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Editors

Lymphoma

Diagnosis and Treatment

Humana Press

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Lymphoma

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Preface

Over the past few years, our understanding of the molecular pathogenesis of malignant lymphomas has led to the improvement in the diagnostic precision and to the identification of a variety of molecular therapeutic targets. Furthermore, new drugs have been approved by the United States and European agencies, resulting in a change of the standard of care of several types of lymphoid malignancies. To provide a timely update on the most important advances in the biology, diagnosis, and therapy of lymphomas, we invited our internationally renowned colleagues to write 23 chapters covering clinically relevant topics. We hope that this book will provide valuable information to our readers.

Houston, Texas — Anas Younes, MD
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The World Health Organization Classification of Lymphoid Neoplasms

1

Alejandra Carvajal-Cuenca, Stefano A. Pileri, and Elias Campo

Abstract

The World Health Organization (WHO) classification of the lymphoid neoplasms updated in 2008 represents a worldwide consensus on lymphoma diagnosis and is based in two major principles: the stratification of neoplasms according to their cell lineage and their derivation of precursor or mature cells and the definition of non-overlapping distinct diseases that are clinically relevant. The identification of these diseases is based on a combination of morphology, immunophenotype, genetic, molecular, and clinical features. In addition to well-defined entities, the classification addresses open issues, such as provisional entities that correspond to categories for which there were insufficient evidence to support its recognition as distinct diseases at the time of publication and borderline categories with overlapping features between large B-cell lymphomas and Burkitt or Hodgkin lymphoma.

Keywords

WHO classification • B-cell lymphoma • T-cell lymphoma • Hodgkin lymphoma

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Introduction

The World Health Organization (WHO) classification of tumors of hematopoietic and lymphoid tissues is based on the Revised European-American Classification of Lymphoid Neoplasms (REAL), published by the International Lymphoma Study Group (ILSG) in 1994. The validation of this proposal in large series of tumors and the publication of the third edition of the WHO classification in 2001 closed a long-lasting history of controversies that surrounded the classification of lymphomas.

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The WHO classification of the lymphoid neoplasms updated in 2008 represents a worldwide consensus on lymphoma diagnosis and is based in two major principles: the stratification of neoplasms according to their cell lineage and their derivation of precursor or mature cells and the definition of non-overlapping distinct diseases that are clinically relevant. The identification of these diseases is based on a combination of morphology, immunophenotype, genetic, molecular, and clinical features [1].

The fourth edition of the WHO classification has integrated new information obtained by different working groups in the last years and has refined definitions of well-recognized diseases, identified new entities, and incorporated new emerging concepts related to the biology of lymphomas. However, the classification has still open issues, such as the provisional entities, that correspond to categories for which there was insufficient evidence to support its recognition as distinct entities at this time. In addition, borderline categories have been created for cases that do not clearly correspond to one well-established entity (Table 1.1) [1, 2].

Table 1.1 WHO Classification of mature lymphoid neoplasms [1]

Precursor lymphoid neoplasms
B lymphoblastic leukemia/lymphoma, NOS
B lymphoblastic leukemia/lymphoma with recurrent genetic abnormalities
T lymphoblastic leukemia/lymphoma
Mature B-cell neoplasms
Chronic lymphocytic leukemia/small lymphocytic lymphoma
B-cell prolymphocytic leukemia
Splenic marginal zone lymphoma
Hairy cell leukemia
Splenic lymphoma/leukemia unclassifiable
Splenic diffuse red pulp small B-cell lymphoma
Hairy cell leukemia variant
Lymphoplasmacytic lymphoma/Waldenström macroglobulinemia
Heavy-chain diseases
Plasma cell neoplasms (Table 1.2)
Extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma)
Nodal marginal zone lymphoma
Pediatric nodal marginal zone lymphoma
Follicular lymphoma
Pediatric follicular lymphoma
Primary cutaneous follicle center lymphoma
Mantle cell lymphoma
Diffuse large B-cell lymphoma (see Table 1.3), NOS
Burkitt lymphoma
Mature T-cell and NK-cell neoplasms
T-cell prolymphocytic leukemia
T-cell large granular lymphocytic leukemia
Chronic lymphoproliferative disorder of NK cell
Aggressive NK-cell leukemia
Systemic EBV-positive T-cell lymphoproliferative disease of childhood
Hydroa vacciniforme-like lymphoma
Adult T-cell leukemia/lymphoma
Extranodal NK-/T-cell lymphoma, nasal type
Enteropathy-associated T-cell lymphoma
Hepatosplenic T-cell lymphoma
Subcutaneous panniculitis-like T-cell lymphoma
Mycosis fungoides
Sézary syndrome
Primary cutaneous CD30-positive T-cell lymphoproliferative disorders
Lymphomatoid papulosis

Precursor Lymphoid Neoplasm

Lymphoblastic leukemia/lymphoma is divided by its B- or T-cell lineage and corresponds to a neoplastic proliferation of small- to medium-sized lymphoblasts with scant cytoplasm, dispersed chromatin, and inconspicuous nucleoli (Fig. 1.1). Patients have bone marrow (BM) and peripheral blood (PB) involvement (acute lymphoblastic leukemia, ALL) and/or primary nodal or extranodal presentation in some cases (lymphoblastic lymphoma, LBL). The term lymphoma is used when a mass lesion is found, with no or minimal PB and BM involvement. Although arbitrary, 25 % or more blasts are the threshold used for the distinction between ALL and LBL. However, the distinction between ALL and LBL is considered to have little clinical or biological relevance.

Table 1.1 (continued)

Primary cutaneous anaplastic large-cell lymphoma
Primary cutaneous gamma-delta T-cell lymphoma
Primary cutaneous CD8-positive aggressive epidermotropic cytotoxic T-cell lymphoma
Primary cutaneous CD4-positive small/medium T-cell lymphoma
Peripheral T-cell lymphoma, NOS
Angioimmunoblastic T-cell lymphoma
Anaplastic large-cell lymphoma, ALK positive
Anaplastic large-cell lymphoma (ALCL), ALK negative
Hodgkin lymphoma
Nodular lymphocyte-predominant Hodgkin lymphoma
Classical Hodgkin lymphoma
Nodular sclerosis classical Hodgkin lymphoma
Lymphocyte-rich classical Hodgkin lymphoma
Mixed cellularity classical Hodgkin lymphoma
Lymphocyte-depleted classical Hodgkin lymphoma

B-Lymphoblastic Leukemia /Lymphoma, NOS

Patients with B-ALL are usually children, most of them showing bone marrow failure and frequent extramedullary involvement of the central nervous system, lymph nodes, spleen, liver, and testis as common sites [3]. Burkitt leukemia/lymphoma should be excluded from this diagnosis. B-LBL correspond approximately to 10 % of lymphoblastic lymphomas, the remainder are of T-cell lineage [4]. B-LBL is generally asymptomatic frequently presenting in the head and neck. Most commonly involved sites are the skin, soft tissue, bone, and lymph nodes. Contrary to T-LBL, mediastinal involvement is rare. B-ALL/B-LBL express B-cell markers (PAX-5, CD19, cytoplasmic CD22, and cytoplasmic CD79a), CD10, and TdT (Fig. 1.1). Clonal *IGH* gene rearrangements are found in most cases, but *TCR* rearrangements are also commonly seen (up to 70 %), and therefore, lineage assignment cannot be established by rearrangement studies only [5]. Cytogenetic abnormalities are frequent and may define specific entities with characteristic phenotypic and prognostic features that are considered separately in the WHO classification. In children, cure rates for B-ALL are high (approximately 80 %), while in adults less than 50 % of patients are cured. Similarly, B-LBL has better prognosis in children than in adults [6].

T-Lymphoblastic Leukemia/Lymphoma

Only 15 % of childhood ALL are of T-cell lineage, but 85–90 % of all lymphoblastic lymphomas are T-LBL. T-ALL presents with a high leukocyte count, often with a large mediastinal mass, lymphadenopathy, and hepatosplenomegaly. T-LBL frequently shows an anterior mediastinal mass (thymic) and may involve any nodal or extranodal site. Involved lymph nodes and thymus generally show extensive effacement of their architecture with a starry-sky pattern resembling Burkitt lymphoma. T-ALL/LBL lymphoblasts are TdT positive, with variable expression of T-cell markers. CD7 and cytoplasmic CD3 are most often positive. Frequent coexpression of CD4 and CD8 is seen in blasts as well as CD10 positivity. Markers that indicate precursor origin are CD99, CD34 and CD1a. Clonal rearrangements of the *TCR* genes are seen nearly in all cases, but 20 % also show clonal *IGH* gene rearrangements [7]. More than half of the cases show cytogenetic abnormalities, most frequently involving the *TCR* loci.

Mature B-Cell Neoplasms

Chronic Lymphocytic Leukemia/Small Lymphocytic Lymphoma (CLL/SLL)

Chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL) is a neoplasm composed of mature small B cells that usually

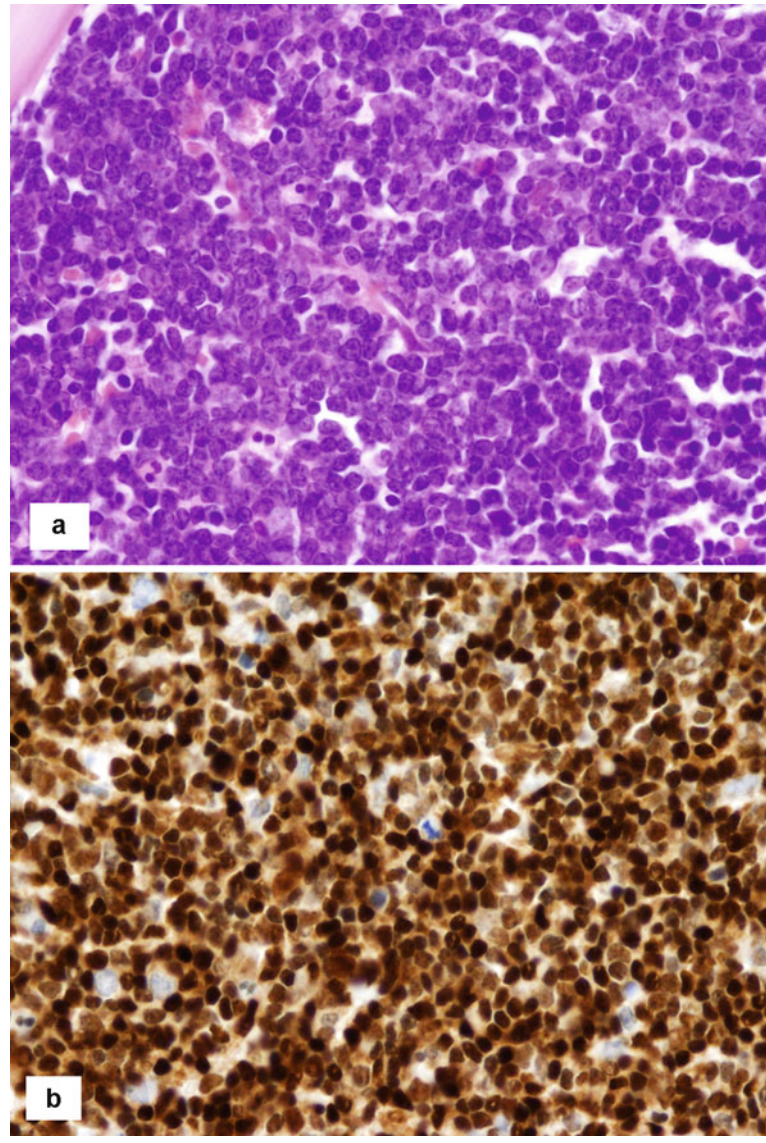


Fig. 1.1 B-lymphoblastic leukemia/lymphoma, NOS. (**a**) Bone marrow diffusely infiltrated by small- to medium-sized lymphoblasts with scant cytoplasm, dispersed chromatin, and inconspicuous nucleoli (H&E, ×400). (**b**) Neoplastic cells show nuclear positivity for TdT (immunoperoxidase staining, 400×)

involves the bone marrow, peripheral blood, lymph nodes, spleen, and liver; but other extranodal sites may occasionally be infiltrated. The presence of 5×10^9/L monoclonal B-lymphocytes with a CLL phenotype in the peripheral blood in the absence of extramedullary tissue involvement is required for diagnosis [8]. The term monoclonal B-cell lymphocytosis (MBL) is used when lower counts of clonal B cells are found in the blood [9]. The diagnosis of CLL may be established with lower lymphocyte counts in patients with cytopenias or disease-related symptoms. The term SLL refers to non-leukemic cases with the same morphology and immunophenotype as CLL.

The tumor cells express B-cell markers with dim CD20 and surface IG and coexpress CD5 and CD23. Atypical cases may have a certain variation in the phenotype with strong IG or CD20 and lack of CD23 or CD5. ZAP-70 is an important prognostic marker of the disease usually associated with an *IGHV*-unmutated CLL

genotype [10]. Expression of CD38 is also considered an adverse prognostic marker.

Bone marrow infiltration is present in almost all cases, with an interstitial, nodular, or diffuse pattern. Involved lymph nodes show usually a diffuse effacement of the architecture. A characteristic feature is the almost constant presence of proliferation centers composed of aggregates of prolymphocytes and paraimmunoblasts (Fig. 1.2). The finding of proliferative tumor cells in these areas together with the presence of follicular dendritic cells and an increased number of CD4+ T cells suggests that these areas have an important role in the pathogenesis of the disease.

Two major CLL subtypes can be recognized based on the mutational status of the IG genes; 40–50 % of the cases show unmutated IG genes (>98 % germline homology), whereas 50–60 % show somatic hypermutations. Patients with

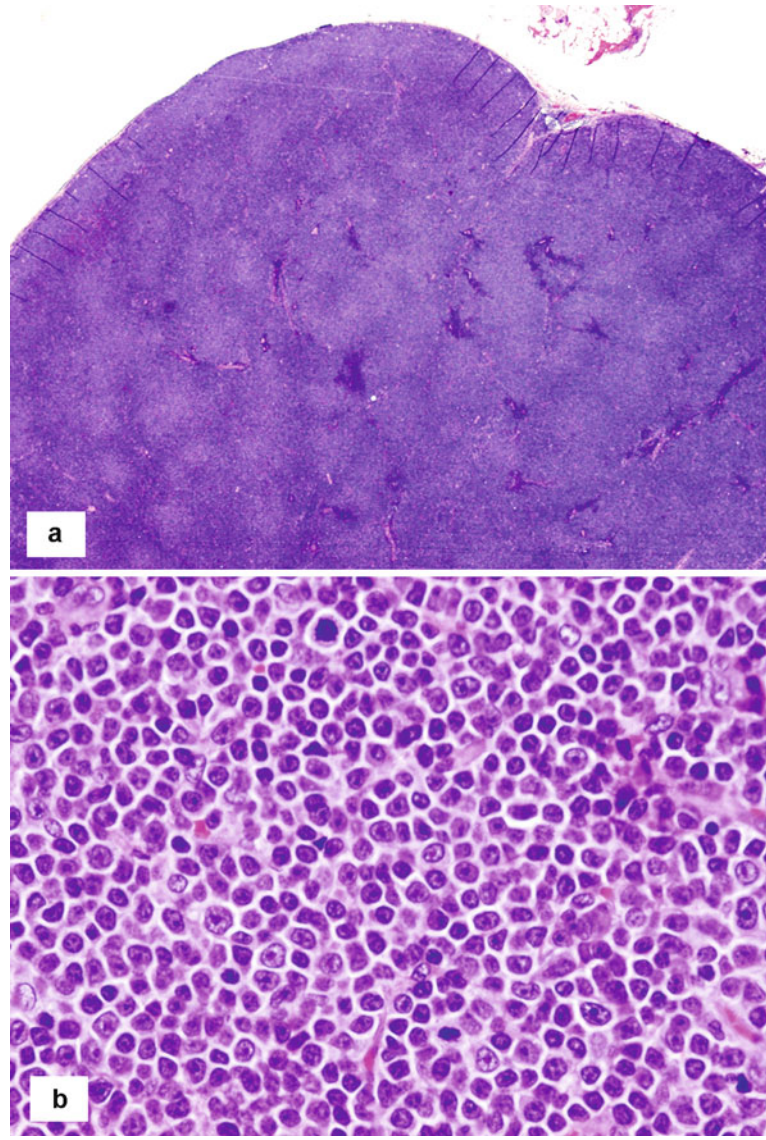


Fig. 1.2 Chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL). (**a**) Lymph node showing a diffuse effacement of the architecture with vaguely nodular pale areas that correspond to the proliferation centers (H&E; ×12.5). (**b**) At higher magnification, proliferation centers are composed of large cells that correspond to prolymphocytes and paraimmunoblasts (H&E, ×400)

unmutated CLL have a more aggressive clinical course. Common cytogenetic alterations in CLL are deletions of chromosome 13q (50 %), trisomy 12 (20 %), 11q deletions (15 %), and 17p deletions (3–7 %). In mutated CLL, 13q deletions are more common, whereas 11q and 17p deletions are more frequently found in unmutated CLL and are associated with worse prognosis. A recent study of whole-genome sequencing of CLL identified recurrent mutations in four genes, *NOTCH1, MYD88, XPO1*, and *KLHL6*. Mutations of *NOTCH1* and *XPO1* were more frequently found in cases of CLL with unmutated *IGHV,* whereas *MYD88* and *KLHL6* mutations were predominantly found in cases of *IGHV*-mutated CLL [11].

Patients may develop clinical symptoms associated with an aggressive evolution of the disease, but the morphological substrate underlying this condition is heterogeneous. Some patients may have expanded proliferation centers with high number of proliferative cells. This situation has been called "accelerated" CLL and seems to have a worse prognosis compared to patients with classical lymph node morphology [12]. Transformation to diffuse large B-cell lymphoma (DLBCL) occurs in 2–8 % of patients (Richter's syndrome) and to classical Hodgkin lymphoma (HL) in <1 %. DLBCL arising in unmutated CLL are usually clonally related to the CLL, whereas DLBCL associated with mutated CLL frequently corresponds to a clonally different lymphoid neoplasm. The prognosis of the clonally related DLBCL is worse with a rapid clinical evolution [1].

B-Cell Prolymphocytic Leukemia

B-cell prolymphocytic leukemia (B-PLL) is defined by the presence of more than 55 % prolymphocytes in PB. The bone marrow (BM) and spleen are frequently involved. Transformed CLL, CLL with increased prolymphocytes, and blastoid MCL carrying t(11;14)(q13;q32) should be excluded from this entity. B-PLL is a very rare disease of elderly patients (median age of 65–69) with a similar male to female distribution [13]. Patients usually show B-symptoms and massive splenomegaly; lymphadenopathy is absent or minimal. A rapidly rising lymphocyte count is seen, usually over 100×10^9/L. Anemia and thrombocytopenias are present in 50 % of patients [14].

Strong surface IgM± IgD and mature B-cell antigens are expressed by B-PLL cells. CD5 and CD23 are positive in only 20–30 % and 10–20 % of cases, respectively. Complex karyotypes are common. Presence of the 17p deletion is found in 50 % of cases and is associated with TP53 gene mutation.

Patients respond poorly to therapies for CLL and have a median survival of 30–50 months. *IgVH* mutations, ZAP-70, and CD38 expression are heterogeneous and, contrary to CLL, cannot be correlated with survival [15].

Splenic Marginal Zone Lymphoma

Splenic marginal zone lymphoma (SMZL) is a B-cell neoplasm composed of small lymphocytes which expand the white pulp, grow around and replace the germinal centers and mantle zones of reactive follicles, and may merge with the neoplastic marginal zones of other follicles (Fig. 1.3). Scattered transformed blasts are present. The red pulp is infiltrated by both small and large cells. Patients usually have a leukemic presentation with splenomegaly and villous lymphocytes in peripheral blood. Splenic hilar lymph nodes and bone marrow are often involved, but extension to peripheral lymph nodes is uncommon [16].

The diagnosis of SMZL requires the exclusion of other lymphoma types, as there are no distinctive phenotypic markers for this entity. Most of the cases show a mature B-cell phenotype and IgM/IgD expression, but other markers more specific of other entities such as CD5, CD23, and CD10 are usually negative. Approximately half of the cases have unmutated IG genes and tend to have deletions of chromosome 7q31–32 and a more unfavorable clinical evolution. Some cases are HCV positive and may respond to antiviral treatment. Clinical course is usually indolent, although some patients may have progressive disease and transformation to a large B-cell lymphoma. Lymphomas with a diffuse infiltrate

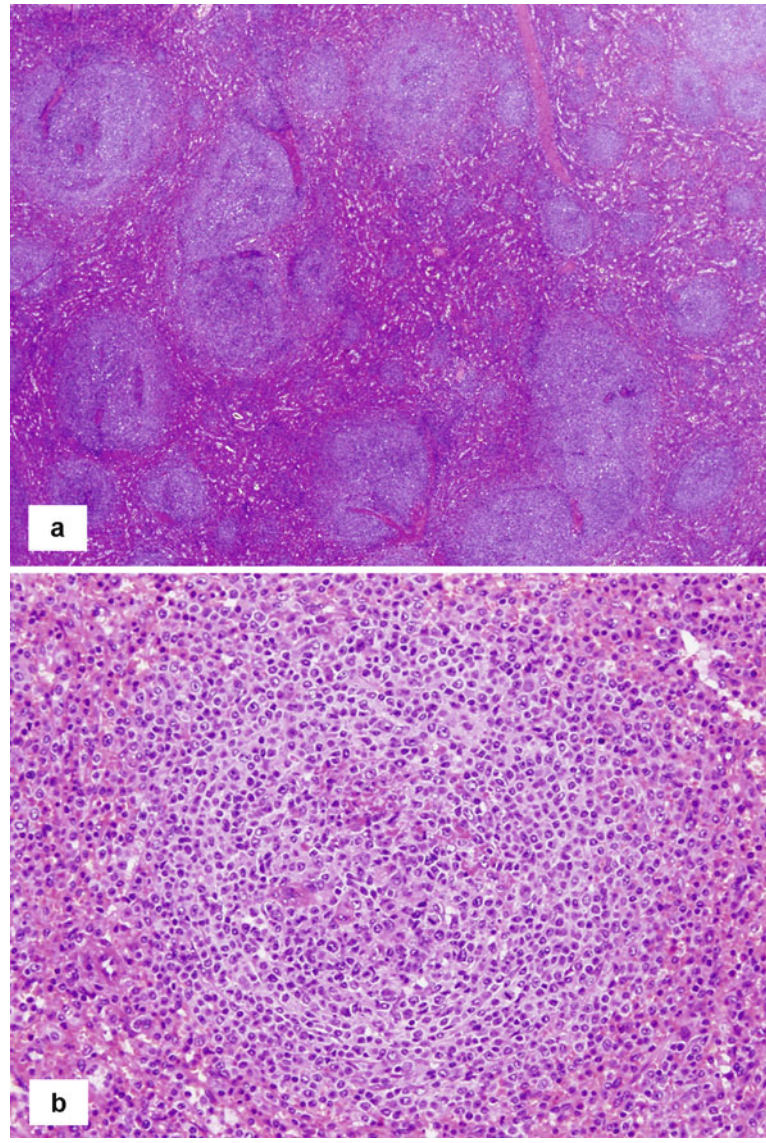


Fig. 1.3 Splenic marginal zone lymphoma. (**a**) The spleen shows a nodular neoplastic infiltration that expands the *white pulp* (H&E, ×12.5). (**b**) At higher magnification, small lymphocytes with abundant pale cytoplasm surround and replace the germinal center (H&E, ×200)

of the red pulp and the hairy cell leukemia variant (HCLv) are included in a provisional category called splenic B-cell lymphoma/leukemia, unclassifiable [1].

Lymphoplasmacytic Lymphoma/ Waldenström Macroglobulinemia

Lymphoplasmacytic lymphoma (LPL) and Waldenström macroglobulinemia (WM) have been redefined in the new classification. LPL is a B-cell neoplasm composed of small lymphocytes, plasmacytoid lymphocytes, and plasma cells. The bone marrow (BM) and sometimes lymph nodes and spleen are involved. Although the detection of a paraprotein usually of IgM type is common, it is not required for diagnosis. WM is defined as LPL with BM involvement and an IgM monoclonal gammopathy of any concentration [17]. As these entities do not have specific markers, it is mandatory to rule out the presence of any other B-cell neoplasm with plasmacytic differentiation.

Table 1.2 Plasma cell disorders

Monoclonal gammopathy of undetermined significance (MGUS)
Plasma cell myeloma
Asymptomatic (smoldering) myeloma
Nonsecretory myeloma
Plasma cell leukemia
Plasmacytoma
Solitary plasmacytoma of bone
Extraosseous (extramedullary) plasmacytoma
Immunoglobulin deposition diseases
Osteosclerotic myeloma (POEMS syndrome)

Plasma Cell Neoplasms

Plasma cell neoplasms comprise a spectrum of lesions characterized by clonal expansion of terminally differentiated B cells, usually heavy-chain class switched, that commonly secrete a single monoclonal immunoglobulin called paraprotein or M-protein (Table 1.2).

The definition of monoclonal gammopathy of undetermined significance (MGUS) includes the presence of <10 % of clonal plasma cells in the bone marrow and <30 g/L of an M-protein and absence of end-organ damage. This process is considered a pre-neoplastic disease since it does not always progress to overt plasma cell neoplasm [1].

Plasma cell myeloma is a bone marrow neoplasm characterized by a multifocal proliferation of plasma cells associated with a seric and/or urine M-protein. Plasma cells in the bone marrow show an interstitial, nodular, or diffuse pattern of infiltration (Fig. 1.4). Some cases may have an asymptomatic presentation, whereas others present with end-organ damage (CRAB: hypercalcemia, renal insufficiency, anemia, bone lesions) [18]. Plasma cell myeloma is positive for plasma cell markers (CD79a, strong CD38, and CD138), but CD19 is nearly always negative. Monotypic cytoplasmic Ig expression and lack of surface Ig is typical. Aberrant expression of CD56 is seen in 75 % of cases (Fig. 1.4), and other markers such as CD117, CD20, CD52, and CD10 can be found in decreasing order of frequency, as well as occasional positivity for myeloid and monocytic markers. Chromosomal abnormalities are seen in >90 % of cases, the most frequent of which is the translocation involving the heavy-chain locus (*IGH*) on chromosome 14q32 and one of the following partners: *cyclin D1* at 11q13, *C-MAF* at 16q23, *FGFR3* at 4p16.3, *cyclin D3* at 6p21, and *MAFB* at 20q11. Together these five translocations are found in 40 % of cases; the remaining cases that lack these alterations are usually hyperdiploid [1].

Plasmacytoma is composed of clonal plasma cells that may occur as a solitary bone lesion or as extraosseous (extramedullary) tissue involvement. Solitary plasmacytoma of bone evolve eventually to plasma cell myeloma or additional solitary or multiple plasmocytomas in two thirds of the patients. On the contrary, extraosseous plasmocytoma follows a relatively indolent clinical course without bone marrow involvement [18]. This suggests a closer relationship to MALT lymphomas than bone marrow plasma cell neoplasms.

Extranodal Marginal Zone Lymphoma of Mucosa-Associated Lymphoid Tissue (MALT Lymphoma)

MALT lymphoma is an extranodal B-cell neoplasm characterized by a heterogeneous population composed of small lymphocytes, marginal zone lymphocytes with cleaved nuclei (centrocyte-like), cells with clear cytoplasm resembling monocytoid B cells, cells with plasmacytic differentiation, and scattered large transformed blasts. This lymphoma expands the marginal zone of reactive follicles and may colonize the germinal centers, but the mantle zones are preserved although they may be attenuated. In most mucosas and glandular organs, the neoplastic cells infiltrate and destroy the epithelium forming lymphoepithelial lesions (Fig. 1.5) [19]. The most common sites of involvement are the gastrointestinal tract, salivary gland, lung, head and neck, ocular adnexa, skin and less frequently thyroid and breast [20].

The lymphoma cells express CD20, CD79a, IgM and less often IgG or IgA; CD5, CD10 and CD23 are negative, although rare cases may be

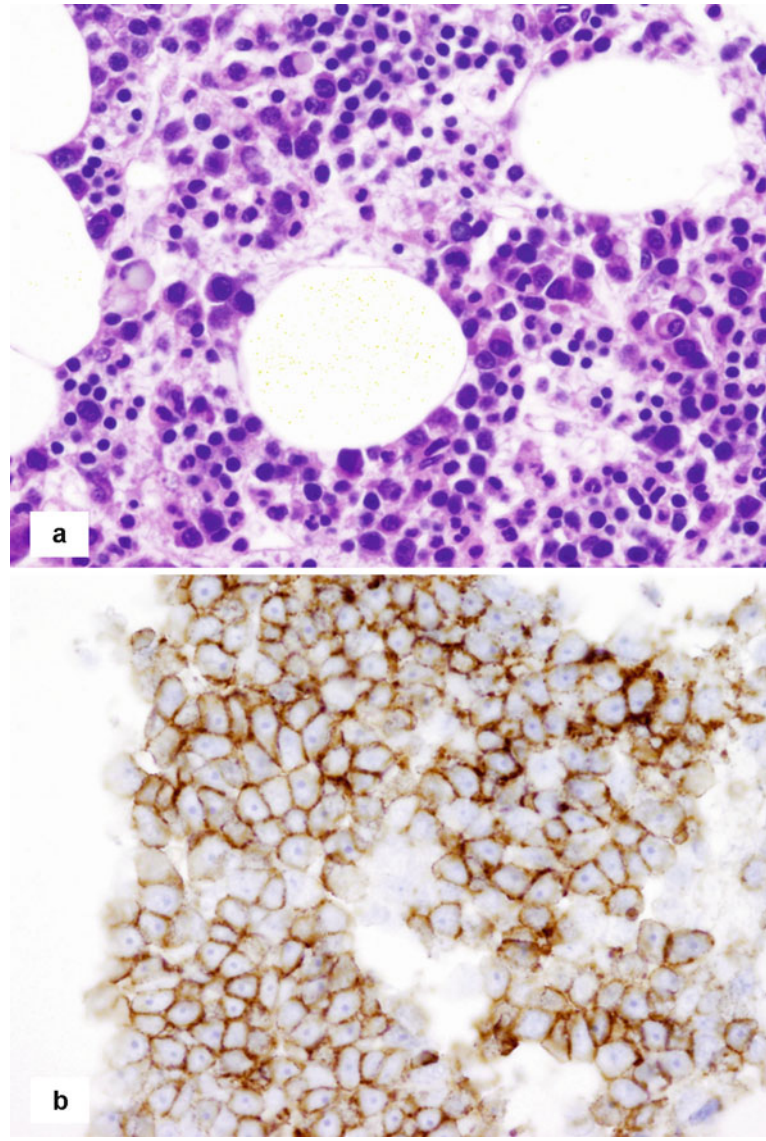


Fig. 1.4 Plasma cell neoplasm. (**a**) Bone marrow with atypical plasma cells with frequent presence of Russell bodies (H&E; ×400). (**b**) Neoplastic plasma cells with aberrant expression for CD56 (immunoperoxidase staining, 400×)

CD5 positive. Four major translocations have been associated with these lymphomas, t(11;18), t(1;14), t(14;18) and t(3;14), leading to the production of a chimeric protein API2-MLT1 or activating *BCL10*, *MLT1* and *FOXP1*, respectively. The t(11;18) translocation is more frequently found in gastric and lung lymphomas, whereas the t(14;18) is more often present in salivary gland and ocular adnexa tumors [1].

MALT lymphomas arise in tissues with a preexisting chronic inflammatory lesion that results in increased extranodal lymphoid reaction induced by infectious, immunologic, or unknown stimuli. Thus, Helicobacter pylori is present in the stomach, Campylobacter jejuni in the immunoproliferative small intestine disease, Chlamydia psitacci in ocular adnexa tumors of certain geographic regions, and Borrelia burgdorferi in some cutaneous MALT lymphomas. Autoimmune disorders of the thyroid gland (Hashimoto disease) and salivary gland (Sjögren syndrome) are preceding lesions of MALT lymphoma in these topographic sites [1].

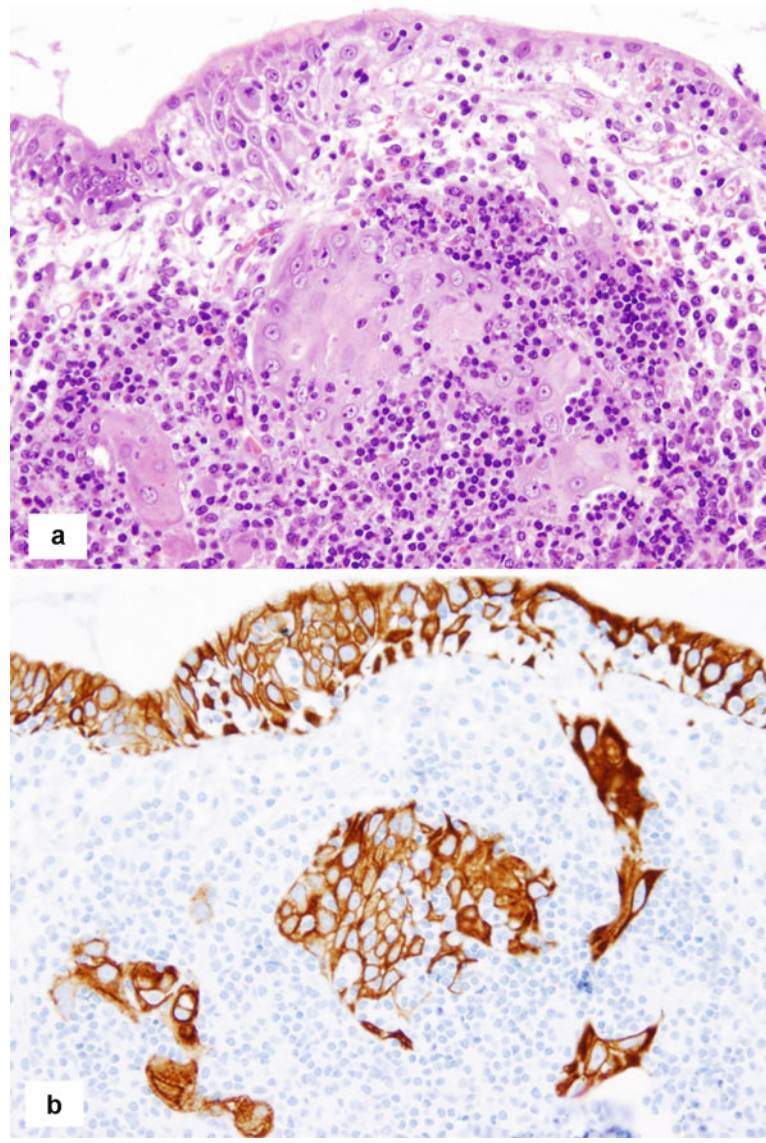


Fig. 1.5 Gastric marginal zone lymphoma of the mucosa-associated lymphoid tissue (MALT lymphoma). (**a**) Gastric mucosa shows neoplastic cells that infiltrate and destroy the glandular epithelium leading to lymphoepithelial lesions (H&E, ×200), observed clearly with cytokeratin staining (Cam 5.2 immunoperoxidase staining, ×200) (**b**)

MALT lymphoma is an indolent tumor that disseminates at a very low rate; it responds to antibiotics for the underlying infectious disease and is sensitive to radiation therapy and local treatment. Recurrences appear after long periods of time and may involve other extranodal sites. Nodal dissemination may induce clinical suspicion of lymphoma and precede the diagnosis of the initial extranodal lesion. Tumors with the t(11;18) appear to be resistant to antibiotic therapy for H. pylori. Transformation to diffuse large B-cell lymphomas may be seen [1].

Nodal Marginal Zone B-Cell Lymphoma

Nodal marginal zone lymphoma (NMZL) is a primary nodal lymphoma that resembles lymph node involvement by extranodal or splenic MZL. Exclusion of an extranodal or splenic lymphoma is required before diagnosis of this entity. The morphology and phenotype is similar to MALT lymphoma, but primary NMZL do not carry the typical translocations of these tumors. Patients may show disseminated disease, and 60–80 % have a survival of more than 5 years

[21]. Some cases may transform to large B-cell lymphomas.

Pediatric nodal marginal zone lymphoma seems to have distinctive clinical and morphological features with a striking male predilection and presenting as an asymptomatic localized tumor. Morphologically, this lymphoma resembles its adult counterpart, but the mantle zones are frequently disrupted by the tumor cell infiltration resembling progressive transformation of the germinal centers. The prognosis of these patients is excellent, and conservative therapy is advised [22].

Follicular Lymphoma

Follicular lymphoma (FL) is composed of germinal center B cells with different proportion of small centrocytes and large centroblasts, which usually has a follicular growth pattern (Fig. 1.6). In western countries, FL is a common lymphoid neoplasm accounting for around 20–30 % of all lymphomas.

FL is graded according to the number of large cells per high power field (hpf): grade 1 has 0–5 large cells, grade 2 has 6–15, and grade 3 has

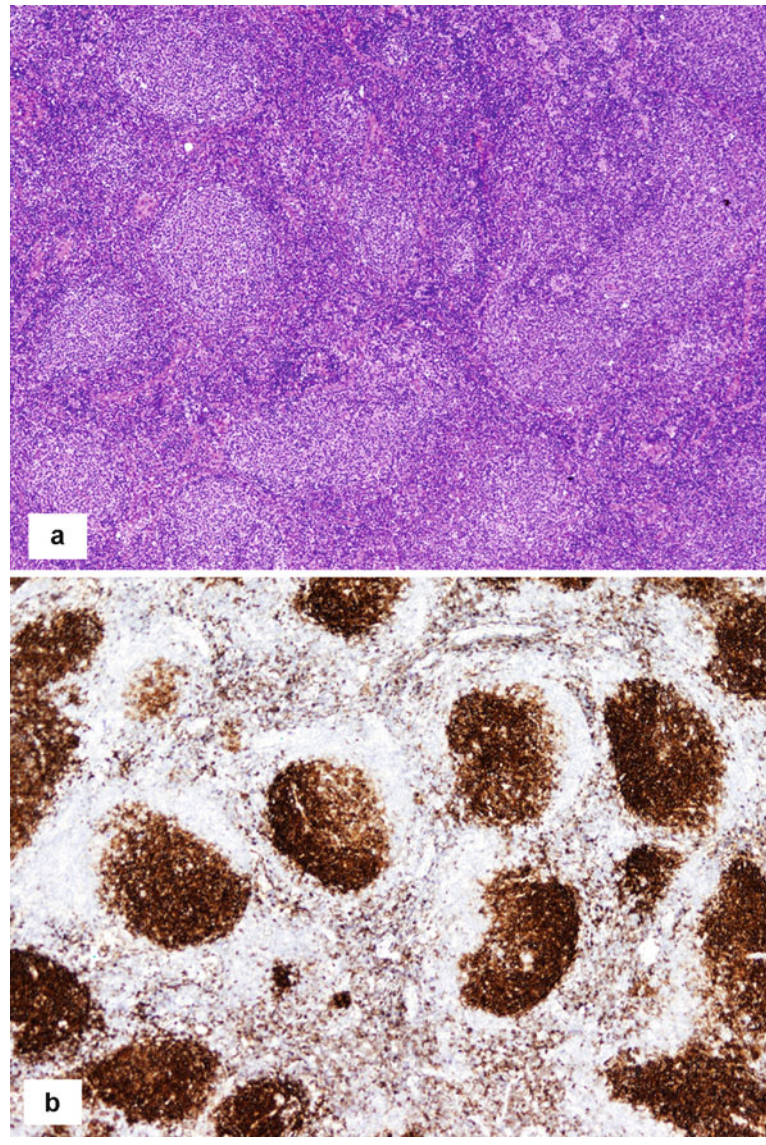


Fig. 1.6 Follicular lymphoma. (**a**) Lymph node infiltrated by tumor cells with a follicular growth pattern. Neoplastic follicles are homogeneous in size and morphology, tend to fusion, and show absence of macrophages (H&E, ×40). (**b**) Neoplastic cells are CD10 positive (immunoperoxidase staining, 40×)

more than 15. The new WHO classification considers grades 1 and 2 as a morphological continuum that does not show relevant clinical or biological differences; this distinction is therefore not mandatory. However, grade 3 tumors are further divided into 3a and 3b according to the presence or absence of small centrocytes; grade 3a shows presence centrocytes, while grade 3b is composed only of large cells. When analyzing the genetic and phenotypic features, FL grade 3a seems more closely related to FL grades 1–2, whereas FL grade 3b to diffuse large B-cell lymphoma (DLBCL). Any diffuse area composed of more than 15 large cells per hpf should be reported as a DLBCL and the percentage of the respective DLBCL and FL component indicated [23].

FL has a mature B-cell immunophenotype with expression of the germinal center markers CD10 (Fig. 1.6) and BCL-6. CD5, CD43, and CD23 are usually negative. IRF4/MUM1, an antigen related to plasma cell differentiation, is also frequently negative in FL grades 1–2. BCL-2 positivity is seen in 85–90 % of grades 1–2 FL but only in 50 % of grade 3 FL. BCL-2 is a very useful marker, as reactive germinal centers are negative. BCL-2 expression reflects the presence of the t(14;18) translocation, the genetic hallmark of this lymphoma that targets the *BCL2* gene. FL grade 3b is less frequently positive for CD10 and BCL-2, and the t(14;18) is only found in 5–40 % of these cases. Furthermore, 40 % of these lymphomas show IRF4/MUM1 expression and carry 3q27 and BCL6 rearrangements in 30–50 % of cases, whereas these aberrations are rare in FL grades 1–3a [1].

Tumor cells are accompanied by a rich microenvironment of different types of T cells and histiocytes that seem to play a major role in determining the biological behavior of FL.

"In situ" FL/intrafollicular neoplasia cases show tumor cells restricted to the germinal centers of the follicles. Only a limited number of the follicles are involved, and usually the atypical cells do not substitute the germinal center completely. The tumor cells are recognized by their strong BCL-2 expression inside the germinal centers and are positive for CD10 and BCL-6. Some of these cases may correspond to an early lymph node involvement by a disseminated FL; another group of patients may develop an overt FL during their follow-up but most patients remain with no evidence of lymphoma. This last group may represent a tissue counterpart of the circulating clonal B cells carrying the t(14;18) translocation commonly detected in healthy individuals. This circulating t(14;18)-positive clone appears to lack additional oncogenic events that are necessary for the development of an overt lymphoma. In the absence of overt FL, patients with these lesions should not be treated for FL [24, 25].

Pediatric follicular lymphoma occurs in children and young adults. A high proportion of these patients show a localized disease, usually involving the head and neck region but also peripheral lymph nodes and extranodal sites such as the testis. Pediatric FL, usually BCL-2 negative, do not carry the t(14;18) and are grade 3. These cases appear to have a good prognosis [26].

Primary intestinal follicular lymphoma occurs in the GI tract, frequently involving the duodenum, and is usually found as an incidental finding. These cases have a conventional morphology, immunophenotype, and genetic features. However, primary intestinal FL expresses IgA, remains localized, and has an excellent prognosis even without treatment [27].

Primary Cutaneous Follicle Center Lymphoma

Primary cutaneous follicle center lymphoma (PCFCL) generally presents in the head and trunk. The tumor has a follicular, follicular and diffuse, or diffuse growth pattern, and the neoplastic cells have a B-cell immunophenotype with BCL-6 expression. CD10 is positive in cases with a follicular pattern but tend to be lost in the diffuse component. BCL-2 expression and the t(14;18) are usually negative. Cutaneous relapses may be seen, but do not indicate progressive disease. PCFCL has an excellent prognosis either in localized or in multifocal skin lesions even with localized therapy [28].

Mantle Cell Lymphoma

Mantle cell lymphoma (MCL) is a B-cell neoplasm generally composed of a monomorphous proliferation of small- to medium-sized cells with irregular nuclear contours (Fig. 1.7). The genetic hallmark of MCL is the t(11;14) that leads to cyclin D1 overexpression (Fig. 1.7). MCL comprises 5–10 % of non-Hodgkin lymphomas and occurs more frequently in males with a median age of 60 years. Most MCL have a disseminated nodal presentation. Although a subgroup of MCL shows exclusive extranodal presentation with leukemic disease, bone marrow and usually spleen involvement. Extranodal involvement is a common finding. Some MCL may have a blastoid or pleomorphic cytology resembling lymphoblasts or DLBCL, respectively. These morphological variants are associated with a higher proliferation rate, complex karyotypes, and worse prognosis. MCL has a vaguely nodular, diffuse, or mantle zone growth pattern. MCL cells express mature B-cell markers with intense CD20 and surface Ig being CD5

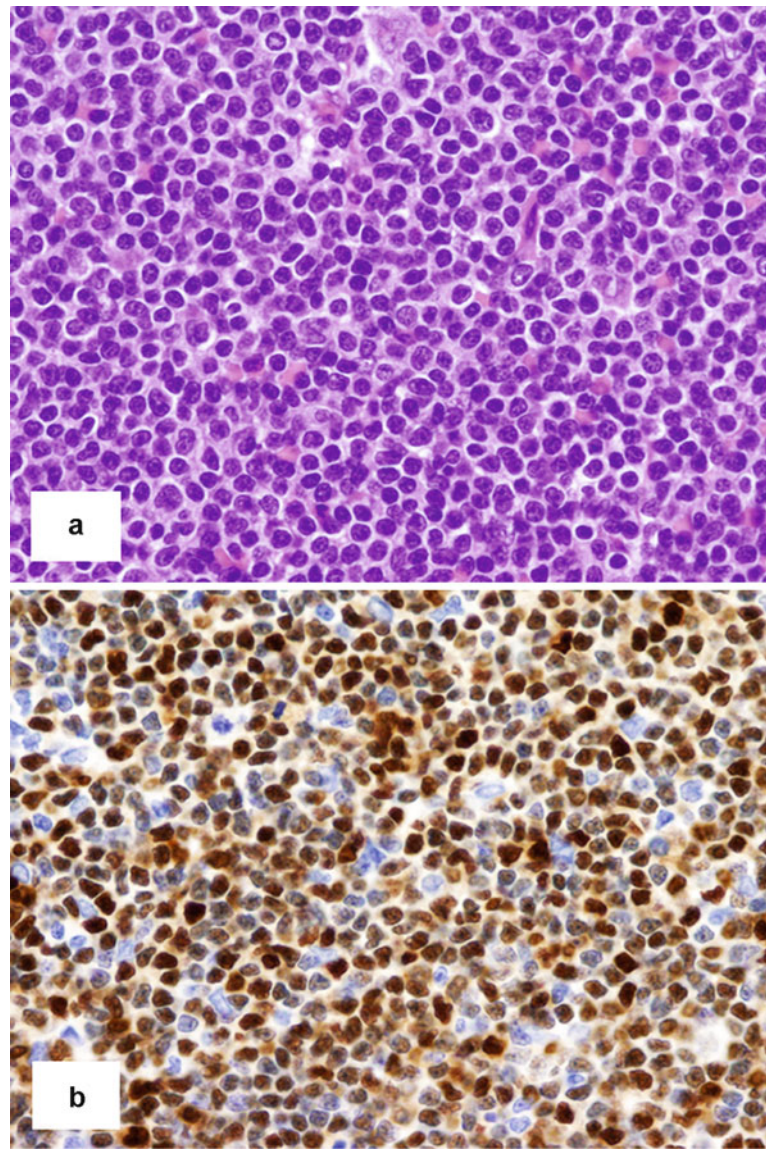
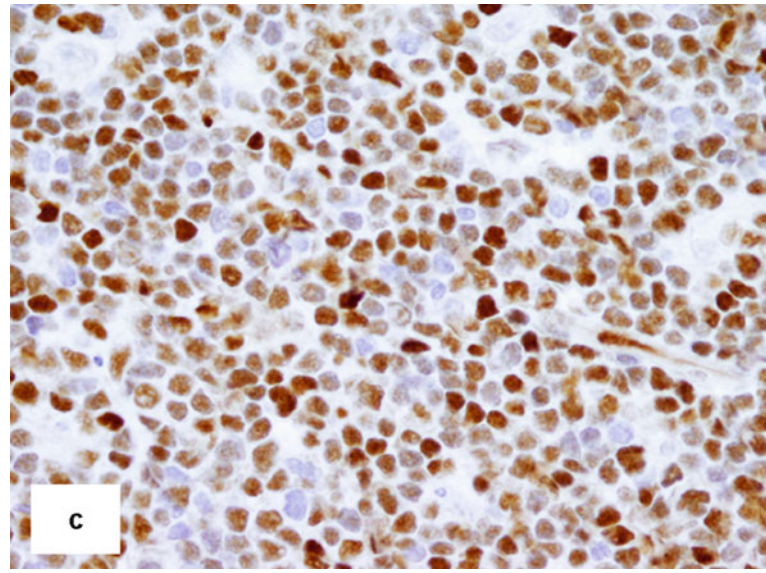


Fig. 1.7 Mantle cell lymphoma, classic type. (**a**) Small- to medium-sized cells with irregular nuclei and dense chromatin (H&E, ×400). (**b**) Neoplastic cells express cyclin D1 and SOX11 (**c**) (immunoperoxidase staining, 400×)

Fig. 1.7 (continued)



and CD43 positive. CD23 and germinal center markers are negative. SOX11, a transcription factor, is positive in 90 % of the cases (Fig. 1.7), whereas most other mature B- and T-cell lymphomas are negative [29–31].

Some cases may show a restricted infiltration of the cyclin D1-positive cells to the inner mantle zones of otherwise reactive follicles. These cells carry the t(11;14) translocation, but they seem to have a limited lymphomagenic potential since most patients do not develop an overt lymphoma after several years of follow-up. This condition has been called "in situ" MCL, but the low, if any, malignant potential has suggested that these lesions should be diagnosed as in situ involvement by mantle cell lymphoma-like cells [32–34].

MCL carries the t(11;14) translocation that targets *CCND1*. Complex karyotypes with many secondary aberrations are common. Cyclin D1-negative cases are rare, and their diagnosis requires a strict morphological and immunophenotypic evaluation. These cases show frequent CCND2 rearrangements with high expression of cyclin D2. SOX11, a transcription factor usually expressed in MCL, is also positive in cyclin D1-negative tumors and is therefore very useful for the recognition of this variant [35].

In general, MCL has poor response to chemotherapy, an aggressive clinical course, and a median survival of 3–5 years. The proliferation rate is considered the best prognostic parameter. Patients presenting with peripheral blood, bone marrow, and sometimes spleen involvement, but without lymphadenopathy, have been reported to have an indolent clinical course even without treatment [29, 36].

Diffuse Large B-Cell Lymphoma, Not Otherwise Specified

Diffuse large B-cell lymphoma (DLBCL) is a very common type of lymphoid neoplasm accounting for 25–30 % of adult non-Hodgkin lymphomas. These lymphomas are very heterogeneous in their clinical, morphological, phenotypic, and molecular aspects. Some particular clinicopathological entities have been identified, but the vast majority of these tumors are still diagnosed in the category of not otherwise specified (NOS) (Table 1.3) [1].

