamma delta T cells with a cytotoxic phenotype. This group includes cases previously called subcutaneous panniculitis-like T-cell lymphoma with a gamma delta phenotype. Gamma delta T-cell lymphomas presenting primarily in mucosal sites (in the past referred to as mucocutaneous gamma delta T-cell lymphoma) are likely unrelated conditions belonging to other site-dependent peripheral T-cell lymphoma entities [150,1299,3850]. The gamma delta T-cell receptor (TCR) may also be expressed by rare cases of otherwise classic mycosis fungoides and lymphomatoid papulosis, which have the same indolent clinical course as cases with an alpha beta T-cell phenotype [2533,2547,3390]. Such cases should be diagnosed as mycosis fungoides or lymphomatoid papulosis, irrespective of TCR expression.

ICD-O code

9726/3

Epidemiology

PCGD-TCLs are rare, accounting for approximately 1% of all cutaneous T-cell lymphomas [4031,4320,4321]. Most cases occur in adults. There is no sex predilection.

Etiology

The distribution of disease reflects the localization of normal gamma delta T cells, which are believed to play a role in host mucosal and epithelial immune responses. Impaired immune function associated with chronic antigen stimulation may predispose individuals to the development of PCGD-TCL [150,3850].

Localization

PCGD-TCLs often present with generalized skin lesions, preferentially affecting the extremities [4031,1493].

Fig. 14.115 Primary cutaneous gamma delta T-cell lymphoma. The epidermis is necrotic.

Fig. 14.116 Primary cutaneous gamma delta T-cell lymphoma. Lesions are clinically diverse, and consist of plaques, without ulceration (A) or with ulceration (B), or tumours (C). The lesion may consist of an indurated plaque with subcutaneous infiltration (D).
Primary cutaneous gamma delta T-cell lymphoma. In this case, the infiltrate is primarily dermal.

Fig. 14.117

Clinical features
The clinical presentation of patients with PCGD-TCL is variable. The disease may be predominantly epidermotropic and present with patches/plaques. Some patients may present with deep dermal or subcutaneous tumours, with or without epidermal necrosis and ulceration [359, 4031, 4321]. The lesions are most often present on the extremities, but other sites may also be affected [4031, 4321]. Dissemination to mucosal and other extranodal sites is frequently observed, but involvement of lymph nodes, spleen, or bone marrow is uncommon. A haemophagocytic syndrome is common, in particular in patients with panniculitis-like tumours [4031, 4321]. B symptoms, including fever, night sweats, and weight loss, occur in most patients.

Imaging
Abnormal PET and/or CT findings are common in sites of active disease [1493].

Microscopy
Three major histological patterns of involvement can be present in the skin: epidermotropic, dermal, and subcutaneous. Often more than one histological pattern is present in the same patient in different biopsy specimens or within a single biopsy specimen [359, 4031, 4321]. Epidermal infiltration may occur as mild epidermotropism to marked pagetoid reticulosis-like infiltrates [359]. Subcutaneous cases may show rimming of fat cells, similar to subcutaneous panniculitis-like T-cell lymphoma of alpha beta origin, but usually show dermal and/or epidermal involvement in addition [4031, 4321]. The neoplastic cells are generally mediumsized to large, with coarsely clumped chromatin [4031]. Large blastic cells with vesicular nuclei and prominent nucleoli are infrequent. Apoptosis and necrosis are common, often with angioinvasion [4031, 4321].

Immunophenotype
The tumour cells characteristically have a gamma delta TCR-positive, beta F1-negative, CD3+, CD2+, CD5-, CD7+/−, CD56+ phenotype with strong expression of cytotoxic proteins, including granzyme B, perforin, and TIA1 [1821, 3495, 4031, 4321]. Most cases lack both CD4 and CD8, although CD8 may be expressed in some cases [4031, 4321]. The gamma delta T-cell phenotype (TCR
Primary cutaneous CD8+ aggressive epidermotropic cytotoxic T-cell lymphoma

Berti E.
Gaulard P.
Willemze R.
Petrella T.
Jaffe E.S.

Definition
This provisional entity is a cutaneous T-cell lymphoma characterized by proliferation of epidermotropic CD8+ cytotoxic T cells and aggressive clinical behaviour. Differentiation from other types of cutaneous T-cell lymphomas with a CD8+ cytotoxic T-cell phenotype is based on the clinical presentation, clinical behaviour, and certain histological features, such as marked epidermotropism with epidermal necrosis.

ICD-O code
9709/3

Epidemiology
This disease is rare, accounting for < 1% of all cutaneous T-cell lymphomas [30, 361,4320]. It occurs mainly in adults. There are no known predisposing factors.

Localization
Most patients present with generalized skin lesions.

Clinical features
Clinically, these lymphomas are characterized by localized ulcerated nodules, tumours, or plaques, or (more commonly) by disseminated eruptive papules, nodules, and tumours showing central ulceration and necrosis [361,3377,3518]. These lymphomas may disseminate to other visceral sites (lungs, testes, CNS, oral mucosa), but lymph nodes are often spared [361,2535,3267,3377].

Microscopy
The histological appearance is very variable, ranging from a lichenoid pattern with marked, pagetoid epidermotropism and subepidermal oedema (disseminated variant) to deeper, more-nodular and less-epidermotropic infiltrates (localized variant). The epidermis may be acanthotic or atrophic, often with necrosis, ulceration, and blister formation [30, 361,3377]. Invasion and destruction of adnexal skin structures are commonly seen. Angiocentricity and angioinvasion may be present [2546,3377]. Tumour cells are small-medium or medium-large, with pleomorphic or blastic nuclei [361].
Immuneophenotype
The tumour cells characteristically have a beta F1-positive, CD3+, CD8+, CD4−, granzyme B-positive, perforin-positive, TIA1+, CD45RA+, CD45RO− phenotype. Most cases lack CD5 and CD2, with variable expression of CD7 [30,317,361,2546,3267,3377,3518]. Most cases are CD30-negative [3267,3377].

Primary cutaneous aggressive epidermotropic CD8+ cytotoxic T-cell lymphoma may be histopathologically and phenotypically indistinguishable from a variant of lymphomatoid papulosis (type D) characterized by self-healing papules and nodules (necrotic lesions) with spontaneous resolution [3473]. Clinical information is also important, to differentiate from other CD8+ cutaneous T-cell lymphomas, including CD8+ mycosis fungoides [3267].

Postulated normal counterpart
A skin-homing, CD8+, cytotoxic T cell of alpha beta type

Genetic profile
The neoplastic T cells show clonal TR gene rearrangements. Specific genetic abnormalities have not been described. EBV is negative [317,2546,3377].

Prognosis and predictive factors
These lymphomas have an aggressive clinical course, with a median survival of 12 months in a recent series [3377]. There is no difference in survival between cases with small or large cell morphology [317], or between cases with localized or diffuse lesions [3377].

Primary cutaneous acral CD8+ T-cell lymphoma

Petrella T.
Gaulard P.
Berti E.
Willemze R.
Jaffe E.S.

Definition
Primary cutaneous acral CD8+ T-cell lymphoma is a rare cutaneous tumour characterized by skin infiltration of clonal atypical medium-sized cytotoxic lymphocytes [3156]. The tumour is clinically characterized by preferential involvement of acral sites (in particular the ears) and by a good prognosis.

ICD-O code 9709/3

Synonym
Indolent cutaneous CD8+ lymphoid proliferation

Epidemiology
The disease affects adults; no paediatric cases have been reported. The median patient age is 53 years, and there is a male predominance, with a male-to-female ratio of 3.2:1 [324,1331,1515,2302,2311,3156,3828,3856,4345,4468].

Etiology
There are currently no clues as to the etiology of primary cutaneous acral CD8+ T-cell lymphoma. A local trigger agent may be suspected by analogy to Helicobacter pylori in gastric MALT lymphoma or Borrelia burgdorferi and Chlamydia psittaci in skin MALT lymphoma [1198], but to date no infectious or toxic candidate has been identified.

Clinical features
Cutaneous lesions are most often an isolated reddish papule or nodule measuring from several millimetres to 3-4 cm, with a history of slow growth over several weeks or months. The most frequent site is the ear (61%), generally the helix or the conch, rarely the lobe. The nose is the second most frequent (22%) site followed by the foot (8%). Other skin sites (eyelids, hands, leg) are anecdotally reported, occasionally, lesions are multiple [1446], and in particular bilateral on the ears [324,3156] and the feet [4345]. Local recurrence after treatment is possible. In rare cases, recurrence may occur at other cutaneous sites [2302,4345].

Microscopy
The tumours are composed of a monotonous dermal proliferation of atypical medium-sized lymphocytes with irregular and frequently folded nuclei and small nucleoli [3156]. A case with signet ring cells has been reported [2311]. Mitoses and apoptotic figures are absent or very rare. Reactive B-cell lymphoid aggregates of follicles may be seen within the atypical infiltrate. Plasma cells, histiocytes, neutro-
phils, and eosinophils are absent or very rare. The epidermis is most often spared, with a grenz zone. Occasionally, minimal insignificant epidermotropism may be seen [1446,1515]. Skin appendages are always spared, and angiotropism, angiodestruction, and necrosis are never seen. The proliferation frequently involves the underlying fat tissue.

Immunophenotype
The tumoural infiltrate is composed of T cells that express CD3, CD8, TIA1, and beta F1. TIA1 displays Golgi dot-like staining. CD4 is always negative. CD2, CD5, and CD7 are regularly positive but one or more of them can be lost or weak. Granzyme B and perforin are generally negative but may occasionally be positive. CD56, CD57, CD30, and TdT are always negative, as are the T follicular helper (TFH) cell markers (CD10, BCL6, PD1, and CXCL13) [1446]. CD68 is frequently positive, displaying as does TIA1, Golgi dot-like staining [4345]. In the vast majority of cases, the Ki-67 proliferation index is very low (<10%). A few typical cases with a high proliferation index have been reported [4468]; however, when the proliferation index is >50%, other CD8+ cutaneous lymphomas should be carefully considered [3377]. Staining for B-cell markers (CD20, CD79a) might reveal reactive B-cell aggregates or follicles. LMP1 and EBV-encoded small RNA (EBER) are negative.

Postulated normal counterpart
The postulated normal counterpart is a skin-homing CD8+ T cell. However, cases that are very similar in terms of morphology, phenotype, and clinical outcome have also been recently described in the gastrointestinal tract [3145] and genital tract [3863], suggesting a new lymphoma entity arising from tissue-resident CD8+ memory T cells.

Genetic profile
The neoplastic T cells show clonal TRG gene rearrangements. Specific genetic abnormalities have not yet been described. EBV is negative.

Prognosis and predictive factors
The tumour has a very good prognosis. Complete remission after surgical excision or radiotherapy is the rule. Local or extra-site skin recurrence may occur. There is no evidence of dissemination to other organs or lymphoid tissue. No chemotherapy is needed. It is important to recognize this disease to avoid overtreatment.

Primary cutaneous CD4+ small/medium T-cell lymphoproliferative disorder
Gaulard P.\nBerti E.\nWillemze R.\nPetrrella T.\nJaffe E.S.

Definition
Primary cutaneous CD4+ small/medium T-cell lymphoproliferative disorder is characterized by a predominance of small to medium-sized CD4+ pleomorphic T cells, and by presentation with a solitary skin lesion in almost all cases, without evidence of the patches and plaques typical of mycosis fungoides. Because these cases have the same clinicopathological features and benign clinical course as cutaneous pseudo-T-cell lymphomas with a nodular growth pattern [323,626,3267], the term...
'primary cutaneous CD4+ small/medium T-cell lymphoproliferative disorder' is preferred, rather than 'primary cutaneous CD4+ small/medium T-cell lymphoma'. Rare cases presenting with widespread skin lesions, large rapidly growing tumours, >30% large pleomorphic T cells, and/or a high proliferative fraction do not belong to this group [1298,1471]. Such cases usually have a more aggressive clinical behaviour and are better classified as peripheral T-cell lymphoma, NOS.

**ICD-O code**
9709/1

**Synonym**
Primary cutaneous CD4+ small/medium T-cell lymphoma (no longer used)

**Epidemiology**
This is a rare disease, accounting for 2% of all cutaneous T-cell lymphomas [4320].

**Localization**
These lesions usually present as a solitary plaque or nodule, most commonly on the face, neck, or upper trunk [317, 323,626,1298,1471,1832,3267].

**Clinical features**
Patients are asymptomatic; a single slow-growing skin lesion is the sole manifestation of disease. In rare cases, multiple lesions are present [323,626]. By definition, there should be no patches typical of mycosis fungoides.

**Microscopy**
These lymphomas show dense, diffuse, or nodular infiltrates within the dermis, with a tendency to infiltrate the subcutis. Epidermotropism may be present focally, but if epidermotropism is conspicuous, the diagnosis of mycosis fungoides should be considered. There is a predominance of small/medium-sized pleomorphic T cells [317,323,626,1298,1471, 1832,3267,3391]. A small proportion (<30%) of large pleomorphic cells may be present [320]. In almost all cases, the atypical CD4+ T cells are admixed with small reactive CD8+ T cells, B cells, plasma cells, and histiocytes (including multinucleated giant cells) [323,626,3391].

**Immunophenotype**
By definition, these proliferations have a CD3-, CD4-, CD8-, CD30- phenotype. Loss of pan-T-cell antigens (except for CD7) is uncommon, and cytotoxic proteins are not expressed [323,626,1471, 3391]. Atypical CD4+ T cells express PD1, BCL6 (variable), and CXCL13, suggesting T follicular helper (TFH) cell derivation [626,3391]. CD10 is usually negative. The Ki-67 proliferation index is low (typically 5%, and at most 20%).

**Postulated normal counterpart**
A skin-homing CD4+ T cell with TFH-cell characteristics

**Genetic profile**
TR genes are clonally rearranged in most cases [626,3391]. Specific genetic abnormalities have not been described. EBV is negative.

**Prognosis and predictive factors**
Patients have an excellent prognosis. Intrallesional steroids, surgical excision, and radiotherapy are preferred modes of treatment [323,626,1471]. Spontaneous remission after biopsy has been reported [626, 1471,1832]. Local recurrences are rare.
Peripheral T-cell lymphoma, NOS

Definition
Peripheral T-cell lymphoma (PTCL), NOS, is a heterogeneous category of nodal and extranodal mature T-cell lymphomas that do not correspond to any of the specifically defined entities of mature T-cell lymphoma in the current classification (Table 14.09). Excluded from this category are tumours with a T follicular helper (TFH) cell phenotype, as defined by the expression of at least two (ideally three) of the following markers: CD10, BCL6, PD1, CXCL13, CXCR5, ICOS, and SAP [34,2231,2496]. PTCL, NOS, nearly always presents in adults, and has an aggressive clinical course.

ICD-O code 9702/3

Synonyms
T-cell lymphoma, NOS; peripheral T-cell lymphoma, pleomorphic small cell; peripheral T-cell lymphoma, pleomorphic medium and large cell; peripheral T-cell lymphoma, large cell; lymphoepithelioid lymphoma; Lennert lymphoma

Epidemiology
These tumours account for approximately 30% of PTCLs in western countries [3365]. Most patients are adults. These lymphomas are very rare in children. The male-to-female ratio is 2:1.

Etiology
The infection of neoplastic cells by EBV is reported in a small number of cases, in which the virus plays a pathogenetic role [178,1068,1505,4298]. More commonly, EBV may be found in background B cells.

Localization
Most patients present with peripheral lymph node involvement, but any site can be affected. Advanced-stage disease is common, with secondary involvement of the bone marrow, liver, spleen, and extranodal tissues [3365]. The peripheral blood is sometimes involved, but leukemic presentation is uncommon [3365].

Extranodal presentations can occur, most commonly in the skin and gastrointestinal tract [3365]. In this setting, the diagnosis of PTCL, NOS, should be made only after more specific entities have been excluded. Other less frequently involved sites include the lungs and CNS [2632].

Clinical features
Patients most often present with lymph node enlargement, and most have...
Fig. 14.134 Peripheral T-cell lymphoma, NOS. A Diffuse infiltrates of large lymphoid cells with pleomorphic, irregular nuclei and prominent nucleoli. B Between the neoplastic cells, there are scattered eosinophils and numerous vessels. C Nuclei are markedly pleomorphic and multilobed. D In some cases, nuclei are round and monomorphic in appearance.

advanced disease with B symptoms (3365). Paraneoplastic features such as eosinophilia, pruritus, or (rarely) hae-mophagocytic syndrome may be seen (3365).

Microscopy
In the lymph node, these lymphomas show paracortical or diffuse infiltrates with effacement of the normal architecture. The cytological spectrum is extremely broad, from polymorphous to monomorphic. Most cases consist of numerous medium-sized and/or large cells with irregular, pleomorphic, hyperchromatic, or vesicular nuclei; prominent nucleoli; and many mitotic figures (1557,1816). Clear cells and Reed–Sternberg–like cells can also be seen. Rare cases have a predominance of small lymphoid cells with atypical, irregular nuclei. Hyperplasia of high endothelial venules and/or follicular dendritic cells and the open marginal sinuses characteristic of angioimmuno-blastic T-cell lymphoma (AITL) are not usually seen (1557,1816). An inflammatory background is often present, including small lymphocytes, eosinophils, plasma cells, large B cells (which may be clonal irrespective of EBV infection) (4258), and clusters of epithelioid histiocytes. Epithelioid histiocytes are particularly numerous in the lymphoepithelioid variant. Extranodal involvement takes the form of diffuse infiltrates composed of similar cells. In the skin, the lymphomatous population tends to infiltrate the dermis and subcutis, often producing nodules, which may undergo central necrosis (3110). Epidermotropism, angiocentricity, and adenoid involvement are sometimes seen (3110).

In the spleen, the pattern is variable, from solitary or multiple fleshy nodules to diffuse white pulp involvement with colonization of the periaortiolar sheaths or, in some cases, predominant infiltration of the red pulp (641).

Variants

Lymphoepithelioid lymphoma
This variant, also known as Lennert lymphoma, shows diffuse or (less commonly) interfolicular growth. Cytologically, it consists predominantly of small cells with slight nuclear irregularities; numerous and sometimes confluent clusters of epithelioid histiocytes; and some larger, more atypical, proliferating blasts. There can be admixed inflammatory cells and scattered Reed–Sternberg–like B cells (usually EBV-positive). High endothelial venules are not prominent. In most cases, the neoplastic cells are CD8-positive and have a cytotoxic profile (34,1321,1569, 4411). This variant may have a somewhat better prognosis than do other forms of PTCL, NOS.

Other variants
The follicular variant included within the PTCL, NOS, category in the 2008 edition of the WHO classification has been moved to the category of AITL and other nodal lymphomas of T follicular helper cell origin in this update. The same is true for a proportion of cases previously designated as the T-zone variant, because they usually have a TFH-cell phenotype (34). Thus, the growth of atypical small T lymphocytes around florid reactive germinal centres (3463,4258) is no longer considered to be a variant of PTCL, NOS, but rather a non-specific
morphological pattern. This pattern may sometimes be mistaken for benign paracortical hyperplasia.

Primary EBV-positive nodal T-cell or NK-cell lymphomas have been reported (178,718,1505,1901). These usually have a monomorphic pattern of infiltration and lack the angiodestruction and necrosis seen in extranodal NK/T-cell lymphoma. They are more common in elderly patients, or in the setting of immune deficiency. For the time being, they are considered a variant of peripheral T-cell lymphoma, NOS, but may be designated as a separate entity with more data.

**Immunophenotype**

PTCL, NOS, is usually characterized by an aberrant T-cell phenotype with frequent downregulation of CD5 and CD7 (4298) (see Table 14.09, p. 403). A CD4+/CD8- phenotype predominates in nodal cases. CD4/CD8 double-positivity or double-negativity is sometimes seen, as is CD8, CD56, and cytotoxic granule expression (e.g. TIA1, granzyme B, and perforin) (4298). T-cell receptor beta (beta F1) is usually expressed, facilitating the distinction from gamma delta T-cell lymphomas and NK-cell lymphomas. CD15 may be positive, and may be coexpressed with CD30 in rare cases (277). Such cases may show features overlapping with those of ALK-negative (ALK-) anaplastic large cell lymphoma (ALCL), but are classified as PTCL, NOS, under the current guidelines. CD15 expression is associated with an adverse prognosis (4298).

![Peripheral T-cell lymphoma, NOS. A Neoplastic cells express CD4. B In the same case, neoplastic cells lack CD8 expression. Note the presence of some reactive T lymphocytes, which serve as an internal control.](image)

![Peripheral T-cell lymphoma, NOS. Neoplastic cells express the cytotoxic marker TIA1.](image)

![Peripheral T-cell lymphoma, NOS. Neoplastic cells show partial and variable expression of CD30.](image)

![Peripheral T-cell lymphoma, NOS. Most neoplastic cells express TBX21.](image)

![Peripheral T-cell lymphoma, NOS. Most neoplastic cells express GATA3.](image)

![Peripheral T-cell lymphoma (PTCL), NOS. Gene expression profiling identifies two main subgroups of PTCL, NOS, characterized by overexpression of TBX21 and GATA3, respectively. The former is associated with a more favourable clinical course, with longer overall survival (OS).](image)
Peripheral T-cell lymphoma, NOS. Note the marked pleomorphism of the neoplastic population. Upper inset: High Ki-67 labelling. Lower inset: Beta F1 staining.

Peripheral T-cell lymphoma, NOS, lymphoepithelioid variant. Neoplastic cells are admixed with prominent epithelioid histiocytic clusters, which tend to obscure the lymphomatous growth (Giemsa stain).

The expression of TBX21 (also known as T-BET) and GATA3 may be relevant for the subclassification of PTCL, NOS (see also Genetic profile and Prognosis and predictive factors) [1775,4253]. The expression of a single TFH-cell marker can sometimes be observed. CD52 is present in 40% of cases [656,3170,3383]. CD30 is detected in >25% of the cells in half of the cases (427,3467) and in some instances is used as a target of immunotoxins [2965,4442], although the cut-off value of CD30 expression required for effective treatment remains elusive [1160,1701]. PTCL, NOS, with high CD30 expression does not seem to respond as well as does ALK- ALCL [3172,3536]. Aberrant expression of CD20 and/or CD79a, as well as of CD15, is occasionally encountered [277,4258,4298]. Proliferation is usually high, and a Ki-67 proliferation index >70% is associated with a worse prognosis [4298].

Postulated normal counterpart
Activated mature T cells, typically of the CD4+ central memory type of the adaptive immune system [1320,3169].

Genetic profile
Antigen receptor genes
TR genes are clonally rearranged in most cases [3365].

Cytogenetic abnormalities and oncogenes
These are usually aberrant neoplasms with complex karyotypes and recurrent chromosomal gains and losses [1573,3365,3992,4472]. Genomic imbalances have been reported, affecting several regions containing members of NF-kappaB signalling and genes involved in cell-cycle control [1573]. The genetic aberrations observed in PTCL, NOS, differ from those of other T-cell lymphomas, such as AITL and ALCL [3992,4472]. Gene expression profiling and microRNA profiling studies have revealed distinctive signatures distinct from those of AITL and ALK- and ALK+ ALCL [31,243,842,905,1774,1775,2184,2364,2617,3169,3172,3202]. Gene expression profiling has also allowed the identification of groups of PTCL, NOS, cases characterized by the expression of TBX21 (also known as T-BET) or GATA3 [1775]. The former group includes some cases with a cytotoxic profile [1775]. Although TBX21 and GATA3 are transcription factors that are master regulators of gene expression profiles in T helper (Th) cells, skewing Th cell polarization into Th1 cell and Th2 cell differentiation pathways, respectively [900,1775], further studies are needed to confirm them as definitive markers of Th1-cell-derived and Th2-cell-derived neoplasms. Immunohistochemical surrogate markers have been used in lieu of gene expression profiling studies, and may have prognostic significance [4253] (see Prognosis and predictive factors). In comparison to normal T lymphocytes, PTCL, NOS, is characterized by the recurrent deregulation of genes involved in relevant cell functions (e.g. matrix deposition, cytoskeleton organization, cell adhesion, apoptosis, proliferation, transcription, and signal transduction) [3169]. The products of these genes might have therapeutic relevance [842,3169]. For example, overexpression of PDGFRA (likely due to an autocrine loop) can herald sensitivity to tyrosine kinase inhibitors [2192,3174]. Although recurrent mutations have been reported in AITL and other nodal PTCLs of TFH-cell origin [523,900,1010A,2264,3040,3176,3395,3485,4238,4428,4429], it is still unclear whether similar alterations are seen in PTCL, NOS. Two publications based on small series of PTCL, NOS, cases [3552,3683] reported different recurrent mutations, findings that do not allow a firm conclusion as to the mutational landscape of the tumour. Two recent studies reported activating mutations or translocations of VAV1 in 10-15% of cases of PTCL, NOS [7A,396A].

Differential diagnosis
Now that the PTCLs with TFH-cell phenotype are excluded from this category, the distinction of PTCL, NOS, from AITL is less of a problem. More problematic
remains the distinction from ALK- ALCL. In fact, CD30 is highly expressed by a subset of PTCL, NOS [427,3467]. On morphological and immunohistochemical grounds, the simultaneous occurrence of hallmark cells (with kidney-shaped or horseshoe-shaped nuclei), strong and uniform CD30 positivity, EMA positivity, and cytotoxic marker expression is characteristic of ALK- ALCL and not observed in PTCL, NOS [1775,3172]. The gene and microRNA signatures of ALK- ALCL are much closer to those of ALK+ ALCL than to those of PTCL, NOS [31,1774,1775,2364,3202]. Importantly, ALK- ALCL carries genomic aberrations (e.g. DUSP22 and TP63 rearrangement) that to date have not been reported in PTCL, NOS [402,836,3069].

Prognosis and predictive factors
These are highly aggressive lymphomas with a poor response to therapy, frequent relapses, and low 5-year overall survival and failure-free survival rates (20–30%) [3365]. The only factors consistently associated with prognosis are stage and International Prognostic Index (IPI) score [3365]. New scoring systems have recently been developed [1282, 4298]. Involvement of the bone marrow and a Ki-67 proliferation index >70% have been proposed as negative prognostic factors, but further confirmation is needed. EBV positivity, NF-kappaB pathway deregulation, a high proliferation signature by gene expression, transformed cells >70%, a GATA3 or cytotoxic profile, and CD30 expression in most or all cells have been found to correlate with a poor prognosis [164,842,1068,2184,3172,3536,4217,4283,4298]. Upfront autologous stem cell transplantation seems to significantly improve both overall and relapse-free survival [855].

Angioimmunoblastic T-cell lymphoma and other nodal lymphomas of T follicular helper cell origin

Since the recognition of T follicular helper (TFH) cells as a unique physiological subset of T helper (Th) cells with a characteristic phenotype, it has been discovered that a subset of peripheral T-cell lymphomas have phenotypic features of TFH cells. These lymphomas are thought to constitute the neoplastic counterpart of TFH cells [905,1470]. The best-studied of these is angioimmunoblastic T-cell lymphoma, in which the neoplastic cells have a TFH-cell gene expression signature and express many of the TFH-cell–associated markers, such as CD10, CXCL13, BCL6, ICOS, CXCR5, SAP, MAF (also called c-MAF), and (in most cases) CD200 [177,1028,1067,1470,2108,2496,3399]. Additionally, a number of nodal peripheral T-cell lymphomas previously classified within the peripheral T-cell lymphoma, NOS, category have recently been shown to have a TFH-cell phenotype [179,906,1010A,1729]. Such cases, including so-called follicular T-cell lymphoma, show some morphological, immunophenotypic, genetic, and clinical overlap with angioimmunoblastic T-cell lymphoma but also have a number of unique, distinctive features as discussed
below. For these reasons, in this update, they have been included under the broader category of nodal lymphomas of TFH-cell origin with angioimmunoblastic T-cell lymphoma, but are summarized separately. Cutaneous T-cell lymphomas and lymphoproliferative disorders expressing TFH-cell markers are excluded from this group of neoplasms.

**Angioimmunoblastic T-cell lymphoma**

**Definition**

Angioimmunoblastic T-cell lymphoma (AITL) is a neoplasm of mature T follicular helper (TFH) cells characterized by systemic disease and a polymorphous infiltrate involving lymph nodes, with a prominent proliferation of high endothelial venules (HEVs) and follicular dendritic cells (FDCs). EBV-positive B cells are nearly always present, and in some cases constitute a significant part of the cellular infiltrate. Recent studies using next-generation sequencing have identified recurrent mutations that help to unify AITL with other T-cell neoplasms derived from TFH cells. The disease is clinically aggressive and seen mainly in older adults.

**ICD-O code**

9705/3

**Synonyms and historical terminology**

Peripheral T-cell lymphoma, angioimmunoblastic lymphadenopathy with dysproteinemia; (obsolete); immunoblastic lymphadenopathy (obsolete); lymphogranulomatosis X (obsolete)

AITL, previously designated as angioimmunoblastic lymphadenopathy, was thought to be an atypical reactive process, with an increased risk of progression to lymphoma. Overwhelming evidence now indicates that AITL arises de novo as a peripheral T-cell lymphoma (PTCL) [901,1013].

**Epidemiology**

AITL occurs in middle-aged and elderly individuals, with a higher incidence in males than in females [901]. It is one of the most common specific subtypes of PTCL, accounting for 15–30% of noncutaneous T-cell lymphomas and 1–2% of all non-Hodgkin lymphomas [904, 3464,4217].

**Etiology**

The strong association with EBV infection suggests a possible role for the virus in the etiology, possibly through antigen drive [1061]. However, the neoplastic T cells are EBV-negative.

**Localization**

The primary site of disease is the lymph node, and virtually all patients present with generalized lymphadenopathy. The spleen, liver, skin, and bone marrow are also frequently involved [901,1013,1169,2767].

**Clinical features**

AITL typically presents with advanced-stage disease, generalized lymphadenopathy, hepatosplenomegaly, systemic symptoms, and polyclonal hypergammaglobulinaemia [1013,2181,2767,3674]. Skin rash, often with pruritus, is frequently present. Other common findings are pleural effusion, arthritis, and ascites. Laboratory findings include circulating immune complexes, cold agglutinins with haemolytic anaemia, positive rheumatoid factor, and anti-smooth muscle antibodies. Patients exhibit immunodeficiency secondary to the neoplastic process. In most cases (75%), expansion of EBV-positive B cells is seen, which is thought to be a consequence of underlying immune dysfunction [92,938,4288].

**Microscopy**

AITL is characterized by partial or total effacement of the lymph node architecture, often with perinodal infiltration but sparing of the peripheral cortical sinuses. Cytologically, the neoplastic T cells of AITL are small to medium-sized lymphocytes, with clear to pale cytoplasm, distinct cell membranes, and minimal cytological atypia. They frequently form small clusters, often adjacent to HEVs. Vascularity is often prominent, with arborization of HEVs in the paracortex. The neoplastic cells are present in a polymorphous inflammatory background containing variable numbers of reactive lymphocytes, histiocytes, plasma cells, and eosinophils. The cellular density varies, and in
some cases there is amorphous interstitial precipitate, producing a hypocellular appearance.

Three overlapping patterns are recognized [177]. In pattern 1, the neoplastic cells surround hyperplastic follicles with well-formed germinal centres, but often lacking well-defined mantle cuffs [3328]. Pattern 1 is difficult to distinguish from reactive follicular hyperplasia, and immunohistochemical stains are necessary to highlight the neoplastic T cells with their characteristic TFH-cell phenotype. In pattern 2, remnants of follicles remain but show regressive changes. The neoplastic cells are more readily identified in the expanded paracortex. In pattern 3, the architecture is totally or subtotally effaced; remnants of regressed follicles may be seen in the outer cortex, displaced by the expanded paracortex. Progression from pattern 1 to pattern 3 in consecutive biopsies has been reported [3387].

In advanced cases, the inflammatory component may be diminished, and the proportion of clear cells and large cells may increase (so-called tumour cell–rich AITL), which may simulate a PTCL, NOS. In such cases, demonstration of a TFH-cell immunophenotype and the presence of expanded FDC meshworks are helpful in diagnosis [178]. In some cases, there may be a prominent infiltrate of reactive epithelioid histiocytes, mimicking a granulomatous reaction and resembling lymphoepithelioid lymphoma [34,178].

The polymorphic infiltrate is frequently associated with increased extrafollicular FDC meshworks, which are most prominent around the HEVs. The neoplastic cells are often arranged in clusters, surrounded by dendritic processes and highlighted by CD21.

Variable numbers of B immunoblasts are usually present in the paracortex, which may be positive or negative for EBV by in situ hybridization for EBV-encoded small RNA (EBER). EBV-positive B cells are present in 80–95% of cases. They range in size, and expansion of B immunoblasts may be prominent [4288,4470]. The EBV-positive B immunoblastic proliferation may progress, either composite with AITL or at relapse, to EBV-positive diffuse large B-cell lymphoma [182,4470]. EBV-positive Reed–Sternberg–like cells of B-cell lineage may be present and may simulate classic Hodgkin lymphoma [178,3265]. In rare cases, EBV-negative Reed–Sternberg–like cells of B-cell lineage may be present [2868]. Plasma cells may be very abundant, in rare cases obscuring the neoplastic T cells [238A, 1736A]. The plasma cells are usually polyclonal, but may be monoclonal in some cases. The expansion of normal B cells and plasma cells in the lesions has been linked to the functional properties of the neoplastic TFH cells [1061].

Immunophenotype

The neoplastic T cells express most pan–T-cell antigens (e.g. CD3, CD2, and CD5) and in the vast majority of cases are positive for CD4. Surface CD3 may be reduced or absent by flow cytometry [679,3684]. Variable numbers of reactive CD8+ T cells are present. Characteristically, the tumour cells show the immunophenotype of normal TFH cells, expressing CD10, CXCL13, ICOS, BCL6, and PD1 (CD279) in 60–100% of cases [177, 905,1026,1470,3399]. This phenotype is helpful in distinguishing AITL from atypical paracortical hyperplasia and other PTCLs [1067,1469], as well as in diagnosing extranodal dissemination [180, 284,2993]. None of these markers is TFH cell–specific, and conversely, although several TFH-cell markers can usually be detected in the neoplastic cells, both

![Fig. 14.148 Angioimmunoblastic T-cell lymphoma](image)

**Fig. 14.148** Angioimmunoblastic T-cell lymphoma. A Pattern 3. Typical cytology of neoplastic T cells with intermediate-sized nuclei and copious pale/clear cytoplasm. B The infiltrate is composed of medium-sized to large lymphoid cells with abundant clear cytoplasm.

![Fig. 14.149 Angioimmunoblastic T-cell lymphoma](image)

**Fig. 14.149** Angioimmunoblastic T-cell lymphoma, pattern 1. CD10 staining highlights neoplastic T cells surrounding the hyperplastic germinal centres.

![Fig. 14.150 Angioimmunoblastic T-cell lymphoma](image)

**Fig. 14.150** Angioimmunoblastic T-cell lymphoma, pattern 3, expressing PD1.
Fig. 14.151 Angioimmunoblastic T-cell lymphoma (AITL). A Multinucleated cells resembling Reed–Sternberg cells. B Low-power view of CD21 immunostaining highlighting marked follicular dendritic cell proliferation entrapping high endothelial venules (pattern 3). C–E The characteristic phenotype of tumour cells expressing CD3 (C), CD10 (D), and CXCL13 (E) (pattern 3). F EBV-positive B-cell proliferation in AITL double stained for CD79a (brown, cytoplasmic staining) and EBV-encoded small RNA (EBER) (blue, nuclear staining).

the extent and the intensity of staining are variable. An inverse correlation tends to be observed between sensitivity and specificity of individual markers, with CXCL13 and CD10 being among the most specific and PD1 and ICOS being more sensitive. B immunoblasts and plasma cells are polytypic; however, secondary EBV-positive B-cell proliferations, including diffuse large B-cell lymphoma, classic Hodgkin lymphoma, and plasmacytoma, may be seen [182, 901,3887]. FDC meshworks expressing CD21, CD23, and CD35 are expanded with an extrafollicular pattern, usually surrounding the HEVs.

Postulated normal counterpart
A CD4+ TFH cell

Genetic profile
TR genes show clonal rearrangements in 75–90% of cases [179,901,3887]. Clonal Ig gene rearrangements are found in about 25–30% of cases [179,3887] and correlate with expanded EBV-positive B cells. Most EBV-infected B cells show ongoing mutation activity while carrying hypermutated Ig genes with destructive mutations, suggesting that in AITL, alternative pathways operate to allow the survival of these mutating so-called ‘forbidden’ (immunoglobulin-deficient) B cells.

At the gene expression level, the molecular profile of AITL is dominated by a strong microenvironment imprint, including overexpression of B-cell-related and FDC-related genes, chemokines and chemokine receptors, and genes related to extracellular matrix and vascular biology. The signature contributed by the neoplastic cells, although quantitatively minor, shows features of normal TFH cells [905,1010A]. By conventional cytogenetic analysis, clonal aberrations (most commonly trisomies of chromosomes 3, 5, and 21; gain of X; and loss of 6q) have been reported in as many as 90% of cases [1013,3558]. Comparative genetic hybridization has shown recurrent gains of 22q, 19, and 11q13 and losses of 13q, whereas trisomies 3 and 5 were identified in only a small number of cases [3992]. Classic and more recently next-generation sequencing studies have identified frequent mutations of genes encoding epigenetic modifiers such as IDH2 (20–30%), TET2 (50–80%), and DNMT3A (20–30%), as well as the small GTPase RHOA (60–70%) [523,2264,2928, 3040,3485,4238,4429]. Among these, IDH2 R172 mutations appear to be specific for AITL, but the others can be seen in other PTCLs, in particular those with a TFH-cell-like immunophenotype. The hotspot RHOA mutation in AITL results in a Gly17Val-mutant, dominant-negative variant of the enzyme [3485,4429]. Several genes encoding components of the T-cell receptor signalling pathway, such as FYN, PLCG1, and CD28, have been found to be mutated in 5–10% of the cases [2254,2488]. Moreover, fusion genes encoding a CTLA4-CD28 hybrid protein consisting of the extracellular domain of CTLA4 and the cytoplasmic region of CD28, likely capable of transforming inhibitory signals into stimulatory signals for T-cell activation, have been identified in >50% of AITLs, and other PTCLs as well [4428]. Rare cases carry t(5;9)(q33;q22), which results in ITK-SYK gene fusion, an alteration initially recognized in association with follicular PTCL [181,3814].

Prognosis and predictive factors
The course of AITL is variable, but overall the prognosis is poor, with a median survival of <3 years in most studies, even when treated aggressively [2767,4217]. No well-defined prognostic factors have been identified. The International Prognostic Index (IPI) and Prognostic Index for T-cell Lymphoma (PIT) are of limited value [901]. Histological features and genetic findings have not been shown to affect clinical course. In multivariate analysis, only male sex, mediastinal lymphadenopathy, and anaemia adversely affected overall survival [2767].
**Follicular T-cell lymphoma**

**Definition**
Follicular T-cell lymphoma (FTCL) is a lymph node-based neoplasm of T-follicular helper (TFH) cells, with a predominantly follicular growth pattern and lacking characteristic features of angioimmunoblastic T-cell lymphoma (AITL) such as proliferation of high endothelial venules or extrafollicular follicular dendritic cells [34,213,906,1729].

**ICD-O code**
9702/3

**Epidemiology**
Like AITL, FTCL is seen in middle-aged and elderly individuals, with a slightly higher incidence in males than in females [1729]. It is a rare neoplasm; the true incidence is unknown, but it likely accounts for < 1% of all T-cell neoplasms.

**Localization**
FTCL is a lymph node-based neoplasm, sometimes involving the skin and bone marrow.

**Clinical features**
The presenting clinical syndrome resembles that of AITL and other nodal peripheral T-cell lymphomas, characterized by advanced-stage disease, generalized lymphadenopathy, splenomegaly, B symptoms, and skin rash. In a subset of patients, laboratory findings typical of AITL (e.g. hypergammaglobulinaemia, eosinophilia, or a positive Coombs test) can be seen [1729]. However, a few patients with localized disease and/or no B symptoms have also been reported [1729].

**Microscopy**
The lymph node architecture is partially or completely effaced by a nodular/follicular proliferation of intermediate-sized monotonous lymphoid cells with round nuclei and abundant pale cytoplasm [906, 1729]. Two distinct growth patterns are recognized: one that mimics follicular lymphoma and one that mimics progressive transformation of germinal centres [1729]. In the follicular lymphoma-like pattern, the neoplastic cells are arranged into well-defined nodules that lack morphological features of normal follicular B cells. In the progressive transformation of germinal centres-like pattern, the neoplastic cells are seen in well-defined aggregates surrounded by numerous small IgD+ mantle zone B cells arranged into large irregular nodules. The interfollicular areas lack the polymorphic infiltrates and vascular proliferation characteristic of AITL. However, scattered immunoblasts can be seen. In a subset of cases, Hodgkin/Reed–Sternberg–like cells, often surrounded by neoplastic T cells, are present [2750].
In a limited number of cases in which consecutive biopsies from different time points were studied, change of morphology from FTCL to typical AITL or vice versa has been observed, suggesting that these two entities may constitute different morphological representations of the same biological process [1729].

Immunophenotype
The neoplastic T cells express the pan-T-cell antigens CD2, CD3, and CD5 (with frequent loss of CD7) and have a CD4+ T helper (Th) cell phenotype. Consistent with a TFH-cell origin, multiple TFH-cell markers (e.g. PD1, CXCL13, BCL6, CD10, and ICOS) are expressed [1729]. Like in AITL, interfollicular CD20+ B immunoblasts, often with EBV reactivity, are present in half of the cases. When Hodgkin/Reed-Sternberg-like large cells are present, they may show phenotypic features of classic Hodgkin lymphoma, expressing CD30, CD15, PAX5 (weakly), and frequently EBV, but lacking other B-cell markers [2668]. Such cells should not be diagnosed as classic Hodgkin lymphoma in the absence of a typical classic Hodgkin lymphoma background. CD21, CD23, and CD35 staining often reveals a retained follicular dendritic cell meshwork structure underlying the follicular lymphoma-like or progressive transformation of germinal centres-like nodular growth pattern.

Genetic profile
FTCLs show clonal TR gene rearrangements in most cases. About 20% of cases carry a t(5;9)(q33;q22) translocation, leading to ITK-SYK fusion [1729,3814]. This translocation appears to be specific for FTCL; it has not been seen in other peripheral T-cell lymphomas, except in a rare case of AITL [181]. Comprehensive genomic profiling of FTCL cases has not been specifically performed, but it is likely that some cases of peripheral T-cell lymphoma with TFH-cell-like immunophenotype showing mutations of TET2, RHOA, and DNMT3A are FTCLs [2264].

Prognosis and predictive factors
The clinical course is not well characterized, due to the rarity of the lesion and the retrospective nature of most studies. The disease appears to have an aggressive course, with half of the patients dying within 24 months of diagnosis [1729].

Nodal peripheral T-cell lymphoma with T follicular helper phenotype
Definition
It has been recognized that a subset of the peripheral T-cell lymphomas classified as NOS have a TFH cell phenotype (i.e. positive for CD4, PD1, CD10, BCL6, CXCL13, and ICOS) and some pathological features of angioimmunoblastic T-cell lymphoma (AITL). The minimum criteria for assignment of TFH-cell phenotype is not very well established, but the detection of at least two (ideally three) of the TFH-cell markers in addition to CD4 is suggested to assign a TFH-cell phenotype to a nodal CD4+ T-cell lymphoma. These neoplasms frequently show a diffuse infiltration pattern without a prominent polymorphic inflammatory background, vascular proliferation, or expansion of follicular dendritic cell meshworks. In some cases, a so-called T-zone pattern may be evident [34]. Genetic studies show that these cases share some of the genetic alterations seen in AITL, including mutations of TET2, DNMT3A, and RHOA [2264,3485]. These phenotypic and genetic characteristics suggest that such cases may be related to AITL and may constitute a tumour cell-rich variant of AITL [178]. However, until further evidence showing that they are biologically and clinically within the spectrum of AITL, it is recommended that such cases are classified as peripheral T-cell lymphoma with a TFH-cell phenotype.

ICD-O code
9702/3
Anaplastic large cell lymphoma, ALK-positive

Definition
ALK-positive (ALK+) anaplastic large cell lymphoma (ALCL) is a T-cell lymphoma consisting of lymphoid cells that are usually large and have abundant cytoplasm and pleomorphic, often horseshoe-shaped nuclei, with a chromosomal translocation involving the ALK gene and expression of ALK protein and CD30. ALCL with comparable morphological and phenotypic features, but lacking ALK rearrangement and the ALK protein, is considered to be a separate category: ALK-negative (ALK−) ALCL. ALK+ ALCL must also be distinguished from primary cutaneous ALCL and from other subtypes of T-cell or B-cell lymphoma with anaplastic features and/or CD30 expression.

ICD-O code 9714/3

Synonym
Ki-1 lymphoma (obsolete)

Epidemiology
ALK+ ALCL accounts for approximately 3% of adult non-Hodgkin lymphomas and 10–20% of childhood lymphomas [3782]. ALK+ ALCL is most frequent in the first three decades of life [335,1151] and shows a male predominance, with a male-to-female ratio of 1.5:1.

Localization
ALK+ ALCL frequently involves both lymph nodes and extranodal sites. The most commonly involved extranodal sites include the skin, bone, soft tissue, lungs, and liver [475,1151,1543,3782]. Involvement of the gut or CNS is rare. Mediastinal disease is less frequent than in classic Hodgkin lymphoma. The estimated incidence of bone marrow involvement is approximately 10% when investigated using H&E staining, but higher (30%) when immunohistochemical stains are used [1239], because bone marrow involvement is often subtle. The small-cell variant of ALK+ ALCL may have a leukaemic presentation with peripheral blood involvement [301,2031,3752].

Clinical features
Most patients (70%) present with advanced (stage III–IV) disease with peripheral and/or abdominal lymphadenopathy, often associated with extranodal infiltrates and involvement of the bone marrow [475,1151,1543]. Most patients (75%) have B symptoms, especially high fever [475,1151]. Rare cases of skin and satellite lymph node involvement by ALK+ ALCL following insect bites have been reported [2200]. The significance remains unclear. Possibly, ALK+ ALCL cells home to these sites of inflammation.

Microscopy
ALK+ ALCLs show a broad morphological spectrum [335,643,949,1138,2031,3181]. However, all cases contain a variable proportion of cells with eccentric, horseshoe-shaped, or kidney-shaped nuclei, often with an eosinophilic region

Fig. 14.159 Anaplastic large cell lymphoma, ALK-positive. Distribution by patient age and sex (N = 386).

A few cases of indolent ALK+ ALCL restricted to the skin have been reported [2996]. Clinical history and staging are required to distinguish such lesions from the aggressive, poor-prognosis secondary cutaneous involvement by systemic ALK+ ALCL.

Fig. 14.160 Anaplastic large cell lymphoma (ALCL), ALK-positive. General features of ALCL, common type. The lymph node architecture is obliterated by malignant cells, and intrasinusoidal cells are observed.
Anaplastic large cell lymphoma (ALCL), ALK-positive. General features of ALCL, common pattern.

Predominant population of large cells with irregular nuclei. Note the large hallmark cells showing eccentric kidney-shaped nuclei. All malignant cells are strongly positive for CD30, EMA, and granzyme B. The hallmark cells are typically large, but smaller cells with similar cytological features may also be seen and can greatly facilitate accurate diagnosis. Depending on the plane of section, some cells may appear to contain nuclear inclusions; however, these are not true inclusions but rather invaginations of the nuclear membrane. Cells with these features have been referred to as doughnut cells.

Morphologically, ALK+ ALCLs range from small-cell neoplasms to the opposite extreme in which very large cells predominate. Several morphological patterns can be recognized. The so-called common pattern accounts for 60% of cases. The tumour cells have abundant cytoplasm that may appear clear, basophilic, or eosinophilic. Multiple nuclei may occur in a wreath-like pattern and may give rise to cells resembling Reed–Sternberg cells. The nuclear chromatin is usually finely clumped or dispersed, with multiple small, basophilic nucleoli. In cases composed of larger cells, the nucleoli are more prominent, but eosinophilic, inclusion-like nucleoli are rarely seen. When the lymph node architecture is only partially effaced, the tumour characteristically grows within the sinuses and thus may resemble a metastatic tumour.

The lymphohistiocytic pattern (10%) shows a predominant population of small to medium-sized neoplastic cells with irregular nuclei. Hallmark cells are always present and are often concentrated around blood vessels. Signet ring–like cells may also be seen rarely. Hallmark cells are always present and are often concentrated around blood vessels. Signet ring–like cells may also be seen rarely. The Hodgkin-like pattern (3%) is characterized by morphological features mimicking nodular sclerosis classic Hodgkin lymphoma.

In 15% of cases, more than one pattern can be seen in a single lymph node biopsy; this is called the composite pattern. Relapses may have morphological features different than those seen initially. Other histological patterns include tumours showing cells with monomorphic, rounded nuclei, either as the predominant population or mixed with more-pleomorphic cells, and cases rich in multinucleated neoplastic giant cells or displaying sarcomatoid features. Occasional cases may have a hypocellular appearance, with a myxoid or oedematous background. Spindle cells may be prominent in such cases and may simulate sarcoma in cases presenting in soft tissue.

The neoplastic cells are usually smaller than in the common pattern, but often cluster around blood vessels and can be highlighted by immunostaining using antibodies to CD30 and/or ALK. Occasionally, the histiocytes show evidence of erythroagocytosis. The small-cell pattern (6–10%) shows a predominant population of small to medium-sized neoplastic cells with irregular nuclei. In some cases, most of the cells have pale cytoplasm and centrally located nuclei; these are referred to as fried-egg cells. Signet ring–like cells may also be seen rarely. Hallmark cells are always present and are often concentrated around blood vessels. This morphological variant of ALCL is often misdiagnosed as peripheral T-cell lymphoma, NOS, by conventional examination. When the peripheral blood is involved, small atypical cells with folded nuclei reminiscent of flower-like cells in addition to rare large cells with blue, vacuolated cytoplasm can be noted in smear preparations.

The Hodgkin-like pattern (3%) is characterized by morphological features mimicking nodular sclerosis classic Hodgkin lymphoma.

Immunophenotype

The tumour cells are positive for CD30 on the cell membrane and in the Golgi region. The strongest immunostaining is seen in the large cells. Smaller
tumour cells may be only weakly positive or even negative for CD30 [335,1138]. In
the lymphohistiocytic and small-cell patterns, the strongest CD30 expression is
also present in the larger tumour cells, which often cluster around blood vessels
[335,1138]. ALK expression is absent from all normal postnatal human tissues
except rare cells in the brain [3250]. For this reason, immunohistochemistry with
specific anti-ALK monoclonal antibodies has supplanted molecular tests for the
diagnosis of ALK+ ALCL. In most cases that have the t(2;5) (NPM1-ALK) trans-
location, ALK staining of large cells is both cytoplasmic and nuclear [335,1138,3782]. In
the small-cell variant, ALK positivity is usually restricted to the nucleus of
tumour cells [335,1138,3782]. In cases with variant translocations, i.e. fusion
of ALK to partners other than NPM1, the ALK staining is usually cytoplasmic and
rarely membranous [335,1138,1147,3782]. Cases with the t(2;5) (NPM1-ALK) trans-
location show aberrant cytoplasmic expression of NPM1, whereas ALK+ ALCLs
carrying variant translocations show the expected nuclear-restricted expression
of NPM1 [1152].

Most ALK+ ALCLs are positive for EMA, but in some cases only a proportion
of malignant cells are positive [335,949]. The great majority of ALK+ ALCLs express
one or more T-cell antigens [335]. However, due to loss of several pan-T-
cell antigens, some cases may have an apparent so-called null-cell phenotype,
but show evidence of a T-cell lineage at the genetic level [1237]. Because no
other differences can be found in cases with a T-cell versus a null-cell phenotype,
T-cell/null-cell ALK+ ALCL is considered a single entity [335,1557]. CD3 (the most
widely used pan-T-cell marker) is negative in >75% of cases [335,413]. CD2,
CD5, and CD4 are more useful and are positive in a significant proportion of cases
(70%). Furthermore, most cases exhibit positivity for the cytotoxic antigens TIA1,
granzyme B, and/or perforin [1237,2111]. CD8 is usually negative, but rare CD8+
cases exist, particularly those with variant morphology [12]. CD43 is expressed
in two thirds of cases, but lacks lineage specificity. Tumour cells are variably posi-
tive for CD45 and CD45RO and strongly positive for CD25 [949]. In the rare cases
in which CD15 expression is observed, only a small proportion of the neoplastic
cells are stained [335]. Tumour cells are

negative for the macrophage-restricted
form of the CD68 antigen recognized
by the PGM1 monoclonal antibody, but
may show granular staining with other,
less-specific anti-CD68 clones, such as
KP1. ALK+ ALCLs are BCL2-negative
[819]. ALCLs are also consistently nega-
tive for EBV, i.e. for EBV-encoded small
RNA (EBER) and LMP1 [465]. A number
of other antigens are expressed in ALCL
but are not of diagnostic value; these
include clusterin [4292], SHP1 phos-
phatase [1682], BCL6, CEBPB [3270],
SERPINA1 [2196], and fascin.

**Differential diagnosis**

A rare, distinct diffuse large B-cell lymphoma with immunoblastic/plasmabo-
tic features expressing the ALK protein may superficially resemble ALK+ ALCL
due to frequent sinusoidal growth pattern. These lymphomas (ALK+ large B-
cell lymphomas) express EMA (as do ALCLs) but lack CD30, and most cases
show a characteristic cytoplasm-restricted granular staining for the ALK protein
[951]. Subsets of non-haematopoietic neo-
plasms, such as rhabdomyosarcoma

![Fig. 14.163 Anaplastic large cell lymphoma, small-cell pattern. A Predominant population of small cells with irregular
nuclei, associated with scattered hallmark cells. Note that large cells predominate around the vessel. B CD30 staining highlights the perivascular pattern. Large cells are strongly positive for CD30, whereas small and medium-sized malignant cells are weakly stained.](image)

![Fig. 14.164 Anaplastic large cell lymphoma, Hodgkin-like pattern. A Tumour nodules are surrounded by broad fibrous bands. B Numerous tumour cells are present, and some resemble lacunar Reed-Sternberg cells. C Tumour cells have cytoplasmic, nuclear, and nucleolar ALK staining, indicating the presence of the t(2;5)(p23;q35) translocation and the NPM1-ALK fusion protein.](image)
ALK-positive ALCL is characterized by the t(2;5) translocation, which results in the fusion of the ALK gene on chromosome 2 with the NPM1 gene on chromosome 5. Other translocations include t(1;2) and t(2;17), each resulting in different staining patterns. ALK+ ALCL must also be distinguished from ALK+ systemic histiocytosis occurring in early infancy, which is characterized by a proliferation of large histiocytes that look morphologically different from ALCL cells, are CD30-negative, and express the CD68 antigen.

Postulated normal counterpart
An activated mature cytotoxic T cell

Genetic profile
Antigen receptor genes
Approximately 90% of ALK+ ALCLs show clonal rearrangement of the TR genes irrespective of whether they express T-cell antigens. The remainder show no rearrangement of TR or IG genes.

Cytogenetic abnormalities and oncogenes
The genes fused with ALK in various chromosomal translocations and the subcellular distribution of NPM1-ALK and ALK variant chimeric proteins are shown in Table 14.10. The most frequent genetic alteration is a translocation, t(2;5)(p23;q35), between the ALK gene on chromosome 2 and the NPM1 gene on chromosome 5. Variant translocations involving ALK and other partner genes on chromosomes 1, 2, 3, 17, 19, 22, and X also occur. FISH using an ALK break-apart probe or karyotyping is not mandatory in routine practice if ALK staining is positive. RT-PCR is usually reserved for detection of minimal residual disease in blood or bone marrow. All of these translocations result in upregulation and aberrant expression of ALK, but the subcellular distribution of ALK staining varies depending on the translocation partner.

The ALK gene encodes a tyrosine kinase receptor belonging to the insulin receptor superfamily, which is normally silent in lymphoid cells. In the t(2;5) (p23;q35) translocation, NPM1 (a housekeeping gene encoding a nucleolar protein) fuses with ALK to produce a chimeric protein in which the N-terminal portion of NPM1 is linked to the intracytoplasmic...
portion of ALK [2753]. In addition to cytoplasmic ALK positivity, cases with t(2;5) show nuclear and nucleolar ALK staining. The latter pattern is due to the transport of the NPM1-ALK fusion protein into the nucleus through formation of heterodimers between wildtype NPM1 and NPM1-ALK. On the other hand, the formation of NPM1-ALK homodimers uses dimerization sites at the N-terminus of NPM1 mimics ligand binding and is responsible for the activation of the ALK catalytic domain and for the oncogenic properties of the ALK protein [3249]. ALK variant translocations also cause ALK activation through self-association and formation of homodimers. In turn, the activation of the ALK catalytic domain (mediated by all translocations) results in the activation of multiple signalling cascades, including the RAS-ERK, JAK/STAT, and PIK-AKT pathways [403]. Comparative genomic hybridization analysis shows that ALK+ ALCLs carry frequent secondary chromosomal imbalances including losses of chromosomes 4, 11q, and 13q and gains of 7, 17p, and 17q [3488]. In addition, this study demonstrates that ALK+ and ALK− ALCLs have a different representation of secondary genetic alterations, supporting the concept that they constitute different biological entities.

### Gene expression profiling

Supervised analysis by class comparison between ALK+ ALCL and ALK− ALCL tumours showed evidence for distinct molecular signatures [2196]. However, the gene expression profile also shows a common signature between the ALK+ and ALK− groups, which facilitates their distinction from other types of peripheral T-cell lymphoma. Some of the genes differentially expressed between the ALK+ and ALK− groups are related to ALK signalling [403]. Among the 117 genes overexpressed in ALK+ ALCL, BCL6, PTPN12, SERPINA1, and CEBPB were the four top genes, being overexpressed with the most significant P values. This overexpression was also confirmed at the protein level for CEBPB, BCL6, and SERPINA1. A robust classifier for ALK+ ALCL also included highly expressed transcripts related to STAT3 regulators (IL6, IL31RA) or targets, cytotoxic molecules, and T helper 17 (Th17) cell–associated molecules [1774].

### Prognosis and predictive factors

No differences have been found between NPM1–ALK–positive tumours and tumours showing ALK variant translocations [1152]. Concurrent MYC rearrangement could be associated with a more aggressive course [2318,2748]. Most cases with small-cell or lymphohistiocytic variant histology do not have the same favourable prognosis as the other ALK+ tumours, because these patients often present with disseminated disease at diagnosis [58,2198]. Risk stratification according to the International Prognostic Index (IPI) is important in assessing prognosis in ALK+ ALCL [1151,1543,3536]. The long-term survival rate associated with ALK+ ALCL approaches 80%, and is better overall than that of ALK− ALCL [1151,1306,1543,3536,3662,3670]. It appears that this difference may be ascribed to the fact that ALK+ ALCL

#### Table 14.10 Translocations and fusion proteins involving ALK at 2p23

<table>
<thead>
<tr>
<th>Chromosomal anomaly</th>
<th>ALK partner</th>
<th>MW of ALK hybrid protein</th>
<th>ALK staining pattern</th>
<th>Percentage of cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>t(2;5)(p23;q35)</td>
<td>NPM1</td>
<td>80 kDa</td>
<td>Nuclear, diffuse cytoplasmic</td>
<td>84%</td>
</tr>
<tr>
<td>t(1;2)(q25;p23)</td>
<td>TPM3</td>
<td>104 kDa</td>
<td>Diffuse cytoplasmic with peripheral intensification</td>
<td>13%</td>
</tr>
<tr>
<td>inv(2)(p23;q35)</td>
<td>ATIC</td>
<td>96 kDa</td>
<td>Diffuse cytoplasmic</td>
<td>1%</td>
</tr>
<tr>
<td>t(2;3)(p23;q12.2)a</td>
<td>TFG Xlong</td>
<td>113 kDa</td>
<td>Diffuse cytoplasmic</td>
<td>&lt;1%</td>
</tr>
<tr>
<td>t(2;17)(p23;q23)</td>
<td>CLTC</td>
<td>250 kDa</td>
<td>Granular cytoplasmic</td>
<td>&lt;1%</td>
</tr>
<tr>
<td>t(X;2)(q11-12;p23)</td>
<td>MSN</td>
<td>125 kDa</td>
<td>Membrane staining</td>
<td>&lt;1%</td>
</tr>
<tr>
<td>t(2;19)(p23;p13.1)</td>
<td>TPM4</td>
<td>95 kDa</td>
<td>Diffuse cytoplasmic</td>
<td>&lt;1%</td>
</tr>
<tr>
<td>t(2;22)(p23;q11.2)</td>
<td>MYH9</td>
<td>220 kDa</td>
<td>Diffuse cytoplasmic</td>
<td>&lt;1%</td>
</tr>
<tr>
<td>t(2;17)(p23;q25)</td>
<td>RNF213</td>
<td>ND</td>
<td>Diffuse cytoplasmic</td>
<td>&lt;1%</td>
</tr>
<tr>
<td>Othersb</td>
<td>ND</td>
<td>ND</td>
<td>Nuclear or cytoplasmic</td>
<td>&lt;1%</td>
</tr>
</tbody>
</table>

MW, molecular weight; ND, not determined.

a Three different fusion proteins (TFG-ALK½, TFG-ALKγ, and TFG-ALKδ) are associated with the t(2;3)(p23;q12.2) translocation that involves TFG.

b Unpublished series of 270 cases of ALK-positive anaplastic large cell lymphoma.

#### Fig. 14.169 Anaplastic large cell lymphoma (ALCL), ALK-positive. In a cohort of 361 patients, the time to treatment failure was shorter for patients with a small-cell (SC) or lymphohistiocytic (LH) component than for patients with ALK− ALCL, common type. From: Lamant L, et al. (2198).
Anaplastic large cell lymphoma, ALK-negative

Definition
ALK-negative (ALK−) anaplastic large cell lymphoma (ALCL) is defined as a CD30+ T-cell neoplasm that is not reproducibly distinguishable on morphological grounds from ALK-positive (ALK+) ALCL, but lacks ALK protein expression. It was included as a provisional entity in the 2008 edition of the WHO classification, but is now considered an accepted entity. ALK− ALCL must be distinguished from primary cutaneous ALCL (C-ALCL), other subtypes of CD30+ T-cell or B-cell lymphoma with anaplastic features, and classic Hodgkin lymphoma (CHL).

ICD-O code 9715/3

Synonyms and historical terminology
ALK− ALCL was included in the 2008 edition of the WHO classification as a provisional entity distinct from ALK+ ALCL, on the basis of important clinical differences including the older median patient age and more-aggressive clinical course of ALK− ALCL [3848]. There were also valid arguments for excluding ALK− ALCL from the category of peripheral T-cell lymphoma (PTCL), NOS [913,1136,1815,3536,3692]. Unfortunately, there are few detailed clinicopathological studies of ALK− ALCL [3939], and although recurrent genetic events have been identified, their role in classification has not been established. Thus, the distinction between PTCL, NOS, and ALK− ALCL is not always straightforward.

Epidemiology
The peak incidence of ALK− ALCL is in adults (aged 40–65 years), unlike ALK+ ALCL, which occurs most commonly in children and young adults, although cases can occur at any age [1136,3782]. There is a modest male preponderance, with a male-to-female ratio of 1.5:1.

Localization
ALK− ALCL involves both lymph nodes and extranodal tissues (e.g. the bone, soft tissue, and skin), although extranodal sites are less commonly involved than in ALK+ ALCL. Cutaneous cases must be distinguished from primary C-ALCL, and cases that involve the gastrointestinal tract must be distinguished from CD30+ enteropathy-associated and other intestinal T-cell lymphomas. Conversely, if a single lymph node is suggestive of ALK− ALCL, clinical history of skin lesions should be sought, to exclude nodal involvement by primary C-ALCL [178,314].

Clinical features
Most patients present with advanced (stage III–IV) disease, with peripheral blood at diagnosis and an early positive minimal residual disease during treatment identify patients at risk of relapse [865]. This could be linked to a poor immune control of the disease, partly reflected by the anti-ALK antibody titre, which is inversely correlated to prognosis [39]. Relapses often remain sensitive to chemotherapy; allogeneic bone marrow transplantation may be effective in refractory cases [2360]. Because ALK is essential for the proliferation and survival of ALK+ ALCL cells, it provides a unique therapeutic target. Promising clinical results have been obtained in small case series of relapsed ALK+ ALCL treated with small-molecule inhibitors of ALK kinase [1285]. CD30-targeting with antibody-drug conjugates is another appealing therapeutic approach for relapsed/refractory ALK+ ALCL [3241].
Microscopy
In most cases, the nodal or other tissue architecture is effaced by solid, cohesive sheets of neoplastic cells. When lymph node architecture is preserved, the neoplastic cells typically grow within sinuses or within T-cell areas, commonly showing a so-called cohesive pattern, which may mimic carcinoma. Absence of these features should suggest a diagnosis of PTCL, NOS (178). Needle biopsies may be inadequate to assess these features. The overall features typically resemble the so-called common pattern described in ALK+ ALCL, and variant morphological patterns are not recognized. Features such as sclerosis and eosinophils may occur, but when present should raise suspicion for CHL. Cases with a confirmed T-cell origin that morphologically resemble CHL are usually best classified as PTCL, NOS (178). Cases resembling CHL may also constitute nodal involvement by lymphomatoid papulosis (1078).

The cytological features of the neoplastic cells show a similar spectrum to ALK+ ALCL, although the small tumour cells seen in the small-cell and lymphohistiocytic variants of ALK− ALCL should not be prominent in ALK− ALCL. Biopsies typically show large pleomorphic cells, sometimes containing prominent nucleoli. Multinucleated cells, including wreath-like cells, may also be present, and mitotic figures are not infrequent. In addition, to a variable degree, hallmark cells with eccentric, horseshoe-shaped, or kidney-shaped nuclei are seen. In most cases of ALK− ALCL, the neoplastic cells are larger and more pleomorphic than those seen in classic ALK+ ALCL, and/or have a higher N:C ratio (1151,2814,3201,3662). A higher N:C ratio may suggest a diagnosis of PTCL, NOS, but in that disorder, abnormal small to medium-sized lymphocytes are often admixed with a morphologically homogeneous neoplastic cell population, and the sheet-like or sinus pattern of infiltration typical of ALCL is absent. Of note, cases of ALK− ALCL with DUSP22-IRF4 rearrangements tend to lack large pleomorphic cells and to have more so-called doughnut cells showing central nuclear pseudoenclaves than do cases lacking DUSP22-IRF4 rearrangements (2029).

Immunophenotype
All tumour cells are strongly positive for CD30, usually most strongly at the cell membrane and in the Golgi region, although diffuse cytoplasmic positivity is also common. Staining should be strong and of equal intensity in all cells, a feature that is important in distinguishing ALK− ALCL from other PTCLs, which can express CD30 in at least a proportion of the cells, and usually with variable intensity. By definition, ALK protein is undetectable. Loss of T-cell markers occurs with greater frequency than is typically seen in PTCL, NOS. In this respect, ALK−ALCL is similar to ALK+ ALCL. However, more than half of all cases express one or more T-cell markers. CD2 and CD3 are found more often than CD5, and CD43 is almost always expressed. CD4 is positive in a significant proportion of cases, whereas CD8+ cases are rare. Many cases express the cytotoxic markers TIA1, granzyme B, and/or perforin.
Fig. 14.173 Anaplastic large cell lymphoma, ALK-negative, with DUSP22 rearrangement. A Densely packed monomorphic large cells. B Strong, uniform staining for CD30.

These markers tend to be absent in cases bearing DUSP22 rearrangements (3069); therefore, although desirable, their expression is not an absolute requirement for the diagnosis of ALK- ALCL. About 43% of cases are positive for EMA, on at least a proportion of malignant cells, a marker that is almost always seen in ALK+ ALCL but only occasionally in PTCL, NOS (3536). Nuclear phospho-STAT3 is expressed in about 43% of cases (836).

In cases that lack all T-cell/cytotoxic markers, CHL rich in neoplastic cells and other large cell malignancies (e.g. embryonal carcinoma) should be ruled out. In this regard, staining for PAX5 is useful, because CHL reveals weak expression of PAX5 in most cases; however, rare cases of ALCL (ALK- or ALK+) may express PAX5 (1175). The demonstration of CD15 should raise suspicion for CHL. However, CD15 may be expressed in some cases of PTCL, NOS, and such cases are generally strongly CD30-positive (277). Whether peripheral T-cell neoplasms expressing both CD30 and CD15 should be classified as ALK- ALCL or PTCL, NOS, remains to be determined. Notably, these cases have a very poor prognosis, clinically more closely resembling PTCL, NOS. ALK- ALCLs are consistently negative for EBV, i.e. for EBV-encoded small RNA (EBER) and LMP1, and the expression of these markers should strongly suggest the possibility of CHL.

ALK- ALCL and ALK+ ALCL lack T-cell receptor proteins, and in this respect tend to differ from PTCL, NOS (413,1322). Clusterin is also commonly expressed in both ALK- and ALK+ ALCL, but rarely in PTCL, NOS (2183,2830,3470); this marker has not been examined extensively in CHL, but was negative in the cases studied (4292).

Differential diagnosis
The principal differential diagnosis of ALK- ALCL is with PTCL, NOS, and CHL. Most cases referred to historically as Hodgkin-like ALCL are now considered to be tumour cell–rich forms of CHL. With complete immunophenotypic and genetic studies, ALK- ALCL can be distinguished from CHL in virtually all cases. In contrast, the distinction between PTCL, NOS, and ALK- ALCL is not always clear-cut, and even experts may disagree on this subject. In general, the WHO classification advocates a conservative approach, recommending the diagnosis of ALK- ALCL only if both the morphology and phenotype are very close to those of ALK+ cases, with the only distinction being the presence or absence of ALK. ALK- ALCL must also be distinguished from primary C-ALCL, which can have a similar phenotype and morphology. Clinical correlation with staging is of paramount importance in this differential. Primary C-ALCL has a much better prognosis than does ALK- ALCL.

Postulated normal counterpart
An activated mature cytotoxic T cell

Genetic profile
Most cases show clonal rearrangement of the TR genes, whether or not they express T-cell antigens.

A constellation of genetic findings, notably recurrent activating mutations of JAK1 and/or STAT3, has been shown to lead to constitutive activation of the JAK/STAT3 pathway in ALK- ALCL (836). Translocations involving tyrosine kinase genes other than ALK also lead to STAT3 activation in a small subset of systemic and cutaneous ALK- ALCLs (836,4171). These genetic events in ALK- ALCL partially explain the biological and pathological similarities to ALK+ ALCL, in which ALK fusion proteins lead to constitutive STAT3 activation (710), and may have therapeutic implications.

DUSP22 rearrangements, i.e. chromosomal rearrangements in or near the DUSP22-IRF4 locus on 6p25.3, occur in about 30% of cases (3069). These are associated with decreased expression of the DUSP22 dual-specificity phosphatase.
gene, with no detectable alteration in expression of the neighbouring IRF4 gene [1174]. The most common partner is the \( FRA7H \) fragile site on 7q32.3. Rearrangements of \( TP63 \) occur in about 8% of cases and encode p63 fusion proteins, most commonly with TBL1XR1, as a result of inv(3)(q26q28) [3069,4161]. Rearrangement of \( DUSP22 \) or \( TP63 \) has not been reported in ALK+ ALCL, but can be seen in a fraction of other PTCLs.

Several studies have shown differences in copy-number abnormalities between ALK- ALCL and both PTCL, NOS, and ALK+ ALCL, including gains of 1q, 6p, 8q, and 12q and losses of 4q, 6q21 (encompassing the \( PRDM1 \) gene, which encodes PRDM1, also known as BLIMP1), 13q, and 17p13 (\( TP53 \)) [402,3488,4472]. Despite their biological and possible prognostic importance (see below), none of the aforementioned genetic abnormalities has an established diagnostic role in distinguishing ALK- ALCL from other entities.

Gene expression studies have shown that ALK- ALCL shares a common molecular signature with ALK+ ALCL, but also shows differential expression of some genes, including a subset related to ALK signalling in the latter entity [403,1774,2196,3991]. In addition, ALK- ALCL has a gene expression profile distinct from that of PTCL, NOS [31,243,1775,3172,3202]. As few as three genes (\( TNFRSF8, BATF \), and \( TMOD1 \)) could distinguish ALK- ALCL from PTCL, NOS, and cases reclassified from PTCL, NOS, to ALK- ALCL on the basis of molecular signature have been confirmed to be ALK- ALCL on pathologically re-review [31,1775].

**Prognosis and predictive factors**

In general, the clinical outcome of ALK- ALCL with conventional therapy is poorer than that of ALK+ ALCL; however, the findings have been variable and may relate in part to patient age, International Prognostic Index (IPI) score, and the genetic heterogeneity in ALK- ALCL [1151, 1306,1543,3536,3938]. A report by the International Peripheral T-cell Lymphoma Project [3536] also showed outcomes differences between PTCL, NOS, and ALK- ALCL. The 5-year failure-free survival rate (36% vs 20%) and overall survival rate (49% vs 32%) were superior in ALK- ALCL compared with PTCL, NOS. Furthermore, restricting the analysis to cases of PTCL, NOS, with high CD30 expression (>80% of cells), which is the group most difficult to differentiate from ALK- ALCL, magnified the difference in 5-year failure-free survival rate and 5-year overall survival rate (19%) compared with ALK- ALCL. A recent study found that ALK- ALCL with \( DUSP22 \) rearrangement was associated with a 5-year overall survival rate similar to that of ALK+ ALCL (90% vs 85%) [3069]. Conversely, the 5-year overall survival rate associated with ALK- ALCL with \( TP63 \) rearrangement was only 17%, compared with 42% in ALK- ALCL with neither \( DUSP22 \) nor \( TP63 \) rearrangement. Loss of \( PRDM1 \) (also known as \( BLIMP1 \)) and/or \( TP53 \) has been associated with poor outcome [402].

---

**Breast implant–associated anaplastic large cell lymphoma**

**Definition**

This provisional entity is a T-cell lymphoma with morphological and immunophenotypic features indistinguishable from those of ALK-negative anaplastic large cell lymphoma (ALCL), arising primarily in association with a breast implant.

**ICD-O code**

9715/3

**Synonym**

Seroma-associated anaplastic large cell lymphoma

**Epidemiology**

Breast implant–associated ALCL is very rare, with an estimated incidence of 1 case per 500 000 to 3 million women with implants [461]. Although a possible etiological relationship between implants and ALCL has been suggested, large studies have not identified an increased risk of lymphoma in women with breast implants [895,2220,2358,2672].

**Localization**

The tumour cells may be localized to the seroma cavity or may involve the pericapsular fibrous tissue, sometimes forming a mass. Locoregional lymph nodes may be involved.

**Clinical features**

The mean patient age is 50 years [2235, 2672,3382]. Most patients present with stage I disease, usually with a peri-implant effusion and less frequently with a mass. As many as 29% of patients have axillary lymphadenopathy, but not all biopsied nodes are positive for tumour. Rare cases present with disseminated disease. The mean interval from implant placement to lymphoma diagnosis is 10.9 years, but the interval varies greatly. No association with the type of implant (silicone vs saline, textured vs smooth) or with breast cancer history has been established.

**Microscopy**

The tumour cells may be identified initially in cytology specimens from aspirated effusion fluid. At capsulectomy, the tumour cells often line the capsule and may show varying degrees of capsular infiltration. In some cases, they extend beyond the capsule to form a mass, which may be palpable in the specimen at the time of gross evaluation [51]. The tumour cells are typically large and pleomorphic, and the so-called hallmark cells seen in other forms of ALCL can usually be identified.
Fig. 14.175. Breast implant-associated anaplastic large cell lymphoma. A Section of the capsule surrounding the breast implant shows a fibrinous exudate, with atypical anaplastic cells in the fluid and exudate. B Seroma fluid contains pleomorphic tumour cells (Wright-Giemsa stain). C Tumour cells are strongly CD30-positive, and are confined to the surface of the exudate without invasion.

Fig. 14.176. Breast implant-associated anaplastic large cell lymphoma. A The tumour cells have pleomorphic nuclear features. This case had invasion of the capsule and underlying breast, shown in B and C. B Some tumours may show invasion of the breast, which is associated with a more aggressive clinical course. C Neoplastic cells with strong staining for CD30 surround a duct in the breast.

Immunophenotype
The phenotype is similar to that of ALK-negative ALCL (51,3382,3911). Tumour cells show strong and uniform expression of CD30, are negative for ALK, and often show incomplete expression of pan-T-cell antigens.

Postulated normal counterpart
An activated mature cytotoxic T cell

Genetic profile
TR genes are clonally rearranged in most cases. Tumours may show complex karyotypes (2246). Recurrent activating JAK1 and STAT3 mutations have been reported (391A).

Prognosis and predictive factors
Most patients have excellent outcomes, often after excision alone. The median overall survival is 12 years (2672). The most important adverse prognostic factor is the presence of a solid mass of tumour cells, which may indicate a need for systemic therapy (2229). In cases restricted to the seroma cavity, the addition of chemotherapy does not appear to affect outcomes (2672).
CHAPTER 15

Hodgkin lymphomas

Nodular lymphocyte predominant Hodgkin lymphoma
Classical Hodgkin lymphoma
Hodgkin lymphomas: Introduction

Definition
Hodgkin lymphomas (HLs) are lymphoid neoplasms usually affecting lymph nodes. They are composed of large dysplastic mononuclear and multinucleated cells surrounded by a variable mixture of mature non-neoplastic inflammatory cells. Abundant band-like and/or more diffuse collagen fibrosis may be present. The neoplastic cells are often ringed by T cells in a rosette-like manner. On the basis of the immunophenotype and morphology of the neoplastic cells and the cellular background, two major types of HL are recognized: nodular lymphocyte predominant HL (NLPHL) and classic HL (CHL).

NLPHL differs from CHL in that the B-cell programme is generally preserved, although there may be partial loss of the B-cell phenotype [477]. Both NLPHL and CHL are characterized by a paucity of neoplastic cells and a rich inflammatory background of non-neoplastic cells, mainly T cells. The features of NLPHL are more fully discussed in a subsequent section.

CHL accounts for approximately 90% of all HLs, with a peak incidence among individuals aged 15–35 years and a second peak in older adults. Patients usually present with peripheral lymphadenopathy, localized to one or two preferentially cervical lymph node-bearing areas. B symptoms consisting of fever, drenching night sweats, and significant body weight loss are present in as many as 40% of patients. Histopathologically, mononuclear Hodgkin cells and multinucleated Reed–Sternberg cells are seen in a cellular background rich in lymphocytes, histiocytes, plasma cells, and/or eosinophils or neutrophils. In >98% of cases, neoplastic cells are derived from mature B cells at the germinal centre stage of differentiation and contain clonal IG gene rearrangements. Treatment advances have resulted in a 5-year survival rate of >90%.

Four histological subtypes are distinguished: nodular sclerosis CHL (NSCHL), lymphocyte-rich CHL (LRCHL), mixed cellularity CHL (MCCHL), and lymphocyte-depleted CHL (LDCHL). These subtypes differ in the character of the microenvironment and the cytological features of the neoplastic cells. They also differ in their clinical features, risk factors, and association with EBV.

Epidemiology
Approximately 90% of HLs are of classic type, and only 10% are NLPHL [2230A]. The age peak of NLPHL patients lies in the fourth and fifth decades of life, but NLPHL is also common in children. It is more common in males than in females. The patient age distributions of the various types of CHL vary greatly. MCCHL has a bimodal age distribution, with a peak in young patients and a second peak in older adults. NSCHL has a peak among individuals aged 15–35 years. There is a male predominance for most CHL subtypes, with the exception of NSCHL, in which the incidence is slightly higher in females. Individuals with a history of infectious mononucleosis have a higher incidence of CHL, in particular the mixed cellularity subtype [2777]. Both familial clustering and geographical clustering have been described [2777]. The age-adjusted annual incidence rate of HL is 2.8 cases per 100,000 population. Unlike that of non-Hodgkin lymphoma, the incidence of HL has not increased over the past decades.

Etiology
EBV has been postulated to play a role in the pathogenesis of CHL. EBV is found in only a proportion of cases, most frequently in MCCHL and LDCHL, but a search for other viruses has been unsuccessful. Loss of immune surveillance in immunodeficiency states, such as HIV infection, may predispose individuals to the development of EBV-associated CHL. In tropical regions and developing countries, as many as 100% of CHL cases are seen.
EBV-positive (125, 276, 1190, 2276, 4277, 4278). However, there are differences between urban and rural areas. For example, NSCHL is more common in urban areas, whereas EBV-positive MCCHL is more common in rural regions (1092). Delayed exposure to childhood infections has been postulated to play a role (1383). It is possible that EBV infection of a B cell replaces one of the genetic alterations necessary for the development of CHL. NLPHL is only rarely positive for EBV (<5% of cases) (1736).

Localization
CHL most often involves lymph nodes of the cervical region (75% of cases), followed by the mediastinal, axillary, and para-aortic regions. Non-axial lymph node groups, such as mesenteric and epitrochlear lymph nodes, are rarely involved. Primary extranodal involvement is rare. More than 60% of patients have localized disease (stage I or II). Approximately 60% of patients, most with NSCHL, have mediastinal involvement. Splenic involvement is common (20%) and is associated with an increased risk of extranodal dissemination. Bone marrow involvement is much less common (5%). Because the bone marrow lacks lymphatics, bone marrow infiltration indicates vascular dissemination of the disease (stage IV). The anatomical distribution varies between the histological subtypes of CHL (3654). NLPHL tends to spare axial lymph node groups, and is more common in peripheral lymph nodes. The mediastinum is uncommonly involved, but mesenteric lymph node involvement may be seen.

Clinical features
Patients with CHL usually present with peripheral lymphadenopathy, localized to one or two lymph node-bearing areas. Mediastinal involvement is most frequently seen in the nodular sclerosis subtype, whereas abdominal involvement and splenic involvement are more common in mixed cellularity cases. B symptoms consisting of fever, drenching night sweats, and significant body weight loss are present in as many as 40% of patients. Most patients with NLPHL are asymptomatic and present with enlargement of peripheral lymph nodes; B symptoms are rare.

Macroscopy
Lymph nodes are enlarged and encapsulated, and show a fish-flesh tumour on cut section. In NSCHL, there is prominent nodularity, dense fibrotic bands, and a thickened capsule. Splenic involvement usually shows scattered nodules within the white pulp. Very large masses are sometime seen; these can demonstrate fibrous bands in the nodular sclerosis
Table 15.01 Differential diagnosis of Hodgkin lymphoma: comparative tumour cell immunophenotypes

<table>
<thead>
<tr>
<th>Marker</th>
<th>NLPHEL</th>
<th>THRLBCL</th>
<th>CHL</th>
<th>DLBCL</th>
<th>ALCL, ALK+</th>
<th>ALCL, ALK-</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD30</td>
<td>- a</td>
<td>- a</td>
<td>+ b</td>
<td>-/±</td>
<td>+ /+</td>
<td>- /-</td>
</tr>
<tr>
<td>CD15</td>
<td>-</td>
<td>-</td>
<td>+/±</td>
<td>-</td>
<td>- /-</td>
<td>-</td>
</tr>
<tr>
<td>CD45</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-/±</td>
<td>+ /+</td>
<td>- /-</td>
</tr>
<tr>
<td>CD20</td>
<td>+</td>
<td>+</td>
<td>-/±</td>
<td>+</td>
<td>+ /+</td>
<td>- /-</td>
</tr>
<tr>
<td>CD79a</td>
<td>+</td>
<td>+</td>
<td>-/±</td>
<td>+</td>
<td>+ /+</td>
<td>- /-</td>
</tr>
<tr>
<td>CD75</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>a</td>
<td>+ /+</td>
<td>- /-</td>
</tr>
<tr>
<td>PAX5</td>
<td>+</td>
<td>+</td>
<td>a</td>
<td>+</td>
<td>+ /+</td>
<td>- /-</td>
</tr>
<tr>
<td>J chain</td>
<td>+/-</td>
<td>+/-</td>
<td>-</td>
<td>-/±</td>
<td>- /-</td>
<td>-</td>
</tr>
<tr>
<td>Ig</td>
<td>+/-</td>
<td>+/-</td>
<td>-</td>
<td>-/±</td>
<td>- /-</td>
<td>-</td>
</tr>
<tr>
<td>OCT2</td>
<td>S+</td>
<td>S+</td>
<td>-/±</td>
<td>+</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>BOB1</td>
<td>+</td>
<td>+</td>
<td>-/±</td>
<td>+</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>CD3</td>
<td>-</td>
<td>-</td>
<td>a</td>
<td>-/±</td>
<td>- /-</td>
<td>-</td>
</tr>
<tr>
<td>CD2</td>
<td>-</td>
<td>-</td>
<td>a</td>
<td>-/±</td>
<td>- /-</td>
<td>-</td>
</tr>
<tr>
<td>Perforin/</td>
<td>-</td>
<td>-</td>
<td>a</td>
<td>-</td>
<td>+ /+</td>
<td>- /-</td>
</tr>
<tr>
<td>granzyme B</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD43</td>
<td>+</td>
<td>-</td>
<td>-/±</td>
<td>+</td>
<td>+/-</td>
<td>+/-</td>
</tr>
<tr>
<td>EMA</td>
<td>+/-</td>
<td>+/-</td>
<td>-/±</td>
<td>a</td>
<td>+/-</td>
<td>+/-</td>
</tr>
<tr>
<td>ALK</td>
<td>-</td>
<td>-</td>
<td>a</td>
<td>-/±</td>
<td>- /-</td>
<td>-</td>
</tr>
<tr>
<td>LMP1</td>
<td>-</td>
<td>-</td>
<td>a</td>
<td>+/-</td>
<td>- /-</td>
<td>-</td>
</tr>
</tbody>
</table>

+ All (or nearly all) cases positive; +/-, majority of cases positive; -/±, minority of cases positive; -, all (or nearly all) cases negative; ALCL, anaplastic large cell lymphoma; CHL, classic Hodgkin lymphoma; DLBCL, diffuse large B-cell lymphoma; Ig, immunoglobulin; n/a, not applicable; NLPHEL, nodular lymphocyte predominant Hodgkin lymphoma; S, strong expression; THRLBCL, T-cell/histiocyte-rich large B-cell lymphoma.

- Positive in fewer than 10% of cases.
- Prominent expression in anaplastic variant and variable expression in mediastinal large B-cell subtype.
- Occasional cases may show focal positivity.
- Present in as many as 40% of cases, but usually expressed on a minority of tumour cells, with variable intensity.
- As many as 10% might be negative.
- The common positivity for IgG and both Ig light chains reflects uptake of these proteins by the tumour cells rather than synthesis.
- Strong expression found in <10% of the cases.
- Rare cases (~10%) may show scattered weak positivity.
- Only a minority of cases are negative.
- Weak expression may be seen in tumour cells in 5% of cases.
- Most frequently positive in DLBCLs with anaplastic morphology.

subtypes. CHL in the thymus can be associated with cystic degeneration and epithelial hyperplasia [2349]. Rare cases can be confined to the thymus.