These tumors are composed of large B-lymphocytes that infiltrate the tissues with a diffuse growth pattern. DLBCL, NOS may show a centroblastic, immunoblastic, or anaplastic

Table 1.3 Diffuse large B-cell lymphoma (DLBCL): variants, subgroups, subtypes, and entities [1]

DLBCL, not otherwise specified (DLBCL, NOS)
Common morphological variants
Centroblastic
Immunoblastic
Anaplastic
Rare morphological variants
Molecular subgroups
Germinal center B-cell-like (GCB)
Activated B-cell-like (ABC)
Immunohistochemical subgroups
CD5-positive DLBCL
Germinal center B-cell-like (GCB)
Non-germinal center B-cell-like (non-GCB)
DLBCL subtypes
T-cell-/histiocyte-rich large B-cell lymphoma
Primary DLBCL of the central nervous system
Primary cutaneous DLBCL, leg type
EBV-positive DLBCL of the elderly
Other lymphomas of large B-cells
Primary mediastinal (thymic) large B-cell lymphoma
Intravascular large B-cell lymphoma
DLBCL associated with chronic inflammation
Lymphomatoid granulomatosis
ALK-positive LBCL
Plasmablastic lymphoma
Large B-cell lymphoma arising in HHV8-associated multicentric Castleman disease
Primary effusion lymphoma
Borderline cases
B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and Burkitt lymphoma
B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and classical Hodgkin lymphoma

cytology (Fig. 1.8). These variants are well recognized and several studies have observed a relationship to different biological, genetic, or clinical features, but they have poor reproducibility among pathologists and a broad overlap that do not permit their use as major classifiers. The tumor cells have a mature B-cell phenotype. CD5 is usually negative, but a subset of DLBCL that express CD5, found particularly in eastern countries, seem to have a more aggressive behavior. Expression of the germinal center markers CD10 and BCL6 has been related to a germinal center (GC) origin, whereas expression of the IRF4/MUM1 antigen has been associated with a non-germinal center activated B-cell (ABC) derivation. Gene expression studies have identified these two subgroups of DLBCL and a more aggressive behavior of the ABC group. However, correlation of gene expression profiles with immunohistochemistry is controversial and makes difficult the use of the latter as a reliable prognostic parameter [1].

Genetically, 20–30 % of DLBCL, NOS carry the t(14;18) translocation with the *BCL2* gene rearrangement. These cases are of the molecular germinal center type and usually express CD10. Translocation of the 3q27 region and *BCL6* rearrangements are found up to 30 % of the cases. *MYC* translocations have been found in up to 10 % of DLBCL and are associated with complex karyotypes and poor outcome. Around 40 % of these *MYC* translocations have a non-*IG* gene as partner, contrary to Burkitt lymphoma in which *MYC* is rearranged with *IG* genes in almost all cases [37].

Gene expression profiling of DLBCL, NOS has identified two major subtypes, the germinal center B-cell-like (GCB) and activated B-cell-like (ABC) DLBCL that express genes related to germinal center cells or activated B cells with secretory function, respectively [37]. The ABC subtype, but not the GCB DLBCL, has a constitutive activation of the NFκ[kappa]B pathway associated with frequent mutations in genes upstream of this pathway such as *CARD11*, *CD79b*, *MYD88,* and *A20*. Differences in genetic and molecular aspects between the GCB and ABC subtypes of DLBCL suggest that they may correspond to distinct clinical entities. Outcome of patients is also different in the two molecular subtypes with 5-year survival rates of 59 % in GCB and 30 % in ABC DLBCL. In addition, late relapses or relapses as FL occur in patients with GCB DLBCL. The prognostic value of these molecular subtypes has been confirmed in patients treated with rituximab-containing regimens.

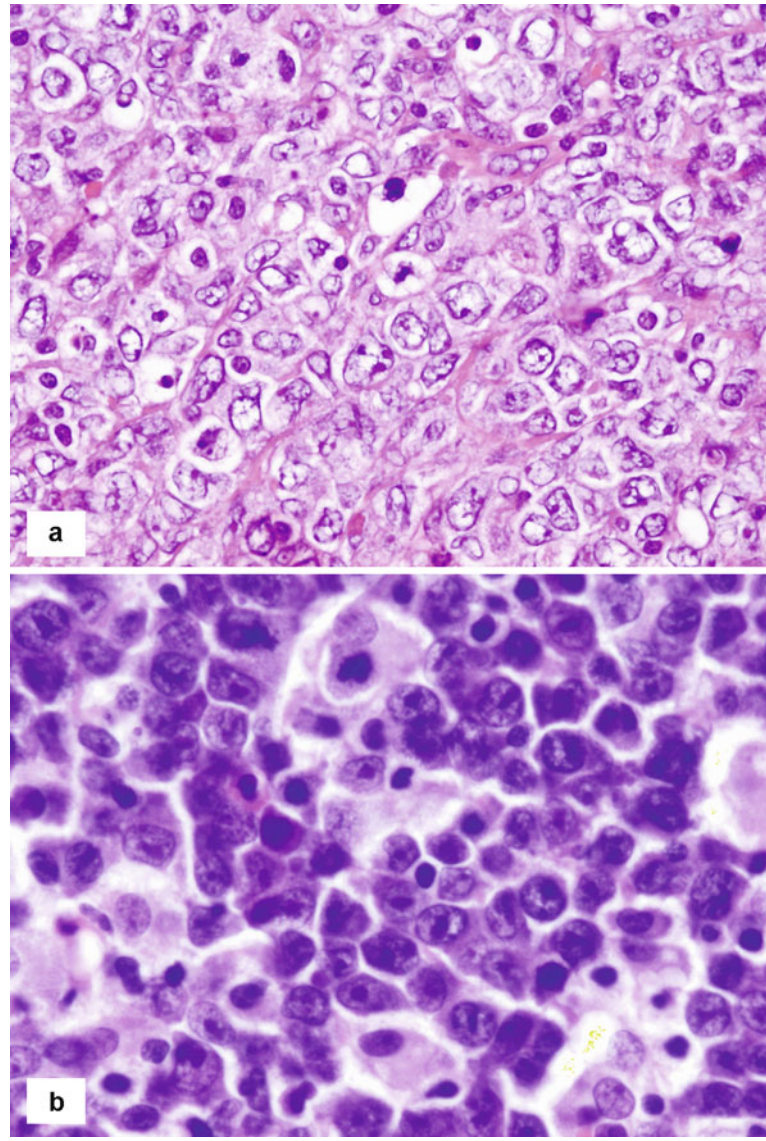


Fig. 1.8 Diffuse large B-cell lymphoma. (**a**) Centroblastic morphology. Note that the neoplastic cells have large nuclei with dispersed chromatin and multiple peripheral nucleoli (H&E, ×400). (**b**) Immunoblastic morphology. Neoplastic cells are large, with round nuclei, central prominent nucleoli, and abundant eccentric cytoplasm (H&E, ×400)

T-Cell-/Histiocyte-Rich Large B-Cell Lymphoma

T-cell-/histiocyte-rich large B-cell lymphoma (THRLBCL) is characterized by scattered large B cells immersed in a T-cell-rich background with frequent presence of histiocytes. Tumor cells express mature B-cell markers and commonly BCL-2 and EMA, but CD30, CD15, and CD138 are negative. The background shows abundant CD3+/CD4+ T cells and CD68-positive histiocytes. T-cell rosettes surrounding tumor B cells are absent. Cases with a similar morphology, but positivity for EBV should be classified as EBV+DLBCL, and patients should be investigated for an immunodeficient status. THRLBCL presents with disseminated disease at diagnosis involving the lymph nodes, spleen, liver, and bone marrow. Therapeutic failure and IPI score are predictors of survival [1].

DLBCL with a Predominant Extranodal Location

The WHO classification recognizes several DLBCL subtypes and entities characterized by a predominant extranodal presentation (Table 1.3).

Primary Mediastinal (Thymic) Large B-Cell Lymphoma

Primary mediastinal (thymic) large B-cell lymphoma (PMBL) seems to originate from a thymic B cell. It occurs predominantly in young women presenting with a large mediastinal mass that frequently invades adjacent structures [38]. A systemic DLBCL with secondary mediastinal involvement has to be excluded. Dissemination outside the mediastinum commonly involves extranodal sites such as the kidney, liver, adrenal, or central nervous system. PMBL is morphologically heterogeneous mainly composed of large cells with abundant pale cytoplasm and round to ovoid nuclei. Reed-Sternberg-like cells are occasionally found, raising the suspicion of Hodgkin lymphoma. Compartmentalizing fibrosis is a common feature in some tumors [39]. PMBL expresses mature B-cell markers, but frequently lacks surface Ig. CD30 positivity is seen in 80 % of cases, although not as uniform and strong as in Hodgkin lymphoma. CD15 is usually negative. Genetically, PMBL has frequent gains of 9p24 and inactivating mutations of SOCS1. PMBL has a distinctive gene expression signature, but has some similarities with Hodgkin lymphoma (HL). In fact, some patients may have a composite HL and PMBL at diagnosis or at relapse or a tumor with intermediate features between both of them (see below). The clinical outcome of PMBL is more favorable than DLBCL, NOS, showing a 5-year survival of 65 %.

Intravascular Large B-Cell Lymphoma

Intravascular large B-cell lymphoma (IVLBCL) is characterized by the selective growth of large B cells within the lumina of capillaries and small- to medium-sized vessels. IVLBCL is rare in western countries, but more common in eastern populations. IVLBCL has a very aggressive clinical course and is frequently diagnosed only at autopsy. Cases with disease limited to the skin have a better prognosis [40].

Primary Cutaneous DLBCL, Leg Type

Primary cutaneous DLBCL, leg type (PCLBCL, leg type), is composed almost exclusively by atypical large B cells and arises most frequently in the skin of lower extremities. These tumors express an activated B-cell phenotype with positivity for IRF4/MUM1 and negativity for CD10. BCL-2 is strongly positive. Genetic features are similar to those found in DLBCL arising at other sites, particularly of the ABC type, but clearly different from those of the PCFCL. Five-year survival is of 50 %, and frequent dissemination to extracutaneous sites is seen [41].

Large-Cell Lymphomas of Terminally Differentiated B Cells

The new WHO classification has included different lymphoma entities that have as a common feature the proliferation of large lymphoid cells with terminally differentiated B-cell phenotype. These cells are characterized by a variable or total lack of CD20 expression and less frequently of CD79a, but express plasma cell-associated antigens such as CD38 and CD138. Most of these tumors are associated with EBV or HHV-8 infection and occur usually in immunosuppressed patients.

ALK-Positive Large B-Cell Lymphoma

ALK-positive large B-cell lymphoma is characterized by a proliferation of large monomorphic immunoblast-like B cells, sometimes with plasmablastic differentiation, that expresses ALK but not CD30 and is EBV negative. These rare tumors present more frequently in young immunocompetent males, usually with nodal involvement and highly aggressive behavior. ALK expression is due to *ALK* rearrangements, particularly with the clathrin (*CLTC*) gene, t(2;17), or less frequently with the nucleophosmin (*NPM*) gene, t(2;5). In contrast to other lymphomas of this group, viral infection and immunodeficiency are not seen [42, 43].

Plasmablastic Lymphoma

Plasmablastic lymphoma (PBL) is a large B-cell neoplasm with immunoblastic morphology and plasma cell immunophenotype. PBL presents in immunosuppressed patients mainly involving extranodal sites such as the oral mucosa and gastrointestinal tract. Most PBL are EBV+ with latency type I (LMP-1 protein negative). HIV infection is the main cause of immunodeficiency, but post-transplant or immunosuppressive treatments are also common. The clinical behavior is very aggressive with poor response to therapy [44, 45].

Primary Effusion Lymphoma

Primary effusion lymphoma (PEL) occurs mainly in immunosuppressed patients. The tumor is composed of large immunoblastic, plasmablastic, or pleomorphic B cells that lack expression of B-cell markers and immunoglobulin but express plasma cell-associated antigens and CD30. These tumors are constantly positive for HHV-8 and also frequently for EBV. PEL usually present as serous effusions without tissue involvement. However, rare cases show extracavitary involvement as extranodal solid tumor masses [46, 47].

Burkitt Lymphoma

Burkitt lymphoma (BL) is a tumor mainly of children and young adults characterized by a diffuse monomorphic proliferation of medium-sized B cells with a mature germinal center B-cell phenotype, negative or very weak BCL-2 expression, high proliferation rate (Ki-67 > 95 %), and presence of the t(8;14) translocation with *MYC* rearrangement. High proliferation is typically associated with a "starry-sky" pattern due to the high number of histiocytes phagocyting apoptotic bodies (Fig. 1.9) [48, 49].

BL is endemic in equatorial Africa and other geographic areas, where BL is associated with EBV infection in almost all cases. Sporadic BL is seen throughout the world, and, in this variant, EBV is detected in 30 % of cases. BL is also seen associated with immunodeficiency, particularly HIV infection. BL mainly involves extranodal sites, particularly as abdominal masses in the ileocecal region, involving jaws and facial bones in endemic areas. A leukemic phase may be seen in patients with bulky disease, and rarely patients may present with a pure leukemic disease (Burkitt leukemia variant), in which CNS tends to be infiltrated. BL is clinically very aggressive but potentially curable with current protocols [1].

Genetically, the t(8;14) is found in the context of a simple karyotype. However, up to 10 % of BL with typical morphology and phenotype may lack this translocation by FISH. On the other hand, DLBCL may also have high proliferation and may carry *MYC* rearrangements, which complicates the diagnosis. Gene expression studies have been useful in establishing differences between BL and DLBCL at molecular level. BL gene expression signature is composed by a group of genes that are targets of the *MYC* gene, high expression of germinal center-related genes, and low expression of NFk[kappa]B target genes and MHC class I genes [37, 50].

B-Cell Lymphoma, Unclassifiable, with Features Intermediate Between DLBCL and Burkitt Lymphoma

Borderline cases between DLBCL and BL comprise rare tumors, occurring predominantly in adults, with a germinal center B-cell phenotype (CD20+, CD10+, BCL6+), presence of *MYC* rearrangements (sometimes with a non-*IG* partner), and high proliferation rate (>90 %). Most of them are aggressive lymphomas with "double-hit" translocations, t(14;18) or less frequently BCL6 rearrangements associated with the t(8;14), and complex karyotypes [51].

Morphologically, these tumors may resemble BL but have an atypical phenotype, such as strong BCL-2 expression, or a typical BL phenotype but an atypical morphology, like blastic nuclear features in the context of negativity for TdT or cyclin D1 negativity. Some of these cases were diagnosed previously as Burkitt-like lymphomas [51, 52].

Gene expression studies have identified a true biologic gray zone between DLBCL and BL. *MYC* rearrangements may occur with an *IG*-gene

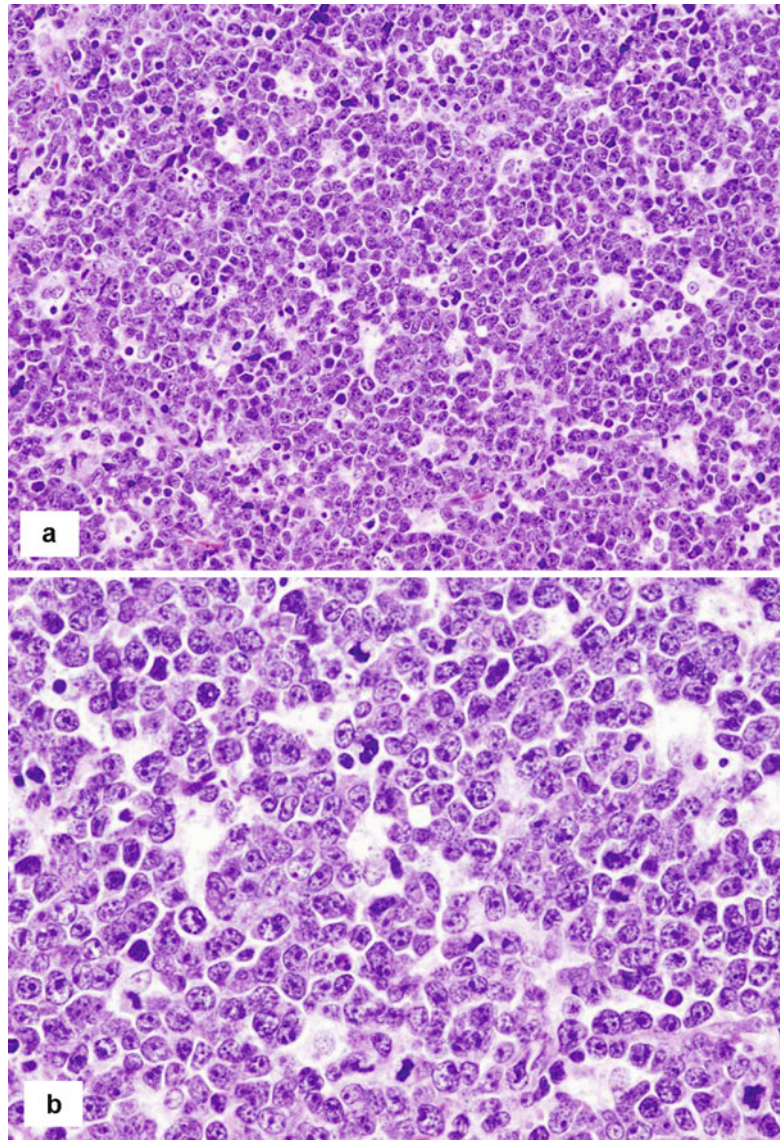


Fig. 1.9 Burkitt lymphoma. (**a**) Diffuse infiltration of medium cells with a starry-sky pattern due to the abundant macrophages with apoptotic bodies (H&E, ×200). (**b**) Monotonous neoplastic infiltrate composed of medium-sized cells with high proliferation (H&E, ×400)

or a non-*IG*-gene partner, and complex karyotypes are common. A strong expression of BCL-2 protein should suggest the presence of a t(14;18) in addition to a t(8;14) ("double hit") [37].

Borderline cases are not considered a specific entity but a working category, because further studies are needed for their precise recognition and clinical management. Lymphomas with typical morphology of DLBCL, high-proliferative activity, and/or presence of t(8;14) should not be included in this category. These lymphomas are very aggressive with poor response to therapy [51].

B-Cell Lymphoma, Unclassifiable, with Features Intermediate Between DLBCL and Classical Hodgkin Lymphoma

This category refers to B-cell lymphomas that have overlapping clinical, morphological, and/or phenotypic features between DLBCL, particularly PMBL, and CHL. These lymphomas predominate in young men presenting with a large mediastinal mass associated in some cases with supraclavicular lymph nodes. Morphologically,

sheets of large, pleomorphic cells in a fibrotic stroma are seen. Lacunar and Hodgkin cells predominate and are associated with sparse inflammatory infiltrate. Strong expression of the complete B-cell program (CD20, CD79a, BOB-1, OCT2, PAX-5) combined with CD30 and CD15 positivity is characteristic. Cases resembling nodular sclerosis classical HL but with uniform expression of CD20 and other B-cell markers and lack of CD15 expression may also be included in this category. Likewise, cases resembling PMBL that lack CD20 and are CD15 positive may favor this diagnosis as well. Sequentially and composite cases of both neoplasms should not be included in this group. The morphological and phenotypic overlapping features of these tumors have been validated by the microarray expression profiling studies as a real biological situation that has similarities and overlapping molecular features between PMBL and HL. Probably, these tumors represent an evidence of plasticity in the differentiation pathways of B cells [53–55].

Mature NK-Cell/T-Cell Neoplasms

Tumors that arise from NK and T cells of peripheral lymphoid organs can be roughly subdivided into three groups: leukemic, extranodal, and nodal.

T-Cell Prolymphocytic Leukemia

T-cell prolymphocytic leukemia (T-PLL) is a rare proliferation of small- to medium-sized T-prolymphocytes (Fig. 1.10a). Patients present with a leukemic picture, usually with hepatosplenomegaly, generalized lymphadenopathy, and BM involvement. Cutaneous infiltration is seen in 20 % of cases. Peripheral blood films show small- to medium-sized lymphoid cells with round to markedly irregular nuclei with visible nucleolus and basophilic non-granular cytoplasm. T-prolymphocytes have a mature T-cell phenotype, with expression of CD2, CD3, CD7, CD52,

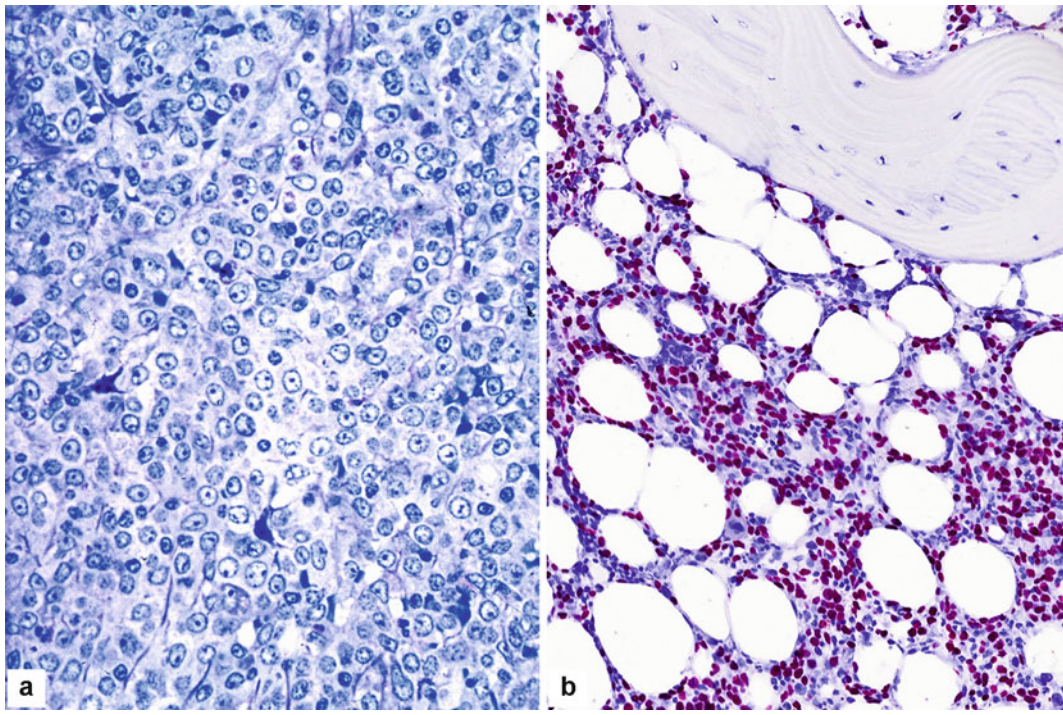


Fig. 1.10 (**a**) T-prolymphocytic leukemia. Neoplastic cells are small- to medium-sized with distinct nucleoli and a narrow rim of cytoplasm grayish at Giemsa (×400). (**b**) Aggressive NK-cell leukemia. Neoplastic cells show extensive interstitial infiltration of the bone marrow as well as clear-cut EBV positivity (EBER in situ hybridization, ×100)

CD4+/CD8− (60 %), CD4+/CD8+ (25 %), and CD4−/CD8+ (15 %). Chromosome abnormalities are seen, with inversion of chromosome 14 being the most common (80 %). Oncogenes *TCL1A* and *TCL1B* are thereby activated, the overexpression of TCL1 representing a poor prognostic marker. Abnormalities in chromosome 8 are also frequently found by FISH studies (70–80 %) as are deletions at 12p13. Clinical course is aggressive, with a median survival of less than a year [1, 56].

Aggressive NK-Cell Leukemia

Aggressive NK-cell leukemia is a rare systemic neoplasm of NK cells associated in most if not all cases with EBV (Fig. 1.10b) and observed mainly in eastern population. Middle-aged individuals are usually affected, with no sex predilection. Commonly involved sites are the PB, BM, liver, and spleen. Complications like coagulopathy, hemophagocytic syndrome (HPS), or multi-organ failure (MOF) are frequent findings. A certain overlap with extranodal NK-/T-cell lymphoma may exist. Leukemic cells show a morphological spectrum from normal large granular lymphocytes to lymphocytes with atypical infolded nuclei, open chromatin, distinct nucleoli, and basophilic cytoplasm. Expression of CD2, CD3ε[epsilon], CD56, and cytotoxic molecules, without surface CD3, is typical, a phenotype identical to that of extranodal NK-/T-cell lymphoma, except that CD16 is frequently positive. T-cell receptor (*TCR*) genes show a germline configuration. Deletions of 6q and 11q have been reported. The majority of cases have a fulminant clinical course [57].

EBV+ T-Cell Lymphoproliferative Disorders of Childhood

Systemic EBV+ T-cell lymphoproliferative disease (LPD) of childhood and hydroa vacciniforme-like lymphoma are the two major types of this disorder. Both occur more frequently in Asia, Mexico, and Central and South America with no sex predilection. In both conditions, there is strong suggestion of a genetic defect in the host immune response to EBV [1].

Systemic EBV+ T-cell LPD of childhood can appear shortly after primary acute EBV infection or in relation with chronic active EBV infection, characterized by a clonal cytotoxic T-cell expansion (Fig. 1.11a). It has rapid evolution, multiple-organ failure, and generally fatal outcome [58].

Hydroa vacciniforme-like lymphoma is a pediatric EBV-positive cutaneous T-cell lymphoma with cytotoxic or less commonly NK-cell phenotype, associated with sensitivity to insect bites and sun exposure. Patients present with a papulovesicular eruption that may be accompanied by systemic symptoms. Recurrent skin lesions may be seen, and systemic involvement is associated with a more aggressive clinical course. Most cases show clonal rearrangements of the *TCR* genes [58].

Adult T-Cell Leukemia /Lymphoma (ATLL)

The distribution of ATLL parallels that of the human T-cell leukemia virus type 1 (HTLV-1), being endemic in southwestern Japan, the Caribbean Basin, and parts of Central Africa. ATLL has a long latency period with exposure to the virus by affected individuals very early in life. Although causally linked to ATLL, HTLV-1 itself is insufficient to cause neoplastic transformation, and additional genetic events are necessary. Patients usually present with multiple lymphadenopathy and PB involvement, the skin being affected in more than half of the cases. Clinical behavior varies from an acute presentation, with usual hypercalcemia and possible lytic bone lesions, to lymphomatous, chronic, or smoldering presentations. The neoplastic proliferation may be of small- to large-sized pleomorphic cells or display anaplastic or even angioimmunoblastic-like morphology (Fig. 1.11b). ATLL-cells are usually positive for CD2, CD3, and CD5 and negative for CD7. Most cases are CD4+/CD8−, but a subset is CD4−/CD8+ or CD4+/CD8+. CD25 is strongly expressed in almost all cases, and FOXP3

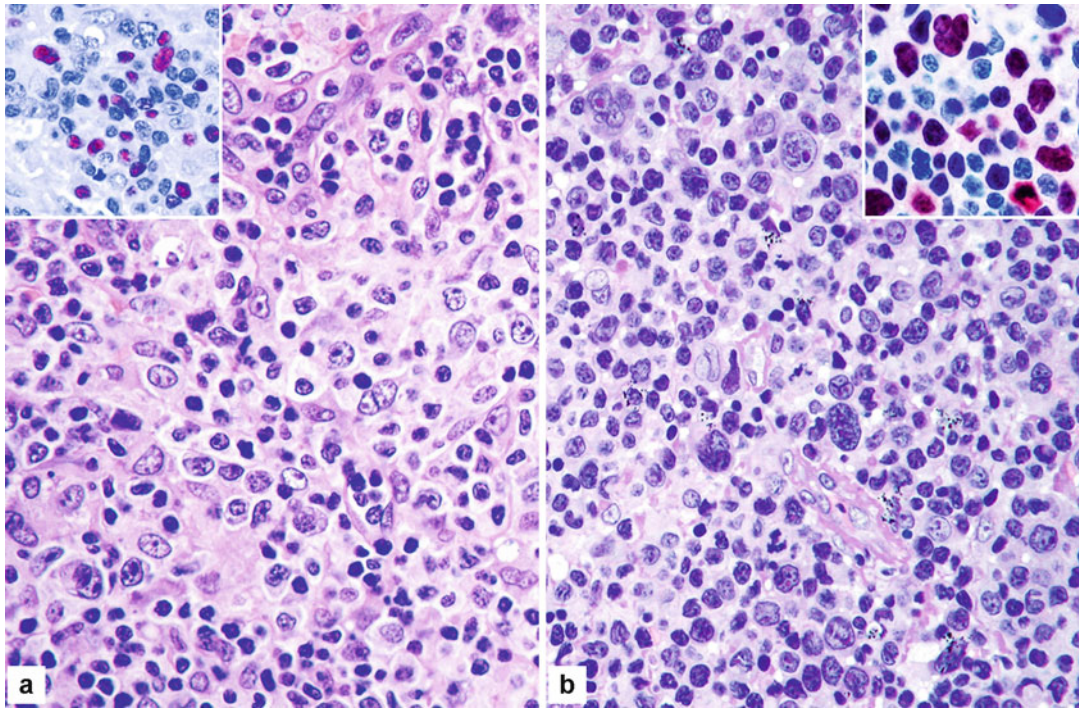


Fig. 1.11 (**a**) EBV+ lymphoproliferative disorder of childhood. Please, note the pleomorphism of the lymphoid proliferation that is admixed with some epithelioid elements (H&E, ×400) and turns EBV positive (inset: EBER in situ hybridization, ×400). (**b**) Adult T-cell lymphoma/leukemia. Neoplastic cells remarkably vary in size and shape, frequently showing a floret-like appearance (H&E, ×400) and Ki-67 expression (inset: immunoalkaline phosphatase technique, ×400)

and CCR4 are frequently positive, all three characteristic markers of regulatory T cells. CD30 may be positive and cytotoxic molecules are absent. TCR genes are clonally rearranged. ATLL has a high proliferative rate, showed by Ki-67 positivity by the neoplastic cells (Fig. 1.11b). Clinical subtypes, age, performance status, seric calcium, and LDH levels are major prognostic indicators. Acute and lymphomatous variants are associated with short survivals, while the chronic and smoldering forms have an indolent course but can progress to an acute phase [1, 59].

Extranodal NK-/T-Cell Lymphoma, Nasal Type (ENK/TCL, NT)

ENK/TCL, NT occurs more frequently in adult males from Asia, Mexico, and Central and South America and is associated with EBV infection, with a type II latency pattern. ENK/TCL, NT typically involves the nasal cavity, nasopharynx, paranasal sinuses, and palate. Most patients show nasal obstruction, epistaxis, or extensive midfacial destruction (the so-called lethal midline granuloma). Extra-nasal sites, including the skin, soft tissue, gastrointestinal tract, and testis may also be involved, while BM involvement is uncommon. Mucosal sites generally have extensive ulceration. The neoplastic infiltrate is diffuse with angiocentric and angiodestructive features with frequent necrosis and apoptotic bodies (Fig. 1.12a). Neoplastic cells may be small, medium-sized, large, or anaplastic with numerous mitotic figures. Phenotypically, they are positive for CD2, CD56 (Fig. 1.12a), CD3ε[epsilon]+, and cytotoxic markers and negative for surface CD3. Occasional cases are CD7+ or CD30+. Noteworthy, CD56 is not specific for ENK/TCL, NT and may be positive in other peripheral T-cell lymphomas (PTCLs), mainly carrying γ[gamma]δ[delta]-TCR. In situ hybridization for EBV-encoded RNA (EBER) is virtually positive in all ENK/TCL, NT, and a diagnosis of ENK/

TCL, NT should be made with great caution if EBER is negative. LMP-1 expression is variable. *TCR* and *IgVH* genes are usually in germline configuration, the former being clonally rearranged in a few cases. The prognosis of ENK/TCL, NT is variable, but generally very poor in cases occurring outside the nasal cavity. Among nasal cases, both IPI and Korean index are provided with predictive value [60]. Notably, survival rates have recently been improved with more intensive regimes including upfront radiotherapy and L-asparaginase [1, 61].

Enteropathy-Associated T-Cell Lymphoma (EATL)

EATL is an intestinal tumor of intraepithelial T lymphocytes. Patients are usually adults presenting with abdominal pain and frequent intestinal perforation. EATL is associated with celiac disease and occurs more often in areas with a high prevalence of this disease, especially in Northern Europe. The tumor usually presents as multiple ulcerating raised mucosal masses with infiltration of the intestinal wall. The jejunum or ileum are the most common involved sites. EATL is usually composed of large lymphoid cells with an inflammatory background of histiocytes and eosinophils and associated with necrosis. The adjacent small intestinal mucosa shows villous atrophy, crypt hyperplasia, increased number of lymphocytes and plasma cells infiltrating the lamina propria, and intraepithelial lymphocytosis. In 10–20 % of cases, EATL is composed of monomorphic medium-sized cells without inflammatory background and rare necrosis (Fig. 1.12b). This monomorphic variant (type II EATL) may occur sporadically and has a broader geographic distribution and no proven relationship with celiac disease. Neoplastic cells are CD3+, CD5−, CD7+,

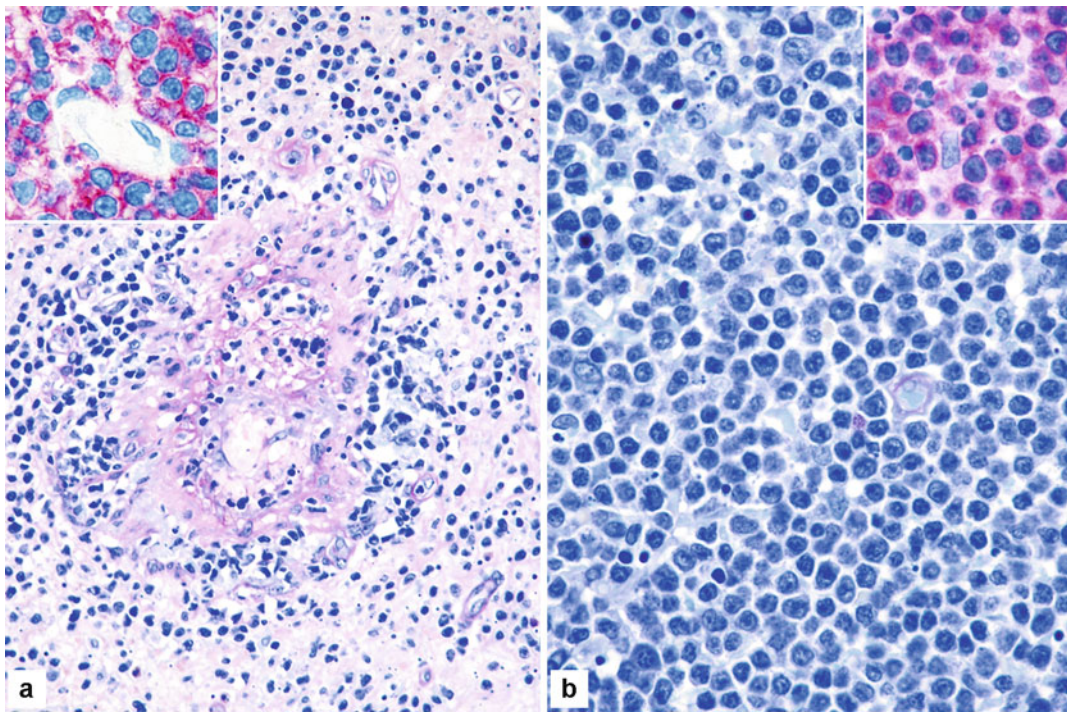


Fig. 1.12 (**a**) Extranodal NK-/T-cell lymphoma, nasal type. The lymphomatous population displays angiocentricity and angioinvasiveness, causing extensive necrosis (Giemsa; ×100), and strong CD56 expression (inset: immunoalkaline phosphatase technique, ×400). (**b**) Enteropathy-associated T-cell lymphoma, type II: neoplastic elements, medium-sized and monotonous (Giemsa; ×400), express TCR γ[gamma]-chain (inset: immunoalkaline phosphatase technique, ×400)

CD8–/+, CD4–, CD103+, and TCR ß[beta]+ and positive for cytotoxic markers, with heterogeneous expression for CD30. Type II EATL has a distinctive immunophenotype (CD3+, CD4–, CD8+, CD56+, and TCRß[beta]+, although TCRγ+ cases have recently been reported (Fig. 1.12b)) [62]. *TCR β[beta]* and *TCR γ[gamma]* genes are clonally rearranged. Poor prognosis with death frequently resulting from abdominal complications is usual [1, 63].

Hepatosplenic T-Cell Lymphoma (HSTL)

HSTL is a rare cytotoxic T-cell neoplasm mostly of the γ[gamma]δ[delta] type seen mainly in adolescents and young males. Up to 20 % of HSTL associates chronic immune suppression, usually in the setting of solid organ transplantation or prolonged antigenic stimulation. It may also occur in patients treated with azathioprine and infliximab for Crohn's disease. Clinical presentation includes systemic symptoms, hepatosplenomegaly, and marked thrombocytopenia. HSTL shows a monotonous infiltrate composed of cells with medium-sized nuclei, dispersed chromatin, inconspicuous nucleoli, and a rim of pale cytoplasm (Fig. 1.13a). In the spleen, the infiltrate involves the red pulp cords and sinuses with atrophy of the white pulp, while the liver and BM show an intra-sinusoidal infiltration pattern. Phenotypically, most cases are CD3+, TCRγ[gamma]δ[delta]1+ (Fig. 1.13a), CD56±, and CD8 and negative for TCRα[alpha]β[beta], CD4, and CD5. Few cases are of α[alpha]β[beta]-type. TIA1 and granzyme-M are expressed, but granzyme-B and perforin are negative. They have clonally rearranged *TCRγ[gamma]* genes. Isochromosome 7q is frequently present. Trisomy 8 and loss of a sex chromosome may also be found. The course is aggressive, with a median survival of less than 2 years [1, 64].

Subcutaneous Panniculitis-Like T-Cell Lymphoma (SPTCL)

SPTCL is a rare lymphoma composed of cytotoxic T cell that preferentially infiltrates subcutaneous tissue and has a slightly predominance in females and a broad age spectrum. According to the new WHO classification, cases expressing γ[gamma]δ[delta]TCR should be excluded from this entity and classified as primary cutaneous γ[gamma]δ[delta] T-cell lymphoma. Approximately 20 % of patients have an associated autoimmune disease, frequently systemic lupus erythematosus. Generally, patients present with multiple subcutaneous nodules in the absence of other involved sites. More than 50 % of patients show systemic symptoms. Cytopenias and abnormal liver function tests are frequent findings, and a hemophagocytic syndrome (HPS) is seen in 15–20 % of cases. Fat lobules are involved with rimming of individual fat cells by neoplastic cells, usually sparing fibrous septa and the overlying dermis and epidermis (Fig. 1.13b). Necrosis and karyorrhexis are common and vascular invasion may be seen. SPTCL-cells have a mature α[alpha]β[beta] T-cell phenotype (β[beta]F1+), usually express CD8+, cytotoxic molecules, and are negative for CD56. Clonal rearrangements of *TCR* genes are present, and EBV sequences are negative. Involvement of lymph nodes and other organs is rare. Five-year overall survival is of 80 %, but patients with HPS have poor prognosis [1, 65].

Mycosis Fungoides (MF) and Sézary Syndrome (SS)

MF is the most frequent primary cutaneous T-cell lymphoma (CTCL) characterized by epidermotropic infiltration of small- to medium-sized T cells with cerebriform nuclei (Fig. 1.14a). Patients are usually male adults/elderly, but it may also be seen in children and adolescents. MF is limited to the skin, with classical evolution of patches, plaques, and tumors. Dissemination to extracutaneous tissues occurs in advanced stages, and progression is slow, over years or sometimes decades. Histological transformation (>25 % blasts in the dermal infiltrates) is found mainly in tumor stages. Neoplastic cells are CD2+, CD3+, TCRß[beta]+, CD5+, CD4+, CD8–, and CD7–. *TCR* genes are clonally rearranged in a variable number of cases. Clinical stage is the single most important prognostic factor. In advanced stages and in cases with histological transformation, the

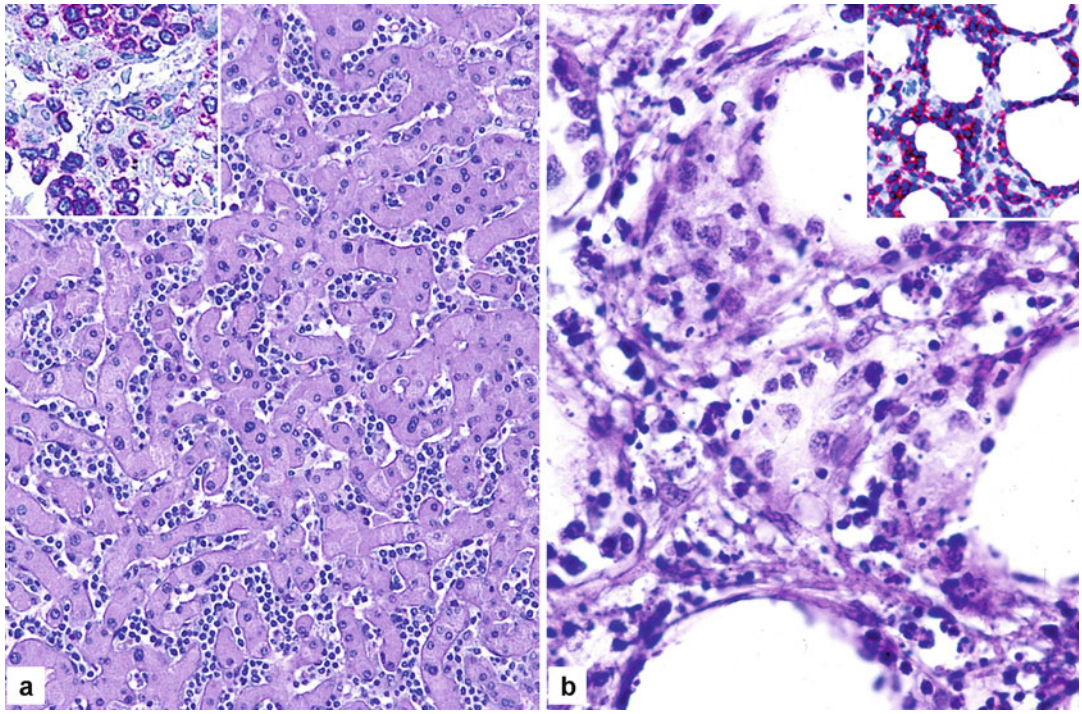


Fig. 1.13 (**a**) Hepatosplenic T-cell lymphoma. Lymphomatous elements diffuse through the liver sinusoids (Giemsa, ×200) and express TCR γ[gamma]-chain in the spleen (inset: immunoalkaline phosphatase technique, ×400). (**b**). Subcutaneous panniculitis-like T-cell lymphoma. Neoplastic cells—often apoptotic—infiltrate the subcutaneous tissue, producing rims around fat lobules (H&E, ×400); riming is underlined by the staining for CD3 (inset: immunoalkaline phosphatase technique, ×200)

prognosis is poor. Rare variants are folliculotropic MF, pagetoid reticulosis, and granulomatous slack skin [28].

Sézary syndrome (SS) is a rare disease of adult males accounting for less than 5 % of CTCLs. SS is defined by the triad of erythroderma, generalized lymphadenopathy, and presence of a clonal expansion of neoplastic T cells with cerebriform nuclei (Sézary cells) in the skin, lymph nodes, and blood. Histological and phenotypic features of SS are similar to those of MF. The clinical course is aggressive with a 5-year overall survival of 10–20 % [28].

Primary Cutaneous CD30-Positive T-Cell Lymphoproliferative Disorders (PCTLPD-CD30+)

These lesions include primary cutaneous anaplastic large-cell lymphoma (C-ALCL) and lymphomatoid papulosis (LyP), which have a spectrum of overlapping histopathologic and phenotypic features between them. Final diagnosis must be established considering clinical appearance and course [1].

C-ALCL is composed of a diffuse, usually non-epidermotropic infiltrate of large cells with an anaplastic, pleomorphic, or immunoblastic morphology, the vast majority of which (>75 %) expresses CD30 (Fig. 1.14b). By definition, C-ALCL should not be preceded or have clinical evidence of MF. Cases of systemic ALCL should also be excluded, as they correspond to a different entity. The most frequent clinical presentation is a solitary nodule, but some patients may have papules located in the trunk, face, extremities, or buttocks that often show ulceration and partial or complete spontaneous regression as seen in LyP. Besides CD30-positivity, C-ALCL cells have an activated CD4+ T-cell phenotype with variable loss of CD2, CD5, and/or CD3 and frequent expression of cytotoxic markers. Contrary to systemic ALCL, C-ALCL expresses CLA, but is

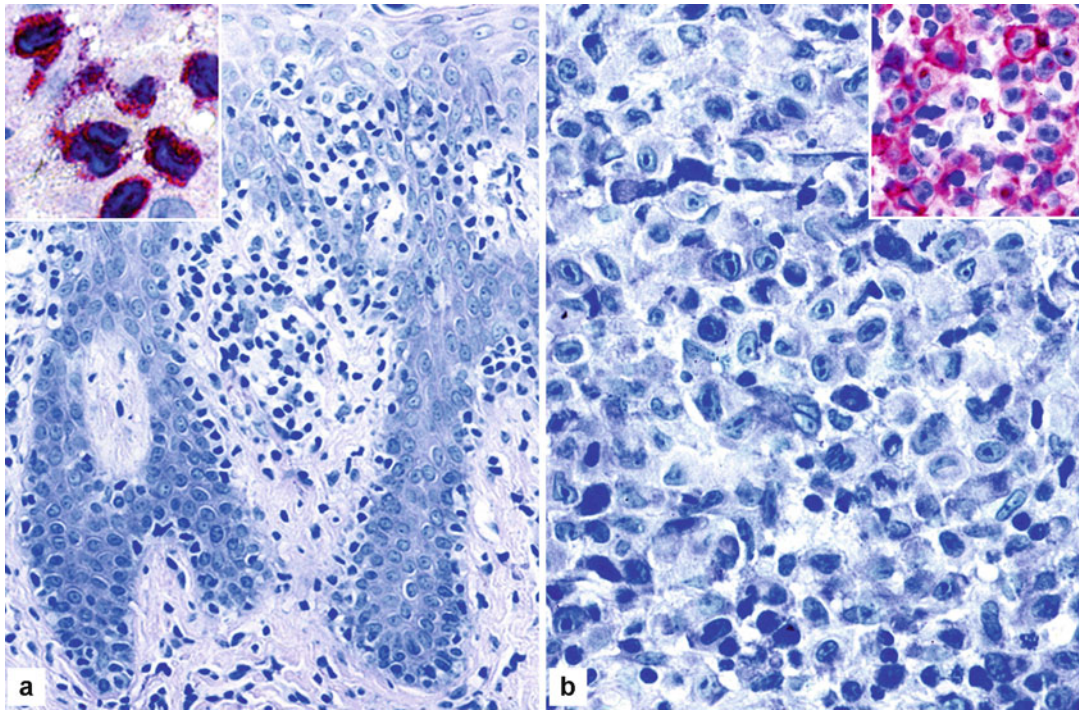


Fig. 1.14 (**a**) Mycosis fungoides, early phase. Lymphomatous elements show evident epidermotropism by producing a "chain" at the dermal-epidermal junction and forming small micro-abscesses with the epidermis (Giemsa, ×100); immunohistochemistry underlines their cerebriform nuclear profile (inset: immunoalkaline phosphatase technique, ×600). (**b**) CD30+ lymphoproliferative disorder of the skin, anaplastic large-cell lymphoma type: the growth turns cohesive and consists of large cells that reveal prominent, inclusion-like nucleoli, a wide rim of cytoplasm grayish at Giemsa (×400) and strong CD30-positivity (inset: immunoalkaline phosphatase technique, ×250)

negative for EMA and ALK. Unlike Hodgkin and Reed-Sternberg cells, CD15 is generally negative in C-ALCL. *TCR* genes are clonally rearranged in most cases. Patients with C-ALCL have usually good prognosis, with a 10-year overall survival rate of approximately 90 %, although frequent local relapses occur [28].

Lymphomatoid papulosis (LyP) has a chronic, recurrent, self-healing clinical presentation, with cutaneous involvement of trunk and extremities of adult males. LyP is characterized by paradoxical eruptions of papular, papulonecrotic, and/or nodular lesions that tend to disappear within 3–12 weeks leaving a scar in some cases. Disease duration is variable, from several months to more than 40 years. Morphologically the large CD30+ cells may have a background of inflammatory cells or predominate as a monotonous population. The phenotype is similar to C-ALCL. About 60 % of LyP lesions show clonally rearranged *TCR* genes. Prognosis of LyP is excellent [28].

Three rare primary cutaneous peripheral T-cell lymphomas with characteristic clinical and histopathologic features have been delineated in the new WHO classification, including primary cutaneous γ[gamma]δ[delta] T-cell lymphoma, primary cutaneous aggressive epidermotropic CD8-positive cytotoxic T-cell lymphoma and primary cutaneous CD4-positive small-medium-sized pleomorphic T-cell lymphoma, the latter two being still provisional entities [1].