Microscopy

In CHL, the lymph node architecture is effaced by variable numbers of Hodgkin/Reed–Sternberg (HRS) cells admixed with a rich inflammatory background. Classic diagnostic Reed–Sternberg cells are large, have abundant slightly basophilic cytoplasm, and have at least two nuclear lobes or nuclei. The nuclei are large and often rounded in contour, with a prominent, often irregular, nuclear membrane; pale chromatin; and usually one prominent eosinophilic nucleolus, with perinuclear clearing (a halo), resembling a viral inclusion. Prototypical Reed–Sternberg cells have at least two nucleoli in two separate nuclear lobes: the so-called owl’s eye appearance. Mononuclear variants are termed Hodgkin cells. Some HRS cells may have condensed cytoplasm and pyknotic reddish nuclei. These variants are known as mummified cells. Many of the neoplastic cells are not prototypical Reed–Sternberg cells. The lacunar Reed–Sternberg variant is characteristic of nodular sclerosis CHL. The neoplastic cells typically constitute only a minority of the cellular infiltrate, amounting to 0.1–10%. The composition of the reactive cellular infiltrate varies according to the histological subtype.

Involvement of secondary sites (bone marrow and liver) is determined based on the identification of atypical mononuclear (CD30+ Hodgkin cells with or without CD15 expression in the appropriate inflammatory background); thus, diagnostic multinucleated Reed–Sternberg cells are not required in a patient with CHL diagnosed at another site [3311]. The neoplastic cells of NLPHEL are referred to as lymphocyte predominant (LP) cells. They have lobed nuclei, usually with smaller basophilic nucleoli than are seen in CHL. They have a rim of pale cytoplasm. The background differs from that of CHL in that it has a predominance of lymphocytes. Epithelioid histiocytes, sometimes in clusters, may be abundant, often around the nodules.

Immunopheonotype

The HRS cells of CHL are positive for CD30 in nearly all cases [3590,3783,3787] and for CD15 [1716,3787,3788] in the majority (75–85%). They are usually negative for CD45 and are consistently negative for J chain, CD75, and macrophage-specific markers such as the PGM1 epitope of the CD68 molecule [724,1143] (Table 15.01). Both CD30 and CD15 are typically present in a membrane pattern, with accentuation in the Golgi area of the cytoplasm; CD15 may be expressed by only a minority of the neoplastic cells and may be restricted to the Golgi region. In 30–40% of cases, CD20 may be detectable but is usually of varied intensity and usually present only on a minority of the neoplastic cells [3565,4504]. The B-cell–associated antigen CD79a is less often expressed. The B-cell nature of HRS cells is further
Classic Hodgkin lymphoma.

A The cytokine receptor CD30 is selectively expressed by the Hodgkin/Reed–Sternberg cells. B The typical membrane and perinuclear dot-like staining of a large Reed–Sternberg cell for CD15 is seen. The small binucleated Reed–Sternberg cell (arrowhead) shows only a very faint labelling. In addition, three neutrophil granulocytes (arrows) are strongly labelled. C Touch imprint of a binucleated Reed–Sternberg cell rimmed by lymphocytes.

Demonstrable in approximately 95% of cases by their expression of the B-cell-specific activator protein PAX5 (also called BSAP) [1238]. The immunostaining of HRS cells for PAX5 is usually weaker than that of reactive B cells, a feature that makes the PAX5+ HRS cells easily identifiable. The transcription factor IRF4/MUM1 is consistently positive in HRS cells, usually at high intensity. In one study, PRDM1 (also known as BLIMP1), the key regulator of plasma cell differentiation, was expressed in only a small proportion of HRS cells in 25% of CHLs [492]. The plasma cell–associated adhesion molecule CD138 is consistently absent [492]. EBV-infected HRS cells express LMP1 and EBNA1 without EBNA2, a pattern characteristic of type II EBV latency [950]. EBV-encoded LMP1 has strong transforming and antiapoptotic potential.

Membranous and less often globular cytoplasmic expression of one or more T-cell antigens by a minority of HRS cells may be encountered in some cases [862,4176]. However, this is often difficult to assess because of the T cells that usually surround the HRS cells. Most T-cell antigen–positive CHL cases have both PAX5 expression and IG gene rearrangement in the HRS cells, so that the expression of T-cell antigens is either aberrant or artefactual [3614,4176]. Expression of EMA is rare and usually weak if present. A further characteristic finding is the absence of the transcription factor OCT2 (also known as POU2F2) in up to 90% of cases in some reports and the absence of its coactivator BOB1 (also known as POU2AF1) in the same proportion [2379]. The transcription factor PU1 is consistently absent from HRS cells [2379,4027]. Most HRS cells express the proliferation-associated nuclear antigen Ki-67 [1332]. CHL cases rich in neoplastic cells may resemble anaplastic large cell lymphoma (ALCL), a T-cell neoplasm. Their identification as CHL is facilitated by demonstrating positivity for PAX5 and absence of EMA and ALK protein [1238,3782]. The detection of EBV-encoded small RNA (EBER) or LMP1 favours CHL over ALCL [1731]. The most difficult differential diagnosis is with diffuse large B-cell lymphoma displaying anaplastic morphology and expressing CD30. There may be a true biological overlap between such cases and CHL, especially in cases with mediastinal disease.

Unlike CHL, NLPHL generally shows preservation of the B-cell programme. The LP cells are positive for CD20, CD79a, PAX5, OCT2, and BOB1. EMA is sometimes positive, but often only in a fraction of the LP cells. IgD (but not IgM) is expressed in LP cells in a subset of cases, most commonly in young males [3231]. However, stains for immunoglobulin light chains are generally negative. CD30 may be weakly expressed in some cases, but CD15 is nearly always negative.

Cytokines and chemokines

CHL is associated with overexpression and an abnormal pattern of cytokines and chemokines and/or their receptors in HRS cells [1613,1751,1897,1899,1937,4120], which likely explains the abundant admixture of inflammatory cells [1876,3944], the fibrosis [1897], and the predominance of T helper 2 (Th2) cells in the infiltrating T-cell population [4120].

Postulated normal counterpart

Classic Hodgkin lymphoma (CHL)
The cellular origin of HRS was unknown for many years, because the cells lack the morphology and immunophenotype of any normal counterpart. However, IG gene rearrangement studies have provided convincing evidence that HRS

Fig. 15.07 Classic Hodgkin lymphoma. A The immunostaining for PAX5 labels the nuclei of the Hodgkin/Reed–Sternberg (HRS) cells weakly and those of the non-neoplastic bystander B cells strongly. B Immunostaining for BOB1 (the coactivator of the octamer-binding transcription factors OCT1 and OCT2) fails to stain the HRS cells in most instances. This is in contrast to the non-neoplastic bystander B cells and plasma cells, which show a moderately strong to strong labelling of their nuclei and in part of their cytoplasm. C Immunostaining for the octamer-binding transcription factor OCT2 is negative in the HRS cells, whereas the non-neoplastic bystander B cells show a nuclear positivity.
cells are clonal and derived from germinal centre B cells despite the frequent absence of B-cell markers other than PAX5. Thus, HRS cells are one of the most extreme examples of discordance between genotype and phenotype [1934, 2495]. The HRS cells of rare CHL cases have been reported to harbour clonally rearranged TR genes, indicating that exceptional cases with morphological features of CHL may be derived from T cells (2798, 3614). Because the neoplastic cells in some cases of peripheral T-cell lymphoma can have an appearance similar to that of HRS cells, the differential diagnosis is challenging. Additionally, some peripheral T-cell lymphomas express CD30 and CD15, making their distinction from CHL even more difficult (277).

Nodular lymphocyte predominant Hodgkin lymphoma (NLPHL)

Due to their expression of B-cell markers, LP cells were more readily identified as B-cell derived [783, 3192, 3193], and this origin was confirmed by rearrangement studies of the IGH gene. These studies also showed that LP cells are clonal expansions of B cells with genotypic features of GCBs, indicating that LP cells constitute a neoplasm of this B-cell type.

Genetic profile

Antigen receptor genes

Rearrangement studies of antigen receptor genes of LP or HRS cells usually provide only reliable results for these cells when DNA of isolated single LP and HRS cells is investigated and not whole tissue DNA.

NLPHL

In all cases LP cells harbour a clonal immunoglobulin gene rearrangement with a high load of somatic mutations and signs of ongoing mutations in the variable (V) region. The rearrangements are usually functional and IG mRNA transcripts are detectable in the LP cells. Consistent with this finding, the transcription factor OCT2 and its coactivator BOB1, which are dominantly involved in the regulation of the expression of IG mRNA, are consistently expressed (3785).

CHL

HRS cells contain clonal immunoglobulin gene rearrangements in more than 98% of cases. The rearranged IGHV genes harbour a high load of somatic hypermutations in the variable (V) region, usually without signs of ongoing mutations. However, the B-cell program is downregulated in CHL, and HRS cells do not express immunoglobulin transcripts. Experimental data from several studies partially explain this defect. In approximately 25% of cases of CHL nonsense mutations in the V region genes were identified, which were proposed to result in the failure of Ig secretion (1934). A major contributory factor is the absent or decreased expression in most cases of CHL of the transcription factors (OCT2, BOB1) that regulate Ig expression (3785). Finally, there is evidence of hypermethylation of multiple genes and pathways in CHL, which may further contribute to the damage to the B-cell programme (2180A). These findings, in conjunction with the study of composite lymphomas consisting of CHL and follicular non-Hodgkin lymphoma, support the assumption that...
Hodgkin lymphomas of B-cell lineage are derived from a GCB [486,2494].

Abnormal gene expression
Despite their derivation from GCBs, HRS cells have lost much of the B-cell–specific expression programme and have acquired B-cell–inappropriate gene products [1083,1838,2178,2498,2560,3594,3786]. In addition, deregulated transcription factors in CHL promote proliferation and abrogate apoptosis in the neoplastic cells. The transcription factor NF-kappaB is constitutively activated in HRS cells, and there is altered activity of the NF-kappaB target genes, which regulate proliferation and survival [1637,1638,1751], the AP1 complex [2559,2560], and the JAK/STAT signalling pathway [3693]. Mutations of the JAK regulator SOCS1 are associated with nuclear STAT5 accumulation in HRS cells, indicating a blockage of the negative feedback loop of the JAK/STAT5 pathway [1879,2625,4297].

EBV infection
The prevalence of EBV in HRS cells varies according to the histological subtype and epidemiological factors. The highest prevalence (~75%) is found in MCCHL and LDCHL and the lowest (5%) in NLPHL [1731,1736]. NSCHL shows an intermediate range of EBV-positivity (10–25%). In resource-poor regions and patients infected with HIV, EBV infection is more frequent, with prevalence approaching 100% in CHL [125,2276,4277,4278]. The prevalent EBV strain also varies across geographical areas. In resource-rich countries, strain 1 prevails; in resource-poor countries, strain 2. Dual infection by both strains is more common in resource-poor countries [466,4277,4278]. It is possible that EBV infection of a B cell replaces one of the genetic alterations necessary for the development of CHL.

Loss of immune surveillance in immunodeficiency settings, such as HIV infection, may predispose individuals to the development of EBV-associated CHL. In tropical regions, resource-poor regions, and patients infected with HIV, as many as 100% of CHL cases are EBV-positive [125,2276,4277,4278]. Most patients with AIDS-associated CHL are EBV-infected. In 1991–1995, AIDS-associated non-Hodgkin lymphomas were 30 times as frequent as were CHLs, but during the period of 2001–2005, the ratio of non-Hodgkin lymphoma cases to CHL cases fell to 7:1. This change might have been influenced by the development of HAART. The risk for the development of CHL appears to be higher in patients with moderately decreased CD4 counts than in patients with very low CD4 counts [1391].

Cytogenetic abnormalities and oncogenes in CHL
Despite their derivation from GCBs, the HRS cells lack much of the B-cell–specific expression programme, and express B-cell–inappropriate gene products, such as CD15, GATA3, TRAF1, ID2, ABF1, JUN, JUNB, AP1, FLIP (CFLAR), JAK/STAT, and STAT5 [1083,1838,2178,2498,2560,3594]. In addition, deregulated transcription factors in CHL promote proliferation and abrogate apoptosis in the neoplastic cells. NF-kappaB–inducing kinase (NIK) is stably expressed in the HRS cells, indicating that NIK and the non-canonical NF-kappaB pathway are very prevalent in CHL [3307]. The transcription factor NF-kappaB is constitutively activated in HRS cells, and there is altered activity of the NF-kappaB target genes, which regulate proliferation and survival [1637,1638,1751], the AP1 complex [2559,2560], and the JAK/STAT signalling pathway [3693]. Mutations of SOCS1, a negative regulator of JAK, are associated with nuclear STAT5 accumulation in HRS cells, indicating a blockage of the negative feedback loop of the JAK/STAT5 pathway [1879,2625,4297]. Despite the frequent overexpression of p53, mutations of TP53 are rare or absent in primary CHL tissue [2711]. CHL has a high incidence of aberrations in the CD274 (also called PDL1) and PDCD1LG2 (also called PDL2) loci at 9p24.1, leading to increased expression of PD1 ligands [3391A]. The 9p24.1 region also includes the JAK2 gene [3453]. JAK2 amplification induces transcription and expression of the PD1 ligand [1436]. Whole-exome sequencing of purified HRS cells has shown the inactivating B2M mutation to be the most frequently detected gene mutation in CHL. This mutation leads to loss of major histocompatibility complex (MHC) class I expression [3334].

Conventional cytogenetic and FISH studies show aneuploidy and hypertetraploidy, consistent with the multinucleation of the neoplastic cells; however, these techniques fail to demonstrate recurrent and specific chromosomal changes in CHL [3524,3557]. However, comparative genomic hybridization reveals recurrent gains of the chromosomal subregions on chromosome arms 2p, 9p, and 12q and distinct high-level amplifications on chromosome bands 4p16, 4q23-24, and 9p23-24 [1880]. The translocations t(14;18) and t(2;5) are absent from HRS cells [1427,2199], but t(14;18) may occur in CHL arising in follicular lymphoma [2811]. One study using interphase cytogenetics found breakpoints in the IGH locus in HRS cells in 17% of CHL cases [2526]. Array comparative genomic hybridization identified copy number alterations in >20% of cases [3780]. Gains in 2p, 9p, 16p, 17q, 19q, and 20q were noted, as were losses of 6q, 11q, and 13q. The affected gene segments harbour genes involved in NF-kappaB signalling, such as REL, IKBKBP, CD40, and MAP3K14.
Genetic susceptibility
Some novel gene loci have been identified as being linked to risk for the development of CHL, irrespective of EBV status (1867,4092). The association between SNP rs6903608 and EBV-negative CHL was limited to the nodular sclerosis histological subtype. Other associations involving HLA class I have been identified in EBV-positive CHLs, mainly of the mixed cellularity subtype (1867). In one study, the allele frequency of HLA-A2 was significantly decreased in the EBV-positive CHL population (1726). Two class II associations were observed to be specific for the EBV-negative population, with an increase of HLA-DR2 and HLA-DR5. HLA-B5 was significantly increased and HLA-DR7 significantly decreased in the total CHL patient population compared with controls. These observations confirm the relevance of histological subtyping of CHL, and in particular the differences between EBV-positive and EBV-negative cases.

Prognosis and predictive factors
CHL
With modern polychemotherapy protocols such as ABVD (i.e. doxorubicin, bleomycin, vinblastine, and dacarbazine) and escalated BEACOPP (bleomycin, etoposide, doxorubicin, cyclophosphamide, vincristine, procarbazine, and prednisone), and with improvements in radiotherapy, CHL is now curable in >85% of cases (790,979). Patients receive stage-adapted treatment after allocation to defined risk groups (early, intermediate, and advanced stages) on the basis of the extent of disease (according to the Ann Arbor system) and the presence or absence of clinical risk factors such as large mediastinal (bulky) mass, extranodal disease, elevated erythrocyte sedimentation rate, and involvement of three (or four) nodal areas. In advanced stages, the International Prognostic Score (IPS), consisting of seven risk factors, correlates with prognosis (1581, 2683). In recent years, interim FDG-PET has been recognized as a valid tool to distinguish between good-risk patients and poor-risk patients requiring more-intensive treatment (70,1281,1581,1744, 1886). In addition to conventional chemotherapy, novel targeted treatment approaches using the histological properties of CHL have become available. The CD30-directed antibody-drug conjugate brentuximab vedotin has already been approved for the treatment of patients with relapsed/refractory CHL, and its combination with conventional chemotherapy is under evaluation (4443). Anti-PD1 antibodies have also been investigated in clinical studies, with promising initial results in patients with relapsed disease (113). Studies using gene expression profiling have identified a signature associated with tumour-infiltrating macrophages associated with an adverse prognosis (3778). In the same study, the authors enumerated macrophages positive for CD68 and correlated the results with clinical outcome. A high content of CD68+ cells correlated with reduced progression-free survival. The significance of macrophage content has been confirmed in some studies (4034,4084), but not in others (98,204,600), and macrophage content is not routinely used as a measure to guide therapy or for prognosis. Notably, a high content of tumour-infiltrating macrophages is a feature of some aggressive forms of CHL, such as the lymphocyte-depleted subtype.

NLPHL
Historically, the treatment of NLPHL has been based on treatment approaches used for CHL. However, approaches have more recently diverged on the basis of clinical observations and differences in underlying biology.
Nodular lymphocyte predominant Hodgkin lymphoma

Definition
Nodular lymphocyte predominant Hodgkin lymphoma (NLPHL) is a B-cell neoplasm usually characterized by a nodular or a nodular and diffuse proliferation of small lymphocytes with single scattered large neoplastic cells known as lymphocyte predominant (LP) or popcorn cells, formerly called L&H cells for lymphocytic and/or histiocytic Reed-Sternberg cell variants. The LP cells are ringed by PD1/CD279+ T cells in almost all instances. In typical cases, the LP cells reside in large nodular meshworks of follicular dendritic cell (FDC) processes that are filled with non-neoplastic lymphocytes (mainly B cells) and histiocytes. They can also grow in an extracellular distribution associated with a diffuse background of T cells. There is increasing evidence that NLPHL cases with a purely diffuse growth pattern overlap with T-cell/histiocyte-rich large B-cell lymphoma (THRLBCL).

ICD-O code 9659/3

Synonyms
Hodgkin lymphoma, lymphocyte predominance, nodular; Hodgkin paragranuloma, NOS (obsolete); Hodgkin paragranuloma, nodular (obsolete)

Epidemiology
NLPHL accounts for approximately 10% of all Hodgkin lymphoma [2230A]. Patients are predominantly male and most are aged 30–50 years.

Localization
NLPHL usually involves cervical, axillary, or inguinal lymph nodes. Mediastinal involvement is rare. Mesenteric lymph node involvement can be seen, unlike in classic Hodgkin lymphoma (CHL). Patients with advanced disease may have involvement of the spleen and bone marrow. Rare cases may have destructive lesions involving bone.

Clinical features
Most patients present with localized peripheral lymphadenopathy (stage I or II). Approximately 20% of patients present with advanced-stage disease [3654].

Microscopy
The lymph node architecture is totally or partially replaced by a nodular, nodular and diffuse, or predominantly diffuse infiltrate consisting of small lymphocytes, histiocytes, epithelioid histiocytes, and intermingled LP cells. A detailed description of growth patterns observed in NLPHL has been given by Fan et al. [1159]. Six distinct immunohistochemical patterns are recognized: pattern A is typical (B-cell-rich) nodular; pattern B is serpiginous nodular; pattern C is nodular with prominent extranodular LP cells; pattern D is T-cell-rich nodular; pattern E is THRLBCL-like, and pattern F is diffuse B-cell-rich. In NLPHLs of patterns A, B, C, and F, the architectural background is composed of variably large spheroidal meshworks of FDCs. A prominence of extranodular LP cells is associated with a propensity to develop a diffuse pattern, with a loss of FDC meshworks, resembling THRLBCL. Such progression to a process with features of THRLBCL is seen more frequently in patients with recurrence [1159]. For lesions that appear totally diffuse on H&E staining, immunostaining is needed to detect the presence of LP cells in association with small B-cells in residual follicular structures. The detection of one such area is sufficient to exclude THRLBCL.

LP cells are large and usually have one large nucleus and scant cytoplasm. The cells have been referred to as popcorn cells due to their nuclei, which are often folded or multilobed. The nucleoli are usually multiple, basophilic, and smaller than those seen in classic Hodgkin/Reed-Sternberg cells. However, some LP cells may contain one prominent nucleolus and/or have more than one nucleus, and thus may be indistinguishable from classic Hodgkin/Reed-Sternberg (HRS) cells on purely cytological grounds. Histiocytes and some polyclonal plasma cells can be found at the margin of the nodules containing LP cells. Neutrophils and...
eosinophils are seldom seen in either the nodular or the diffuse regions. Occasionally, there is reactive follicular hyperplasia (with or without progressive transformation of germinal centres) adjacent to the NLPHL lesions (91,1557). It is uncertain whether the progressively transformed germinal centres are preneoplastic. However, the vast majority of patients with reactive hyperplasia and progressive transformation of germinal centres do not develop Hodgkin lymphoma (1207, 2994). A rim of reactive lymphoid tissue may be seen peripherally to the dominant nodular lesion. Sclerosis is infrequently present in primary biopsies (7%), but can be found more frequently in recurrences (44%). Remnants of small germinal centres are infrequently present in the nodules of NLPHL, a finding more typical of nodular lymphocyte-rich CHL (91,1159).

Immunophenotype

LP cells are positive for CD20, OCT2, CD75, CD79a, BOB1, PAX5, and CD45 in all or nearly all cases (783,3192,3193, 3785). Staining for OCT2 and CD75 is strong and highlights the presence of LP cells; admixed small mantle zone B cells are only weakly positive (472, 3785). J chain is present in most cases and EMA in >50% (91,724,3218,3784) (Table 15.01, p.426). LP cells are positive for BCL6 (1139), but CD10 is absent. Unlike the HRS cells in CHL, LP cells co-express OCT2, BOB1, and activation-induced cytidine deaminase (2762,3785); Staining for immunoglobulin light and/or heavy chains is variable (3565,3566). In 9–27% of cases, the LP cells are IgD-positive, but negative for IgM (1451,3231). The expression of IgD is more common in young males (3231). LP cells lack CD15 and CD30 in nearly all instances. However, CD30+ large cells, which usually constitute reactive immunoblasts unrelated to the LP cells, may be seen (91). Infrequently, the LP cells show weak expression of CD30, and rare cases with CD15 positivity have been reported (4175). As revealed by their nuclear positivity for Ki-67, LP cells are usually in cycle.

FDC meshworks highlighted by CD21 or other FDC-associated antigens are seen in the patterns A, B and C described by Fan et al. (1159). The FDC meshworks are predominantly filled with small bystander B cells and a varying number of T cells of the TFH type typically expressing PD1/CD279 and/or CD57 (3219). PD1+ T cells form rosettes around LP cells in all NLPHL cases with a nodular or a nodular and diffuse growth pattern, and to a lesser and variable extent in the diffuse areas (2822). The rosette formation by PD1+ T cells can therefore serve as a useful additional diagnostic feature. The T cells in NLPHL express molecules such as MAF (also called c-MAF), BCL6, IRF4/MUM1, and CD134, consistent with a subset of germinal centre T cells, but they do not produce IL2 or IL4 (175). Cells positive for TIA1 and CD40 ligand are usually absent, whereas T cells double-positive for CD4 and CD8 detected by flow cytometry are frequent (882,3281). In diffuse growth patterns, the presence of CD4+CD8+/CD57+/PD1+ T cells favours NLPHL, whereas a total absence of small B cells, low numbers of CD57+ T cells, and a dominant presence of CD8+ cells and TIA1+...
cells favour primary THRLBCL. Strongly stained LP cells in association with weakly stained B cells are best identified by OCT2 or PAX5 immunostaining.

Postulated normal counterpart
A germinal centre B cell at the centroblastic stage of differentiation

Genetic profile
LP cells harbour clonally rearranged IG (IGHV) genes [2493,2947]. The clonal rearrangements are usually not detectable in whole-tissue DNA but only in the DNA of isolated single LP cells. The IGHV genes carry a high load of somatic mutations, and also show signs of ongoing mutations. The rearrangements are usually functional, and IG mRNA transcripts are detectable in the LP cells of most cases [2493]. EBV infection detected by EBV-encoded small RNA (EBER) may be found in the LP cells in 3–5% of cases in both children and adults [1736]. EBV positivity might be higher in Asia [653]. EBV may also be present in bystander lymphocytes [91]. BCL6 rearrangements (involving IG genes, IKAROS family genes, ABR, and other partner genes) are present in about half of NLPHLs [174,3343,4341]. Aberrant somatic hypermutations were found in 80% of NLPHL cases, most frequently in PAX5, but also in PIM1, RHOH (also called TTF), and MYC [2359]. Mutations of SGK1, DUSP2, and JUNB are also reported in about half of NLPHLs [1575].

Relationship between NLPHL and THRLBCL
LP cells by gene expression profiling are similar to the cells of THRLBCL and CHL [477]. They show partial loss of their B-cell phenotype, and deregulation of many apoptosis regulators and putative oncogenes. The only investigations that point to distinct pathogeneses of NLPHL and primary THRLBCL are two comparative genomic hybridization studies in which more and different genomic aberrations were identified in NLPHL than in THRLBCL [1246,1247]. Such different genetic patterns were not found in the gene expression profiling study of micro-dissected tumour cells of typical cases of NLPHL and THRLBCL [1570,1571]. These studies revealed, in addition to a common expression of BCL6, CD75, EMA, J chain, and PU1, an identical expression of BAG6 (also called BAT3), HIGD1A, and UBD (also called FAT10) in the tumour cells of cases with a nodular, a nodular and diffuse, and a diffuse growth in NLPHL and primary THRLBCL, suggesting the possibility that THRLBCL may represent a variant or extension of NLPHL. This speculation is supported by the observation that NLPHL can show a THRLBCL-like transformation, which is indistinguishable from primary THRLBCL. It is likely that the distinction between NLPHL and THRLBCL does not lie in different genomic alterations and the immunophenotype of the tumour cells, but rather in the different cellular composition of the microenvironment as described above.

Genetic susceptibility
An increased familial risk of NLPHL has been noted in some families [3466].
However, the specific genetic factors have not been identified. NLPHL has also been identified in patients with Hermansky-Pudlak syndrome type 2 [2391]. The affected patients in that study exhibited NK-cell and T-cell defects. An increased risk is also seen in patients with autoimmune lymphoproliferative syndrome with mutations in FAS [3810], which may also be linked to defective immune surveillance.

Prognosis and predictive factors
NLPHL in its nodular and its nodular and diffuse forms develops slowly, with fairly frequent relapses. It usually remains responsive to therapy and thus is rarely fatal. The prognosis of patients with stage I or II disease is very good, with a 10-year overall survival rate >80% [980, 2888]. It is not yet clear whether immediate therapy is necessary to achieve this favourable prognosis; in some countries (e.g. France), stage I disease (especially in children) is not treated after the resection of the affected lymph node [3125]. Histopathological variants characterized by LP cells outside B-cell nodules, B-cell depletion of the microenvironment, or THRLBCL-like transformation (patterns C, D, E and F as described by Fan et al., Figure 15.17) are associated more often with advanced disease and a higher relapse rate compared with that of typical NLPHL [1572]. Therefore, it is useful to note these variant features in the diagnostic report. Clinically recognized advanced stages have an unfavourable prognosis [980]. Progression to diffuse large B-cell lymphoma has been reported in approximately 3–5% of cases [723,1538,2660]. The neoplastic cells in such cases may resemble LP cells or may have centroblastic or immunoblastic features. However, they keep their typical immunophenotype (strong coexpression of CD20, OCT2, and CD75). In some cases, diffuse large B-cell lymphoma was found to precede NLPHL [1159]. The large B-cell lymphomas associated with NLPHL, if localized, generally have a good prognosis [1538]. A clonal relationship between NLPHL and the associated diffuse large B-cell lymphoma has been demonstrated [1452,1575,4310]. Bone marrow involvement is rare in NLPHL and raises the possibility of THRLBCL, or THRLBCL-like transformation, in particular if the characteristic microenvironment is absent. Cases of NLPHL with bone marrow involvement are clinically aggressive [2003]. Advanced-stage NLPHL responds poorly to the chemotherapy regimens traditionally used for CHL, but responds better to the CHOP chemotherapy regimen plus rituximab (R-CHOP), or regimens used for aggressive B-cell lymphomas [4387].
Classic Hodgkin lymphoma (CHL) is a monoclonal lymphoid neoplasm derived from B cells, composed of mononuclear Hodgkin cells and multinucleated Reed-Sternberg cells in a background containing a variable mixture of non-neoplastic reactive immune cells, including small lymphocytes, eosinophils, neutrophils, histiocytes, and plasma cells. On the basis of the characteristics of the reactive infiltrate and to a certain extent the morphology of the Hodgkin/Reed-Sternberg (HRS) cells, four histological subtypes have been distinguished: nodular sclerosis CHL, lymphocyte-rich CHL, mixed cellularity CHL, and lymphocyte-depleted CHL. The HRS cells in all forms of CHL share similar immunophenotypic features, with reduced expression of most B-cell antigens (CD20, CD79a, PAX5) and positive staining for CD30 and CD15 in most cases. The association with EBV varies across subtypes, being most commonly positive (i.e. in as many as 75% of cases) in mixed cellularity CHL and lymphocyte-depleted CHL. The four subtypes of CHL also differ in their epidemiological features, clinical presentation, and prevalence of systemic symptoms, as discussed in the following text. These observations suggest potential differences in underlying biology and pathogenesis. It is customary to subclassify CHL as one of the four subtypes, but with limited biopsy material, precise subclassification is not always feasible, and the diagnosis of CHL, NOS, is sometimes made. It remains important to distinguish these cases from nodular lymphocyte predominant Hodgkin lymphoma.

**Definition**

Nodular sclerosis classic Hodgkin lymphoma (NSCHL) is a subtype of classic Hodgkin lymphoma (CHL) characterized by collagen bands that surround at least one nodule, and by HRS cells with lacunar-type morphology.

**ICD-O code**

9663/3

**Synonyms**

Hodgkin disease, nodular sclerosis, NOS; Hodgkin lymphoma, nodular sclerosis, grade 1 (9665/3); Hodgkin lymphoma, nodular sclerosis, grade 2 (9667/3); Hodgkin lymphoma, nodular sclerosis, cellular phase (9664/3)

**Epidemiology**

NSCHL accounts for approximately 70% of all CHLs in Europe and the USA. However, the rate varies greatly among other geographical regions; NSCHL is more common in resource-rich than in resource-poor areas, and the risk is highest among those with high socioeconomic status [762]. The incidence of NSCHL is similar in males and females, and peaks among individuals aged 15–34 years [184,2759].

**Localization**

Mediastinal involvement occurs in 80% of cases, bulky disease in 54%, splenic and/or lung involvement in 8–10%, bone involvement in 5%, bone marrow involvement in 3%, and liver involvement in 2% [782,3654].

**Clinical features**

Most patients present with Ann Arbor stage II disease. B symptoms are encountered in approximately 40% of cases [3654] and are more frequent with advanced-stage disease.

**Macroscopy**

The cut surface of lymph nodes typically shows a nodular configuration, with cellular nodules surrounded by dense fibrosis. With higher-grade lesions (grade 2), central areas of necrosis may be evident. Following therapy, a persistent mass lesion may be present, with diffuse fibrotic replacement and no viable involvement by Hodgkin lymphoma. Such lesions may persist radiologically, but should not be negative by PET, confirming the absence of active disease.

---

**Fig. 15.21** Nodular sclerosis classic Hodgkin lymphoma. A CT shows a large anterior mediastinal mass. B Chest X-ray of the same patient shows a mediastinal mass exceeding one third of the chest diameter.
Fig. 15.22 Nodular sclerosis classic Hodgkin lymphoma. In this excised mediastinal mass, cellular nodules with a more yellowish-tan appearance are surrounded by dense fibrosis, which is white in colour.

Microscopy
The lymph nodes have a nodular growth pattern, with nodules surrounded by collagen bands (nodular sclerosis). The broad fibroblast-poor collagen bands surround at least one nodule. This fibrosing process is usually associated with a thickened lymph node capsule. The lymphoma contains a highly variable number of HRS cells, small lymphocytes, and other non-neoplastic inflammatory cells. The HRS cells tend to have more-segmented nuclei with smaller lobes, less prominent nucleoli, and a larger amount of cytoplasm than do HRS cells in other types of CHL. In formalin-fixed tissues, the cytoplasm of the HRS cells frequently shows retraction of the cytoplasmic membrane, so that the cells seem to be sitting in lacunae. These cells have therefore been designated lacunar cells. Lacunar cells may form cellular aggregates, which may be associated with necrosis and a histiocytic reaction, resembling necrotizing granulomas. When aggregates are very prominent, the term ‘syncytial variant’ has been used. Eosinophils, histiocytes, and (to a lesser extent) neutrophils are often numerous [3178]. Grading according to the proportion of HRS cells or the characteristics of the background infiltrate (e.g. the number of eosinophils) may predict prognosis in some settings, but is not necessary for routine clinical purposes [1630,2430,4141,4210]; it may serve a research purpose in protocol studies.

Fig. 15.23 Nodular sclerosis classic Hodgkin lymphoma (NSCHL). This lymph node was obtained following successful treatment for NSCHL. The lymph node shadow persisted on X-ray. Histological examination showed nodules composed of dense collagen, without evidence of Hodgkin/Reed–Sternberg cells. The internodular regions contained a scant inflammatory infiltrate of lymphocytes and plasma cells in an oedematous background.

Fig. 15.24 Thymic cyst arising with thymic involvement by Hodgkin lymphoma. Cystic degeneration of the thymus gland is common with involvement by nodular sclerosis classic Hodgkin lymphoma.

Fig. 15.25 Nodular sclerosis classic Hodgkin lymphoma, grade 2. Capsular fibrosis is present. Central areas of necrosis are noted in the nodular infiltrate.

Fig. 15.26 Nodular sclerosis classic Hodgkin lymphoma, grade 2. Lacunar Hodgkin/Reed–Sternberg cells palisade around central necrotic area containing numerous neutrophils.

Fig. 15.27 Nodular sclerosis classic Hodgkin lymphoma with aberrant CD3. The neoplastic cells are positive for CD3. Expression of PAX5 (not shown) and negative studies for TR gene rearrangement helped to confirm the diagnosis.

Fig. 15.28 Nodular sclerosis classic Hodgkin lymphoma. This case had aberrant expression of CD2, a finding usually seen in grade 2 disease. In this case, the CD2 antigen appears to be partially expressed on the outer surface of the cell membrane, suggestive of adsorption.

Fig. 15.29 Nodular sclerosis classic Hodgkin lymphoma. Lacunar cells show artificial retraction of the cytoplasm upon fixation. The nucleoli are often smaller than those seen in classic Reed–Sternberg cells.
Immunophenotype
The malignant cells exhibit a CHL phenotype; however, association with EBV as demonstrated by EBV-encoded small RNA (EBER) or the EBV-encoded LMP1 is less frequent (10-25%) than in mixed cellularity CHL (1612,1614,4285,4289). CD30 is expressed in nearly all cases, but CD15 may be negative in 15-25% of cases. PAX5 is weakly positive in nearly all cases, CD20 may be variably expressed, but is usually weak and only on a subset of the neoplastic cells. CD79a is positive in approximately 10% of cases [4086]. Approximately 5% of NSCHLs abnormally express T-cell antigens [4083], most commonly cases of high histological grade (grade 2). The T-cell antigens most often expressed are CD4 and CD2, and less commonly CD3; positive cases are associated with shorter overall and event-free survival compared with CHL negative for T-cell antigens [4176]. A pitfall in such cases is misdiagnosis as ALK-negative anaplastic large cell lymphoma. Nearly all cases (>90%) with aberrant T-cell antigen expression are positive for PAX5, consistent with a diagnosis of CHL. Gene rearrangement studies help to confirm the diagnosis, being negative for clonal rearrangement of TR genes and often positive for clonal rearrangements of IG. The basis for the aberrant T-cell antigen expression is unknown. In some cases, the aberrant T-cell antigen appears to be adsorbed to the surface of the neoplastic cells, but in other cases it appears to be a product of cell synthesis, and staining may also be observed in the Golgi region.

Grading
A grading system for NSCHL was proposed by the British National Lymphoma Investigation (BNLI). Two histological grades were proposed, which in some series show correlation with clinical features (2429,4313). According to these criteria, nodular sclerosis is classified as grade 2 if >25% of the nodules show pleomorphic or reticular lymphocyte depletion; if >80% of the nodules show features of the fibrohistiocytic variant; or if >25% of the nodules show numerous bizarre, anaplastic-appearing Hodgkin cells without lymphocyte depletion. When these criteria are applied, approximately 15-25% of cases are classified as grade 2. In some older series, higher-grade cases of nodular sclerosis were referred to as the lymphocyte-depleted subtype of nodular sclerosis [965]. Grading is not mandatory for clinical purposes, but has been investigated as a prognostic feature in some clinical trials. However, the importance of grading is declining due to advances in therapy, which obscure the differences seen in less-effectively treated patients (2539,4141). The fibrohistiocytic variant of NSCHL may mimic a reactive process or a mesenchymal neoplasm. Fibroblasts and histiocytes are abundant, and the HRS cells may be difficult to identify without immunohistochemical staining. In such cases, PAX5 and CD30 are valuable, because CD20 is typically negative. In the syncytial variant of nodular sclerosis, the lacunar cells form cohesive nests in the centres of the nodules. Necrosis may or may not be present, but if prominent should prompt consideration for grade 2 disease. The syncytial variant may prompt consideration for anaplastic large cell lymphoma or even a non-lymphoid neoplasm [588]. Positivity for PAX5 is helpful in ruling out anaplastic large cell lymphoma. However, some cases of CHL may have a cytotoxic phenotype [163].

Prognosis and predictive factors
Overall, the prognosis of NSCHL is better than that of other types of CHL [70]. Massive mediastinal disease is an adverse prognostic factor [3745]. In the modern era, grading of NSCHL is less significant as an independent risk factor than in the past [4141]. However, grade may be more relevant in patients with advanced-stage disease, whereas it has little impact in patients with localized disease.
**Lymphocyte-rich classic Hodgkin lymphoma**

Anagnostopoulos I.
Piris M.A.
Isaacson P.G.
Jaffe E.S.
Stein H.

**Definition**
Lymphocyte-rich classic Hodgkin lymphoma (LRCHL) is a subtype of classic Hodgkin lymphoma (CHL) characterized by scattered Hodgkin/Reed-Sternberg (HRS) cells and a nodular or (less often) diffuse cellular background consisting of small lymphocytes, with an absence of neutrophils and eosinophils.

**ICD-O code**
9651/3

**Synonyms**
Hodgkin disease, lymphocytic-histiocytic predominance (obsolete); Hodgkin disease, lymphocyte predominance, diffuse (obsolete); Hodgkin disease, lymphocyte predominance, NOS (obsolete)

**Epidemiology**
LRCHL accounts for approximately 5% of all CHLs, occurring at a frequency slightly less than that of nodular lymphocyte predominant Hodgkin lymphoma (NLPHL). The median patient age is similar to that of NLPHL and significantly older than seen in other subtypes of CHL [980]. The male-to-female ratio is 2:1 [3654].

**Localization**
Peripheral lymph nodes are typically involved. Mediastinal involvement and bulky disease are uncommon [980,3654].

**Clinical features**
Most patients present with stage I or II disease. B symptoms are rare. The clinical features are similar to those of NLPHL, with the exception that multiple relapses seem to occur less frequently [980]. Patients are also older than those with NLPHL or the nodular sclerosis subtype.

**Microscopy**
There are two growth patterns: the common nodular pattern [91] and the rare diffuse pattern [91]. The nodules of the nodular variant encompass most of the involved tissue, so that the T-zone is attenuated. The nodules are composed of small lymphocytes, with an absence of neutrophils and eosinophils.

**Fig. 15.33** Lymphocyte-rich classic Hodgkin lymphoma, nodular variant. CD30 staining highlights the presence of Hodgkin/Reed-Sternberg cells. They are located within or at the peripheral margin of the follicular mantles, but not within the germinal centres.

**Fig. 15.34** Lymphocyte-rich classic Hodgkin lymphoma, nodular variant. Cellular nodules are composed of small lymphocytes surrounding regressed germinal centres.

**Fig. 15.35** Lymphocyte-rich classic Hodgkin lymphoma, nodular variant. Classic Reed-Sternberg cells are surrounded by small lymphocytes.

**Fig. 15.36** Lymphocyte-rich classic Hodgkin lymphoma. CD20 immunostain identifies small lymphocytes and is negative in HRS cells.

**Fig. 15.37** Lymphocyte-rich classic Hodgkin lymphoma. HRS cells in perifollicular region are highlighted by CD30 immunostain.

**Fig. 15.38** Lymphocyte-rich classic Hodgkin lymphoma. CD3-positive T cells form a rosette around an HRS cell.

**Fig. 15.39** Lymphocyte-rich classic Hodgkin lymphoma. CD15 immunostain of HRS cells.
lymphocytes and may harbour germinal centres, which are usually eccentrically located and relatively small or regressed. The HRS cells are predominantly found within the nodules, but consistently outside of the germinal centres. A proportion of the HRS cells may resemble lymphocyte predominant (LP) cells or mononuclear lacunar cells. This subtype can easily be confused with NLPHL. In the past, approximately 30% of cases initially diagnosed as NLPHL were later found to be LRCHL [91]. The demonstration of an immunophenotype typical of classic HRS cells is essential in making this distinction. Eosinophils and/or neutrophils are absent from the nodules, or if present, are located in the interfollicular zones and are few in number. In rare instances, the LRCHL-typical nodules may be surrounded by fibrous bands associated with randomly distributed HRS cells in T-cell–rich zones. Typing of these cases as nodular sclerosis CHL might be more appropriate. In some cases, sequential biopsies have shown nodular sclerosis CHL, implying a possible relationship between the two subtypes of CHL; this is further inferred by the finding of HRS cells within expanded follicular mantle zones in some cases of nodular sclerosis CHL [1780]. Coexistence of LRCHL and mixed cellularity CHL occurs but is rare.

In diffuse LRCHL cases, the small lymphocytes of the cellular background may be admixed with histiocytes with or without epithelioid features.

**Immunophenotype**

The immunophenotype of the neoplastic cells and their microenvironment display a mixture of features of NLPHL and CHL. The atypical cells in LRCHL show the same immunophenotype (CD30+, CD15+/−, IRF4/MUM1+, PAX5+/−, CD20−/+, J chain−, CD75−, PU1−, EBV/LMP1+ for EBV-harbouring cases) as the HRS cells in the other subtypes of CHL. Thus, the distinction of LRCHL from NLPHL is possible by immunophenotyping in nearly all instances [91]. The expression of B-cell transcription factors such as OCT2, BOB1, and BCL6 has been found to be more frequent in LRCHL than in the other CHL subtypes [2821]. Rosettes with a T follicular helper (TFH) cell immunophenotype (PD1/CD279+, CD57−/−) surrounding the neoplastic cells are present in as many as 50% of cases [2821]. The small lymphocytes in the nodules have the features of mantle cells (i.e. positivity for IgM and IgD). Thus, the nodules predominantly constitute expanded mantle zones. At least some of them contain eccentrically located, usually small, germinal centres, which are highlighted by a dense meshwork of CD21+ follicular dendritic cells. Because intact germinal centres are infrequent in NLPHL, this feature is helpful in differential diagnosis.
Mixed cellularity classic Hodgkin lymphoma

Weiss L.M.
Poppema S.
Jaffe E.S.
Stein H.

Definition
Mixed cellularity Hodgkin lymphoma (MCCHL) is a subtype of classic Hodgkin lymphoma (CHL) characterized by classic Hodgkin/Reed-Sternberg (HRS) cells in a diffuse mixed inflammatory background. Fine interstitial fibrosis may be present, but fibrous bands are absent and capsular fibrosis is usually absent. This subtype of CHL is frequently (i.e. in ~75% of cases) associated with EBV.

ICD-O code
9652/3

Fig. 15.43 Mixed cellularity classic Hodgkin lymphoma. A The mixed cellular infiltrate does not contain fibrotic bands. B A typical binucleated Reed-Sternberg cell in a mixed cellular infiltrate with lymphocytes, macrophages, and eosinophils is visible.

Fig. 15.44 Mixed cellularity classic Hodgkin lymphoma. CD30-negative histiocytes, with a pronounced epithelioid differentiation, forming clusters, predominate. CD30 immunostaining highlights the presence of a large Reed-Sternberg cell and a small mononuclear variant.

Prognosis and predictive factors
With modern risk-adjusted treatment, overall and progression-free survival rates are slightly better than those of the other subtypes of CHL and similar to those of NLPHL, except that relapses are more common in NLPHL than in LRCHL [91,980,3654].
**Lymphocyte-depleted classic Hodgkin lymphoma**

Benharroch D.
Jaffe E.S.
Stein H.

**Definition**

Lymphocyte-depleted classic Hodgkin lymphoma (LDCHL) is a diffuse form of classic Hodgkin lymphoma (CHL) rich in Hodgkin/Reed–Sternberg (HRS) cells and/or depleted of non-neoplastic lymphocytes. Histiocytes are usually abundant. Prior to the introduction of modern immunohistochemical studies, LDCHL was often mistaken for other entities, mainly for aggressive forms of other B-cell or T-cell lymphomas (1930). When stringent diagnostic criteria are applied, LDCHL accounts for <2% of all Hodgkin lymphomas. It is more common in developing countries and is also seen with HIV infection. Like mixed cellularity CHL, it is frequently positive for EBV (i.e. in ~75% of cases). Most patients present with advanced-stage disease and B symptoms (2042).

**ICD-O code**

9653/3

**Synonyms**

Hodgkin lymphoma disease, lymphocyte depletion, NOS; classic Hodgkin lymphoma, lymphocyte depletion, NOS; Hodgkin lymphoma, lymphocyte depletion, diffuse fibrosis (9654/3); Hodgkin lymphoma, lymphocyte depletion, reticular (9655/3)

**Epidemiology**

This is the rarest CHL subtype; it accounts for <1% of cases in western countries (2042), but may be more common in the developing world (334). About 60–75% of patients are male and the age ranges from 30 to 71 years. Others have described LDCHL to an equal extent in women (3695). This subtype is often associated with HIV infection. HIV prevalence was found in 3.8% of Hodgkin lymphoma patients, including 15.1% of LDCHL patients (3649).

**Localization**

LDCHL has a predilection for the retroperitoneal lymph nodes, abdominal organs, and bone marrow. Peripheral lymphadenopathy may also be seen (2042).

**Clinical features**

Widespread involvement (including of the subdiaphragmatic region and bone marrow at diagnosis), with B symptoms, supports an aggressive behaviour. However, peripheral lymph nodes are also affected (334,3695).

**Microscopy**

Although the appearance of LDCHL is highly variable, a unifying feature is the relative predominance of HRS cells and the scarcity of background lymphocytes in relation to the neoplastic cells (2484). Two patterns are seen. In one, there is diffuse fibrosis, in which prominent fibroblastic proliferation is seen. However, well-formed fibrous bands are absent. In this variant, the tumour microenvironment contains numerous histiocytes and some small lymphocytes, but usually...
lacks significant numbers of plasma cells or eosinophils. The second pattern is rich in neoplastic cells, often with anaplastic and pleomorphic features (3695). These two variants correspond to the two subtypes initially described in the Lukes–Butler classification, diffuse fibrosis and reticular (2412).

**Immunophenotype**
The immunophenotype is similar to that of other forms of CHL. EBV/LMP1 is frequently positive. Coexpression of CD30 and PAX5 helps to differentiate LDCHL from ALK-negative anaplastic large cell lymphoma. Either OCT2 or BOB1 may be expressed in HRS cells, but usually not both. CD79a is typically negative (3695). Strong expression of B-cell markers such as CD20 and CD79a should prompt consideration for a diagnosis of EBV-positive diffuse large B-cell lymphoma, in which HRS-like cells may be seen.

**Genetic profile**
IGH gene rearrangement shows B-cell clonality, which can be more readily detected due to the paucity of normal B cells and relative abundance of tumour cells (3695).

**Prognosis and predictive factors**
Patients with LDCHL have more adverse risk factors than do patients with other forms of CHL (1947,2042). SEER data indicate a 5-year survival rate of 48.8% (65). However, outcome has been less adverse in prospective clinical trials. Patients with LDCHL are more likely to have advanced-stage disease and B symptoms. However, with effective therapy, complete remission was achieved in 82% of patients with LDCHL, versus in 93% of patients with other Hodgkin subtypes (2042). At 5 years, survival was significantly worse, with progression-free survival rates of 71% versus 85% (P <0.001) and overall survival rates of 83% versus 92% respectively (P = 0.0018). Patients who underwent more-intensive therapy regimens appeared to fare better (2042), suggesting that patients with LDCHL benefit from treatment with dose-intensive treatment strategies.
CHAPTER 16

Immunodeficiency-associated lymphoproliferative disorders
Lymphoproliferative diseases associated with primary immune disorders

Definition
Lymphoproliferative diseases associated with primary immune disorders (PIDs) are lymphoid proliferations that arise in the setting of immune deficiency due to a primary immunodeficiency or immunoregulatory disorder. Because the pathology and pathogenesis of the >60 PIDs are heterogeneous, the manifestations of these lymphoproliferative diseases are highly variable. The PIDs most frequently associated with lymphoproliferative disorder are ataxia-telangiectasia (AT), Wiskott–Aldrich syndrome (WAS), common variable immunodeficiency (CVID), severe combined immunodeficiency (SCID), X-linked lymphoproliferative disease (XLP), Nijmegen breakage syndrome (NBS), hyper-IgM syndrome (HIgM), and autoimmune lymphoproliferative syndrome (ALPS).

Epidemiology
Patients with PIDs have an increased incidence of lymphomas (1878,3635). Age-specific mortality rates for all neoplasms in patients with PIDs are 10–200 times the expected rates for the general population. However, given that PIDs are rare, the overall occurrence of PID-associated lymphoproliferative disorder is low, accounting for 2.4% of all paediatric lymphoma cases (144). With the exception of CVID, these diseases present primarily in the paediatric age group. They are more common in males than in females, primarily because several of the primary genetic abnormalities are X-linked, for example, XLP, SCID, and HIgM (1871). It should be kept in mind that children can present with a lymphoproliferation without the underlying immunodeficiency being known. Especially in polymorphous lesions, detection of EBV may indicate an underlying immune deficiency.

Etiology
The cause of the lymphoproliferative disorder is related to the underlying primary immune defect (2897). EBV is involved in most PID-associated lymphoid proliferations (4136). In these cases, defective T-cell immune surveillance to EBV is believed to be the primary mechanism (1628,3073,3251). The absence of T-cell control may be complete (resulting in fatal infectious mononucleosis) or partial (resulting in other lymphoproliferative disorders) (1823). WAS is a complex immune disorder, with defects in function of T cells, B cells, neutrophils, and macrophages. T-cell dysfunction is significant, and tends to increase in severity during the course of the disease.

HIgM results from mutations in the gene for CD40 or CD40 ligand, which affect interactions between T cells and B cells and impair effective differentiation of B cells into class-switched plasma cells (1767). In ALPS, mutations in FAS or FASLG (and rarely other abnormalities) may contribute directly to lymphoid proliferations, through the accumulation of lymphoid cells that fail to undergo apoptosis, resulting in the accumulation of CD4− and CD8− cells in the peripheral blood and lymphoid tissues (2331,3711). In ALPS, the severity of the apoptotic defect correlates directly with the risk of development of lymphoproliferative disorder (1822). The importance of FAS mutations in causing lymphoproliferative disorder is supported by the fact that sporadic FAS mutations are associated with lymphomas in the absence of immune abnormalities (1483).

In AT, an abnormal DNA repair mechanism due to mutations of ATM can contribute to the development of lymphoma, leukaemia, and other neoplasms (1090). In these cases, non-leukaemic T-cell clones that have translocations involving the TR genes similar to those seen in overt leukaemias can be detected in the peripheral blood. B-cell non-Hodgkin lymphoma, Hodgkin lymphoma, and lymphoblastic leukaemia occur at a high rate and earlier age than do carcinomas in AT. T-cell prolymphocytic leukaemias are rarer than initially reported. Prognosis is poor, but patients may benefit from treatment, with an improved survival (3823).

NBS also results from defects in DNA repair due to mutations in the NBN gene (also called NBS1), resulting in many chromosomal breaks and translocations, including in antigen receptor genes. In patients with NBS, lymphoproliferative disorder is the most common neoplasm (565,2880,4158). In patients with CVID, marked lymphoid hyperplasia may occur in the lungs and gastrointestinal tract (3507), a setting in which more aggressive-lymphoproliferative disorder or overt lymphoma may develop (959).
Table 16.01 Clinical features of the primary immune disorders (PIDs)

<table>
<thead>
<tr>
<th>Type of PID</th>
<th>Frequency (among all PIDs)</th>
<th>Genes or proteins implicated</th>
<th>Most common abnormalities</th>
<th>Most common associated lymphoproliferative disorders (percentage)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Combined T-cell and B-cell immunodeficiencies</td>
<td>9–18%</td>
<td></td>
<td>Recurrent severe bacterial, fungal, and viral infections, including opportunistic infections; skin rash</td>
<td>EBV-associated lesions, fatal IM (nearly 100%)</td>
</tr>
<tr>
<td>Severe combined immunodeficiency</td>
<td>1–5%</td>
<td>Gamma chain of IL2R, IL4R, IL7R, IL9R, IL15R, IL21R; JAK3 kinase; IL7R, CD45, CD3-delta or CD3-epsilon; RAG1/2, Artemis, ADA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD40 ligand and CD40 deficiencies (hyper-IgM syndrome)</td>
<td>1–2%</td>
<td>CD40 ligand (CD40L, CD154) or CD40</td>
<td>Neutropenia, thrombocytopenia, haemolytic anaemia, biliary tract and liver disease, opportunistic infections</td>
<td>EBV-associated lesions (DLBCL, Hodgkin lymphoma), large granular lymphocytic leukaemia</td>
</tr>
<tr>
<td>Predominantly antibody PIDs</td>
<td>53–72%</td>
<td>Unknown</td>
<td>Bacterial infections (lung, gastrointestinal tract), autoimmune cytopenias, granulomatous disease (lung, liver)</td>
<td>EBV-associated lesions (DLBCL, Hodgkin lymphoma), extranodal marginal zone lymphoma, small lymphocytic lymphoma, lymphoplasmyocytic lymphoma, PTCL (rare) (2–7%)</td>
</tr>
<tr>
<td>Common variable immunodeficiency</td>
<td>21–31%</td>
<td>Unknown</td>
<td>EBV-associated lesions (DLBCL, Hodgkin lymphoma), lymphomatoid granulomatosis (3–9%)</td>
<td></td>
</tr>
<tr>
<td>Other well-defined immunodeficiency syndromes</td>
<td>5–22%</td>
<td></td>
<td>E</td>
<td></td>
</tr>
<tr>
<td>Wiskott–Aldrich syndrome</td>
<td>1–3%</td>
<td>WAS (also called WASP)</td>
<td>Thrombocytopenia, small platelets, eczema, autoimmune disease, bacterial infections</td>
<td>EBV-associated lesions (DLBCL, Hodgkin lymphoma), lymphomatoid granulomatosis (3–9%)</td>
</tr>
<tr>
<td>Ataxia-telangiectasia</td>
<td>2–8%</td>
<td>ATM</td>
<td>Ataxia, telangiectasias, increased AFP, increased sensitivity to ionizing radiation</td>
<td>Non-leukaemic clonal T-cell proliferations, DLBCL, BL, T-PLL, T-ALL, Hodgkin lymphoma (10–30%)</td>
</tr>
<tr>
<td>Nijmegen breakage syndrome</td>
<td>1–2%</td>
<td>NBN (nibrin, also called NBS1)</td>
<td>Microcephaly, progressive mental retardation, sensitivity to ionizing radiation, predisposition to cancer</td>
<td>DLBCL, PTCL, T-ALL/LBL, Hodgkin lymphoma (28–36%)</td>
</tr>
<tr>
<td>Diseases of immune dysregulation</td>
<td>1–3%</td>
<td></td>
<td>EBV-triggered abnormalities (fatal IM, hepatitis, aplastic anaemia), lymphoma</td>
<td>EBV-associated lesions (BL, DLBCL) (nearly 100%)</td>
</tr>
<tr>
<td>X-linked lymphoproliferative disease</td>
<td>&lt;1%</td>
<td>SH2D1A</td>
<td>Defective lymphocyte apoptosis, splenomegaly, adenopathy, autoimmune cytopenias, recurrent infections</td>
<td>NLPHL, CHL, DLBCL, BL, PTCL (rare) (CHL, DLBCL, and BL may be EBV+ or EBV−) (3–10%)</td>
</tr>
<tr>
<td>Autoimmune lymphoproliferative syndrome (Canale-Smith syndrome)</td>
<td>&lt;1%</td>
<td>FAS (type 1a), FASLG (type 1b), CASP10 (caspase 10; type 2a), or CASP8 (caspase 8; type 2b)</td>
<td>EBV-associated lesions (BL, DLBCL) (nearly 100%)</td>
<td></td>
</tr>
</tbody>
</table>

ADA, adenosine deaminase; AFP, alpha-fetoprotein; BL, Burkitt lymphoma; CHL, classic Hodgkin lymphoma; DLBCL, diffuse large B-cell lymphoma; IM, infectious mononucleosis; NLPHL, nodular lymphocyte predominant Hodgkin lymphoma; PTCL, peripheral T-cell lymphoma; T-ALL, T-lymphoblastic leukaemia; T-ALL/LBL, T-lymphoblastic leukaemia/lymphoma; T-PLL, T-cell prolymphocytic leukaemia.

a Data compiled from reports of several national and international registries.
b Percentages, where provided, indicate the approximate proportion of patients in whom lymphoproliferative disorder develops.

Localization
The presentation depends on the underlying disease. More often, lymphoproliferative disorders present in extranodal sites, most commonly the gastrointestinal tract, lungs, and CNS.

Clinical features
Patients often present with symptoms resembling those of infection or neoplasia (i.e., fever, fatigue, and infectious mononucleosis–like syndromes). In some diseases, such as ALPS and XLP, the lymphoid proliferation is the first sign of the underlying immune defect, but in most patients, the diagnosis of PID has already been established because of other manifestations (Table 16.01).
Microscopy
As in other immune deficiency states, lymphoid proliferations in patients with primary immunodeficiency include reactive hyperplasias, polymorphous lymphoid infiltrates similar to those seen in the post-transplant setting, and frank lymphomas that do not differ from those in immunocompetent hosts. The type and frequency of each lesion differ among the PIDs [4099] (see Table 16.01, p. 445).

Non-neoplastic lesions
Primary EBV infection in PID may result in fatal infectious mononucleosis, characterized by a highly polymorphous proliferation of lymphoid cells showing evidence of plasmacytoid and immunoblastic differentiation. Reed-Sternberg-like cells may be seen. This condition is primarily seen in patients with XLP (Duncan disease) [1372] and SCID [3251]. The abnormal B-cell proliferation is systemic, involving both lymphoid and non-lymphoid organs, most commonly the terminal ileum. Haemophagocytic syndrome is frequent, and is most readily identified in bone marrow aspirates. In CVID, waxing and waning lymphoproliferations may occur in lymph nodes and extranodal sites, with variable morphology (including follicular hyperplasia) and paracortical expansion with many EBV-positive cells, often including large atypical cells that may resemble Reed–Sternberg cells. CVID is characterized by nodular lymphoid hyperplasia in the gastrointestinal tract; clonality testing may detect clonal B-cell populations that are a sign of more aggressive disease but may also be self-limited [2227, 3507, 4128]. In ALPS, expansions of double-negative (CD4+/CD8−), alpha beta CD45RA+, CD45RO− T cells in peripheral blood, lymph nodes, spleen, and other tissues are the hallmark of the disease. T-cell expansion can be very marked, and the T cells may have slightly immature chromatin, which can lead to a mistaken diagnosis of T-cell lymphoma, especially when (as is usual) the patient does not carry a pre-existing diagnosis of ALPS [2331]. Follicular hyperplasia is often prominent, and progressively transformed germinal centres may be seen [2331].

HlgM is characterized by circulating peripheral blood B cells that bear only IgM and IgD. Germinat centres are absent in lymph nodes. IgM-producing plasma cells often accumulate, most commonly in extranodal sites, such as the gastrointestinal tract, liver, and gallbladder. These lesions may be so extensive as to be fatal, without progression to clonal lymphoproliferative disorder.

Lymphomas
Lymphomas occurring in patients with PIDs do not generally differ in their morphology from those occurring in immunocompetent hosts. Lymphomatoid granulomatosis, an EBV-driven proliferation of B cells associated with a marked T-cell infiltration, is increased in frequency in patients with WAS [1754]. The most common sites of involvement are the lungs, skin, brain, and kidneys. Diffuse large B-cell lymphoma is the most common type of lymphoma seen in PIDs in general; classic Hodgkin lymphoma and Burkitt lymphoma [4007], as well as peripheral T-cell lymphoma [3146], also occur. In AT, and to a lesser extent in NBS, T-cell lymphomas and leukemias are more common than B-cell neoplasms [3910]. Rare cases of true peripheral T-cell lymphoma have been
seen in patients with ALPS [3146,3810]. Both T-lymphoblastic leukaemia/lymphoma and T-cell prolymphocytic leukaemia have been reported in PIDs.

**Hodgkin lymphoma**

Hodgkin lymphoma–like lymphoproliferations resembling those seen in the setting of methotrexate therapy, as well as lymphoproliferative disorder with all morphological and phenotypic features of classic Hodgkin lymphoma, has been reported in patients with WAS or AT [1090,3476]. In ALPS, nodular lymphocyte predominant Hodgkin lymphoma, classic Hodgkin lymphoma, and T-cell/histiocyte-rich large B-cell lymphoma have been described [2331].

**Precursor lesions**

The underlying PID is the principal precursor lesion leading to the development of lymphoproliferative disorder. This morphological spectrum is accompanied by an increasing dominant clonal population: from clearly polyclonal, to oligoclonal, to monoclonal. However, monoclonal expansions, particularly if they are minor clones, do not necessarily progress to major persistent clonal lesions [2227]. Nevertheless, the detection of a dominant clone indicates a more aggressive disease [4128].

**Immunophenotype**

**Non-neoplastic proliferations**

In ALPS, there is expansion of a distinctive CD3+, CD4−, CD8−, CD45RA+, CD45RO− naive T-cell population in the peripheral blood and bone marrow. The T cells may express CD57 but not CD25. Increased numbers of CD5+ polyclonal B cells may also be seen [2331]. HlgM is characterized by peripheral blood B cells that bear only IgM and IgD.

**Neoplasms**

Most of the lymphomas in patients with PID are of B-cell lineage, and thus express B-cell antigens corresponding to their differentiation stage. EBV infection of B cells often leads to downregulation of B-cell antigens. Thus, CD20, CD19, and CD79a may be negative or expressed on only some of the neoplastic cells in EBV-positive lymphoproliferative disorder. Similarly, EBV leads to the expression of CD30 in most cases. In patients with EBV-positive lymphoproliferative disorder resulting from defective immune surveillance, the latency genes including LMP1 may be expressed. In cases showing evidence of plasmacytoid differentiation, monotypic cytoplasmic immunoglobulin may be identified. The immunophenotypes of the specific B-cell and T-cell lymphomas in PIDs do not differ from those of the same lymphomas in immunocompetent patients.
Genetic profile

Antigen receptor genes

Because lymphoproliferative disorder in PID is a spectrum from reactive to aggressive lymphoproliferations, the proliferations can be polyclonal, oligoclonal, or monoclonal. Fatal infectious mononucleosis is generally polyclonal; overt lymphomas such as diffuse large B-cell lymphoma and Burkitt lymphoma have clonal IG heavy and light chain gene rearrangement (1090,2227,3507). There is only limited experience with T-cell clonality tests in the setting of PIDs.

Cytogenetic abnormalities and oncogenes

Genetic alterations may be directly related to the primary immune defect, such as FAS mutation in patients with ALPS, mutations of the SH2D1A gene encoding for SAP/SLAM in XLP, and many chromosomal breaks in NBS (1371, 3898). Other abnormalities may occur in the course of lymphoproliferative disorder. In AT, in addition to mutations of ATM, inversions and translocations of the TR genes on chromosomes 7 and 14 are common. Consequently, these often show breakpoints at 14q11, 7q35, 7p13 and chromosomal rearrangements/translocations including inv(7)(p13q35), t(7;7) (p13;q35), and t(7;14)(p13;q11), as well as t(14;14)(q11;q32), which can involve the IGH gene locus (3910). Such translocations may also involve the TCL1A gene in 14q32.1 and other oncogenes leading to T-cell lymphoproliferative diseases, including T-cell prolymphocytic leukaemia and pre-T lymphoblastic leukaemia/lymphoma.

Prognosis and predictive factors

The prognosis is related to both the underlying PID and the type of lymphoproliferative disorder. The immunological status of the host is an important risk factor (547). The lymphoid proliferations in ALPS are often self-limiting. Lymphoid hyperplasias in CVID may be indolent. Most of the other lymphoproliferative disorders in patients with PID are aggressive. However, given the wide variety of underlying conditions and ensuing lymphoproliferative disorders, the prognosis must be evaluated in each case individually, although clonality testing may be of additional value (4126). In patients with EBV-driven infectious mononucleosis, a haemophagocytic syndrome may be the primary cause of death, usually associated with marked pancytopenia, liver dysfunction, coagulopathy, and further infectious complications.

Treatment is determined on the basis of both the nature of the neoplastic process and the underlying genetic defect. In general, less-aggressive therapy is needed than in patients without PID. Allogeneic bone marrow transplantation has been used in patients with WAS, SCID, and HIgM (1066,1508). Because the EBV-driven B-cell expansion in lymphomatoid granulomatosis is often not autonomous, it may respond to immunoregulatory therapy using interferon alfa 2b (4332); however, like in post-transplant lymphoproliferative disorder, anti-CD20 directed therapy is more important.
Lymphomas associated with HIV infection

Definition
Lymphomas that develop in HIV-positive patients are predominantly aggressive B-cell lymphomas. In a proportion of cases, they are considered AIDS-defining conditions and are the initial manifestation of AIDS. These disorders are heterogeneous and include lymphomas usually diagnosed in immunocompetent patients, as well as lymphomas seen much more often in the setting of HIV infection. The most common HIV-associated lymphomas include Burkitt lymphoma, diffuse large B-cell lymphoma (DLBCL; often involving the CNS), primary effusion lymphoma (PEL), and plasmablastic lymphoma. Classic Hodgkin lymphoma (CHL) is also increased in the setting of HIV infection.

Epidemiology
The incidence of all subtypes of non-Hodgkin lymphoma is increased 60–200 times in HIV-positive patients. Before combination antiretroviral therapy (cART) was available, primary CNS lymphoma and Burkitt lymphoma were increased approximately 1000 times in comparison with the general population [345, 2285]. Since the introduction of cART, the incidence of non-Hodgkin lymphoma has decreased by 50%, mainly due to decreased CNS lymphoma and the immunoblastic histological subtype [1107]. Moreover, the decreased incidence of most AIDS-associated non-Hodgkin lymphomas after cART introduction is consistent with improved CD4 counts [363,373]. cART is also associated with increased survival (with a 75% decrease in mortality), although lymphomas now account for a higher proportion of first AIDS-defining illnesses [974,1107]. In contrast, the risk of HIV-associated CHL has remained stable, and burden of disease has increased. The incidence of CHL is about 50 cases per 100,000 person-years among HIV-infected individuals, 5–20 times the incidence in the general population [3649]. Because CHL incidence is lower among patients with severe immunosuppression than among those with moderate immune defect, the relatively increased CHL incidence could be related to improvements in CD4 counts [374,770]. Lymphocyte predominant Hodgkin lymphoma can occur in HIV-infected individuals, but there is no known association.

Etiology
Lymphomas in HIV-infected patients are heterogeneous, reflecting several pathogenetic mechanisms: chronic antigen stimulation, genetic abnormalities, cytokine deregulation, and the role of EBV and HHV8 [555,557]. HIV-related lymphomas are consistently monoclonal and are characterized by a number of common genetic abnormalities of MYC and BCL6, as well as tumour suppressor genes [1272,4096]. The polyclonal or oligoclonal nature of some HIV-related lymphoid proliferations suggests a multistep lymphomagenesis. B-cell stimulation, hypergammaglobulinaemia, and persistent generalized lymphadenopathy preceding the development of these lymphomas probably reflect the role of chronic antigenic stimulation and impaired immune response. Disruption of the cytokine network, leading to high serum levels of IL6 and IL10, is a feature of HIV-related lymphomas associated with EBV or HHV8. EBV is identified in the neoplastic cells of approximately 40% of HIV-related lymphomas, but the detection of EBV varies considerably with the site of presentation and the histological subtype. EBV infection is found in 80–100% of primary CNS lymphomas [536] and PELs, in 80% of DLBCLs with immunoblastic features, and in 30–50% of Burkitt lymphomas [1531]. Nearly all CHL cases in the setting of HIV infection are associated with EBV [191,3754]. HHV8 is specifically associated with PEL, which usually occurs in the late stages of the disease, in the setting of profound immunosuppression [623].

Prevention
Despite the therapeutic advances with cART, there is still no effective immunization or cure for HIV infection. Diversity caused by circulating recombinant viral forms is a major challenge, which has
Towards a higher rate of opportunistic infection than in other immunodeficiency states, including after transplantation. In addition, there are unusual lymphomas that occur more specifically (though not exclusively) in patients with AIDS.

**Localization**

HIV-related lymphomas display a marked propensity to involve extranodal sites, in particular the gastrointestinal tract, CNS (less frequently since cART), liver, and bone marrow. The peripheral blood is rarely involved except in cases of Burkitt lymphoma, which may even present as acute leukaemia. Unusual sites such as the oral cavity, jaw, and body cavities are often involved. Other extranodal sites include lung, skin, testis, heart, and breast. Lymph nodes are involved in about one third of reported cases at presentation (365), but since the cART era, nodal involvement accounts for half of all cases (1643).

**Clinical features**

Most patients present with advanced clinical stage; bulky disease with a high tumour burden is frequent. Lactate dehydrogenase is usually markedly elevated. There is a significant relationship between the subtype of lymphoma and the HIV disease status. DLBCL more often occurs in the setting of longstanding AIDS and is associated with a trend towards a higher rate of opportunistic infections and lower CD4+ T-cell counts (mean: <100 x 10^6/L). In contrast, Burkitt lymphoma and Hodgkin lymphoma occur in less immunodeficient patients, with a shorter mean interval between the diagnosis of HIV seropositivity and lymphoma and significantly higher CD4+ T-cell counts (>200 x 10^6/L) (363, 553).

**Microscopy**

In HIV-positive patients, there is a spectrum of lymphoid proliferations, some of which resemble aggressive B-cell lymphomas that develop sporadically in the absence of HIV infection. Others, like the polymorphous lymphoid proliferations, may resemble those seen in other immunodeficiency states, including after transplantation. In addition, there are unusual lymphomas that occur more specifically (though not exclusively) in patients with AIDS.

**Lymphomas also occurring in immunocompetent patients**

**Burkitt lymphoma**

Burkitt lymphoma accounts for 20–30% of all HIV-associated lymphomas, and is more common in the setting of HIV infection than in other immunodeficiency states. The gastrointestinal tract is the most common site of presentation, but in patients with AIDS, Burkitt lymphoma can present in unusual sites including the bone marrow. Burkitt lymphoma occurs in patients with CD4+ cell cut-offs above the threshold for AIDS, suggesting that onset may require functional CD4+ cells. There is a diffuse, monotonous, and cohesive proliferation of intermediate-sized cells with round nucleoli and basophilic cytoplasm, which appears vacuolated on imprints. Mitoses are frequent, as are tingible body macrophages imparting the starry-sky appearance, which is characteristic (3310). In some cases, there may be greater variation in cell size and shape, previously termed Burkitt-like lymphoma. In most HIV-related Burkitt lymphoma cases, the cells reveal plasmacytoid differentiation, which is peculiar to patients with AIDS. They are characterized by medium-sized cells with an eccentric nucleus, as many as four nucleoli, and a small rim of amphophilic cytoplasm. The cells may contain cytoplasmic immunoglobulin. EBV-encoded small RNA (EBER) is positive in about 30–50% of cases, whereas EBV LMP1 is negative (881). Tumour cells have features of the small non-cleaved cells found in germinal centres, and are positive for CD20, CD10, and BCL6, but negative for BCL2. The Ki-67 proliferation index approaches 100%. The cells are positive for MYC with immunohistochemical stains, and the MYC translocation can usually be demonstrated by cytogenetics or FISH.

**DLBCL**

Patients with HIV-related DLBCL usually present with extranodal or disseminated disease; nodal presentation is less common than in the immunocompetent population. Most patients have high-stage disease (stage III or IV). The most common sites of extranodal disease are the CNS, gastrointestinal tract, bone marrow, and liver. There may be involvement of unusual sites, such as the heart or anorectal region. HIV-related DLBCL may be of the germinal centre B-cell type or the activated B-cell type, with the germinal centre B-cell type being more common. EBV is present in about 30% of cases, and together with expression of MYC, correlates with an impaired 2-year survival in patients with DLBCL treated in the cART era (630, 659, 660, 1056). Most HIV-related DLBCLs consist of centroblasts, which are large cells with round or oval nuclei and two or more nucleoli, often aligned along the nuclear membrane. Mitoses may be frequent, but they usually...
Plasmablastic lymphoma

Plasmablastic lymphoma accounts for about 2% of all HIV-related lymphomas [554]. The blastoid morphology and immunophenotype suggest that these cells retain the blastoid appearance of immunoblasts or centroblasts, but have acquired the antigen profile of plasma cells. They may occur at all ages but are rare in paediatric patients. The median patient age at presentation is 38 years (577). The cell or origin is considered to be an activated B cell after somatic hypermutation and class-switch recombination. About 60–70% of cases are positive for EBER in the absence of EBNA2 (compared with about 50% in immunocompetent patients with plasmablastic lymphoma) [2755]. There is expression of MYC in about 50% of cases, which correlates with MYC translocation or sometimes amplification. There are no translocations involving BCL2, BCL6, MALT1, or PAX5. EBV LMP1 is usually negative (type I EBV latency), although type III latency has been recorded [578]. Extranodal presentation is most frequent in the oral cavity or jaw. For this reason, it is sometimes referred to as plasmablastic lymphoma of the oral cavity type. Other sites of involvement include the gastrointestinal tract, skin, abdomen, retroperitoneum, and soft tissue of the extremities [578]. Patients do not usually have a monoclonal gammopathy, but may have advanced clinical stage, with B symptoms and bone marrow involvement. Histologically, plasmablastic lymphoma is characterized by sheet-like proliferation of large cells with immunoblastic or plasmablastic appearance, including central round or oval nuclei with prominent nucleoli and moderately abundant amphophilic cytoplasm. The nuclei may be eccentrically located with a perinuclear clearing or so-called hof. In the oral cavity, the cells may have a more centroblastic appearance but retain the plasmablastic phenotype [936]. There are frequent apoptotic cells, but a starry-sky pattern is rare. The neoplastic cells are negative or weakly positive for CD45 and usually negative for B-cell markers, including CD19, CD20, and PAX5, but most cases are positive for CD79a, IRF4 (also called MUM1), PRDM1 (also called BLIMP1), CD38, and CD138. Intracytoplasmic IgG may be detected in some patients. There may be aberrant expression of T-cell markers, including CD2 and
CD4. The Ki-67 (MIB1) proliferation index is almost 100%.

**Hodgkin lymphoma**

The incidence of CHL may have increased since the introduction of cART, suggesting that a threshold of CD4+ cells may be required for the pathogenesis, and that cART does not provide protection from developing CHL (1657,2218). In the era before cART, most cases were of the mixed-cellularity or lymphocyte-depleted subtypes. Likely due to improved immunity with anti-HIV therapy, nodular sclerosis CHL now accounts for nearly 50% of cases (3649). HIV-associated CHL may have an atypical clinical presentation with advanced-stage bone marrow or liver involvement, as well as non-contiguous spread to multiple nodal groups. In HIV-related Hodgkin lymphoma, the Hodgkin/Reed–Sternberg cells are positive for EBER in 80–100% of cases; the cells express a type II EBV latency pattern in which expression of EBV-encoded genes is limited to EBNA1 and latent membrane proteins (LMP1 and LMP2) (1624). Both these proteins have oncogenic potential, including the activation of the NF-kappaB pathway. In HIV-related CHL, there may be decreased nodal CD4+ T cells and lack of CD4+ rosetting around Hodgkin/Reed–Sternberg cells (1574).

Other lymphomas

Cases of marginal zone lymphoma and lymphoma of mucosa-associated lymphoid tissue have been described in both paediatric and adult patients with HIV infection (1376,2593,3945). Rare cases of NK/T-cell lymphomas have been reported, including mycosis fungoides, anaplastic large cell lymphoma, and naso- type NK/T-cell lymphoma (159,365,501,546,1266,1492,1667,1857,4008). There is also an increased risk of lymphoplasmacytic lymphoma and lymphoblastic leukaemia (1370).

**Lymphomas occurring more specifically in HIV-positive patients**

PEL, plasmablastic lymphoma, and HHV8-positive DLBCL, NOS, occur more specifically in HIV-positive patients.

**Lymphomas occurring in other immunodeficient states**

Polymorphic lymphoid proliferations resembling post-transplant lymphoproliferative disorder may be seen in adults and also in children, but are much less common than in the post-transplant setting, accounting for <5% of HIV-associated lymphomas. The mean patient age at presentation is 38 years, similar to those of other HIV-related non-Hodgkin lymphomas. They may present in lymph nodes as well as extranodal sites. These conform to the criteria of polymorphic B-cell post-transplant lymphoproliferative disorder. The infiltrates contain a range of lymphoid cells, from small cells (often with plasmacytoid features) to immunoblasts, with scattered large bizarre cells expressing CD30. EBV is often present, but some cases are EBV-negative (2030,2519,2804,3901). A clonal B-cell population is present in most cases, and there may be an oligoclonal background, suggesting variable numbers of clonal cells within a polymorphic background. Clonal EBV infection has been demonstrated. They generally lack structural alterations in MYC, BCL6, the RAS family of genes, and TP53 (2804).

**Prognosis and predictive factors**

Before the cART era, the outcome of patients with lymphoma and HIV infection was closely related to the severity of immunodeficiency (430). HIV-directed therapy can now reduce the impact of HIV-related prognostic factors and allow curative therapy for most patients with aggressive lymphoma (278,2333,3580). The achievement of complete remission is the most important prognostic factor with respect to survival (4351). Expression of MYC in DLBCL from HIV-positive patients is associated with increased 2-year mortality (660). In HIV-associated DLBCL, the stromal immune reaction may also influence patient survival (661). Patients with HIV have reduced stromal CD4+ and FOXP3+ T cells, and increased density of stromal macrophages. A higher density of infiltrating CD8+ T cells may be associated with reduced mortality from lymphoma (661). Plasmablastic lymphoma is associated with early relapses, and chemotherapy resistance with an inferior overall survival compared with DLBCL and Burkitt lymphoma (278,578). In Burkitt lymphoma, use of modified CODOX-M (cyclophosphamide, vincristine, doxorubicin, and high-dose methotrexate) regimens results in survival rates similar to those seen in studies that excluded HIV-positive patients (2906). On the other hand, PEL usually has a very poor prognosis, with a low complete remission rate. CHL should be treated with curative intent, and has outcomes comparable to those in the non-HIV population (4069). There is a need for increased supportive care and coincident cART in the HIV-infected population (791).
Post-transplant lymphoproliferative disorders

Definition
Post-transplant lymphoproliferative disorders (PTLDs) are lymphoid or plasmacytic proliferations that develop as a consequence of immunosuppression in a recipient of a solid organ or stem cell allograft. They constitute a spectrum ranging from usually EBV-driven polyclonal proliferations to EBV-positive or EBV-negative proliferations indistinguishable from a subset of B-cell or (less often) T/NK-cell lymphomas that occur in immunocompetent individuals. The monomorphic and classic Hodgkin lymphoma types of PTLD are further categorized as in non-immunosuppressed patients, according to the lymphoma they resemble. With the rare exception of EBV-positive MALT lymphomas, indolent B-cell lymphomas (e.g., follicular lymphoma and EBV-negative MALT lymphomas in allograft recipients) are designated as they are in the immunocompetent host and not considered a type of PTLD. The standardized incidence ratios for chronic lymphocytic leukaemia / small lymphocytic lymphoma, follicular lymphoma, mantle cell lymphoma, and splenic/nodal marginal zone lymphoma are not increased in solid organ transplant recipients, and those for lymphoplasmacytic lymphoma, marginal zone lymphoma, and mucosa-associated lymphoid tissue type lymphoma are only moderately elevated (763,2051). The presence of rare EBV-positive cells in a lymphoid/plasmacytic proliferation, in the absence of other diagnostic features, is insufficient for the diagnosis of a PTLD. There are four major categories of PTLD, with all but the polymorphic group requiring further subcategorization (Table 16.02). Their criteria are summarized in Table 16.03. Patients may have more than one type of PTLD in a single site or at separate sites. Cases that fulfill the criteria for EBV-positive mucocutaneous ulcer should be separately designated. It is important to diagnose cases as PTLD and then indicate what type because of the prognostic and therapeutic implications. Given the intralvesional heterogeneity of many PTLDs, the importance of architectural features in their categorization, and the need in some cases for extensive ancillary studies, excisional biopsy is preferred over fine-needle aspiration or core needle biopsies whenever feasible.

Epidemiology
The characteristics of PTLD appear to differ somewhat across institutions, probably as a result of different patient populations, allograft types, and immunosuppressive regimens. A variety of risk factors have been identified (521,522,2980), but the most important risk factor for EBV-driven PTLD is EBV seronegativity at the time of transplantation (522,720,4270). Among adult and paediatric solid organ recipients, the frequency of PTLD correlates, in part, with the intensity and type of the immunosuppressive regimen, although no single immunosuppressive agent is uniquely responsible. Among adults, patients receiving renal allografts have the lowest frequency of PTLD (generally <1%); those with hepatic and cardiac allografts have an intermediate risk (approximately 1–5%); and those receiving heart–lung, lung, or intestinal allografts have the highest frequency (≥5%) (236,521,522,2980). In children, for any given organ, the incidence is much higher (966,4271), with most cases being associated with post-transplantation primary EBV infection (4271). This is consistent with the finding of an increased incidence of PTLD among EBV-seronegative organ transplant recipients (720). The incidence of PTLD is reported to rise again in patients aged >50 years (1106,3262). Lack of prior cytomegalovirus exposure is also a risk factor in some series. Additional host factors such as genetic polymorphisms may also impact the risk for PTLD (2756,3345).

In general, stem cell allograft recipients have a low risk of PTLD (~1–2%); the risk of early-onset PTLD (<1 year) is highest with unrelated or HLA-mismatched related donors, selective T-cell depletion of donor bone marrow, and use of antithymocyte globulin or anti-CD3 monoclonal antibodies. The risk of PTLD in these patients increases for those with two or more of these risk factors (851). PTLD-like lesions are rare after autologous stem cell transplantation; they may be associated with additional high-dose immunosuppressive regimens and are best considered iatrogenic immunodeficiency-associated lymphoproliferative disorders rather than PTLD (2832).

Etiology
Most PTLDs are associated with EBV infection, and appear to constitute EBV-induced monoclonal or, less often, polyclonal B-cell or monoclonal T-cell proliferations that occur in a setting of decreased T-cell immune surveillance (629,767,1205,1245,2053,2819,3845). EBV positivity is best demonstrated using in situ hybridization for EBV-encoded small RNA (EBER); EBV LMP1 immunostaining

Table 16.02 Categories of post-transplant lymphoproliferative disorder (PTLD)

<table>
<thead>
<tr>
<th>Non-destructive PTLD</th>
<th>Polymorphic PTLD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasmacytic hyperplasia</td>
<td>(classify according to lymphoma they resemble)</td>
</tr>
<tr>
<td>Infectious mononucleosis</td>
<td>B-cell neoplasms</td>
</tr>
<tr>
<td>Florid follicular hyperplasia</td>
<td>Diffuse large B-cell lymphoma</td>
</tr>
<tr>
<td></td>
<td>Burkitt lymphoma</td>
</tr>
<tr>
<td></td>
<td>Plasma cell myeloma</td>
</tr>
<tr>
<td></td>
<td>Plasma cytoma</td>
</tr>
<tr>
<td></td>
<td>Other</td>
</tr>
<tr>
<td></td>
<td>T-cell neoplasms</td>
</tr>
<tr>
<td></td>
<td>Peripheral T-cell lymphoma, NOS</td>
</tr>
<tr>
<td></td>
<td>Hepatosplenic T-cell lymphoma</td>
</tr>
<tr>
<td></td>
<td>Other</td>
</tr>
</tbody>
</table>

a The ICD-0 codes for these lesions are the same as those for the respective lymphoid or plasmacytic neoplasm.
b Indolent small B-cell lymphomas arising in transplant recipients are not included among the PTLDs, with the exception of EBV-positive marginal zone lymphomas (see text).
is less sensitive. Most EBV-positive cases exhibit a type III EBV latency pattern, but a moderate number show a type II pattern, and fewer show a type I pattern, although not all of the cells in a given case show the same pattern [1405,3011]. Evidence of lytic EBV infection can also be documented in more than half of the cases and has been associated with plasmacytic differentiation [1405]. About 20–40% of PTLDs are EBV-negative, with some series reporting an even higher proportion of cases. Approximately two thirds of the T-cell PTLDs are EBV-negative [47,385, 1205,2239,2756,2848,3845]. Furthermore, the proportion of EBV-negative PTLDs has increased since PTLDs were first being reported [2848]. EBV-negative PTLDs are more common in adults, tend to occur later after transplantation, and are more likely to be monomorphic compared with EBV-positive cases [1355,2848]. Although data are limited, EBV-negative cases appear to have a gene expression profile similar to that of diffuse large B-cell lymphoma occurring in immunocompetent hosts [2754]. Differences in the regulation of BCL2 family proteins between EBV-positive and EBV-negative PTLD have also been reported [1354]. HHV8-associated PTLDs have been reported, including post-transplant primary effusion lymphoma [1030,1935, 2571]; however, the etiology of the vast majority of EBV-negative PTLDs is unknown. Some may be due to EBV that is no longer detectable [3760], some due to other unknown viruses, and some due to chronic antigenic stimulation, including by the transplant itself [385]. Two gene expression profiling studies support a non-viral etiology for the EBV-negative cases, although other studies have failed to find differences between EBV-positive and EBV-negative PTLDs [832,2754, 4100]. The EBV-negative cases are still considered to represent PTLD, and some may respond to decreased immunosuppression [2848]. The majority (>90%) of PTLDs in solid organ recipients are of host origin, and only a minority of donor origin. Donor-origin PTLDs appear to be most common in liver and lung allograft recipients, and frequently involve the allograft [147, 632,2225,3756,4290]. In contrast, most PTLDs in stem cell allograft recipients are of donor origin, as would be expected, given that successful engraftment results in an immune system that is nearly exclusively of donor origin [4507].