Peripheral T-Cell Lymphoma, Not Otherwise Specified (PTCL, NOS)

PTCL, NOS is a heterogeneous category that includes nodal and extranodal mature T-cell lymphomas, which do not fulfil the criteria to be

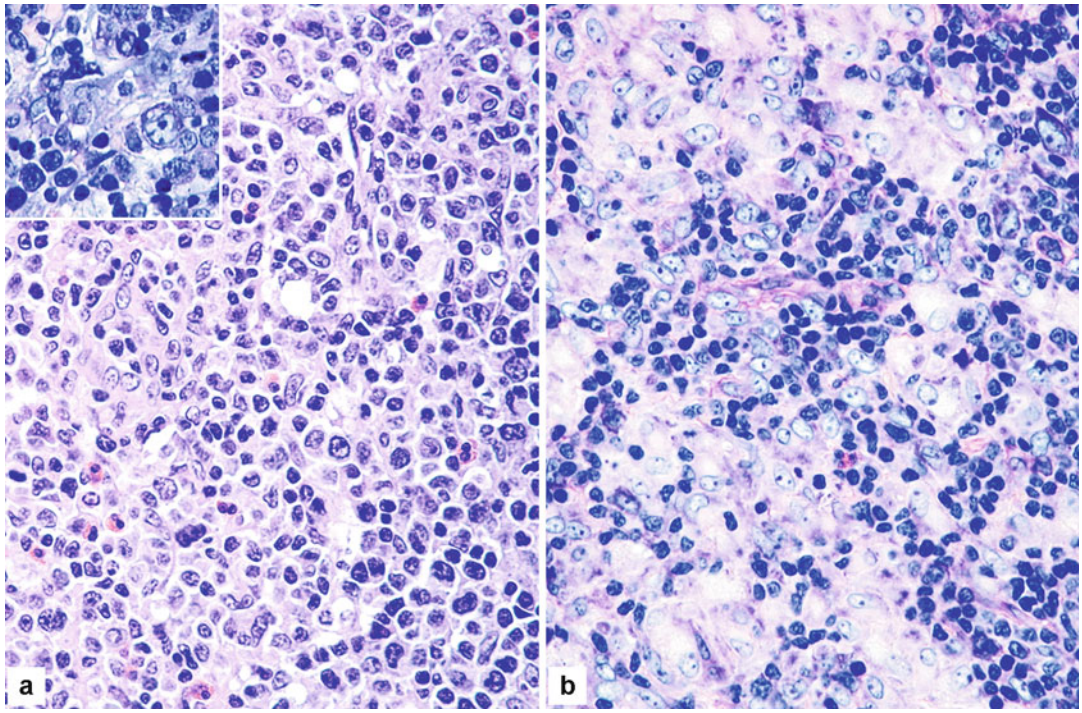


Fig. 1.15 (**a**) Peripheral T-cell lymphoma, not otherwise specified. The lymphomatous population, admixed with some reactive elements such as eosinophils, is characterized by marked pleomorphism with bean-shaped to cerebriform nuclei (H&E, ×400; inset: Giemsa, ×600). (**b**) Peripheral T-cell lymphoma, not otherwise specified, Lennert's type. Please, note the huge amount of epithelioid elements as well as the more monotonous appearance of neoplastic cells with exceptional blasts (Giemsa, ×400)

classified in one of the specific entities of T-cell lymphomas. It represents approximately 30 % of PTCLs in western countries. Patients are generally adults with a slight male predominance, showing peripheral lymphadenopathy and B-symptoms. Generalized disease includes the BM, liver, spleen, and extranodal tissue involvement. Leukemic presentation is uncommon. PTCL, NOS has a wide cytological spectrum from highly polymorphous to monotonous. Most cases show medium-sized cells and/or large cells with irregular, pleomorphic nuclei; prominent nucleoli; and numerous mitotic figures. Clear cells and Reed-Sternberg-like cells may be present. Most cases show an inflammatory background (Fig. 1.15a). The differential diagnosis with angioimmunoblastic T-cell lymphoma (AITL) must include extensive immunophenotyping. PTCL, NOS usually has an aberrant T-cell phenotype with frequent loss of T-cell markers CD5 and CD7. A CD4+/CD8− phenotype is more frequent in nodal cases. CD4/CD8 double positivity or double negativity is sometimes seen, as is CD8, CD30, CD56, and/or cytotoxic granule expression. CD52 is negative in 60 % of cases. Some cases carry follicular T helper cell (FTH) markers: [66] they differ from angioimmunoblastic T-cell lymphoma (see below) because of the lack of follicular dendritic cell (FDC) and high-endothelial venule (HEV) hyperplasia [67]. Aberrant CD20 and/or CD79a expression is occasionally found. Proliferation is high and Ki-67 rates over 70 % are associated with poor prognosis. *TCR* genes are clonally rearranged. PTCL, NOS is a highly aggressive tumor with poor response to therapy, frequent relapses, and a 5-year overall survival of 20–30 %. IPI is the only factor consistently associated with prognosis. Recently, new scoring systems have been proposed. Three morphological variants of PTCL, NOS are recognized, lymphoepithelioid/Lennert's type (Fig. 1.15b), follicular, and T-zone [1, 68].

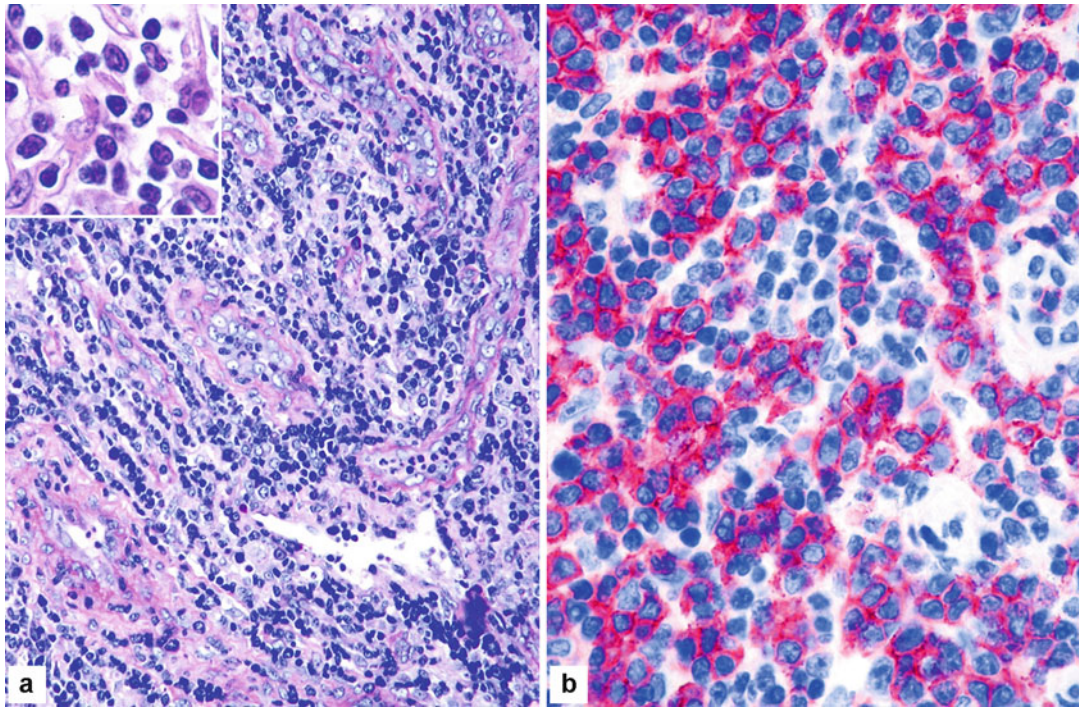


Fig. 1.16 (**a**) Angioimmunoblastic T-cell lymphoma. At low power, the growth shows rather low cellular density and abundant branching epithelioid venules (Giemsa, ×100); at higher magnification, lymphomatous elements reveal a slightly irregular nuclear profile and an evident rim of clear cytoplasm (H&E, 400). (**b**) On immunohistochemistry, neoplastic cells show strong PD1 positivity (immunoalkaline phosphatase technique, ×400)

Angioimmunoblastic T-Cell Lymphoma (AITL)

AITL is characterized by systemic disease and nodal involvement by a polymorphous infiltrate with prominent proliferation of HEV and FDC (Fig. 1.16a). AITL occurs in middle-aged/elderly individuals of either sex and represents 15–20 % of all PTCLs. Most patients present with advanced stage disease, systemic symptoms, generalized lymphadenopathy, hepatosplenomegaly, polyclonal hypergammaglobulinemia, circulating immune complexes, cold agglutinins with hemolytic anemia, positive rheumatoid factor, and anti-smooth muscle antibodies. Skin rash is frequently observed and BM is often involved. Other common findings are pleural effusion, arthritis, and ascites. Partial effacement of the lymph node architecture is characteristic, as is sparing of the peripheral cortical sinuses. The neoplastic cells are small to medium in size, with clear to pale cytoplasm, and mild cytological atypia. Small clusters of neoplastic cells are seen around hyperplastic HEV and FDC and have a polymorphic background of small reactive lymphocytes, eosinophils, plasma cells, and histiocytes. EBV+ B-immunoblasts are usually present. Neoplastic cells are CD4-positive and carry at least three of the FTH-associated markers CD10, BCL6, CXCL13, ICOS, PD-1, and SAP (Fig. 1.16b) [66]. Most cases have clonally rearranged *TCR* genes. Clonal *IgVH* gene rearrangements are present in about 25–30 % of cases and correlate with the expanded EBV+ B cells. AITL has an aggressive clinical course with a median survival of less than 3 years. Large B-cell lymphoma (often but not invariably EBV+) development may occur [1, 69].

Anaplastic Large Cell Lymphoma, ALK Positive (ALCL, ALK+)

ALCL, ALK+ is composed of large neoplastic cells with abundant cytoplasm and pleomorphic

kidney-/horseshoe-shaped nuclei (hallmark cells), with a translocation involving the *ALK* gene and expression of ALK protein (Fig. 1.17a) and CD30. The latter may be the target of the humanized monoclonal antibody conjugated with monomethyl auristin E, brentuximab vedotin [70]. ALCL that are morphologically and phenotypically similar but lacking the *ALK* rearrangement and ALK protein expression are considered a distinct category (ALCL/ALK−). ALCL, ALK+ is more prevalent in the first three decades of life with a male predominance. Lymph nodes and extranodal sites are involved. Stages III–IV diseases and B-symptoms are present in most patients. Morphology may be variable, but the "common pattern" that represents 60 % of cases consists of large tumor cells, commonly with hallmark appearance, that tend to grow within the sinuses, resembling a metastatic tumor. Approximately 10 % of cases may have a "lymphohistiocytic pattern" characterized by few tumor cells accompanied by reactive histiocytes. The "small cell variant" (5–10 %) with small- to medium-sized neoplastic cells with irregular nuclei and, in some cases, presence of small cells with abundant cytoplasm ("fried egg cells") seems to have a worse prognosis. "Hodgkin-lymphoma pattern" is described in 3 % of ALCL, ALK+ cases, resembling nodular sclerosis classical Hodgkin lymphoma, in which neoplastic cells are ALK positive but CD15 negative. Relapses may present with different morphological features as seen in the initial tumor. Cases with t(2;5)/NPM-ALK translocation show cytoplasmic and nuclear ALK staining. In cases with variant translocations, ALK staining may be membranous or cytoplasmic. ALCL, ALK+ cells are CD30+, frequently EMA+ with expression of cytotoxic markers and one or more T-cell antigens, although some may have "null" phenotype. EBV is negative. *TCR* gene is clonally rearranged. The most frequent translocation is t(2;5)(p23;q35) involving *ALK* and nucleophosmin (*NPM*) genes. Variant translocations show fusion of *ALK* gene with other partners on chromosomes 1, 2, 3, 17, 19, 22, and X. All these aberrations result in up-regulation of the ALK protein that plays a pivotal role in the process of lymphomagenesis [71]. They also cause distinct subcellular distribution of the fusion protein as shown by immunohistochemistry. However, none of them affects the disease behavior. Different chromosomal and gene expression profiles are seen in ALCL, ALK+; ALCL/ALK−; and PTCL, NOS. IPI may be used as a prognostic factor. Five-year overall survival is around 80 % [1, 72].

Anaplastic Large Cell Lymphoma, ALK Negative (ALCL/ALK−)

ALCL/ALK− is a provisional entity, composed of anaplastic large CD30+ cells (Fig. 1.17b), most of which express both T-cell-associated and cytotoxic markers. Differential diagnosis must be done with C-ALCL, other CD30+ T- or B-cell lymphomas with anaplastic features, and CHL. ALCL/ALK− is more common in adults with a slight male predominance. Lymph nodes are more frequently involved than extranodal sites. Most patients have advanced-stage disease, with lymphadenopathy and B-symptoms. Usually, the architecture of lymph nodes or other tissues is effaced by solid, cohesive sheets of neoplastic cells. In the lymph node, neoplastic cells expand the sinuses or the T-cell areas, mimicking metastatic carcinoma. PTCL, NOS and CHL must be excluded, but immunophenotypic and molecular studies may identify these entities. In this respect, BSAP/PAX5 staining is a useful tool: CHL shows weak positivity in the majority of cases, but BSAP/PAX5 is negative in ALCL/ALK−. By contrast, the distinction between PTCL, NOS and ALCL/ALK− is not always straightforward. Diagnosis of ALCL/ALK- is favored when homogeneous strong CD30-staining on the cell membrane and Golgi area of all neoplastic cells is seen, as well as complete loss of T-cell markers and lack of NFATc2 expression [73]. Most cases have clonal *TCR* gene rearrangement and EBV is negative. Recently, it has been reported that about one third of ALCL/ALK− carry t(6;7)(p25.3;q32.3) translocations [74]. The overall survival rate for ALCL/ALK− is shorter than ALCL, ALK+ (49 % vs. 80 %), but better than PTCL, NOS (49 % vs. 32 %) [1, 75].

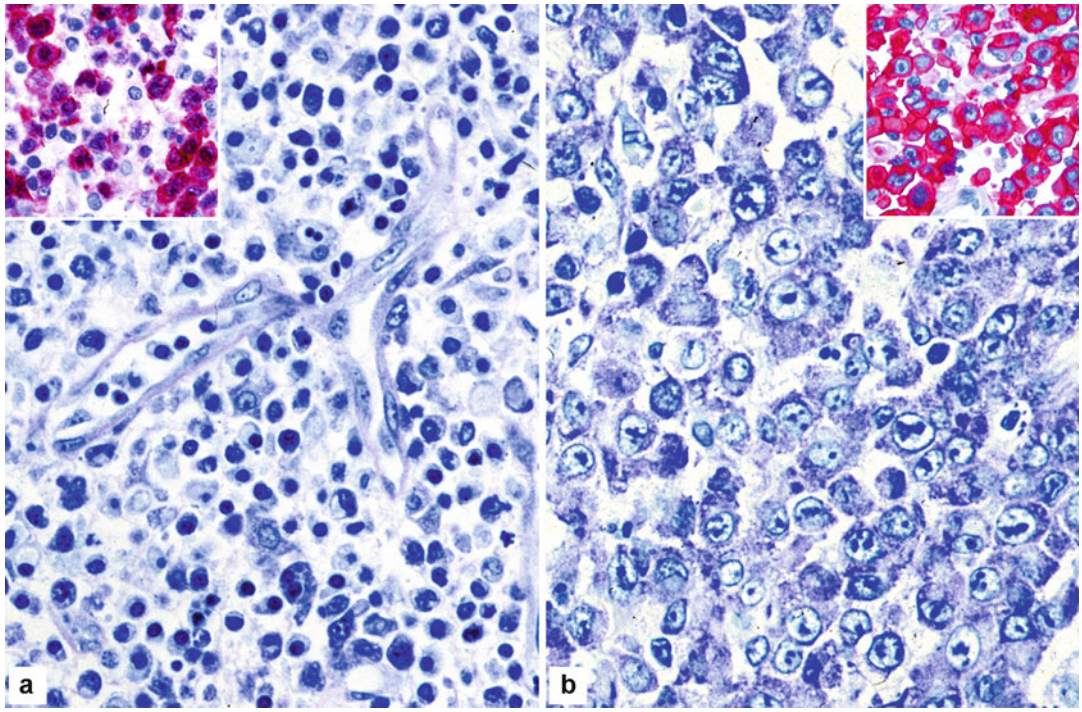


Fig. 1.17 (**a**) ALK+ anaplastic large cell lymphoma, lymphohistiocytic variant. Neoplastic cells with variable size and shape (from small to hallmark) are almost obscured by a huge histiocytic component (Giemsa, ×400); the staining for the ALK protein allows their easy identification: please, note that the positivity occurs at both the nuclear and cytoplasmic level indicating the presence of t(2;5) (inset: immunoalkaline phosphatase technique, ×400). (**b**) ALK- anaplastic large-cell lymphoma. The lymphomatous population growths cohesively, has prominent, inclusion-like nucleoli, reveals a large rim of cytoplasm *grayish violet* at Giemsa (×400) and turns strongly CD30-positive (inset: immunoalkaline phosphatase technique, ×250)

Hodgkin Lymphoma (HL)

HL accounts for approximately 30 % of all lymphomas. Irrespectively of the subclassification, HL is characterized by the following features: (a) usual onset in lymph nodes, preferentially in the cervical region; (b) predilection for young adults; (c) small number of mononucleated and multinucleated tumor cells (Hodgkin and Reed-Sternberg cells or HRS cells), within a background of abundant inflammatory cells; and (d) tumor cells that are often surrounded by rosettes of T lymphocytes. Biologically and clinically HL are divided in two disease entities: nodular lymphocyte-predominant HL (NLPHL) and classical HL (CHL) [1].

NLPHL is a monoclonal B-cell lymphoma characterized by a nodular or nodular and diffuse proliferation of scattered large multilobated neoplastic cells ("popcorn" or LP cells) (Fig. 1.18a). Tumor cells reside in follicles with a large FDC meshwork filled with nonneoplastic lymphocytes and histiocytes. Some morphological variants of this prototypic pattern have been described: serpiginous/interconnected, nodular with prevalent extra-nodular LP cells, T-cell-rich-nodular, and THRBCL-like [76]. One third of the cases diagnosed as NLPHL in the past were lymphocyte-rich CHL. It is currently unclear whether a diffuse NLPHL exists, and its distinction from TCRBCL is not always evident. NLPHL represents nearly 5 % of HL. Patients are generally males in the fourth decade of life. LP cells are most frequently CD20+, CD79a+, EMA+, BCL6+, and CD45+. Contrary to HRS cells of CHL, tumor cells are positive for Oct-2, BOB.1, and Ig light and/or heavy chains but lack CD15, CD30, and EBV infection. In more than

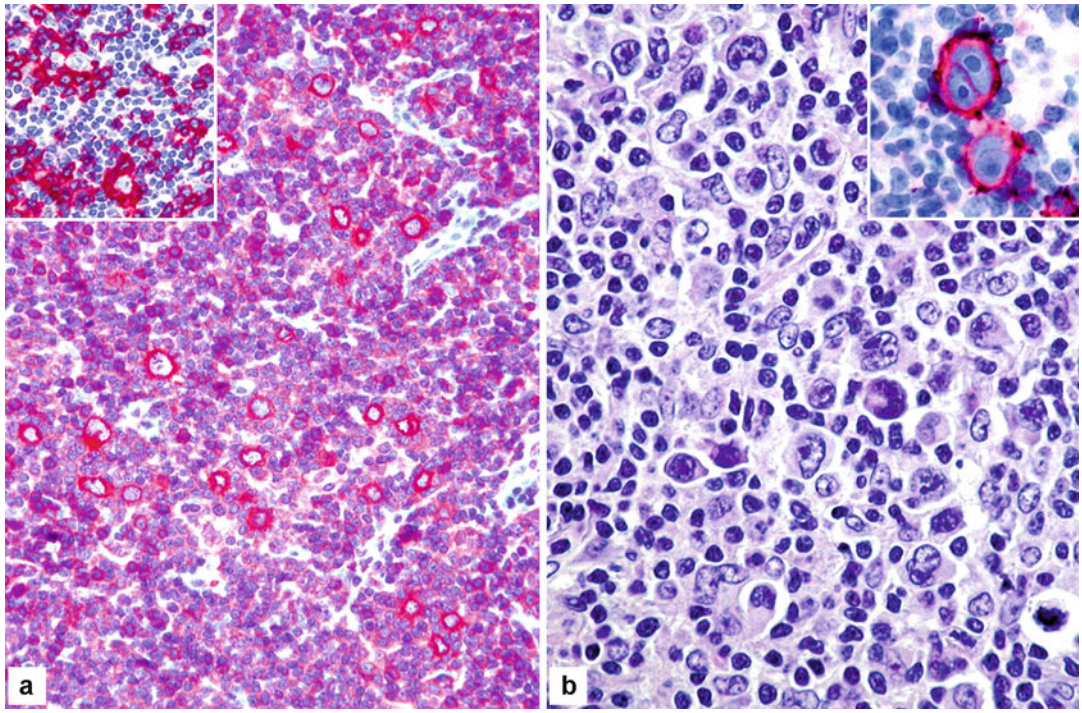


Fig. 1.18 (**a**) Lymphocyte-predominant Hodgkin lymphoma. LP cells express IgD as do most small B lymphocytes that form the nonneoplastic cellular milieu along with rosettes of PD1-positive T lymphocytes (inset) (immunoalkaline phosphatase technique, ×100). (**b**) Classical Hodgkin lymphoma, lymphocyte rich. Typical Hodgkin and Reed-Sternberg cells are admixed with small lymphocytes and some epithelioid elements (H&E, ×250); please, note the strong CD30 expression by tumoral cells (inset: immunoalkaline phosphatase technique, ×400)

20 % of cases, LP cells are IgD+ (Fig. 1.18a), IgM− and CD27−: the patients are usually young males [77]. The tumor cells are ringed by follicular helper T-cells CD3+ CD4+, PD-1+ (Fig. 1.18a), and (to a lesser extent) CD57+. LP cells show clonally rearranged *IgVH* genes with a high load of somatic mutations that may be ongoing. Aberrant somatic hypermutations of *PAX5*, *PIM1*, *RhoH/TTF,* and *MYC* genes are reported in 80 % of cases. NLPHL has an indolent clinical course despite frequent relapses and usually remains responsive to therapy. Patients with advanced stages of disease have an unfavorable prognosis. Progression to large B-cell lymphoma-like lesions may occur in 3–5 % of cases [78].

CHL is a monoclonal lymphoid neoplasm (almost always derived from B cells) composed of HRS cells within a background of reactive lymphocytes, eosinophils, neutrophils, histiocytes, plasma cells, fibroblasts, and collagen fibers in variable proportions (Fig. 1.18b). Four histological subtypes are distinguished based on the characteristics of the inflammatory background: lymphocyte rich (LRCHL), nodular sclerosis (NSCHL), mixed cellularity (MCCHL), and lymphocyte depleted (LDCHL). These histological subtypes of HRSC have identical immunophenotypic and genetic features, whereas clinical features and EBV association may be different. NSCHL is characterized by the presence of a distinctive variant of HRSC, i.e., the lacunar cell. CHL represents 95 % of all HLs, presenting a bimodal age curve in resource-rich countries, with a peak at 15–35 years of age and a second peak late in life. Cervical lymph nodes are frequently involved, and in approximately 60 % of patients (usually with NSCHL) a mediastinal mass is present. HRS cells are few in number, typically between 0.1 and 10 % of the cellular infiltrate. The composition of the reactive milieu varies according to the subtype. HRS cells show CD30 expression in nearly all cases (Fig. 1.18b) and

CD15 expression in most. The former can represent the target for the in vivo administration of brentuximab vedotin [70]. CD20 may be detected in 30–40 % of cases, but with a heterogeneous staining pattern. The B-cell nature of neoplastic cells is corroborated in about 95 % of cases by their BSAP/PAX5 expression. BSAP/PAX5 staining is usually weaker than that of reactive B cells. EBV + HRS cells show a type 2 latency pattern (LMP-1+, EBNA-2−). Characteristically, the transcription factors Oct-2 and BOB.1 are absent in the majority of cases (90 %), while PU.1 is consistently absent. Great attention has recently been paid to the characteristics of tumor microenvironment, since this may influence both the prognosis and response to therapy [79]. RS cells show clonal *IgVH* gene rearrangements in more than 98 % of cases and clonal *TCR* gene rearrangements in rare instances. The rearranged *IgVH* genes show a high load of somatic hypermutations, generally not ongoing. CHL is curable in more 85 % of cases with current treatment protocols. The response after two ABVD courses at FDG-PET imaging is an important prognostic indicator [1, 80, 81].

References

1. Swerdlow S, Campo E, Harris N, et al. WHO classification of tumours of haematopoietic and lymphoid tissues. 4th ed. Lyon: International Agency for Research on Cancer; 2008.
2. Campo E, Swerdlow SH, Harris NL, et al. The 2008 WHO classification of lymphoid neoplasms and beyond: evolving concepts and practical applications. Blood. 2011;117:5019–32.
3. Lin P, Jones D, Dorfman DM, Medeiros LJ. Precursor B-cell lymphoblastic lymphoma: a predominantly extranodal tumor with low propensity for leukemic involvement. Am J Surg Pathol. 2000;24:1480–90.
4. Borowitz MJ, Croker BP, Metzgar RS. Lymphoblastic lymphoma with the phenotype of common acute lymphoblastic leukemia. Am J Clin Pathol. 1983;79:387–91.
5. van der Velden VH, Brüggemann M, Hoogeveen PG, et al. TCRB gene rearrangements in childhood and adult precursor-B-ALL: frequency, applicability as MRD-PCR target, and stability between diagnosis and relapse. Leukemia. 2004;18:1971–80.
6. Maitra A, McKenna RW, Weinberg AG, Schneider NR, Kroft SH. Precursor B-cell lymphoblastic lymphoma. A study of nine cases lacking blood and bone marrow involvement and review of the literature. Am J Clin Pathol. 2001;115:868–75.
7. Pilozzi E, Muller-Hermelink HK, Falini B, et al. Gene rearrangements in T-cell lymphoblastic lymphoma. J Pathol. 1999;188:267–70.
8. Hallek M, Cheson BD, Catovsky D, et al. Guidelines for the diagnosis and treatment of chronic lymphocytic leukemia: a report from the International Workshop on Chronic Lymphocytic Leukemia updating the National Cancer Institute-Working Group 1996 guidelines. Blood. 2008;111:5446–56.
9. Rawstron AC, Bennett FL, O'Connor SJ, et al. Monoclonal B-cell lymphocytosis and chronic lymphocytic leukemia. N Engl J Med. 2008;359:575–83.
10. Crespo M, Bosch F, Villamor N, et al. ZAP-70 expression as a surrogate for immunoglobulin-variable-region mutations in chronic lymphocytic leukemia. N Engl J Med. 2003;348:1764–75.
11. Puente XS, Pinyol M, Quesada V, et al. Whole-genome sequencing identifies recurrent mutations in chronic lymphocytic leukaemia. Nature. 2011;475:101–5.
12. Gine E, Martinez A, Villamor N, et al. Expanded and highly active proliferation centers identify a histological subtype of chronic lymphocytic leukemia ("accelerated" chronic lymphocytic leukemia) with aggressive clinical behavior. Haematologica. 2010;95:1526–33.
13. Melo JV, Catovsky D, Galton DA. The relationship between chronic lymphocytic leukaemia and prolymphocytic leukaemia. I. Clinical and laboratory features of 300 patients and characterization of an intermediate group. Br J Haematol. 1986;63:377–87.
14. Krishnan B, Matutes E, Dearden C. Prolymphocytic leukemias. Semin Oncol. 2006;33:257–63.
15. Del Giudice I, Davis Z, Matutes E, et al. IgVH genes mutation and usage, ZAP-70 and CD38 expression provide new insights on B-cell prolymphocytic leukemia (B-PLL). Leukemia. 2006;20:1231–7.
16. Berger F, Felman P, Thieblemont C, et al. Non-MALT marginal zone B-cell lymphomas: a description of clinical presentation and outcome in 124 patients. Blood. 2000;95:1950–6.
17. Owen RG, Treon SP, Al-Katib A, et al. Clinicopathological definition of Waldenstrom's macroglobulinemia: consensus panel recommendations from the Second International Workshop on Waldenstrom's Macroglobulinemia. Semin Oncol. 2003;30:110–5.
18. International Myeloma Working Group. Criteria for the classification of monoclonal gammopathies, multiple myeloma and related disorders: a report of the International Myeloma Working Group. Br J Haematol. 2003;121:749–57.
19. Isaacson PG, Du MQ. MALT lymphoma: from morphology to molecules. Nat Rev Cancer. 2004;4:644–53.
20. Thieblemont C, Bastion Y, Berger F, et al. Mucosa-associated lymphoid tissue gastrointestinal and nongastrointestinal lymphoma behavior: analysis of 108 patients. J Clin Oncol. 1997;15:1624–30.
21. Arcaini L, Paulli M, Burcheri S, et al. Primary nodal marginal zone B-cell lymphoma: clinical features and prognostic assessment of a rare disease. Br J Haematol. 2007;136:301–4.

22. Taddesse-Heath L, Pittaluga S, Sorbara L, et al. Marginal zone B-cell lymphoma in children and young adults. Am J Surg Pathol. 2003;27: 522–31.
23. Harris NL, Jaffe ES, Diebold J, et al. World Health Organization classification of neoplastic diseases of the hematopoietic and lymphoid tissues: report of the Clinical Advisory Committee meeting-Airlie House, Virginia, November 1997. J Clin Oncol. 1999;17:3835–49.
24. Cong P, Raffeld M, Teruya-Feldstein J, et al. In situ localization of follicular lymphoma: description and analysis by laser capture microdissection. Blood. 2002;99:3376–82.
25. Jegalian AG, Eberle FC, Pack SD, et al. Follicular lymphoma in situ: clinical implications and comparisons with partial involvement by follicular lymphoma. Blood. 2011;118:2976–84.
26. Lorsbach RB, Shay-Seymore D, Moore J, et al. Clinicopathologic analysis of follicular lymphoma occurring in children. Blood. 2002;99:1959–64.
27. Shia J, Teruya-Feldstein J, Pan D, et al. Primary follicular lymphoma of the gastrointestinal tract: a clinical and pathologic study of 26 cases. Am J Surg Pathol. 2002;26:216–24.
28. Willemze R, Jaffe ES, Burg G, et al. WHO-EORTC classification for cutaneous lymphomas. Blood. 2005;105:3768–85.
29. Campo E, Raffeld M, Jaffe ES. Mantle-cell lymphoma. Semin Hematol. 1999;36:115–27.
30. Jares P, Colomer D, Campo E. Genetic and molecular pathogenesis of mantle cell lymphoma: perspectives for new targeted therapeutics. Nat Rev Cancer. 2007;7:750–62.
31. Mozos A, Royo C, Hartmann E, et al. SOX11 expression is highly specific for mantle cell lymphoma and identifies the cyclin D1-negative subtype. Haematologica. 2009;94:1555–62.
32. Carvajal-Cuenca A, Sua LF, Silva NM, et al. In situ mantle cell lymphoma: clinical implications of an incidental finding with indolent clinical behavior. Haematologica. 2012;97:270–8.
33. Roullet MR, Martinez D, Ma L, et al. Coexisting follicular and mantle cell lymphoma with each having an in situ component: a novel, curious, and complex consultation case of coincidental, composite, colonizing lymphoma. Am J Clin Pathol. 2010;133: 584–91.
34. Nodit L, Bahler DW, Jacobs SA, Locker J, Swerdlow SH. Indolent mantle cell lymphoma with nodal involvement and mutated immunoglobulin heavy chain genes. Hum Pathol. 2003;34:1030–4.
35. Salaverria I, Royo C, Carvajal-Cuenca A, et al. CCND2 rearrangements are the most frequent genetic events in cyclin D1- mantle cell lymphoma. Blood. 2013;121:1394–402.
36. Fernandez V, Salamero O, Espinet B, et al. Genomic and gene expression profiling defines indolent forms of mantle cell lymphoma. Cancer Res. 2010;70: 1408–18.
37. Hummel M, Bentink S, Berger H, et al. A biologic definition of Burkitt's lymphoma from transcriptional and genomic profiling. N Engl J Med. 2006;354: 2419–30.
38. Lazzarino M, Orlandi E, Paulli M, et al. Treatment outcome and prognostic factors for primary mediastinal (thymic) B-cell lymphoma: a multicenter study of 106 patients. J Clin Oncol. 1997;15:1646–53.
39. Paulli M, Strater J, Gianelli U, et al. Mediastinal B-cell lymphoma: a study of its histomorphologic spectrum based on 109 cases. Hum Pathol. 1999;30:178–87.
40. Ferreri AJ, Campo E, Seymour JF, et al. Intravascular lymphoma: clinical presentation, natural history, management and prognostic factors in a series of 38 cases, with special emphasis on the 'cutaneous variant'. Br J Haematol. 2004;127:173–83.
41. Vermeer MH, Geelen FA, van Haselen CW, et al. Primary cutaneous large B-cell lymphomas of the legs. A distinct type of cutaneous B-cell lymphoma with an intermediate prognosis. Dutch Cutaneous Lymphoma Working Group. Arch Dermatol. 1996;132:1304–8.
42. Delsol G, Lamant L, Mariame B, et al. A new subtype of large B-cell lymphoma expressing the ALK kinase and lacking the 2; 5 translocation. Blood. 1997;89:1483–90.
43. Reichard KK, McKenna RW, Kroft SH. ALK-positive diffuse large B-cell lymphoma: report of four cases and review of the literature. Mod Pathol. 2007;20:310–9.
44. Colomo L, Loong F, Rives S, et al. Diffuse large B-cell lymphomas with plasmablastic differentiation represent a heterogeneous group of disease entities. Am J Surg Pathol. 2004;28:736–47.
45. Delecluse HJ, Anagnostopoulos I, Dallenbach F, et al. Plasmablastic lymphomas of the oral cavity: a new entity associated with the human immunodeficiency virus infection. Blood. 1997;89:1413–20.
46. Nador RG, Cesarman E, Chadburn A, et al. Primary effusion lymphoma: a distinct clinicopathologic entity associated with the Kaposi's sarcoma-associated herpes virus. Blood. 1996;88:645–56.
47. Chadburn A, Hyjek E, Mathew S, et al. KSHV-positive solid lymphomas represent an extra-cavitary variant of primary effusion lymphoma. Am J Surg Pathol. 2004;28:1401–16.
48. Shiramizu B, Barriga F, Neequaye J, et al. Patterns of chromosomal breakpoint locations in Burkitt's lymphoma: relevance to geography and Epstein-Barr virus association. Blood. 1991;77:1516–26.
49. Chan JK, Banks PM, Cleary ML, et al. A revised European-American classification of lymphoid neoplasms proposed by the International Lymphoma Study Group. A summary version. Am J Clin Pathol. 1995;103:543–60.
50. Dave SS, Fu K, Wright GW, et al. Molecular diagnosis of Burkitt's lymphoma. N Engl J Med. 2006;354: 2431–42.
51. Chuang SS, Ye H, Du MQ, et al. Histopathology and immunohistochemistry in distinguishing Burkitt lymphoma from diffuse large B-cell lymphoma with very high proliferation index and with or without a starry-sky pattern: a comparative study with EBER and FISH. Am J Clin Pathol. 2007;128:558–64.
52. Nakamura F, Tatsumi E, Tani K, et al. Coexpression of cell-surface immunoglobulin (sIg), terminal

deoxynucleotidyl transferase (TdT) and recombination activating gene 1 (RAG-1): two cases and derived cell lines. Leukemia. 1996;10:1159–63.
53. Garcia JF, Mollejo M, Fraga M, et al. Large B-cell lymphoma with Hodgkin's features. Histopathology. 2005;47:101–10.
54. Traverse-Glehen A, Pittaluga S, Gaulard P, et al. Mediastinal gray zone lymphoma: the missing link between classic Hodgkin's lymphoma and mediastinal large B-cell lymphoma. Am J Surg Pathol. 2005;29:1411–21.
55. Savage KJ, Monti S, Kutok JL, et al. The molecular signature of mediastinal large B-cell lymphoma differs from that of other diffuse large B-cell lymphomas and shares features with classical Hodgkin lymphoma. Blood. 2003;102:3871–9.
56. Matutes E, Brito-Babapulle V, Swansbury J, et al. Clinical and laboratory features of 78 cases of T-prolymphocytic leukemia. Blood. 1991;78:3269–74.
57. Suzuki R, Suzumiya J, Nakamura S, et al. Aggressive natural killer-cell leukemia revisited: large granular lymphocyte leukemia of cytotoxic NK cells. Leukemia. 2004;18:763–70.
58. Suzuki K, Ohshima K, Karube K, et al. Clinicopathological states of Epstein-Barr virus-associated T/NK-cell lymphoproliferative disorders (severe chronic active EBV infection) of children and young adults. Int J Oncol. 2004;24:1165–74.
59. Shimoyama M. Diagnostic criteria and classification of clinical subtypes of adult T-cell leukaemia-lymphoma. A report from the Lymphoma Study Group (1984–87). Br J Haematol. 1991;79:428–37.
60. Au WY, Weisenburger DD, Intragumtornchai T, et al. Clinical differences between nasal and extranasal natural killer/T-cell lymphoma: a study of 136 cases from the International Peripheral T-Cell Lymphoma Project. Blood. 2009;113:3931–7.
61. Chan JK. Natural killer cell neoplasms. Anat Pathol. 1998;3:77–145.
62. Garcia-Herrera A, Song JY, Chuang SS, et al. Nonhepatosplenic gammadelta T-cell lymphomas represent a spectrum of aggressive cytotoxic T-cell lymphomas with a mainly extranodal presentation. Am J Surg Pathol. 2011;35:1214–25.
63. Wright DH. Enteropathy associated T cell lymphoma. Cancer Surv. 1997;30:249–61.
64. Cooke CB, Krenacs L, Stetler-Stevenson M, et al. Hepatosplenic T-cell lymphoma: a distinct clinicopathologic entity of cytotoxic gamma delta T-cell origin. Blood. 1996;88:4265–74.
65. Kumar S, Krenacs L, Medeiros J, et al. Subcutaneous panniculitic T-cell lymphoma is a tumor of cytotoxic T lymphocytes. Hum Pathol. 1998;29:397–403.
66. Laurent C, Fazilleau N, Brousset P. A novel subset of T-helper cells: follicular T-helper cells and their markers. Haematologica. 2010;95:356–8.
67. Agostinelli C, Hartmann S, Klapper W. Peripheral T-cell lymphomas with follicular T-helper phenotype: a new basket or a distinct entity? Revising Karl Lennert's personal archive. Histopathology. 2011;59:679–91.
68. Rizvi MA, Evens AM, Tallman MS, Nelson BP, Rosen ST. T-cell non-Hodgkin lymphoma. Blood. 2006;107:1255–64.
69. Dogan A, Attygalle AD, Kyriakou C. Angioimmunoblastic T-cell lymphoma. Br J Haematol. 2003;121:681–91.
70. Younes A, Bartlett NL, Leonard JP, et al. Brentuximab vedotin (SGN-35) for relapsed CD30-positive lymphomas. N Engl J Med. 2010;363:1812–21.
71. Chiarle R, Voena C, Ambrogio C, Piva R, Inghirami G. The anaplastic lymphoma kinase in the pathogenesis of cancer. Nat Rev Cancer. 2008;8:11–23.
72. Falini B, Pileri S, Zinzani PL, et al. ALK+ lymphoma: clinico-pathological findings and outcome. Blood. 1999;93:2697–706.
73. Piva R, Agnelli L, Pellegrino E, et al. Gene expression profiling uncovers molecular classifiers for the recognition of anaplastic large-cell lymphoma within peripheral T-cell neoplasms. J Clin Oncol. 2010;28:1583–90.
74. Feldman AL, Dogan A, Smith DI, et al. Discovery of recurrent t(6;7)(p25.3;q32.3) translocations in ALK-negative anaplastic large cell lymphomas by massively parallel genomic sequencing. Blood. 2011;117:915–9.
75. Falini B. Anaplastic large cell lymphoma: pathological, molecular and clinical features. Br J Haematol. 2001;114:741–60.
76. Fan Z, Natkunam Y, Bair E, Tibshirani R, Warnke RA. Characterization of variant patterns of nodular lymphocyte predominant Hodgkin lymphoma with immunohistologic and clinical correlation. Am J Surg Pathol. 2003;27:1346–56.
77. Prakash S, Fountaine T, Raffeld M, Jaffe ES, Pittaluga S. IgD positive L&H cells identify a unique subset of nodular lymphocyte predominant Hodgkin lymphoma. Am J Surg Pathol. 2006;30:585–92.
78. Anagnostopoulos I, Hansmann ML, Franssila K, et al. European Task Force on Lymphoma project on lymphocyte predominance Hodgkin disease: histologic and immunohistologic analysis of submitted cases reveals 2 types of Hodgkin disease with a nodular growth pattern and abundant lymphocytes. Blood. 2000;96:1889–99.
79. Steidl C, Connors JM, Gascoyne RD. Molecular pathogenesis of Hodgkin's lymphoma: increasing evidence of the importance of the microenvironment. J Clin Oncol. 2011;29:1812–26.
80. Shimabukuro-Vornhagen A, Haverkamp H, Engert A, et al. Lymphocyte-rich classical Hodgkin's lymphoma: clinical presentation and treatment outcome in 100 patients treated within German Hodgkin's Study Group trials. J Clin Oncol. 2005;23:5739–45.
81. Colby TV, Hoppe RT, Warnke RA. Hodgkin's disease: a clinicopathologic study of 659 cases. Cancer. 1982;49:1848–58.

Genomic Analysis of B-Cell Lymphomas

2

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Abstract

Technical progress has enabled molecular analysis of lymphoma samples on a genomic scale. Research on many fronts is now providing a comprehensive picture of the lymphoma cell's genomic sequence, identifying its mutations and allelic derangements, and the epigenetic regulation and transcription of that genome. Macromolecules of other types are also being profiled, including noncoding RNA, proteins, and other non-nucleic acid metabolites. Furthermore, whether by design or inadvertent inclusion, elements in the tumor microenvironment are often included and provide important insights into key processes such as angiogenesis, stromal reaction, and interaction with the host immune system. The wealth of data generated by these studies also brings challenges in interpretation, for which a variety of analytical techniques are used. This chapter selectively covers the development and current status of genomic analyses of B-cell lymphomas, some of the prominent findings, and the prospects for clinical application.

Keywords

Genomic analysis • Gene expression profiling • Comparative genomic hybridization • Genome sequencing • Noncoding RNA • Epigenetic regulation • Proteomics • Metabolomics

Introduction

In little more than 10 years, technologies for genomic-scale molecular analysis have revolutionized the study of lymphomas as much as in any type of cancer. Although this review is organized by the type of technology involved, some familiarity with the technologies (and the subject of lymphoma) is assumed, so that the presentation can focus more on the findings rather than the methods. Special attention will be paid to the subject of clinical translation of genomic technologies and the knowledge they have fostered.

Molecular analysis is already a standard in lymphoma diagnosis and treatment. Examples are tests for specific biomarkers (hemoglobin,

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lactate dehydrogenase) that are part of prognostic indices; immunohistochemistry (IHC) to detect individual proteins (CD20, CD30, NPM-ALK) for diagnostic stratification or confirmation of their presence as therapeutic targets; and certain genetic tests (e.g., rearrangement of c-myc or bcl2 [1]) with prognostic significance. For a variety of reasons (cost, regulatory issues, clinical trial design), focused "single-analyte" testing for specific biomarkers, as opposed to genomic-scale technologies, will likely dominate clinical molecular analysis in the near term, even though the latter will surely be the engine of discovery. However, this chapter will not discuss limited-scale testing for specific analytes.

Certain neoplasms of B-cell origin are not included in this chapter: pre-B acute lymphoblastic leukemia, chronic lymphocytic leukemia/small lymphocytic lymphoma, and multiple myeloma. Finally, apologies are made to those whose work has not been included.

Gene Expression Profiling of Diffuse Large B-Cell Lymphoma

Gene expression profiling (GEP) by microarray hybridization was the first analytical technology to be applied on a genome-wide scale. The first type of lymphoma to be studied by GEP was *diffuse large B-cell lymphoma* (*DLBCL*), which is also presented here first because it illustrates many of the topics relevant to GEP of other types of lymphoma. DLBCL was previously considered to be a single disease, although doubts were raised by the disparity in clinical outcomes between patients after standard treatment. Since germinal center B (GCB) cells were thought to be the normal counterpart of DLBCL tumor cells, Alizadeh et al. used a custom array enriched for genes expressed by GCB cells and found that roughly half of DLBCL primary tumors resembled normal GCB cells [2]. This observation has been repeatedly verified and is supported by other genomic approaches presented below. The remaining half of primary DLBCL tumors resembled normal blood B cells activated in vitro by cross-linking of their B-cell receptor (BCR) and thus were labeled as belonging to an activated B-cell (ABC) type. In addition to the biological differences between the ABC and GCB DLBCL types implied by their different gene expression profiles, outcomes with standard CHOP chemotherapy are significantly worse for ABC-DLBCL patients, even when adjusted for risk according to the International Prognostic Index (IPI) score [3, 4].

It is still uncertain what is the true normal counterpart of ABC-DLBCL, but it is nonetheless now widely accepted that DLBCL encompasses two distinct types of lymphoma, differing in apparent cell of origin (COO), biological regulatory programs (such as signaling pathways), genetic abnormalities [5], and clinical outcome with CHOP-based chemotherapy [6]. This illustrates the obvious: genomic analysis can see beyond the limitations of morphologic analysis and standard IHC and provides additional information beyond clinical prognostic indices such as the IPI. However, these non-genomic techniques have proven value in clinical practice and should be supplemented rather than replaced by genomic-based studies until years of experience prove that is safe to do so.

The finding that DLBCL encompasses two different types of lymphoma was an example of *class discovery*, in which GEP provides a rational basis for subdividing a group of samples. (It should be noted that the following discussion applies to genomic profiling methods other than GEP.) If there are phenotypic differences of importance between classes, such as differences in clinical outcome or likely susceptibility to a proposed novel therapy, it is therefore desirable to be able to assign future cases to these classes, the exercise known as *class prediction*. Class discovery and class prediction are obviously interrelated, in that features distinguishing groups are the basis of class prediction. Such features are often referred to simply as *biomarkers*, because they may be discovered and used for prediction (and other purposes) without understanding their biological role. The high dimensionality of the GEP data, in which samples typically express more than 10,000 genes at levels above the detection threshold, invariably dwarfs the number of samples in any research study. There

is thus a high potential for false-positive findings in biomarker identification and a related risk of data overfitting in class discovery. How best to perform class discovery and class prediction continues to be an active subject of research, and a discussion of methods is outside the scope of this chapter. The reader is referred elsewhere for detailed presentations of solutions such as controlling the false discovery rate [7], significant analysis of microarrays [8], validation strategies, and Bayesian class prediction [9].

Outcome prediction is another major goal of GEP analysis. This is also interrelated with class discovery and class prediction, in that (1) whether putative classes have different outcomes is one of their important features to determine and helps to validate the distinction if present and (2) the clinical utility of class prediction depends heavily on having different outcomes. Outcome prediction starts with outcome correlation, i.e., finding genes, signatures (groups of genes), or classes that correlate with significant differences in outcome. There are many different ways to do this, and statistical concerns and solutions (e.g., overfitting and cross-validation, respectively) apply. In the original study defining ABC and GCB types of DLBCL, finding outcome differences was a secondary goal; i.e., the types were first identified by COO, then differences in outcome were found and used to validate the distinction and its clinical importance and the power of GEP to add to the IPI [2]. However, later studies have had outcome prediction as a primary goal. Staudt and colleagues have relied heavily on the use of signatures of genes that are similarly expressed across the spectrum of samples, established computationally by hierarchical clustering [10]. It is assumed that genes within a signature are coexpressed or similarly regulated and therefore reflect the activity of only one or a few biological processes or the proportion within mixed samples of a particular cell type. To the extent that this is true, using multiple genes to calculate a single average value for a signature is preferable to using values of a few individual genes, because (1) few genes are truly specific for one process or cell type and (2) it reduces the effect of noise. Signatures are also one method to achieve dimension reduction in GEP data, reducing the number of statistical tests in downstream analysis.

In addition to their potential as biomarkers of class or clinical outcome, genes implicated by GEP can serve as indicators or mediators of *underlying biological processes*. Such processes can be targeted therapeutically and ultimately constitute another category of biomarkers. Biomarkers identified merely by correlation with outcome are nonspecific in their therapeutic implications; e.g., a biomarker of poor outcome suggests that a different therapy should be tried, but may not indicate what that therapy should be. Biomarkers of biological processes, although more difficult to identify, may ultimately indicate the therapy that is best for the patient. This is the vision of "personalized medicine," although it is far from being realized. The first step, perhaps the most difficult, is to infer the underlying biological process from GEP findings. This has become more systematic in recent years, through the use of *gene set analysis techniques* such as GSEA (gene set enrichment analysis) [11]. In brief, these methods use a statistical test to determine whether genes belonging to a gene set are differentially expressed between two groups of samples, greater than expected by chance. In GSEA, the entire spectrum of expressed genes is employed, after rank ordering for the relative differences between two sample groups, and gene sets are examined for their nonrandom distribution across the spectrum; this has the advantage of not requiring the establishment of those genes which are differentially expressed, based on exceeding some threshold of statistical significance. With gene set analysis, researchers no longer need to stare at a list of differentially expressed genes and hope for inspiration; bioinformatic tools like GSEA can formally suggest what may be the processes involved. An alternative approach, exemplified by the Connectivity Map project, can even bypass the need to identify and validate the processes involved [12]. In correlating GEP findings in samples with those obtained from cell lines treated with drugs, potential therapies may emerge from drugs that produce effects opposite to the GEP features of

samples, as has been shown in studies of drug resistance. However, the power of gene set analysis and drug response methods is obviously dependent on the quality and breadth of gene sets and drug response data; although these are already extensive, they are frequently limited in their ability to provide insight in research investigations. Continued curation of the world's output of GEP data, and incorporation of new information such as target genes determined by chromatin immunoprecipitation and high-throughput sequencing (ChIP-Seq), is necessary to improve the interpretation of GEP data.

The first example of process or pathway identification by GEP in DLBCL sprang from the informal observation that many known target genes of the NFkB and pathway were highly expressed in ABC-DLBCL primary tumors and cell lines. Functional investigations confirmed that what is now known as the classical NFkB pathway is constitutively active in ABC-DLBCL lines and showed that this is essential for their survival in vitro [13]. Screening based on RNA interference found that NFkB activation in ABC-DLBCL lines relied on the CARD11/MALT1/BCL10 (CBM) complex, a known mediator of antigen receptor signaling in lymphocytes [14]. The discovery that 10 % of primary ABC-DLBCL tumors had acquired somatic mutations in a single domain of CARD11 and that these mutations spontaneously activated or enhanced CARD11's ability to activate NFkB, provided compelling evidence that constitutive NFkB activity was not an artifact of ABC-DLBCL cell lines, but a process that was positively selected in vivo [15]. Further investigations of the NFkB pathway in ABC-DLBCL showed that activation originates in chronic signaling by the B-cell receptor (BCR) [16], augmented by MyD88 and Toll-receptor pathway signaling [17]. The obvious therapeutic implication of these results is that inhibiting the NFkB pathway may be effective against ABC-DLBCL, which is less often cured by CHOP chemotherapy. Many groups and companies are working toward this end, but clinical translation is far from complete. The NFkB pathway is fundamental to many normal processes such as immunity, so there is considerable potential for side effects even with a "targeted" therapy that inhibits NFkB. It is possible that inhibiting the specific upstream pathway of NFkB activation in ABC-DLBCL will reduce side effects, and trials of specific BTK inhibitors are in progress [18]. As proof of principle, however, it may already be cited that inhibition with bortezomib of the proteasome, which includes NFkB inhibition among its effects, selectively potentiates chemotherapy effectiveness in ABC-DLBCL [19]. Several other pathways of therapeutic promise in DLBCL, implicated by genomic studies, are beyond the scope of this chapter but should be mentioned: BCL6 [20], STAT3 [21], PI3K [22, 23], and EZH2 [24].

Primary lymphoma samples used for GEP typically include the entire tumor, not just the neoplastic B cells, and therefore GEP data entail a requirement, and provide an opportunity, to consider what they may implicate in tumor biology besides what concerns neoplastic cells. A large study of DLBCL confirmed that the outcome of ABC-DLBCL was still worse after the addition of rituximab (anti-CD20) to CHOP, indicating that the ABC-GCB distinction (as quantified by a "germinal-center B-cell" signature) is predictive [4]. However, two signatures attributable to nonneoplastic tumor elements were also found and had independent predictive power; together, the 3-signature index added to the predictive power of the IPI. The favorable "stromal-1" signature reflected extracellular matrix deposition and histiocytic infiltration, while the unfavorable "stromal-2" signature reflected tumor blood-vessel density. These results add to the wealth of evidence that outcomes in lymphoma are influenced by *host factors*, and GEP has indicated those genes which appear to be most important as biomarkers or mediators of these factors.