### Localization

Involvement of lymph node, gastrointestinal tract, lungs, and liver is common, but disease can occur at almost any site in the body [1205,2819,3130,4271]. The CNS is involved uncommonly, either as the only site of disease or in association with multiorgan involvement [575,1119]. In solid organ transplant recipients, PTLD can involve the allograft, which can cause diagnostic confusion because rejection and infection can result in a similar clinical picture. Allograft involvement appears more frequently in early-onset, EBV-positive disease and is most common in lung and intestinal transplant recipients [236,1355,2980,3296]. The non-destructive PTLDs often present with tonsillar and/or adenoid involvement but can also occur

<table>
<thead>
<tr>
<th>Pathological type of PTLD</th>
<th>Histopathology</th>
<th>Immunophenotype/ in-situ hybridization</th>
<th>Genetics</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Plasmacytic hyperplasia</strong></td>
<td>Absent</td>
<td>Predominantly small lymphocytes and plasma cells</td>
<td>Pol or very small mcl B-cell population(s)</td>
</tr>
<tr>
<td><strong>Infectious mononucleosis</strong></td>
<td>Absent</td>
<td>Admixed small lymphocytes, plasma cells, and immunoblasts</td>
<td>Pol or very small mcl B-cell population(s); may have clonal/oligoclonal TR genes</td>
</tr>
<tr>
<td><strong>Florid follicular hyperplasia</strong></td>
<td>Absent</td>
<td>Prominent hyperplastic germinal centres</td>
<td>Pol B cells and admixed T cells; EBV±</td>
</tr>
<tr>
<td><strong>Polymorphic</strong></td>
<td>Present</td>
<td>Full spectrum of lymphoid maturation seen, not fulfilling criteria for NHL</td>
<td>Pol ± mcl B cells and admixed T cells; most EBV+</td>
</tr>
<tr>
<td><strong>Monomorphic</strong></td>
<td>Usually present</td>
<td>Fulfils criteria for an NHL (other than one of the indolent B-cell neoplasms) or plasma cell neoplasm</td>
<td>Varies based on type of neoplasm they resemble; EBV more variable than in other categories</td>
</tr>
<tr>
<td><strong>CHL</strong></td>
<td>Present</td>
<td>Fulfils criteria for CHL</td>
<td>Similar to other CHL; EBV+</td>
</tr>
</tbody>
</table>

CHL, classic Hodgkin lymphoma; NHL, non-Hodgkin lymphoma; mcl, monoclonal; pcl, polyclonal. Monoclonality and polyclonality are only inferred when finding monotypic or polytypic light chain expression.

*EBV-positive MALT lymphomas at least of skin/subcutaneous tissues should be considered a type of PTLD.*
at other sites. EBV-positive MALT lymphoma PTLDs most typically present in cutaneous or subcutaneous tissues [1368]. The plasmacytoma lesions may have nodal or, more commonly, extranodal presentations, usually without bone marrow involvement [1951,3352,4042]. Some cases have a myeloma-like presentation, including osteolytic bone lesions [1951,4042]. Overt bone marrow involvement by polymorphic PTLDs (P-PTLDs) and monomorphic PTLDs (M-PTLDs) is present in about 15–20% of cases, but peripheral blood is rarely involved [2697]. The presence of occasional small lymphoid aggregates or rare EBV-positive cells is not sufficient to diagnose PTLD in the marrow. Bone marrow allograft recipients tend to present with widespread disease involving nodal and extranodal sites, including liver, spleen, gastrointestinal tract, and lungs [3130,3636,4507].

Clinical features
The clinical features of PTLD are highly variable and correlate to some extent with the type of allograft and morphologically defined categories. PTLD frequently presents in the first year after transplantation, especially in EBV-seronegative recipients who acquire early post-transplant EBV infection, often from the donor. This pattern of presentation is particularly common in children. However, the median time to PTLD in some studies, especially those of adult populations, is several years, and as many as 15–25% of cases occur >10 years after the transplant [931,1122,2819,3130]. There is some evidence for an increase in prevalence of late-onset disease [720], although this may in part reflect the ever-expanding population of patients at risk as the number of long-term survivors of transplantation increases. EBV-negative PTLD and T/NK-cell PTLD tend to present later (with median times to occurrence of 4–5 years and ~6 years, respectively), although T-cell PTLDs following haematopoietic stem cell or bone marrow transplantation occur significantly earlier [851,1625,2239,2849,3845,3999].

PTLD presentations vary greatly. Some PTLDs are found incidentally, some present with very vague non-specific symptoms such as fever and malaise, and some present with infectious mononucleosis–like findings. Others present with tonsillar or adenoid enlargement, lymphadenopathy or tumorous masses, often at extranodal sites, sometimes with organ-specific dysfunction and occasionally with widely disseminated disease. A viral septic shock–like picture is another rare presentation.

Prognosis and predictive factors
Although overall mortality rates of 25–60% are still quoted [2756], newer therapeutic strategies appear to be associated with a better overall outcome. The non-destructive PTLDs (previously termed early lesions) tend to regress with reduction in immune suppression; if this can be accomplished without graft rejection, the prognosis is excellent, particularly in children [2385,2849,4462]. However, some infectious mononucleosis–like PTLDs can be fatal. P-PTLDs and even a significant minority of M-PTLDs may also regress with reduction in immune suppression [3346,3769,4271]. Some factors that have been reported to be associated with a lack of response to decreased immunosuppression include elevated lactate dehydrogenase, organ dysfunction, multiorgan involvement, advanced stage, bulky disease, and older patient age [3346,4066]. Clinical caution is required because rebound acute or chronic rejection is frequently observed during reduction of immunosuppression and can lead to graft loss and death [4271]. A proportion of P-PTLDs and
Immunodeficiency-associated lymphoproliferative disorders

more-numerous M-PTLDs fail to regress, and require additional therapies such as monoclonal antibodies directed against B-cell antigens (most commonly anti-CD20), sometimes together with chemotherapy [741,794,1099,1404,1474]. The anti-CD30 antibody brentuximab vedotin has also been used, with mixed results [47,1634]. Surgical excision or sometimes radiation therapy are other important therapeutic modalities in localized cases. Adoptive T-cell immunotherapy is another therapeutic approach [1031, 1545, 1629]. Although no comparative clinical trials have been reported for Burkitt lymphoma PTLDs, cessation of immunosuppression and immediate use of multidrug (immuno)chemotherapy likely offer the best outcomes for this aggressive PTLD [3168,4495]. Plasmacytomatous PTLDs have a variable outcome, but many do well, sometimes with very limited therapy [981,3142,3205,3352,4042]. The myelomatous lesions and cases with osteolytic bone lesions are not expected to regress with decreased immunosuppression and have a poor prognosis, although they may respond to myeloma-type therapy [2053,4042]. T/NK-cell PTLDs are also typically aggressive, particularly those of hepatosplenic type, with the exception of those of large granular lymphocyte type, which typically do very well [3845,3999]. Nevertheless, some T-cell PTLDs do respond to reconstitution of the patient's immune system. Classic Hodgkin lymphoma PTLDs are generally treated with conventional classic Hodgkin lymphoma therapeutic regimens, with good results. Risk factors for adverse outcome vary greatly across studies. Some of the reported factors that have been associated with an adverse prognosis include multiple sites of disease (perhaps not in children), advanced stage, involvement of the CNS, bone marrow and serous effusions, older patient age at diagnosis, elevated lactate dehydrogenase, and hypoalbuminaemia [522,741,981,1099,1120,1356,2240,2435,4061,4271]. The combination of lytic EBV infection and type III EBV latency has also been associated with an adverse prognosis, as well as with early onset [1405]. Although EBV negativity in PTLD, and even among the T/NK-cell PTLDs, has been reported to be an adverse prognostic indicator, not all studies document a survival difference [741,3845]. PTLD of donor origin in solid organ transplant recipients and the overlapping group of PTLD localized to the allograft have better than average prognoses, although in T-cell PTLD, graft involvement has been reported to be an adverse prognostic indicator [3999]. PTLDs with oncogene abnormalities are also considered to be more aggressive. Whether P-PTLD does better than M-PTLD is controversial. Overall, the mortality of PTLD is much greater in bone marrow allograft recipients than in solid organ allograft recipients. It should also be remembered that, although uncommon, there may be progression from non-destructive to polymorphic and from polymorphic to monomorphic PTLD, sometimes with documented additional molecular abnormalities [3168,4377]. Serial monitoring of EBV DNA levels in whole blood, peripheral blood mononuclear cell preparations, or plasma is often used to help predict the risk for PTLD and the onset of PTLD, as well as to follow PTLD, including to guide preemptive therapy in some patients. Its use appears most helpful in solid organ transplant recipients who are seronegative at transplantation, particularly children. However, it should be noted that EBV-positive PTLD can develop in the absence of high viral loads, high viral loads are not predictive in all settings, and a fall in load may not always predict treatment response. A rapid fall in EBV viral load is almost invariably when anti-B cell monoclonal antibodies are given as part of the treatment regimen, irrespective of the type of PTLD localized to the allograft and the onset of PTLD, as well as with early onset [1405]. Although EBV negativity in PTLD, and even among the T/NK-cell PTLDs, has been reported to be an adverse prognostic indicator, not all studies document a survival difference [741,3845]. PTLD of donor origin in solid organ transplant recipients and the overlapping group of PTLD localized to the allograft have better than average prognoses, although in T-cell PTLD, graft involvement has been reported to be an adverse prognostic indicator [3999].

Non-destructive post-transplant lymphoproliferative disorders

Definition
Non-destructive post-transplant lymphoproliferative disorders are defined as lymphoid proliferations in an allograft recipient characterized by architectural preservation of the involved tissue and an absence of features that would be diagnostic of a malignant lymphoma. In most cases, they form mass lesions. These PTLDs must be distinguished from lymphoid proliferations with other known explanations and from other non-specific chronic inflammatory processes.

Fig. 16.15 Florid follicular hyperplasia post-transplant lymphoproliferative disorder in a 20-year-old man, 10 years after heart transplantation. The lesion, which had only rare EBV+ cells, regressed after the patient's immunosuppression was reduced and did not recur. A Endoscopic image of ileocaecal mass. B,C Biopsies showing a variably dense infiltrate composed of organized lymphoid tissue with scattered germinal centres, many small lymphocytes, some plasma cells, and infrequent transformed cells.
Because cases of plasmacytic hyperplasia (PH) and florid follicular hyperplasia are histologically non-specific, the diagnosis requires the formation of a mass lesion and/or significant EBV positivity. This group of PTLDs were formerly known as early lesions; however, this term has been deleted due to confusion with the group of PTLDs that occur early after transplantation. In fact, a series of so-called early PTLDs from 2005–2007 were diagnosed at a median of 50 months after transplantation (2849).

Clinical features
PH and infectious mononucleosis (IM) PTLDs tend to occur at a younger age than the other PTLDs, and are often seen in children or in adult solid organ recipients who have not had a prior EBV infection (629,2385,2849). Cases of florid follicular hyperplasia PH also occur most commonly in children (2849,4101). These non-destructive lesions involve lymph nodes or tonsils and adenoids more often than true extranodal sites (2848,2385). They often regress spontaneously with reduction in immunosuppression or may be successfully treated by surgical excision; however, IM-like lesions can be fatal. In some cases, polymorphic or monomorphic PTLD may follow one of the non-destructive type lesions (2819,4377).

Microscopy
PH is characterized by numerous plasma cells, small lymphocytes, and generally infrequent bland-appearing immunoblasts, whereas IM PTLD has the typical morphological features of IM, with paracortical/interfollicular expansion and numerous immunoblasts in a background of T cells and plasma cells. Florid follicular hyperplasia is a mass lesion with marked follicular hyperplasia that does not suggest IM (4101). Criteria for the distinction of these non-destructive PTLDs from other reactive lymphoid infiltrates are not well defined and rest on the extent of the proliferation, clinical correlation, and the presence or absence of EBV.

Immunophenotype
Immunophenotypic studies show an admixture of polytypic B cells, plasma cells, and T cells without phenotypic aberrancy. EBV is present in many of the reported cases of PH and florid follicular hyperplasia (2053,2849,4101). These diagnoses should be made only with great caution in EBV-negative cases, due to the non-specificity of the histological/immunophenotypic findings. IM PTLDs are typically EBV+ with EBV LMP1+ immunoblasts (2385).

Genetic profile
Clonally rearranged IG genes are not expected in PH, although small clonal populations may be demonstrated with Southern blot analysis using probes to the terminal repeat region of EBV. Some IM PTLDs may have small monoclonal or oligoclonal populations. The significance of oligoclonality or a small clonal band in these cases is unknown (2053,4377). Florid follicular hyperplasia does not usually demonstrate clonal B cells but, as is also reported in IM PTLD, rarely demonstrates simple clonal cytogenetic abnormalities (2849,4101).

Polymorphic post-transplant lymphoproliferative disorders
Definition
Polymorphic post-transplant lymphoproliferative disorders (P-PTLDs) are composed of a heterogeneous population of immunoblasts, plasma cells, and small and intermediate-sized lymphoid cells that efface the architecture of lymph nodes or form destructive extranodal masses and do not fulfill the criteria for any of the recognized types of lymphoma described in immunocompetent hosts. There are no established criteria for the proportion of transformed cells/immunoblasts that may be present in P-PTLD. Distinction from cases of infectious mononucleosis PTLD with marked architectural distortion may be difficult. More problematic is the distinction of some P-PTLDs from monomorphic PTLDs (M-PTLDs). The criteria for the distinction of P-PTLDs from M-PTLDs that have plasmacytic differentiation are not well defined. PTLDs that fulfill the criteria for T-cell/histiocyte-rich large B-cell lymphoma or EBV-positive diffuse large B-cell lymphoma, NOS, which may appear polymorphic, are best considered a form of M-PTLD, because they would be diagnosed as lymphoma in a non-transplant patient. Of great importance, cases of monomorphic T-cell PTLD, which can also appear very polymorphic, must not be confused with P-PTLD. Some PTLDs that appear polymorphic but fulfill the criteria for EBV-positive mucocutaneous ulcer should be so-designated (for a more detailed description, see Other iatrogenic immunodeficiency-associated lymphoproliferative disorders, p. 462) (1565). The mucocutaneous ulcer type of PTLDs characteristically lack peripheral blood EBV DNA and do well with reduced/alkalized immunosuppression with or without rituximab (1565).

ICD-O code
9971/1

Clinical features
The reported frequency of P-PTLDs varies widely, but they account for a minority of PTLDs in most studies. However, in children P-PTLD is generally more common and frequently follows post-transplantation primary EBV infection (4271). The clinical presentation of P-PTLDs is not distinguishable from that of PTLDs in general, although they have been
in the bone marrow in some patients with P-PTLD [2068]. They are more common in children than in adults. The clinical significance of these aggregates, which are not always EBV-positive, is uncertain.

**Immunophenotype**

Immunophenotypic studies demonstrate B cells and a variable proportion of heterogeneous T cells that are usually moderately numerous and sometimes predominate (1062,2819). Light chain class restriction does not exclude the diagnosis and, when present, may be focal, or with the presence of different clonal populations in the same or different sites [2819]. The presence of clear-cut light chain class restriction must be noted in the diagnostic report, because some of these cases could also be classified as monomorphic diffuse large B-cell lymphoma PTLD with plasmacytic differentiation or as plasma cell neoplasm with increased transformed cells. Prominent CD30 expression is common, but unlike in most cases of classic Hodgkin lymphoma, the CD30+ Reed-Sternberg-like cells are CD20- and CD15- (694,4159).

Most cases of P-PTLD contain numerous cells positive for EBV-encoded small RNA (EBER). Detection of EBV by in situ hybridization for EBER is a useful tool in the differential diagnosis of PTLD versus rejection in allografts.

**Genetic profile**

P-PTLDs are expected to demonstrate clonally rearranged IG genes, although the clones are less predominant than in M-PTLD (766,1936,2053,2378). EBV terminal repeat analysis is the most sensitive method for demonstrating clonal populations in the EBV-positive cases, but is not generally performed. In some reported cases, tumours at different sites in the same patient may be clonally distinct [628]. About 75% of P-PTLDs are reported to have mutated IGH genes without ongoing mutations, and the remainder are unmutated (550). Significant T-cell clones are not expected. Clonal cytogenetic abnormalities may be present although less commonly detected than in B-cell M-PTLD [1010,4101]. Comparative genomic hybridization studies also demonstrate abnormalities in some P-PTLDs, including some recurrent abnormalities also seen in M-PTLD (3207). BCL6 somatic hypermutations are present in a subset of cases, as is aberrant promoter

**Microscopy**

Unlike the three types of non-destructive PTLD lesions, P-PTLDs show effacement of the underlying tissue architecture (1258,1541). However, unlike many lymphomas, they show the full range of B-cell maturation, from immunoblasts to plasma cells, with small and medium-sized lymphocytes and cells with irregular nuclear contours, some of which represent the typically prominent T-cell component. There may be areas of geographical necrosis and scattered large, bizarre cells that not infrequently resemble Reed-Sternberg cells (atypical immunoblasts). Numerous mitoses may be present. Some cases have areas that appear more monomorphic in the same or other tissues; thus, there may be a continuous spectrum between these lesions and M-PTLD. Other P-PTLDs have features that more closely resemble those of Hodgkin lymphoma. Some of these cases were previously referred to as Hodgkin-like. Variously sized, but usually small, lymphoid aggregates with or without plasma cell clusters are seen.
methyltransferase, but other mutations are only uncommonly detected (550,622,410). Nevertheless, it has been reported that P-PTLD does not segregate from non-germinal centre M-PTLD based on gene expression profiling (4100).

**Monomorphic post-transplant lymphoproliferative disorders (B- and T/NK-cell types)**

**Introduction**

Monomorphic post-transplant lymphoproliferative disorders (M-PTLDs), which make up about 60–80% of all PTLDs in most studies, fulfil the criteria for one of the B-cell or T/NK-cell neoplasms that are recognized in immunocompetent hosts and described elsewhere in this volume. The only exception to this is that the small B-cell lymphoid neoplasms are not designated as PTLD, except for the EBV-positive lymphoidplasmacytic proliferations that typically occur in skin/subcutaneous tissue, which fulfil the criteria for extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma) (1368).

The M-PTLDs should be designated as PTLD in the diagnostic line of the pathology report, and then further categorized based on the classification of lymphomas arising in immunocompetent hosts. Although the term monomorphic PTLD reflects the fact that many cases are composed of a monotonous proliferation of transformed lymphoid cells or plasmacytic cells, there may be significant pleomorphism, variability of cell size, and many admixed T cells within a given case. In addition, because polymorphic PTLD and M-PTLDs of B-cell origin form a spectrum, their distinction can become blurred, particularly with the recognition of polymorphic EBV-positive diffuse large B-cell lymphomas that arise in the absence of primary or secondary immunodeficiency or in association with age-related immune senescence. A predominance of large transformed cells/immunoblasts and abnormalities in oncogenes and tumour suppressor genes favour the diagnosis of M-PTLD, but are not required findings (2053). As noted above, cases that fulfil the criteria for EBV-positive mucocutaneous ulcer should be separately designated and not diagnosed as M-PTLD even if there are many atypical and transformed B cells and monoclonality (1565).

M-PTLDs can be further categorized as either monomorphic B-cell PTLDs or monomorphic T/NK-cell PTLDs.

**Monomorphic B-cell PTLD**

**Definition**

The monomorphic B-cell PTLDs are monoclonal transformed B-lymphocytic or plasmacytic proliferations that fulfill the criteria for a diffuse large B-cell lymphoma, or less often a Burkitt lymphoma or a plasma cell neoplasm. The latter may have all the features of an extranodal plasmacytoma with involvement of the gastrointestinal tract, lymph nodes or other extranodal sites or much less often of plasma cell myeloma. EBV+ extranodal marginal zone lymphomas of mucosa-associated lymphoid tissue (MALT lymphomas) (3142). A plasmablastic lymphoma PTLD must then be excluded. The EBV+ cutaneous/subcutaneous MALT lymphoma PTLDs resemble other MALT lymphomas that have plasmacytic differentiation, with many lymphoid cells with abundant pale cytoplasm, follicles with follicular colonization and at least focally prominent plasma cells. Some cases have been diagnosed as plasma cell PTLD consistent with the concept that plasma cytomas (immunocytomas) of skin are considered to represent MALT lymphomas. Rare cases at extracutaneous sites that might be otherwise similar have also been reported.

**Immunophenotype**

The lesions, other than those resembling plasma cell neoplasms, have B-cell-associated antigen expression (CD19, CD20, CD79a), sometimes with demonstrable monotypic immunoglobulin (often with expression of gamma or alpha heavy chain) in paraffin sections and more often if flow cytometric studies are performed. Many cases are CD30+, with or without anaplastic morphology. Most M-PTLDs are of non-germinal centre type based on immunohistochemistry. The EBV-positive cases usually have a non-germinal centre phenotype (CD10+, BCL6+, IRF4/MUM1+) even though only a minority are CD138 positive, whereas the EBV-negative cases are more likely to have a germinal centre type phenotype (CD10-, BCL6-, IRF4/MUM1-, CD138-; 60% of cases in one study). The Burkitt PTLDs, which are often EBV-positive, however, are CD10+. The myeloma or plasmacytoma-PTLDs, which may be EBV-positive or -negative, are phenotypically similar to those in immunocompetent patients. Although only a limited number of cases have been reported, the majority of the EBV+ MALT PTLDs have been IgA+.
Fig. 16.19 Monomorphic B-cell PTLDs. A Liver biopsy showing partial replacement by diffuse large B-cell lymphoma, immunoblastic variant. B Diffuse large B-cell lymphoma, centroblastic variant, EBV-negative, showing large transformed cells, many of which have peripheral nucleoli, consistent with centroblasts. There are also admixed immunoblasts. C Burkitt lymphoma showing mononuclear, medium-sized cells with multiple nucleoli, basophilic cytoplasm and numerous mitoses. D Monomorphic B-cell PTLD with a polymorphic background. Note the admixture of many pleomorphic transformed cells with small lymphocytes and occasional plasma cells. The diagnosis of an M-PTLD is based on the very prominent light chain class restricted large transformed cells associated in part with many T-cells.

Genetic profile
Clonal IG gene rearrangement is present in virtually all cases, and the majority contain EBV genomes, which, if present, are in clonal episomal form. PTLDs at different sites may have different clones as well as when they ‘relapse’. Most cases have somatically mutated IGHV with a minority showing ongoing mutations. However, some cases have IGHV loci inactivated related to clonal selection as seen in CHL. Caution in interpreting T-cell clonality studies is advised as monoclonal T-cell receptor rearrangements in the absence of a T-cell neoplasm have been reported in about half of B-PTLD particularly when a prominent CD8+ T-cell population is present. Of interest, this finding was not observed in DLBCL associated with HIV or in immunocompetent hosts. Consistent with the phenotypic findings, EBV+ PTLDs are of activated B-cell type, but, in contrast, 45% of the EBV-negative cases are of germinal centre type. As in non-PTLD DLBCL, oncogene abnormalities (RAS, TP53 mutations and/or MYC rearrangements) may be found, and BCL6 gene somatic hypermutation is common; however, BCL6 translocations are uncommon. Aberrant promoter hypermethylation and aberrant somatic hypermutation also occur in M-PTLD. Cytogenetic abnormalities are common, and are more frequent than in the non-destructive or polymorphic PTLD. While some recurrent abnormalities are reported in PTLD such as breaks involving the 1q11-q21 region, 8q24.1, 3q27, 16p13, 14q32, 11q23-24 and trisomies 9, 11, 7, X, 2 and 12, different studies find different common abnormalities. Comparative genomic hybridization and single nucleotide polymorphism (SNP) studies demonstrate additional gains and losses, although no individual abnormality is very common. Although some of these are shared with DLBCL in immunocompetent hosts, differences are observed as well. Differences from HIV-associated DLBCL are also observed [3361A]. EBV-negative monomorphic PTLD frequently lack expression of the cyclin dependent kinase inhibitor CDKN2A (p16INK4a). Although the majority of Burkitt lymphoma PTLD do have IG/MYC translocations, 3 of 7 EBV-negative post-transplant molecularly defined Burkitt lymphomas had the same 11q abnormalities seen in the new provisional entity of Burkitt-like lymphoma with 11q aberrations raising the possibility that these cases are more frequent in the post-transplant setting.

Fig. 16.20 Monomorphic B-cell PTLD with a polymorphic background (EBER ISH for EBV). The pleomorphic large cells and some smaller ones are EBER-positive.
Monomorphic T/NK-cell PTLD

Definition
Monomorphic T/NK-cell PTLDs (T/NK-PTLD) include PTLDs that fulfill the criteria for any of the T- or natural killer (NK) cell lymphomas. In North America and western Europe, these lesions constitute no more than 15% of PTLDs. They include almost the entire spectrum of T- and NK-cell neoplasms, with the largest group being peripheral T-cell lymphoma, NOS, followed by hepatosplenic T-cell lymphoma, which together make up slightly more than 10% of T-PTLDs. Other types of T/NK-PTLDs include T-cell large granular lymphocyte leukaemia, adult T-cell leukaemia/lymphoma (ATLL), extranodal NK/T cell lymphoma, nasal type, mycosis fungoides/Sézary syndrome, primary cutaneous anaplastic large cell lymphoma, other anaplastic large cell lymphomas and even rare cases of T-lymphoblastic leukaemia/Lymphoma. In some instances T-cell PTLDs have occurred with, or subsequent to, other types of PTLDs. Very rarely aggressive NK-cell PTLDs also occur.

Clinical features
Clinical presentation depends on the type of T/NK-cell neoplasm. Most cases present at extranodal sites, sometimes with associated lymphadenopathy. The more common sites of involvement include the PB or BM, spleen, skin, liver, gastrointestinal tract and lung.

Microscopy
The morphologic features of T/NK-PTLD do not differ from those of the same T/NK-cell lymphomas in immunocompetent hosts. It is critical to distinguish T-cell large granular lymphocyte leukaemias from the other T/NK-PTLDs.

Immunophenotype
T/NK-PTLDs show expression of pan-T-cell and sometimes NK-associated antigens. Depending on the specific type, they may express CD4 or CD8, CD30, ALK and either alpha beta or gamma delta T-cell receptors. About one third of cases are EBV-positive. Cases of ATLL are associated with HTLV-1.

Genetic profile
Cases of T-cell origin have clonal T-cell receptor gene rearrangement. Caution is advised as clonal or oligoclonal CD8+ T-cells may be seen following bone marrow transplantation or in IM and clonal T-cell rearrangements are also reported in M-PTLD of B-cell origin. Chromosomal abnormalities are common and similar to those seen in T/NK-cell neoplasms in the immunocompetent host such as i(7) (q10) and +8 in most of the hepatosplenic T-cell lymphomas. Oncogene mutations, such as in TP53, are also reported in a high proportion of T/NK-PTLDs.
**Classic Hodgkin lymphoma post-transplant lymphoproliferative disorder**

**Definition**

Classic Hodgkin lymphoma (CHL) post-transplant lymphoproliferative disorder (PTLD), the least common major form of PTLD, is almost always EBV-positive, and should fulfill the diagnostic criteria for CHL (see Chapter 15: Hodgkin lymphomas, p. 423). These lesions are usually of the mixed-cellularity type and have a type II EBV latency pattern as is typical in immunocompetent hosts. Because Reed–Sternberg–like cells may be seen in non-destructive, polymorphic, and some monomorphic PTLDs, the diagnosis of CHL must be based on both classic morphological and immunophenotypic features, preferably including both expression of both CD15 and CD30 (2820,3436). Although CD15-negative CHLs occur, caution is advised in making the diagnosis of CHL PTLD, because these cases must be distinguished from the other types of PTLD that include Reed–Sternberg–like cells, which are most typically EBV+, CD45+, CD15−, and CD20+ and often present in association with small and intermediate-sized EBV+ lymphoid cells (694,1034,1417, 2820,3198,3304). CHL PTLD is more likely to show B-cell antigen expression than is CHL in immunocompetent hosts (23). Rare cases may follow other types of PTLD. Although the distinction of PTLD with some Hodgkin-like features, such as prominent Reed–Sternberg–like cells, from CHL PTLD may be difficult in some cases, cases that do not clearly fulfill the criteria for CHL are best classified as either polymorphic PTLD or monomorphic PTLD, depending on their overall morphological features (3198,3304).

**ICD-O code** 9650/3

---

**Other iatrogenic immunodeficiency-associated lymphoproliferative disorders**

**Definition**

The other iatrogenic immunodeficiency-associated lymphoproliferative disorders are lymphoid proliferations or lymphomas that arise in patients treated with immunosuppressive drugs for autoimmune disease or conditions other than in the post-transplant setting. They constitute a spectrum ranging from polymorphic proliferations resembling polymorphic post-transplant lymphoproliferative disorders (PTLDs) to cases that fulfill the criteria for diffuse large B-cell lymphoma (DLBCL) or other B-cell lymphomas, such as EBV-positive DLBCL, peripheral T/NK-cell lymphoma, and classic Hodgkin lymphoma (CHL). EBV-positive mucocutaneous ulcer is a specific type of immunosuppression-associated lymphoproliferative disorder due to iatrogenic immunosuppression or age-related immune senescence that often has Hodgkin-like features and typically a self-limited, indolent course (1018). Iatrogenically related lymphomas occurring in treated haematological malignancies are not covered here (16).

**Epidemiology**

The frequency of these disorders is not well known, and it is difficult to determine how many are directly related to the iatrogenic immunosuppression rather than the underlying disorder or chance alone. However, their prevalence may be on the rise due to the increased number of patients receiving immunosuppression therapy. It is likely that the risk and type of lymphoproliferative disorders that develop in this setting vary depending on the type of immunosuppressive agent, the degree of immune deficiency, and the nature of the underlying disorder being treated, such as rheumatoid arthritis, inflammatory bowel disease, psoriasis and psoriatic arthritis, systemic lupus erythematosus, and other autoimmune disorders (528,1478,1583, 23). Rare cases may follow other types of the degree of inflammatory activity (219, 1665). Methotrexate was the first reported immunosuppressive agent associated with lymphoproliferative disorders in this setting (1910,3109,3498), predominantly in patients being treated for rheumatoid arthritis. Most studies have failed to show a significant increased lymphoma risk in patients with rheumatoid arthritis treated with TNF inhibitors or receiving other biological response modifiers, although cases of large B-cell lymphoma and classic Hodgkin lymphoma have been reported in these patients (471,504,2387,2389, 2510,2673,4352,4355). Although there is concern that patients with Crohn disease or inflammatory bowel disease treated with infliximab and other TNF antagonists (adalimumab and etanercept) are at increased risk for hepatosplenic T-cell lymphoma (HSTL), other studies have shown no increased risk (1054), or an increased incidence only when patients were also receiving a thiopurine or in patients only receiving a thiopurine (1626, 2091,2426,3414,3824,4355).
Etiology
Although some of these other iatrogenic lymphoproliferative disorders are associated with EBV, like in many PTLDs, the frequency of EBV infection is very variable (1703,3498). Overall, about 40% of lymphoproliferative disorders in rheumatoid arthritis patients treated with methotrexate are EBV-positive, with EBV detected more frequently in Hodgkin lymphoma (~80%) than in DLBCL (~25–60%) or other B-cell lymphoma types (1752,2065,2673,4403). EBV is almost always found in polymorphic lymphoproliferative disorders and in lymphoproliferative disorders that have been reported to have Hodgkin-like features in this setting (1703,1752,3498). EBV is not seen in HSTL. The degree and duration of immunosuppression likely plays a role in the development of EBV-positive lymphoproliferative disorders. However, the degree of inflammation and/or chronic antigenic stimulation as well as the patient's genetic background may also be important determinants of the risk and type of lymphoproliferative disorder (219,220). For example, patients with rheumatoid arthritis are estimated to have a 2-fold to 20-fold increased risk of lymphoma even in the absence of methotrexate therapy (220,1155,3989), a risk which might be increased in patients receiving methotrexate or tacrolimus and may have an altered EBV-host balance (527,1583). Spontaneous regression of these lymphoproliferative disorders in some cases after drug withdrawal underscores the putative pathogenic role of methotrexate or other immunosuppressive drugs in these lymphoproliferative disorders (219).

Table 16.04 Characteristics of methotrexate-associated lymphoproliferative disorders (LPDs), including EBV-positive mucocutaneous ulcer (EBVMCU), in 274 cases with details reported. Compiled from the literature; see text for references.

<table>
<thead>
<tr>
<th>Type</th>
<th>Total</th>
<th>EBV</th>
<th>Extranaodal</th>
<th>Regress</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>B-cell lymphomas</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DLBCL</td>
<td>159</td>
<td>45/108</td>
<td>66/90</td>
<td>35/115</td>
</tr>
<tr>
<td>Polymorphic/lymphoplasmacyclic infiltrates</td>
<td>27</td>
<td>12/17</td>
<td>6/6</td>
<td>10/14</td>
</tr>
<tr>
<td>Follicular lymphoma</td>
<td>11</td>
<td>2/10</td>
<td>2/5</td>
<td>3/8</td>
</tr>
<tr>
<td>Burkitt lymphoma</td>
<td>3</td>
<td>1/3</td>
<td>0/1</td>
<td>0/3</td>
</tr>
<tr>
<td>MZL/MALT lymphoma</td>
<td>3</td>
<td>0/3</td>
<td>3/3</td>
<td>1/1</td>
</tr>
<tr>
<td>Lymphoplasmacyclic lymphoma</td>
<td>2</td>
<td>0/2</td>
<td>--</td>
<td>0/2</td>
</tr>
<tr>
<td>CLL/SLL</td>
<td>1</td>
<td>0/1</td>
<td>0/1</td>
<td>0/1</td>
</tr>
<tr>
<td>MCL</td>
<td>1</td>
<td>0/1</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td><strong>T-cell LPDs/lymphomas</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PTCL</td>
<td>7</td>
<td>0/4</td>
<td>0/1</td>
<td>3/5</td>
</tr>
<tr>
<td>Extranodal NK/T-cell lymphoma, nasal type</td>
<td>2</td>
<td>2/2</td>
<td>2/2</td>
<td>1/1</td>
</tr>
<tr>
<td>Other T-cell LPD</td>
<td>1</td>
<td>1/1</td>
<td>1/1</td>
<td>1/1</td>
</tr>
<tr>
<td>Hodgkin lymphoma</td>
<td>42</td>
<td>19/23</td>
<td>2/19</td>
<td>11/25</td>
</tr>
<tr>
<td>Hodgkin-like lesions&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6</td>
<td>6/8</td>
<td>3/5</td>
<td>6/6</td>
</tr>
<tr>
<td>EBVMCU&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9</td>
<td>9/9</td>
<td>9/9</td>
<td>5/6</td>
</tr>
<tr>
<td>Total</td>
<td>274</td>
<td>97/190</td>
<td>94/143</td>
<td>76/188</td>
</tr>
</tbody>
</table>

CLL/SLL, chronic lymphocytic leukaemia/ small lymphocytic lymphoma; DLBCL, diffuse large B-cell lymphoma; MALT, mucosa-associated lymphoid tissue; MCL, mantle cell lymphoma; MZL, marginal zone lymphoma; PTCL, peripheral T-cell lymphoma.

<sup>a</sup> Likely correspond to EBV-positive B-cell LPDs with Hodgkin-like cells, and may include EBV-positive cases presenting in oropharynx, skin, or gastrointestinal tract, which might represent the newly recognized EBVMCU.

<sup>b</sup> Only cases designated as EBVMCU occurring in patients receiving methotrexate are included here.

Fig 16.23 Polymorphic lymphoproliferative disorder with Hodgkin lymphoma–like features in a patient with rheumatoid arthritis treated with methotrexate. A Note the polymorphous infiltrate, with lymphocytes, histiocytes, and Reed–Sternberg-like cells. B In situ hybridization for EBV-encoded small RNA (EBER) showing numerous positive large cells as well as many positive small lymphocytes.
Infliximab has been reported to induce clonal expansion of gamma delta T cells in patients with Crohn disease [1985].

Localization
Of the cases reported in patients receiving methotrexate, 40–50% have been extranodal, occurring at sites such as the gastrointestinal tract, skin, liver and spleen, lung, kidney and adrenal gland, thyroid gland, bone marrow, CNS, gingiva, and soft tissue [74,1703,1787,2065,2945,3498]. As is the case for HSTL in other settings, the spleen, liver, and bone marrow are the most common sites of involvement of HSTL in patients with Crohn disease receiving immunomodulators [2426,3414].

Clinical features
The clinical features are the same as those seen in immunocompetent patients with similar-appearing lymphomas.

Microscopy
The distribution of histological types of iatrogenic lymphoproliferations in non-transplantation settings appears to differ from that seen in other immunodeficiency settings, with a probable increase in the frequency of Hodgkin lymphoma and lymphoid proliferations with Hodgkin-like features, such as EBV-positive mucocutaneous ulcer. Among patients treated with methotrexate, the reported cases are most commonly DLBCL (35–60%) and CHL (12–25%), with less frequent cases of follicular lymphoma (3–10%), Burkitt lymphoma, extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma), and peripheral T-cell lymphoma [1703,1752,1908,2387,2509,3498]. Polymorphic or lymphoplasmacytic infiltrates resembling polymorphic PTLD have been described in as many as 20% of cases in this setting (1752). Peripheral T-cell lymphomas occurring in this setting seem to have a common extranodal presentation, a cytotoxic profile, and may include extranodal NK/T-cell lymphomas [2079,3870]. Among CHLs, the mixed-cellularity subtype is more frequent than nodular sclerosis, with a significant proportion that cannot be further classified [2387]. Lesions containing Reed–Sternberg–like cells but not fulfilling the criteria for Hodgkin lymphoma, which in the past were referred to as Hodgkin-like lesions, have been reported [1911], with some likely constituting the recently recognized EBV-positive mucocutaneous ulcer [1018] and others more closely resembling a polymorphic PTLD. HSTL in patients who have been treated with infliximab is indistinguishable from HSTL arising in immunocompetent or post-transplant patients.

Immunophenotype
The immunophenotype of the lymphoproliferative disorder does not appear to differ from that of the lymphomas in non-immunosuppressed hosts, which they resemble. Among methotrexate-associated DLBCLs, the majority have an activated-B-cell immunophenotype, especially EBV-positive cases. EBV-positive methotrexate-associated DLBCLs commonly express CD30 [2065,4403]. Immunophenotype is a useful tool in the distinction from lymphoproliferative disorders that may have some Hodgkin lymphoma–like features, but which should not be considered to represent CHL, the large cells most typically being CD20+/CD30+/CD15− and CD20+/CD30+/CD15+, respectively. EBV is variably positive, with type II latency (LMP1-positive and EBNA2-negative) more common than type III (LMP1-positive, EBNA2-positive) [1752,4403].

Prognosis and predictive factors
A significant proportion of patients with methotrexate-associated lymphoproliferative disorder have shown at least partial regression in response to drug withdrawal (Table 16.04). Although most responses have occurred in EBV-positive cases [1703,1752,1764,2387,3498,4403], a proportion of EBV-negative cases also respond [1752,2065]. A variable proportion of DLBCLs (as many as ~40%) have regressed, while most require cytotoxic therapy. The proportion of polymorphic lymphoproliferative disorder patients with regression after withdrawal is higher. In recent studies of methotrexate-associated lymphoproliferative disorder in rheumatoid arthritis patients, the 5-year overall survival rate was >70%. Spontaneous regression following methotrexate withdrawal has been associated with EBV positivity and a non-DLBCL type of lymphoproliferative disorder, whereas patient age >70 years and DLBCL type are predictive of a shorter survival [1752,4014]. Early lymphocyte recovery after methotrexate withdrawal may be predictive of good response [1764]. A moderate number of patients whose lymphoproliferative disorder initially regresses after discontinuation of methotrexate later relapse and then require chemotherapy [1703,1752]. Regression after discontinuation of drug seldom occurs in patients who develop lymphoproliferative disorder following the administration of TNF blockers. Like in individuals without overt immunodeficiency, cases of HSTL in patients treated with infliximab plus thiopurine have a very aggressive clinical course, with most survivors treated with allogeneic bone marrow transplantation [2426,3414].
CHAPTER 17

Histiocytic and dendritic cell neoplasms

Histiocytic sarcoma
Tumours derived from Langerhans cells
Indeterminate dendritic cell tumour
Interdigitating dendritic cell sarcoma
Follicular dendritic cell sarcoma
Fibroblastic reticular cell tumour
Disseminated juvenile xanthogranuloma
Erdheim–Chester disease
Histiocytic and dendritic cell neoplasms

**Introduction**

Pileri S.A.  
Jones D.M.  
Jaffe R.  
Jaffe E.S.  
Facchetti F.

**Definition**

Histiocytic neoplasms are derived from mononuclear phagocytes (macrophages and dendritic cells) or histiocytes. Dendritic cell tumours are related to several lineages of accessory antigen-presenting cells (dendritic cells) that have a role in phagocytosis, processing, and presentation of antigen to lymphoid cells.

**Epidemiology**

Tumours of histiocytes are among the rarest tumours affecting lymphoid tissues, probably accounting for < 1% of tumours presenting in the lymph nodes or soft tissue [1165,3180]. Because several of these tumour types were poorly recognized until recently, their true incidence remains to be determined. Historically, some large cell lymphomas of B-cell or T-cell type were thought to be histiocytic or reticulum cell sarcomas on purely morphological grounds, but only a small number have proven to be of true macrophage or dendritic cell origin. Some of the regulatory disorders, such as macrophage activation and haemophagocytic syndromes, can have large numbers of histiocytes, but these are non-neoplastic. No sex, racial, or geographical predilection has been described (Table 17.01).

**Histogenesis**

The cellular counterparts of this group of neoplasms consist of myeloid-derived macrophages, myeloid-derived dendritic cells, and stromal-derived dendritic cells. The myeloid-derived macrophages and dendritic cells constitute divergent lines of differentiation from bone marrow precursors, although transdifferentiation or hybrid differentiation states likely occur. Histiocytic and dendritic cell neoplasms tend to reproduce the morphological, phenotypic, and ultrastructural characteristics of terminally differentiated elements. In line with this, blastic plasmacytoid dendritic cell (PDC) neoplasms is excluded from this section on histiocytic and dendritic cell neoplasms, and is discussed after the acute myeloid leukaemias and related precursor neoplasms, because it stems from a cell that acquires terminal differentiation and dendritic appearance following activation (see Chapter 9, Blastic plasmacytoid dendritic cell neoplasm, p. 173).

Intriguingly, during the past few years, several publications have highlighted the fact that, irrespective of their supposed normal counterparts (myeloid-derived macrophages or myeloid-derived dendritic cells), some of these neoplasms are associated with or preceded by a malignant lymphoma (e.g. follicular lymphoma, chronic lymphocytic leukaemia, B-lymphoblastic leukaemia/lymphoma or T-lymphoblastic leukaemia/lymphoma, and peripheral T-cell lymphoma) [678, 859,860,1172,3313,3633]. Under these circumstances, they carry the same TR or IG rearrangements and chromosomal aberrations as lymphoid neoplasms, consistent with transdifferentiation [678, 859,860,1172,3313,3633]. The histiocytic and lymphoid neoplasm may share a common progenitor [479], but one that has already undergone IG rearrangement. To date, neither comprehensive gene expression profiling nor next-generation sequencing studies have been carried out, with the exception of studies based on canine models [399] and small series of follicular dendritic cell (FDC) sarcomas [1460,1569A,2184A, 2383A]. A next generation sequencing study of 13 FDC sarcomas revealed recurrent loss-of-function alterations in tumour suppressor genes involved in the negative regulation of NF-kappaB activation (38% of cases) and cell-cycle progression (31% of cases) [1460]. The possible occurrence of BRAF V600E mutation has been reported in the setting of histiocytic sarcoma, Langerhans cell histiocytosis.