Should the *ABC-GCB distinction* always be made for DLBCL, and how? For clinical trials, it seems imperative to make this distinction, so as to be able to determine the efficacy in each DLBCL type. As shown by the results of combining bortezomib with chemotherapy [19], the relative outcomes for the types depend on the therapy. The need to determine DLBCL type is

especially great for trials of newer targeted agents, which may be effective in only one type, and some trials of targeted agents in DLBCL are now being restricted to patients with only one of the types, based on prospective GEP. However, the reality is that many DLBCL trials do not provide for making the ABC-GCB distinction, or for evaluating or discovering other potential biomarkers of outcome, even in retrospect. There is a "chicken and egg" quality to this omission: making the ABC-GCB distinction is unlikely to enter routine clinical practice until a choice of therapeutic alternatives depends on it and that in turn is unlikely to happen until clinical trials include this distinction in their analysis of results. In the meantime it could be argued that the predictive significance of the ABC-GCB distinction for CHOP-based chemotherapy is worth knowing for complex clinical decision making, but it is not routinely available.

There is not yet a standard way for making the ABC-GCB distinction, but many reasonable methods exist. Using multiple genes to make the ABC-GCB distinction is preferable because it reduces the effect of noise on individual samples and may accurately reflect the "big picture" despite aberrations in individual genes. This requires an algorithm to handle the measurements made on multiple genes, which is specific for the platform and probe sequences employed. Bayesian and shrunken centroid approaches have been used to address the complexity of multigene classification schemes [9, 25]. Once created, an algorithm is relatively easy to use, but using multiple genes creates opportunities for hybrid results or mixed patterns, confounding the dichotomous outcomes that are preferred in clinical decision making. Furthermore, microarray technology and its interpretation do not conform easily to traditional regulatory standards applied by the FDA and required for CLIA certification as a clinical laboratory test, and there are perceptions that they are too complicated and costly for clinical application. Therefore, there have been several efforts to make the ABC-GCB distinction in DLBCL, or even to bypass it altogether for the purpose of outcome prediction, using a minimum number of genes. Notable examples have used six [26] and even only two [27] genes, with excellent outcome prediction and even validation in other datasets than the ones from which the genes were originally chosen.

For clinical application, COO or outcome predictors need to be measured by an FDA- and CLIA-approved technology, preferably using routinely preserved formalin-fixed, paraffin-embedded (FFPE) samples. Most efforts to develop such predictors have focused on the measurement of certain gene products, i.e., proteins, by IHC. With GEP as the "gold standard" for distinguishing ABC and GCB DLBCL cases, several algorithms have been developed using IHC as a surrogate [28, 29]. Consistency and reproducibility have been good with these algorithms, and it can be argued that IHC has advantages in (1) measuring proteins, which are more proximal effectors of biological processes (and whose expression is often affected by posttranslational mechanisms not apparent by GEP), and (2) allowing attribution of expression to the neoplastic cells. However, some studies have questioned the reliability of IHC algorithms, especially on the basis of their performance at predicting outcome [30]. Another technique for measuring individual genes is quantitative real-time PCR (qRT-PCR), more analogous to GEP because it quantifies transcripts, which was used for a 6-gene predictive signature [26]. Other emerging techniques for measuring mRNA transcripts, especially suited to FFPE samples and not employing amplification, are the quantitative nuclease protection assay (qNPA) [31] and the Nanostring nCounter [32]. However, of relevance to these and other methods using algorithms based on only a small number of genes, statistical concerns remain, such as in setting "cutoff" values for IHC [33].

There is no doubt, even from the example of DLBCL alone, that GEP is a very powerful scientific tool and hypothesis generator. However, acceptance of GEP as a clinical decision-making tool is made more difficult by the very thing that makes it so useful as a research tool: the simultaneous measurement of the expression of so many genes. This ultimately gives GEP more potential for clinical application in lymphoma than just the determination of subtype and prediction of

response to therapy. Perhaps the prime example is the determination of active or aberrant pathways that might be targeted by personalized therapy in the future, especially if such pathways are not highly correlated with lymphoma type or subtype. Similar to making the ABC-GCB distinction, determination of pathway activity is likely better to be based on multiple genes than on only a few. However, we are far from having the knowledge base or analytical tools to make the interpretation of GEP results a definitive basis for clinical decisions, comparable to the confidence with which radiographic results or histopathologic decisions are acted upon. It is also possible that GEP is not the best approach for determining pathway activity; other approaches, such as proteomics and metabolomics, are more direct and are discussed below. Much work remains to be done on how to perform and interpret GEP for it to become a comprehensive method for determining the active pathways in a lymphoma and the basis for personalized therapy.

GEP in Other Types of Lymphoma

Primary Mediastinal B-Cell Lymphoma (PMBL)

PMBL was included in some GEP studies of DLBCL, despite its distinctive clinical features (predominant mediastinal involvement, young age, and female predilection) and morphologic features (clear cells and sclerosis). One study showed that slightly more than half of cases diagnosed as PMBL, based on clinical and morphologic features, expressed a signature of genes distinct from non-mediastinal cases of DLBCL [34]. In other words, GEP had again exceeded the limitations of morphology and immunostaining and defined a separate type of DLBCL. The PMBL signature was found largely to be expressed by cell lines of classical Hodgkin's lymphoma (cHL) and selectively confirmed in microdissected Reed-Sternberg cells from primary cHL tumors. The similarity of PMBL to cHL has been confirmed by other genomic studies. However, there are also substantial differences between the gene expression profiles of PMBL and cHL; for example, PMBL uniformly expresses B-cell differentiation markers that are characteristically absent in cHL. The most likely normal counterpart of PMBL is thymic B cells, which highly express MAL, a gene also expressed highly by PMBL and cHL.

Hodgkin's Lymphoma

Both types of Hodgkin's lymphoma, cHL and the nodular lymphocyte predominant type (NLPHL), are remarkable for a relative paucity of neoplastic cells and a prominent background of immune cells. They are included in this chapter because of their derivation from B cells and their occasional diagnostic confusion with other types of B-cell lymphoma. GEP studies on whole tumors have shown that prognostic genes and signatures are contributed by both microenvironmental and neoplastic cells [35, 36], and insights provided by all types of genomic studies of cHL have been recently reviewed [37]. In one study, a macrophage signature by GEP was correlated with failure of primary and secondary treatment, and this was validated by IHC for a macrophage marker (CD68) in a larger, independent set of patients with cHL [38]. Brune et al. microdissected the putative neoplastic cells of NLPHL, the so-called "L&H" (lymphocytic and histiocytic) cells, for GEP after RNA amplification [39]. This showed that despite the consistent preservation of certain B-cell differentiation features often lost in R-S cells, L&H cells showed a closer relatedness to R-S cells and the putative neoplastic cells of T-cell rich B-cell lymphoma, a poorly defined entity, than to neoplastic cells of DLBCL, follicular lymphoma, or Burkitt's lymphoma. The profile of L&H cells was also notable for reduced likelihood of apoptosis, increased expression of genes involved in immunosuppression (galectins, cathepsin B, MMP9, CD59, MHC class I genes, and annexin A2) or extracellular matrix remodeling, and activation of the NFkB and ERK pathways.

Follicular Lymphoma (FL)

Dave et al. performed a large GEP study of whole primary tumor samples of FL patients treated with a variety of chemotherapy regimens, none of whom received now-standard rituximab [10]. A multistep search for predictive factors in a "training set" of half the samples found multiple predictive signatures, composed of hierarchically clustered genes that alone had at least some predictive power. Two of these signatures in combination provided an optimal predictor of survival in the remaining half of samples ("test set"). A favorable signature (immune response 1, IR-1) was attributed to tumor-infiltrating T lymphocytes, while an unfavorable signature (IR-2) was attributed to tumor-associated macrophages. The latter finding is reminiscent of findings in cHL, but genes in IR-2 are markers of macrophage attributes rather than macrophage number. Genes in IR-1 were also markers of the quality of T cells rather than the number of total T cells or subsets. This study has been followed by numerous IHC studies of FL, which continue to underscore the importance of the host immune system to outcome in FL but also show its complexity. For example, a follicular pattern of tumor infiltration by regulatory T cells (T_{regs}) is an IPI-independent adverse predictor of overall and progression-free survival [40]. Also, macrophages are a favorable predictive factor in rituximab-treated patients, in contrast to the findings of [41].

These findings on the immune system in FL merit considerable discussion. At face value, they suggest that variation in characteristics of the neoplastic FL cells has no impact on survival, in contrast to findings of GEP studies of all other types of non-Hodgkin's lymphoma (NHL). This is very unlikely for two general reasons, the first of which is that other types of genomic studies clearly show that tumor cell genotype influences outcome in FL. The second is that if outcome in FL is indeed entirely determined by the state of the host immune response, that state may be in turn determined by influences from neoplastic cells. However, the nature of those putative influences is unknown and not apparent from the gene expression profile of the whole tumor, and single-nucleotide polymorphism (SNP) studies suggest that the contraction and course of FL are in part influenced by the germline state of host immune response genes [42]. The failure of the study of Dave et al. to detect predictive factors attributable to neoplastic FL cells may be due to their use of whole tumors, rather than purified tumor cells, since the use of mixed samples reduces the ability of GEP to detect changes in a particular cell type within the mixture [43]. This was implied by a recent study which performed GEP separately on purified B-cell and non-B-cell fractions from FL tumors, with the goal of comparing microenvironmental "cross talk" between these fractions in FL and that in normal lymph nodes [44]. This was a small study that did not perform correlation with clinical outcome, and no observations about the neoplastic cells were reported, but the study found that tumor-infiltrating T cells in FL were enriched for T-follicular helper cells. Since sorting of cells is unlikely to become a routine technique for FL analysis, it may ultimately be that IHC will be a better method for studying expressed genes than whole-tumor GEP, because results can be attributed to individual cell types.

Mantle Cell Lymphoma (MCL)

MCL differs considerably from other NHL types in its gene expression profile, but relatively little molecular insight has been gained from GEP alone regarding the pathobiology of MCL. Nonetheless, Rosenwald et al. did find that a signature of genes related to cell proliferation was inversely correlated with survival [45]. At first this may seem intuitive, but it has not been observed in many other types of NHL. The most distinctive molecular feature of MCL is the t(11;14) translocation, juxtaposing cyclin D1 to the immunoglobulin heavy chain locus and resulting in its upregulation. An effect on proliferation is to be expected, because cyclin D1 functions to promote the G1/S phase transition, and cyclin D1 mRNA levels were positively correlated with the proliferation signature. It was originally not clear why there is such variation in cyclin D1 mRNA

levels, given the high frequency of the t(11;14) translocation in MCL. Occasional MCL cases lack the t(11;14) translocation but are otherwise indistinguishable from MCL in general; most of these are found to have point mutations or truncations in the cyclin D1 gene that increase its mRNA stability, resulting in increased cyclin D1 protein similar to translocation [46]. Further correlations with proliferation (negative) and survival (positive) were shown with mRNA levels of the ARK(p14)/INK4A (p16) gene cluster, largely determined by mutation or deletion at the genomic locus; this is also to be expected, since these genes inhibit the G1/S transition. Still other MCL cases lack cyclin D1 protein expression. Many of these have upregulation of cyclin D2 or D3, but SOX11 expression was found to be the most distinctive characteristic of these cases [47]. It was also observed that lack of SOX11 expression characterizes the minority of MCL patients who have much longer survival, independent of the proliferation signature [48]. Recent GEP studies of MCL have benefited from correlation with DNA copy number arrays, described below [49].

Burkitt's Lymphoma (BL)

As originally described, "endemic" BL (eBL) is associated with a particular translocation involving c-myc, Epstein-Barr virus in tumor cells, and highly characteristic clinical and morphologic features. However, tumors with high similarity are observed sporadically in the general population (sBL) and frequently in immunocompromised patients (HIV-BL), and morphologically defined DLBCL may have some of the genetic features of BL. A prominent study found that "classical" or "atypical" BL, as defined by morphology, c-myc translocation, and IHC features (Ki-67 in >90 % of cells, indicative of a high proliferation rate, and expression of CD10 and/or Bcl-6), can be consistently distinguished by GEP from standard DLBCL types, based on a core signature including many target genes of c-myc [50]. This signature was also present in a subset of cases of DLBCL with atypical features, suggesting a "molecular diagnosis" of BL. Among patients with a molecular diagnosis of BL, those who received intensive chemotherapy regimens had much better outcomes than those treated with CHOP or CHOP-like regimens, indicating the clinical significance of this distinction. A later study confirmed that all types of BL cases have GEP features readily distinguishing them from other lymphoma types [51]. The eBL and HIV-BL subtypes were nearly identical, but there was a small set of consistent differences between eBL and sBL. These differences were enriched for targets of certain miRNAs expressed in BL and for genes regulated by RBL2, a tumor suppressor of the retinoblastoma family involved in cell cycle control.

High-Throughput Sequencing (HTS)

Also known as "next-generation" or "solid-phase" sequencing, HTS is producing another revolution in understanding lymphomas. It should be noted that HTS can be applied to cellular RNA corresponding to mRNA, in the technique known as RNA-Seq. The enumeration of transcripts provides a "digital" equivalent to hybridization-based GEP discussed above and is potentially more accurate and sensitive, although the analysis of raw data is far more complicated than from arrays [52]. RNA-Seq has unique advantages such as being informative about alternative splicing and detecting mutations and fusion genes produced by translocations, but these alone may not routinely justify its increased cost and complexity [53]. HTS also underlies the newest methods for three genomic approaches discussed below: detection of microRNA, DNA methylation, and genomic alterations. However, here will be discussed the unique application of HTS: *the detection of genetic mutations*.

HTS has been more extensively applied to nonlymphoid tumors to date, but early results from HTS of hematopoietic malignancies suggest that certain observations hold true for cancers in general. Distinguishing "driver" from "passenger" mutations is a challenge, but not an insurmountable one, using principles such as recurrence in multiple patients, association with

disease subtype (e.g., ABC vs. GCB DLBCL) or prognosis, clustering in "hot spots," favored expression of the mutated allele, and predicted effect on protein function [54]. It is important to have germline DNA for comparison, so as to be able to distinguish deviations from the consensus human genome in the tumor that are single-nucleotide polymorphisms (SNPs) from those which are somatic tumor-specific mutations, although SNPs themselves may have important roles in disease susceptibility and/or course. Mutations are also best interpreted with respect to the biological processes, such as signaling pathways, that they are likely to affect; in other words, in a group of tumors of a given type, mutations may be scattered across multiple genes involved in a particular process, to such an extent that they may appear sporadic, but are actually recurrent for that process due to biological selection. Finally, there are technical aspects to consider in the choice of HTS approach (whole-genome, whole-exome, RNA-Seq, etc.), affecting cost, sensitivity, and accuracy of mutation detection, not discussed here.

Two recent articles provided the first large-scale HTS-based summaries of the mutation-altered "genomic landscape" of DLBCL [55, 56]. As has been observed in other tumors, mutated genes with characteristics of being recurrent drivers in DLBCL tend to be *epigenetic regulators*. Prominent among these was MLL2, one of six methyltransferases affecting lysine residue 4 of histone H3 (H3K4). Inactivating mutations were not found in normal centroblasts, but were present in 32 % of DLBCL and 89 % of FL cases. MLL2 is one of several epigenetic regulators including MLL3, EZH2, and MLL5, mutations of which in DLBCL appear to be mutually exclusive. MLL2 is likely a tumor suppressor, because it tends to be biallelically inactivated. Another mutated epigenetic regulator was MEF2, a recruiter of histone deacetylators and transferases, which has specific loss-of-function mutations in 12 % of FL and 25 % of DLBCL (all GCB subtype) cases. Yet another is CREBBP (CBP/p300), a transcriptional coactivator and histone acetylator. CREBBP has monoallelic inactivating mutations in 18 % of DLBCL cases, and single-copy loss in others, from both subtypes. CREBBP is only the most common of several histone-modifying genes inactivated in DLBCL, the functional and molecular consequences of which are hard to predict. In other words, the discovery of mutations in epigenetic regulators may have raised more questions than it has answered. However, already it is known that decreased CBP/p300 leads to increased BCL6 and decreased p53, and predicts sensitivity to HDAC inhibitor therapy.

Not to be overlooked is that these studies confirmed the presence of several, *subtype-associated mutations* previously found in DLBCL. Although involvement of these genes is therefore not a novel finding revealed by HTS, they must be included in considering the genomic landscape of DLBCL, especially since their mutations have already been studied as to functional consequences. Examples include the histone methyltransferase EZH2, found to have a heterozygous Y641F mutation in 18 % of DLBCL cases and lines solely of the GCB-DLBCL subtype [57]. In vitro functional studies show that the heterodimeric enzyme, composed of WT and mutant proteins, has greater overall activity in the complete trimethylation of the target lysine residue [58, 59]. Highly characteristic NFkB-activating or NFkB-enhancing mutations are found in ABC-DLBCL cases in the BCR signal transducing genes CD79A and CD79B [16] (24 % overall) and the Toll-receptor pathway signal transducer MyD88 (39 %) [17]. These promote constitutive NFkB activity, as do inactivating mutations in the negative regulator A20 found in >50 % of ABC-DLBCL cases [60]. Various forms of inactivation (mutations and loss) affect PRDM1/BLIMP1, a master regulator in B-cell maturation to plasma cells, exclusively and in a high proportion of ABC-DLBCL cases. These changes are particularly significant in that recent studies in genetically engineered mice show that the combination of NFkB activation and BLIMP inactivation predictably leads to an ABC-like form of DLBCL in mice [61].

Plans are underway for further HTS to be performed on approximately 500 cases of DLBCL, as part of The Cancer Genome Atlas (TCGA)

initiative. Almost by definition, there is a diminishing marginal utility to the extent to which this will reveal new recurrent driver mutations, but it will surely provide a more exact picture. However, as for other TCGA initiatives, the greater benefit may come from the concurrent performance of other genomic analyses such as GEP, providing a more complete and integrated assessment of the biology of individual tumors. The power of integrated analysis is discussed in the next section.

Array-Based Comparative Genomic Hybridization (aCGH)

Gene copy number variation (CNV) has been detected in several types of lymphomas over the past decade, using arrays of increasing precision. This technology may ultimately be supplanted by HTS, but has already provided much useful information, from which a number of conclusions may be drawn. First, it is clear that the genome of lymphoma cells, even in primary tumors, is extensively altered by CNV. This is consistent with abundant evidence of alteration in DNA repair mechanisms in lymphoma, e.g., in nonhomologous end joining [62], as well as the mutagenic and translocation-promoting effects of activation-induced cytidine deaminase (AID) in lymphoid malignancies [63]. Second, newer SNP genotyping arrays that detect particular alleles have shown that loss of heterozygosity (LOH) is additionally extensive in lymphoma, expanding the spectrum of copy-neutral genomic abnormalities beyond mutations and translocations. Third, comparing CNV from different tumors of a given type reveals minimal recurrent regions of loss or gain, presumably containing critical oncogenes or tumor suppressor genes. An aCGH study of DLBCL found recurrent regions that were relatively subtype-specific and contained genes of interest further implicated by various types of evidence [5]. ABC-specific genes of interest were the transcription factor SpiB, highly expressed in primary tumors and shown by knockdown studies to be essential to ABC-DLBCL lines; the cell-cycle regulatory INK4a/ARF locus, often deleted with adverse effects on survival; and the oncogene FOXP1, characteristically highly expressed in ABC-DLBCL and often amplified by trisomy 3 or smaller gains. Other examples in which recurrently altered regions have led to the discovery of critical genes include PMBL and 9p24 [64, 65], and FL and TNFRSF14 [66].

Knowledge of recurrent regions of CNV alone is not always revealing of critical genes, as even relatively small regions may contain many candidate genes. Bioinformatic methods for correlating aCGH results with GEP results have increased the likelihood that candidate genes are actually critical genes [67]. Applied to lymphoma, aCGH-GEP correlation has implicated FOXM1 as a critical gene and potential therapeutic target in DLBCL, FL, and B-cell chronic lymphocytic leukemia [68]. Applied to MCL alone, aCGH-GEP correlation found several outcome-predictive genes within common altered regions, perhaps due to their correlation with proliferation [49]. This study also found that regions of LOH frequently overlap with commonly altered regions, suggesting another potential mechanism of dysregulation, and that inactivation of the Hippo signaling pathway could contribute to MCL pathogenesis.

Profiling of Noncoding RNA

Regulation by noncoding RNA, chiefly microRNA (miRNA), is important to many aspects of normal lymphoid biology and varies according to stages of B-cell development [69, 70]. It is therefore not surprising that expression of miRNAs appears to distinguish various types of lymphoma and correlates with clinical outcome. One of the earliest miRNAs discovered, miR-17–92, was found to be recurrently amplified exclusively in GCB-DLBCL (12.5 % of cases) and to cooperate with myc in expression of myc target genes and transformation of mouse B cells [5]. Early large-scale measurement of miRNAs was performed with arrays and by qRT-PCR, assaying for defined miRNAs. Zhang et al. were among the first to show that various types of NHL have distinctive miRNA profiles, largely corresponding to their normal counterparts in B-cell differentiation but

with some differences [70]. Malumbres et al. found that miRNA profiles of DLBCL could distinguish the ABC and GCB subtypes and correlated with outcome [69], which was confirmed in a larger study [71]. Di Lisio et al. found a distinctive profile of increased and decreased miRNAs distinguishing MCL from normal lymphoid tissues; the significance of several of these miRNAs was shown by correlation with mRNA levels of predicted targets, functional studies in cell lines, and correlation with clinical outcome [72]. Genome-scale measurement of miRNAs will increasingly be done by HTS, especially since not all species have been described. This was illustrated by Jima et al., who found 333 known miRNAs in a survey of normal and malignant B cells but an additional 286 candidate novel miRNAs [73].

Much of the research on miRNAs in lymphoma to date has focused on their prognostic significance, but clearly they are more than just biomarkers. MiRNAs are attractive therapeutic targets because a single miRNA can regulate the expression of many proteins; in theory, a single miRNA-based therapeutic could accomplish what would require many agents at the protein level. Dysregulation of miRNA in cancer includes both abnormal upregulation of oncogenic miRNAs (oncomirs) and decreased expression of miRNAs regarded as tumor suppressors. Therapeutics are being developed to both oppose (antagomirs) and restore miRNAs accordingly and have shown promise in animal models at preventing or shrinking metastatic lesions [74, 75]. Therefore, we can expect that miRNA research in lymphoma may become increasingly of therapeutic relevance.

Epigenetic Profiling

Epigenetic silencing of certain genes, typically ones associated with tumor suppression or differentiation, is a hallmark of cancer. Silencing is accomplished by a variety of means, such as histone modifications, and as noted above, HTS of DLBCL was most remarkable for mutations in histone-modifying epigenetic regulators. Chromatin immunoprecipitation, most informative when combined with HTS (ChIP-Seq), is a powerful tool for studying the functions of epigenetic regulators and is already being applied to specific instances, e.g., to find genes likely dysregulated through mutated EZH2 [24]. DNA methylation of promoter CpG islands, the epigenetic modification that has been most studied on a genomic scale, correlates inversely with gene expression and is often related to the activity of histone modifiers. In general, studies comparing DNA methylation patterns of cell lines and primary tumors of NHL to normal B cells show extensive differences [76, 77]. Target genes of EZH2 are frequently hypermethylated in DLBCL and cHL, in contrast to the exclusive patterns of EZH2 activity and cytosine methylation normally found in GCB cells [24]. DNA methylation patterns correlated highly with the mRNA-based discrimination between ABC and GCB DLBCL primary tumors, and methylation-based ABC-GCB distinction could be based on as few as 16 genes [78]. DNA methylation patterns also distinguished PMBL, cHL, and "gray zone" cases [79]. Abnormal states of methylation, particularly hypomethylation, were found not only to distinguish MCL cells from normal B cells but also to provide bases for rational therapy [80] and to correlate with outcome; tumor suppressor genes like CDKN2B, HOXD8, MLF1, and PCDH8 are hypermethylated in MCL but can be derepressed by treatment with demethylating agents [81].

Proteomic Profiling

Profiling at the protein level is still difficult to accomplish on a genomic scale but will be attractive if the technical challenges can be overcome. As compared to nucleic acids, proteins are more proximally the effectors of abnormal physiology and exclusively the direct targets of current therapeutics. Furthermore, proteins are subject to a variety of posttranslational modifications that are functionally significant but often not predictable from profiling of nucleic acids. Of particular interest is protein phosphorylation at specific

residues, indicating the activity of signaling pathways. For example, Akt phosphorylation was a negative predictor of outcome in DLBCL patients treated with R-CHOP, independent of the IPI and other molecular markers [82]. Akt phosphorylation also illustrates the therapeutic significance of protein studies, because it suggests that Akt inhibitor therapy might be beneficial. Furthermore, protein status (in particular, posttranslational modifications) can be very dynamic, and so protein profiling has been productively used to study subjects such as the response to chemotherapy or radiation [83, 84].

IHC is routinely applied in pathology laboratories but is semiquantitative at best and limited to single analytes. ELISA or similar solution-based immunodetection methods are quantitative and more sensitive, but still limited to small numbers of analytes. Antibody-based detection has large-scale potential, as evidenced by a report of array-based profiling of over 5,000 proteins in cell lines [85], and can detect specific posttranslational modifications. Batch proteomic profiling with reverse-phase protein arrays has been insightful in studies of acute myeloid leukemia [86] and the PI3K signaling pathway [87]. However, immunodetection methods are obviously dependent on the production and validation of antibodies with requisite specificity. Mass spectrometry (MS) is capable of genomic-scale protein detection and identification, is sensitive to posttranslational modifications, and is potentially capable of detecting any type of molecular species. MS is expensive and limited in throughput, in part because of the need to simplify complex mixtures by means such as 2-D gel electrophoresis, surface desorption, phosphotyrosine enrichment, or liquid chromatography (LC). Proteomic profiling is therefore far from direct clinical application, but in the research setting, it has been used to make a number of significant observations about lymphoma, many of which suggest a clinically feasible role for measurement of single proteins [88]. Examples include differences between proteomic signatures of MCL, small lymphocytic lymphoma, and marginal zone lymphoma; [89] phosphorylation of BCR signaling intermediates in MCL, suggesting a role for BCR signaling in this type of lymphoma as well; [90] identification of galectin-1 as a predictive biomarker for relapsed/refractory disease in cHL [91], consistent with its predicted role as a mediator of escape from immune surveillance; [92] identification of extracellular matrix proteins overexpressed in cHL as compared to reactive lymphoid tissues; [93] and proteins overexpressed by endothelial cells within nodal and extranodal sites of B-cell lymphoma in mice as compared with their levels in corresponding normal host organs, suggesting novel targets for inhibiting tumor neovasculature [94].

Metabolic Profiling

Profiling analytes other than genes and their protein products pushes the boundary of "genomic" profiling, but indirectly these are the result of abnormalities of genes and their expression and are potentially very informative. Altered metabolism is a topic of great interest in cancer, whose generality may extend to lymphoma. The utility of ^{18}FDG-PET scanning in lymphoma is due to the high rate of aerobic glycolysis by lymphoma, known as the Warburg effect, which is actively being pursued as a therapeutic target in cancer [95]. Specific examples of potential therapeutic benefit of targeting glycolysis in lymphoma have been identified in preclinical studies [96, 97]. The presumption may be that ^{18}FDG-PET scanning will be used to monitor glycolysis-targeting therapy, but emerging technologies suggest alternative ways to monitor tumor metabolism. High-resolution proton nuclear magnetic resonance (^{1}H NMR) spectroscopy of fresh lymphoma samples ex vivo was able to distinguish 14 DLBCL samples from 17 FL samples with 86 % sensitivity and 76 % specificity, principally based on relatively increased alanine in DLBCL and taurine in FL [98]. Studies in other cancers and other diseases have progressed far beyond this, principally using NMR and/or MS to identify and quantify small biomolecules of various types other than nucleic acids and proteins: amino acids and metabolites, carbohydrates, lipids, etc. Profiling of plasma and urine for metabolites is

now an active area of epidemiologic research, [99] and the methods developed for biomarker detection in other cancers [100] may ultimately prove useful in noninvasive studies of lymphoma. When ^{1}H NMR and LC-MS were used to profile medium from cultured B cells and BCR-transfected myeloma cell lines, profound changes in secreted metabolites were observed during phases of proliferation and antibody secretion, associated with BCR stimulation and induced plasma cell differentiation [101]. There is therefore reason to believe that metabolic studies will be useful for lymphoma diagnosis and treatment in the future.

Conclusion

Genomic profiling of lymphoma samples has provided many valuable insights into the biology of lymphoma, with considerable therapeutic implications. It can be expected that this will continue and that it will move more closely to clinical applications. Adaptively randomized trials guided by biomarker studies [102, 103] may soon provide opportunities for genomic discoveries to impact the development of personalized therapy.

References

1. Obermann EC, Csato M, Dirnhofer S, Tzankov A. BCL2 gene aberration as an IPI-independent marker for poor outcome in non-germinal-centre diffuse large B cell lymphoma. J Clin Pathol. 2009;62:903–7.
2. Alizadeh AA, Eisen MB, Davis RE, Ma C, Lossos IS, Rosenwald A, et al. Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. Nature. 2000;403:503–11.
3. Rosenwald A, Wright G, Chan WC, Connors JM, Campo E, Fisher RI, et al. The use of molecular profiling to predict survival after chemotherapy for diffuse large-B-cell lymphoma. N Engl J Med. 2002;346:1937–47.
4. Lenz G, Wright G, Dave SS, Xiao W, Powell J, Zhao H, et al. Stromal gene signatures in large-B-cell lymphomas. N Engl J Med. 2008;359:2313–23.
5. Lenz G, Wright GW, Emre NC, Kohlhammer H, Dave SS, Davis RE, et al. Molecular subtypes of diffuse large B-cell lymphoma arise by distinct genetic pathways. Proc Natl Acad Sci USA. 2008;105:13520–5.
6. Lenz G, Staudt LM. Aggressive lymphomas. N Engl J Med. 2010;362:1417–29.
7. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J R Stat Soc Ser B. 1995;57:289–300.
8. Tusher VG, Tibshirani R, Chu G. Significance analysis of microarrays applied to the ionizing radiation response. Proc Natl Acad Sci USA. 2001;98:5116–21.
9. Wright G, Tan B, Rosenwald A, Hurt EH, Wiestner A, Staudt LM. A gene expression-based method to diagnose clinically distinct subgroups of diffuse large B cell lymphoma. Proc Natl Acad Sci USA. 2003;100:9991–6.
10. Dave SS, Wright G, Tan B, Rosenwald A, Gascoyne RD, Chan WC, et al. Prediction of survival in follicular lymphoma based on molecular features of tumor-infiltrating immune cells. N Engl J Med. 2004;351:2159–69.
11. Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA, et al. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. Proc Natl Acad Sci USA. 2005;102:15545–50.
12. Lamb J, Crawford ED, Peck D, Modell JW, Blat IC, Wrobel MJ, et al. The Connectivity Map: using gene-expression signatures to connect small molecules, genes, and disease. Science. 2006;313:1929–35.
13. Davis RE, Brown KD, Siebenlist U, Staudt LM. Constitutive nuclear factor kappaB activity is required for survival of activated B cell-like diffuse large B cell lymphoma cells. J Exp Med. 2001;194:1861–74.
14. Ngo VN, Davis RE, Lamy L, Yu X, Zhao H, Lenz G, et al. A loss-of-function RNA interference screen for molecular targets in cancer. Nature. 2006;441:106–10.
15. Lenz G, Davis RE, Ngo VN, Lam L, George TC, Wright GW, et al. Oncogenic CARD11 mutations in human diffuse large B cell lymphoma. Science. 2008;319:1676–9.
16. Davis RE, Ngo VN, Lenz G, Tolar P, Young R, Romesser PB, et al. Chronic active B cell receptor signaling in diffuse large B cell lymphoma. Nature. 2010;463:88–92.
17. Ngo VN, Young RM, Schmitz R, Jhavar S, Xiao W, Lim KH, et al. Oncogenically active MYD88 mutations in human lymphoma. Nature. 2011;470(7332):115–9.
18. Honigberg LA, Smith AM, Sirisawada M, Vernera E, Lourya D, Changa B, et al. The Btk inhibitor PCI-32765 blocks B cell activation and is efficacious in models of autoimmune disease and B cell malignancy. Proc Natl Acad Sci USA. 2010;107:13075–80.
19. Dunleavy K, Pittaluga S, Czuczman MS, Dave SS, Wright G, Grant N, et al. Differential efficacy of bortezomib plus chemotherapy within molecular subtypes of diffuse large B-cell lymphoma. Blood. 2009;113:6069–76.
20. Cerchietti LC, Ghetu AF, Zhu X, Da Silva GF, Zhong S, Matthews M, et al. A small-molecule inhibitor of BCL6 kills DLBCL cells in vitro and in vivo. Cancer Cell. 2010;17:400–11. 2858395.
21. Scuto A, Kujawski M, Kowolik C, Krymskaya L, Wang L, Weiss LM, et al. STAT3 inhibition is a therapeutic strategy for ABC-like diffuse large B-cell lymphoma. Cancer Res. 2011;71:3182–8. 3085657.
22. Kloo B, Nagel D, Pfeifer M, Grau M, Duwel M, Vincendeau M, et al. Critical role of PI3K signaling

for NF-kappaB-dependent survival in a subset of activated B-cell-like diffuse large B-cell lymphoma cells. Proc Natl Acad Sci USA. 2011;108:272–7. 3017191.
23. Zhao MY, Auerbach A, D'Costa AM, Rapoport AP, Burger AM, Sausville EA, et al. Phospho-p70S6K/p85S6K and cdc2/cdk1 are novel targets for diffuse large B-cell lymphoma combination therapy. Clin Cancer Res. 2009;15:1708–20.
24. Velichutina I, Shaknovich R, Geng H, Johnson NA, Gascoyne RD, Melnick AM, et al. EZH2-mediated epigenetic silencing in germinal center B cells contributes to proliferation and lymphomagenesis. Blood. 2010;116:5247–55. 3012542.
25. Blenk S, Engelmann J, Weniger M, Schultz J, Dittrich M, Rosenwald A, et al. Germinal center B cell-like (GCB) and activated B cell-like (ABC) type of diffuse large B cell lymphoma (DLBCL): analysis of molecular predictors, signatures, cell cycle state and patient survival. Cancer Inform. 2007;3:399–420. 2675856.
26. Malumbres R, Chen J, Tibshirani R, Johnson NA, Sehn LH, Natkunam Y, et al. Paraffin-based 6-gene model predicts outcome in diffuse large B-cell lymphoma patients treated with R-CHOP. Blood. 2008;111:5509–14. 2424149.
27. Alizadeh AA, Gentles AJ, Alencar AJ, Liu CL, Kohrt HE, Houot R, et al. Prediction of survival in diffuse large B-cell lymphoma based on the expression of 2 genes reflecting tumor and microenvironment. Blood. 2011;118:1350–8. 3152499.
28. Hans CP, Weisenburger DD, Greiner TC, Gascoyne RD, Delabie J, Ott G, et al. Confirmation of the molecular classification of diffuse large B-cell lymphoma by immunohistochemistry using a tissue microarray. Blood. 2004;103:275–82.
29. Choi WW, Weisenburger DD, Greiner TC, Piris MA, Banham AH, Delabie J, et al. A new immunostain algorithm classifies diffuse large B-cell lymphoma into molecular subtypes with high accuracy. Clin Cancer Res. 2009;15:5494–502.
30. Salles G, de Jong D, Xie W, Rosenwald A, Chhanabhai M, Gaulard P, et al. Prognostic significance of immunohistochemical biomarkers in diffuse large B-cell lymphoma: a study from the Lunenburg Lymphoma Biomarker Consortium. Blood. 2011;117:7070–8.
31. Rimsza LM, Leblanc ML, Unger JM, Miller TP, Grogan TM, Persky DO, et al. Gene expression predicts overall survival in paraffin-embedded tissues of diffuse large B-cell lymphoma treated with R-CHOP. Blood. 2008;112:3425–33.
32. Kulkarni MM. Digital multiplexed gene expression analysis using the NanoString nCounter system. Curr Protoc Mol Biol. 2011. doi:10.1002/0471142727.mb25b10s94. Chapter 25, Unit25B 10.
33. Tzankov A, Zlobec I, Went P, Robl H, Hoeller S, Dirnhofer S. Prognostic immunophenotypic biomarker studies in diffuse large B cell lymphoma with special emphasis on rational determination of cut-off scores. Leuk Lymphoma. 2010;51:199–212.
34. Rosenwald A, Wright G, Leroy K, Yu X, Gaulard P, Gascoyne RD, et al. Molecular diagnosis of primary mediastinal B cell lymphoma identifies a clinically favorable subgroup of diffuse large B cell lymphoma related to Hodgkin lymphoma. J Exp Med. 2003;198:851–62. 2194208.
35. Bertucci F, Chetaille B, Xerri L. Gene expression profiling for in silico microdissection of Hodgkin's lymphoma microenvironment and identification of prognostic features. Adv Hematol. 2011;2011:485310. 3004394.
36. Sanchez-Espiridion B, Montalban C, Lopez A, Menarguez J, Sabin P, Ruiz-Marcellan C, et al. A molecular risk score based on 4 functional pathways for advanced classical Hodgkin lymphoma. Blood. 2010;116:e12–7.
37. Derenzini E, Younes A. Predicting treatment outcome in classical Hodgkin lymphoma: genomic advances. Genome Med. 2011;3:26. 3129642.
38. Steidl C, Lee T, Shah SP, Farinha P, Han G, Nayar T, et al. Tumor-associated macrophages and survival in classic Hodgkin's lymphoma. N Engl J Med. 2010;362:875–85. 2897174.
39. Brune V, Tiacci E, Pfeil I, Doring C, Eckerle S, van Noesel CJ, et al. Origin and pathogenesis of nodular lymphocyte-predominant Hodgkin lymphoma as revealed by global gene expression analysis. J Exp Med. 2008;205:2251–68. 2556780.
40. Farinha P, Al-Tourah A, Gill K, Klasa R, Connors JM, Gascoyne RD. The architectural pattern of FOXP3-positive T cells in follicular lymphoma is an independent predictor of survival and histologic transformation. Blood. 2010;115:289–95.
41. Kelley T, Beck R, Absi A, Jin T, Pohlman B, Hsi E. Biologic predictors in follicular lymphoma: importance of markers of immune response. Leuk Lymphoma. 2007;48:2403–11.
42. Cerhan JR, Wang S, Maurer MJ, Ansell SM, Geyer SM, Cozen W, et al. Prognostic significance of host immune gene polymorphisms in follicular lymphoma survival. Blood. 2007;109:5439–46.
43. Bryant PA, Smyth GK, Robins-Browne R, Curtis N. Detection of gene expression in an individual cell type within a cell mixture using microarray analysis. PLoS One. 2009;4:e4427.
44. Pangault C, Ame-Thomas P, Ruminy P, Rossille D, Caron G, Baia M, et al. Follicular lymphoma cell niche: identification of a preeminent IL-4-dependent T(FH)-B cell axis. Leukemia. 2010;24:2080–9.
45. Rosenwald A, Wright G, Wiestner A, Chan WC, Connors JM, Campo E, et al. The proliferation gene expression signature is a quantitative integrator of oncogenic events that predicts survival in mantle cell lymphoma. Cancer Cell. 2003;3:185–97.
46. Wiestner A, Tehrani M, Chiorazzi M, Wright G, Gibellini F, Nakayama K, et al. Point mutations and genomic deletions in CCND1 create stable truncated cyclin D1 mRNAs that are associated with increased proliferation rate and shorter survival. Blood. 2007;109:4599–606. 1885523.
47. Mozos A, Royo C, Hartmann E, De Jong D, Baro C, Valera A, et al. SOX11 expression is highly specific

for mantle cell lymphoma and identifies the cyclin D1-negative subtype. Haematologica. 2009;94:1555–62. 2770966.
48. Fernandez V, Salamero O, Espinet B, Sole F, Royo C, Navarro A, et al. Genomic and gene expression profiling defines indolent forms of mantle cell lymphoma. Cancer Res. 2010;70:1408–18.
49. Hartmann EM, Campo E, Wright G, Lenz G, Salaverria I, Jares P, et al. Pathway discovery in mantle cell lymphoma by integrated analysis of high-resolution gene expression and copy number profiling. Blood. 2010;116:953–61. 2924229.
50. Dave SS, Fu K, Wright GW, Lam LT, Kluin P, Boerma EJ, et al. Molecular diagnosis of Burkitt's lymphoma. N Engl J Med. 2006;354:2431–42.
51. Piccaluga PP, De Falco G, Kustagi M, Gazzola A, Agostinelli C, Tripodo C, et al. Gene expression analysis uncovers similarity and differences among Burkitt lymphoma subtypes. Blood. 2010;117:3596–608.
52. Garber M, Grabherr MG, Guttman M, Trapnell C. Computational methods for transcriptome annotation and quantification using RNA-seq. Nat Methods. 2011;8:469–77.
53. Malone JH, Oliver B. Microarrays, deep sequencing and the true measure of the transcriptome. BMC Biol. 2011;9:34. 3104486.
54. Greenman C, Wooster R, Futreal PA, Stratton MR, Easton DF. Statistical analysis of pathogenicity of somatic mutations in cancer. Genetics. 2006;173:2187–98. 1569711.
55. Pasqualucci L, Trifonov V, Fabbri G, Ma J, Rossi D, Chiarenza A, et al. Analysis of the coding genome of diffuse large B-cell lymphoma. Nat Genet. 2011;43:830–7.
56. Morin RD, Mendez-Lago M, Mungall AJ, Goya R, Mungall KL, Corbett RD, et al. Frequent mutation of histone-modifying genes in non-Hodgkin lymphoma. Nature. 2011;476:298–303. 3210554.
57. Morin RD, Johnson NA, Severson TM, Mungall AJ, An J, Goya R, et al. Somatic mutations altering EZH2 (Tyr641) in follicular and diffuse large B-cell lymphomas of germinal-center origin. Nat Genet. 2010;42:181–5. 2850970.
58. Yap DB, Chu J, Berg T, Schapira M, Cheng SW, Moradian A, et al. Somatic mutations at EZH2 Y641 act dominantly through a mechanism of selectively altered PRC2 catalytic activity, to increase H3K27 trimethylation. Blood. 2011;117:2451–9. 3062411.
59. Sneeringer CJ, Scott MP, Kuntz KW, Knutson SK, Pollock RM, Richon VM, et al. Coordinated activities of wild-type plus mutant EZH2 drive tumor-associated hypertrimethylation of lysine 27 on histone H3 (H3K27) in human B-cell lymphomas. Proc Natl Acad Sci USA. 2010;107:20980–5. 3000297.
60. Compagno M, Lim WK, Grunn A, Nandula SV, Brahmachary M, Shen Q, et al. Mutations of multiple genes cause deregulation of NF-kappaB in diffuse large B-cell lymphoma. Nature. 2009;459:717–21. 2973325.
61. Calado DP, Zhang B, Srinivasan L, Sasaki Y, Seagal J, Unitt C, et al. Constitutive canonical NF-kappaB activation cooperates with disruption of BLIMP1 in the pathogenesis of activated B cell-like diffuse large cell lymphoma. Cancer Cell. 2010;18:580–9. 3018685.
62. Roddam PL, Allan JM, Dring AM, Worrillow LJ, Davies FE, Morgan GJ. Non-homologous end-joining gene profiling reveals distinct expression patterns associated with lymphoma and multiple myeloma. Br J Haematol. 2010;149:258–62.
63. Gruber TA, Chang MS, Sposto R, Muschen M. Activation-induced cytidine deaminase accelerates clonal evolution in BCR-ABL1-driven B-cell lineage acute lymphoblastic leukemia. Cancer Res. 2010;70:7411–20. 2948648.
64. Rui L, Emre NC, Kruhlak MJ, Chung HJ, Steidl C, Slack G, et al. Cooperative epigenetic modulation by cancer amplicon genes. Cancer Cell. 2010;18:590–605. 3049192.
65. Green MR, Monti S, Rodig SJ, Juszczynski P, Currie T, O'Donnell E, et al. Integrative analysis reveals selective 9p24.1 amplification, increased PD-1 ligand expression, and further induction via JAK2 in nodular sclerosing Hodgkin lymphoma and primary mediastinal large B-cell lymphoma. Blood. 2010;116:3268–77. 2995356.
66. Cheung KJ, Johnson NA, Affleck JG, Severson T, Steidl C, Ben-Neriah S, et al. Acquired TNFRSF14 mutations in follicular lymphoma are associated with worse prognosis. Cancer Res. 2010;70:9166–74.
67. Akavia UD, Litvin O, Kim J, Sanchez-Garcia F, Kotliar D, Causton HC, et al. An integrated approach to uncover drivers of cancer. Cell. 2010;143:1005–17. 3013278.
68. Green MR, Aya-Bonilla C, Gandhi MK, Lea RA, Wellwood J, Wood P, et al. Integrative genomic profiling reveals conserved genetic mechanisms for tumorigenesis in common entities of non-Hodgkin's lymphoma. Genes Chromosomes Cancer. 2011;50:313–26.
69. Malumbres R, Sarosiek KA, Cubedo E, Ruiz JW, Jiang X, Gascoyne RD, et al. Differentiation stage-specific expression of microRNAs in B lymphocytes and diffuse large B-cell lymphomas. Blood. 2009;113:3754–64.
70. Zhang J, Jima DD, Jacobs C, Fischer R, Gottwein E, Huang G, et al. Patterns of microRNA expression characterize stages of human B-cell differentiation. Blood. 2009;113:4586–94.
71. Montes-Moreno S, Martinez N, Sanchez-Espiridion B, Diaz Uriarte R, Rodriguez ME, Saez A, et al. miRNA expression in diffuse large B-cell lymphoma treated with chemoimmunotherapy. Blood. 2011;118:1034–40.
72. Di Lisio L, Gomez-Lopez G, Sanchez-Beato M, Gomez-Abad C, Rodriguez ME, Villuendas R, et al. Mantle cell lymphoma: transcriptional regulation by microRNAs. Leukemia. 2010;24:1335–42.
73. Jima DD, Zhang J, Jacobs C, Richards KL, Dunphy CH, Choi WW, et al. Deep sequencing of the small RNA transcriptome of normal and malignant human B cells identifies hundreds of novel microRNAs. Blood. 2010;116:e118–27. 3012600.

74. Ma L, Reinhardt F, Pan E, Soutschek J, Bhat B, Marcusson EG, et al. Therapeutic silencing of miR-10b inhibits metastasis in a mouse mammary tumor model. Nat Biotechnol. 2010;28:341–7. 2852471.
75. Valastyan S, Chang A, Benaich N, Reinhardt F, Weinberg RA. Activation of miR-31 function in already-established metastases elicits metastatic regression. Genes Dev. 2011;25:646–59. 3059837.
76. Choi JH, Li Y, Guo J, Pei L, Rauch TA, Kramer RS, et al. Genome-wide DNA methylation maps in follicular lymphoma cells determined by methylation-enriched bisulfite sequencing. PLoS One. 2010;5(9):e13020. 2947499.
77. Shi H, Guo J, Duff DJ, Rahmatpanah F, Chitima-Matsiga R, Al-Kuhlani M, et al. Discovery of novel epigenetic markers in non-Hodgkin's lymphoma. Carcinogenesis. 2007;28:60–70.
78. Shaknovich R, Geng H, Johnson NA, Tsikitas L, Cerchietti L, Greally JM, et al. DNA methylation signatures define molecular subtypes of diffuse large B-cell lymphoma. Blood. 2010;116:e81–9. 2993635.
79. Eberle FC, Rodriguez-Canales J, Wei L, Hanson JC, Killian JK, Sun HW, et al. Methylation profiling of mediastinal gray zone lymphoma reveals a distinctive signature with elements shared by classical Hodgkin's lymphoma and primary mediastinal large B-cell lymphoma. Haematologica. 2011;96:558–66. 3069233.
80. Leshchenko VV, Kuo PY, Shaknovich R, Yang DT, Gellen T, Petrich A, et al. Genomewide DNA methylation analysis reveals novel targets for drug development in mantle cell lymphoma. Blood. 2010;116:1025–34. 2938124.
81. Enjuanes A, Fernandez V, Hernandez L, Navarro A, Bea S, Pinyol M, et al. Identification of methylated genes associated with aggressive clinicopathological features in mantle cell lymphoma. PLoS One. 2011;6:e19736. 3095614.
82. Hasselblom S, Hansson U, Olsson M, Toren L, Bergstrom A, Nilsson-Ehle H, et al. High immunohistochemical expression of p-AKT predicts inferior survival in patients with diffuse large B-cell lymphoma treated with immunochemotherapy. Br J Haematol. 2010;149:560–8.
83. Mactier S, Henrich S, Che Y, Kohnke PL, Christopherson RI. Comprehensive proteomic analysis of the effects of purine analogs on human Raji B-cell lymphoma. J Proteome Res. 2011;10:1030–42.
84. Jiang Y, Liu X, Fang X, Wang X. Proteomic analysis of mitochondria in Raji cells following exposure to radiation: implications for radiotherapy response. Protein Pept Lett. 2009;16:1350–9.
85. Fagerberg L, Stromberg S, El-Obeid A, Gry M, Nilsson K, Uhlen M, et al. Large-scale protein profiling in human cell lines using antibody-based proteomics. J Proteome Res. 2011;10:4066–75.
86. Carter BZ, Qiu YH, Zhang N, Coombes KR, Mak DH, Thomas DA, et al. Expression of ARC (apoptosis repressor with caspase recruitment domain), an anti-apoptotic protein, is strongly prognostic in AML. Blood. 2011;117:780–7. 3035072.
87. Creighton CJ, Fu X, Hennessy BT, Casa AJ, Zhang Y, Gonzalez-Angulo AM, et al. Proteomic and transcriptomic profiling reveals a link between the PI3K pathway and lower estrogen-receptor (ER) levels and activity in ER+ breast cancer. Breast Cancer Res. 2010;12:R40. 2917035.
88. Boyd RS, Dyer MJ, Cain K. Proteomic analysis of B-cell malignancies. J Proteomics. 2010;73:1804–22.
89. Rolland D, Bouamrani A, Houlgatte R, Barbarat A, Ramus C, Arlotto M, et al. Identification of proteomic signatures of mantle cell lymphoma, small lymphocytic lymphoma, and marginal zone lymphoma biopsies by surface enhanced laser desorption/ionization-time of flight mass spectrometry. Leuk Lymphoma. 2011;52:648–58.
90. Pighi C, Gu TL, Dalai I, Barbi S, Parolini C, Bertolaso A, et al. Phospho-proteomic analysis of mantle cell lymphoma cells suggests a pro-survival role of B-cell receptor signaling. Cell Oncol (Dordr). 2011;34:141–53. 3063577.
91. Kamper P, Ludvigsen M, Bendix K, Hamilton-Dutoit S, Rabinovich GA, Moller MB, et al. Proteomic analysis identifies galectin-1 as a predictive biomarker for relapsed/refractory disease in classical Hodgkin lymphoma. Blood. 2011;117:6638–49.
92. Juszczynski P, Ouyang J, Monti S, Rodig SJ, Takeyama K, Abramson J, et al. The AP1-dependent secretion of galectin-1 by Reed Sternberg cells fosters immune privilege in classical Hodgkin lymphoma. Proc Natl Acad Sci USA. 2007;104:13134–9. 1936978.
93. Kischel P, Waltregny D, Greffe Y, Mazzucchelli G, De Pauw E, de Leval L, et al. Identification of stromal proteins overexpressed in nodular sclerosis Hodgkin lymphoma. Proteome Sci. 2011;9:63. 3200160.
94. Schliemann C, Roesli C, Kamada H, Borgia B, Fugmann T, Klapper W, et al. In vivo biotinylation of the vasculature in B-cell lymphoma identifies BST-2 as a target for antibody-based therapy. Blood. 2011;115:736–44.
95. Dang CV, Hamaker M, Sun P, Le A, Gao P. Therapeutic targeting of cancer cell metabolism. J Mol Med (Berl). 2011;89:205–12.
96. Meynet O, Beneteau M, Jacquin MA, Pradelli LA, Cornille A, Carles M, et al. Glycolysis inhibition targets Mcl-1 to restore sensitivity of lymphoma cells to ABT-737-induced apoptosis. Leukemia. 2012;26(5):1145–7.
97. Le A, Cooper CR, Gouw AM, Dinavahi R, Maitra A, Deck LM, et al. Inhibition of lactate dehydrogenase a induces oxidative stress and inhibits tumor progression. Proc Natl Acad Sci USA. 2011;107:2037–42. 2836706.
98. Barba I, Sanz C, Barbera A, Tapia G, Mate JL, Garcia-Dorado D, et al. Metabolic fingerprinting of fresh lymphoma samples used to discriminate between follicular and diffuse large B-cell lymphomas. Exp Hematol. 2009;37:1259–65.
99. Bictash M, Ebbels TM, Chan Q, Loo RL, Yap IK, Brown IJ, et al. Opening up the "black box": metabolic phenotyping and metabolome-wide association studies in epidemiology. J Clin Epidemiol. 2010;63:970–9.

100. Corona G, Rizzolio F, Giordano A, Toffoli G. Phamaco-metabolomics: an emerging "omics" tool for the personalization of anticancer treatments and identification of new valuable therapeutic targets. J Cell Physiol. 2012;227(7):2827–31.
101. Garcia-Manteiga JM, Mari S, Godejohann M, Spraul M, Napoli C, Cenci S, et al. Metabolomics of B to plasma cell differentiation. J Proteome Res. 2011;10:4165–76.
102. Barker AD, Sigman CC, Kelloff GJ, Hylton NM, Berry DA, Esserman LJ. I-SPY 2: an adaptive breast cancer trial design in the setting of neoadjuvant chemotherapy. Clin Pharmacol Ther. 2009;86:97–100.
103. Jones D. Adaptive trials receive boost. Nat Rev Drug Discov. 2010;9:345–8.

Molecular Profiling of Peripheral T-Cell Lymphomas

3

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Abstract

Based on their own experience and knowledge of the literature, the authors revise the pathobiological characteristics of peripheral T-cell lymphomas (PTCL) by focusing on the most recent data available as far as gene expression profile (GEP) analyses are concerned.

First, GEP studies provided important insight into the histogenesis, molecular pathogenesis, and targeted treatments of different PTCL subtypes. For example, it was clearly shown that angioimmunoblastic T-cell lymphoma (AITL) corresponds to T follicular helper (TFH) lymphocytes and presents consistent deregulation of genes involved in angiogenesis. Noteworthy, targeting some of them, such as VEGF/VEGFR2, may represent an innovative and effective therapeutic strategy. Secondly, it was shown that PTCLs/not otherwise specified (PTCL/NOS) include at least three different subset characterized by specific cellular derivation (T-central memory, T-cytotoxic, and TFH) and possibly different outcome. Besides that, notably, all PTCLs/NOS present with constant deregulation of certain molecules, including the PDGFRA, which represents a suitable therapeutic target in this setting. Finally, both ALK+ and ALK– ALCLs have been shown to be distinct from the other PTCLs, possibly constituting separate entities. Remarkably, the molecular profile of the ALK+ forms largely relies on the activation of ALK and its downstream STAT3, while other tyrosine kinases are probably activated in the ALK– ones.

Keywords

Peripheral T-cell lymphoma (PTCL) • Cutaneous T-cell lymphoma (CTCL) • T-cell gene receptor (TCR) • Stem cell transplantation • Chemotherapy

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Background

In 1994, the Revised European–American Lymphoma (REAL) classification introduced new standards in the lymphoma field [1]. In particular, it stated for the first time that a classification of lymphoid tumors should consist in a list of "real" entities, each defined by the amalgamation of cell morphology, phenotype, molecular genetics, clinical data, and identification of a normal counterpart, if possible [1]. After a validation trial [2], the REAL classification was adopted by the World Health Organization (WHO) as guidelines for lymphoma diagnosis and therapy [3]. On such occasion, its methodology was extended to all tumors of the hematopoietic system [3]. According to patients' survival without any treatment, non-Hodgkin lymphomas (NHLs) are classified as indolent (survival measurable in years) and aggressive (survival measurable in months).

Peripheral T-cell lymphomas (PTCLs) belong to the aggressive lymphoma group with a few exceptions (see below) [4]. They represent approximately 12 % of all lymphoid neoplasms [4, 5]. Their incidence varies in different countries and races, being higher in HTLV-1 endemic areas (Asia, Caribbean basin, and some parts of the United States) [4, 5]. PTCLs are a heterogeneous group of tumors that can be roughly subdivided into specified and not otherwise specified (NOS) forms [5, 6]. In particular, the latter—corresponding to about 35 % of T-cell lymphomas—cannot be further classified on the basis of morphology, phenotype, and conventional molecular studies [4]. Usually, they occur in the fifth to sixth decade of life, without sex predilection [7–10]. Although PTCLs/NOS can present as isolated disease, they more often have a widespread dissemination (stages III–IV) with nodal, skin, liver, spleen, bone marrow, and peripheral blood involvement [7–10]. B symptoms are recorded in about 45 % of cases at diagnosis. A hemophagocytic syndrome may also be encountered [7–10].

The tumor morphology is highly variable, comprising cells of different size and shape [4]. PTCLs/NOS may contain prominent reactive components, including small lymphocytes, eosinophils, plasma cells, histiocytes, and epithelioid elements [4].

Immunohistochemistry does generally show T-cell-associated molecule expression, although the phenotypic profile is aberrant in about 80 % of cases [11].

Clonal rearrangements of T-cell receptor encoding genes are generally detected [12]. The karyotype is aberrant in more than 80 % of cases and often characterized by complex abnormalities. However, only a few specific alterations have not been identified, such as t(2;5) and variants and iso7q [13]. Recently, some recurrent lesions have been documented by comparative genomic hybridization and SNPs analysis [14, 15].

On clinical grounds, PTCLs/NOS are among the most aggressive non-Hodgkin lymphomas (NHL). In the majority of cases, the response to conventional chemotherapy is indeed frustrating, with relapse free and overall survival (OS) rates at five years below 30 % [5].

Besides the PTCL/NOS category, histological classification remains anyway a basic prognostic indicator in the PTCL setting [5, 6, 8, 16]. First, nodal and extranodal entities are clinically well distinct, as extranodal tumors, and specially the cutaneous forms, often display a relatively good outcome [5]. In addition, among nodal PTCLs, the distinction between anaplastic large-cell lymphoma (ALCL) and other entities as well as the distinction of ALK^+ and ALK^- cases among ALCLs retain a significant prognostic impact [5, 6, 8, 16]. In fact, ALK^+ ALCL, particularly when occurring in children and young adults, has a significantly better clinical outcome if compared with all other forms [5, 6]. Importantly, it was recently suggested to include ALK^- ALCL within the PTCLs/NOS basing on the lack of evidences of clear biological differences between them. However, new clinical and molecular findings demonstrated that ALK^- ALCL and PTCL/NOS are distinct entities, also presenting with different clinical outcome [6, 17, 18].

In addition to the basic distinction of the different entities, in those last years, several attempts have been made in order to further characterize the molecular pathology of PTCLs and identify reliable prognostic indicators to be offered to

clinicians. Indeed, novel insights have been provided by GEP studies, especially as far as tumor histogenesis, molecular pathogenesis, and possibly new, targeted therapies are concerned. On the other hand, novel and more refined prognostic indicators have been proposed, which may help in patients stratification.

Gene Expression Profiling of Peripheral T-Cell Lymphoma

The pathobiology of PTCLs has been neglected for a long time. The main reasons for that probably relied on the relative rarity of the disease, as well as the extreme difficulty to culture these neoplastic cells ex vivo. However, in the last few years, a new interest on PTCLs did emerge. Specially, these lymphomas have been then the object of different studies based on the application of high-throughput technologies, and several reports dealt with the GEP of the different subtypes [17–32]. In particular, on one hand, some authors focused on specific topics, that is, the GEP of mycosis fungoides, ALK^+ and ALK^- ALCLs, angioimmunoblastic lymphomas (AITL), γ[gamma]δ[delta]-T-cell lymphomas, adult T-cell lymphoma/leukemia (ATLL), and extranodal NK/T lymphoma nasal type, respectively [19, 25, 26, 28–31]. On the other hand, others analyzed larger collections of PTCLs of the NOS, AITL, and ALCL types [17, 18, 20, 23, 24, 32]. However, some of these studies suffer of limitations that vary from the usage of chips with a restricted number of genes [20, 23, 24] to the lack of a reliable normal counterpart for comparison [20, 24]. Specifically, Martinez-Delgado et al. reported that PTCL/NOS corresponds to a heterogeneous group of tumors, whose GEP is difficult to interpret due to the significant amount of infiltrating reactive cells. According to this study, the most relevant information provided by GEP pertains the expression level of genes belonging to the NFκ[kappa]B pathway (see below) [20]. Ballester et al. [23] found that the GEP could discriminate among PTCLs of the NOS, AITL, and ALCL types, although the former did not share a single profile. Using a multi-class predictor, the authors separated their cases into three molecular subgroups called U1, U2, and U3. The U1 gene expression signature included genes known to be associated with poor outcome in other tumors, such as *CCND2*. The U2 subgroup was associated with overexpression of genes involved in T-cell activation, including *NFKB1* and *BCL2*. The third group was mainly defined by the overexpression of genes involved in the IFN/JAK/STAT pathway and comprised most histiocyte-rich tumors. This finding suggests that the signatures recorded by Ballester et al. might be at least in part influenced by reactive components. Nevertheless, at present, it is not defined yet whether the presence of specific reactive components may significantly affect the tumor behavior in the field of PTCL/NOS, as it appeared in the case of some B-cell-derived lymphomas (namely, follicular and Hodgkin lymphomas) [33, 34] and possibly AITL (see below) [32].

Subsequently, Piccaluga et al. [17] have published a GEP study based on the analysis of 28 PTCLs/NOS, all corresponding to lymph node biopsies and containing an amount of neoplastic cells that exceeded the 70 % value of the whole examined population. The mRNA extracted from these cases was hybridized on the HG U133 2.0 *Plus* gene chip. The obtained results were compared with those of 6 AITLs, 6 ALCLs (2 ALK^+ and 4 ALK^-), and 20 samples of normal T-lymphocytes, purified from the peripheral blood and tonsil and corresponding to the main T-cell subsets ($CD4^+$, $CD8^+$, resting, and activated). Thus, the study of Piccaluga et al. significantly differs from most previous reports [20, 23, 24] in terms of methodology and selection criteria. In addition, it provides for the first time the rationale for possible targeted therapies in PTCL/NOS by offering clear evidence of their effectiveness ex vivo. In particular, the GEP detected by Piccaluga et al. [17] indicates that PTCLs/NOS are distinct from the other lymphoid malignancies and normal T-lymphocytes, establishing a clear relationship between PTCL/NOS and normal cellular counterparts and providing the basis for a better understanding of their pathogenesis.

More recently, Iqbal and Colleagues analyzed a large series of PTCLs, collected within the International T-Cell Lymphoma Project [32]. Importantly, they could build a robust molecular classifier for ATLL, AITL, and ALK$^+$ ALCL. On the other hand, PTCLs/NOS were confirmed to have a more heterogeneous profile possibly related to those of the normal counterparts. In addition, importantly, this study provided novel evidences on AITL and PTCL/NOS prognostication (see below). Additional, important, information have been then offered by Piva et al. [18]. In their study, the authors mainly focused on the molecular pathogenesis of ALCLs but also established the relationship between ALCL and PTCL/NOS. Importantly, they showed that ALCLs are molecularly distinct from PTCL/NOS, thus flattening the diffuse, though not biologically based, proposal of including ALK$^-$ ALCL within the group of PTCL/NOS. Consistently, in the course of their analysis, Piccaluga et al. [17] already found that all ALCLs tended to cluster together, irrespectively of their ALK positivity or negativity, though in a more limited number of cases. In all, this suggests that—besides the occurrence or not of a translocation involving the ALK gene at 2p23—these tumors share a set of deregulated pathways. On this respect, Feldman et al. [35] have recently shown by massive parallel genomic sequencing and FISH analyses that about 30 % of ALK- ALCLs carry the t(6;7)(p25.3;q32.2) translocation with downregulation of the DUSP22 gene and upregulation of MIR29 microRNAs, producing downstream effects similar to t(2;5) and variants. Nevertheless, it is possible to clearly differentiate ALK$^+$ and ALK$^-$ cases basing on GEP, as shown by different authors [18, 25, 32]. To this regard, in particular, the strong biological relevance of the ALK/STAT3 signaling in characterizing the global molecular profile of ALK+ ALCL was demonstrated [18].

Histogenesis of PTCLs

In the REAL and subsequent WHO classifications of lymphomas, the recognition of the non-neoplastic cellular counterpart is regarded as a main factor contributing to the definition of the single disease entity. However, differently from the field of B-NHLs, the vast majority of PTCLs have not yet definitely associated to a normal counterpart, mainly due to the complexity of T-cell compartment, as well as the bizarre morphology and largely aberrant phenotype of the neoplastic elements. Nevertheless, the recent GEP studies provided evidences, which may be the basis for a future histogenetic classification of these tumors. First, robust data were generated supporting the concept that AITL cells correspond to follicular T-helper (TFH) cells [26, 28]. Specifically, De Leval and colleagues studied 18 AITL cases and, by gene set enrichment analysis (GSEA) [36], found that AITL signature is significantly enriched in molecules characteristic of normal TFH, including *CXCL13, BCL6, PDCD1, CD40L*, and *NFATC1* [26]. At the same time, Piccaluga et al., by using a different algorithm, also showed that the GEP of AITL is definitely related to that of TFH lymphocytes [28]. Noteworthy, both the studies proved that such feature is largely restricted to AITL cases [26, 28], though a small fraction of PTCLs/NOS, more often characterized by clear cell cytology, presence of blastic EBV$^+$ B-cells, and, sometimes, follicular architecture [4], also presents with TFH molecular pattern [26]. Importantly, GEP results were validated by the immunohistochemical demonstration of TFH markers, such as CD10, BCL6, CXCL13, PD1, CCR5, SAP, and ICOS, in AITL [26, 28, 37–41], and were largely in keeping with the observations previously made by Rüdiger et al. [42]. Subsequently, the expression of the same molecules was confirmed in follicular PTCL/NOS [43]. Noteworthy, on the practical ground, when immunohistochemistry is used for the defining the TFH phenotype, at least three markers have to be detected [41] (PiccalugaPP et al., Expert Reviews in Hematology 2011, in press), in order to overcome the puzzling effect of phenotypic aberrancies, typical of PTCLs [11].

As far as PTCLs/NOS are concerned, GEP results suggested that these tumors are more closely related to activated rather than resting

T-cells [17]. Interestingly, this was partially independent from the expression of classical T-cell activation markers [17]; in addition, apparently, this is partially independent from the activation of T-cell receptor (TCR) signaling, indicating the alternative contribution of other mechanism, such as tyrosine-kinase activation (see below) (Piccaluga PP et al., personal observation 2012). As in normal mature T-lymphocytes, it was possible to identify two main subgroups of PTCL/NOS, with GEPs related to either CD4 or CD8 elements [17]. Notably, this characteristic did not correspond to the immunophenotype with regard to the expression of the single CD4 and CD8 molecules [17], reflecting the aberrancy in tumor phenotypes [11]. Importantly, the existence of these two molecularly distinct subgroups of PTCL/NOS was later confirmed by the International T-cell Lymphoma Project study [32]. Remarkably, the latter also suggested that cases with cytotoxic molecular profile might be provided with a worse prognosis (see below).

Finally, ALCLs, according to GEP, appeared to be related to either T_{H17} [32] (Piccaluga, unpublished 2013) or T_{H1} lymphocytes (Piccaluga, unpublished 2013).

Overall, the recognition of normal counterparts for different PTCLs has both biological and practical relevance. On one hand, in fact, it provides the basis for the recognition of cellular abnormalities and comprehension of the interaction with the microenvironment (i.e., the relationship of TFH-derived neoplastic cells and follicular dendritic cells, mast cells, etc., in AITL). On the other, it can be used in clinics for easier differential diagnosis, by applying new cell-specific markers (i.e., a panel of TFH-associated markers for the distinction of AITL and PTCL/NOS) [41].

Molecular Pathogenesis

Besides histogenetic information, different GEP studies provided relevant insights into the functional alterations of PTCLs. First, a careful comparison of PTCL/NOS with the closest normal cellular counterparts revealed, in fact, the extensive deregulation of genes, which control functions that are typically damaged in malignant cells, such as matrix remodeling, cell adhesion, transcription regulation, proliferation, and apoptosis. In particular, the analysis of Piccaluga et al. [17] might explain the dissemination pattern of PTCL/NOS, with frequent extranodal and bone marrow involvement and spread to peripheral blood [4], by showing the upregulation of *FN1*, *LAMB1*, *COL1A2*, *COL3A1*, *COL4A1*, *COL4A2*, and *COL12A1*, that is, of genes which promote local invasion and metastasis in different types of human cancers [44–46]. In addition, it revealed the deregulation of genes involved in apoptosis (e.g., *MOAP1*, *ING3*, *GADD45A*, and *GADD45B*) [47–53] and chemoresistance (such as *CYR61* and *NNMT*) [44–46, 54–65], which may be responsible for the poor response to conventional chemotherapy. Secondly, a couple of GEP studies suggested the possible deregulation of NF-kappa B (NFkB) pathway in a certain number of PTCL/NOS cases [20, 21, 23]. Indeed, it was shown that 30–40 % of cases present with nuclear localization (i.e., activation) of NFkB elements and peculiar GEP [66]. Noteworthy, it was then demonstrated that a fraction of PTCL/NOS presents with REL locus abnormalities, including amplifications and translocations, finally leading to NFkB constitutive activation [15]. Interestingly, on the other hand, downregulation of *BCL10* (an upstream activator of NFkB in human lymphocytes) was reported to occur in PTCL/NOS [17, 67] (personal observation, unpublished 2012) with consequent NFkB shutoff. However, it is still debated whether (1) cases with or without NFkB activation have a different clinical outcome and (2) whether anti-NFkB approaches can be eventually effective.

Finally, different studies characterized an aberrant tyrosine kinase (TK) signaling in PTCLs [17, 18, 22, 31]. In particular, Piccaluga et al. showed that PTCLs/NOS constantly express the *PDGRA* gene and present with consistent phosphorylation (i.e., activation) of the encoded protein [17, 22]. Remarkably, our group also indicated that the activation of PDGFRA is sustained by an autocrine stimulation, as later on demonstrated also for T-prolymphocytic leukemia

[68]. Noteworthy, it was subsequently showed that also other T/NK-derived tumors present with such phenomenon with relevant therapeutic implications (see below) [31]. Moreover, Piva et al. demonstrated that STAT3 activation induced by ALK is a major contributor to ALK$^+$ ALCL molecular signature [18].

Importantly, immunohistochemistry was largely adopted in order to provide in situ validation of the genomic data by showing correspondence between mRNA and protein expression, as seen, for example, with *PDGFRA* [17, 22] and *BCL10* [17, 67]. In addition, by comparison with normal tissues, immunohistochemistry allowed the identification of staining patterns corresponding to the synthesis of ectopic or paraphysiologic products by neoplastic cells. On the other hand, the phenotypic test highlighted the possibility that some of the results obtained by gene expression profiling may depend on nonneoplastic cellular components present in the analyzed sample, as seen for caldesmon [17].

Targeted Therapy

Tyrosine-Kinase Inhibitors

Basing on the evidence of tyrosine-kinase deregulation in different subtypes of PTCLs, the application of TK inhibitors (TKI) has been tested ex vivo [17, 31]. In particular, Piccaluga et al. [17] designed experiments aiming to test the sensitivity of PTCL/NOS cells to different TKI, including imatinib mesylate. The results obtained were of interest, with about 50 % cytotoxic effect seen at 48 h with a 1 μmol concentration. Notably, imatinib exerted a limited effect on the viability of normal lymphocytes. On the other hand, Huang et al. treated with imatinib T/NK-tumor-derived cell lines and obtained analogue results [31]. Importantly, following such observation, some clinical trials were initiated and the effectiveness of TKI was demonstrated in vivo in PTCL/NOS patients. Finally, as far as ALCL are concerned, consistently with GEP data, Chiarle et al. clearly showed that targeting ALK$^+$ and its downstream STAT3 is an effective strategy in ALK$^+$ ALCL [69, 70]. In addition, PDGFRs inhibition was shown to be effective in this setting as well, inducing clinical responses in vivo.

Histone Deacetylase Inhibitors

Interestingly, GEP analyses provided evidence for the silencing of genes, possibly regulated by epigenetic mechanisms such as acetylation (e.g., *GADD45A* and *GADD45B*), and suggested to test histone deacetylase inhibitors (HDACi) against PTCL/NOS primary cells and cell lines [17]. Notably, these compounds induced a dramatic reduction in cell viability, with G0–G1 cell cycle arrest and apoptosis at therapeutic concentrations, suggesting a possible role for this class of drugs in PTCL/NOS therapy. Noteworthy, this idea was also supported by some clinical preliminary observations [71]. Interestingly, the association of HDACi and daunorubicin apparently had a slight additive effect, as already observed in other settings [72]. Notably, the triple combination of TKI, HDACi, and anthracyclines produced a remarkable effect on cell viability: it might represent a promising option for future therapeutic applications. More recently, the effectiveness of associations of HDACi and demethylating agents was suggested by ex vivo studies.

Antiangiogenetic Therapy

Increased angiogenesis is a major characteristic of AITL. However, its molecular basis has been unknown for a long time. Recently, a couple of studies documented the upregulation of the *VEGF* gene in this tumor [26, 28]. Importantly, immunohistochemistry, extensively applied to a large series of cases on tissue microarrays, demonstrated that VEGF is mainly expressed by the neoplastic elements [28] and not only by the abundant vascular component, as initially proposed [26]. Remarkably, it was further shown that AITL cells do also express a VEGF receptor, VEGFR2/KDR [28], suggesting the intriguing hypothesis of an autocrine/paracrine stimulation also in this setting. In addition, it suggested the possible AITL sensitivity to antiangiogenetic drugs, such as thalidomide and bevacizumab.

Indeed, several reports have then documented their positive activity in AITL cases [73–78].

Monoclonal Antibodies

During the last few years, therapeutic monoclonal antibodies (MoAb) have become a major component of anti-lymphoma approaches. However, as far as PTCLs are concerned, a significant limitation emerged differently from what was seen in B-NHL. In particular, PTCLs were demonstrated to extensively present with the aberrant expression of T-cell-associated molecules [11, 17]. This phenomenon is indeed relevant in the clinical practice. In fact, some antigens against which MoAbs have been designed, such as CD4 [79] and, specially, CD52 [80, 81], are frequently downregulated in T-cell tumors [11, 17, 82–84]. Based on these findings, different authors agreed that the estimation of CD52 expression may provide a rationale for the selection of patients with higher probability of responding to alemtuzumab, by avoiding the risk of unwanted toxicity [82]. Similar considerations will have to be applied to other antigens/MoAbs available in the future. Notably, durable objective responses and tumor regression in relapsed and refractory ALCL patients have recently been reported by brentuximab vedotin (SGN-35), which is a CD30-specific humanized monoclonal antibody conjugated with the antitubulin agent monomethyl auristin E [85]. Such approach has expanded what a previous experience published in 1992 by Falini et al. [86].

Prognostication

In the last few years, some of the studies dealing with the GEP of nodal PTCLs tried to provide for novel insight into PTCL prognostication. First, as mentioned, a few reports suggested that PTCLs/NOS may present with up- or downregulation of NFkB molecules [20, 21, 23], with possible prognostic relevance [21, 23]. In particular, cases with higher levels of NFkB-related molecules or other evidence of NFκ[kappa]B activity showed a better median overall survival (25 months, range 0–124 months, vs. 12 months, range 0–19 months; $p=0.032$) [21, 23]. This observation was then confirmed by another Spanish group, the 5-year OS being 45 % vs. 0 %, in $NFkB^+$ and $NFkB^-$ cases, respectively ($p=0.04$) [67]. However, all these studies included a relatively limited number of cases, by mixing different histotypes [21, 67], or cases with prominent nonneoplastic components [23], which might have influenced, at least in part the results.

In addition, basing on GEP obtained from 35 nodal PTCL cases (23 PTCLs/NOS and 12 AITLs), it was suggested that overexpression of genes involved in a so-called proliferation signature was associated significantly with shorter survival of patients [27]. This proliferation signature included genes commonly associated with the cell cycle, such as *CCNA*, *CCNB, TOP2A*, and *PCNA* [27]. Notably, this evidence of high proliferation as a possible adverse prognostic factor was definitely in line with what was reported by Went et al. [11] and what was observed within the ITCLP [87], highlighting the importance of such parameter.

Finally, our group, basing on GEP analyses, indicated that PTCLs/NOS could be subclassified according to their histogenesis. In particular, at least two subgroups were described, derived from activated helper and cytotoxic elements, respectively [17]. Importantly, such finding was recently confirmed by Iqbal et al. [32]. Intriguingly, in this report, it was also suggested that the cytotoxic profile might be associated with unfavorable outcome, though this evidence was based on a limited series and warrants further validation. On the other hand, a possible more favorable outcome for PTCL cases with helper phenotype had been also previously suggested by others [11, 88, 89].

Overall, GEP studied provided evidences that molecular features may be useful in defining the prognosis of PTCL patients. However, no complete explanation has been offered as far as the molecular bases of drug resistance are concerned. Notably, our group described for the first time the expression of molecules associated to drug resistance in solid tumors such as *CYR61* and *NNMT* in PTCL/NOS [17]. Furthermore, more recently, Rodríguez-Antona et al. found that a high

expression of cytochrome P450 3A (CYP3A), an enzyme involved in the inactivation of chemotherapy drugs, was associated to poor response to the standard PTCL chemotherapy, suggesting that CYP3A could be useful as a predictor of response [90]. Indeed, the molecular classification of PTCLs and the identification of key events in their molecular pathology will be probably the basis for future prognostication and targeted treatment in this field as in the case of DLBCL [91, 92].

Conclusion

PTCLs have represented for a long time an orphan pathology. This can be explained by their relatively low incidence (i.e., anyway higher than that of a "common" tumor, such as mantle cell lymphoma), the difficulties encountered in their analysis, and their dismal prognosis. During the last few years, however, a great deal of interest has developed shedding new light on the pathobiology of these tumors and leading to the proposal of more effective prognosticators. In particular, though IPI is somehow effective for PTCL prognostication, novel more refined and possibly disease-specific prognosticators have been explored, and several models including clinical-pathological and molecular features have been proposed, their validation process being now ongoing. In addition, innovative therapeutic schedules have been recently proposed, based on the application of the newly developed microarray techniques. The morning of a new era seems quite close that will actually dissipate the shadows, which have wrapped PTCLs for several decades.

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References

1. Harris NL, Jaffe ES, Stein H, et al. A revised European-American classification of lymphoid neoplasms: a proposal from the International Lymphoma Study Group. Blood. 1994;84(5):1361–92.
2. A clinical evaluation of the International Lymphoma Study Group classification of non-Hodgkin's lymphoma. The Non-Hodgkin's Lymphoma Classification Project. Blood 1997;89(11):3909–18.
3. Swerdlow Steven H, International Agency for Research on Cancer. WHO classification of tumors of hematopoietic and lymphoid tissues. IVth ed. Lyon: IARC; 2008.
4. Pileri S, Ralfkiaer E, Weisenburger D, et al. Peripheral T-cell lymphoma, not otherwise specified. In: Swerdlow S, Campo E, Harris NL, et al., editors. WHO classification of tumors of hematopoietic and lymphoid tissues. IVth ed. Lyon: IARC; 2008. p. 429.
5. Vose J, Armitage J, Weisenburger D. International peripheral T-cell and natural killer/T-cell lymphoma study: pathology findings and clinical outcomes. J Clin Oncol. 2008;26(25):4124–30.
6. Savage KJ, Harris NL, Vose JM, et al. ALK– anaplastic large-cell lymphoma is clinically and immunophenotypically different from both ALK+ ALCL and peripheral T-cell lymphoma, not otherwise specified: report from the International Peripheral T-Cell Lymphoma Project. Blood. 2008;111(12):5496–504.
7. Evens AM, Gartenhaus RB. Treatment of T-cell non-Hodgkin's lymphoma. Curr Treat Options Oncol. 2004;5(4):289–303.
8. Lopez-Guillermo A, Cid J, Salar A, et al. Peripheral T-cell lymphomas: initial features, natural history, and prognostic factors in a series of 174 patients diagnosed according to the R.E.A.L. Classification. Ann Oncol. 1998;9(8):849–55.
9. Gisselbrecht C, Gaulard P, Lepage E, et al. Prognostic significance of T-cell phenotype in aggressive non-Hodgkin's lymphomas. Groupe d'Etudes des Lymphomes de l'Adulte (GELA). Blood. 1998;92(1):76–82.
10. Effect of age on the characteristics and clinical behavior of non-Hodgkin's lymphoma patients. The Non-Hodgkin's Lymphoma Classification Project. Ann Oncol 1997;8(10):973–8.
11. Went P, Agostinelli C, Gallamini A, et al. Marker expression in peripheral T-cell lymphoma: a proposed clinical-pathologic prognostic score. J Clin Oncol. 2006;24(16):2472–9.
12. Rudiger T, Weisenburger DD, Anderson JR, et al. Peripheral T-cell lymphoma (excluding anaplastic large-cell lymphoma): results from the Non-Hodgkin's Lymphoma Classification Project. Ann Oncol. 2002;13(1):140–9.
13. Zettl A, Rudiger T, Konrad MA, et al. Genomic profiling of peripheral T-cell lymphoma, unspecified, and anaplastic large T-cell lymphoma delineates novel recurrent chromosomal alterations. Am J Pathol. 2004;164(5):1837–48.
14. Oshiro A, Tagawa H, Ohshima K, et al. Identification of subtype-specific genomic alterations in aggressive adult T-cell leukemia/lymphoma. Blood. 2006;107(11):4500–7.
15. Hartmann S, Gesk S, Scholtysik R, et al. High resolution SNP array genomic profiling of peripheral T cell lymphomas, not otherwise specified, identifies a subgroup with chromosomal aberrations affecting the REL locus. Br J Haematol. 2010;148(3):402–12.

16. Ascani S, Zinzani PL, Gherlinzoni F, et al. Peripheral T-cell lymphomas. Clinico-pathologic study of 168 cases diagnosed according to the R.E.A.L. Classification. Ann Oncol. 1997;8(6):583–92.
17. Piccaluga PP, Agostinelli C, Califano A, et al. Gene expression analysis of peripheral T cell lymphoma, unspecified, reveals distinct profiles and new potential therapeutic targets. J Clin Invest. 2007;117(3):823–34.
18. Piva R, Agnelli L, Pellegrino E, et al. Gene expression profiling uncovers molecular classifiers for the recognition of anaplastic large-cell lymphoma within peripheral T-cell neoplasms. J Clin Oncol. 2010;28(9):1583–90.
19. Tracey L, Villuendas R, Dotor AM, et al. Mycosis fungoides shows concurrent deregulation of multiple genes involved in the TNF signaling pathway: an expression profile study. Blood. 2003;102(3):1042–50.
20. Martinez-Delgado B, Melendez B, Cuadros M, et al. Expression profiling of T-cell lymphomas differentiates peripheral and lymphoblastic lymphomas and defines survival related genes. Clin Cancer Res. 2004;10(15):4971–82.
21. Martinez-Delgado B, Cuadros M, Honrado E, et al. Differential expression of NF-kappaB pathway genes among peripheral T-cell lymphomas. Leukemia. 2005;19(12):2254–63.
22. Piccaluga PP, Agostinelli C, Zinzani PL, Baccarani M, Dalla Favera R, Pileri SA. Expression of platelet-derived growth factor receptor alpha in peripheral T-cell lymphoma not otherwise specified. Lancet Oncol. 2005;6(6):440.
23. Ballester B, Ramuz O, Gisselbrecht C, et al. Gene expression profiling identifies molecular subgroups among nodal peripheral T-cell lymphomas. Oncogene. 2006;25(10):1560–70.
24. Mahadevan D, Spier C, Della Croce K, et al. Transcript profiling in peripheral T-cell lymphoma, not otherwise specified, and diffuse large B-cell lymphoma identifies distinct tumor profile signatures. Mol Cancer Ther. 2005;4(12):1867–79.
25. Lamant L, de Reynies A, Duplantier MM, et al. Gene-expression profiling of systemic anaplastic large-cell lymphoma reveals differences based on ALK status and two distinct morphologic ALK+ subtypes. Blood. 2007;109(5):2156–64.
26. de Leval L, Rickman DS, Thielen C, et al. The gene expression profile of nodal peripheral T-cell lymphoma demonstrates a molecular link between angioimmunoblastic T-cell lymphoma (AITL) and follicular helper T (TFH) cells. Blood. 2007;109(11):4952–63.
27. Cuadros M, Dave SS, Jaffe ES, et al. Identification of a proliferation signature related to survival in nodal peripheral T-cell lymphomas. J Clin Oncol. 2007;25(22):3321–9.
28. Piccaluga PP, Agostinelli C, Califano A, et al. Gene expression analysis of angioimmunoblastic lymphoma indicates derivation from T follicular helper cells and vascular endothelial growth factor deregulation. Cancer Res. 2007;67(22):10703–10.
29. Miyazaki K, Yamaguchi M, Imai H, et al. Gene expression profiling of peripheral T-cell lymphoma including gammadelta T-cell lymphoma. Blood. 2009;113(5):1071–4.
30. Pise-Masison CA, Radonovich M, Dohoney K, et al. Gene expression profiling of ATL patients: compilation of disease-related genes and evidence for TCF4 involvement in BIRC5 gene expression and cell viability. Blood. 2009;113(17):4016–26.
31. Huang Y, de Reynies A, de Leval L, et al. Gene expression profiling identifies emerging oncogenic pathways operating in extranodal NK/T-cell lymphoma, nasal-type. Blood. 2010;115(6):1226–37.
32. Iqbal J, Weisenburger DD, Greiner TC, et al. Molecular signatures to improve diagnosis in peripheral T-cell lymphoma and prognostication in angioimmunoblastic T-cell lymphoma. Blood. 2010;115(5):1026–36.
33. Dave SS, Wright G, Tan B, et al. Prediction of survival in follicular lymphoma based on molecular features of tumor-infiltrating immune cells. N Engl J Med. 2004;351(21):2159–69.
34. Steidl C, Lee T, Shah SP, et al. Tumor-associated macrophages and survival in classic Hodgkin's lymphoma. N Engl J Med. 2010;362(10):875–85.
35. Feldman AL, Dogan A, Smith DI, et al. Discovery of recurrent t(6;7)(p25.3;q32.3) translocations in ALK-negative anaplastic large cell lymphomas by massively parallel genomic sequencing. Blood. 2011; 117(3):915–9.
36. Watson A, Mazumder A, Stewart M, Balasubramanian S. Technology for microarray analysis of gene expression. Curr Opin Biotechnol. 1998;9(6):609–14.
37. Dupuis J, Boye K, Martin N, et al. Expression of CXCL13 by neoplastic cells in angioimmunoblastic T-cell lymphoma (AITL): a new diagnostic marker providing evidence that AITL derives from follicular helper T cells. Am J Surg Pathol. 2006; 30(4):490–4.
38. Grogg KL, Attygale AD, Macon WR, Remstein ED, Kurtin PJ, Dogan A. Expression of CXCL13, a chemokine highly upregulated in germinal center T-helper cells, distinguishes angioimmunoblastic T-cell lymphoma from peripheral T-cell lymphoma, unspecified. Mod Pathol. 2006;19(8):1101–7.
39. Roncador G, Garcia Verdes-Montenegro JF, Tedoldi S, et al. Expression of two markers of germinal center T cells (SAP and PD-1) in angioimmunoblastic T-cell lymphoma. Haematologica. 2007;92(8):1059–66.
40. Marafioti T, Paterson JC, Ballabio E, et al. The inducible T-cell co-stimulator molecule is expressed on subsets of T cells and is a new marker of lymphomas of T follicular helper cell-derivation. Haematologica. 2010;95(3):432–9.
41. Laurent C, Fazilleau N, Brousset P. A novel subset of T-helper cells: follicular T-helper cells and their markers. Haematologica. 2010;95(3):356–8.
42. Rudiger T, Geissinger E, Muller-Hermelink HK. 'Normal counterparts' of nodal peripheral T-cell lymphoma. Hematol Oncol. 2006;24(4):175–80.
43. Huang Y, Moreau A, Dupuis J, et al. Peripheral T-cell lymphomas with a follicular growth pattern are derived from follicular helper T cells (TFH) and may show

overlapping features with angioimmunoblastic T-cell lymphomas. Am J Surg Pathol. 2009;33(5):682–90.
44. Tapper J, Kettunen E, El-Rifai W, Seppala M, Andersson LC, Knuutila S. Changes in gene expression during progression of ovarian carcinoma. Cancer Genet Cytogenet. 2001;128(1):1–6.
45. Sado Y, Kagawa M, Naito I, et al. Organization and expression of basement membrane collagen IV genes and their roles in human disorders. J Biochem (Tokyo). 1998;123(5):767–76.
46. van den Boom J, Wolter M, Kuick R, et al. Characterization of gene expression profiles associated with glioma progression using oligonucleotide-based microarray analysis and real-time reverse transcription-polymerase chain reaction. Am J Pathol. 2003;163(3):1033–43.
47. Jin S, Tong T, Fan W, et al. GADD45-induced cell cycle G2-M arrest associates with altered subcellular distribution of cyclin B1 and is independent of p38 kinase activity. Oncogene. 2002;21(57):8696–704.
48. Papa S, Zazzeroni F, Bubici C, et al. Gadd45 beta mediates the NF-kappa B suppression of JNK signalling by targeting MKK7/JNKK2. Nat Cell Biol. 2004;6(2):146–53.
49. Chen F, Lu Y, Zhang Z, et al. Opposite effect of NF-kappa B and c-Jun N-terminal kinase on p53-independent GADD45 induction by arsenite. J Biol Chem. 2001;276(14):11414–9.
50. Hirose T, Sowa Y, Takahashi S, et al. p53-independent induction of Gadd45 by histone deacetylase inhibitor: coordinate regulation by transcription factors Oct-1 and NF-Y. Oncogene. 2003;22(49):7762–73.
51. Tan KO, Tan KM, Chan SL, et al. MAP-1, a novel proapoptotic protein containing a BH3-like motif that associates with Bax through its Bcl-2 homology domains. J Biol Chem. 2001;276(4):2802–7.
52. Nagashima M, Shiseki M, Pedeux RM, et al. A novel PHD-finger motif protein, p47ING3, modulates p53-mediated transcription, cell cycle control, and apoptosis. Oncogene. 2003;22(3):343–50.
53. Gunduz M, Ouchida M, Fukushima K, et al. Allelic loss and reduced expression of the ING3, a candidate tumor suppressor gene at 7q31, in human head and neck cancers. Oncogene. 2002;21(28):4462–70.
54. Lee MS, Hanspers K, Barker CS, Korn AP, McCune JM. Gene expression profiles during human CD4+ T cell differentiation. Int Immunol. 2004;16(8): 1109–24.
55. Chtanova T, Newton R, Liu SM, et al. Identification of T cell-restricted genes, and signatures for different T cell responses, using a comprehensive collection of microarray datasets. J Immunol. 2005;175(12):7837–47.
56. Chtanova T, Tangye SG, Newton R, et al. T follicular helper cells express a distinctive transcriptional profile, reflecting their role as non-Th1/Th2 effector cells that provide help for B cells. J Immunol. 2004;173(1):68–78.
57. Hosack DA, Dennis Jr G, Sherman BT, Lane HC, Lempicki RA. Identifying biological themes within lists of genes with EASE. Genome Biol. 2003;4(10):R70.
58. Han JS, Macarak E, Rosenbloom J, Chung KC, Chaqour B. Regulation of Cyr61/CCN1 gene expression through RhoA GTPase and p38MAPK signaling pathways. Eur J Biochem. 2003;270(16):3408–21.
59. Leu SJ, Liu Y, Chen N, Chen CC, Lam SC, Lau LF. Identification of a novel integrin alpha 6 beta 1 binding site in the angiogenic inducer CCN1 (CYR61). J Biol Chem. 2003;278(36):33801–8.
60. Schober JM, Lau LF, Ugarova TP, Lam SC. Identification of a novel integrin alphaMbeta2 binding site in CCN1 (CYR61), a matricellular protein expressed in healing wounds and atherosclerotic lesions. J Biol Chem. 2003;278(28):25808–15.
61. Tsai MS, Bogart DF, Castaneda JM, Li P, Lupu R. Cyr61 promotes breast tumorigenesis and cancer progression. Oncogene. 2002;21(53):8178–85.
62. Tsai MS, Hornby AE, Lakins J, Lupu R. Expression and function of CYR61, an angiogenic factor, in breast cancer cell lines and tumor biopsies. Cancer Res. 2000;60(20):5603–7.
63. Lin MT, Chang CC, Chen ST, et al. Cyr61 expression confers resistance to apoptosis in breast cancer MCF-7 cells by a mechanism of NF-kappaB-dependent XIAP up-regulation. J Biol Chem. 2004;279(23):24015–23.
64. Kassem H, Sangar V, Cowan R, Clarke N, Margison GP. A potential role of heat shock proteins and nicotinamide N-methyl transferase in predicting response to radiation in bladder cancer. Int J Cancer. 2002; 101(5):454–60.
65. Xu J, Capezzone M, Xu X, Hershman JM. Activation of nicotinamide N-methyltransferase gene promoter by hepatocyte nuclear factor-1{beta} in human papillary thyroid cancer cells. Mol Endocrinol. 2005; 19(2):527–39.
66. Piccaluga P, Agostinelli C, Righi S, et al. Expression of classical NF-kappa B pathway molecules in peripheral t-cell lymphoma not otherwise specified. In: 10th international conference on malignant lymphoma, Lugano, 4–6 June 2008, p. 231.
67. Briones J, Moga E, Espinosa I, et al. Bcl-10 protein highly correlates with the expression of phosphorylated p65 NF-kappaB in peripheral T-cell lymphomas and is associated with clinical outcome. Histopathology. 2009;54(4):478–85.
68. Yang J, Liu X, Nyland SB, et al. Platelet-derived growth factor mediates survival of leukemic large granular lymphocytes via an autocrine regulatory pathway. Blood. 2010;115(1):51–60.
69. Chiarle R, Simmons WJ, Cai H, et al. Stat3 is required for ALK-mediated lymphomagenesis and provides a possible therapeutic target. Nat Med. 2005;11(6):623–9.
70. Chiarle R, Martinengo C, Mastini C, et al. The anaplastic lymphoma kinase is an effective oncoantigen for lymphoma vaccination. Nat Med. 2008;14(6):676–80.
71. Duvic M, Talpur R, Ni X, et al. Phase 2 trial of oral vorinostat (suberoylanilide hydroxamic acid, SAHA) for refractory cutaneous T-cell lymphoma (CTCL). Blood. 2007;109(1):31–9.
72. Sanchez-Gonzalez B, Yang H, Bueso-Ramos C, et al. Antileukemia activity of the combination of an

anthracycline with a histone deacetylase inhibitor. Blood. 2006;108(4):1174–82.
73. Strupp C, Aivado M, Germing U, Gattermann N, Haas R. Angioimmunoblastic lymphadenopathy (AILD) may respond to thalidomide treatment: two case reports. Leuk Lymphoma. 2002;43(1):133–7.
74. Bruns I, Fox F, Reinecke P, et al. Complete remission in a patient with relapsed angioimmunoblastic T-cell lymphoma following treatment with bevacizumab. Leukemia. 2005;19(11):1993–5.
75. Dogan A, Ngu LS, Ng SH, Cervi PL. Pathology and clinical features of angioimmunoblastic T-cell lymphoma after successful treatment with thalidomide. Leukemia. 2005;19(5):873–5.
76. Ramasamy K, Lim Z, Pagliuca A, Salisbury JR, Mufti GJ, Devereux S. Successful treatment of refractory angioimmunoblastic T-cell lymphoma with thalidomide and dexamethasone. Haematologica. 2006;91(8 Suppl):ECR44.
77. Aguiar Bujanda D. Complete response of relapsed angioimmunoblastic T-cell lymphoma following therapy with bevacizumab. Ann Oncol. 2008;19(2):396–7.
78. Gottardi M, Danesin C, Canal F, et al. Complete remission induced by thalidomide in a case of angioimmunoblastic T-cell lymphoma refractory to autologous stem cell transplantation. Leuk Lymphoma. 2008;49(9):1836–8.
79. Kim YH, Duvic M, Obitz E, et al. Clinical efficacy of zanolimumab (HuMax-CD4): two phase 2 studies in refractory cutaneous T-cell lymphoma. Blood. 2007;109(11):4655–62.
80. Enblad G, Hagberg H, Erlanson M, et al. A pilot study of alemtuzumab (anti-CD52 monoclonal antibody) therapy for patients with relapsed or chemotherapy-refractory peripheral T-cell lymphomas. Blood. 2004;103(8):2920–4.
81. Gallamini A, Zaja F, Patti C, et al. Alemtuzumab (Campath-1H) and CHOP chemotherapy as first-line treatment of peripheral T-cell lymphoma: results of a GITIL (Gruppo Italiano Terapie Innovative nei Linfomi) prospective multicenter trial. Blood. 2007;110(7):2316–23.
82. Piccaluga PP, Agostinelli C, Righi S, Zinzani PL, Pileri SA. Expression of CD52 in peripheral T-cell lymphoma. Haematologica. 2007;92(4):566–7.
83. Rodig SJ, Abramson JS, Pinkus GS, et al. Heterogeneous CD52 expression among hematologic neoplasms: implications for the use of alemtuzumab (CAMPATH-1H). Clin Cancer Res. 2006;12(23):7174–9.
84. Chang ST, Lu CL, Chuang SS. CD52 expression in non-mycotic T- and NK/T-cell lymphomas. Leuk Lymphoma. 2007;48(1):117–21.
85. Younes A, Bartlett NL, Leonard JP, et al. Brentuximab vedotin (SGN-35) for relapsed CD30-positive lymphomas. N Engl J Med. 2011;363(19):1812–21.
86. Falini B, Bolognesi A, Flenghi L, et al. Response of refractory Hodgkin's disease to monoclonal anti-CD30 immunotoxin. Lancet. 1992;339(8803):1195–6.
87. Weisenburger DD, Savage KJ, Harris NL, et al. Peripheral T-cell lymphoma, not otherwise specified: a report of 340 cases from the International Peripheral T-cell Lymphoma Project. Blood. 2011;117(12):3402–8.
88. Bekkenk MW, Vermeer MH, Jansen PM, et al. Peripheral T-cell lymphomas unspecified presenting in the skin: analysis of prognostic factors in a group of 82 patients. Blood. 2003;102(6):2213–9.
89. Kojima H, Hasegawa Y, Suzukawa K, et al. Clinicopathological features and prognostic factors of Japanese patients with "peripheral T-cell lymphoma, unspecified" diagnosed according to the WHO classification. Leuk Res. 2004;28(12):1287–92.
90. Rodriguez-Antona C, Leskela S, Zajac M, et al. Expression of CYP3A4 as a predictor of response to chemotherapy in peripheral T-cell lymphomas. Blood. 2007;110(9):3345–51.
91. Agostinelli C, Piccaluga PP, Went P, et al. Peripheral T cell lymphoma, not otherwise specified: the stuff of genes, dreams and therapies. J Clin Pathol. 2008;61(11):1160–7.
92. Lenz G, Staudt LM. Aggressive lymphomas. N Engl J Med. 2010;362(15):1417–29.

Small Lymphocytic Lymphoma/ Chronic Lymphocytic Leukemia

4

Emili Montserrat

Abstract

Small lymphocytic lymphoma (SLL)/chronic lymphocytic leukemia (CLL) is due to the accumulation of mature B cell with a distinctive immunophenotype. SLL/CLL is extremely heterogeneous from the biologic and clinical points of view, with different clinico-biologic forms of the disease being recognized. Management of patients with SLL/CLL is based on an individualized approach that takes into account both patient's and disease's characteristics. In the last decades, important progress has been made in the treatment of CLL/SLL, resulting in an important improvement in patients' outlook. Although the cure of the disease is elusive, further progress in therapy based on treatments targeting disease-specific pathogenic pathways is already on the horizon.

Keywords

Small lymphocytic lymphoma • Chronic lymphocytic leukemia • Diagnosis • Prognosis • Treatment

Introduction

Small lymphocytic lymphoma (SLL) and chronic lymphocytic leukemia (CLL) are due to the accumulation in bone marrow, peripheral blood, and lymphoid tissues of monoclonal B lymphocytes with a distinct immunophenotype. SLL and CLL are considered as the same mature B-cell neoplasm that mainly differ in the extent to which the tumor involves lymphoid tissue (SLL) or blood (CLL) [1]. Within the SLL/CLL spectrum, about 10 % of cases present as SLL and 90 % as CLL.

SLL/CLL is a heterogeneous disorder from both the biologic and the clinical points of view. The median survival of patients with SLL/CLL is around 10 years, but the individual prognosis is highly variable, ranging from a few months to a normal life span. In spite of important progress in its therapy, SLL/CLL is incurable.

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Epidemiology

The median age of patients is 72 years. Males are more affected than females (1.5:1). Most patients are older than 65 years, and only 10–15 % are under the age of 50 years. The incidence of the disease is 4–5/100,000 persons/year and increases dramatically with age to more than 30/100,000 in people older than 80 years [2, 3]. In Japan, China, and other Eastern countries, as well as in Africa, SLL/CLL seems to constitute an infrequent disorder [4, 5].