Fig. 17.01 Schematic diagram of the origin of histiocytic and dendritic cells. Macrophages and dendritic cells (DCs; antigen-presenting cells) are derived from a common bone marrow precursor. In contrast, follicular dendritic cells are thought to be of non-haematopoietic origin.
  
\[+,\ most\ if\ not\ all\ cells\ positive;\ -,\ all\ cells\ negative;\ -/+,\ a\ minority\ of\ cells\ positive;\ v,\ variable\ intensity.\]

466 Histiocytic and dendritic cell neoplasms
FDC sarcoma, and disseminated juvenile xanthogranuloma [1366]. In Erdheim-Chester disease, activating mutations in MAPK pathway genes, most notably BRAF V600E, as well as NRAS mutation, can be detected. Recurrent mutations in the PI3K pathway gene have also been described [71].

Monocytes, macrophages, and histiocytes
Metchnikoff, considered the father of the macrophage, coined the term 'phagocytosis' in 1883, postulating a central role for the process in the body's innate defence against infection [1412,2648]. The histiocytes/macrophages are derived from bone marrow–derived monocytes. Following migration/maturation in tissues, they participate in the innate response with proinflammatory and anti-inflammatory cytokine effects, as well as in particulate removal and tissue reconstitution [1412,1413]. They are derived largely from the circulating peripheral blood monocyte pool that migrates through blood vessel walls to reach its site of action, but local proliferation also contributes [3303]. Histiocytic tumours are closely related to the monocytic tumours from which their precursors are derived. The distinction between a leukaemic infiltrate of monocytic origin and histiocytic sarcoma can sometimes be difficult on morphological grounds alone.

Macrophages display phagocytosis under some conditions of activation; at this stage, there is heightened expression of lysosomal enzymes that can be demonstrated by histochemistry, including non-specific esterases and acid phosphatase. Phagocytic activity is not a prominent feature of histiocytic malignancy but is a cardinal feature of the haemophagocytic syndromes. The haemophagocytic macrophage activation syndromes are an important group of non-neoplastic proliferative disorders that need to be differentiated from true histiocytic neoplasms, and are far more common. The haemophagocytic syndromes are the result of genetic or acquired disorders in the regulation of macrophage activation. The familial haemophagocytic lymphohistiocytosis is due to genetically determined inability to regulate macrophage killing by NK and/or T cells due to mutations in perforin or in its packaging, export, or release. Acquired or secondary causes of the haemophagocytic macrophage activation syndromes follow certain infections, most notably by EBV and a wide variety of other infectious agents, as well as some malignancies, rheumatic disorders, and multiorgan failure [1834]. The characteristic cytopenias of the haemophagocytic syndromes are most likely due to bone marrow suppression by the cytokine storm, because the bone marrow is often hypercellular at the outset.

Myeloid-derived dendritic cells
Dendritic cells or antigen-presenting cells are found in various sites and at different
Histiocytic and dendritic cell neoplasms

Overgrowth) may be seen in Castleman nodes and express SMA. Hyperplasia they ensheath the postcapillary venules and become associated with bone marrow stromal progenitors (i.e. stromal and other mediators. In lymph nodes, they mature into secondary B-cell follicles, trap and store antigen on the cell surface as immunocomplexes for long periods of time. FDCs appear to be closely related to bone marrow stromal progenitors with features of myofibroblasts. They are resident within primary and secondary B-cell follicles, trap and present antigen to B cells. FDCs can store antigen on the cell surface as immune complexes for long periods of time. FDCs appear to be closely related to bone marrow stromal progenitors with features of myofibroblasts. They are of mesenchymal origin and express SMA. Hyperplasia of fibroblastic reticular cells (i.e. stromal overgrowth) may be seen in Castleman disease, and tumours of fibroblastic reticular cells arise in lymph nodes and have features of myofibroblastic tumours and closely related neoplasms.

Prognosis and predictive factors

Because there are few phenotypic markers unique for dendritic or macrophage histiocytes, investigators should use a panel appropriate to the cell in question (Table 17.02, p. 467) and rigorously exclude other cell lineages (i.e. T-cell, B-cell, and NK-cell lineages, but also stromal, melanocytic, and epithelial lineages) by immunophenotypic and molecular means. It is also worth mentioning that some leukaemias and anaplastic large cell lymphomas can be accompanied in lymph nodes by an exuberant histiocytic response that may obscure the neoplastic cells. Consistent with their rarity, the treatment of histiocytic and dendritic cell neoplasms is extremely variable, no specific trials being available. Excisional biopsies should be performed whenever possible, needle aspirates being indeed proscribed. Accurate staging is mandatory, because the distinction between localized and systemic forms impacts therapeutic decisions. Localized forms are usually surgically resected; the utility of radiotherapy and/or chemotherapy as adjuvant is debated. The prognosis is relatively favourable, even in cases of relapse, which has been reported in at least one quarter of patients. In contrast, widespread disease requires chemotherapy and has a poor prognosis overall.

Histiocytic sarcoma

Weiss L.M. Pileri S.A. Chan J.K.C. Fletcher C.D.M.

Definition

Histiocytic sarcoma is a malignant proliferation of cells showing morphological and immunophenotypic features of mature tissue histiocytes. Neoplastic proliferations associated with acute monocytic leukaemia are excluded.

ICD-O code

9755/3

Synonym

True histiocytic lymphoma (obsolete)

Epidemiology

Histiocytic sarcoma is a rare neoplasm, with only limited numbers of reported series of bona fide cases. There is a wide patient age range, from infancy to old age; however, most cases occur in adults. A male predilection is found in some studies but not others. Some cases are associated with prior or metachronous low-grade lymphoma, usually follicular lymphoma, but also chronic lymphocytic leukaemia/small lymphocytic lymphoma.

Fig. 17.02 Histiocytic sarcoma. Diffuse effacement of architecture by a large-cell proliferation that is indistinguishable from a diffuse large B-cell lymphoma by conventional histopathology.

468 Histiocytic and dendritic cell neoplasms
Etiology
The etiology is unknown. A subset of cases occur in patients with mediastinal germ cell tumours, most commonly malignant teratoma, with or without a yolk sac tumour component [952]. Because teratocarcinoma cells may differentiate along haematopoietic lines in vitro [844], histiocytic neoplasms may arise from pluripotent germ cells. Other cases may be associated with malignant lymphoma, either preceding or subsequent, or with myelodysplasia and leukaemia [1172, 1176,3180,4486].

Localization
Most cases present in extranodal sites [1688,3180,4216], most commonly the intestinal tract, skin, and soft tissue. Others present with lymphadenopathy. Rare patients have a systemic presentation, with multiple sites of involvement, sometimes referred to as malignant histiocytosis [585,3180,4331].

Clinical features
Patients may present with a solitary mass, but systemic symptoms, such as fever and weight loss, are relatively common [1688,3180,4216]. Skin manifestations may range from a benign-appearing rash to solitary lesions to innumerable tumours on the trunk and extremities. Patients with intestinal lesions often present with intestinal obstruction. Hepatosplenomegaly and associated pancytopenia may occur. The bone may show lytic lesions [1688, 3180,4216].

Microscopy
The tumour consists of a diffuse non-cohesive proliferation of large cells (>20 μm), but a sinusoidal distribution may be seen in the lymph nodes, liver, and spleen. The proliferating cells may be monomorphic or (more commonly) pleomorphic. The individual neoplastic cells are usually large and round to oval in shape; however, focal areas of spindling (sarcomatoid areas) may be observed. The cytoplasm is usually abundant and eosinophilic, often with some fine vacuoles. Haemophagocytosis occurs occasionally in the neoplastic cells. The nuclei are generally large, round to oval or irregularly folded, and often eccentrically placed; large multinucleated forms are commonly seen. The chromatin pattern is usually vesicular, and atypia varies from mild to marked. Immunostaining is essential for distinction from other large cell neoplasms, such as large cell lymphoma, melanoma, and carcinoma. A variable number of reactive cells may be seen, including small lymphocytes, plasma cells, benign histiocytes, and eosinophils. Sometimes the neoplastic cells are obscured by a heavy inflammatory infiltrate including many neutrophils, mimicking an inflammatory lesion; this feature is particularly common in histiocytic sarcoma involving the CNS [699].

Ultrastructure
The neoplastic cells show abundant cytoplasm with numerous lysosomes. Birbeck granules and cellular junctions are not seen.

Immunophenotype
By definition, there is expression of one or more histiocytic markers, including CD163, CD68 (KP1 and PGM1), and lysozyme, with typical absence of Langerhans cell (CD1a, langerin), follicular dendritic cell (CD21, CD35), and myeloid cell (CD13, MPO) markers [1688,3180,4216]. Both CD68 and lysozyme show granular cytoplasmic staining. The lysozyme staining is accentuated in the Golgi region. CD163 staining is in the cell membrane and/or cytoplasm. Rarely, weak expression of CD15 occurs [3180]. In addition,
CD45, CD45RO, and HLA-DR are usually positive. There may be expression of S100 protein, but this is usually weak and focal [3180]. There is no positivity for specific B-cell and T-cell markers. CD4 is often positive with cytoplasmic staining. These tumours are devoid of HMB45, EMA, and keratin. The Ki-67 proliferation index is variable [3180].

Postulated normal counterpart
A mature tissue histiocyte

Genetic profile
A subset of cases of histiocytic sarcoma have clonal Ig rearrangements [680], particularly when there is an association with low-grade B-cell lymphoma, most likely constituting examples of transdifferentiation [1540,3294,4216]. In cases associated with t(14q18)-positive follicular lymphoma, identical chromosomal breakpoints in the BCL2 locus may be present in both neoplasms [1172]. In one study, 5 of 8 cases were found to have BRAFV600E mutations [1386], although another study found the mutation to be absent in 3 cases [1553]. Rare cases arising in mediastinal germ cell tumour show isochromosome 12p, identical to the genetic change in the germ cell tumour [2865].

Prognosis and predictive factors
Histiocytic sarcoma is usually an aggressive neoplasm, with poor response to therapy, although some exceptions have been reported [1688]. Most patients (60-80%) die of progressive disease, reflecting the high clinical stage at presentation (stage III/IV) in the majority (70%) of patients [3180,4216]. Patients with clinically localized disease and small primary tumours have a more favourable long-term outcome [1688,3180].

Tumours derived from Langerhans cells
Weiss L.M.
Jaffe R.
Facchetti F.

Definition
Tumours derived from Langerhans cells (LCs) are divided into two main subgroups, according to the degree of cytomorphological atypia and clinical aggressiveness: LC histiocytosis and LC sarcoma. Both subgroups maintain the phenotypic profile and ultrastructural features of LCs. Rare cases can be difficult to assign to one category or the other; these cases require further clinicopathological studies to clarify their nature.

Langerhans cell histiocytosis
Definition
Langerhans cell histiocytosis (LCH) is a clonal neoplastic proliferation of Langerhans-type cells that express CD1a, langerin, and S100 protein and show Birbeck granules by ultrastructural examination.

ICD-O codes
LCH, NOS 9751/1
LCH, monostotic 9751/1

Synonyms
Langerhans cell granulomatosis; solitary lesion: histiocytosis X, eosinophilic granuloma (obsolete); multiple lesions: Hand-Schüller-Christian disease (obsolete); cases with disseminated or visceral involvement: Letterer-Siwe disease (obsolete); Langerhans cell histiocytosis, unifocal (9752/3) (obsolete); Langerhans cell histiocytosis, multifocal (9753/3) (obsolete); Langerhans cell histiocytosis, disseminated (9754/3) (obsolete)

Epidemiology
The annual incidence is about 5 cases per 1 million population, with most cases occurring in childhood. There is a male predilection, with a male-to-female ratio of 3.7:1 [3180]. The disease is more common in White populations of northern European descent and rare in Black populations. Primary LCH of the lung is almost always a disease of smokers [857]. Rare cases may be associated with follicular lymphoma [4302].

Etiology
An IL1 loop model has been proposed for the pathogenesis, based on the finding of high levels of the tyrosine phosphatase SH1 in lesional tissues and increased levels of IL17A in peripheral blood and lesional tissues, particularly in patients with multiorgan disease [2787].

Localization
The disease can be localized to a single site, can occur in multiple sites within a single system (usually bone), or can be more disseminated and multisystem (2668,4009). The dominant sites of involvement in the solitary form are bone and adjacent soft tissue (skull, femur, vertebra, pelvic bones, and ribs).
less commonly, lymph node, skin, and lung. Multifocal lesions are largely confined to bone and adjacent soft tissue. In multisystem disease, the skin, bone, liver, spleen, and bone marrow are the preferential sites of involvement. The gonads and kidney appear to be spared even in disseminated cases.

Clinical features

Patients with unifocal disease are usually older children or adults who most commonly present with a lytic bone lesion eroding the cortex. Solitary lesions at other sites present as mass lesions or enlarged lymph nodes. Patients with unisystem multifocal disease are usually young children who present with multiple or sequential destructive bone lesions, often associated with adjacent soft tissue masses. Skull and mandibular involvement is common. Diabetes insipidus follows cranial involvement. Patients with multisystem involvement are infants who present with fever, cytopenias, skin and bone lesions, and hepatosplenomegaly. Pulmonary disease in childhood is clinically variable. There is an association between LCH and T-lymphoblastic leukaemia, with the leukaemia-associated TR gene rearrangement present in the LCH cells; this has been considered a transdifferentiation phenomenon.

Microscopy

The key feature is the LCH cells. These are oval; about 10–15 μm; and recognized by their grooved, folded, indented, or lobed nuclei with fine chromatin, inconspicuous nucleoli, and thin nuclear membranes. Nuclear atypia is minimal, but mitotic activity is variable and can be high without atypical forms. The cytoplasm is moderately abundant and slightly eosinophilic. Unlike epidermal Langerhans cells or dermal perivascular cells, LCH cells are oval in shape and devoid of dendritic cell processes. The characteristic milieu includes a variable number of eosinophils, histiocytes (both multinucleated LCH forms and osteoclast-type cells, especially in bone), neutrophils, and small lymphocytes. Plasma cells are usually sparse. Occasionally, eosinophilic abscesses with central necrosis, rich in Charcot–Leyden crystals, may be found. In early lesions, LCH cells predominate, along with eosinophils and neutrophils. In late lesions, the LCH cells are decreased in number, with increased foamy macrophages and fibrosis. Involved lymph nodes have a sinus pattern with secondary infiltration of the paracortex. Spleen shows nodular red pulp involvement. Liver involvement has strong preference for intrahepatic biliary involvement with progressive sclerosing cholangitis. Bone marrow biopsy is preferred to aspiration for documentation of bone marrow involvement.

Large clusters or sheets of LCH cells accompanied by eosinophils can be found within other lesions (lymphomas and sarcomas). It remains to be determined whether these constitute a local reactive phenomenon or a transdifferentiation process.

Ultrastructure

The ultrastructural hallmark is the cytoplasmic Birbeck granules, whose presence can be confirmed by langerin expression. The Birbeck granule has a tennis-racket shape, and is 200–400 nm long and 33 nm wide, with a zipper-like appearance.
Langerhans cell histiocytosis. This lymph node biopsy shows extensive involvement of the sinuses (A) and paracortical regions (B).

**Immunophenotype**

LCH consistently expresses CD1a, langerin (also called CD207), and S100 protein [714,2110]. Langerin and CD1a staining may be particularly useful in detecting bone marrow involvement (2017). In addition, the cells are positive for vimentin, CD68, and HLA-DR. CD45 expression and lysozyme content is low. B-cell and T-cell lineage markers (except for CD4), CD30, and follicular dendritic cell markers are absent. The Ki-67 proliferation index is highly variable [3180]. Expression of PDL1 is seen in many cases [1308].

**Postulated normal counterpart**

A mature Langerhans cell.

**Genetic profile**

LCH has been shown to be clonal by X-linked androgen receptor gene (HUMARA) assay, except in some adult pulmonary lesions [4330,4449, 4451]. About 30% of cases have detectable clonal IGH, IGK, or TR rearrangements, including some cases with both T-cell and B-cell gene rearrangements [681]. Approximately 50% of cases harbour BRAF V600E mutation [215,489]. BRAF V600E mutation has also been identified in 28% of pulmonary cases, suggesting that at least many of these cases constitute a clonal proliferation [3381]. In addition, about 25% of cases are associated with somatic MAP2K1 mutations, almost always occurring in BRAF germline cases [469,2852]. Other BRAF germline cases may have somatic ARAF mutations [2851].

**Genetic susceptibility**

Familial clustering has shown a high concordance rate for identical twins but not dizygotic twins, and no vertical inheritance [145]. Rare familial cases are reported [141,143]. There is a suggestion that interferon gamma and IL4 polymorphisms affect susceptibility to LCH and might be responsible for some of the clinical variation [893].

**Prognosis and predictive factors**

The clinical course is related to staging of the disease at presentation, with ≥99% survival for unifocal disease and 66% mortality for young children with multisystem involvement who do not respond promptly to therapy [1268,2668,4009]. Involvement of the bone marrow, liver, or lung is considered a high-risk factor [2668,4009]. Progression from initial focal disease to multisystem involvement can occur, most commonly in infants. Patient age, per se, is a less important indicator than is extent of disease [2668, 4009]. BRAF V600E mutation does not seem to affect prognosis [2612]. Systemic and (rarely) multifocal disease can be complicated by haemophagocytic syndrome [1166].
Langerhans cell sarcoma

Definition
Langerhans cell (LC) sarcoma is a high-grade neoplasm with overtly malignant cytological features and the LC phenotype.

ICD-O code 9756/3

Epidemiology
LC sarcoma is rare [327,401,3180], and almost all reported cases are in adults. The median patient age is 41 years (range: 10–72 years). Rare cases may be associated with follicular lymphoma [4302].

Etiology
Merkel cell polyomavirus sequences have been identified in a subset of cases [2786].

Localization
The skin and underlying soft tissue are the most common sites of involvement. Multiorgan involvement can affect the lymph nodes, lung, liver, spleen, and bone [401,1202,3180].

Clinical features
Most cases are extranodal (involving skin and bone) and multifocal; high-stage (III–IV) disease is seen in 44%. Only 22% of cases are primarily nodal. Hepatosplenomegaly is noted in 22% and pancytopenia in 11%.

Microscopy
The most prominent feature is the overtly malignant cytology of a pleomorphic tumour, and only the phenotype and/or ultrastructure reveal the LC derivation. Chromatin is clumped and nucleoli are conspicuous. Some cells may have the complex grooves of the LC histiocytosis cell, a key clue to the diagnosis. The mitotic rate is high, usually >50 mitoses per 10 high-power fields. Rare eosinophils may be admixed.

Ultrastructure
Birbeck granules are present, whereas desmosomes/junctional specializations are absent [3180].

Immunophenotype
The immunophenotype is identical to that of LC histiocytosis, although staining for individual markers can be focal.

Postulated normal counterpart
A mature Langerhans cell

Genetic profile
At least one case has been found to harbour the BRAF V600E mutation [678].

Prognosis and predictive factors
LC sarcoma is an aggressive, high-grade malignancy, with >50% mortality from progressive disease.
Indeterminate dendritic cell tumour

Weiss L.M.
Chan J.K.C.
Fletcher C.D.M.

Definition
Indeterminate dendritic cell tumour, also known as indeterminate cell histiocytosis, is a neoplastic proliferation of spindled to ovoid cells with phenotypic features similar to those of normal indeterminate cells, the alleged precursor cells of Langerhans cells. These neoplasms are extraordinarily rare [104,360,412,651,2076,3407,3672,4160,4174,4363,4374]. There may be an association with low-grade B-cell lymphoma [4160].

ICD-O code 9757/3

Localization
Patients typically present with one or (more commonly) multiple generalized papules, nodules, or plaques on the skin. Less often, primary lymph node or splenic disease has been reported.

Clinical features
Systemic symptoms are usually not present.

Microscopy
The lesions are usually based in the dermis, but may extend into the subcutaneous fat. The infiltrate is diffuse, consisting of cells resembling Langerhans cells, with irregular nuclear grooves and clefts. Cytoplasm is typically abundant and usually eosinophilic. Multinucleated giant cells may be present. In some cases, there may be spindling of the cells. The mitotic rate varies widely from case to case. An accompanying eosinophilic infiltrate is usually not present.

Ultrastructure
By definition, these cells lack Birbeck granules on ultrastructural examination. There can be complex interdigitating cell processes, but desmosomes are lacking.

Immunophenotype
The proliferating cells consistently express S100 protein and CD1a. Langerin is negative. They are negative for specific B-cell and T-cell markers, CD30, the histiocytic marker CD163, and the follicular dendritic cell markers CD21, CD23, and CD35. They are variably positive for CD45, CD68, lysozyme, and CD4. The Ki-67 proliferation index is highly variable.

Cell of origin
Normal indeterminate cells, the postulated precursors of Langerhans cells

Genetic profile
One case has been shown to be clonal by human androgen receptor gene assay (4374). One case has been shown to harbour BRAF V600E mutation (2916).

Prognosis and predictive factors
The clinical course has been highly variable, ranging from spontaneous regression to rapid progression. There are no known prognostic factors. One case was associated with the development of acute myeloid leukaemia (4174).
Interdigitating dendritic cell sarcoma

Weiss L.M.
Chan J.K.C.

Definition
Interdigitating dendritic cell (IDC) sarcoma is a neoplastic proliferation of spindle to ovoid cells with phenotypic features similar to those of IDCs.

ICD-O code 9757/3

Synonym
Interdigitating dendritic cell tumour

Epidemiology
IDC sarcoma is an extremely rare neoplasm, with most studies constituting single case reports or very small series [104, 1179, 2411, 2659, 2812, 2813, 3180, 3431, 4286]. The largest series to date have consisted of 4 cases [1269, 3180, 3186]. The reported cases have occurred predominantly in adults, although one paediatric series has been reported [3186]. There is a slight male predominance. Occasional cases have been associated with low-grade B-cell lymphoma, and rare cases have been associated with T-cell lymphoma [1172, 1269].

Localization
The presentation can vary widely. Solitary lymph node involvement is most common, but extranodal presentations, in particular in the skin and soft tissue, have also been reported.

Clinical features
Patients usually present with an asymptomatic mass, although systemic symptoms, such as fatigue, fever, and night sweats, have been reported. Rarely, there may be generalized lymphadenopathy, splenomegaly, or hepatomegaly.

Microscopy
The lesional tissue in lymph nodes is present in a paracortical distribution with residual follicles. The neoplastic proliferation usually forms fascicles, a storiform pattern, and whorls of spindled to ovoid cells. Sheets of round cells are occasionally found. The cytoplasm of the neoplastic cells is usually abundant and slightly eosinophilic, and often has an indistinct border. The nuclei also appear spindled to ovoid, and may show indentations; occasional multinucleated cells may be seen. The chromatin is often vesicular, with small to large, distinct nucleoli. Cytological atypia varies from case to case, although the mitotic rate is usually low (<5 mitoses per 10 high-power fields). Necrosis is usually not present. There are often numerous admixed lymphocytes, and less commonly, plasma cells. The histological appearance is sometimes indistinguishable from that of a follicular dendritic cell sarcoma, and phenotyping is necessary for precise diagnosis.

Ultrastructure
The neoplastic cells show complex interdigitating cell processes, but well-formed desmosomes are not present. Scattered lysosomes may be present, but Birbeck granules are not seen.

Immunophenotype
The neoplastic cells consistently express S100 protein and vimentin, with CD1a and langerin being negative. They are usually positive for fascin and (variably) weakly positive for CD68, lysozyme, and CD45. Strong nuclear staining for p53 may be present. They are negative for markers of follicular dendritic cells (CD21, CD23, and CD35), MPO, CD34, specific B-cell-associated and T-cell-associated antigens, CD30, EMA, and cytokeratins. The Ki-67 proliferation index is usually 10–20% (median: 11%) [1179]. The admixed small lymphocytes are almost always of T-cell lineage, with near absence of B cells.

Postulated normal counterpart
Interdigitating dendritic cell (IDC)
Fig. 17.17 Interdigitating dendritic cell sarcoma. A Note the paracortical pattern of tumour growth in the lymph node. B There are scattered small lymphocytes throughout the lesion. C The CD21 stain is negative on the tumour cells, but labels follicular dendritic cells in residual follicles. D In contrast, the stain for S100 protein is strongly positive in the tumour cells.

Genetic profile
A subset of cases of IDC sarcoma have clonal IG rearrangements, particularly when there is an association with low-grade B-cell lymphoma, most likely constituting examples of transdifferentiation [680,1172]. In cases associated with t(14;18)-positive follicular lymphoma, identical chromosomal breakpoints affecting the BCL2 locus are present in both neoplasms [1172]. The TR genes are in a germline configuration [4286]. At least one case has been shown to harbour somatic BRAF V600E mutation [2916].

Prognosis and predictive factors
The clinical course is generally aggressive, with about half of all patients dying of the disease. Commonly affected visceral organs include the liver, spleen, kidney, and lung. Stage may be an important prognostic factor; however, histological features have not been correlated with clinical outcome.

Follicular dendritic cell sarcoma
Chan J.K.C.
Pileri S.A.
Fletcher C.D.M.
Weiss L.M.
Grogg K.L.

ICD-O code
9758/3

Synonyms
Dendritic reticulum cell tumour (no longer recommended); follicular dendritic cell tumour

Epidemiology
FDC sarcoma is a rare neoplasm [104, 645,2695,3135,3180,4286]. There is a wide patient age range, with an adult predominance (median patient age: 50 years) [3180,3543]. The sex distribution is about equal [3180].

Etiology
A proportion of cases appear to arise in the setting of hyaline-vascular Castleman disease, sometimes with a recognizable intermediary phase of FDC proliferation outside the follicles [638,645]. The Castleman disease lesion may be found concurrent with the FDC sarcoma, or may precede the latter by several years.

Localization
FDC sarcoma presents with lymph node disease in 31% of cases, extranodal disease in 58%, and both nodal and extranodal disease in 10% [3543]. Nodal disease most often affects the cervical nodes. A wide variety of extranodal sites can be affected, most commonly tonsil, gastrointestinal tract, soft tissue, mediastinum, retroperitoneum, omentum, and lung [1670,3543]. Common sites for metastasis include lymph nodes, lung, and liver [737].

Clinical features
Patients most often present with a slow-growing, painless mass lesion, although patients with abdominal disease may present with abdominal pain. The tumours are often large, with a mean size of 7 cm [3543]. Most patients have localized disease at presentation [3543]. Systemic symptoms are uncommon. Rare patients have paraneoplastic pemphigus [2250,2536,4240].

Microscopy
The neoplasm consists of spindled to ovoid cells forming fascicles, storiform arrays, whorls (sometimes reminiscent of the 360° pattern observed in meningioma), diffuse sheets, or vague nodules. The individual neoplastic cells generally show indistinct cell borders and a moderate amount of eosinophilic cytoplasm. The
Follicular dendritic cell sarcoma. This mass occurred in the soft tissue and has the appearance of a sarcoma.

nuclei are oval or elongated, with vesicular finely dispersed chromatin, small but distinct nucleoli, and a delicate nuclear membrane. The nuclei tend to be unevenly spaced, with areas showing clustering. Nuclear pseudoinclusions are common. Binucleated and multinucleated tumour cells are often seen. Although the cytological features are usually relatively bland, significant cytological atypia may be found in some cases. The mitotic rate is usually 0–10 mitoses per 10 high-power fields, although the more pleomorphic cases can show much higher mitotic rates (>30 mitoses per 10 high-power fields), easily found atypical mitoses, and coagulative necrosis.

The tumour is typically lightly infiltrated by small lymphocytes, which can sometimes be aggregated around the blood vessels as well [642]. Less common morphological features include epithelioid tumour cells with hyaline cytoplasm, clear cells, oncocytic cells, myxoid stroma, fluid-filled cystic spaces, prominent fibrovascular septa, and admixed osteoclastic giant cells [642,645,3135,3136]. Uncommonly, there is a nodular growth pattern, with the large neoplastic cells scattered in a background of small B lymphocytes [2390]. Rare cases may also show jigsaw puzzle–like lobulation and perivascular spaces, mimicking thymoma or carcinoma showing thymus-like element [737].

Ultrastructure
The neoplastic cells have elongated nuclei, often with cytoplasmic invaginations. There are characteristically numerous long, slender cytoplasmic process-
Two studies examining the transcriptional profile of FDC sarcoma have revealed: 1) a peculiar immunological microenvironment enriched in TFH and Treg populations, with special reference to the inhibitory immune receptor PD1 and its ligands PD-L1 and PD-L2, and 2) the highly specific expression of the genes encoding for FDCSP and SRGN [1460B,1460C]. Conventional FDC sarcomas are negative for EBV, whereas the inflammatory pseudotumour-like variant consistently shows EBV in the neoplastic cells [697].

Prognosis and predictive factors
FDC sarcoma is usually treated by complete surgical excision, with or without adjuvant radiotherapy or chemotherapy. A pooled analysis of the literature showed local recurrence and distant metastasis rates of 28% and 27%, respectively [3543], but the true figures are likely to be higher, because these adverse events are sometimes delayed many years. The 2-year survival rates for early, locally advanced, and distant metastatic diseases are 82%, 80%, and 42%, respectively [3543]. Some patients may die from refractory paraneoplastic pemphigus.

Large tumour size (≥6 cm), coagulative necrosis, high mitotic count (≥5 mitoses per 10 high-power fields), and significant cytological atypia are associated with a worse prognosis [645,3543].

Inflammatory pseudotumour-like follicular/fibroblastic dendritic cell sarcoma

Inflammatory pseudotumour-like follicular/fibroblastic dendritic cell (FDC/FRC) sarcoma occurs predominantly in young to middle-aged adults, with a marked female predilection [697]. It typically involves the liver or spleen, but both sites may be simultaneously involved [134,697,3619]. Rarely, it selectively involves the gastrointestinal tract in the form of a polyloid lesion [3042]. Patients are asymptomatic or present with abdominal distension or pain, sometimes accompanied by systemic symptoms [683,697,733].

Histologically, the neoplastic spindled cells are dispersed within a prominent lymphoplasmacytic infiltrate. The nuclei usually show a vesicular chromatin pattern and small but distinct nucleoli. Nuclear atypia is highly variable; usually most cells are bland-looking, but some cells with enlarged, irregularly folded or hyperchromatic nuclei are almost always found. Some tumour cells may even resemble Reed–Sternberg cells [3619]. Necrosis and haemorrhage are often present, and may be associated with a histiocytic or granulomatous reaction. The blood vessels frequently show fibrinoid deposits in the walls. In occasional cases, the tumour may be masked by massive infiltrates of eosinophils or numerous epithelioid granulomas [2310].

The neoplastic cells are often positive for follicular dendritic cell markers, such as CD21 and CD35, with the staining ranging from extensive to very focal. However, in some cases, they may be negative for follicular dendritic cell markers but express SMA, raising the possibility of fibroblastic reticular cell differentiation. Still other cases are positive for all these markers or lack these markers [129,697,733,1399,2293]. Both FDC and FRC share a common mesenchymal origin, with plasticity in the immunophenotype. The neoplastic cells are consistently associated with EBV [134,697], which is present in a monoclonal episomal form [3619]. Outcome data are limited, but
Fibroblastic reticular cell tumour

Weiss L.M.
Chan J.K.C.
Fletcher C.D.M.

Definition
Fibroblastic reticular cell tumour is very rare. Tumours diagnosed as cytokeratin-positive interstitial reticulum cell tumours probably constitute the same entity [104, 639].

ICD-O code 9759/3

Synonyms
Cytokeratin-positive interstitial reticulum cell tumour; fibroblastic dendritic cell tumour

Localization
This tumour can occur in the lymph nodes, spleen, or soft tissue [104, 639, 1415, 1874].

Microscopy
The tumour is histologically similar to follicular dendritic cell sarcoma or interdigitating dendritic cell sarcoma, but lacks the immunophenotypic profile of these tumour types. There are often interspersed delicate collagen fibres.

Ultrastructure
Ultrastructurally, the spindle cells show delicate cytoplasmic extensions and features similar to those of myofibroblasts (i.e. filaments with occasional fusiform densities, well-developed desmosomal attachments, rough endoplasmic reticulum, and basal lamina–like material).

Immunophenotype
The tumour cells are variably immunoreactive for SMA, desmin, cytokeratin (in a dendritic pattern), and CD68.

Prognosis and predictive factors
Available data regarding outcome are extremely limited. The clinical outcome is variable. Some patients die of the disease.
Disseminated juvenile xanthogranuloma

Brousse N. Pileri S.A. Haroche J. Dagna L. Jaffe R. Fletcher C.D.M. Jaffe E.S. Harris N.L.

Definition
Disseminated juvenile xanthogranuloma (JXG) is characterized by a proliferation of histiocytes similar to those of the dermal JXG, commonly having a foamy (xanthomatous) component with Touton-type giant cells. There is evidence for clonality in some instances. Erdheim–Chester disease is distinguished from JXG in the current classification of histiocytic neoplasms [1101].

Synonyms
Benign cephalic histiocytosis; progressive nodular histiocytosis; generalized (non-lipidaemic) eruptive histiocytosis (skin); xanthoma disseminatum (skin plus mucosa lesions); Erdheim–Chester disease (adult form with bone and lung involvement)

Epidemiology
Solitary dermal JXG is vastly more common than other forms and does not progress to more-disseminated forms [925]. Most deep, visceral, and disseminated forms occur by the age of 10 years, half within the first year of life [1836].

Etiology
There is a known association with neurofibromatosis type 1; patients with both are at slightly higher risk of juvenile myelomonocytic leukaemia [4508]. Patients with both Langerhans cell disease (i.e. Langerhans cell histiocytosis; LCH) and JXG are also encountered. To date, no cases of JXG bearing mutations of BFAF or other genes involved in the MAPK pathway have been described [634,1553].

Localization
The skin and soft tissues are affected most commonly. Disseminated forms commonly affect the mucosal surfaces, in particular within the upper aerodigestive tract. When skin is involved, there seems to be a predilection for the head and neck region [925]. The CNS, dura, and pituitary stalk can be affected, as can eye, liver, lung, lymph node, and bone marrow. Retroperitoneal and peri-aortic involvement is principally noted in Erdheim–Chester disease [925,1256,1836].

Clinical features
Skin lesions other than the common papular solitary form are small (1–2 mm) and multiple. Soft tissue lesions can be large, and the lesions present as mass effect. Optic lesions can cause glaucoma. Like in LCH, CNS and pituitary lesions can cause diabetes insipidus, seizures, hydrocephalus, and mental status changes [925,1256,1836]. Unlike in LCH, liver involvement does not target the biliary system or lead to sclerosing cholangitis [1826]. There is some capacity for lesions to slowly regress. JXG appears to be benign, but a concomitant macrophage activation syndrome can lead to cytopenias, liver damage, and (in the systemic forms) death.

Microscopy
The JXG cell is small and oval, sometimes slightly spindled with a bland round to oval nucleus without grooves and with pink cytoplasm. Touton cells are less common at non-dermal sites. The cells become progressively lipidized (xanthomatous). A mixed inflammatory component is variable. Variants include epithelioid cells with glassy cytoplasm. The ultrastructural features are histiocytic, without distinguishing features [4465].

Immunophenotype
In common with macrophages, cells express vimentin, surface CD14, CD68 (PGM1) in a coarse granular pattern, CD163 in a surface and cytoplasmic pattern, and stabilin-1 (MS-1 antigen). Factor XIIIa staining is common but not universal. Fascin stains the cell cytoplasm and SI 100 is positive in <20% of the cases. However, none of these markers is specific for JXG. CD1a and langerin are negative [654,2104,3513,4465].

Cell of origin
The cell of origin is uncertain. Despite their macrophage phenotype, the cells of disseminated JXG have been postulated (on the basis of shared factor XIIIa and fascin immunostaining) to originate from a dermal/interstitial dendritic cell, but these characteristics have limited specificity [2104].

Genetic profile
No consistent cytogenetic or molecular genetic change has been identified. IG and TR rearrangements are germline. There is evidence for clonality in some instances [1835]. Unlike in Erdheim–Chester disease, there are no reported cases of JXG bearing BFAF mutations [634,1553].

Genetic susceptibility
An association with neurofibromatosis type 1 is known in some cases.

Prognosis and predictive factors
All clinical forms are benign, although multiple lesions in brain, dura, or pituitary can cause local consequences and even death. Systemic forms that involve liver and bone marrow have been treated with LCH-type therapy.
Erdheim–Chester disease

Brousse N.
Pileri S.A.
Haroche J.
Dagna L.

Jaffe R.
Fletcher C.D.M.
Jaffe E.S.
Harris N.L.

Definition
Erdheim–Chester disease (ECD) is a clonal systemic proliferation of histiocytes, commonly having a foamy (xanthomatosus) component, and containing Touton giant cells (considered to be a non–Langerhans cell histiocytosis). It was first described as lipoid granulomatosis in 1930 [693]. The name Erdheim–Chester disease was coined in 1972 to recognize the contribution to the discovery of the condition by Erdheim, who was Chester’s mentor [693]. Diagnosis of ECD is based on clinical features, imaging, and histology [693,1101].

ICD-O code 9749/3

Synonyms
Lipogranulomatosis; lipoid granulomatosis; lipid (cholesterol) granulomatosis; polyostotic sclerosing histiocytosis

Epidemiology
ECD is a rare condition. To date, <1000 cases have been reported. The mean patient age at diagnosis is 55–60 years, but rare paediatric cases (i.e. in patients aged < 15 years) have also been reported. The male-to-female ratio is 3:1.

Localization
Virtually any organ or tissue can be infiltrated by ECD. Skeletal involvement occurs in >95% of cases. Cardiovascular involvement probably occurs in at least half of all patients, but is likely underdiagnosed. One third of patients have retroperitoneal involvement. CNS involvement, diabetes insipidus, and/or exophthalmos occur in 20–30% of patients. CNS involvement may occur due to tissue infiltration by histiocytes or degenerative alterations typically affecting the cerebellum, with involvement due to degenerative alterations being much more difficult to treat. Xanthelasma, generally involving the eyelids or periorbital spaces, is the most common cutaneous manifestation.

Clinical features
The clinical course of ECD depends on the extent and distribution of the disease. Some cases, with lesions limited to the bone, are asymptomatic; others, with multisystemic disease, may follow an aggressive, rapid clinical course. In one third of patients, bone involvement may cause mild pain that starts at any time during the course of the disease and usually affects the distal limbs. Cardiovascular involvement may be asymptomatic and detected incidentally by MRI or CT. The most common cardiovascular alteration corresponds to circumferential soft tissue sheathing of the thoracic and abdominal aorta and large arteries, infiltration of the right atrium or auricu-

Imaging
Characteristic features on imaging studies are bilateral and symmetrical cortical osteosclerosis of the diaphyseal and metaphyseal parts of the long bones on X-ray and/or symmetrical and abnormally intense labelling of the distal ends of the long bones of the legs (and in some cases, arms) on 99Tc bone scintigraphy. These findings are highly suggestive of ECD. Occasionally, osteolytic lesions are also seen. PET/CT has a high specificity for the diagnosis of bone involvement by ECD. Cardiovascular involvement manifests as circumferential sheathing of the thoracic or abdominal aorta (so-called

Fig. 17.34 Erdheim–Chester disease (ECD). A Radiograph showing a lytic and sclerotic lesion in the distal femur and the proximal tibia. There is destruction of the anterior femoral cortex, with an impacted pathological fracture through the lesion and an anterolateral soft tissue mass extending from the destroyed femur (arrow). Reprinted from Rosier RN and Rosenberg AE [3416A]. B Abdominal CT from a patient with ECD showing a soft tissue infiltrate surrounding the aorta and kidneys (white arrows). There is a sclerotic lesion in the vertebra (black arrow). Reprinted from Mills JA et al. [2665A].

Fig. 17.35 Retroperitoneal Erdheim–Chester disease. The infiltrate is made up of foamy histiocytes and Touton cells admixed with scattered small lymphocytes in a fibrous tissue.
coated aorta) and large arteries, pericardial effusion (sometimes leading to tamponade), infiltration of the right atrium or auriculoventricular groove, and diffuse pleural thickening. Retroperitoneal involvement may be prominent around the renal capsule (producing the characteristic so-called hairy kidney appearance) and ureters.

Microscopy
There is tissue infiltration by histiocytes, generally with single small nuclei and foamy (xanthomatous) cytoplasm. Other histiocytes, with compact eosinophilic cytoplasm, may also be present. A few multinucleated histiocytes with a central ring of nuclei (Touton cells) are frequently observed. Fibrosis is present in most cases and is sometimes abundant. Reactive small lymphocytes, plasma cells, and neutrophils are also frequently present. The infiltrate may easily be misdiagnosed as a reactive process.

Immunophenotype
ECD histiocytes express three molecules common to macrophages: CD14 (a monocyte or macrophage receptor that binds lipopolysaccharide), CD68 (a largely lysosomal macrosialin), and CD163 (a haemoglobin- and haptoglobin-scavenging receptor). In addition, the cells express factor XIIIa (a tissue transglutaminase) and fascin (an actin-bundling protein), both of which are typical of interstitial and interdigitating dendritic cells. The abnormal histiocytes lack S100, CD1a, and langerin, which are all markers of Langerhans cells. However, cases focally positive for S100 have been reported [1101]. The characteristic immunophenotype is shared by all members of the xanthogranuloma family of histiocytoses. As many as 20% of patients with ECD also have Langerhans cell histiocytosis lesions, and infiltration by ECD and Langerhans cell histiocytosis may be present within the same biopsies [1101]. Cases of ECD with mutated 

Prognosis and predictive factors
ECD is a chronic disease. The disease outcome correlates with sites of involvement; patients with CNS disease or multisystemic disease have a worse outcome [149A,974A]. Disease activity is assessed by clinical examination, imaging, and C-reactive protein values, but no disease activity score has been established. PET scans are very useful for assessments of ECD activity [149C]. Histology is not predictive of evolution. ECD may respond to therapy with interferon alpha, but many cases are refractory, especially those with CNS and cardiovascular involvement [544A,1552A]. Vemurafenib, an inhibitor of BRAF that is approved for treating patients with metastatic melanoma and BRAF V600 mutations, has recently been used, with promising results [544A,1552A,1553A]. The reported 5-year overall survival rate of patients treated with interferon therapy is 68% [149B]. Older series, perhaps with less-effective therapy, reported 43% of patients alive after an average follow-up of 32 months [4192A].
Contributors

Dr Cem AKIN
Department of Internal Medicine
Division of Allergy and Immunology
University of Michigan
1150 West Medical Center Drive
5520-B, MSRB-1, Ann Arbor MI 48109-5600
USA
Tel. +1 734 647 6234; +1 734 936 5634
Fax +1 734 763 4151
cemakin@umich.edu; cakin@partners.org

Dr Ioannis ANAGNOSTOPOULOS
Institute of Pathology, Campus Mitte
Charité – Universitätsmedizin Berlin
Charitéplatz 1
10117 Berlin
GERMANY
Tel. +49 30 450 536 026
Fax +49 30 450 536 914
ioannis.anagnostopoulos@charite.de

Dr Katsuyuki AOZASA
Department of Pathology (C3)
Osaka University Graduate School of Medicine
2-2 Yarindaoka
Suita, Osaka 565-0871
JAPAN
Tel. +81 6 6879 3710
Fax +81 6 6679 3713
aozasa@molpath.med.osaka-u.ac.jp

Dr Daniel A. ARBER
Department of Pathology
University of Chicago
5841 S. Maryland Avenue,
S327, MC 3083
Chicago, IL 60637
USA
Tel. +1 773 702 0647
Fax +1 773 834 5414
darber@uchicago.edu

Dr Michele BACCARANI
University of Bologna
GIMEMA CML Working Party
Via Adolfo Albertazzi 16/2
40137 Bologna
ITALY
Tel. +39 051 349845
Fax +39 051 349845
michele.baccarani@unibo.it

Dr Marie-Christine BÉNÉ
Service d’Hématologie Biologique
Centre Hospitalier Universitaire (CHU) Nantes
9, Quai Moncoux
44000 Nantes
FRANCE
Tel. +33 2 40 08 41 89
Fax +33 2 83 44 60 22
mariebene@gmail.com

Dr Barbara J. BAIN
Department of Haematology
Imperial College Faculty of Medicine
St Mary’s Hospital
Praed Street
UNITED KINGDOM
Tel. +44 20 3312 6806
Fax +44 20 3312 2390
b.bain@ic.ac.uk

Dr Daniel BENHARROCH
Department of Pathology
Soroka Medical Center
Ben Gurion University
Beer Sheva, 84101
ISRAEL
Tel. +972 8 640 0920
Fax +972 8 623 2770
benaroch@bgc.ac.il

Dr John M. BENNETT
Departments of Medicine and Pathology
University of Rochester Medical Center
James P. Wilmot Cancer Center
601 Elmwood Avenue
Rochester NY 14642
USA
Tel. +1 585 275 4915
Fax +1 585 442 0039
john_bennett@urmc.rochester.edu

Dr Emilio BERTI
Department of Dermatology
Fondazione IRCCS Ca’ Granda
Ospedale Maggiore Policlinico
Via Pace 9
20122 Milan
ITALY
Tel. +39 2 5503 5107; +39 2 5503 5200
Fax +39 2 5032 0779
emilio.berti@unimib.it; emilio.berti@gmail.com

Dr Govind BHAGAT
Division of Hematopathology
Department of Pathology and Cell Biology
Columbia University Medical Center
VC14-228, 630 West 168th Street
New York NY 10032
USA
Tel. +1 212 342 1323
Fax +1 212 305 2301
gb96@cumc.columbia.edu

Dr Gunnar BIRGEGÅRD
Department of Hematology
Uppsala University Hospital
SE-751 85 Uppsala
SWEDEN
Tel. +46 18 61 14 412
Fax +46 18 50 92 97
gunnar.birgegard@medsci.uu.se

# Indicates disclosure of interests
Declaration of interests

Dr Akın reports receiving personal consulting fees from Novartis.