Ethiopathogenesis

The cause of SLL/CLL is unknown. There is no demonstrated, clear-cut relationship between ambiental factors and SLL/CLL, and a long-pursued infectious/inflammatory origin of the disease has never been proved. First-degree relatives of patients with CLL have an estimated four to six-fold higher possibility of presenting CLL than the normal population. Moreover, around 10 % of first-degree relatives of patients with CLL present in their blood a monoclonal B-cell population whose immunophenotype is very similar, if not identical, to that observed in CLL (see monoclonal B-cell lymphocytosis, MBL, in section "Diagnosis"). This, coupled with differences in the incidence of the disease according to the race, points out to a genetic predisposition [6]. Interestingly, in familial cases, the diagnosis tends to be made at an earlier age in each subsequent generation ("anticipation phenomenon") [7]. Genome-wide association studies have identified susceptibility loci for risk of familial CLL, for example, at 16q24.1 and 6q21.3 [8, 9].

A detailed analysis of the complex biology of SLL/CLL is beyond the scope of this chapter (see Chaps. 1 and 2 and references [10–15] for reviews). CLL is characterized by the accumulation of a monoclonal subpopulation of antigen-experienced, activated B cells. The cell of origin of CLL is a matter of debate. Immunophenotypically, CLL cells express surface membrane immunoglobulin (SmIg), usually of IgM or both IgM and IgD types, in small amounts and a single Ig light chain (κ[kappa] or λ[lamda]). They also express CD5, HLADR, and B-cell antigens (e.g., CD19, CD20); in most cases they are CD23+, whereas CD22 and CD79b are infrequently or weakly expressed. Altogether, these cells immunophenotypically resemble those normally present in the mantle zone of lymphoid follicles. Because of this, it has been generally accepted that CLL can have its origin in the mantle zone of lymphoid follicles. However, it has been recently demonstrated that CLL hematopoietic stem cells (CLL-HSC) may play a crucial role in the ethiopathogenesis of the disease [16]. CLL-HSCs cell would produce, first, a high number of polyclonal B cells; subsequently, B-cell clones would be selected and expanded giving origin to an MBL-like picture. Next, accumulation of genetic alterations might cause transformation of a small proportion of MBL clones into CLL, plausible candidates for this effect being the deletion of miR15 and miR16 in chromosome 13q14 [17–20].

The majority of CLL cells are arrested in the G0–G1 phase of the cell cycle, but there is also a fraction of cells that actively multiply in the proliferation centers ("pseudofollicles") of lymph nodes [21]. The neoplastic B lymphocytes from CLL express large amounts of antiapoptotic BCL2 and MCL1 proteins, whereas the proapoptotic BCLX proteins are decreased. This, together with the interaction of the neoplastic cells with the "microenvironment" (an admixture of T cells, "nurse-like" cells, and macrophages) in lymph nodes and bone marrow through several soluble factors, leads to the accumulation of leukemic cells in the organism. Finally, *IGVH* genes can be either unmutated or mutated [22, 23]. Since somatic mutation of the *IGVH* genes takes place in the germinal center of lymphoid follicles, CLL can be either a tumor of pre-germinal-center B cells or a tumor of post-germinal B cells. As discussed later (see section "Prognosis"), although these two forms share an almost identical genetic signature as determined by microarrays, they have different clinical behavior; because of this, CLL is considered to be a single disease with two variants (i.e., mutated, unmutated) [24]. As the biology of CLL unfolds, other forms or variants will be surely described.

Clinical Features

Currently, most patients are diagnosed while asymptomatic on the occasion of a blood analysis performed for trivial or routine reasons. As a result, clinical features at diagnosis have significantly changed as compared to those observed in older series in patients diagnosed due to symptomatic disease. The main demographic, clinical, and biologic features in 699 unselected patients from the Hospital Clinic of Barcelona diagnosed between 1995 and 2010 are shown in Table 4.1.

In contrast to patients with lymphoma, malaise, and fatigue, general symptoms (i.e., fever, night sweats, weight loss) are rare at diagnosis. Painless generalized peripheral lymphadenopathy (i.e., neck, axillae, inguinal) is frequent in symptomatic cases. In contrast, mediastinal and retroperitoneal lymphadenopathy are infrequently seen. On some occasions, the investigation of bacterial or herpes virus infections may lead to the diagnosis. Rarely, the disease is discovered during the diagnostic workup of an autoimmune hemolytic anemia (AIHA) or, even less frequently, an immune thrombocytopenia (ITP).

A peculiar feature in some patients is severe reactions to insect, mainly mosquitoes, bites [25]. Rarely, CLL may involve extra-hematological tissues such as skin, liver, kidney, or central nervous system (CNS); in such cases, however, disease transformation needs to be ruled out. In addition, vasculitis, hypercalcemia, and nephrotic syndrome are occasionally observed. Spontaneous regression of the disease can be observed in 1 % of patients per year [26, 27].

Table 4.1 Main clinical and laboratory characteristics of 699 patients with CLL diagnosed between 1995 and 2010 at the Hospital Clinic, Barcelona

	N = 699
Age, years; median (range)	64 (28–97)
Patients >65 years	49 %
Patients <50 years	19 %
Sex, males	60 %
Clinical stage (Binet)	
A	81 %
B	13 %
C	6 %
B-symptoms	6 %
ECOG	
0	88 %
1	8 %
2	3 %
3	1 %
Hb (g/dl), median (range)	13.6 (4.5–18.5)
Hb < 11 g/dl	7 %
Platelets (×1,000/μl), median (range)	201 (11—573)
Platelets <100,000/μl	3 %
WBC count (×1,000/μl), median (range)	29.8 (3.2—461)
WBC count >50.000/μl	12 %
Lymphadenopathy[a]	40 %
Splenomegaly[a]	14 %
Hepatomegaly[a]	5 %
DAT (Coombs) positive	7 %
LDH >450 U/l	11 %
Beta2m. >2.5 g/l	48 %
ZAP-70 positive	35 %
CD38 positive	33 %
FISH	
Normal	30 %
Del13q	34 %
+12	14 %
Del11q	10 %
Del17p	5 %
IGVH unmutated	55 %

[a]Lymphadenopathy, splenomegaly, and hepatomegaly as clinically assessed

Laboratory Features

The hallmark of the disease is an increased WBC count with a high percentage (80–90 %) of small, mature-looking lymphocytes (see section "Diagnosis"). In patients diagnosed on the occasion of a routine analysis, anemia is found in less than 10 % of the patients. Importantly, anemia is not always due to the infiltration of the bone marrow by the disease; other causes such as autoimmunity, iron, folic acid, or vitamin B12 deficiency need to be taken into account. Likewise, a marked thrombocytopenia (e.g., <20,000/μl) should raise the possibility of an immune-mediated origin, particularly in the absence of anemia. Hypoglobulinemia is frequent (30 % of patients) and tends to worsen over the course of the disease. Serum immunofixation can demonstrate an M component (usually of the IgM type) in around 10–15 % of patients. A positive

DAT test is observed in around 5 % of the patients at the time of diagnosis, with clinically apparent AIHA being less frequent.

The lymph nodes show involvement by small lymphocytes and, as a distinctive feature, proliferation centers ("pseudofollicles"). The bone marrow displays a variable degree of infiltration by the disease; in contrast to follicular lymphoma, there is no paratrabecular infiltration.

In disparity with what is observed in lymphoma, reciprocal balanced chromosomal translocations are extremely rare. However, chromosomal deletions and amplifications can be detected by fluorescence in situ hybridization (FISH) in up to 90 % of patients [28–36]. The most relevant genetic abnormality are del(13q) as isolated abnormality (14–60 % of cases), del(11q) (10–32 %), trisomy 12 (11–18 %), del (17p) (3–27 %), and del (6q) (2–9 %), depending on the time point at which the study is performed and whether or not the disease is resistant to therapy. Deletions of 17p convey dysfunction of the *TP53* gene. Importantly, 4–5 % of patients may have a *TP53* mutation in the absence of del(17p). Likewise, deletions and mutations of chromosome 11q may imply loss of the *ATM* function. Translocations are rare but cases of t(14;19)(q32;q13) involving the *Ig* gene and BCL3 loci can be observed [28–37].

The application of high-resolution genomic techniques allows demonstration of abnormalities that cannot be detected with either FISH or standard karyotyping. For example, around 10–15 % of patients can present mutations of *NOTCH-1* or of the RNA splicing factor *SF3B1*, which are associated with disease transformation, resistance to fludarabine therapy, and short survival. Moreover, *NOTCH-1* mutations predominate in patients with trisomy 12 [38–46].

Cytogenetic abnormalities, using the hierarchical model proposed by Döhner et al. [31], correlate with several clinical and biologic features which can be summarized as follows:

- Deletion 13q (isolated): early, nonprogressive disease, good prognosis
- Deletion 11q: younger, male patients with bulky lymphadenopathy in both peripheral and abdominal regions, aggressive disease, *SF3B1* mutations, poor response to monotherapy, short progression-free survival
- Deletions 17p/mutations *TP53*: progressive disease resistant to conventional therapy
- Trisomy 12: atypical morphology (e.g., increased percentage of prolymphocytes) and immunophenotype (e.g., FMC7 positivity, CD5 negativity, strong SmIg and CD38) on neoplastic lymphocytes, *NOTCH-1* mutations
- Deletion 6q: blood lymphocytes presenting lymphoplasmacytoid features

Recently discovered genetic abnormalities also show important clinical and treatment correlates [38–46]:

- *NOTCH-1* mutations: disease transformation, resistance to therapy, poor prognosis
- *SF3B1* mutations: poor prognosis

Complications

Patients with CLL may develop several complications of which physicians need to be aware [47].

Autoimmune Cytopenias

AIHA occurs in around 10 % of patients and ITP in 5–7 %. AIHA can appear before the diagnosis of CLL is made, spontaneously over the course of the disease or be triggered by treatment. Most patients with CLL and AIHA have anemia with positive DAT in the context of reticulocytosis, raised bilirubin, and low haptoglobins serum levels; serum LDH is less discriminating since it may be elevated due to active CLL. Moreover, "DAT negative AIHA" can be seen, particularly in association with therapy. Likewise, reticulocytosis may not be striking in the context of a bone marrow overwhelmed by leukemic cells or in patients under therapy [48–51].

ITP is most commonly an incidental finding on a routine blood count [50, 51]. Diagnosing ITP may pose difficulties, particularly because there is no sensitive and specific diagnostic test. Nevertheless, thrombocytopenia can be considered as immune mediated when there is a sudden profound fall in platelets (>50 % fall to a platelet count <100,000/μl) in the absence of splenomegaly, infection, or chemotherapy and with abundant

megakaryocytes in the bone marrow. In advanced disease, anemia usually occurs before thrombocytopenia; hence, isolated thrombocytopenia is more likely to be immune in origin. Response to corticosteroids may be the final, post hoc, diagnostic test. On some occasions AIHA and ITP are found together (i.e., Evan's syndrome).

Erythroblastopenia is defined by the lack, or maturation arrest, of red blood cell precursors in bone marrow, anemia, and low absolute reticulocyte count. In the presence of anemia, the reticulocyte percentage can be misleadingly normal, the reticulocyte percentage corrected according to the hematocritic, and the absolute reticulocyte count being more informative. The bone marrow shows characteristic defects of erythroblast maturation. In the presence of a bone marrow heavily infiltrated by lymphocytes, the identification of red cell precursors can be difficult. In this setting, an anti-glycophorin immunohistochemistry may facilitate the identification of red cell precursors. Importantly, PRCA can be seen in association with other immune cytopenias, particularly AIHA. In addition, any patient with CLL and anemia with a low reticulocyte count should be evaluated for viral infections which can be associated with PRCA, namely, cytomegalovirus, Epstein–Barr virus, and parvovirus [50, 51].

Disease Transformation

CLL can transform into B-cell diffuse large cell lymphoma (DLCL) (Richter's syndrome, RS), the risk being of around 10 % at 10 years from diagnosis. RS is extremely heterogeneous in its biology and prognosis [52–54]. Because of its prognostic implications, the most important distinction is between clonally related and clonally unrelated transformation. RS, particularly when clonally related, is frequently accompanied by the acquisition of genetic alterations (e.g., *C-MYC*, *TP53*, and *NOTCH-1* mutations) which may account for refractoriness to treatment.

Disease transformation should be suspected whenever the patient suffers an abrupt worsening of the general status, fever, enlarging lymph nodes, or extranodal involvement; also, a sudden and rapidly rising serum LDH is an important clue to suspect disease transformation. However, the diagnosis may be difficult because RS can be localized in an isolate organ (e.g., spleen, lymph nodes of a given territory, extralymphatic tissue). Moreover, the diagnosis needs to be confirmed by biopsy. In this regard, and in contrast to uncomplicated CLL, PET/CT can show areas of hyperactivity corresponding to the transformed tissue and be therefore of help in guiding the site to be biopsied [55]. In some cases, EBV infection can be demonstrated in the involved tissue. The prognosis of RS is generally poor and highly depends on whether the disease is clonally related or unrelated to CLL (median survival 14 vs. 62 months, respectively) [54].

Besides DLCL, cases of transformation into Hodgkin's lymphoma can be observed, their prognosis being better than that of transformation into DLCL [56].

Second Neoplasias

Around 10–15 % of patients with CLL present other cancers, the risk being significantly higher (relative risk, 2) than in the general population. Melanoma, lung carcinoma, lymphoma, Kaposi sarcoma, and CNS and gastrointestinal tumors are the cancer types most frequently observed [57–60]. Patients with CLL have a substantially increased risk for a rare skin tumor known as Merkel cell carcinoma and vice versa [61]. There is no relationship between the characteristics of the disease and its treatment and the incidence of secondary solid tumors. Physicians treating patients with CLL should keep in mind the possibility of a second tumor whenever a given patient presents with unexpected, unusual symptoms.

Infections

Infections are very frequent and a common cause of death [62–64]. Their pathogenesis is multifactorial, including hypogammaglobulinemia, immunosuppression, and treatment-related myelotoxicity. With chlorambucil, most infections are bacterial and frequently involve the respiratory tract. The pathogenesis of infections

with purine analogs is related to the quantitative and qualitative T-cell abnormalities induced by these agents, with herpes virus infections being very frequent. Infections by *Pneumocystis*, *Listeria*, *Mycobacteria*, *Aspergillus*, and *Candida* can be also observed [62–64]. The use of alemtuzumab is frequently complicated (10–25 % of patients) by CMV reactivation, which deserves close monitoring and preemptive treatment. Likewise reactivation of HBV and HCV infection may occur under therapy with immunosuppressive drugs. Chronic HBV carriers, as defined by a positive surface antigen, undergoing therapy should receive prophylactic treatment to prevent HBV reactivation.

Diagnosis

Chronic Lymphocytic Leukemia

The diagnosis of CLL is based on the presence in blood of more than 5,000 monoclonal B-cell lymphocytes/μl with a distinctive immunophenotype (i.e., CD5+, CD19+, CD20−/+, CD23) persisting for at least 3 months. The clonality of the circulating B lymphocytes needs to be confirmed by flow cytometry [1, 65].

The leukemia cells seen in the blood smear are characteristically small, mature-looking lymphocytes with a narrow border of cytoplasm and a dense nucleus lacking discernible nucleoli and having partially aggregated chromatin. CLL cells are extremely fragile and can become partially broken during the preparation of a blood smears for staining; these cells are known as "basket," "smudge" of "Gumprecht cells" and, although unspecific, are typical of CLL.

Immunophenotypically, CLL cells co-express the CD5 antigen and B-cell surface antigens CD19, CD20, and CD23. The levels of surface immunoglobulin and CD20 are characteristically low compared to normal B cells. Each clone of leukemia cells is restricted to expression of either kappa or lambda immunoglobulin light chains.

Bone marrow aspiration and biopsy is not required to establish the diagnosis. However, it may be necessary in selected cases, for example, to assess the origin of blood cytopenias (bone marrow "failure" vs. immune, "peripheral" mechanisms).

Usually, the diagnosis is straightforward and does not present difficulties. However, non-Hodgkin's lymphoma in leukemic phase, particularly mantle cell lymphoma, lymphoplasmacytoid lymphoma (immunocytoma), and marginal zone lymphoma, can mimic CLL. Although in CLL a small percentage (e.g., 10–20 %) of atypical lymphocytes (e.g., prolymphocytes, lymphoplasmacytoid cells, cleaved cells or centrocytes) can be present in blood, the diagnosis of "atypical" CLL should not be accepted without having excluded a lymphoma in leukemic phase. The presence of more than 55 % prolymphocytes is a feature of prolymphocytic leukemia, a disorder which however in most cases corresponds to mantle cell lymphoma in leukemic phase. Immunophenotyping of the leukemic cells is useful in the differential diagnosis. In particularly difficult cases, the biopsy of involved lymph nodes, bone marrow, as well as genetic and molecular studies can be of help (Table 4.2).

Small Lymphocytic Lymphoma

The diagnosis of SLL requires the presence of lymphadenopathy, organomegaly, cytopenias, or other disease-related features with <5,000 monoclonal B lymphocytes/μl in blood. SLL cells show the same immunophenotype as CLL. The diagnosis should be confirmed by the histopathologic evaluation of a lymph node or another tissue biopsy [1, 60]. Some cases initially diagnosed as SLL can evolve over time to CLL.

Monoclonal B-Cell Lymphocytosis

In absence of lymphadenopathy, organomegaly, cytopenias, and clinical symptoms, the presence of fewer than 5,000 monoclonal B lymphocytes/μl in blood is defined as MBL [65–69]. Importantly, MBL always precedes but does not always progress to CLL, which occurs in only 1–2 % cases per year. Different forms of MBL are recognized, namely, (1) CLL-like, (2) atypical CLL, and (3) CD5-negative MBL.

Table 4.2 CLL and other B-cell chronic lymphoproliferative disorders: immunophenotype, genetic, and molecular characteristics

	SMIG	CD20	CD5	CD10	CD23	CD11C	CD25	CD103	Other
CLL	–/+	–/+	+	–	+	–/+	–	–	CD19(+), FMC7(–)
PL	+	+	–/+	–/+	–/+	–	+	–	TP53 mutations
HCL	+	+	–	–	–	+	+	+	Annexin A1 (+), DBA44(+), T-bet[a] TRAP[b], t(2;6), t(2;7) BRAFF (V600E) mutated
LPL	+	+	–	–	–		+/–	–	Ig cytoplasmic (+), trisomy 4, MYD88 mutations
SMZL	+	+	–/+	–	–/+	–/+	–/+	–	Bcl-2(+); del(7q32)
MCL	+	+	+	–	–	–	–/+	–	t(11;14)(q13;q32); cyclin D1(+); SOX11 (+)
FL	+	+	–	+/–	–/+	–	+	–	t(14;18); Bcl-2 +

CLL chronic lymphocytic leukemia, *PL* prolymphocytic leukemia, *HCL* Hairy cell leukemia, *LPL* lymphoplasmacytic lymphoma, *SMZL* splenic marginal zone lymphoma, *MCL* mantle cell lymphoma, *FL* follicular lymphoma, *FMC7* CD20 epitope

[a]Monoclonal antibodies for immunohistochemical studies

[b]TRAP, tartrate-resistant acid phosphatase

Prognosis

Survival of patients with CLL is highly variable. There are patients in whom the disease runs a rapidly evolving clinical course and die shortly after diagnosis, while others have a survival not different from that of the general population and eventually die because of causes not related to CLL.

Clinical staging systems, which roughly reflect the progressive accumulation of lymphocytes in the organism over time, are the backbone for prognostication [70, 71] (Table 4.3; Fig. 4.1). Clinical stages, however, have some limitations. First, the majority of patients are currently diagnosed in asymptomatic, early stage, this limiting their prognostic value; second, clinical stages do not identify progressive and indolent forms of the disease (i.e., patients who are likely to have symptoms and require therapy vs. patients with stable disease not requiring treatment); third, patients are assigned to have advanced clinical stage based on the presence of anemia or thrombocytopenia, regardless of the origin of the cytopenia. Importantly, autoimmune cytopenia does not necessarily confer poor prognosis to patients with CLL. In recent series, patients with immune cytopenia at diagnosis (stage C "immune") have been shown to have a better outcome than those in whom cytopenia is due to bone marrow failure (stage C "infiltrative") [48, 49]; fourth, and more importantly, because of the favorable impact of more effective therapies on patients' outcome, clinical stages have lost part of their robustness in predicting survival (Fig. 4.1).

In addition to clinical stages, a plethora of prognostic factors have been described, some of them showing independent prognostic significance from disease stage; these parameters are good predictors of the likelihood of disease progression, response to therapy, and survival. Among them the most reliable are blood lymphocyte doubling time, cytogenetics, mutational status of *IGVH* genes, ZAP-70 and CD38 expression in leukemic lymphocytes, and serum β_2-microglobulin levels (reviewed in [11, 14, 75, 76]).

A rapid blood lymphocyte doubling time (i.e., <12 months) indicates progressive disease and is a criterion for starting therapy. Genetic lesions are extremely important to predict patients' outcome (reviewed in [52, 53]). Patients with normal karyotype or deletion 13q as isolated abnormality have an excellent prognosis, whereas those with complex karyotype, deletion 17p, or mutations of *TP53* have poor prognosis. Patients with low ZAP-70 expression (<20 %) or low CD38 expression (<30 %) in leukemic lymphocytes have a much better outlook than those with high ZAP-70 or CD38 expression [77, 78]. Importantly, patients with I*GVH* mutations have a more aggressive disease

Table 4.3 Clinical staging systems

System	Stage	Criteria	Median survival (years)
RAI			
Low risk	0	Lymphocytosis	>15
Intermediate risk	I	Lymphadenopathy	
	II	Spleen or liver enlargement	5–8
High risk	III	Hb < 11 g/dl	3–5
	IV	Platelets < 100,000/μl	
Binet			
Low risk	A	<3 lymphoid areas[a] enlarged	>15
Intermediate risk	B	>*3* lymphoid areas[a] enlarged	6–8
High risk	C	Hb < 10 g/dl or platelets < 100,000/μl	3–5

[a]Lymphoid areas considered are lymphadenopathy (either uni- or bilateral) in the following regions: (1) cervical, (2) axillae, (3) inguinal, (4) spleen, and (5) liver (all of them clinically assessed). Survival of patients with CLL has been improving over the last years (see Fig. 4.1)

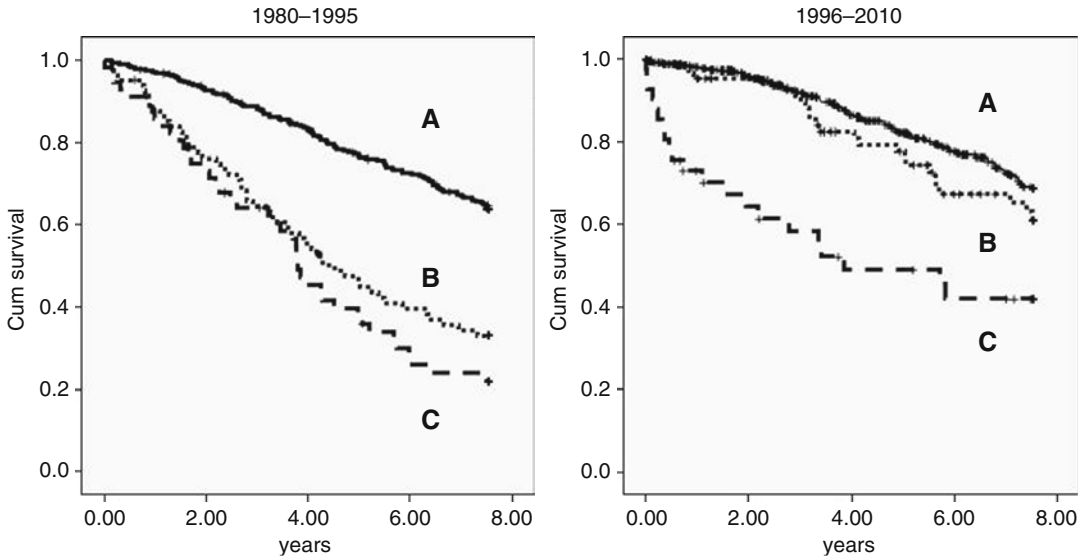

Fig. 4.1 Survival of patients with CLL according to Binet stage and period of diagnosis. Hospital Clinic, Barcelona. Period 1980–1995: estimated 6-year survival; stage *A* (*n* = 377) 72 %, stage *B* (*n* = 82) 40 %, stage *C* (*n* = 56). Period 1996–2010: stage *A* (*n* = 570) 80 %, stage *B* (*n* = 90) 70 %, stage *C* (*n* = 41) 40 %. Even with the caveat of different follow-up times, differences, particularly for stages B and C, are striking. See also references [72–74]

than those with unmutated I*GVH* genes. Keeping with this observation, "mutated" forms are usually ZAP-70 and CD38 negative and show no genetic aberrations; in contrast, "unmutated" cases tend to be ZAP-70 and CD38 positive and present genetic lesions [22, 23]. Patients that use VH3.21 *IGVH* genes, however, have poor prognosis independently on whether they are mutated or unmutated [79]. *NOTCH-1* and *SF3B1* mutations, which are observed in around 10 and 15 % of patients, respectively, also predict poor prognosis [37–46].

Because of practical and economic reasons, to study on a routine basis all the prognostic factors described above would be unrealistic (and probably more confusing than informative). In daily practice, prognosis of patients with CLL can reliably be made by considering only some of these parameters

(e.g., clinical stage, *IGVH* mutational status, ZAP-70 expression, blood lymphocyte doubling time, and serum β_2-microglobulin levels). Genetic studies, particularly the analysis of *TP53* aberrations and 11q deletions, are important to select therapy (see section "Treatment") and should be performed before advising treatment. Finally, it is important to keep in mind that the best prognostic factors can only complement, but not replace, medical expertise and a sound clinical judgement.

Treatment

Treatment Indications

Therapy is only justified when any of the following features is present [65]:

- Evidence of progressive marrow failure as manifested by the development of, or worsening of, anemia and/or thrombocytopenia.
- Massive (i.e., at least 6 cm below the left costal margin) or progressive or symptomatic splenomegaly.
- Massive nodes (i.e., at least 10 cm in longest diameter) or progressive or symptomatic lymphadenopathy.
- Progressive lymphocytosis with an increase of more than 50 % over a 2-month period or lymphocyte doubling time (LDT) of less than 6 months. LDT can be obtained by linear regression extrapolation of absolute lymphocyte counts obtained at intervals of 2 weeks over an observation period of 2–3 months. In patients with initial blood lymphocyte counts of less than 30,000/μl, LDT should not be used as a single parameter to define a treatment indication. In addition, factors contributing to lymphocytosis (or lymphadenopathy) other than CLL (e.g., infections) should be excluded.
- Autoimmune anemia and/or thrombocytopenia that does not respond to corticosteroids or other standard therapy.
- Constitutional symptoms, defined as any one or more of the following disease-related symptoms or signs:
  - Unintentional weight loss of 10 % or more within the previous 6 months
  - Significant fatigue (i.e., ECOG PS 2 or worse; inability to work or perform usual activities)
  - Fever higher than 100.5 °F or 38.0 °C for 2 or more weeks without other evidence of infection
  - Night sweats for more than 1 month without evidence of infection

Of note, a marked hypogammaglobulinemia or increased WBC counts are not by themselves sufficient to initiate treatment. However, in patients reaching extremely high WBC counts (e.g., >250,000/μl), a short course of chlorambucil or fludarabine can be appropriate to prevent leukostasis, although this is exceedingly uncommon in CLL, and also, and perhaps more importantly, to reduce the anxiety that a steadily increasing WBC count may cause to the patient.

Baseline Studies Before Therapy

Before starting therapy, patients need to be submitted to a complete evaluation including [65, 80]:

- History and physical examination with a careful palpation of all lymph node areas, spleen, and liver. Imaging studies (e.g., abdominal ultrasound, computed tomography (CT) scans) are not part of the examinations required to define clinical stage and should only be performed if clinically indicated or in the framework of clinical trials.
- Complete blood cell count and differential count.
- Coombs test (DAT).
- Serum chemistry including renal and liver function tests, serum LDH, and immunoglobulins.
- Serology for hepatitis B and C viruses and CMV (the later prior alemtuzumab or allogeneic stem cell transplantation).
- FISH analysis (13q−, trisomy 12, 11q−, 17p−).

Other explorations to be considered are:

- Bone marrow aspirate/biopsy. Although bone marrow biopsy is not required for diagnosis, it is strongly recommended prior to initiating myelosuppressive therapies and for the diagnostic evaluation of unclear cytopenias.
- Imaging studies as clinically indicated.

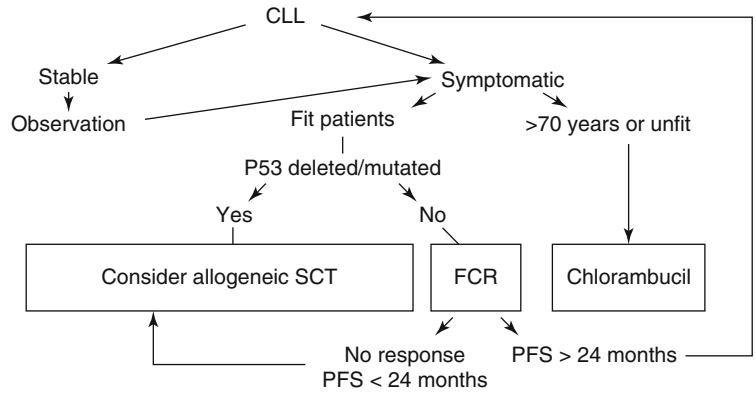

Fig. 4.2 Treatment algorithm for patients with CLL not included in trials

Treatment Approach

Whenever possible, patients requiring therapy should be included in clinical trials. For patients not entering into studies, current, evidence-based treatment approaches are discussed below and schematically presented in Fig. 4.2. Since patients with deletion of 17p or mutations of *TP53* do not respond to standard therapy and have very poor prognosis, it is highly advisable to exclude these aberrations before deciding therapy. Likewise, deletion of 11q should be also investigated since patients with this abnormality respond better to chemoimmunotherapy than to other treatments and have shorter progression-free survival.

Frontline Therapy

It is accepted that patients with asymptomatic, nonprogressive disease should not be treated. This notion, however, derives from studies in which treatment revolved around alkylating agents. Whether patients in early stage could gain benefit from newer and more effective therapies is being investigated in clinical trials.

In contrast, the majority of patients with intermediate (Rai I–II, Binet B) and virtually all patients with advanced stage (Rai III and IV, Binet C) require therapy based on the criteria mentioned above (see section "Treatment Indications"), although a fraction of them may run a relatively indolent course and do not require therapy unless the disease progresses.

The last two decades have witnessed an important improvement in CLL therapy. Table 4.4 summarizes results of key clinical trials that led to identifying chemoimmunotherapy (the combination of an anti-CD20 monoclonal antibody with purine analogs-based chemotherapy) as current treatment of choice [72, 73, 81, 82].

In 1999, the MD Anderson Cancer Center Group developed the combination of fludarabine, cyclophosphamide, and rituximab (FCR). The overall and CR rates were 73 and 25 % for previously treated patients and 95 and 72 % for treatment-naïve patients, the best ever reported results in CLL therapy [74, 83]. The central role of FCR in the management of patients with CLL was confirmed by two clinical trials in previously untreated and treated patients, respectively. Results from the German CLL Study Group showed that FCR was superior to FC regarding overall response rate (95.1 % vs. 88.4 %), CR rate (44.1 % vs. 21.8 %), and progression-free survival (PFS) (median, 51.8 vs. 32.8 months). Importantly, a small but significant difference was also observed in survival (87.2 % vs. 82.5 % at 3 years), this being the first time in the history of CLL treatment in which a given therapy demonstrated such effect [74, 83]. Similarly, FCR has been shown to result in a higher response rate and a longer PFS than FC in previously treated patients [84]. Thereafter, several observational studies have confirmed the superiority of chemoimmunotherapy over chemotherapy alone in CLL treatment [85–87]. The superiority of FCR over FC is particularly evident in patients with poor prognosis biologic features, such as del(11q) or unmutated

TABLE 4.4 CLL treatment: main clinical trials in untreated patients leading to the concept of chemoimmunotherapy as current standard therapy

	Treatment	Median age, years	*N*	ORR (%)	CR (%)	PFS, months	OS (%)
Rai et al. [81]	Fludarabine vs. chlorambucil	64	170	63	20	20	Median 55 months
		62	181	37	4	14	Median, 56 months
Catovsky et al. [72]	Chl vs. F vs. FC	65	387	72	7	20	
		64	194	80	15	23	
		65	196	94	38	43	
Hallek et al. [73]	FCR vs. FC	61	409	95	44	52	87 (3-year)*
		61	408	85	22	33	82.5 (3-year)

*$p < 0.05$

IGVH genes; however, patients with aberrations of *TP53* do not respond well.

Some modifications of the FCR regimen have been investigated. R-FCM, which consists on FCR + mitoxantrone, resulted in a high number of responses in two phase II trials from Barcelona and Houston, respectively [88, 89]. In other studies FC has been combined with either cladribine or pentostatin, or FCR has been given along with alemtuzumab ([89–94] and reviewed in [95]). Treatment results with all these combinations, however, are similar to those obtained with FCR.

Importantly, FCR and similar treatments make it possible to achieve not only a high CR rate but also molecular CR (i.e., with no detectable residual disease), an important fact because response to therapy is the most important prognostic factor once patients need therapy, the better the response the longer the life expectancy [96].

Unfortunately, not all patients can be safely treated with FCR. Thus, older patients (e.g., >70 years), patients with important comorbidity, poor performance status, abnormal renal function (i.e., creatinine clearance <60 ml/min.), or active HBV or HCV infection constitute a high-risk population that should not be treated with FCR. Although age by itself is not a criterion for avoiding FCR, the proportion of patients older than 70 years that can be treated with FCR is quite small because of chronic diseases. This is an important limitation since the majority of patients with CLL are older. Current studies comparing FCR with F + bendamustine (FB) should determine whether FB is as effective as FCR and less toxic.

Myelotoxicity is the most severe side effect related to FC or FCR treatment, making it necessary to administer antibiotics, G-CSF, and treatment dose and schedule modifications in many cases. Patients treated with rituximab can present late-onset neutropenias, although these are usually asymptomatic and self-limited. More important is the higher, although small, risk of myelodysplasia/acute leukemia, as well as that of multifocal progressive leukoencephalopathy [97, 98].

Treatment of patients which cannot receive FCR constitutes a challenge. For many decades chlorambucil was the only available treatment for CLL. The overall response rate achieved with this treatment ranges from 37 to 60 %, with less than 5 % complete responses (CR). The German CLL Study Group compared the efficacy of chlorambucil with fludarabine in patients older than 65 years, showing that although fludarabine produced a significantly higher OR and CR rates than chlorambucil, there were no differences in PFS [99]. More recently, bendamustine was compared to chlorambucil in previously treated patients younger than 65 years. The results showed a significantly higher OR and CR rates and longer PFS in patients allocated to bendamustine, although no differences in survival were observed [100]. Whether chlorambucil will be replaced by bendamustine, FC or other combinations in the near future remains to be seen.

Treatment of Patients Refractory or in Relapse

Importantly, disease relapse by itself is not a criterion to restart therapy; patients should present symptoms or signs of disease progression before

considering further therapy. There are not widely accepted rules for second-line treatment, which should be decided based on the modality of previous therapy and the response to it. A fraction of patients (about 20–40 %), usually harboring abnormalities of the *TP53* gene, fail to respond to FCR or similar regimens ("refractory" disease). These patients have a very poor prognosis (median survival <24 months) and whenever possible should be offered allogeneic stem cell transplantation, the only therapy capable of overcoming the poor prognostic significance of *TP53* aberrations and other high-risk biomarkers through its graft- vs.-leukemia effect [101–109].

Alemtuzumab (Campath 1H) is an anti-CD52 monoclonal antibody which can be transiently effective in patients with abnormalities of *TP53*, particularly when combined with corticosteroids, with a response rate of around 80 %. Alemtuzumab, however, is not effective in patients with large lymphadenopathy (e.g., >5 cm), is highly immunosuppressive, and carries a substantial risk of opportunistic infections, including cytomegalovirus (CMV) reactivation [110–112]. Also, alemtuzumab can jeopardize the results of allogeneic stem cell transplantation by increasing, through its immunosuppressive effect, the relapse rate. For all these reasons, the role of alemtuzumab in the treatment of patients with *TP53* abnormalities should not be overrated. Rituximab and high-dose metilprednisolone may also produce a relatively high response rate, although of short duration, and the infection risk is high [113].

Similarly to patients unresponsive to FCR or similar regimens, those whose response is inferior to 24–36 months have very poor prognosis, and salvage therapy with a lymphoma-type regimen (e.g., R-CHOP, R-DHAP) followed by allogeneic stem cell transplantation may be the best treatment option.

In contrast, patients who progress after monotherapy (chlorambucil, fludarabine) or FC usually respond well to FCR, the longer the disease progression interval, the better the results [114, 115].

New Agents and Treatment Approaches

Recent progress in the understanding of the biology of SLL/CLL is allowing the development of new compounds able to target metabolic pathways specifically involved in the pathogenesis of the disease (reviewed in [116–118]). Although an extensive review of these agents is beyond the scope of this chapter, compounds with proved effectiveness and already in clinical use are ofatumumab [119–124], bendamustine [125–128], and lenalidomide [129–132], particularly in combination with other drugs; other agents which are in advanced phases of development are GA-101 (an anti-CD20 monoclonal antibody) [133], cyclin-dependent kinase inhibitors (e.g., flavopiridol) [134, 135], BCR signal transduction inhibitors (e.g., GS-1101, PCI 32765) [136–139], and anti-BCL2 molecules (e.g., ABT-26 or navitoclax) [140].

Finally, different strategies to recapitulate the graft vs. tumor effect mediated by T cells have been investigated, although in general with little success. Recently, Porter et al. obtained dramatic responses, including two CR and a good PR, in three heavily pretreated and refractory patients treated with autologous T cells engineered to present anti-CD19 chimeric antigen receptor (CAR-T cells) [141]; these impressive results deserve further investigation.

Treatment of Complications

Most patients with AIHA or ITP respond to corticosteroids, while cyclosporine is the treatment of choice for patients with pure red cell aplasia. Rituximab and thrombopoietin analogs can be used in selected cases of ITP not responding to corticosteroids [28, 31, 32]. Resistance of immune cytopenia to conventional therapy is an indication for treating the underlying CLL [65, 82].

Infections are frequent because of the immune defects that characterize CLL (e.g., hypogammaglobulinemia, T-cell subsets abnormalities) and which are further exaggerated by the immunosuppressive effect treatment. Prophylactic

intravenous immunoglobulin has no impact on overall survival and is therefore not recommended on a routine basis [82]. Antibiotic, antiviral, or antifungal prophylaxis should be used in selected patients with recurrent infections and/or very high risk of developing infections (e.g., alemtuzumab treatment). Because of their abnormal immune function, patients do not mount an appropriate immune response upon vaccination. There is no general consensus on the use of G-CSF and antibiotic and antiviral prophylaxis, but these agents may be useful in heavily pretreated patients and those receiving purine analogs regimens; cotrimoxazole prevents the development of infections by pneumocystis [44, 45, 82]. Finally, erythropoietin may be useful to treat anemia unresponsive to other measures.

Disease transformation has a dismal prognosis (median survival <12 months) [33–35]. There are no large controlled studies regarding the best treatment approach. A rituximab-containing regimen (e.g., R-CHOP, R-DHAP) should be used for remission induction. Poor prognostic features include the presence of aberrations of *TP53* and refractoriness to treatment. Allogeneic stem cell transplantation should be considered in fit patients with an available donor. The transformation of the disease into HD represents a separate entity, and conventional chemotherapy against HD often achieves long-lasting remissions [37].

Response Evaluation

Response evaluation includes a careful physical examination and a blood cell count. A marrow biopsy is recommended for the proper definition of CR and is mandatory in clinical trials. Chest X-ray and an abdominal ultrasound or CT should be performed, if abnormal prior to therapy. Detection of minimal residual disease (MRD) has prognostic impact. Patients who have become MRD negative after the end of treatment have significantly longer response duration and survival. However, MRD studies are only justified within clinical trials [65, 82].

References

1. Müller-Hermelink HK, Montserrat E, Catovsky D, Campo E, Harris NL, Stein H, et al. Chronic lymphocytic leukaemia/small lymphocytic lymphoma. In: Swerdlow SH, Campo E, Harris NL, editors. WHO classification of tumours of the haematopoietic and lymphoid tissues. Lyon: IARC Press; 2008. p. 188–90.
2. Altekruse SF KC, Krapcho M, Neyman N, et al., editors. SEER Clinical Statistics Review, 1975–2007. National Cancer Institute, Bethesda; 2010.
3. Sant M, Allemani C, Tereanu C, HAEMACARE Working Group, et al. Incidence of hematologic malignancies in Europe by morphologic subtype: results of the HAEMACARE project. Blood. 2010; 116:3724–34.
4. Nishiyarna H, Mokuno J, lnoue T. Relative frequency and mortality rate of various types of leukemia in Japan. Jpn J Cancer Res. 1969;60:71–81.
5. Finch SC, Linet MS. Chronic leukaemias. Baillieres Clin Haematol. 1992;5:27–56.
6. Goldin LR, Slager SL, Caporaso NE. Familial chronic lymphocytic leukemia. Curr Opin Hematol. 2010;17:350–5.
7. Goldin LR, Sgambati M, Marti GE, et al. Anticipation in familial chronic lymphocytic leukemia. Am J Hum Genet. 1999;65:265–9.
8. Crowther-Swanepoel D, Houlston RS. Genetic variation and risk of chronic lymphocytic leukaemia. Semin Cancer Biol. 2010;20:363–9. Epub 2010 Sep 15.
9. Slager SL, Rabe KG, Achenbach SJ, et al. Genome-wide association study identifies a novel susceptibility locus at 6p21.3 among familial CLL. Blood. 2011;117:1911–6.
10. Chiorazzi N, Rai KR, Ferrarini M. Chronic lymphocytic leukemia. N Engl J Med. 2005;352:804–15.
11. Dighiero G, Hamblin T. Chronic lymphocytic leukaemia. Lancet. 2008;371:1017–29.
12. Chiorzazzi N, Ferrarini M. Cellular origin(s) of chronic lymphocytic leukemia: cautionary notes and additional considerations and possibilities. Blood. 2011;117:1781–91.
13. Klein U, Dalla-Favera R. New insights into the pathogenesis of chronic lymphocytic leukemia. Semin Cancer Biol. 2010;20:377–83.
14. Zenz T, Mertens D, Küpper R, Döhner H, Stilgenbauer S. From pathogenesis to treatment of chronic lymphocytic leukemia. Nat Rev Cancer. 2010;10:36–50.
15. Stevenson FK, Krysov S, Davies AJ, Steele AJ, Packham G. B-cell receptor signaling in chronic lymphocytic leukemia. Blood. 2011;118:4313–20.
16. Kikushige Y, Ishikawa F, Miyamoto T, et al. Self-renewing hematopoietic stem cell is the primary target in pathogenesis of human chronic lymphocytic leukemia. Cancer Cell. 2011;20:246–59.
17. Fazi C, Scarfò L, Pecciarini L, et al. General population low-count CLL-like MBL persist over time without clinical progression, though carrying the same cytogenetic abnormalities of CLL. Blood. 2011;118:6618–25.

18. Calin GA, Dumitru CD, Shimizu M, et al. Frequent deletions and down-regulation of micro-RNA genes miR15 and miR16 at 13q14 in chronic lymphocytic leukemia. Proc Natl Acad Sci USA. 2002;99:15524–9.
19. Calin GA, Croce CM. Chronic lymphocytic leukemia: interplay between non-coding RNAs and protein-coding genes. Blood. 2009;114:4761–70.
20. Klein U, Lia M, Crespo M, et al. The DLEU2/miR-15a/16–1 cluster controls B cell proliferation and its deletion leads to chronic lymphocytic leukemia. Cancer Cell. 2010;17:28–40.
21. Messmer BT, Messmer D, Allen SL, et al. In vivo measurements document the dynamic cellular kinetics of chronic lymphocytic leukemia B cells. J Clin Invest. 2005;115:755–64.
22. Damle RN, Wasil T, Fais F, et al. Ig V gene mutation status and CD38 expression as novel prognostic indicators in chronic lymphocytic leukemia. Blood. 1999;94:1840–7.
23. Hamblin TJ, Davis Z, Gardiner A, Oscier DG, Stevenson FK. Unmutated Ig V(H) genes are associated with a more aggressive form of chronic lymphocytic leukemia. Blood. 1999;94:1848–54.
24. Rosenwald A, Alizadeh AA, Widhopf G, et al. Relation of gene expression phenotype to immunoglobulin mutation genotype in B cell chronic lymphocytic leukaemia. J Exp Med. 2001;194:1639–47.
25. Weed RI. Exaggerated delayed hypersensitivity to mosquito bites in chronic lymphocytic leukemia. Blood. 1965;26:257–68.
26. Ribera JM, Viñolas N, Urbano Ispizua A, et al. 'Spontaneous' complete remissions in chronic lymphocytic leukemia: report of three cases and review of the literature. Blood Cells. 1987;12:471–9.
27. Del Giudice I, Chiaretti S, Tavolaro S, et al. Spontaneous regression of chronic lymphocytic leukemia: clinical and biologic features of 9 cases. Blood. 2009;16:638–46.
28. Dohner H, Stilgenbauer S, Fischer K, Bentz M, Lichter P. Cytogenetic and molecular cytogenetic analysis of B cell chronic lymphocytic leukemia: specific chromosome aberrations identify prognostic subgroups of patients and point to loci for candidate genes. Leukemia. 1997;11:S19–24.
29. Döhner H, Stilgenbauer S, James MR, et al. 11q deletions identify a new subset of B-cell chronic lymphocytic leukemia characterized by extensive nodal involvement and inferior prognosis. Blood. 1997;89:2516–22.
30. Stilgenbauer S, Bullinger L, Benner A, et al. Incidence and clinical significance of 6q deletions in B cell chronic lymphocytic leukemia. Leukemia. 1999;13:1331–4.
31. Döhner H, Stilgenbauer S, Benner A, et al. Genomic aberrations and survival in chronic lymphocytic leukemia. N Engl J Med. 2000;343:1910–6.
32. Zenz T, Eichhorst B, Busch R, et al. TP53 mutation and survival in chronic lymphocytic leukemia. J Clin Oncol. 2010;28:4473–9.
33. Gonzalez D, Martinez P, Wade R, et al. Mutational status of the TP53 gene as a predictor of response and survival in patients with chronic lymphocytic leukemia: results from the LRF CLL4 trial. J Clin Oncol. 2011;29:2223–9.
34. Montserrat E, Moreno C. Genetic lesions in chronic lymphocytic leukemia: clinical implications. Curr Opin Oncol. 2009;21:609–14.
35. Zenz T, Mertens D, Döhner H, Stilgenbauer S. Importance of genetics in chronic lymphocytic leukemia. Blood Rev. 2011;25:131–7.
36. Rossi D, Cerri M, Deambrogi C, et al. The prognostic value of TP53 mutations in chronic lymphocytic leukemia is independent of del17p13: implications for overall survival and chemorefractoriness. Clin Cancer Res. 2009;15:995–1004.
37. Rinaldi A, Mian M, Kwee I, et al. Genome-wide DNA profiling better defines the prognosis of chronic lymphocytic leukaemia. Br J Haematol. 2011;154:590–9.
38. Sportoletti P, Baldoni S, Cavalli L, et al. NOTCH1 PEST domain mutation is an adverse prognostic factor in B-CLL. Br J Haematol. 2010;151:404–6.
39. Puente XS, Pinyol M, Quesada V, et al. Whole-genome sequencing identifies recurrent mutations in chronic lymphocytic leukaemia. Nature. 2011;475:101–5.
40. Fabbri G, Rasi S, Rossi D, et al. Analysis of the chronic lymphocytic leukemia coding genome: role of NOTCH1 mutational activation. J Exp Med. 2011;208:1389–401.
41. Rossi D, Bruscaggin A, Spina V, et al. Mutations of the SF3B1 splicing factor in chronic lymphocytic leukemia: association with progression and fludarabine-refractoriness. Blood. 2011;118:6904–8.
42. Rossi D, Radi S, Fabbri V, et al. Mutations of NOTCH1 are an independent predictor of survival in chronic lymphocytic leukemia. Blood. 2012;119:521–9.
43. Wang L, Lawrence MS, Wan Y, et al. SF3B1 and other novel cancer genes in chronic lymphocytic leukemia. N Engl J Med. 2011;365:2497–506.
44. Balatti V, Bottoni A, Palamarchuk A, et al. NOTCH1 mutations in CLL associated with trisomy 12. Blood. 2012;119:329–31.
45. Del Giudice G, Rossi D, Chiaretti S, et al. NOTCH1 mutations in +12 chronic lymphocytic leukemia (CLL) confer an unfavorable prognosis, induce a distinctive transcriptional profiling and refine the intermediate prognosis of +12 CLL. Haematologica. 2012;97:437–41.
46. Quesada V, Conde L, Villamor N, et al. Exome sequencing identifies recurrent mutations of the splicing factor SF3B1 gene in chronic lymphocytic leukemia. Nat Genet. 2012;44:47–52.
47. Dearden C. Disease-specific complications of chronic lymphocytic leukemia. Hematology Am Soc Educ Program Hematol. 2008:450–6.
48. Zent CS, Ding W, Schwager SM, et al. The prognostic significance of cytopenia in chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL). Br J Haematol. 2008;141:615–21.
49. Moreno C, Hodgson K, Ferrer G, et al. Autoimmune cytopenia in chronic lymphocytic leukemia: prevalence, clinical associations, and prognostic significance. Blood. 2010;116:4771–6.