Dr Arber reports having received personal consulting fees from United States Diagnostic Standards.

Dr Baccarani reports receiving personal consulting and speaking fees from Ariad, Bristol-Myers Squibb, Novartis, and Pfizer.

Dr Béné reports having received travel and lodging support from Celgene, Beckman Coulter, Mundipharma, Roche and Chugai. She participated in COST EuGESMA, which covered travel and lodging expenses. She has received personal consulting fees from Beckman Coulter, and as head of the Harmonemia project also received travel and lodging support from Beckman Coulter.

Dr Bennett reports receiving personal consulting fees from Celgene and providing unrenumerated consulting services to GlaxoSmithKline, Onconova Therapeutics, and Amgen.

Dr Birgégård reports that the Department of Medical Sciences at Uppsala University has received research funding from Shire. He also reports having received speaking fees from Shire and Renapharma AB, and consulting fees from Shire.

Dr Borowitz reports receiving research support from Bristol-Myers Squibb, Amgen, and MedImmune, and having received research support from Beckman Coulter. He reports receiving non-financial research support from Becton, Dickinson and Company.

Dr Brouss reports having received personal speaking fees from Roche, Kephen, and Acuitude.

Dr Chadburn reports receiving personal consulting fees from Clarient.

Dr Wing Chung Chan reports owning intellectual property rights in a patent (held by the United States National Cancer Institute) on the classification of B-cell lymphoma using gene expression signatures.

Dr Cook reports having received personal consulting fees from Medical Marketing Economics.

Dr de Jong reports receiving personal consulting fees from Millennium Pharmaceuticals and Celgene.

Dr Dogan reports receiving personal consulting fees from Janssen Pharmaceuticals.

Dr Escribano reports receiving travel support from Thermo Fisher Scientific Brazil.

Dr Falini reports having applied for a patent on the clinical use of nucleophosmin protein (NPM1) mutants.

Dr Feldman reports owning intellectual property rights in United States patents (held by Mayo Clinic) on detecting IRF4, DUSP22, or FLJ43663 polypeptide expression and on detecting TBL1XR1 and TP63 translocations.

Dr Fend reports having received travel support from Roche and Janssen Pharmaceuticals.

Dr Gascoyne reports receiving personal speaking fees and research support from Seattle Genetics. He reports receiving personal consulting fees from Celgene, Seattle Genetics, Genentech, Roche, and Janssen Pharmaceuticals.

Dr Gatterman reports receiving personal consulting fees and research support from Novartis and Celgene. He reports receiving personal speaking fees and research support from Novartis.

Dr Germing reports that the MDS Registry, through the University of Düsseldorf, has received funding from Novartis, Celgene, Chugai, Amgen, and Johnson & Johnson. He reports receiving personal speaking fees from Celgene and Johnson & Johnson.

Dr Ghia reports receiving personal consulting fees from Gilead Sciences, Pharmacyclics, Merck, MedImmune, Roche, and AbbVie. He reports receiving personal speaking fees from Gilead Sciences and research support from Roche and GlaxoSmithKline.

Dr Hasseltian reports having received personal consulting fees from Sanofi and Incyte.

Dr Hellström-Lindberg reports receiving unrestricted research support from Celgene.

Dr Inghirami reports having received personal consulting fees from Menarini Diagnostics, Celgene, and Seattle Genetics.

Dr Elaine S. Jaffe reports receiving personal speaking fees from the French and Australian Divisions of the International Academy of Pathology.

Dr Ronald Jaffe reports receiving personal consulting fees from GlaxoSmithKline.

Dr Jones reports that he is a director and shareholder at Quest Diagnostics.

Dr Kadin reports receiving personal consulting fees from Allergan.

Dr Kinney reports that the University of Texas Health Science Center at San Antonio has received fees from Seattle Genetics for her consulting services.

Dr Kovrigina reports receiving travel support and personal consulting fees from Novartis.

Dr Kyle reports being on monitoring committees or boards for Celgene, Novartis, Merck, Bristol-Myers Squibb, Aeterna Zentaris (Keryx), Onyx Pharmaceuticals (Amgen), and Pharmacyclics. He reports receiving personal consulting fees from Binding Site.

Dr List reports receiving personal consulting fees from Cell Therapeutics and Celgene. He reports having received research support from Celgene.

Dr Macon reports receiving personal consulting fees from Seattle Genetics.

Dr Niemeyer reports that the University of Freiburg receives fees from Celgene for her consulting services.

Dr Orazi reports receiving personal consulting fees from Incyte and speaking fees from Incyte and Novartis. He reports that the Department of Pathology and Laboratory Medicine at Weill Cornell Medical College has a contract with GlaxoSmithKline to review histology slides from patients with chronic idiopathic thrombocytopenic purpura who have received long-term recombinant thrombopoietin therapy.

Dr Piris reports receiving personal speaking fees from Takeda.

Dr Porwit reports receiving meeting travel support from Beckman Coulter and personal speaking fees from Novartis.

Dr Radich reports receiving honoraria from Novartis, Bristol-Myers Squibb, Ariad, and Pfizer. He reports that the Fred Hutchinson Cancer Research Center receives non-financial research support from Novartis.

Dr Siebert reports receiving non-financial research support from Abbott.

Dr Stein reports receiving technical research support from HistoGene.

Dr Swerdlow reports having received personal consulting fees from Centocor R&D, Travel Destinations Management Group, and Johnson & Johnson.

Dr Thiele reports having received personal consulting fees from Sanofi, Incyte and Novartis.

Dr Vardiman reports that his department at the University of Chicago has received fees from Celgene for his consulting services.

Dr Whittaker reports that his research unit at St John’s Institute of Dermatology receives fees from Actelion, Yaeuon Therapeutics, and ProStrakan for his consulting services. He reports that his unit has also received fees for his consulting services from Johnson & Johnson.
Clinical Advisory Committee (Leukaemias)

Dr Cem Akin
Allergy and Immunology
Brigham and Women's Hospital
Chestnut Hill, MA 02467, USA

Dr Mario Cazzola #
Policlinico San Matteo
University of Pavia
27100 Pavia, Italy

Dr Jason Gollob
Stanford University
Cancer Center
Stanford, CA 94305-5821, USA

Dr Tomoki Naoe
Graduate School of Medicine
Nagoya University
Nagoya 466-8550, Japan

Dr Robert J. Arceci
Phoenix Children's Hospital
College of Medicine
University of Arizona
Phoenix, AZ 85004, USA

Dr Hartmut Döhner
Internal Medicine
University of Ulm
89081 Ulm, Germany

Dr Peter L. Greenberg
Stanford University
Cancer Center
Stanford CA 94305-5821, USA

Dr Charlotte M. Niemeyer
Department of Pediatrics and Adolescent Medicine
University of Freiburg
79106 Freiburg, Germany

Dr Michele Baccarani
Policlinico S. Orsola
University of Bologna
40138 Bologna, Italy

Dr James R. Downing
St. Jude Children's Research Hospital
Memphis, TN 38105, USA

Dr David Grimwade
Guy’s Hospital
London SW15 2RG, UK

Dr Jerald P. Radich
Fred Hutchinson Cancer Research Center
Seattle, WA 98109, USA

Dr Tiziano Barbui
Research Foundation
Ospedale Papa Giovanni XXIII
24127 Bergamo, Italy

Dr Elihu H. Estey
University of Washington
School of Medicine
Seattle Cancer Care Alliance
Seattle, WA 98109, USA

Dr Rüdiger Hehlmann
Medical Faculty Mannheim
University of Heidelberg
68169 Mannheim, Germany

Dr Martin S. Tallman
Memorial Sloan Kettering Cancer Center
New York, NY 10065, USA

Dr Giovanni Barosi
Fondazione IRCCS
Policlinico San Matteo
27100 Pavia, Italy

Dr Brunangelo Falini
University of Perugia
Polo di Monteluce
06123 Perugia, Italy

Dr Eva Hellström-Lindberg
Department of Medicine, Karolinska Institutet
SE-171 77 Stockholm, Sweden

Dr Ayalew Tefferi
Mayo Clinic
Rochester, MN 55905, USA

Dr John M. Bennett
University of Rochester
Medical Center
Rochester, NY 14642, USA

Dr Pierre Fenaux
Hôpital Saint-Louis
Université Paris 7
75475 Paris, France

Dr Richard A. Larson
Department of Medicine
Hematology/Oncology
University of Chicago
Chicago, IL 60637, USA

Dr Hwei-Fang Tien
National Taiwan University Hospital
Taipei, Taiwan, China

Dr Clara D. Bloomfield #
James Cancer Hospital
and Solove Research Institute
Ohio State University
Columbus, OH 43210, USA

Dr Robin Foà
Division of Hematology
Sapienza University of Rome
00161 Rome, Italy

Dr Ross Levine
Memorial Sloan Kettering Cancer Center
New York, NY 10021, USA

Dr Alan K. Burnett
Cardiff University School of Medicine
Cardiff CF14 FXN, UK

Dr Ulrich Germing
Klinik für Hämatologie, Onkologie
und Klinische Immunologie
Heinrich Heine University
40225 Düsseldorf, Germany

Dr Alan F. List
Hematology/Oncology
Moffitt Cancer Center
Tampa, FL 33612, USA

Dr William Carroll
NYU Cancer Institute
New York, NY 10016-6402,
USA

Dr Heinz Gisslinger
Medical University of Vienna
1090 Vienna
Austria

Dr Luca Malcovati
Dept of Molecular Medicine
Policlinico San Matteo
27100 Pavia, Italy

A meeting with members of the Clinical Advisory Committees took place at the Gleacher Center, University of Chicago, 31 March to 1 April, 2014.

494 Clinical Advisory Committees
Clinical Advisory Committee (Lymphomas)

Dr Ranjana Advani #
Stanford University
Stanford, CA 94305-5821, USA

Dr Paolo Ghia
Università Vita-Salute
San Raffaele
20132 Milan, Italy

Dr Michael Link
Stanford University School of Medicine
Palo Alto, CA 94304-1812, USA

Dr Wyndham H. Wilson
Lymphoid Malignancy Branch
National Cancer Institute,
Bethesda, MD 20892, USA

Dr Kenneth C. Anderson
Harvard Medical School
Dana-Farber Cancer Institute
Boston, MA 02115-6013, USA

Dr Michele Ghielmini #
Oncology Institute of Southern Switzerland
6500 Bellinzona, Switzerland

Dr Armando López-Guillermo
Hospital Clinic
University of Barcelona
08036 Barcelona, Spain

Dr Anas Younes
Memorial Sloan Kettering Cancer Center
New York, NY 10021, USA

Dr Wing Y. Au
Queen Mary Hospital
University of Hong Kong
Hong Kong SAR, China

Dr John Gribben
London School of Medicine
London, E1 4NS
UK

Dr Michael Pfundschuh
Department of Medicine I
Saarland University
D-66421 Homburg, Germany

Dr Andrew Zelenetz #
Memorial Sloan Kettering Cancer Center
New York, NY 10065, USA

Dr Peter Leif Bergsagel
Department of Medicine
Mayo Clinic
Scottsdale, AZ 85259, USA

Dr Anton Hagenbeek
Department of Haematology
Academic Medical Center
1105 AZ Amsterdam,
The Netherlands

Dr Steven Rosen
Robert H. Lurie Comprehensive Cancer Center
Northwestern University
Chicago, IL 60611-3008, USA

Dr Pier-Luigi Zinzani
Institute of Hematology Oncology
University of Bologna
40138 Bologna, Italy

Dr Joseph Connors
BC Cancer Agency
Vancouver Centre
Vancouver, BC V5Z 4E6, Canada

Dr Peter Johnson
Southampton General Hospital
School of Medicine
Southampton S016 6YD, UK

Dr Gilles Andre Salles #
Hématologie
Centre Hospitalier Lyon-Sud
69495 Pierre-Bénite, France

Dr Emanuele Zucca
Oncology Institute of Southern Switzerland
6500 Bellinzona, Switzerland

Dr Francesco d’Amore
Dept of Hematology
Aarhus University Hospital
Aarhus, Denmark

Dr Brad S. Kahl
University of Wisconsin
UW Carbone Cancer Center
Madison, WI 53705-2275, USA

Dr Kensei Tobinai
National Cancer Center Hospital
Tokyo 104-0045, Japan

Dr Martin Dreyling
University Hospital
Ludwig Maximilian University
81377 Munich, Germany

Dr Eva Kimby
Karolinska Institutet
Huddinge University Hospital
14186 Huddinge, Sweden

Dr Steven P. Treon
Harvard Medical School
Dana-Farber Cancer Institute
Boston, MA 02115, USA

Dr Arnold S. Freedman
Harvard Medical School
Dana-Farber Cancer Institute
Boston, MA 02115-6013, USA

Dr Ann S. LaCasce
Harvard Medical School
Dana-Farber Cancer Institute
Boston, MA 02115-6013, USA

Dr Julie M. Vose
Oncology/Hematology
Nebraska Medical Center
Omaha, NE 68198-7680, USA

Dr Jonathan Friedberg
James P. Wilmot Cancer Institute
University of Rochester
Rochester, NY 14642, USA

Dr John P. Leonard
New York-Presbyterian Hospital
Weill Cornell Medical Center
New York, NY 10803, USA

Dr Rein Willemze
Department of Dermatology
Leiden University Medical Center
2300 RC Leiden
The Netherlands

# Co-chair
IARC/WHO Committee for the International Classification of Diseases for Oncology (ICD-O)

Dr Daniel A. ARBER
Department of Pathology
University of Chicago
5841 S. Maryland Avenue, S327, MC 3063
Chicago IL 60637
USA
Tel. +1 773 702 0647
Fax +1 773 834 5414
darber@uchicago.edu

Dr Paul KLEIHUES
Faculty of Medicine
University of Zurich
Pestalozzistrasse 5
8032 Zurich
SWITZERLAND
Tel. +41 44 362 21 10
Fax +41 44 251 06 65
dk@pathol.uzh.ch

Dr Leslie H. SOBIN
Frederick National Laboratory for Cancer Research - Cancer Human Biobank
National Cancer Institute
6110 Executive Blvd, Suite 250
Rockville MD 20852
USA
Tel. +1 301 443 7947
Fax +1 301 402 9325
leslie.sobin@nih.gov

Dr Freddie Bray
Section of Cancer Surveillance
International Agency for Research on Cancer
150 Cours Albert Thomas
69372 Lyon
FRANCE
Tel. +33 4 72 73 84 53
Fax +33 4 72 73 86 96
brayf@iarc.fr

Dr Hiroko OHGAKI
Section of Molecular Pathology
International Agency for Research on Cancer
150 Cours Albert Thomas
69372 Lyon
FRANCE
Tel. +33 4 72 73 85 34
Fax +33 4 72 73 86 98
ohgakih@iarc.fr

Dr Robert JAKOB
Data Standards and Informatics
World Health Organization (WHO)
20 Avenue Appia
1211 Geneva 27
SWITZERLAND
Tel. +41 22 791 58 77
Fax +41 22 791 48 94
jakobr@who.int

Dr Marion PIÑEROS
Section of Cancer Surveillance
International Agency for Research on Cancer
150 Cours Albert Thomas
69372 Lyon
FRANCE
Tel. +33 4 72 73 84 18
Fax +33 4 72 73 86 96
pinerosm@iarc.fr

Dr Brian ROUS
National Cancer Registration Service
Eastern Office
Victoria House, Capital Park
CB21 5XB, Fulbourn, Cambridge
UNITED KINGDOM
Tel. +44 1 223 213 625
Fax +44 1 223 213 571
brian.rous@phe.gov.uk

Mrs April FRITZ
A. Fritz and Associates, LLC
21361 Crestview Road
Reno NV 89521
USA
Tel. +1 775 636 7243
Fax +1 888 891 3012
april@afritz.org

Dr Leslie H. SOBIN
Frederick National Laboratory for Cancer Research - Cancer Human Biobank
National Cancer Institute
6110 Executive Blvd, Suite 250
Rockville MD 20852
USA
Tel. +1 301 443 7947
Fax +1 301 402 9325
leslie.sobin@nih.gov

Dr Steven H. SWERDLow
Division of Hematopathology
UPMC Presbyterian
200 Lothrop Street, Room G-335
Pittsburgh PA 15213
USA
Tel. +1 412 647 5191
Fax +1 412 647 4008
swerdlowsh@upmc.edu
Sources of figures and tables

Sources of figures

1.01 Vardiman J.W.
1.02 Vardiman J.W.
1.03 A,B Vardiman J.W.
1.04 Goessgen J.
1.05 Porwit A.

2.01 A–C Vardiman J.W.
2.02 A–D Vardiman J.W.
2.03 A,B,D Thiele J.
2.04 A,B Vardiman J.W.
2.04 C Thiele J.
2.05–2.07 Vardiman J.W.
2.08 A,B Le Beau M.M.
2.09 Melo J.V.
2.10 A,B Vardiman J.W.

2.12 A,C,D Vardiman J.W.

2.13 A,B Thiele J.
2.14 A–D Thiele J.
2.15 Vardiman J.W.
2.16 A Vardiman J.W.
2.16 B–D Thiele J.
2.17 Vardiman J.W.
2.18 Kvasnicka H.M.
2.19 A–C Thiele J.
2.20 A–D Thiele J.
2.21 A,B Thiele J.
2.22 A,B Thiele J.
2.23 A Thiele J.
2.24 A–C Thiele J.
2.25 A–D Thiele J.
2.26 A,B Kvasnicka H.M.
2.27 A,B Vardiman J.W.
2.28 Vardiman J.W.

2.29 Vardiman J.W.
2.30 A,B Kvasnicka H.M.
2.31 Kvasnicka H.M.
2.32 A,B Medenica M.
3.01 A,B University of Chicago, USA (deceased)
3.01 C–3.03 Longley J.B. Department of Dermatology, University of Wisconsin School of Medicine and Public Health, Madison, WI, USA
3.04 A,B Vardiman J.W.
3.05 Bruning R.D.
3.06 A Jaffe E.S.
3.06 B Vardiman J.W.
3.07–3.10 Vardiman J.W.
3.11 A,B Horny H.-P.
4.01 Bain B.J.
4.02 Bain B.J.
4.03 A–C Bain B.J.
4.04 A–C Vardiman J.W.
4.05 Horny H.-P.
5.01 A,B Vardiman J.W.
5.01 C Orazi A.
5.02 Vardiman J.W.
5.03 A–C Vardiman J.W.
5.03 D Orazi A.
5.04 A–C Vardiman J.W.
5.05 A,B Vardiman J.W.
5.06–5.07 Vardiman J.W.
5.08 A–C Vardiman J.W.
5.09 A,B Vardiman J.W.
5.10 Baumann I.
5.11 A,B Vardiman J.W.
5.12 Niemeyer C.M.
5.13 A,B Vardiman J.W.
5.13 C,D Huszain A.
5.14 A–D Vardiman J.W.
5.15 A–C Bueso-Ramos C.

7.01 A,B Vardiman J.W.
7.02 A–C Vardiman J.W.
8.01 Arber D.A.
8.02 Bruning R.D.
8.03 Flandrin G.
8.04 A,B Laboratoire Central d’Hematopathologie Hôpital Necker Paris, France
8.05 A Hirsch B.
8.06 Bruning R.D.
8.07 Falini B.
8.08 A,B Hirsch B.
8.09 A,B Bruning R.D.
8.10 A,B Vardiman J.W.
8.11 Arber D.A.
8.12 Bruning R.D.
8.13 A–C Falini B.
8.14 A–D Falini B.
8.15 A–C Falini B.


Republithed with permission from American Society of Hematology.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>8.01</td>
<td>Hirsch B. Dept. of Laboratory Medicine and Pathology University of Minnesota Medical School Minneapolis, MN, USA, Susana Raimondi Cytogenetics Laboratory Department of Pathology St. Jude Children's Research Hospital Memphis, TN, USA, Soheli Meschineri Hutchinson Cancer Research Center University of Washington School of Medicine Seattle, WA, USA, Nyla Heerema Department of Pathology The Ohio State University Columbus, OH, USA, Carroll</td>
</tr>
</tbody>
</table>
References


207. Batastos CD, Nairer TM, Ruppert AS et al. (2003). BAALC expression predicts clinical outcome of de novo acute myeloid leukemia patients with normal cytogenetics; a
References
immunological status may be more predictive of the outcome than other criteria. Histopathology. 38:146–59, PMID:12107265
514 References


56 References
cytogenetic abnormality in myelofibrosis with myeloid metaplasia. Br J Haematol. 130:229–32. PMID:16023451


References 521
References 523


demonstrates unique cytomorphological, cytogenetic, molecular and prognostic features. Leukemia. 23:304-43. PMID:19194466
References 529


expression, and karyotypic abnormalities in 210 pediatric and adult cases. Am J Clin Pathol. 111:467–76. PMID:10191766


two follicular lymphomas: implications for lymphomagenesis. Leukemia. 47:1523-34. PMID:16596283
lymphoma: a study of 778 cases with comparison of marginal zone lymphoma and monocytoid B-cell hyperplasia. Mod Pathol. 11:564–9. PMID:17355836
plasmacytoid

dendritic

cells

feature for differentiating
from

subcutaneous

is

a

lupus

helpful

panniculitis

and occupational risk factors for follicuiar lym¬

(2007). Chromosome 5q deletion and epi¬

PMID:24723457

phoma:

genetic suppression of the gene encoding

the

InterLymph

Non-Hodgkin

Lym¬

T-cell


phoma Subtypes Project. J Natl Cancer Inst

alpha-catenin (CTNNA1) in myeloid cell trans¬


CD94 1A transcripts characterize lymphoblastic

Monogr. 2014:26^0, PMID:25174024

formation, Nat Med. 13:78-83. PMID:17159988

PMID:23600665

lymphoma/leukemia of immature natural killer


2321. Licht JD (2001). AML1 and the AML1-

cell origin with distinct clinical features. Blood.


hyperplasia

Pure erythroid leukemia: a reassessment of

ETO

106:3567-74. PMID:16046525

mimicking invasive squamous cell carcinoma

the entity using the 2008 World Health Organ¬
ization classification. Mod Pathol. 24:375-83.

lymphoma.

of

panniculitis-like


HIstopathology.

fusion

t(8;21)

protein
AML.

in

the

pathogenesis

Oncogene.

20:5660-79.

Pseudoepitheliomatous


in extranodal natural killerTT-cell lymphoma: a

PMID:11607817

De novo CD5+ Burkitt lymphoma/leukemia. Am

report of 34 cases. HIstopathology. 67:404-9.

PMID:21102413


J Clin Pathol. 112:828-35. PMID:10587706

PMID:25619876


mon phenotypes of acute myelogenous leu-


2356. Link DC, Schuettpelz LG, Shen D, et al.

Cutaneous

!

kemia: basophilic, mast cell, eosinophilic, and


immunoglobulin


lymphoma: a case report and review of the

myeloid dendritic ceil subtypes: a review. Blood

deposition disease: the disease spectrum. J

susceptibility mutation through whole-genome

literature.

'

Cells Mol Dis. 35:370-83. PMID:16203163

Am Soc Nephrol. 12:1482-92. PMID:11423577

sequencing of a patient with therapy-related

PMID:25015867

I


AML. JAMA. 305:1568-76. PMID:21505135

2373. Liu YJ (2005). IPC: professional type 1

‘

(2000). How to restrict liver biopsy to high-risk

Characterization of CEBPA mutations in acute

2357. Lipford EH Jr, Margolick JB, Longo DL,

interferon-producing

patients in early-stage Hodgkin’s disease. Ann

myeloid leukemia: most patients with CEBPA

et al. (1988). Angiocentric immunoproliferative


I

Renal

monoclonal

intravascular
Am

J

Clin

natural
Pathol.

killer-celi

cells and

plasmacytoid

Hematol. 79:73-8. PMID:10741918

mutations have bialleiic mutations and show a

lesions: a clinicopathologic spectrum of post-

23:275-306. PMID:15771572

2324. Liebross RH, Ha CS, Cox JD, et al.

distinct immunophenotype of the leukemic cells.


!


Clin Cancer Res. 11:1372-9. PMID:15746035

PMID:3263153

Sites of specific B cell activation in primary and

I

and

radiother¬


2358.

McLaughlin

secondary responses to T cell-dependent and


and follicular dendritic cell proliferative lesions

JK (2009). Breast implants and lymphoma

T cell-independent antigens, Eur J Immunol.

PMiD:9719116

in Castleman’s disease of hyaline-vascular

risk: a review of the epidemiologic evidence

21:2951-62, PMID:1748148

2326. Lierman E, Folens C, Stover EH, et


al. (2006). Sorafenib is a potent inhibitor of

21:1295-306. PMID:9351567

PMID:19319041

NPMI-mutated

FIPILI-PDGFRalpha and the imatinib-resist-


2359. Liso A, Capello D, Marafioti T, et al,

monocytic or myeloid origin exhibit distinct

ant FIPILI-PDGFRalpha T674I mutant. Blood.

al.


immunophenotypes.

108:1374-6. PMID:16645167

involving extramedullary sites: morphologic and

tumor cells of nodular-lymphocyte-predomi¬

PMID:23601747

•


immunophenotypic findings in 44 patients. Am J


!.

al. (2004). Distinct gene signatures of tran-

Surg Pathol. 27:1104-13. PMID:12883242

108:1013-20, PMID:16614247

(2013). Retinoic acid and arsenic trioxide for

I

sient and acute megakaryoblastic leukemia


in Down syndrome. Leukemia. 18:1617-23.

(2012). Prognostic value of MYC rearrange¬

Haploidentical

369:111-21. PMID:23841729

PMID: 15343346

ment in cases of B-cell lymphoma, unclassifia-

transplantation

anaplastic


ble, with features intermediate between diffuse


treat juvenile myelomonocytic leukemia. Blood.

;

.
•

1

'

prognostic factors

Comprehensive

mlRNA

following

sequence

analysis

(2003).

Waldenstrom

macroglobulinemia

Lipworth

L, Tarone RE,

hypermutation

peripheral-blood
for

in

stem-cell

ALK-positive

acute

myeloid

Leuk

leukemia

Res.

of

37:737-41.

large B-cell lymphoma and Burkitt lymphoma.

PMID:14761818

125:1083-90. PMID:25564399

reveals survival differences in diffuse large

Cancer. 118:1566-73. PMID:21882178


(


Lenalidomide in the myelodysplastic syndrome

genetic analysis of lymphoid tumors arising

i

PMID:25723320

(2000). Precursor B-cell lymphoblastic lym¬


after


phoma: a predominantly extranodal tumor with

355:1456-65. PMID:17021321

135:977-87. PMID:2556930

•

Systemic

mastocytosis

in

342

consecutive

organ

transplantation.

Am

J

Pathol.

low propensity for leukemic involvement. Am J

2362. Lister TA, Crowther D, Sutcliffe SB, et al.

2379.

’ adults: survival studies and prognostic factors.

Surg Pathol. 24:1480-90. PMID:11075849


1

Blood. 113:5727-36. PMID:19363219

Loddenkemper

C,

Anagnostopoulos


cuss the evaluation and staging of patients with

enhancer activity and expression of BOB.1/

; 2330. Urn MS, Beaty M, Sorbara L, et al.

(2003). Diffuse large B-cell lymphoma occurring

Hodgkin’s disease: Cotswolds meeting. J Clin

OBF.1, Oct2, PU.1, and immunoglobulin in

I

(2002). T-cell/histiocyte-rich large B-cell lym-

in patients with lymphoplasmacytic lymphoma/

Oncol. 7:1630-6. PMID:2809679

reactive B-cell populations, B-cell non-Hodgkin

I

phoma: a heterogeneous entity with derivation

Waldenstrom macroglobulinemia. Clinicopatho-


logic features of 12 cases. Am J Clin Pathol.


202:60-9. PMID:14694522

120:246-53. PMID:12931555

loproliferative disorder associated with Down


syndrome:

(2014). Clinical features of de novo acute mye¬


Lymphoplasmacytic

and

immune lymphoproliferative syndrome. Am J

non-marginal zone lymphomas with plasma-

9:1432-9. PMID:7658708

Pathol. 153:1541-50. PMID:9811346

26:1458-66. PMID:12409722

lymphoma

and

other

morphologic,

cytogenetic

immunophenotypic

manifestations.

Leukemia.

loid leukemia with concurrent DNMT3A, FLT3
and NPM1 mutations. J Hematol Oncol. 7:74.

cytic differentiation. Am J Clin Pathol. 136:195-


PMID:25281355


210. PMID:21757593

(2013). MicroRNA expression profiling identifies

2381. Loh ML, Sakai DS, Flotho C, et al. (2009),

(

(2005). AIDS-related Burkitt's lymphoma ver-


molecular signatures associated with anaplas¬

Mutations in CBL occur frequently in juvenile

I

sus diffuse large-ceil lymphoma in the pre-

Flow cytometric immunophenotypic analysis

tic large cell lymphoma. Blood. 122:2083-92,

myelomonocytic leukemia. Blood. 114:1859-

I

highly active antiretroviral therapy (HAART)

:

of 306 cases of multiple myeloma. Am J Clin

PMID:23801630

63, PMID:19571318

; and HAART eras: significant differences in sur-

Pathol. 121:482-8. PMID:15080299


■


(2012). Plasmablastic lymphoma of the elderly:

(2004). Mutations in PTPN11

23:4430-8. PMID:15883411

prevalence of JAK2, MPL, and CALR mutations

a clinicopathological comparison with age-re¬


i


in Chinese patients with BCR-ABL1-negative

lated

'!

Prognostic factors in HIV-related diffuse large¬


lymphoproliferative

cell lymphoma: before versus after highly active

144:165-71. PMID:26071474

61:1183-97, PMID:22958176


2349. Lindfors KK, Meyer JE, Dedrick CG, et

2366.

Epstein-Barr

Liu

H,

virus-associated
disorder.

B

cell

HIstopathology,

103:2325-31. PMID:14644997
2383. Lohr JG, Stojanov P, Carter SL, et
al.

Ruskon-Fourmestraux

A,

implicate the

(2014).

Widespread

genetic

neity in multiple myeloma:

I

PMID:16230675

al. (1985). Thymic cysts in mediastinal Hodgkin


targeted

j


disease. Radiology. 156:37-41. PMID:4001419

of t(11:18) positive gastric mucosa-associated

PMID:24434212

1

(2007). Low IPSS score and bone marrow


lymphoid tissue

Helicobacter

2384. Lohr JG, Stojanov P, Lawrence MS,

j

hypocellularity in MDS patients predict hema-

ogy of double-hit B-ceil lymphomas. Curr Opin


et al. (2012). Discovery and prioritization of

!

tological responses to antithymocyte globulin.

Hematol, 19:299-304. PMID:22504522

PMID:11197361

somatic mutations in diffuse large B-cell lym¬

Leukemia. 21:1436^1. PMID:17507999

2351. Lindsley RC, Mar BG, Mazzola E, et al.


phoma (DLBCL) by whole-exome sequenc¬

2335. Lima M, Almeida J, Dos Anjos Teixeira

(2015). Acute myeloid leukemia ontogeny is

CD30+ cutaneous lymphoproliferative disor¬


M, et al. (2003). TCRalphabeta+/CD4+ large

defined by distinct somatic mutations. Blood.

ders: the Stanford experience in lymphomatoid

PMID:22343534

granular lymphocytosis: a new clonal T-cell

125:1367-76. PMID:25550361

papulosis and primary cutaneous anaplastic

2384A.

lymphoproliferative

2352. Lindstrom MS, Wiman KG (2002). Role


(2000). T-cell-rich large B-cell lymphoma in
children and adolescents: a clinicopathologic

disorder.

Am

J

Pathol.

lymphoma to

therapy.

Cancer

heteroge¬

implications for
Cell.

Lones MA, Cairo MS,

25:91-101,

Perkins SL

163:763-71. PMiD:12875995

of genetic and epigenetic changes in Burkitt

49:1049-58. PMID:14639383

2336. Lima M, Almeida J, Montero AG, et

lymphoma.


report of six cases from the Children’s Cancer

al.

PMID:12191637

Chronic neutropenia mediated by fas ligand.


(2004).

Clinicobiological,

immunopheno-

Linet

Semin
MS,

Cancer
Harlow

Biol.
SD,

12:381-7.

typic, and molecular characteristics of mon¬

2353.

oclonal CD56-/+dim chronic natural killer cell

JK (1987). A case-control study of multiple

McLaughlin

Blood. 95:3219-22. PMID:10807792

PMID:10820362


2385. Lones MA, Mishalani S, Shintaku IP, et

large granular lymphocytosis. Am J Pathol.

myeloma in whites: chronic antigenic stimula¬

(2013). Follicular lymphomas in children and

al. (1995). Changes in tonsils and adenoids in

165:1117-27. PMID:15466379

tion, occupation, and drug use. Cancer Res,

young adults: a comparison of the pediatric var¬

children with posttransplant lymphoprolifera¬


47:2978-81. PMID:3567914

iant with usual follicular lymphoma. Am J Surg

tive disorder: report of three cases with early

SF3B1 mutations in patients with myelodys-


Pathol. 37:333^3. PMID:23108024

involvement of Waldeyer’s ring. Hum Pathol.

plastic syndromes: the mutation is stable during

(2014). Medical history, lifestyle, family history.

2370. Liu TX, Becker MW, Jelinek J, et al.

26:525-30. PMID:7750936

References

541




cell cytoplasmic light chain ratio in differentiating between multiple myeloma and monoclonal gammopathy of undetermined significance. Leuk Lymphoma. 6:491–3. PMID:1297481


546 References


Nakamura Y, Watanabe N, Yamahara A, et al. (2009). A leukemic plasmacytic dendritic cell line, PD0505, with the ability to secrete IFN-α/α by stimulation via Toll-like receptors and present antigens to naive T cells. Leuk Res. 33:1224-31. PMID:19315303
3241. Prevost S, Hamilton-Dutoit S, Audouin...
References
**References**

557


distinguishes cases with underlying PLZF/RARA gene rearrangements. Blood. 96:1287–96. PMID:10942230
3493. Salaverria I, Martin-Guerrero I, Wago...
References
10:895-902. PMID:19717091

de I’Adulte trials. J Clin Oncol. 30:3939-46,


3656. Shimada K, Matsue K, Yamamoto K,

PMID:23045585

(2007). Therapy-related myelodysplastic syn¬

PMID:11110676

et al. (2008). Retrospective analysis of intra¬

3670A. Siddiqi

vascular large B-cell lymphoma treated with

son KQ, et al. (2016). Characterization of

not be clinically relevant. Am J Clin Pathol.

al.

rituximab-containing chemotherapy as reported

a variant of t(14;18) negative nodai diffuse

127:197-205. PMID:17210514

Budd-Chiari syndrome and portal vein throm¬

by the IVL study group in Japan. J Clin Oncol.

follicular

26:3189-95. PMID:18506023

sion,


STAT6

(2010). Central nervous system involvement in

PMID:26965583

Clones of Undetermined Significance, T-Cell

man E, et al. (2006). Myeloproliferative disease

intravascular large B-cell lymphoma: a retro¬


LGL Leukemias, and T-Cell Immunoclones. Am

in the pathogenesis and survival of Budd-Chi¬


Hodgkin’s lymphoma in Pakistan: a clinico-ep-

J Clin Pathol. 144:137-44. PMID:26071471

ari

101:1480-6. PMID:20412122

idemiological study of 658 cases at a cancer


PMID:17145613

3657A. Shimada A, Taki T, Tabuchi K, et al.


Consistent patterns of allelic loss in natural


(2006). KIT mutations, and not FLT3 internal

7:651-5. PMID:17250446

killer cell lymphoma. Am J Pathol, 157:1803-9,

(2011). GWAS of follicular lymphoma reveals

tandem duplication, are strongly associated


PMID:11106552

allelic heterogeneity at 6p21.32 and suggests

with a poor prognosis in pediatric acute myeloid

(1996).

histiocytosis-a


shared genetic susceptibility with diffuse large

leukemia with 1(8:21): a study of the Japanese

clinicopathological entity with features of both

Comparative genomic hybridization analysis of


Childhood AML


natural killer cell lymphoma/leukemia. Recogni¬

PMID:21533074


134:525-32, PMID:8731682

tion of consistent patterns of genetic alterations.


Am J Pathol. 155:1419-25. PMID:10550295

(1986).

physiology and management of inherited bone


3690. Skibola CF, Berndt SI, Vijai J, et al,

report of 13 cases with a review of the litera¬

marrow failure syndromes. Blood Rev. 24:101-

65:5-29. PMID:25559415

(2014).

study

ture. Hematol Oncol. 4:307-13. PMID:3549511

22. PMID:20417588


identifies five susceptibility loci for follicular


3659. Shimizu H, Yokohama A, Hatsumi N,


lymphoma outside the HLA region. Am J Hum

(2003). Expression of B-cell-attracting chemok-

et al. (2014). Philadelphia chromosome-pos¬

thy (AILD)-type T-cell lymphoma: prognostic

Genet, 95:462-71. PMID:25279986

ine 1 (CXCL13) by malignant lymphocytes and

itive mixed phenotype acute leukemia in the

impact of clinical observations and laboratory

3691. Skibola CF, Bracci PM, Nieters A, et al,

vascular endothelium in primary central nerv¬


findings at presentation. Ann Oncoi. 6:659-64.

(2010). Tumor necrosis factor (TNF) and lym-


PMID:24750307

PMID:8664186

photoxin-alpha (LTA) polymorphisms and risk

PMID:12393412

3660. Shimoyama M (1991). Diagnostic criteria

3675. Sieniawski M, Angamuthu N, Boyd K,

of non-Hodgkin lymphoma in the InterLymph


and classification of clinical subtypes of adult

et al. (2010). Evaluation of enteropathy-as¬

Consortium. Am

171:267-76.

(2014). The ambiguous boundary between

T-cell leukaemia-lymphoma. A report from the

sociated T-cell lymphoma comparing stand¬

PMID:20047977

EBV-related hemophagocytic lymphohistiocy-

Lymphoma Study Group (1984-87). Br J Hae¬

ard therapies with a novel regimen including

3692. Skinnider BF, Connors JM, Sutcliffe SB,

tosis and systemic EBV-driven T cell lymph¬

matol. 79:428-37. PMID:1751370

autologous stem cell transplantation. Blood.

et al. (1999). Anaplastic large cell lymphoma:

oproiiferative disorder, Int J Clin Exp Pathol,

3661. Shimoyama Y, Yamamoto K, Asano N, et

115:3664-70. PMID:20197551

a clinicopathologic analysis. Hematol Oncol.

7:5738-49. PMID:25337215

al. (2008). Age-related Epstein-Barr virus-asso¬


17:137-48. PMID:10725869

3708. Smith ML, Cavenagh JD, Lister TA, et

ciated B-cell lymphoproiiferative disorders: spe¬

(2008). Effect of a gluten-free diet on the risk

3693. Skinnider BF, Elia AJ, Gascoyne RD,

al. (2004), Mutation of CEBPA in familial acute

cial references to lymphomas surrounding this

of enteropathy-associated


newly recognized

in celiac disease.

Cooperative

Study

Group.

clinicopathologic disease.

IN,

Friedman J,

lymphoma

with

1p36/TNFRSF14
mutations.

CD23

expres¬

abnormalities,

Mod

Pathol.

Indeterminate

cell

Angioimmunoblastic

Dig

Barry-Hol-

and

29:570-81.

lymphadenopa-

drome:

morphologic

subclassification

may

3702. Smalberg JH, Arends LR, Valla DC, et
(2012).

Myeloproliferative

neoplasms

in


bosis: a meta-analysis. Blood,

(2015). Spectrum of Clonal Large Granular

PMID:23043069

Lymphocytes (LGLs) of ap T Cells: T-Cell

3703. Smalberg JH, Darwish Murad S, Braak-

Genome-wide

J

association

Epidemiol.

syndrome.

120:4921-8.

Haematologica.

91:1712-3.

Non-secretory multiple myeloma: a

T-cell

lymphoma

et al. (2002). Signal transducer and activa¬

Dis Sci.

53:972-6.

tor of transcription 6 is frequently activated in

PMID:15575056

Hodgkin and Reed-Sternberg cells of Hodgkin


Cancer Sci. 99:1085-91. PMID:18429953

PM1D:17934841

3662. Shiota M, Nakamura S, Ichinohasama R,


Evaluation of WHO criteria for diagnosis of

3694.

Kats-

patients with therapy-related myelodysplasia

expressing the novel chimeric protein p80NPM/

polycythemia

man-Kuipers JE, et al. (2014), Cooperativity

and myeloid leukemia: the University of Chicago

vera:

a

prospective

analysis.

Skokowa

J,

Steinemann

D,


Blood. 122:1881-6. PM1D:23900239

of RUNX1 and CSF3R mutations in severe

series. Blood. 102:43-52. PMID:12623843

86:1954-60. PMID:7655022


congenital neutropenia: a unique pathway in


3663. Shiozawa E, Yamochi-Onizuka T, Taki-

(2015). Cancer incidence and metolachlor use


Immunoglobulin gene rearrangement in immu-

moto M, et al. (2007). The GCB subtype of

in the Agricultural Health Study: An update. Int J

PMID:24523240

noproliferative small intestinal disease (IPSID).

diffuse large B-cell lymphoma is less frequent

Cancer. 137:2630-43. PMID:26033014

3695. Slack GW, Ferry JA, Hasserjian RP,

J Clin Pathol. 40:1291-7. PMID:3121678

in Asian countries. Leuk Res. 31:1579-83.


et al. (2009). Lymphocyte depleted Hodgkin


PMID:17448534

diagnosed

lymphoblastic

lymphoma: an evaluation with immunopheno-

(1997).


leukemia: update on prognostic factors and

typing and genetic analysis. Leuk Lymphoma.

features of an autoimmune lymphoproiifer¬

(2007). Lack of nucleophosmin mutation in

treatment.

50:937^3. PMID:19456461

ative

patients with myelodysplastic syndrome and

PMID:12799535


lymphocyte

acute myeloid leukemia with chromosome 5


(2014). CD30 expression in de novo diffuse

PMID:9028957

abnormalities.

(1999). Abnormal clones of T cells producing

large B-cell lymphoma: a population-based

3712. Snuderl M, Kolman OK, Chen YB, et al.

Leuk Lymphoma. 48:2141^.

childhood
Curr

Opin

acute

Hematol.

10:290-6.

Clincal,

syndrome

immunologic,
associated

apoptosis.

and

with

Blood.

genetic
abnormal

89:1341-8,

PMID:17990177

interleukin-5 in idiopathic eosinophilia. N Engl J


(2010). B-cell lymphomas with concurrent IGH-


Med. 341:1112-20. PMID:10511609

167:608-17. PMID:25135752

BCL2 and MYC rearrangements are aggressive

(2014).

Identification of pre-leukaemic hae¬

3681. Simon TA, Thompson A, Gandhi KK, et

3697. Slager SL, Caporaso NE, de Sanjose S,

neoplasms with clinical and pathologic features

matopoietic stem cells in acute leukaemia.

al. (2015). Incidence of malignancy in adult

et al. (2013). Genetic susceptibility to chronic

distinct from Burkitt lymphoma and diffuse large

Nature. 506:328-33. PMID:24522528

patients with rheumatoid arthritis: a meta-analy¬

lymphocytic leukemia. Semin Hematol, 50:296-

B-cell lymphoma. Am J Surg Pathol. 34:327-


sis. Arthritis Res Then 17:212. PMID:26271620

302. PMID:24246697

40. PMID:20118770

MERRF: a model disease for understanding the

3682. Simonitsch-Klupp I, Hauser I, Ott G, et


3713. Sobin LH, Gospodarowicz MK, Wittekind

principles of mitochondrial genetics. Rev Neurol

al. (2004). Diffuse large B-cell lymphomas with


Ch, editors. (2009). International Union against

(Paris). 147:431-5. PMID:1962048

plasmablastic/plasmacytoid features are asso¬

in patients with myelodysplasia treated with

Cancer (UlCC): TNM classification of malignant


ciated with TP53 deletions and poor clinical out¬

immunosuppressive


therapy.

J

Clin

Oncol.

Correlations between BCL6 rearrangement and

come. Leukemia. 18:146-55. PMID:14603341

26:2505-11. PMID:18413642


outcome in patients with diffuse large B-cell


3699. Slovak ML, Bedell V, Popplewell L, et al.

Chronic myelomonocytic leukemia monocytes

lymphoma treated with CHOP or R-CHOP,


Concurrent Mutations in ATM and

(2002). 21q22 balanced chromosome aberra¬

uniformly display a population of monocytes

Haematologica. 95:96-101. PMID:19797725

Genes Associated with Common y Chain Sig¬

tions in therapy-related hematopoietic disor¬

with CDIIc underexpression. Am J Clin Pathol.

3668. Shustik J, Quinn M, Connors JM, et

naling in Peripheral T Cell Lymphoma. PLoS

ders: report from an international workshop.

140:686-92. PMID:24124148

al. (2011). Follicular non-Hodgkin lymphoma

One. 10:e0141906. PMID:26536348

Genes

33:379-94.


Chromosomes

Cancer.

grades 3A and 3B have a similar outcome and


PMID:11921272

Mechanisms of regulatory T-cell suppression -

appear incurable with anthracycline-based ther¬

(2014). Peripheral blood sCD3D CD4D T cells:

3700. Slovak ML, Gundacker H, Bloomfield

a diverse arsenal for a moving target. Immunol¬


a useful diagnostic tool in angioimmunoblastic

CD, et al. (2006). A retrospective study of 69

ogy. 124:13-22. PMID:18346152

3669. Sibaud V, Beylot-Barry M, Thiebaut R,


patients with t(6;9)(p23;q34) AML emphasizes


et al. (2003). Bone marrow histopathologic

PMID:23798351

the need for a prospective, multicenter initiative

(1984), Prognostic discrimination in “good-risk"

and molecular staging in epidermotropic T-cell


for rare ‘poor prognosis' myeloid malignancies.

chronic granulocytic leukemia. Blood. 63:789-


Increased incidence of monoclonal gammopa-

Leukemia. 20:1295-7. PMID:16628187

99. PMID:6584184

PMID: 12645344

thy of undetermined significance in blacks and

3701. Slovak ML, Kopecky KJ, Cassileth PA,


its age-related differences with whites on the

et al. (2000). Karyotypic analysis predicts out¬

(2010). JAK2(V617F) mutation in myelodys¬

(2012). Long-term outcome of adults with sys¬

basis of a study of 397 men and one woman in

come of preremission and postremission ther¬

plastic syndrome (MDS) with del(5q) arises

temic anaplastic large-cell lymphoma treated


apy in adult acute myeloid leukemia: a South¬

in genetically discordant clones. Leuk Res.

within the Groupe d'Etude des Lymphomes

PMID:2246554

west

34:821-3. PMID:19819015

Oncology

Group/Eastern

Cooperative

References

563


References


A marginal zone phenotype in follicular lymphoma with \[14;18\] is associated with a high risk of myelodysplasia. Blood. 101:3407–12. PMID:12325207


The consistent association between Epstein-Barr virus and Hodgkin's disease in children in Kyushu, Blood. 87:3828–38. PMID:8611709


Subject index

Numbers
5q minus syndrome 115

A
Activated B-cell subtype (ABC subtype) 291, 293-297
Achromobacter xylosoxidans 198
Acute basophilic leukaemia 164
Acute erythraemic leukaemia 187
Acute erythraemic myeloblastosis 161
Acute erythroid leukaemia 134, 141, 150, 151
Acute granulocytic leukaemia 18, 84, 86, 134, 141, 150
Acute monocytic leukaemia 18, 131, 162, 163, 166
Acute monoblastic leukaemia 160
Acute myeloblastic leukaemia 156
Acute myelomonocytic leukaemia 159
Acute myeloid leukaemia 159
Acute myeloid leukaemia associated with Down syndrome 170
Acute myeloid leukaemia, CBF-beta/MLL fusion 132
Acute myeloid leukaemia, inv(16) (p13;q22) 132
Acute myeloid leukaemia, M6 type 161
Acute myeloid leukaemia, NOS 132
Acute myeloid leukaemia, t(8;21) (q22;q22) 132
Acute myeloid leukaemia, t(8;11) (q22;q11-12) 134
Acute myeloid leukaemia with balanced translocations/inversions 130
Acute myeloid leukaemia with germline CEBPA mutation 124
Acute myeloid leukaemia with inv(16) (p13.1;q22) 132
Acute myeloid leukaemia with inv(16) (p13.1;q22) or t(16;16)(p13.1;q22); CBFB-MYH11 132
Acute myeloid leukaemia with minimal differentiation 156
Acute myeloid leukaemia with multilineage dysplasia 150
Acute myeloid leukaemia with myelodysplasia-related changes 117, 150, 151
Acute myeloid leukaemia with maturation 158
Acute myeloid leukaemia without maturation 157
Acute myeloid leukaemia with prior myelodysplastic syndrome 150
Acute myeloid leukaemia with t(8;21)(q22;q22.1); RUNX1-RUNX1T1 130
Acute myeloid leukaemia with t(9;11)(p21.3;q23.3) 136
Acute myelomonocytic leukaemia 159
Acute myelomonocytic leukaemia with abnormal eosinophils 132
Acute non-lymphocytic leukaemia 156
Acute panmyelosis, NOS 161
Acute promyelocytic leukaemia (APL) with t(15;17)(q22;q11-12) 134
Acute promyelocytic leukaemia, M2 type 161
Acute promyelocytic leukaemia, NOS 132
Acute promyelocytic leukaemia, PML-RARA 134
Acute promyelocytic leukaemia, PML-RAR-alpha 134
Acute promyelocytic leukaemia, PML-RARA 134
Adult T-cell lymphoma/leukaemia (ATLL) 194, 196-168, 363-367, 403, 461
Age-related EBV-positive lymphoproliferative disorder 304
Aggressive NK-cell leukaemia/lymphoma 353-355
Aggressive systemic mastocytosis (ASM) 63, 66, 67, 69
Agranulocytic myeloid metaplasia 44
Agranular CD4+ CD66+ haematodermic neoplasm/tumour 174
Agranular CD4+ NK leukaemia 174
AHN See Associated haematological neoplasm
AIM1 371
ALK + ALCL 403, 407, 413, 415-421
ALK - ALCL 403, 406, 407, 417-421
ALK+ anaplastic large cell lymphoma 403
ALK- anaplastic large cell lymphoma 403
ALK-positive 194, 195, 291, 293, 319-321, 396, 413, 414, 416-418
ALK-negative 418-420
Aneuploidy 360
Anaplastic large cell lymphoma, ALK-positive 413
Anaplastic large cell lymphoma, ALK-negative 407
Angiocentric cutaneous T-cell lymphoma of childhood 361
Angiocentric immunoproliferative lesion 312
Angiocentric lymphoproliferative lesion 312
Angiocentric T-cell lymphoma 368
Angiogenesis 317
Angiogenetic myeloid metaplasia 44
Subject index

Atypical chronic myeloid leukaemia, BCR-ABL1-like 208
B-cell lymphoma/leukaemia 174
B-PLL 200-202
B-lymphoblastic leukaemia/lymphoma (B-ALL/LBL) 23, 72, 77-79, 181, 196, 200, 203, 205-208, 273, 335, 336, 466
B-lymphoblastic leukaemia/lymphoma, BCR-ABL1-like 208
B-lymphoblastic leukaemia/lymphoma, not otherwise specified (NOS) 200
B-lymphoblastic leukaemia/lymphoma with hyperdiploidy 205
B-lymphoblastic leukaemia/lymphoma with hypodiploidy 206
B-lymphoblastic leukaemia/lymphoma with iAMP21 208
B-lymphoblastic leukaemia/lymphoma with recurrent genetic abnormalities 203
B-lymphoblastic leukaemia/lymphoma with t(1;19)(q23;p13.3); TCF3-PBX1 207
B-lymphoblastic leukaemia/lymphoma with t(5;14)(q31.1;q32.1); IGHL/LIL3 206
B-lymphoblastic leukaemia/lymphoma with t(9;22)(q34.1;q11.2); BCR-ABL1 203
B-lymphoblastic leukaemia/lymphoma with t(12;21)(p13.2;q22.1); ETV6-RUNX1 204
B-lymphoblastic leukaemia/lymphoma with t(v;11q23.3); KMT2A-rearranged 203
B/myeloid mixed-phenotype acute leukaemia (MPAL); B/myeloid MPAL, NOS 184, 185
BOB1 308, 315, 343, 426-428, 432, 439, 442
Body cavity-based lymphoma 323
Bone marrow mastocytosis 67
Borrelia burgdorferi 198, 240
B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma and Burkitt lymphoma 335, 336
B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma and classic Hodgkin lymphoma 342
B-cell prolymphocytic leukaemia (B-PLL) 217, 220, 222, 229
BCOR 104, 105, 347
BCR-ABL1 rearrangement 43, 49, 53, 182
BCR-FGRF1 78
BCR-JAK2 23
Benign cephalic histiocytosis 480
BIN2-PDGFRB 77
Birbeck granules 469-471, 473-475, 477
BIRC3-MALT1 fusion 194, 225, 240, 262
Blastic cell leukaemia 182
Blastic NK leukaemia/lymphoma 174
Blastic plasmacytoid dendritic cell neoplasm (BPDCN) 174
B-LBL 200-202
B-cell lymphoma/leukaemia 174
Atypical chronic myeloid leukaemia, BCR/ABL1-negative 87
Atypical chronic myeloid leukaemia, Philadelphia chromosome negative 87
Autoimmune lymphoproliferative syndrome (Canale-Smith syndrome) 445
Autosomal dominant familial MDS/AML syndrome 125

B
B2M mutation 429
B acute lymphoblastic leukaemia/lymphoma with t(12;21)(p13;q22), TEL/AML1 (ETV6-RUNX1) 204
B acute lymphoblastic leukaemia/lymphoma (B-ALL) 182, 200-210, 212
Basophilic leukaemia 164
B-cell acute lymphoblastic leukaemia 200
B-cell lymphoma rich in T cells and simulating B-cell acute lymphoblastic leukaemia 200
B-PLL 222, 223
BRAF V600E 226, 228, 231, 466, 467, 470, 472-474, 476, 477, 482
Breast implant-associated anaplastic large cell lymphoma 421
B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma and classic Hodgkin lymphoma 342
B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma and classic Hodgkin lymphoma 342
B-cell prolymphocytic leukaemia (B-PLL) 217, 220, 222, 229
BCOR 104, 105, 347
BCR-ABL1 rearrangement 43, 49, 53, 182
BCR-FGRF1 78
BCR-JAK2 23
Benign cephalic histiocytosis 480
BIN2-PDGFRB 77
Birbeck granules 469-471, 473-475, 477
BIRC3-MALT1 fusion 194, 225, 240, 262
Blastic cell leukaemia 182
Blastic NK leukaemia/lymphoma 174
Blastic plasmacytoid dendritic cell neoplasm (BPDCN) 174
B-LBL 200-202
B-cell lymphoma/leukaemia 174
Atypical chronic myeloid leukaemia, BCR/ABL1-negative 87
Atypical chronic myeloid leukaemia, Philadelphia chromosome negative 87
Autoimmune lymphoproliferative syndrome (Canale-Smith syndrome) 445
Autosomal dominant familial MDS/AML syndrome 125

C
CAEBV See Chronic active EBV infection (CAEBV) of T-cell or NK-cell type, systemic form 358
Chronic eosinophilic leukaemia (CEL) 54, 55
Chronic erythroaemia 39
Chronic granulocytic leukaemia, BCR-ABL1-positive 30
Chronic granulocytic leukaemia, Philadelphia chromosome-positive (Ph+) 30
Chronic granulocytic leukaemia, Philadelphia chromosome-positive (Ph+) 30
Chronic idiopathic myelofibrosis 44
Chronic idiopathic myelofibrosis 44
Chronic idiopathic myelofibrosis 44
Chronic idiopathic myelofibrosis 44
Chronic idiopathic myelofibrosis 44
Chronic lymphocytic leukaemia, B-cell type 216
Chronic lymphocytic leukaemia (CLL) 220-222, 224, 237
Chronic lymphocytic leukaemia/small lymphocytic lymphoma (CLL/SLL) 216
Chronic lymphoid leukaemia 216
Chronic lymphoproliferative disorders of NK cells (CLPD-NKs) 351
Chronic myelodysplastic/myeloproliferative disease 35
Chronic myelogenous leukaemia, Philadelphia chromosome-positive (Ph+) 30
Chronic myelogenous leukaemia, Philadelphia chromosome-positive (Ph+) 30
Chronic myelogenous leukaemia, BCR-ABL1-negative 87
Chronic myelogenous leukaemia, BCR-ABL1-positive 30, 31, 32, 33, 35
Chronic myeloid leukaemia (CML) 30, 31, 34, 36
Chronic myeloid leukaemia (CML) 76, 82, 83-85
Chronic myelomonocytic leukaemia in
Cytotoxic agents implicated in therapy-related myeloid neoplasms 153
Franklin disease 238
French-American-British (FAB) classification 86,132, 156-162,
FTCL See Follicular T-cell lymphoma
Fulminant EBV-positive T-cell lymphoproliferative disorder of childhood 355
Fulminant haemophagocytic syndrome in children 355

G
Gamma heavy chain disease 238, 239
GATA1 116, 127, 165, 169-171
GATA2 116, 120, 122-124, 126, 127, 138, 144, 212
Gamma heavy chain disease 239
Fulminant haemophagocytic syndrome in children 355

H
HACE1 371
Hand-Schüller-Christian disease 470
Hairy cell leukaemia variant 230
Hairy cell leukaemia 227, 228, 229, 231
Hairy cell leukaemia variant 230
Hand-Schüller-Christian disease 470
HCL See Hairy cell leukaemia
Heavy chain deposition disease (HCDD) 255
Heavy chain diseases 237, 241
HECW1-PDGFRB 77
Hepatosplenic T-cell lymphoma (HSTL) 195, 381, 453
HERV-K-FGRF1 78
HGaL 191, 264, 269, 293
HGBL 335, 336, 338, 340, 341
HHV8-associated lymphoproliferative disorders 321, 325
HHV8-positive diffuse large B-cell lymphoma 291, 325, 327, 329
HHV8-positive diffuse large B-cell lymphoma, NOS 327, 329
HHV8-positive germinotropic lymphoproliferative disorder (GLPD) 328, 329
High-grade B-cell lymphoma (HGBL) 291, 297, 335, 340, 341
High-grade B-cell lymphoma with MYC and/or BCL2 and/or BCL6 rearrangements 291, 297, 335
High hyperdiploid acute lymphoblastic leukaemia 205
HIP1-PDGFRB 77
Histiocyte-rich-T-cell-rich large B-cell lymphoma 298
Histiocytic neoplasms 466
Histiocytosis X 470
HIV-associated lymphomas 449, 450, 452
Hodgkin disease, lymphocyte predominance, diffuse 438
Hodgkin disease, lymphocyte predominance, NOS 438
Hodgkin disease, lymphocytic-histiocytic predominance 438
Hodgkin disease, nodular sclerosis, NOS 435
Hodgkin-like anaplastic large cell lymphoma 342
Hodgkin lymphoma, diffuse, lymphocyte depletion, NOS 441
Hodgkin lymphoma, lymphocyte depletion, diffuse fibrosis 441
Hodgkin lymphoma, lymphocyte predominance 431
Hodgkin lymphoma, nodular sclerosis, cellular phase 435
Hodgkin lymphoma, nodular sclerosis, grade 1 435
Hodgkin lymphoma, nodular sclerosis, grade 2 435
Hodgkin paragranuloma, nodular 431
Hodgkin paragranuloma, NOS 431
Hodgkin/Reed-Sternberg (HRS) cells 426, 427, 431, 435, 438, 440, 441
H. pylori 259, 260
H. pylori antigens 259
HRS cells See Hodgkin/Reed-Sternberg cells
Hodgkin/Reed-Sternberg (HRS) cells 426, 431, 435, 438, 440, 441
Hodgkin lymphoma, lymphocyte depletion, NOS 441
Hodgkin lymphoma, lymphocyte depletion, diffuse fibrosis 441
Hodgkin lymphoma, lymphocyte predominance 431
Hodgkin lymphoma, nodular sclerosis, cellular phase 435
Hodgkin lymphoma, nodular sclerosis, grade 1 435
Hodgkin lymphoma, nodular sclerosis, grade 2 435
Hodgkin paragranuloma, nodular 431
Hodgkin paragranuloma, NOS 431
Hodgkin/Reed-Sternberg (HRS) cells 426, 427, 431, 435, 438, 440, 441
H. pylori 259, 260
H. pylori antigens 259
Hodgkin lymphoma, lymphocyte depletion, NOS 441
Hodgkin lymphoma, lymphocyte depletion, diffuse fibrosis 441
Hodgkin lymphoma, lymphocyte predominance 431
Hodgkin lymphoma, nodular sclerosis, cellular phase 435
Hodgkin lymphoma, nodular sclerosis, grade 1 435
Hodgkin lymphoma, nodular sclerosis, grade 2 435
Hodgkin paragranuloma, nodular 431
Hodgkin paragranuloma, NOS 431
Hodgkin/Reed-Sternberg (HRS) cells 426, 427, 431, 435, 438, 440, 441
H. pylori 259, 260
H. pylori antigens 259
HRS cells See Hodgkin/Reed-Sternberg cells
In situ follicular neoplasia (ISFN) 274, 275
In situ mantle cell lymphoma 285, 290
Intravascular large B-cell lymphoma 291, 317, 318
Intravascular large B-cell lymphoma 291, 317, 318
Intravascular lymphomatosis 317
IPSS 96, 98, 104-106, 107, 109, 111-113
IRF4 rearrangement 270, 278-281, 291, 394, 395
ISFN See In situ follicular neoplasia
ISM 66
Isochromosome 7q 382

J
JAK2 SV617F 22, 23, 38, 39, 41, 43, 45, 49, 53, 89, 93, 94, 96, 115
JAK2 SV617F mutation 22, 45, 89, 93, 94, 96
JAK3 91, 171, 347, 371, 378, 445
Juvenile chronic myelomonocytic leukaemia (JMML) 77, 89-92
JAK3 91, 171, 347, 371, 378, 445
JAK3 91, 171, 347, 371, 378, 445
Juvenile myelomonocytic leukaemia (JMML) 77, 89-92, 123
JXG 480

K
Kahler disease 243
KANK1-PDGFRB 77
KAP1 171
Kaposi sarcoma 323, 325, 326, 328
Kaposi sarcoma-associated herpesvirus 323, 325
KAT6A-CREBBP 131
Ki-1 lymphoma 413
KIR genes 352
KIT SD816V mutation 63, 66-69
KAT6A-CREBBP 131
Kaposi sarcoma 323, 325, 326, 328
Kaposi sarcoma-associated herpesvirus 323, 325
KMT2D 234, 271, 272, 279, 288, 295, 371
KMT2A-MLLT1 fusion 204
KMT2A-MLLT1 137, 204, 211
KMT2A-MLLT10 131
KMT2A-MLLT11 131
KMT2A-rearranged 136, 183, 203, 204
KMT2D 234, 271, 272, 279, 288, 295, 371

L
Langerhans cell granulomatosis 470
Langerhans cell histiocytosis (LCH) 470
Langerhans cell (LC) sarcoma 473
Large B-cell lymphoma with IRF4 rearrangement 280
LBC 280, 314, 319, 321, 322
LCHL 424, 425, 429, 441, 442
Lennert lymphoma 403, 404
Lethal midline granuloma 368, 369
Letterer-Siwe disease 470
Leukaemic non-nodal mantle cell lymphoma 290
Leukaemic reticuloendotheliosis 226
LG4 syndrome 119
Light and heavy chain deposition disease (LHDD) 255
Lipid (cholesterol) granulomatosis 481
Lipogranulomatosis 481
Lipo- granulomatosis 481
LMO2 191, 211, 264, 269, 293, 302
LPL 195, 232-236
LRCHL 424, 425, 438-440
LRRFP1-1FGFR1 78
LYG See Lymphomatoid granulomatosis
Lymphocyte-depleted CHL 424, 425, 435, 440
Lymphocyte-depleted classic Hodgkin lymphoma (LDCHL) 441
Lymphocyte predominant Hodgkin lymphoma (NLPHL) 424
Lymphocyte-rich classic Hodgkin lymphoma (LRCHL) 424, 425, 432, 435, 438, 439
Lymphoepithelioid lymphoma 403, 404, 409
Lymphogranulomatosis 408
Lymphomas associated with HIV infection 449
Lymphomatoid granulomatosis (LYG) 291, 312, 313, 446
Lymphomatoid papulosis (LyP) 392, 393-395
Lymphomatosis cerebri 300
Lymphoplasmacytic lymphoma (LPL) 232, 233, 234, 236, 463
Lymphoproliferative diseases associated with primary immune disorders (PID) 444
LyP See Lymphomatoid papulosis
M2, NOS 158
M4 86, 159
M5 86, 160
M6 86, 160
M1 86, 160
Maculopapular cutaneous mastocytosis 62, 65
MALT1 194, 225, 234, 240, 262, 272, 302, 303, 451
MALT lymphoma 259
Mantle cell lymphoma 285-289
Mantle cell leukaemia 10, 62, 68, 69
Mast cell sarcoma (MCS) 62, 69, 530, 536
Mastocytosis 22, 62
Mature T follicular helper (TFH) cells 408
MBL 220, 221
MCAS See Mast cell activation syndrome
MCCHL 424, 425, 429, 440
MCD 325-329
MCS See Mast cell sarcoma
MDS/AML 122-128
MDS-EB 100, 101, 104, 113, 114
MDS-MLD 101, 107, 108, 111-113
MDS/MPN-S-T 93, 95
MDS/MPN-U 95, 96
MDS-RS 93, 94, 101, 106-113
MDS-SLD 101, 106-109
MDS-UM 101, 116
MECOM 36, 136-138, 149
Mediastinal diffuse large cell lymphoma with sclerosis 314
Mediastinal grey-zone lymphoma (MGZL) 342
Mediterranean lymphoma 240
Medullary plasmacytoma 243
MEF2B 271, 295
Mega-karyocytic leukaemia 162
Mega-karyocytic lineage 50, 169-171
Mega-karyocytic myelosclerosis 44
Methotrexate 302, 307, 350, 447, 452, 463, 464
MGUS See IgM monoclonal gamopathy of undetermined significance
MGZL See Mediastinal grey-zone lymphoma
Mixed cellularity classic Hodgkin lymphoma (MCCHL) 424, 425, 429, 435, 437, 439-441
Mixed myeloproliferative/myelodysplastic syndrome, unclassifiable 95
Mixed-phenotype acute leukaemia 20, 78, 89, 140, 157, 168, 180, 181, 183-187, 211, 212
Mixed-phenotype acute leukaemia (MPAL) with t(v;11q23.3) 183
Mixed-phenotype acute leukaemia, NOS, rare types 186
Mixed-phenotype acute leukaemia with gene rearrangements 179
Mixed-phenotype acute leukaemia, B/myeloid, NOS 184
Mixed-phenotype acute leukaemia, T/myeloid, NOS 185
Mixed-phenotype acute leukaemia with MLL rearrangement 183
Mixed-phenotype acute leukaemia with rare types 186
Mixed-phenotype acute leukaemia with t(9;22)(q34.1;q11.2); BCR-ABL1 182
Mixed-phenotype acute leukaemia with t(v;11q23.3); KMT2A-rearranged 183
MLH1 177
MML rearranged 183
MLLT1 137, 204, 211
Monoclonal gammopathy of undetermined significance (MGUS) 241
Monoclonal B-cell lymphocytosis (MBL) 220
Monoclonal gammopathy, NOS 241
Monoclonal gammopathy of undetermined significance 37, 38, 233, 236, 241, 242, 244, 248, 249, 255, 256, 258
Monoclonal immunoglobulin (Ig) deposition diseases 254
Monoclonal light chain and heavy chain deposition diseases 250
Monoclonally rearranged TR genes 357, 359
Monoclonal rearrangement of the TR genes 360
Monocytic leukemia, NOS 241
Monocytic lymphoma 263
Monocytoid B-cell lymphoma 263
Myelodysplastic syndrome (MDS) 23, 24, 98, 106, 116, 122, 151, 109, 111, 113, 115, 116
Myelodysplastic syndrome (MDS), unclassifiable (MDS-U) 116
Myelodysplastic syndrome (MDS) with excess blasts (MDS-EB) 100, 113, 114
Myelodysplastic syndrome (MDS) with multilineage dysplasia (MDS-MLD) 111
Myelodysplastic syndrome (MDS) with ring sideroblasts (MDS-RS) 109
Myelodysplastic syndrome (MDS) with single lineage dysplasia (MDS-SLD) 106, 107, 108
Myelodysplastic syndrome with 5q deletion 115
Myelodysplastic syndrome with excess blasts and erythroid predominance 114
Myelodysplastic syndrome with excess blasts and fibrosis 114
Myelodysplastic syndrome with fibrosis 103, 105
Myelodysplastic syndrome with isolated del(5q) 115
Myelodyplastic syndrome with ring sideroblasts 109
Myelofibrosis/sclerosis with myeloid metaplasia 44
Myelofibrosis with myeloid metaplasia 44, 45, 49
Myeloid and lymphoid neoplasms with PDGFRα rearrangement 73
Myeloid leukemia associated with Down syndrome 169, 170
Myeloid/lymphoid neoplasms with FGFR1 rearrangement 77, 210
Myeloid/lymphoid neoplasms with PCM1-JAK2 76, 79
Myeloid/lymphoid neoplasms with PDGFRA rearrangement 73
Myeloid/myeloid neoplasms with PDGFRB rearrangement 75
Myeloid neoplasms with germline ANKRD26 mutation 125
Myeloid neoplasms with germline DDX41 mutation 125
Myeloid neoplasms with germline ETV6 mutation 126
Myeloid neoplasms with germline GATA2 mutation 123, 126
Myeloid neoplasms with germline RUNX1 mutation 125
Myeloid neoplasms with PDGFRB rearrangement 76
Myeloid sarcoma 25, 27, 49, 77, 78, 90, 130, 136, 140, 143, 154, 167, 168
Myelomonocytic leukemia 243
Myelomonocytic leukemia, NOS 244
Myeloproliferative neoplasm (MPN) 30, 39, 44, 50, 54, 57, 75, 82, 95, 117, 167
Myeloproliferative neoplasm (MPN), unclassifiable (MPN-U) 57, 58, 63, 86, 95, 96
Myeloproliferative neoplasm (MPN) with excess blasts (MDS-EB) 100, 113, 114
Myeloproliferative neoplasm (MPN) with multilineage dysplasia (MDS-MLD) 111
Myeloproliferative neoplasm (MPN) with ring sideroblasts (MDS-RS) 109
Myeloproliferative neoplasm (MPN) with single lineage dysplasia (MDS-SLD) 106, 107, 108
Myeloproliferative syndrome with 5q deletion 115
Myeloproliferative syndrome with excess blasts and erythroid predominance 114
Myeloproliferative syndrome with excess blasts and fibrosis 114
Myeloproliferative syndrome with fibrosis 103, 105
Myeloproliferative syndrome with isolated del(5q) 115
Myeloproliferative syndrome with ring sideroblasts 109
Myelofibrosis/sclerosis with myeloid metaplasia 44
Myelofibrosis with myeloid metaplasia 44, 45, 49
Myeloid and lymphoid neoplasms with PDGFRα rearrangement 73
Myeloid leukemia associated with Down syndrome 169, 170
Myeloid/lymphoid neoplasms with FGFR1 rearrangement 77, 210
Myeloid/lymphoid neoplasms with PCM1-JAK2 76, 79
Myeloid/lymphoid neoplasms with PDGFRA rearrangement 73
Myeloid/myeloid neoplasms with PDGFRB rearrangement 75
Myeloid neoplasms with germline ANKRD26 mutation 125
Myeloid neoplasms with germline DDX41 mutation 125
Myeloid neoplasms with germline ETV6 mutation 126
Myeloid neoplasms with germline GATA2 mutation 123, 126
Myeloid neoplasms with germline RUNX1 mutation 125
Myeloid neoplasms with PDGFRB rearrangement 76
Myeloid sarcoma 25, 27, 49, 77, 78, 90, 130, 136, 140, 143, 154, 167, 168
Myelomonocytic leukemia 243
Myelomonocytic leukemia, NOS 244
Myeloproliferative neoplasm (MPN) 30, 39, 44, 50, 54, 57, 75, 82, 95, 117, 167
Myeloproliferative neoplasm (MPN), unclassifiable (MPN-U) 57, 58, 63, 86, 95, 96
MYO18A-PDGFRB 77
N
NDE1-PDGFRB 77
NF1 20, 23, 89-92, 125, 138
Nijmegen breakage syndrome (NBS) 444
NIP-PDGFRB 77
NK-cell large granular lymphocyte lymphocytosis 351
NK-lymphoblastic leukemia/lymphoma 213
NLFPL See Nodal lymphocyte predominant Hodgkin lymphoma
NMZL 263-265
Nodal marginal zone lymphoma (NMZL) 263
Nodal peripheral T-cell lymphoma with TFH phenotype 403
Nodal peripheral T-cell lymphoma with T follicular helper phenotype 412
Nodal lymphocyte predominant Hodgkin lymphoma (NLPHL) 424, 431, 432-434
Nodal sclerosis classic Hodgkin lymphoma (NSCHL) 197, 315, 343, 424-426, 435, 439, 440, 452
Nodal sclerosis, grade I 435
Non-destructive post-transplant lymphoproliferative disorders 456
Non-IgM monoclonal gammopathy of undetermined significance 241
Noonan syndrome 89, 90, 92, 123
Non-secretory myeloma 250
NOTCH1 184, 212, 217-221, 230, 286, 376
NOTCH2 225
NPM1-ALK fusion 319, 415-417
NPM1-TYK2 gene fusion 396
NMZL 263-265
Nodal marginal zone lymphoma (NMZL) 263
Nodal peripheral T-cell lymphoma with TFH phenotype 403
Nodal peripheral T-cell lymphoma with T follicular helper phenotype 412
Nodal lymphocyte predominant Hodgkin lymphoma (NLPHL) 424, 431, 432-434
Nodal sclerosis classic Hodgkin lymphoma (NSCHL) 197, 315, 343, 424-426, 435, 439, 440, 452
Nodal sclerosis, grade I 435
Non-destructive post-transplant lymphoproliferative disorders 456
Non-IgM monoclonal gammopathy of undetermined significance 241
Noonan syndrome 89, 90, 92, 123
Non-secretory myeloma 250
NOTCH1 184, 212, 217-221, 230, 286, 376
NOTCH2 225
NPM1-ALK fusion 319, 415-417
NPM1-TYK2 gene fusion 396
NSCHL 424, 425, 429, 435-437, 440
NUP98-KDM5A 131
NUP98-KDM5A 131
O
OCT2 308, 315, 343, 426-428, 432-434, 439, 442
Oedematous, scarring vasculitic panniculitis 361
Osseous plasmacytoma 251
Osteosclerotic myeloma 257
Other iatrogenic immunodeficiency-associated lymphoproliferative disorders 457, 482
Overlap syndrome, unclassifiable 95
Overt primary myelofibrosis 48
Owl's eye appearance 425, 426
P
Paediatric nodal marginal zone lymphoma (NMZL) 264, 265
Paediatric-type follicular lymphoma (PTFL) 278, 279
Sézary disease 390
Sézary syndrome (SS) 365-387, 390, 391, 461
SF3B1 mutation 93, 94, 96, 101, 104, 106-113, 115
SH2B3 91, 171
Shwachman-Diamond syndrome 99, 119, 120, 124, 127, 128
SLL 191, 194, 195, 216-220, 463
Smouldering (asymptomatic) plasma cell myeloma 249
Smouldering systemic mastocytosis 66, 67, 69
SMZL 195, 223-225, 229, 230
Solitary mastocytoma of skin 65
Solitary myeloma 251
Solitary plasmacytoma of bone (SPB) 250
SOS1 91, 92
SPB 250, 251, 253
SPECC1-PDGFRB 77
Splenic B-cell lymphoma/leukaemia, unclassifiable 229
Splenic B-cell marginal zone lymphoma 223
Splenic red pulp small B-cell lymphoma (SRPL) 229
Splenic lymphoma with circulating villous lymphocytes 223
Splenic lymphoma with villous lymphocytes 223, 229
Splenic marginal zone lymphoma (SMZL) 223, 224, 225, 229
Splenic marginal zone lymphoma, diffuse variant 229
Splenic red pulp lymphoma with numerous basophilic villous lymphocytes 229
SPTBN1-PDGFRB 77
SPTCL See Subcutaneous panniculitis-like T-cell lymphoma
SRSF2 26, 44, 45, 54, 58, 68, 82, 86, 104, 105, 110, 113, 114, 145
SS See Sézary syndrome
SSBP2-PDGFRB 75
STAG2 26, 104, 105, 113, 145
STAT3 351, 352
Stem cell acute leukaemia 182
Stem cell leukaemia 77, 182
Subcutaneous panniculitis-like T-cell lymphoma (SPTCL) 383, 384, 461, 467
Systemic EBV-positive T-cell lymphoma of childhood 355-357
Systemic light chain disease 254
Systemic mastocytosis with an associated haematological neoplasm (AHN) 62, 66, 68

T
T acute lymphoblastic leukaemia 209
T-ALL/LBL See T-lymphoblastic leukaemia/lymphoma
TAM 169-171
TBL1XPR1 295, 296, 421
T-cell and NK-cell lymphomas 193, 197, 198, 361
T-cell large granular lymphocytic leukaemia (T-LGLL) 107, 348, 349, 352, 382, 461
T-cell large granular lymphocytosis 348
T-cell leukaemia 194, 196, 210, 211, 346, 347, 363-367, 403, 461
T-cell lymphoproliferative disease of granular lymphocytes 348
T-cell prolymphocytic leukaemia (T-PLL) 210, 346, 347, 445, 447, 461
T-cell rich B-cell lymphoma 298
T-cell-rich/histiocyte-rich large B-cell lymphoma 298
T-cell-rich large B-cell lymphoma 298
T cells with cerebriform nuclei (Sézary cells) 390
TCF3 202, 207, 211, 333, 340
TCF3-HLF 202
TCF3-PBX1 207
TCL1 347
TEMPO syndrome 241, 257, 258
Testicular follicular lymphoma 268
Testicular follicular lymphoma 268
Therapy-related acute myeloid leukaemia, alkylating agent-related 153
Therapy-related acute myeloid leukaemia, epipodophyllotoxin-related 153
Therapy-related acute myeloid leukaemia, NOS 153
Therapy-related myeloid neoplasms 27, 82, 87, 93, 95, 99, 130, 153
THRLBCL See T-cell/histiocyte-rich large B-cell lymphoma
Thrombocytopenia 5 (germline ETV6 mutation) 126
TKI therapy 30, 32-34, 36
T-LGLL 348-350
T-lymphoblastic leukaemia/lymphoma (T-ALL/LBL) 72-74, 78, 79, 168, 192, 196, 209, 445, 447, 461, 466
T/myeloid mixed-phenotype acute leukaemia (MPAL) 185
T/myeloid mixed-phenotype acute leukaemia, NOS 186
TNFAIP3 262, 271, 288, 304, 310, 316
TNFRSF14 268, 271, 272, 275, 277, 279, 295
TP53BP1-PDGFRB 77
T-PLL See T-cell prolymphocytic leukaemia
TPM3-PDGFRB 77
TRD rearrangement 382
TRG rearrangement 140, 347, 350, 378, 380, 395, 399, 401
TRIM24-PDGFRB 78
Type II enteropathy-associated T-cell lymphoma 377
Type I interferon 174, 175, 176
T-zone hyperplasia 403

U
U2AF1 26, 104, 105, 110, 152
Undifferentiated leukaemia 157, 180, 182, 187
Urticaria pigmentosa 62, 63, 65

V
VAV1 367, 406

W
Waldenström macroglobulinaemia 232-234, 236
Warts, hypogammaglobulinaemia, immunodeficiency, and myelokathexis (WHIM syndrome) 234
WAS See Wiskott-Aldrich syndrome
WASP See Wiskott-Aldrich syndrome
WASP-associated lymphoproliferative disease (XLP) 312, 314, 444

X
Xanthoma disseminatum 480
X-chromosome inactivation 472
X-linked androgen receptor gene (HUMARA) assay 56
X-linked lymphoproliferative disease (XLP) 312, 314, 334

Z
ZBTB16-RARA 135, 136
ZEB2 75, 177
ZEB2-PDGFRB 75
ZMYM2-PDGFRB 75
ZRSR2 26, 104, 110, 153
ZRP3BP1-PDGFRB 77
ZTBP1-PDGFRB 77
ZTBPB16-RARA 135, 136
ZEB2 75, 177
ZEB2-PDGFRB 75
ZMYM2-PDGFRB 75
ZRSR2 26, 104, 110, 153
### List of abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>AIDS</td>
<td>acquired immunodeficiency syndrome</td>
</tr>
<tr>
<td>ATP</td>
<td>adenosine triphosphate</td>
</tr>
<tr>
<td>CHOP</td>
<td>cyclophosphamide, hydroxydaunorubicin, oncovin (vincristine), prednisone</td>
</tr>
<tr>
<td>CI</td>
<td>confidence interval</td>
</tr>
<tr>
<td>CNS</td>
<td>central nervous system</td>
</tr>
<tr>
<td>CT</td>
<td>computed tomography</td>
</tr>
<tr>
<td>DNA</td>
<td>deoxyribonucleic acid</td>
</tr>
<tr>
<td>EBV</td>
<td>Epstein-Barr virus</td>
</tr>
<tr>
<td>EORTC</td>
<td>European Organisation for Research and Treatment of Cancer</td>
</tr>
<tr>
<td>FISH</td>
<td>fluorescence in situ hybridization</td>
</tr>
<tr>
<td>FLAIR</td>
<td>fluid-attenuated inversion recovery</td>
</tr>
<tr>
<td>GDP</td>
<td>guanosine diphosphate</td>
</tr>
<tr>
<td>GTP</td>
<td>guanosine-5’-triphosphate</td>
</tr>
<tr>
<td>H&amp;E</td>
<td>haematoxylin and eosin</td>
</tr>
<tr>
<td>HHV</td>
<td>human herpesvirus</td>
</tr>
<tr>
<td>HIV</td>
<td>human immunodeficiency virus</td>
</tr>
<tr>
<td>HLA</td>
<td>human leukocyte antigen</td>
</tr>
<tr>
<td>HTLV-1</td>
<td>human T-lymphotropic virus type 1</td>
</tr>
<tr>
<td>ICD-O</td>
<td>International Classification of Diseases for Oncology</td>
</tr>
<tr>
<td>Ig</td>
<td>immunoglobulin</td>
</tr>
<tr>
<td>LOH</td>
<td>loss of heterozygosity</td>
</tr>
<tr>
<td>MAPK</td>
<td>mitogen-activated protein kinase</td>
</tr>
<tr>
<td>MHC</td>
<td>major histocompatibility complex</td>
</tr>
<tr>
<td>MRI</td>
<td>magnetic resonance imaging</td>
</tr>
<tr>
<td>mRNA</td>
<td>messenger ribonucleic acid</td>
</tr>
<tr>
<td>N:C ratio</td>
<td>nuclear-to-cytoplasmic ratio</td>
</tr>
<tr>
<td>NK cell</td>
<td>natural killer cell</td>
</tr>
<tr>
<td>NOS</td>
<td>not otherwise specified</td>
</tr>
<tr>
<td>PAS</td>
<td>periodic acid–Schiff</td>
</tr>
<tr>
<td>PCR</td>
<td>polymerase chain reaction</td>
</tr>
<tr>
<td>PET</td>
<td>positron emission tomography</td>
</tr>
<tr>
<td>RB</td>
<td>retinoblastoma</td>
</tr>
<tr>
<td>RBC</td>
<td>red blood cell</td>
</tr>
<tr>
<td>RNA</td>
<td>ribonucleic acid</td>
</tr>
<tr>
<td>RT-PCR</td>
<td>reverse transcriptase polymerase chain reaction</td>
</tr>
<tr>
<td>SEER</td>
<td>Surveillance, Epidemiology, and End Results</td>
</tr>
<tr>
<td>SNP</td>
<td>single nucleotide polymorphism</td>
</tr>
<tr>
<td>TdT</td>
<td>terminal deoxynucleotidyl transferase</td>
</tr>
<tr>
<td>TNM</td>
<td>tumour, node, metastasis</td>
</tr>
<tr>
<td>WBC</td>
<td>white blood cell</td>
</tr>
</tbody>
</table>

**A note on gene and protein nomenclature:** Throughout this volume, we have used the Human Genome Organisation (HUGO) Gene Nomenclature Committee (HGNC) Guidelines ([http://www.genenames.org/](http://www.genenames.org/)) for citing genes and proteins. For immunoglobulin (IG) and T-cell receptor (TR) alleles, we have used the nomenclature assigned by the ImMunoGeneTics (IMGT) Nomenclature Committee ([http://www.imgt.org/](http://www.imgt.org/)), as recommended by HGNC.
WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues is a Revised 4th Edition Volume of the WHO series on histological and genetic typing of human tumours. This authoritative, concise reference book provides an international standard for oncologists and pathologists and will serve as an indispensable guide for use in the design of studies monitoring response to therapy and clinical outcome.

Diagnostic criteria, pathological features, and associated genetic alterations are described in a strictly disease-oriented manner. Sections on all recognized neoplasms and their variants further include new ICD-O codes, epidemiology, clinical features, macroscopy, prognosis and predictive factors.

This classification, prepared by 132 authors from 23 countries, contains about 1300 colour images and tables, and more than 4500 references.