50. Hodgson K, Ferrer G, Montserrat E, Moreno C. Chronic lymphocytic leukemia and autoimmunity: systematic review. Haematologica. 2011;96:752–61.
51. Hodgson K, Ferrer G, Pereira A, Moreno C, Montserrat E. Autoimmune cytopenia in chronic lymphocytic leukaemia: diagnosis and treatment. Br J Haematol. 2011;154:14–22.
52. Robertson LE, Pugh W, O'Brien S, et al. Richter's syndrome: a report on 39 patients. J Clin Oncol. 1993;11:1985–9.
53. Rossi D, Spina V, Forconi F, et al. Molecular history of Richter syndrome: origin from a cell already present at the time of chronic lymphocytic leukemia diagnosis. Int J Cancer. 2012;130:3006–10.
54. Rossi D, Spina V, Deambrogi C, et al. The genetics of Richter syndrome reveals disease heterogeneity and predicts survival after transformation. Blood. 2011;117:3391–401.
55. Bruzzi JF, Macapinlac H, Tsimberidou AM, et al. Detection of Richter's transformation of chronic lymphocytic leukemia by PET/CT. J Nucl Med. 2006;47:1267–73.
56. Bockorny B, Codreanu I, Dasanu CA. Hodgkin lymphoma as Richter Transformation in chronic lymphocytic leukaemia: a retrospective analysis of world literature. Br J Haematol. 2012;156:50–66.
57. Hisada M, Biggar RJ, Greene MH, et al. Solid tumors after chronic lymphocytic leukemia. Blood. 2001;98:1979–81.
58. Tsimberidou AM, Wen S, McLaughlin P, et al. Other malignancies in chronic lymphocytic leukemia/small lymphocytic lymphoma. J Clin Oncol. 2009;27:904–10.
59. Morton LM, Curtis RE, Linet MS, et al. Second malignancy risks after non-Hodgkin's lymphoma and chronic lymphocytic leukemia: differences by lymphoma subtype. J Clin Oncol. 2010;28:4935–44.
60. Royle JA, Baade PD, Joske D, et al. Second cancer incidence and cancer mortality among chronic lymphocytic leukaemia patients: a population-based study. Br J Cancer. 2011;105:1076–81.
61. Kaae J, Hansen AV, Biggar RJ, et al. Merkel cell carcinoma: incidence, mortality, and risk of other cancers. J Natl Cancer Inst. 2010;102:793–801.
62. Molica S. Infections in chronic lymphocytic leukemia: risk factors and impact on survival, and treatment. Leuk Lymphoma. 1994;13:203–14.
63. Morrison VA. Infectious complications of chronic lymphocytic leukaemia: pathogenesis, spectrum of infection, preventive approaches. Best Pract Res Clin Haematol. 2010;23:145–53.
64. Young JA. Epidemiology and management of infectious complications of contemporary management of chronic leukemias. Infect Disord Drug Targets. 2011;11:3–10.
65. Hallek M, Cheson BD, Catovsky D, et al. Guidelines for the diagnosis and treatment of chronic lymphocytic leukemia: a report from the international Workshop on Chronic Lymphocytic Leukemia updating the National Cancer Institute-Working Group 1996 guidelines. Blood. 2008;111:5446–56.
66. Rawstron AC, Bennett FL, O'Connor SJ, et al. Monoclonal B-cell lymphocytosis and chronic lymphocytic leukemia. N Engl J Med. 2008;359:575–83.
67. Landgren O, Albitar M, Ma W, et al. B-cell clones as early markers for chronic lymphocytic leukemia. N Engl J Med. 2009;360:659–67.
68. Nieto WG, Almeida J, Romero A, Primary Health Care Group of Salamanca for the Study of MBL, et al. Increased frequency (12 %) of circulating chronic lymphocytic leukemia-like B-cell clones in healthy subjects using a highly sensitive multicolor flow cytometry approach. Blood. 2009;114:33–7.
69. Shanafelt TD, Ghia P, Lanasa MC, Landgren O, Rawstron AC. Monoclonal B-cell lymphocytosis (MBL): biology, natural history and clinical management. Leukemia. 2010;24:512–20.
70. Rai KR, Sawitsky A, Cronkite EP, et al. Clinical staging of chronic lymphocytic leukemia. Blood. 1975;46:219–34.
71. Binet JL, Auquier A, Dighiero G, et al. A new prognostic classification of chronic lymphocytic leukemia derived from a multivariate survival analysis. Cancer. 1981;48:198–206.
72. Catovsky D, Richards S, Matutes E, UK National Cancer Research Institute (NCRI) Haematological Oncology Clinical Studies Group; NCRI Chronic Lymphocytic Leukaemia Working Group, et al. Assessment of fludarabine plus cyclophosphamide for patients with chronic lymphocytic leukaemia (the LRF CLL4 Trial): a randomised controlled trial. Lancet. 2007;370:230–9.
73. Hallek M, Fischer K, Fingerle-Rowson G, International Group of Investigators; German Chronic Lymphocytic Leukaemia Study Group, et al. Addition of rituximab to fludarabine and cyclophosphamide in patients with chronic lymphocytic leukaemia: a randomised, open-label, phase 3 trial. Lancet. 2010;376:1164–74.
74. Keating MJ, O'Brien S, Albitar M, et al. Early results of a chemoimmunotherapy regimen of fludarabine, cyclophosphamide, and rituximab as initial therapy for chronic lymphocytic leukemia. J Clin Oncol. 2005;23:4079–88.
75. Moreno C, Montserrat E. New prognostic markers in chronic lymphocytic leukemia. Blood Rev. 2008;4:211–9.
76. Cramer P, Hallek M. Prognostic factors in chronic lymphocytic leukemia-what do we need to know? Nat Rev Clin Oncol. 2011;8:38–47.
77. Crespo M, Bosch F, Villamor N, et al. ZAP-70 expression as a surrogate for immunoglobulivariable-region mutations in chronic lymphocytic leukemia. N Engl J Med. 2003;348:1764–75.
78. Wiestner A, Rosenwald A, Barry TS, et al. ZAP-70 expression identifies a chronic lymphocytic leukemia subtype with unmutated immunoglobulin genes, inferior clinical outcome, and distinct gene expression profile. Blood. 2003;101:4944–51.
79. Tobin G, Thunberg U, Johnson A. Somatically mutated Ig VH3–21 genes characterize a new subset of chronic lymphocytic leukemia. Blood. 2002;99:2262–4.

80. Byrd JC, Peterson BL, Morrison VA, et al. Randomized phase 2 study of fludarabine with concurrent versus sequential treatment with rituximab in symptomatic, untreated patients with B cell chronic lymphocytic leukemia: results from Cancer and Leukemia Group B 9712. Blood. 2003;101:6–14.
81. Rai KR, Peterson BL, Appelbaum FR, et al. Fludarabine compared with chlorambucil as primary therapy for chronic lymphocytic leukemia. N Engl J Med. 2000;343:1750–7.
82. Eichhorst B, Dreyling M, Robak T, Montserrat E, Hallek M. Chronic lymphocytic leukemia: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol. 2011;22(Supplement 6):vi50–4.
83. Tam CS, O'Brien S, Wierda W, et al. Long-term results of the fludarabine, cyclophosphamide, and rituximab regimen as initial therapy of chronic lymphocytic leukemia. Blood. 2008;112:975–80.
84. Robak T, Dmoszynska A, Solal-Céligny P, et al. Rituximab plus fludarabine and cyclophosphamide prolongs progression-free survival compared with fludarabine and cyclophosphamide alone in previously treated chronic lymphocytic leukemia. J Clin Oncol. 2010;28:1756–65.
85. Abrisqueta P, Pereira A, Rozman C, et al. Improving survival in patients with chronic lymphocytic leukemia (1980–2008): the Hospital Clinic of Barcelona experience. Blood. 2009;114:2044–50.
86. Kristinsson SY, Dickman PW, Wilson WH, Caporaso N, Björkholm M, Landgren O. Improved survival in chronic lymphocytic leukemia in the past decade: a population-based study including 11,179 patients diagnosed between 1973–2003 in Sweden. Haematologica. 2009;94:1259–65.
87. Danese MD, Griffiths RI, Gleeson M, et al. An observational study of outcomes after initial infused therapy in Medicare patients diagnosed with chronic lymphocytic leukemia. Blood. 2011;117:3505–13.
88. Bosch F, Abrisqueta P, Villamor N, et al. Rituximab, fludarabine, cyclophosphamide, and mitoxantrone: a new, highly active chemoimmunotherapy regimen for chronic lymphocytic leukemia. J Clin Oncol. 2009;27:4578–84.
89. Faderl S, Wierda W, O'Brien S, Ferrajoli A, Lerner S, Keating MJ. Fludarabine, cyclophosphamide, mitoxantrone plus rituximab (FCM-R) in frontline CLL <70 years. Leuk Res. 2010;34:284–8.
90. Kay NE, Geyer SM, Call TG, et al. Combination chemoimmunotherapy with pentostatin, cyclophosphamide, and rituximab shows significant clinical activity with low accompanying toxicity in previously untreated B chronic lymphocytic leukemia. Blood. 2007;109:405–11.
91. Reynolds C, Di Bella N, Lyons RM, et al. A Phase III trial of fludarabine, cyclophosphamide, and rituximab vs. pentostatin, cyclophosphamide, and rituximab in B-cell chronic lymphocytic leukemia. Invest New Drugs. 2012;30:1232–40.
92. Robak T, Blonski JZ, Gora-Tybor J, Polish Leukemia Group (PALG CLL2), et al. Cladribine alone and in combination with cyclophosphamide or cyclophosphamide plus mitoxantrone in the treatment of progressive chronic lymphocytic leukemia: report of a prospective, multicenter, randomized trial of the Polish Adult Leukemia Group (PALG CLL2). Blood. 2006;108:473–9.
93. Robak T, Jamroziak K, Gora-Tybor J, et al. Comparison of cladribine plus cyclophosphamide with fludarabine plus cyclophosphamide as first-line therapy for chronic lymphocytic leukemia: a phase III randomized study by the Polish Adult Leukemia Group (PALG-CLL3 Study). J Clin Oncol. 2010;28:1863–9.
94. Montillo M, Tedeschi A, Petrizzi VB, et al. An open-label, pilot study of fludarabine, cyclophosphamide, and alemtuzumab (FCC) in relapsed/refractory patients with B-cell chronic lymphocytic leukemia. Blood. 2011;118:4079–85.
95. Tam CS, Keating MJ. Chemoimmunotherapy for chronic lymphocytic leukemia. Nat Rev Clin Oncol. 2010;7:521–32.
96. Montserrat E. Treatment of chronic lymphocytic leukemia: achieving minimal residual disease—negative status as a goal [editorial]. J Clin Oncol. 2005;23:2884–5.
97. Smith MR, Neuberg D, Flin IW. Incidence of therapy-related myeloid neoplasia after initial therapy for chronic lymphocytic leukemia with fludarabine-cyclophosphamide versus fludarabine: long-term follow-up of US Intergroup Study E2997. Blood. 2011;118:3525–7.
98. D'Souza A, Wilson J, Mukherjee S, Jaiyesimi I. Progressive multifocal leukoencephalopathy in chronic lymphocytic leukemia: a report of three cases and review of the literature. Clin Lymphoma Myeloma Leuk. 2010;10(1):E1–9.
99. Eichhorst BF, Busch R, Stilgenbauer S, et al., German CLL Study Group (GCLLSG). First-line therapy with fludarabine compared with chlorambucil does not result in a major benefit for elderly patients with advanced chronic lymphocytic leukemia. Blood. 2009;114:3382–91.
100. Knauf WU, Lissichkov T, Aldaoud A, et al. Phase III randomized study of bendamustine compared with chlorambucil in previously untreated patients with chronic lymphocytic leukemia. J Clin Oncol. 2009;27:4378–84.
101. Dreger P, Corradini P, Kimby E, et al., Chronic Leukemia Working Party of the EBMT. Indications for allogeneic stem cell transplantation in chronic lymphocytic leukemia: the EBMT transplant consensus. Leukemia. 2007;21:12–7.
102. Montserrat E, Moreno C, Esteve J, Urbano-Ispizua A, Gine E, Bosch F. How I treat refractory CLL. Blood. 2006;107:1276–83.
103. Moreno C, Villamor N, Colomer D, et al. Allogeneic stem-cell transplantation may overcome the adverse prognosis of unmutated VH gene in patients with chronic lymphocytic leukemia. J Clin Oncol. 2005;23:3433–8.

104. Caballero D, Garcia-Marco JA, Martino R, et al. Allogeneic transplant with reduced intensity conditioning regimens may overcome the poor prognosis of B-cell chronic lymphocytic leukemia with unmutated immunoglobulin variable heavy-chain gene and chromosomal abnormalities (11q- and 17p-). Clin Cancer Res. 2005;11:7757–63.
105. Schetelig J, Thiede C, Bornhauser M, et al. Evidence of a graft-versus-leukemia effect in chronic lymphocytic leukemia after reduced-intensity conditioning and allogeneic stem-cell transplantation: the Cooperative German Transplant Study Group. J Clin Oncol. 2003;21:2747–53.
106. Schetelig J, van Biezen A, Brand R, et al. Allogeneic hematopoietic stem-cell transplantation for chronic lymphocytic leukemia with 17p deletion: a retrospective European Group for Blood and Marrow Transplantation Analysis. J Clin Oncol. 2008;26:5094–100.
107. Sorror ML, Storer BE, Sandmaier BM, et al. Five-year follow up of patients with advanced chronic lymphocytic leukemia treated with allogeneic hematopoietic cell transplantation after nonmyeloablative conditioning. J Clin Oncol. 2008;26:4912–20.
108. Dreger P, Döhner H, Ritgen M, et al. Allogeneic stem cell transplantation provides durable disease control in poor-risk chronic lymphocytic leukemia: long-term clinical and MRD results of the German CLL Study Group CLL3X trial. Blood. 2010;116:2438–47.
109. Gribben JG, Hosing C, Maloney DG. Stem cell transplantation for indolent lymphoma and chronic lymphocytic leukemia. Biol Blood Marrow Transplant. 2011;17(1 Suppl):S63–70.
110. Keating MJ, Flinn I, Jain V, et al. Therapeutic role of alemtuzumab (Campath-1H) in patients who have failed fludarabine: results of a large international study. Blood. 2002;99:3554–61.
111. Hillmen P, Skotnicki AB, Robak T, et al. Alemtuzumab compared with chlorambucil as first-line therapy for chronic lymphocytic leukemia. J Clin Oncol. 2007;25:5616–23.
112. Osterborg A, Foà R, Bezares RF, et al. Management guidelines for the use of alemtuzumab in chronic lymphocytic leukemia. Leukemia. 2009;23:1980–8.
113. Castro JE, Sandoval-Sus JD, Bole J, Rassenti L, Kipps TJ. Rituximab in combination with high dose methylprednisolone for the treatment of fludarabine refractory high-risk chronic lymphocytic leukemia. Leukemia. 2008;22:2048–53.
114. Keating MJ, Wierda WG, Tam CS, et al. Long term outcome following treatment failure of FCR chemoimmunotherapy as initial therapy for chronic lymphocytic leukemia [abstract]. Blood (ASH Annu Meet Abstr). 2009;114:Abstract 2381.
115. Badoux XC, Keating M, Wang X, et al. Fludarabine, cyclophosphamide, and rituximab chemoimmunotherapy is highly effective treatment for relapsed patients with CLL. Blood. 2011;117:3016–24.
116. Delgado J, Briones J, Sierra J. Emerging therapies for patients with advanced chronic lymphocytic leukaemia. Blood Rev. 2009;23:217–24.
117. Burger JA, Ghia P, Rosenwald A, Caligaris-Cappio F. The microenvironment in mature B-cell malignancies: a target for new treatment strategies. Blood. 2009;114:3367–75.
118. Gribben JG, O'Brien S. Update on therapy of chronic lymphocytic leukemia. J Clin Oncol. 2011;29:544–50.
119. Coiffier B, Lepretre S, Pedersen LM, et al. Safety and efficacy of ofatumumab, a fully human monoclonal anti-CD20 antibody, in patients with relapsed or refractory B-cell chronic lymphocytic leukemia: a phase 1–2 study. Blood. 2008;111:1094–100.
120. Wierda WG, Kipps TJ, Mayer J, et al. Hx-CD20–406 Study Investigators. Ofatumumab as single-agent CD20 immunotherapy in fludarabine-refractory chronic lymphocytic leukemia. J Clin Oncol. 2010;28:1749–55.
121. Lemery SJ, Zhang J, Rothmann MD, et al. U.S. Food and Drug Administration approval: ofatumumab for the treatment of patients with chronic lymphocytic leukemia refractory to fludarabine and alemtuzumab. Clin Cancer Res. 2010;16:4331–8.
122. Gravanis I, Ersbøll J, Skovlund E, Abadie E, Marti M, Pignatti F. The European Medicines Agency review of ofatumumab (Arzerra®) for the treatment of chronic lymphocytic leukemia in patients refractory to fludarabine and alemtuzumab: summary of the scientific assessment of the European medicines agency committee for medicinal products for human use. Oncologist. 2010;15:1335–43.
123. Wierda WG, Kipps TJ, Dürig J, on behalf of the 407 Study Investigators, et al. Chemoimmunotherapy with O-FC in previously untreated patients with chronic lymphocytic leukemia. Blood. 2011;117:6450–8.
124. Wierda WG, Padmanabhan S, Chaib GW, Gupta IV, Lisby S, Osterborg A, et al. Ofatumumab is active in patients with fludarabine-refractory CLL irrespective of prior rituximab: results from the phase 2 international study. Blood. 2011;118:5126–9.
125. Bergmann MA, Goebeler ME, Herold M, et al. Efficacy of bendamustine in patients with relapsed or refractory chronic lymphocytic leukemia: results of a phase I/II study of the German CLL Study Group. Haematologica. 2005;90:1357–64.
126. Cheson BD, Rummel MJ. Bendamustine: rebirth of an old drug. J Clin Oncol. 2009;27:122.
127. Fischer K, Cramer P, Busch R, et al. Bendamustine combined with rituximab in patients with relapsed and/or refractory chronic lymphocytic leukemia: a multicenter phase II trial of the German Chronic Lymphocytic Leukemia Study Group. J Clin Oncol. 2011;29:3559–66.
128. Iannitto E, Morabito F, Mancuso S, Gentile M, et al. Bendamustine with or without rituximab in the treatment of relapsed chronic lymphocytic leukaemia: an Italian retrospective study. Br J Haematol. 2011;153:351–7.
129. Chanan-Khan A, Miller KC, Musial L, Lawrence D, Padmanabhan S, Takeshita K, et al. Clinical efficacy of lenalidomide in patients with relapsed or refractory chronic lymphocytic leukemia: results of a phase II study. J Clin Oncol. 2006;24:5343–9.

130. Ferrajoli A, Lee BN, Schlette EJ, et al. Lenalidomide induces complete and partial remissions in patients with relapsed and refractory chronic lymphocytic leukemia. Blood. 2008;111:5291–7.
131. Chen CI, Bergsagel PL, Paul H, et al. Single-agent lenalidomide in the treatment of previously untreated chronic lymphocytic leukemia. J Clin Oncol. 2011;29:1175–81.
132. Badoux C, Keating MJ, Wen S, et al. Lenalidomide as initial therapy of elderly patients with chronic lymphocytic leukemia. Blood. 2011;118:3489–98.
133. Bologna L, Gotti E, Manganini M, et al. Mechanism of action of type II, glycoengineered, anti-CD20 monoclonal antibody GA101 in B-chronic lymphocytic leukemia whole blood assays in comparison with rituximab and alemtuzumab. J Immunol. 2011;186:3762–9.
134. Phelps MA, Lin TS, Johnson AJ, et al. Clinical response and pharmacokinetics from a phase 1 study of an active dosing schedule of flavopiridol in relapsed chronic lymphocytic leukemia. Blood. 2009;113:2637–45.
135. Lin TS, Ruppert AS, Johnson AJ, et al. Phase II study of flavopiridol in relapsed chronic lymphocytic leukemia demonstrating high response rates in genetically high-risk disease. J Clin Oncol. 2009;27:6012–8.
136. Herman SEM, Gordon AL, Hertlein E, et al. Bruton tyrosine kinase represents a promising therapeutic target for treatment of chronic lymphocytic leukemia and is effectively targeted by PCI-32765. Blood. 2011;117:6287–96.
137. Lannutti BJ, Meadows SA, Herman SEM, et al. CAL-101, a p110δ selective phosphatidylinositol-3- kinase inhibitor for the treatment of B-cell malignancies, inhibits PI3K signaling and cellular viability. Blood. 2011;117:591–4.
138. Herman SEM, Gordon AL, Wagner AJ, et al. Phosphatidylinositol 3-kinase-δ inhibitor CAL-101 shows promising preclinical activity in chronic lymphocytic leukemia by antagonizing intrinsic and extrinsic cellular survival signals. Blood. 2010;116:2078–88.
139. O'Brien S, Burger JA, Coutre SE, et al. The BTK inhibitor PCI-32765 is highly active and tolerable in patients with poor-risk CLL: interim results from a phase Ib/II study. Ann Oncol (ICML Meet Abstr) 2011;22(Suppl 4):122.
140. Roberts AW, Seymour JF, Brown JR, et al. Substantial susceptibility of chronic lymphocytic leukemia to BCL2 inhibition: Results of a phase I study of navitoclax in patients with relapsed or refractory disease. J Clin Oncol. 2012;30:488–96.
141. Porter DL, Levine BL, Kalos M, Bagg A, June CH. Chimeric antigen receptor–modified T Cells in chronic lymphoid leukemia. N Engl J Med. 2011;365:725–33.

Waldenström's Macroglobulinemia/ Lymphoplasmacytic Lymphoma

5

Steven P. Treon and Giampaolo Merlini

Abstract

Waldenström's macroglobulinemia (WM) is included in the World Health Organization classification as the lymphoplasmacytic lymphoma. It is a rare type of non-Hodgkin lymphoma (NHL) with distinct clinicopathological features resulting from the accumulation of clonally related B lymphocytes, lymphoplasmacytic cells, and plasma cells which secrete a monoclonal IgM protein. Unlike other types of NHL, WM is rarely associated with lymphadenopathy or splenomegaly. WM has a chronic clinical course and treatment options are usually different from other types of indolent B-cell lymphoma. In this chapter, we will review the most recent data on the biology of WM and current treatment strategies.

Keywords

Waldenstrom's macroglobulinemia • Lymphoplasmacytic lymphoma • Myd88 • Igm neuropathy • Cryoglobulinemia • Cold agglutinins • Rituximab • Bortezomib • Bendamustine

Introduction

Waldenström's macroglobulinemia (WM) is a distinct clinicopathological entity resulting from the accumulation, predominantly in the bone marrow, of clonally related lymphocytes, lymphoplasmacytic cells, and plasma cells which secrete a monoclonal IgM protein [1]. This condition is considered to correspond to the lymphoplasmacytic lymphoma (LPL) as defined by the World Health Organization classification system [2]. Most cases of LPL are WM, with less than 5 % of cases made up of IgA, IgG, and nonsecreting LPL.

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Epidemiology

WM is an uncommon disease, with a reported age-adjusted incidence rate of 3.4 per million among males and 1.7 per million among females in the United States and a geometrical increase with age [3]. The incidence rate for WM is higher among Caucasians, with African descendants representing only 5 % of all patients. The incidence of WM may be higher among individuals of Ashkenazi Jewish decent [4]. Genetic factors appear to be an important to the pathogenesis of WM. A common predisposition for WM with other malignancies has been raised [4, 5], with numerous reports of familiar clustering of individuals with WM alone and with other B-cell lymphoproliferative diseases [6–10]. In a large single center experience, 26 % of 924 consecutive patients with WM had a first- or second-degree relative with either WM or another B-cell disorder [5]. Frequent familiar association with other immunological disorders in healthy relatives, including hypogammaglobulinemia and hypergammaglobulinemia (particularly polyclonal IgM), autoantibody (particularly to thyroid) production, and manifestation of hyperresponsive B cells have also been reported [10, 11]. Increased expression of the *bcl-2* gene with enhanced B-cell survival may underlie the increased immunoglobulin synthesis in familial WM [10]. The role of environmental factors in WM remains to be clarified, but chronic antigenic stimulation from infections, certain drug, and Agent Orange exposures remains suspect. An etiological role for hepatitis C virus (HCV) infection has been suggested, though in one study no association could be established using both serological and molecular diagnostic studies for HCV infection in a hundred consecutive WM patients [12, 13].

Biology

Cytogenetics

Chromosome 6q deletions encompassing 6q21–25 have been observed in up to half of WM patients and at a comparable frequency among patients with and without a familial history [7, 14–16]. The presence of 6q deletions has been suggested to discern patients with WM from those with IgM monoclonal gammopathy of unknown significance (MGUS) and to have potential prognostic significance including impact on progression-free survival following treatment response though others have reported no prognostic significance to the presence of 6q deletions in WM [14, 16, 17]. Other abnormalities by cytogenetic or FISH analyses include deletions in 13q14, TP53 and ATM, and trisomies 4, 12, and 18 [17, 18]. IgH rearrangements are uncommon in WM and may be helpful in discerning cases of WM from IgM myeloma wherein IgH switch region rearrangements are a prominent feature [19].

Mutation in MYD88

A highly recurrent somatic mutation (MYD88 L265P) has recently been identified in WM patients by paired tumor/normal whole genome sequencing and subsequent confirmation by Sanger sequencing [20]. MYD88 L265P was expressed in tumor cells from 91 % of LPL cases, which included patients with IgM (WM) and IgG secreting LPL. By comparison, MYD88 L265P was absent in myeloma samples, including IgM myeloma, and was expressed in a small subset (6.5 %) of MZL patients, who surprisingly had many WM-related features. Of particular interest in this study was the absence of MYD88 L265P in nearly all cases of IgM MGUS examined. In the sole patient in whom MYD88 L265P was identified, subsequent disease evolution occurred. The expression of MYD88 L265P in familial and sporadic WM patients at the same frequency in this study is also worthy of note. These findings appear to denote that acquisition of MYD88 L265P is a common transforming event for WM, regardless of familial predisposition. Importantly, knockdown of MYD88 decreased survival of MYD88 L265P expressing WM cells, whereas survival was more enhanced by knock-in of MYD88 L265P versus wild-type MYD88. The discovery of a mutation in MYD88 is of

significance given its role as an adaptor molecule in Toll-like receptor (TLR) and interleukin-1 receptor (IL-1R) signaling [21]. All TLRs except for TLR3 use MYD88 to facilitate their signaling. Following TLR or IL-1R stimulation, MYD88 is recruited to the activated receptor complex as a homodimer which then complexes with IRAK4 and activates IRAK1 and IRAK2 [22–24]. Tumor necrosis factor receptor associated factor 6 is then activated by IRAK1 leading to NF-κ[kappa]B activation via Iκ[kappa]Bα phosphorylation [25]. Use of inhibitors of MYD88 pathway led to decreased IRAK1 and Iκ[kappa]Bα phosphorylation as well as survival of MYD88 L265P expressing WM cells. These observations are of particular relevance to WM since NF-κ[kappa]B signaling is important for WM growth and survival [26].

Nature of the Clonal Cell

The WM bone marrow B-cell clone shows intraclonal differentiation from small lymphocytes with large focal deposits of surface immunoglobulins, to lymphoplasmacytic cells, to mature plasma cells that contain intracytoplasmic immunoglobulins [27]. Clonal B cells are detectable among blood B lymphocytes, and their number increases in patients who fail to respond to therapy or who progress [28]. These clonal blood cells present the peculiar capacity to differentiate spontaneously, in in vitro culture, to plasma cells. This is through an interleukin-6 (IL-6)-dependent process in IgM MGUS and mostly an IL-6-independent process in WM patients [29]. All these cells express the monoclonal IgM present in the blood and a variable percentage of them also express surface IgD. The characteristic immunophenotypic profile of the lymphoplasmacytic cells in WM includes the expression of the pan B-cell markers CD19, CD20, CD22, CD79, and FMC7.2 [30–32]. Expression of CD5, CD10, and CD23 may be found in 10–20 % of cases and does not exclude the diagnosis of WM [33].

The phenotype of lymphoplasmacytic cells in WM cell suggests that the clone is a postgerminal center B cell. This indication is further strengthened by the results of the analysis of the nature (silent or amino acid replacing) and distribution (in framework or CDR regions) of somatic mutations in Ig heavy- and light-chain variable regions performed in patients with WM [34, 35]. This analysis showed a high rate of replacement mutations, compared with the closest germline genes, clustering in the CDR regions and without intraclonal variation. Subsequent studies showed a strong preferential usage of VH3/JH4 gene families, no intraclonal variation, no evidence for any isotype-switched transcripts [36, 37]. These data indicate that WM may originate from an IgM^+ and/or IgM^+ IgD^+ memory B cell. Normal IgM^+ memory B cells localize in bone marrow, where they mature to IgM-secreting cells [38].

Bone Marrow Microenvironment

Increased numbers of mast cells are found in the bone marrow of WM patients, wherein they are usually admixed with tumor aggregates [2, 32, 39]. The role of mast cells in WM has been investigated in one study wherein coculture of primary autologous or mast cell lines with WM LPC resulted in dose-dependent WM cell proliferation and/or tumor colony formation, primarily through CD40 ligand (CD40L) signaling. Furthermore, WM cells through elaboration of soluble CD27 (sCD27), induced the upregulation of CD40L on mast cells derived from WM patients and mast cell lines suggesting a microenvironmental support system [39, 40]. High levels of CXCR4 and VLA-4 have also been observed in WM cells [41]. In blocking experiments studies, CXCR4 was shown to support migration of WM cells, while VLA-4 contributed to adhesion of WM cells to bone marrow stromal cells.

Clinical Features

The clinical and laboratory findings at the time of diagnosis of WM in one large institutional study are presented in Table 5.1. Unlike most

Table 5.1 Clinical and laboratory findings for 149 consecutive newly diagnosed patients with the consensus panel diagnosis of WM presenting to the Dana-Farber Cancer Institute

	Median	Range	Institutional normal reference range
Age (year)	59	34–84	NA
Gender (male/female)	85/64		NA
Bone marrow involvement	30 %	5–95 %	NA
Adenopathy	16 %		NA
Splenomegaly	10 %		NA
IgM (mg/dL)	2,870	267–12,400	40–230
IgG (mg/dL)	587	47–2,770	700–1,600
IgA (mg/dL)	47	8–509	70–400
Serum viscosity (cp)	2.0	1.4–6.6	1.4–1.9
Hct (%)	35.0 %	17.2–45.4 %	34.8–43.6
Plt ($\times 10^9$/L)	253	24–649	155–410
Wbc ($\times 10^9$/L)	6.0	0.3–13	3.8–9.2
B_2M (mg/dL)	3.0	1.3–13.7	0–2.7
LDH	395	122–1,131	313–618

NA not applicable

Table 5.2 Physicochemical and immunological properties of the monoclonal IgM protein in Waldenstrom's macroglobulinemia

Properties of IgM monoclonal protein	Diagnostic condition	Clinical manifestations
Pentameric structure	Hyperviscosity	Headaches, blurred vision, epistaxis, retinal hemorrhages, leg cramps, impaired mentation, intracranial hemorrhage
Precipitation on cooling	Cryoglobulinemia (type I)	Raynaud's phenomenon, acrocyanosis, ulcers, purpura, cold urticaria
Autoantibody activity to myelin-associated glycoprotein (MAG), ganglioside M1 (GM1), sulfatide moieties on peripheral nerve sheaths	Peripheral neuropathies	Sensorimotor neuropathies, painful neuropathies, ataxic gait, bilateral foot drop
Autoantibody activity to IgG	Cryoglobulinemia (type II)	Purpura, arthralgias, renal failure, sensorimotor neuropathies
Autoantibody activity to red blood cell antigens	Cold agglutinins	Hemolytic anemia, Raynaud's phenomenon, acrocyanosis, livedo reticularis
Tissue deposition as amorphous aggregates	Organ dysfunction	Skin: bullous skin disease, papules, Schnitzler's syndrome GI: diarrhea, malabsorption, bleeding Kidney: proteinuria, renal failure (light-chain component)
Tissue deposition as amyloid fibrils (light-chain component most commonly)	Organ dysfunction	Fatigue, weight loss, edema, hepatomegaly, macroglossia, organ dysfunction of involved organs: heart, kidney, liver, peripheral sensory and autonomic nerves

indolent lymphomas, splenomegaly and lymphadenopathy are prominent in only a minority of patients (<15 %). Purpura is frequently associated with cryoglobulinemia and more rarely with AL amyloidosis, while hemorrhagic manifestations and neuropathies are multifactorial (see later). The morbidity associated with WM is caused by the concurrence of two main components: tissue infiltration by neoplastic cells and, more importantly, the physicochemical and immunological properties of the monoclonal IgM. As shown in Table 5.2, the monoclonal IgM

can produce clinical manifestations through several different mechanisms related to its physicochemical properties, nonspecific interactions with other proteins, antibody activity, and tendency to deposit in tissues [42–44].

Morbidity Mediated by the Effects of IgM

Hyperviscosity Syndrome

Blood hyperviscosity is affected by increased serum IgM levels leading to hyperviscosity-related complications [45]. The mechanisms behind the marked increase in the resistance to blood flow and the resulting impaired transit through the microcirculatory system are rather complex [45–47]. The main determinants are (1) a high concentration of monoclonal IgMs, which may form aggregates and may bind water through their carbohydrate component, and (2) their interaction with blood cells. Monoclonal IgMs increase red cell aggregation (*rouleaux* formation) and red cell internal viscosity while also reducing deformability. The possible presence of cryoglobulins can contribute to increasing blood viscosity as well as to the tendency to induce erythrocyte aggregation. Serum viscosity is proportional to IgM concentration up to 30 g/L and then increases sharply at higher levels. Plasma viscosity and hematocrit are directly regulated by the body. Increased plasma viscosity may also contribute to inappropriately low erythropoietin production, which is the major reason for anemia in these patients [48]. Clinical manifestations are related to circulatory disturbances that can be best appreciated by ophthalmoscopy, which shows distended and tortuous retinal veins, hemorrhages, and papilledema [49]. Symptoms usually occur when the monoclonal IgM concentration exceeds 50 g/L or when serum viscosity is >4.0 centipoises (cp), but there is a great individual variability, with some patients showing no evidence of hyperviscosity even at 10 cp [45]. The most common symptoms are oronasal bleeding, visual disturbances due to retinal bleeding, and dizziness that may rarely lead to coma. Heart failure can be aggravated, particularly in the elderly, owing to increased blood viscosity, expanded plasma volume, and anemia. Inappropriate transfusion can exacerbate hyperviscosity and may precipitate cardiac failure.

Cryoglobulinemia

In up to 20 % of WM patients, the monoclonal IgM can behave as a cryoglobulin (type I), but it is symptomatic in 5 % or less of the cases [50]. Cryoprecipitation is mainly dependent on the concentration of monoclonal IgM; for this reason plasmapheresis or plasma exchange is commonly effective in this condition. Symptoms result from impaired blood flow in small vessels and include Raynaud's phenomenon, acrocyanosis, and necrosis of the regions most exposed to cold such as the tip of the nose, ears, fingers, and toes, malleolar ulcers, purpura, and cold urticaria. Renal manifestations may occur but are infrequent.

Autoantibody Activity

Monoclonal IgM may exert its pathogenic effects through specific recognition of autologous antigens, the most notable being nerve constituents, immunoglobulin determinants, and red blood cell antigens.

IgM-Related Neuropathy

The presence of peripheral neuropathy has been estimated to range from 5 to 38 % in WM patients [51–55]. The nerve damage is mediated by diverse pathogenetic mechanisms: IgM antibody activity toward nerve constituents causing demyelinating polyneuropathies; endoneurial granulofibrillar deposits of IgM without anti-

body activity, associated with axonal polyneuropathy; occasionally by tubular deposits in the endoneurium associated with IgM cryoglobulin; and, rarely, by amyloid deposits or by neoplastic cell infiltration of nerve structures [56]. Half of the patients with IgM neuropathy have a distinctive clinical syndrome that is associated with antibodies against a minor 100-kDa glycoprotein component of nerve, myelin-associated glycoprotein (MAG). Anti-MAG antibodies are generally monoclonal IgMκ, and usually also exhibit reactivity with other glycoproteins or glycolipids that share antigenic determinants with MAG [57–59]. The anti-MAG-related neuropathy is typically distal and symmetrical, affecting both motor and sensory functions; it is slowly progressive with a long period of stability [52, 60]. Most patients present with sensory complaints (paresthesias, aching discomfort, dysesthesias, or lancinating pains), imbalance and gait ataxia, owing to lack proprioception, and leg muscles atrophy in advanced stage. Patients with predominantly demyelinating sensory neuropathy in association with monoclonal IgM to gangliosides with disialosyl moieties, such as GD1b, GD3, GD2, GT1b, and GQ1b, have also been reported [61, 62]. Anti-GD1b and anti-GQ1b antibodies were significantly associated with predominantly sensory ataxic neuropathy. These antiganglioside monoclonal IgMs present core clinical features of chronic ataxic neuropathy with variably present ophthalmoplegia and/or red blood cell cold agglutinating activity. The disialosyl epitope is also present on red blood cell glycophorins, thereby accounting for the red cell cold agglutinin activity of anti-Pr2 specificity [63, 64]. Monoclonal IgM proteins that bind to gangliosides with a terminal trisaccharide moiety, including GM2 and GalNac-GD1A, are associated with chronic demyelinating neuropathy and severe sensory ataxia, unresponsive to corticosteroids [65]. Antiganglioside IgM proteins may also cross-react with lipopolysaccharides of C*ampylobacter jejuni*, whose infection is known to precipitate the Miller Fisher syndrome, a variant of the Guillain–Barré syndrome [66]. This finding indicates that molecular mimicry may play a role in this condition. Antisulfatide monoclonal IgM proteins, associated with sensory/sensorimotor neuropathy, have been detected in 5 % of patients with IgM monoclonal gammopathy and neuropathy [67]. Motor neuron disease has been reported in patients with WM and monoclonal IgM with anti-GM1 and sulfoglucuronyl paragloboside activity [68]. POEMS (polyneuropathy, organomegaly, endocrinopathy, M protein, and skin changes) syndrome is rarely associated with WM [69].

Cold Agglutinin Hemolytic Anemia

Monoclonal IgM may present with cold agglutinin activity, i.e., it can recognize specific red cell antigens at temperatures below physiological, producing chronic hemolytic anemia. This disorder occurs in <10 % of WM patients [70] and is associated with cold agglutinin titers >1:1,000 in most cases. The monoclonal component is usually an IgMκ[kappa] and reacts most commonly with I/i antigens, with complement fixation and activation [71, 72]. Mild chronic hemolytic anemia can be exacerbated after cold exposure but rarely does hemoglobin drop below 70 g/L. The hemolysis is usually extravascular (removal of C3b opsonized cells by the reticuloendothelial system, primarily in the liver) and rarely intravascular from complement destruction of red blood cell (RBC) membrane. The agglutination of RBCs in the cooler peripheral circulation also causes Raynaud's syndrome, acrocyanosis, and livedo reticularis. Macroglobulins with the properties of both cryoglobulins and cold agglutinins with anti-Pr specificity have been reported. These properties may have as a common basis the immune binding of the sialic acid-containing carbohydrate present on red blood cell glycophorins and on Ig molecules. Several other macroglobulins with various antibody activities toward autologous antigens (i.e., phospholipids, tissue and plasma proteins, etc.) and foreign ligands have also been reported.

Tissue Deposition

The monoclonal protein can deposit in several tissues as amorphous aggregates. Linear deposition of monoclonal IgM along the skin basement membrane is associated with bullous skin disease [73]. Amorphous IgM deposits in the dermis determine the so-called IgM storage papules on the extensor surface of the extremities—macroglobulinemia cutis [74]. Deposition of monoclonal IgM in the lamina propria and/or submucosa of the intestine may be associated with diarrhea, malabsorption, and gastrointestinal bleeding [75, 76]. It is well known that kidney involvement is less common and less severe in WM than in multiple myeloma, probably because the amount of light chain excreted in the urine is generally lower in WM than in myeloma and because of the absence of contributing factors, such as hypercalcemia, although cast nephropathy has also been described in WM [77]. On the other hand, the IgM macromolecule is more susceptible to being trapped in the glomerular loops where ultrafiltration presumably contributes to its precipitation, forming subendothelial deposits of aggregated IgM proteins that occlude the glomerular capillaries [78]. Mild and reversible proteinuria may result and most patients are asymptomatic. The deposition of monoclonal light chain as fibrillar amyloid deposits (AL amyloidosis) is uncommon in patients with WM [79]. Clinical expression and prognosis are similar to those of other AL patients with involvement of the heart (44 %), kidneys (32 %), liver (14 %), lungs (10 %), peripheral/autonomic nerves (38 %), and soft tissues (18 %). However, the incidence of cardiac and pulmonary involvement is higher in patients with monoclonal IgM than with other immunoglobulin isotypes. The association of WM with reactive amyloidosis (AA) has been documented rarely [80, 81]. Simultaneous occurrence of fibrillary glomerulopathy, characterized by glomerular deposits of wide non-congophilic fibrils and amyloid deposits, has been reported in WM [82].

Manifestations Related to Tissue Infiltration by Neoplastic Cells

Tissue infiltration by neoplastic cells is rare and can involve various organs and tissues, from the bone marrow (described later) to the liver, spleen, lymph nodes, and possibly the lungs, gastrointestinal tract, kidneys, skin, eyes, and central nervous system. Pulmonary involvement in the form of masses, nodules, diffuse infiltrate, or pleural effusions is relatively rare, since the overall incidence of pulmonary and pleural findings reported for WM is only 3–5 % [83–85]. Cough is the most common presenting symptom, followed by dyspnea and chest pain. Chest radiographic findings include parenchymal infiltrates, confluent masses, and effusions. Malabsorption, diarrhea, bleeding, or obstruction may indicate involvement of the gastrointestinal tract at the level of the stomach, duodenum, or small intestine [86–89]. In contrast to multiple myeloma, infiltration of the kidney interstitium with lymphoplasmacytoid cell has been reported in WM [90], while renal or perirenal masses are not uncommon [91]. The skin can be the site of dense lymphoplasmacytic infiltrates, similar to that seen in the liver, spleen, and lymph nodes, forming cutaneous plaques and, rarely, nodules [92]. Chronic urticaria and IgM gammopathy are the two cardinal features of the Schnitzler syndrome, which is not usually associated initially with clinical features of WM [93], although evolution to WM is not uncommon. Thus, close follow-up of these patients is warranted. Invasion of articular and periarticular structures by WM malignant cells is rarely reported [94]. The neoplastic cells can infiltrate the periorbital structures, lacrimal gland, and retro-orbital lymphoid tissues, resulting in ocular nerve palsies [95, 96]. Direct infiltration of the central nervous system by monoclonal lymphoplasmacytic cells as infiltrates or as tumors constitutes the rarely observed Bing–Neel syndrome, characterized clinically by confusion, memory loss, disorientation, and motor dysfunction (reviewed in Civit et al. [97]).

Laboratory Investigations and Findings

Hematological Abnormalities

Anemia is the most common finding in patients with symptomatic WM and is caused by a combination of factors: mild decrease in red cell survival, impaired erythropoiesis, hemolysis, moderate plasma volume expansion, and blood loss from the gastrointestinal tract. Blood smears are usually normocytic and normochromic, and rouleaux formation is often pronounced. Electronically measured mean corpuscular volume may be elevated spuriously owing to erythrocyte aggregation. In addition, the hemoglobin estimate can be inaccurate, i.e., falsely high, because of interaction between the monoclonal protein and the diluent used in some automated analyzers [98]. Leukocyte and platelet counts are usually within the reference range at presentation, although patients may occasionally present with severe thrombocytopenia. As reported above, monoclonal B-lymphocytes expressing surface IgM and late-differentiation B-cell markers are uncommonly detected in blood by flow cytometry. A raised erythrocyte sedimentation rate is almost constantly observed in WM and may be the first clue to the presence of the macroglobulin. The clotting abnormality detected most frequently is prolongation of thrombin time. AL amyloidosis should be suspected in all patients with nephrotic syndrome, cardiomyopathy, hepatomegaly, or peripheral neuropathy. Diagnosis requires the demonstration of green birefringence under polarized light of amyloid deposits stained with Congo red.

Biochemical Investigations

High-resolution electrophoresis combined with immunofixation of serum and urine is recommended for identification and characterization of the IgM monoclonal protein. The light chain of the monoclonal IgM is κ[kappa] in 75–80 % of patients. A few WM patients have more than one M component. The concentration of the serum monoclonal protein is very variable but in most cases lies within the range of 15–45 g/L. Densitometry should be adopted to determine IgM levels for serial evaluations because nephelometry is unreliable and shows large intralaboratory as well as interlaboratory variation. The presence of cold agglutinins or cryoglobulins may affect determination of IgM levels and, therefore, testing for cold agglutinins and cryoglobulins should be performed at diagnosis. If present, subsequent serum samples should be analyzed under warm conditions for determination of serum monoclonal IgM level. Although Bence Jones proteinuria is frequently present, it exceeds 1 g/24 h in only 3 % of cases. While IgM levels are elevated in WM patients, IgA and IgG levels are most often depressed and do not demonstrate recovery even after successful treatment suggesting that patients with WM harbor a defect which prevents normal plasma cell development and/or Ig heavy chain rearrangements [99, 100].

Serum Viscosity

Because of its large size (almost 1,000,000 Da), most IgM molecules are retained within the intravascular compartment and can exert an undue effect on serum viscosity. Therefore, serum viscosity should be measured if the patient has signs or symptoms of hyperviscosity syndrome. Fundoscopy remains an excellent indicator of clinically relevant hyperviscosity. Among the first clinical signs of hyperviscosity is the appearance of peripheral and midperipheral dot and blot-like hemorrhages in the retina, which are best appreciated with indirect ophthalmoscopy and scleral depression [49]. In more severe cases of hyperviscosity, dot, blot, and flame-shaped hemorrhages can appear in the macular area along with markedly dilated and tortuous veins with focal constrictions resulting in "venous sausaging" as well as papilledema.

Bone Marrow Findings

The bone marrow is always involved in WM. Central to the diagnosis of WM is the demonstration, by trephine biopsy, of bone marrow infiltration by a lymphoplasmacytic cell population constituted by small lymphocytes with evidence of plasmacytoid/plasma cell differentiation. The pattern of bone marrow infiltration may be diffuse, interstitial, or nodular, showing usually an intertrabecular pattern of infiltration. A solely paratrabecular pattern of infiltration is unusual and should raise the possibility of follicular lymphoma [1]. The bone marrow infiltration should routinely be confirmed by *immunophenotypic studies* (flow cytometry and/or immunohistochemistry) showing the following profile: $sIgM^+CD19^+CD20^+CD22^+CD79^+$ [30–32]. Up to 20 % of cases may express either CD5, CD10 or CD23 [33]. In these cases, care should be taken to satisfactorily exclude chronic lymphocytic leukemia and mantle cell lymphoma [1]. "Intranuclear" periodic acid-Schiff (PAS)-positive inclusions (Dutcher-Fahey bodies) [101] consisting of IgM deposits in the perinuclear space, and sometimes in intranuclear vacuoles, may be seen occasionally in lymphoid cells in WM. An increased number of mast cells, usually in association with the lymphoid aggregates, is commonly found in WM, and their presence may help in differentiating WM from other B-cell lymphomas [1, 2].

Other Investigations

Magnetic resonance imaging (MRI) of the spine in conjunction with computed tomography (CT) of the abdomen and pelvis are useful in evaluating the disease status in WM [102]. Bone marrow involvement can be documented by MRI studies of the spine in over 90 % of patients, while CT of the abdomen and pelvis demonstrated enlarged nodes in 43 % of WM patients [102]. Lymph node biopsy may show preserved architecture or replacement by infiltration of neoplastic cells with lymphoplasmacytoid, lymphoplasmacytic, or polymorphous cytological patterns. The residual disease after high-dose chemotherapy with allogeneic or autologous stem cell rescue can be monitored by polymerase chain reaction (PCR)-based methods using primers specific for the monoclonal Ig variable regions.

Prognosis

Waldenström's macroglobulinemia typically presents as an indolent disease though considerable variability in prognosis can be seen. The median survival reported in several large series has ranged from 5 to 10 years [103–109], though in a recent study of 436 consecutive patients with WM, the median overall survival from time of diagnosis was in excess of 10 years [110]. Age is consistently an important prognostic factor (>60–70 years) [103, 104, 106, 109], though it is often impacted by unrelated morbidities. Anemia, which can be multifactorial, is an adverse prognostic factor in WM, with hemoglobin levels of <9–12 g/dL associated with decreased survival in several series [103–105, 109]. Cytopenias have also been regularly identified as a significant predictor of survival. The number of cytopenias in a given patient may predict survival [104]. Serum albumin levels have correlated with survival in WM patients in certain but not all studies using multivariate analyses [104, 107]. High serum beta-2 microglobulin (>3–3.5 g/dL) levels [105, 107, 109], high serum IgM M-protein (>7 g/dL) [109], low serum IgM M-protein (<4 g/dL) [107], the presence of cryoglobulins [103], and the presence of a familial disease background [110] have also been reported to confer adverse outcomes. The presence of 6q deletion as an adverse marker remains controversial [14, 16]. A few prognostic scoring systems have been proposed (Table 5.3). While the use of prognostic markers and/or scoring systems to make therapeutic decisions remains to be clarified [106], patients with familial disease predisposition show better outcomes following bortezomib-based therapy [110].

Table 5.3 Prognostic scoring systems in Waldenstrom's macroglobulinemia

Study	Adverse prognostic factors	Number of groups	Survival
Gobbi et al. [103]	Hb <9 g/dL Age >70 year Weight loss Cryoglobulinemia	Prognostic factors 2–4 prognostic factors	Median: 48 months Median: 80 months
Morel et al. [104]	Age >65 year Albumin <4 g/dL Number of cytopenias: Hb <12 g/dL Platelets <150 × 10^9/L Wbc <4 × 10^9/L	Prognostic factors 2 prognostic factors 3–4 prognostic factors	5 years: 87 % 5 years: 62 % 5 years: 25 %
Dhodapkar et al. [105]	β[beta]$_2$M >3 g/dL Hb <12 g/dL IgM <4 g/dL	β[beta]$_2$M <3 mg/dL + Hb >12 g/dL β[beta]$_2$M <3 mg/dL + Hb <12 g/dL β[beta]$_2$M >3 mg/dL + IgM >4 g/dL β[beta]$_2$M >3 mg/dL + IgM <4 g/dL	5 years: 87 % 5 years: 63 % 5 years: 53 % 5 years: 21 %
Application of International Staging System Criteria for Myeloma to WM Dimopoulos et al. [107]	Albumin <3.5 g/dL β[beta]$_2$M >3.5 mg/L	Albumin >3.5 g/dL + β[beta]$_2$M <3.5 mg/dL Albumin <3.5 g/dL + β[beta]$_2$M <3.5 or β[beta]$_2$M 3.5–5.5 mg/dL β[beta]$_2$M >5.5 mg/dL	Median: NR Median: 116 months Median: 54 months
International Prognostic Scoring System for WM Morel et al. [109]	Age >65 year Hb <11.5 g/dL Platelets <100 × 10^9/L β[beta]$_2$M >3 mg/L IgM >7 g/dL	Prognostic factors[a] 2 prognostic factors[b] 3–5 prognostic factors	5 years: 87 % 5 years: 68 % 5 years: 36 %

[a]excluding age
[b]or age >65

Treatment of Waldenström's Macroglobulinemia

Treatment Indications

Consensus guidelines on indications for treatment initiation were formulated as part of the Second International Workshop on Waldenström's Macroglobulinemia [106]. Initiation of therapy should not be based on the IgM levels since this may not correlate with either disease burden nor symptomatic status [111, 112]. Initiation of therapy is appropriate for patients with constitutional symptoms, such as recurrent fever, night sweats, fatigue due to anemia, or weight loss. The presence of progressive, symptomatic lymphadenopathy or splenomegaly provides additional reasons to begin therapy. The presence of anemia with a hemoglobin value of <10 g/dL or a platelet count <100 × 10^9/L on this basis of disease is also a reasonable indication for treatment initiation. Certain complications of WM, such as hyperviscosity syndrome, symptomatic sensorimotor peripheral neuropathy, systemic amyloidosis, renal insufficiency, or symptomatic cryoglobulinemia, are also indications for therapy.

Treatment Options

A precise therapeutic algorithm for therapy of WM remains to be defined given the paucity of randomized clinical trials. Active agents include alkylators (chlorambucil, cyclophosphamide), nucleoside analogues (cladribine, fludarabine), monoclonal antibodies (rituximab, ofatumumab,

alemtuzumab), bortezomib, thalidomide, everolimus, and bendamustine [111, 112]. Combination therapy particularly with rituximab has been associated with improved clinical outcomes. Individual patient considerations, including the presence of cytopenias, need for more rapid disease control, age, and candidacy for autologous transplant therapy, should be taken into account in making the choice of a first-line agent. For patients who are candidates for autologous transplant therapy, exposure to continuous chlorambucil or nucleoside analogue therapy should be limited given potential for stem cell damage. The use of nucleoside analogues may also increase risk for histological transformation to diffuse large B-cell lymphoma as well as myelodysplasia and acute myelogenous leukemia [113].

Chlorambucil

Oral alkylating drugs, alone and in combination therapy with steroids, have been extensively evaluated in the upfront treatment of WM. The greatest experience with oral alkylator therapy has been with chlorambucil, which has been administered on both a continuous (i.e., daily dose schedule) as well as an intermittent schedule. Patients receiving chlorambucil on a continuous schedule typically receive 0.1 mg/kg/day, while on the intermittent schedule patients will typically receive 0.3 mg/kg for 7 days, every 6 weeks. In a prospective randomized study, Kyle et al. [114] reported no significant difference in the overall response rate between these schedules, although interestingly the median response duration was greater for patients receiving intermittent versus continuously dosed chlorambucil (46 vs. 26 months). Despite the favorable median response duration in this study for use of the intermittent schedule, no difference in the median overall survival was observed. Moreover, an increased incidence for development of myelodysplasia and acute myelogenous leukemia with the intermittent (3 of 22 patients) versus the continuous (0 of 24 patients) chlorambucil schedule prompted the authors of this study to express preference for use of continuous chlorambucil dosing. The use of steroids in combination with alkylator therapy has also been explored. Dimopoulos and Alexanian [115] evaluated chlorambucil (8 mg/m^2) along with prednisone (40 mg/m^2) given orally for 10 days, every 6 weeks, and reported a major response (i.e., reduction of IgM by greater than 50 %) in 72 % of patients. Non-chlorambucil-based alkylator regimens employing melphalan and cyclophosphamide in combination with steroids have also been examined by Petrucci et al. [116] and Case et al. [117] producing slightly higher overall response rates and response durations, although the benefit of these more complex regimens over chlorambucil remains to be demonstrated. Facon et al. [118] have evaluated parameters predicting for response to alkylator therapy. Their studies in patients receiving single-agent chlorambucil demonstrated that age 60, male sex, symptomatic status, and cytopenias (but, interestingly, not high tumor burden and serum IgM levels) were associated with poor response to alkylator therapy. Additional factors to be taken into account in considering alkylator therapy for patients with WM include necessity for more rapid disease control given the slow nature of response to alkylator therapy as well as consideration for preserving stem cells in patients who are candidates for autologous transplant therapy.

Nucleoside Analogues

Both cladribine and fludarabine have been extensively evaluated in untreated as well as previously treated WM patients. Cladribine administered as a single agent by continuous intravenous infusion, by 2-h daily infusion, or by subcutaneous bolus injections for 5–7 days has resulted in major responses in 40–90 % of patients who received primary therapy, while in the salvage setting, responses have ranged from 38 to 54 % [111–125]. Median time for achievement of response following cladribine ranged from 1.2 to 5 months in these studies. The overall response rate with daily infusional fludarabine therapy administered mainly on 5-day schedules in previously untreated and treated WM patients has ranged from 38 to 100 % and 30–40 %, respectively [105, 126–132], which are on par with the response data for cladribine. Median time to achievement of response for fludarabine was also on par with cladribine at 3–6 months. In general,

response rates and durations of responses have been greater for patients receiving nucleoside analogues as first-line agents, although in several of the above studies wherein both untreated and previously treated patients were enrolled, no substantial difference in the overall response rate was reported. Myelosuppression commonly occurred following prolonged exposure to either of the nucleoside analogues, as did lymphopenia with sustained depletion of both $CD4^+$ and $CD8^+$ T-lymphocytes observed in WM patients 1 year following initiation of therapy. Treatment-related mortality due to myelosuppression and/or opportunistic infections attributable to immunosuppression occurred in up to 5 % of all treated patients in some series with either nucleoside analogue. Factors predicting for response to nucleoside analogues in WM included age at start of treatment (<70 years), pretreatment hemoglobin >95 g/L, platelets >75,000/mm3, disease relapsing off therapy, patients with resistant disease within the first year of diagnosis, and a long interval between first-line therapy and initiation of a nucleoside analogue in relapsing patients. There are limited data on the use of an alternate nucleoside analogue to salvage patients whose disease relapsed or demonstrated resistance off cladribine or fludarabine therapy [125, 126]. Three of four (75 %) patients responded to cladribine to salvage patients who progressed following an unmaintained remission to fludarabine, whereas only one of ten (10 %) with disease resistant to fludarabine responded to cladribine [125]. However, Lewandowski et al. [132] reported a response in two of six patients (33 %) and disease stabilization in the remaining patients to fludarabine, in spite of an inadequate response or progressive disease following cladribine therapy. The combination of nucleoside analogues with cyclophosphamide and/or rituximab has been investigated and discussed below.

The safety of nucleoside analogues has been the subject of investigation in several recent studies. Thomas et al. recently reported their experiences in harvesting stem cells in 21 patients with symptomatic WM in whom autologous peripheral blood stem cell collection was attempted. Autologous stem cell collection succeeded on the first attempt in 14/15 patients who received non-nucleoside analogue-based therapy versus 2/6 patients who received a nucleoside analogue [133]. The long-term safety of nucleoside analogues in WM was recently examined by Leleu et al. [113] in a large series of WM patients. A sevenfold increase in transformation to an aggressive lymphoma and a threefold increase in the development of acute myelogenous leukemia/myelodysplasia were observed among patients who received a nucleoside analogue versus other therapies for their WM. A recent metanalysis by Leleu et al. [134] of several trials utilizing nucleoside analogues in WM patients, which included patients who had previously received an alkylator agent, showed a crude incidence of 6.6–10 % for development of disease transformation and 1.4–8.9 % for development of myelodysplasia or acute myelogenous leukemia. None of the studied risk factors, i.e., gender, age, family history of WM or B-cell malignancies, typical markers of tumor burden and prognosis, type of nucleoside analogue therapy (cladribine vs. fludarabine), time from diagnosis to nucleoside analogue use, nucleoside analogue treatment as primary or salvage therapy, as well as treatment with an oral alkylator (i.e., chlorambucil), predicted for the occurrence of transformation or development of myelodysplasia/acute myelogenous leukemia for WM patients treated with a nucleoside analogue.

Monoclonal Antibodies

Rituximab is a chimeric monoclonal antibody which targets CD20, a widely expressed antigen on lymphoplasmacytic cells in WM [135]. The use of rituximab at standard dosimetry (i.e., 4 weekly infusions at 375 mg/m^2) induces major responses in approximately 27–35 % of previously treated and untreated patients [136, 137]. However, patients who achieved even minor responses benefited from rituximab as evidenced by improved hemoglobin and platelet counts and reduction of lymphadenopathy and/or splenomegaly [136]. The median time to treatment failure in these studies was found to range from 8 to 27+ months. Studies evaluating an extended rituximab schedule consisting of 4 weekly courses at 375 mg/m^2/week, repeated 3 months later by another

4-week course, have demonstrated higher major response rates of 44–48 %, with time to progression estimates of 16+ to 29+ months [138, 139].

In many WM patients, a transient increase of serum IgM (IgM flare) may be noted immediately following initiation of rituximab treatment [140–142]. The IgM flare may be related to the release of interleukin-6 by bystander immune in response to the binding of rituximab to Fcγ[gamma]RIIA receptors and also occurs in response to intravenous immunoglobulin administration in WM patients [143]. The IgM flare in response to rituximab does not herald treatment failure, and while most patients will return to their baseline serum IgM level by 12 weeks, some patients may flare for months despite having tumor responses in their bone marrow. Patients with baseline serum IgM levels of >50 g/dL or serum viscosity of >3.5cp may be particularly at risk for a hyperviscosity-related event and in such patients plasmapheresis should be considered or rituximab omitted for the first few cycles of therapy until IgM levels decline to safer levels [110]. Because of the decreased likelihood of response in patients with higher IgM levels as well as the possibility that serum IgM and viscosity levels may abruptly rise, rituximab monotherapy should not be used as sole therapy for the treatment of patients at risk for hyperviscosity symptoms.

Time to response after rituximab is slow and exceeds 3 months on the average. The time to best response in one study was 18 months [139]. Patients with baseline serum IgM levels of <60 g/dL are more likely to respond, irrespective of the underlying bone marrow involvement by tumor cells [138, 139]. A recent analysis of 52 patients who were treated with single-agent rituximab has indicated that the objective response rate was significantly lower in patients who had either low serum albumin (<35 g/L) or elevated serum monoclonal protein (>40 g/LM-spike). Furthermore, the presence of both adverse prognostic factors was related with a short time to progression (3.6 months). Moreover patients who had normal serum albumin and relatively low serum monoclonal protein levels derived a substantial benefit from rituximab with a time to progression exceeding 40 months [144].

The genetic background of patients may also be important for determining response to rituximab. A correlation between polymorphisms at amino acid position 158 in the Fcγ[gamma]RIIIa receptor (CD16) and rituximab response has been observed in WM patients. WM patients who carry a valine amino acid (either in a homozygous or heterozygous pattern) at this polymorphic site had a fourfold higher major response rate to rituximab versus patients who expressed phenylalanine in a homozygous pattern [145]. The attainment of better categorical responses, i.e., very good partial response or complete response following rituximab-based therapy, appears also dependent on the presence of at least one valine amino acid at Fcγ[gamma]RIIIa-158 [146].

Ofatumumab is a fully humanized CD20-directed monoclonal antibody that targets the small loop of CD20, a target which is different than that of rituximab. A 59 % overall response rate was observed in a series of 37 symptomatic WM patients following ofatumumab administration, which included untreated and previously treated patients [147]. Responses were higher among rituximab-naïve patients. An IgM flare with symptomatic hyperviscosity was also observed in 2 patients in this series who required plasmapheresis. Ofatumumab has also been successfully administered to WM patients who demonstrated intolerance to rituximab [147, 148].

The activity of alemtuzumab has also been investigated in WM patients given the broad expression of CD52 [135]. The WMCTG recently reported a multicenter study in symptomatic WM patients, whose median prior therapies was 2 (range 0–5), and 43 % had refractory disease [149]. Patients received alemtuzumab intravenously at 30 mg three times weekly for up to 12 weeks, after test dosing, and received hydrocortisone, acyclovir, and bactrim or equivalent prophylaxis. The overall response rate in this series was 75 % and included major responses in 36 % of patients. With a median follow-up of 64 months, the median time to progression was 14.5 months. Hematological and infectious complications, including CMV reactivation were more common in previously treated patients and

indirectly associated with 3 deaths. Long-term follow-up revealed late-onset idiopathic thrombocytopenia in 4 patients at a median of 13.6 months following therapy and contributed to one death. High rates of response with the use of alemtuzumab were also observed by Owen et al. [150] who reported their preliminary experience in a small series of heavily pretreated WM patients. The median number of prior therapies in this series was 4, and similar to this study patients received up to 12 weeks of therapy (at 30 mg IV three times weekly) following initial dose escalation. Among the 7 patients treated with alemtuzumab, 5 achieved a partial response and one a complete response. Disseminated aspergillus and mycobacterial infections contributed to 2 deaths in this series.

Bortezomib

Bortezomib is a proteasome inhibitor which has been extensively investigated in WM. In a multicenter study of the WMCTG, 27 patients received up to 8 cycles of bortezomib at 1.3 mg/m^2 on days 1, 4, 8, and 11 [151]. All but one patient had relapsed/or refractory disease. Following therapy, median serum IgM levels declined from 4,660 to 2,092 mg/dL ($p<0.0001$). The overall response rate was 85 %, with 10 and 13 patients achieving minor (<25 % decrease in IgM) and major (<50 % decrease in IgM) responses. Responses were prompt and occurred at median of 1.4 months. The median time to progression for all responding patients in this study was 7.9 (range 3–21.4+) months, and the most common grade III/IV toxicities occurring in >5 % of patients were sensory neuropathies (22.2 %), leukopenia (18.5 %), neutropenia (14.8 %), dizziness (11.1 %), and thrombocytopenia (7.4 %). Importantly, sensory neuropathies resolved or improved in nearly all patients following cessation of therapy. As part of an NCI-Canada study, Chen et al. [152] treated 27 patients with both untreated (44 %) and previously treated (56 %) diseases. Patients in this study received bortezomib utilizing the standard schedule until they either demonstrated progressive disease or 2 cycles beyond a complete response or stable disease. The overall response rate in this study was 78 %, with major responses observed in 44 % of patients. Sensory neuropathy occurred in 20 pts, 5 with grade >3, and occurred following 2–4 cycles of therapy. Among the 20 patients developing a neuropathy, 14 patients resolved and one patient demonstrated a one-grade improvement at 2–13 months. In addition to the above experiences with bortezomib monotherapy in WM, Dimopoulos et al. [153] observed major responses in 6 of 10 (60 %) previously treated WM patients, while Goy et al. [154] observed a major response in 1 of 2 WM patients who were included in a series of relapsed or refractory patients with non-Hodgkin's lymphoma (NHL). The combination of bortezomib with steroids and/or rituximab has also been investigated and is discussed below.

Immunomodulatory Agents

Thalidomide as monotherapy and in combination with dexamethasone and/or clarithromycin has been examined in WM. Dimopoulos et al. [155] demonstrated a major response in five of 20 (25 %) previously untreated and treated patients who received single-agent thalidomide. Dose escalation from the thalidomide start dose of 200 mg daily was hindered by the development of side effects, including the development of peripheral neuropathy in five patients obligating discontinuation or dose reduction. Low doses of thalidomide (50 mg orally daily) in combination with dexamethasone (40 mg orally once a week) and clarithromycin (250 mg orally twice a day) have also been examined, with 10 of 12 (83 %) previously treated patients demonstrating at least a major response [156]. However, in a follow-up study by Dimopoulos et al. [157] using a higher thalidomide dose (200 mg orally daily) along with dexamethasone (40 g orally once a week) and clarithromycin (500 mg orally twice a day), only two of ten (20 %) previously treated patients responded. Thalidomide, as well as lenalidomide, has also been investigated in combination with rituximab and these studies are discussed below.

Bendamustine

Bendamustine is a recently approved agent for the treatment of relapsed/refractory indolent non-Hodgkin's lymphoma (NHL). Bendamustine has structural similarities to both alkylating agents and purine analogues [158]. Bendamustine in combination with rituximab has been investigated in both previously untreated and relapsed/refractory WM patients and is discussed below.

Everolimus

Everolimus is an oral inhibitor of the mTOR pathway, which is approved for the treatment of renal cell carcinoma. The Akt-mTOR-p70 pathway is active in WM, and inhibition of this pathway leads to apoptosis of primary WM cells and WM cell lines [159, 160]. Fifty patients with a median of 3 prior therapies were treated with everolimus in a joint Dana Farber/Mayo Clinic study [161]. The overall response rate was 70 %, with 42 % of patients attaining a major response. The progression-free survival at 12 months was estimated to be 62 %. Grade 3 or higher related toxicities were observed in 56 % of patients with cytopenias constituting the most common toxicity. Pulmonary toxicity occurred in 10 % of patients. Dose reductions due to toxicity occurred in 52 % of patients.

A clinical trial examining the activity of everolimus in previously untreated patients with WM was completed by the WMCTG [162]. While 67 % of patients achieved at least a minor response by consensus criteria which rely on paraprotein reduction, IgM discordance to underlying disease burden was seen in up to half of patients on this upfront study. Cytopenias, particularly anemia and thrombocytopenia, were common, and pneumonitis occurred in 15 % of patients.

Combination Strategies

Because rituximab is an active and a nonmyelosuppressive agent, its combination with various chemotherapeutic agents has been extensively explored in WM. The combination of CHOP (cyclophosphamide, doxorubicin, vincristine, prednisone) with rituximab (CHOP-R) was investigated in a randomized frontline study by the German Low Grade Lymphoma Study Group (GLSG) involving 69 patients, most of whom had WM [163]. The addition of rituximab to CHOP resulted in a higher overall response rate (94 % vs. 67 %) and median time to progression (63 vs. 22 months) in comparison to patients treated with CHOP alone. Dimopoulos et al. [164] investigated the combination of rituximab, dexamethasone, and oral cyclophosphamide (RCD) as primary therapy in 72 patients with WM. At least a major response was observed in 74 % of patients in this study, and the 2-year progression-free survival was 67 %. Therapy was well tolerated, though one patient died of interstitial pneumonia. In the salvage setting, the use of CHOP-R has been investigated in relapsed/refractory WM patients [165]. Among 13 evaluable patients, 10 patients achieved a major response (77 %) including 3 CR and 7 PR, and 2 patients achieved a minor response. In a retrospective study, Ioakimidis et al. [166] examined the outcomes of symptomatic WM patients who received CHOP-R, CVP-R, or CP-R. Baseline characteristics for all 3 cohorts were similar for age, prior therapies, bone marrow involvement, hematocrit, platelet count, and serum beta 2 microglobulin, though serum IgM levels were higher in patients treated with CHOP-R. The overall response rates to therapy were comparable for all three treatments: CHOP-R (96 %), CVP-R (88 %), and CP-R (95 %), though more CRs were observed among patients treated with either CVP-R or CHOP-R. Comparison of adverse events for these regimens showed a higher incidence for neutropenic fever as well as treatment-related neuropathy in patients receiving CHOP-R and CVP-R versus CPR. These results suggest that in WM, the use of doxorubicin and vincristine may be omitted in order to minimize treatment-related complications.

Combination therapy with nucleoside analogues has been investigated as both first-line and salvage therapy in WM. Weber et al. [167] administered rituximab along with cladribine and cyclophosphamide to 17 previously untreated patients

with WM. At least a partial response was documented in 94 % of WM patients including a complete response in 18 %. With a median follow-up of 21 months, no patient has relapsed. Laszlo et al. [168] recently evaluated the combination of subcutaneous cladribine with rituximab in 29 WM patients with either untreated or previously treated disease. Intended therapy consisted of rituximab on day 1 followed by subcutaneous cladribine 0.1 mg/kg for 5 consecutive days, administered monthly for 4 cycles. With a median follow-up of 43 months, the overall response rate observed was 89.6 %, with seven complete responses (CR), 16 partial responses, and three minor responses. Response activity was similar between untreated and previously treated patients. No major infections were observed despite the lack of antimicrobial prophylaxis. In a study by the WMCTG, the combination of rituximab and fludarabine was administered to 43 WM patients, 32 (75 %) of whom were previously untreated [169]. The overall response rate was 95.3 %, and 83 % of patients achieved a major response. The median time to progression was 51.2 months in this series, and was longer for those patients who were previously untreated and for those achieving at least a very good partial response. Hematological toxicity was common, particularly neutropenia and thrombocytopenia. Two deaths occurred in this study due to non-pneumocystis carinii pneumonia. Secondary malignancies including transformation to aggressive lymphoma and development of myelodysplasia or AML were observed in 6 patients in this series. The addition of rituximab to fludarabine and cyclophosphamide has also been explored in the salvage setting by Tam et al. [170] wherein 4 of 5 patients demonstrated a response. Hensel et al. [171] administered rituximab along with pentostatin and cyclophosphamide to 13 patients with untreated and previously treated WM or lymphoplasmacytic lymphoma. A major response was observed in 77 % of patients. The addition of alkylating agents to nucleoside analogues has also been explored in WM. Weber et al. [167] administered two cycles of oral cyclophosphamide along with subcutaneous cladribine to 37 patients with previously untreated WM. At least a partial response was observed in 84 % of patients and the median duration of response was 36 months. Dimopoulos et al. [172] examined fludarabine in combination with intravenous cyclophosphamide and observed partial responses in 6 of 11 (55 %) patients with either primary refractory disease or who relapsed on treatment. The combination of fludarabine plus cyclosphosphamide (FC) was also evaluated in a recent study by Tamburini et al. [173] involving 49 patients, 35 of whom were previously treated. Seventy-eight percent of the patients in this study achieved a response and median time to treatment failure was 27 months. Hematological toxicity was commonly observed and three patients died of treatment-related toxicities. Two interesting findings in this study were the development of acute leukemia in 2 patients, histologic transformation to diffuse large cell lymphoma in one patient, and 2 cases of solid malignancies (prostate and melanoma) as well as failure to mobilize stem cells in 4 of 6 patients. Tedeschi et al. [174] recently completed a multicenter study on fludarabine, cyclophosphamide, and rituximab (FCR) in symptomatic WM patients with untreated or relapsed/refractory disease to one line of chemotherapy. Treatment consisted of rituximab at 375 mg/m^2 on day 1, fludarabine at 25 mg/m^2, and cyclophosphamide at 250 mg/m^2 by intravenous administration on days 2–4 every 4 weeks. Forty-three patients were accrued to this study. The overall response rate was 89 %, with 83 % of patients attaining a major remission and 14 % a complete response. Prolonged neutropenia was observed in up to a third of patients. With a median follow-up of 15 months, the median progression-free survival for this study has not been reached.

The combination of bortezomib, dexamethasone, and rituximab (BDR) has been investigated as primary therapy in patients with WM by the WMCTG. An overall response rate of 96 %, major response rate of 83 %, and complete attainment in 22 % was observed with BDR [175]. The updated median progression-free survival in this study was >56.1 months. The incidence of grade 3 neuropathy was 30 % in this study which utilized a twice a week schedule for bortezomib

administration at 1.3 mg/m^2. Peripheral neuropathy from bortezomib was reversible in most patients in this study following discontinuation of therapy, and patients benefitted with pregabalin. An increased incidence of herpes zoster was also observed with BDR prompting the use of prophylactic antiviral therapy. An alternative schedule for bortezomib administration (i.e., weekly at 1.6 mg/m^2) in combination with rituximab and/or dexamethasone has been investigated in several studies with overall response rates of 80–90 % [176–178]. A lower incidence of peripheral neuropathy was observed in two studies using once a week bortzomib [172, 178]. The impact of once versus twice a week bortezomib administration on progression-free survival remains to be clarified.

The combination of immunomodulator agents (thalidomide, lenalidomide) with rituximab was investigated by the WMCTG. Thalidomide was administered at 200 mg daily for 2 weeks, followed by 400 mg daily and thereafter for 1 year. Patients received four weekly infusions of rituximab at 375 mg/m^2 beginning 1 week after initiation of thalidomide, followed by four additional weekly infusions of rituximab at 375 mg/m^2 beginning at week 13. The overall and major response rate was 72 and 64 %, respectively, and the median time to progression was 38 months in this series [179]. Dose reduction and/or discontinuation of thalidomide was common and mainly attributed to treatment-related neuropathy. The investigators concluded in this study that lower doses of thalidomide (i.e., 50–100 mg/day) should be considered in this patient population. The combination of lenalidomide with rituximab was investigated by the WMCTG using lenalidomide at 25 mg daily on a syncopated schedule wherein therapy was administered for 3 weeks, followed by a 1-week pause for an intended duration of 48 weeks [180]. Patients received 1 week of therapy with lenalidomide, after which rituximab (375 mg/m^2) was administered weekly on weeks 2–5, then 13–16. The overall and major response rates in this study were 50 and 25 %, respectively, and a median TTP for responders was 18.9 months. In two patients with bulky disease, significant reduction in extramedullary disease was observed. However, an acute decrease in hematocrit was observed during first 2 weeks of lenalidomide therapy in 13/16 (81 %) patients with a median absolute decrease in hematocrit of 4.8 %, resulting in anemia-related complications and hospitalizations in 4 patients. Despite dose reduction, most patients in this study continued to demonstrate aggravated anemia with lenalidomide. There was no evidence of hemolysis or more general myelosuppression with lenalidomide in this study. Therefore, the mechanism for lenalidomide-related anemia in WM patients remains to be determined, and the use of this agent among WM patients should be avoided.

The use of bendamustine in combination with rituximab was explored by Rummel et al. [181] in the frontline therapy of WM. As part of a randomized study, patients received 6 cycles of bendamustine plus rituximab (Benda-R) or CHOP-R. A total of 546 patients were enrolled in this study for indolent NHL patients and included 40 patients with WM. Patients on the Benda-R arm received bendamustine at 90 mg/m^2 on days 1 and 2 and rituximab at 375 mg/m^2 on day 1 with the frequency of 4 weeks for each cycle. The overall response rate was 96 % for Benda-R and 94 % for CHOP-R-treated patients. With a median observation period of 26 months, 20/23 (87 %) Benda-R versus 9/17 (53 %) CHOP-R-treated WM patients remain free of progression. Importantly, Benda-R was associated with a lower incidence of grade 3 or 4 neutropenia, infectious complications, and alopecia. In the salvage setting, the outcome of 30 WM patients with relapsed/refractory disease who received bendamustine alone or with a CD20-directed antibody was reported by Treon et al. [182]. An overall response rate of 83.3 % and a median progression-free survival of 13.2 months were reported in this study. Overall, therapy was well tolerated though prolonged myelosuppression occurred in patients who received prior nucleoside analogue therapy.

Maintenance Therapy

A role for maintenance rituximab in WM patients following response to a rituximab-containing

regimen was raised in a study examining the outcome of 248 WM rituximab-naïve patients who were either observed or received maintenance rituximab [183]. In this retrospective study, categorical responses improved in 16/162 (10 %) of observed patients and in 36/86 (41.8 %) of patients who received maintenance rituximab following induction therapy. Both progression-free (56.3 vs. 28.6 months) and overall survivals (>120 vs. 116 months) were longer in patients who received maintenance rituximab. Improved progression-free survival was evident despite previous treatment status, induction with rituximab alone or in combination therapy. Best serum IgM response was lower and hematocrit higher in those patients receiving maintenance rituximab. Among patients receiving maintenance rituximab, an increased number of infectious events, predominantly sinusitis and bronchitis, were observed, though were mainly grade 1 or 2.

High-Dose Therapy and Stem Cell Transplantation

The use of stem cell transplantation (SCT) therapy has also been explored in patients with WM. Desikan et al. [184] reported their initial experience of high-dose chemotherapy and autologous stem cell transplant, which has more recently been updated by Munshi et al. [185]. Their studies involved eight previously treated WM patients between the ages of 45 and 69 years who received either melphalan at 200 mg/m^2 or melphalan at 140 mg/m^2 with total body irradiation. Stem cells were successfully collected in all eight patients, although a second collection procedure was required for two patients who had extensive previous nucleoside analogue exposure. There were no transplant-related mortalities and toxicities were manageable. All eight patients responded, with 7 of 8 patients achieving a major response and one patient achieving a complete response with durations of response raging from 5+ to 77+ months. Dreger et al. [186] investigated the use of the DEXA-BEAM (dexamethasone, BCNU, etoposide, cytarabine, melphalan) regimen followed by myeloablative therapy with cyclophosphamide, and total body irradiation and autologous stem cell transplantation in seven WM patients, which included four untreated patients. Serum IgM levels declined by >50 % following DEXA-BEAM and myeloablative therapy for 6 of 7 patients, with progression-free survival ranging from 4+ to 30+ months. All three evaluable patients who were previously treated also attained a major response in a study by Anagnostopoulos et al. [187] wherein WM patients received various preparative regimens and demonstrated event-free survivals of 26+, 31, and 108+ months. Tournilhac et al. [188] recently reported the outcome of 18 WM patients in France who received high-dose chemotherapy followed by autologous stem cell transplantation. All patients were previously treated with a median of three (range 1–5) prior regimens. Therapy was well tolerated with an improvement in response status observed for seven patients (six PR to CR, one SD to PR), while only one patient demonstrated progressive disease. The median event-free survival for all nonprogressing patients was 12 months. Tournilhac et al. [188] have also reported the outcome of allogeneic transplantation in ten previously treated WM patients (ages 35–46) who received a median of three prior therapies, including three patients with progressive disease despite therapy. Two of three patients with progressive disease responded, and an improvement in response status was observed in five patients. The median event-free survival for nonprogressing, evaluable patients was 31 months. Concerning in this series was the death of three patients owing to transplantation related toxicity. Anagnostopoulos et al. [189] have also reported on a retrospective review of WM patients who underwent either autologous or allogeneic transplantation and whose outcomes were reported to the International Blood and Marrow Transplant Registry. Seventy-eight percent of patients in this cohort had two or more previous therapies, and 58 % of them were resistant to their previous therapy. The relapse rate at 3 years was 29 % in the allogeneic group and 24 % in the autologous

group. Nonrelapse mortality however was 40 % in the allogeneic group and 11 % in the autologous group in this series.

Kyriakou et al. [190] reported on the outcome of WM patients in the European Bone Marrow Transplant (EBMT) registry who received either an autologous or allogeneic SCT. Among 158 patients receiving an autologous SCT, which included primarily relapsed or refractory patients, the 5-year progression-free and overall survival rate were 39.7 and 68.5 %, respectively. Nonrelapse mortality at 1 year was 3.8 %. Chemorefractory disease and the number of prior lines of therapy at time of the autologous SCT were the most important prognostic factor for progression-free and overall survival. The achievement of a negative immunofixation after autologous SCT had a positive impact on progression-free survival. When used as consolidation at first response, autologous transplantation provided a progression-free survival of 44 % at 5 years. In the allogeneic SCT experience from the EBMT, the long-term outcome of 86 WM patients was reported by Kyriakou [191]. A total of 86 patients received allograft by either myeloablative ($n=37$) or reduced-intensity ($n=49$) conditioning. The median age of patients in this series was 49 years, and 47 patients had three or more previous lines of therapy. Eight patients failed prior autologous SCT. Fifty-nine patients (68.6 %) had chemotherapy-sensitive disease at the time of allogeneic SCT. Nonrelapse mortality at 3 years was 33 % for patients receiving a myeloablative transplant and 23 % for those who received reduced-intensity conditioning. The overall response rate was 75.6 %. The relapse rates at 3 years were 11 % for myeloablative and 25 % for reduced-intensity conditioning recipients. Five-year progression-free and overall survival for WM patients who received a myeloablative allogeneic SCT were 56 and 62 % and for patients who received reduced-intensity conditioning were 49 and 64 %, respectively. The occurrence of chronic graft-versus-host disease was associated with improved progression-free survival and suggested the existence of a clinically relevant graft-versus-WM effect in this study.

Response Criteria in Waldenstrom's Macroglobulinemia

As part of the International Workshops on WM, consensus panels developed guidelines for uniform response criteria in WM [192, 193]. The category of minor response was adopted at the Third International Workshop of WM, given that clinically meaningful responses were observed with newer biological agents and is based on >25 to <50 % decrease in serum IgM level, which is used as a surrogate marker of disease in WM. At the Sixth International Workshop on WM, the categorical response of very good partial response (VGPR), i.e., 90 % reduction in IgM levels, was adopted given reports of improved clinical outcome associated with VGPR or better response achievement [146, 169, 175, 190]. In distinction, the term major response is used to denote a response of >50 % in serum IgM levels and includes partial or better responses [193]. Response categories and criteria for progressive disease in WM based on consensus recommendations are summarized in Table 5.4 [194].

An important concern with the use of IgM as a surrogate marker of disease is that it can fluctuate, independent of tumor cell killing, particularly with biologically targeted agents such as rituximab, bortezomib, and everolimus [140–142, 151, 162, 195]. Rituximab induces a spike or flare in serum IgM levels which can occur when used as monotherapy and in combination with other agents including cyclophosphamide, nucleoside analogues, thalidomide, and lenalidomide, and last for several weeks to months [140–142, 166, 179, 180, 196], whereas bortezomib and everolimus can suppress IgM levels independent of tumor cell killing in certain patients [151, 162, 196]. Moreover, Varghese et al. [197] showed that in patients treated with selective B-cell depleting agents such as rituximab and alemtuzumab, residual IgM-producing plasma cells are spared and continue to persist, thus potentially skewing the relative response and assessment to treatment. Therefore, in circumstances where the serum IgM levels appear out of context with the clinical progress of the patient, a bone marrow biopsy should

Table 5.4 Summary of updated response criteria adopted at the Sixth International Workshop on Waldenstrom's macroglobulinemia [194]

Complete response	CR	IgM in normal range and disappearance of monoclonal protein by immunofixation; no histological evidence of bone marrow involvement, and resolution of any adenopathy/organomegaly (if present at baseline), along with no signs or symptoms attributable to WM. Reconfirmation of the CR status is required by repeat immunofixation studies
Very good partial response	VGPR	A >90 % reduction of serum IgM and decrease in adenopathy/organomegaly (if present at baseline) on physical examination or on CT scan. No new symptoms or signs of active disease
Partial response	PR	A >50 % reduction of serum IgM and decrease in adenopathy/organomegaly (if present at baseline) on physical examination or on CT scan. No new symptoms or signs of active disease
Minor response	MR	A >25 % but <50 % reduction of serum IgM. No new symptoms or signs of active disease
Stable disease	SD	A <25 % reduction and <25 % increase of serum IgM without progression of adenopathy/organomegaly, cytopenias, or clinically significant symptoms due to disease and/or signs of WM
Progressive disease	PD	A >25 % increase in serum IgM by protein confirmed by a second measurement or progression of clinically significant findings due to disease (i.e., anemia, thrombocytopenia, leukopenia, bulky adenopathy/organomegaly) or symptoms (unexplained recurrent fever >38.4 °C, drenching night sweats, >10 % body weight loss, or hyperviscosity, neuropathy, symptomatic cryoglobulinemia, or amyloidosis) attributable to WM

be considered inorder to clarify the patient's underlying disease burden. Soluble CD27 may serve as an alternative surrogate marker in WM and remains a faithful marker of disease in patients experiencing a rituximab-related IgM flare as well as plasmapheresis [40, 198].

References

1. Owen RG, Treon SP, Al-Katib A, Fonseca R, Greipp PR, McMaster ML, et al. Clinicopathological definition of Waldenström's macroglobulinemia: consensus panel recommendations from the Second International Workshop on Waldenström's macroglobulinemia. Semin Oncol. 2003;30:110–5.
2. Swerdlow SH, Campo E, Harris NL, et al. World Health Organization classification of tumours of haematopoietic and lymphoid tissues. Lyon: IARC Press; 2008.
3. Groves FD, Travis LB, Devesa SS, Ries LA, Fraumeni Jr JF. Waldenström's macroglobulinemia: incidence patterns in the United States, 1988–1994. Cancer. 1998;82:1078–81.
4. Hanzis C, Ojha RP, Hunter Z, Manning R, Lewicki M, Brodsky P, et al. Associated malignancies in patients with Waldenström's macroglobulinemia and their Kin. Clin Lymphoma Myeloma Leuk. 2011;11:88–92.
5. Varettoni M, Tedesci A, Arcaini L, et al. Risk of second cancers in Waldenstrom macroglobulinemia. Ann Oncol. 2012;23:411–5.
6. Renier G, Ifrah N, Chevailler A, et al. Four brothers with Waldenstrom's macroglobulinemia. Cancer. 1989;64:1554–9.
7. Treon SP, Hunter ZR, Aggarwal A, et al. Characterization of familial Waldenstrom's macroglobulinemia. Ann Oncol. 2006;17:488–94.
8. Kristinsson SY, Bjorkholm M, Goldin LR, et al. Risk of lymphoproliferative disorders among first-degree relatives of lymphoplasmacytic lymphoma/Waldenstrom's macroglobulinemia patients: a population-based study in Sweden. Blood. 2008;112:3052–6.
9. McMaster ML, Csako G, Giambarresi TR, et al. Long-term evaluation of three multiple-case Waldenstrom's macroglobulinemia families. Clin Cancer Res. 2007;13:5063–9.
10. Ogmundsdottir HM, Sveinsdottir S, Sigfusson A, Skaftadottir I, Jonasson JG, Agnarsson BA. Enhanced B cell survival in familial macroglobulinaemia is associated with increased expression of Bcl-2. Clin Exp Immunol. 1999;117:252–60.
11. Ogmundsdottir HM, Steingrimsdottir H, Haraldsdottir V. Familial paraproteinemia: hyper-responsive B-cells as endophenotype. Clin Lymphoma Myeloma Leukemia. 2011;11:82–4.
12. Silvestri F, Barillari G, Fanin R, Zaja F, Infanti L, Patriarca F, et al. Risk of hepatitis C virus infection, Waldenström's macroglobulinemia, and monoclonal gammopathies. Blood. 1996;88:1125–6.

13. Leleu X, O'Connor K, Ho A, Santos DD, Manning R, Xu L, et al. Hepatitis C viral infection is not associated with Waldenstrom's macroglobulinemia. Am J Hematol. 2007;82:83–4.
14. Schop RF, Kuehl WM, Van Wier SA, Ahmann GJ, Price-Troska T, Bailey RJ, et al. Waldenström macroglobulinemia neoplastic cells lack immunoglobulin heavy chain locus translocations but have frequent 6q deletions. Blood. 2002;100:2996–3001.
15. Ocio EM, Schop RF, Gonzalez B, et al. 6q deletion in Waldenstrom's macroglobulinemia is associated with features of adverse prognosis. Br J Haematol. 2007; 136:80–6.
16. Chang H, Qi C, Trieu Y, et al. Prognostic relevance of 6q deletion in Waldenstrom's macroglobulinemia. Clin Lymph Myeloma. 2009;9:36–8.
17. Nguyen-Khac F, Lejeune J, Chapiro E, et al. Cytogenetic abnormalities in a cohort of 171 patients with Waldenström macroglobulinemia before treatment: clinical and biological correlations. Blood 2010;116: Abstract 801.
18. Rivera AI, Li MM, Beltran G, Krause JR. Trisomy 4 as the sole cytogenetic abnormality in a Waldenstrom macroglobulinemia. Cancer Genet Cytogenet. 2002; 133:172–3.
19. Avet-Loiseau H, Garand R, Lode L, Robillard N, Bataille R. 14q32 translocations discriminate IgM multiple myeloma from Waldenstrom's macroglobulinemia. Semin Oncol. 2003;30:153–5.
20. Treon SP, Xu L, Zhou Y, et al. Whole genome sequencing reveals a widely expressed mutation (MYD88 L265P) with oncogenic activity in Waldenstrom's macroglobulinemia. Blood. 2011;118: Abstract 300.
21. Watters T, Kenny EF, O'Neill LAJ. Structure, function and regulation of the Toll/IL-1 receptor adaptor proteins. Immunol Cell Biol. 2007;85:411–9.
22. Cohen L, Henzel WJ, Baeuerie PA. IKAP is a scaffold protein of the IkappaB kinase complex. Nature. 1998;395:292–6.
23. Loiarro M, Gallo G, Fanto N, et al. Identification of critical residues of the MYD88 death domain involved in the recruitment of downstream kinases. J Biol Chem. 2009;284:28093–281023.
24. Lin SC, Lo YC, Wu H. Helical assembly in the MYD88-IRAK4-IRAK2 complex in TLR/IL-1R signaling. Nature. 2010;465:885–91.
25. Kawagoe T, Sato S, Matsushita K, et al. Sequential control of Toll-like receptor dependent responses by IRAK1 and IRAK2. Nat Immunol. 2008;9:684–91.
26. Leleu X, Eeckhoute J, Jia X, et al. Targeting NF-kappaB in Waldenstrom macroglobulinemia. Blood. 2008;111:5068–77.
27. Preud'homme JL, Seligmann M. Immunoglobulins on the surface of lymphoid cells in Waldenström's macroglobulinemia. J Clin Invest. 1972;51:701–5.
28. Smith BR, Robert NJ, Ault KA. In Waldenstrom's macroglobulinemia the quantity of detectable circulating monoclonal B lymphocytes correlates with clinical course. Blood. 1983;61:911–4.
29. Levy Y, Fermand JP, Navarro S, Schmitt C, Vainchenker W, Seligmann M, et al. Interleukin 6 dependence of spontaneous in vitro differentiation of B cells from patients with IgM gammopathy. Proc Natl Acad Sci USA. 1990;87:3309–13.
30. Owen RG, Barrans SL, Richards SJ, O'Connor SJ, Child JA, Parapia LA, et al. Waldenström macroglobulinemia. Development of diagnostic criteria and identification of prognostic factors. Am J Clin Pathol. 2001;116:420–8.
31. Feiner HD, Rizk CC, Finfer MD, Bannan M, Gottesman SR, Chuba JV, et al. IgM monoclonal gammopathy/Waldenström's macroglobulinemia: a morphological and immunophenotypic study of the bone marrow. Mod Pathol. 1990;3:348–56.
32. San Miguel JF, Vidriales MB, Ocio E, Mateo G, Sanchez-Guijo F, et al. Immunophenotypic analysis of Waldenstrom's macroglobulinemia. Semin Oncol. 2003;30:187–95.
33. Hunter ZR, Branagan AR, Manning R, Patterson CJ, Santos DD, Tournilhac O, et al. CD5, CD10, CD23 expression in Waldenstrom's Macroglobulinemia. Clin Lymph. 2005;5:246–9.
34. Wagner SD, Martinelli V, Luzzatto L. Similar patterns of V kappa gene usage but different degrees of somatic mutation in hairy cell leukemia, prolymphocytic leukemia, Waldenström's macroglobulinemia, and myeloma. Blood. 1994;83:3647–53.
35. Aoki H, Takishita M, Kosaka M, Saito S. Frequent somatic mutations in D and/or JH segments of Ig gene in Waldenström's macroglobulinemia and chronic lymphocytic leukemia (CLL) with Richter's syndrome but not in common CLL. Blood. 1995;85:1913–9.
36. Shiokawa S, Suehiro Y, Uike N, Muta K, Nishimura J. Sequence and expression analyses of mu and delta transcripts in patients with Waldenström's macroglobulinemia. Am J Hematol. 2001;68:139–43.
37. Sahota SS, Forconi F, Ottensmeier CH, Provan D, Oscier DG, Hamblin TJ, et al. Typical Waldenström macroglobulinemia is derived from a B-cell arrested after cessation of somatic mutation but prior to isotype switch events. Blood. 2002;100:1505–7.
38. Paramithiotis E, Cooper MD. Memory B lymphocytes migrate to bone marrow in humans. Proc Natl Acad Sci USA. 1997;94:208–12.
39. Tournilhac O, Santos DD, Xu L, Kutok J, Tai YT, Le Gouill S, et al. Mast cells in Waldenstrom's Macroglobulinemia support lymphoplasmacytic cell growth through CD154/CD40 signaling. Ann Oncol. 2006;17:1275–82.
40. Ho A, Leleu X, Hatjiharissi E, Tournilhac O, Xu L, O'Connor K, et al. Patterson, Anderson KC., Treon pp. CD27–CD70 interactions in the pathogenesis of Waldenstrom's Macroglobulinemia. Blood. 2008;112:4683–9.
41. Ngo HT, Leleu X, Lee J, Jia X, et al. SDF-1/CXCR4 and VLA-4 interaction regulates homing in Waldenstrom macroglobulinemia. Blood. 2008;112: 150–8.

42. Merlini G, Farhangi M, Osserman EF. Monoclonal immunoglobulins with antibody activity in myeloma, macroglobulinemia and related plasma cell dyscrasias. Semin Oncol. 1986;13:350–65.
43. Farhangi M, Merlini G. The clinical implications of monoclonal immunoglobulins. Semin Oncol. 1986;13:366–79.
44. Marmont AM, Merlini G. Monoclonal autoimmunity in hematology. Haematologica. 1991;76:449–59.
45. Mackenzie MR, Babcock J. Studies of the hyperviscosity syndrome. II. Macroglobulinemia. J Lab Clin Med. 1975;85:227–34.
46. Gertz MA, Kyle RA. Hyperviscosity syndrome. J Intens Care Med. 1995;10:128–41.
47. Kwaan HC, Bongu A. The Hyperviscosity syndromes. Semin Thromb Hemost. 1999;25:199–208.
48. Singh A, Eckardt KU, Zimmermann A, Gotz KH, Hamann M, Ratcliffe PJ, et al. Increased plasma viscosity as a reason for inappropriate erythropoietin formation. J Clin Invest. 1993;91:251–6.
49. Menke MN, Feke GT, McMeel JW, Branagan A, Hunter Z, Treon SP. Hyperviscosity-related retinopathy in Waldenstrom's macroglobulinemia. Arch Opthalmol. 2006;124:1601–6.
50. Merlini G, Baldini L, Broglia C, Comelli M, Goldaniga M, Palladini G, et al. Prognostic factors in symptomatic Waldenström's macroglobulinemia. Semin Oncol. 2003;30:211–5.
51. Dellagi K, Dupouey P, Brouet JC, Billecocq A, Gomez D, Clauvel JP, et al. Waldenström's macroglobulinemia and peripheral neuropathy: a clinical and immunologic study of 25 patients. Blood. 1983;62:280–5.
52. Nobile-Orazio E, Marmiroli P, Baldini L, Spagnol G, Barbieri S, Moggio M, et al. Peripheral neuropathy in macroglobulinemia: incidence and antigen-specificity of M proteins. Neurology. 1987;37:1506–14.
53. Nemni R, Gerosa E, Piccolo G, Merlini G. Neuropathies associated with monoclonal gammapathies. Haematologica. 1994;79:557–66.
54. Ropper AH, Gorson KC. Neuropathies associated with paraproteinemia. N Engl J Med. 1998;338:1601–7.
55. Treon SP, Hanzis CA, Ioakimidis LI, et al. Clinical characteristics and treatment outcome of disease-related peripheral neuropathy in Waldenstrom's macroglobulinemia. Proc Am Soc Clin Oncol 2010; 28: Abstract 8114.
56. Vital A. Paraproteinemic neuropathies. Brain Pathol. 2001;11:399–407.
57. Latov N, Braun PE, Gross RB, Sherman WH, Penn AS, Chess L. Plasma cell dyscrasia and peripheral neuropathy: identification of the myelin antigens that react with human paraproteins. Proc Natl Acad Sci USA. 1981;78:7139–42.
58. Chassande B, Leger JM, Younes-Chennoufi AB, Bengoufa D, Maisonobe T, Bouche P, et al. Peripheral neuropathy associated with IgM monoclonal gammopathy: correlations between M-protein antibody activity and clinical/electrophysiological features in 40 cases. Muscle Nerve. 1998;21:55–62.
59. Weiss MD, Dalakas MC, Lauter CJ, Willison HJ, Quarles RH. Variability in the binding of anti-MAG and anti-SGPG antibodies to target antigens in demyelinating neuropathy and IgM paraproteinemia. J Neuroimmunol. 1999;95:174–84.
60. Latov N, Hays AP, Sherman WH. Peripheral neuropathy and anti-MAG antibodies. Crit Rev Neurobiol. 1988;3:301–32.
61. Dalakas MC, Quarles RH. Autoimmune ataxic neuropathies (sensory ganglionopathies): are glycolipids the responsible autoantigens? Ann Neurol. 1996;39: 419–22.
62. Eurelings M, Ang CW, Notermans NC, Van Doorn PA, Jacobs BC, Van den Berg LH. Antiganglioside antibodies in polyneuropathy associated with monoclonal gammopathy. Neurology. 2001;57:1909–12.
63. Ilyas AA, Quarles RH, Dalakas MC, Fishman PH, Brady RO. Monoclonal IgM in a patient with paraproteinemic polyneuropathy binds to gangliosides containing disialosyl groups. Ann Neurol. 1985;18:655–9.
64. Willison HJ, O'Leary CP, Veitch J, Blumhardt LD, Busby M, Donaghy M, et al. The clinical and laboratory features of chronic sensory ataxic neuropathy with anti-disialosyl IgM antibodies. Brain. 2001;124:1968–77.
65. Lopate G, Choksi R, Pestronk A. Severe sensory ataxia and demyelinating polyneuropathy with IgM anti-GM2 and GalNAc-GD1A antibodies. Muscle Nerve. 2002;25:828–36.
66. Jacobs BC, O'Hanlon GM, Breedland EG, Veitch J, Van Doorn PA, Willison HJ. Human IgM paraproteins demonstrate shared reactivity between Campylobacter jejuni lipopolysaccharides and human peripheral nerve disialylated gangliosides. J Neuroimmunol. 1997;80:23–30.
67. Nobile-Orazio E, Manfredini E, Carpo M, Meucci N, Monaco S, Ferrari S, et al. Frequency and clinical correlates of antineural IgM antibodies in neuropathy associated with IgM monoclonal gammopathy. Ann Neurol. 1994;36:416–24.
68. Gordon PH, Rowland LP, Younger DS, Sherman WH, Hays AP, Louis ED, et al. Lymphoproliferative disorders and motor neuron disease: an update. Neurology. 1997;48:1671–8.
69. Pavord SR, Murphy PT, Mitchell VE. POEMS syndrome and Waldenström's macroglobulinaemia. J Clin Pathol. 1996;49:181–2.
70. Crisp D, Pruzanski W. B–cell neoplasms with homogeneous cold-reacting antibodies (cold agglutinins). Am J Med. 1982;72:915–22.
71. Pruzanski W, Shumak KH. Biologic activity of cold-reacting autoantibodies (first of two parts). N Engl J Med. 1977;297:538–42.
72. Pruzanski W, Shumak KH. Biologic activity of cold-reacting autoantibodies (second of two parts). N Engl J Med. 1977;297:583–9.
73. Whittaker SJ, Bhogal BS, Black MM. Acquired immunobullous disease: a cutaneous manifestation of IgM macroglobulinaemia. Br J Dermatol. 1996;135: 283–6.

74. Daoud MS, Lust JA, Kyle RA, Pittelkow MR. Monoclonal gammopathies and associated skin disorders. J Am Acad Dermatol. 1999;40:507–35.
75. Gad A, Willen R, Carlen B, Gyland F, Wickander M. Duodenal involvement in Waldenström's macroglobulinemia. J Clin Gastroenterol. 1995;20:174–6.
76. Case records of the Massachusetts General Hospital. Weekly clinicopathological exercises. Case 3–1990. A 66-year-old woman with Waldenström's macroglobulinemia, diarrhea, anemia, and persistent gastrointestinal bleeding. N Engl J Med. 1990;322: 183–92.
77. Isaac J, Herrera GA. Cast nephropathy in a case of Waldenström's macroglobulinemia. Nephron. 2002;91:512–5.
78. Morel-Maroger L, Basch A, Danon F, Verroust P, Richet G. Pathology of the kidney in Waldenström's macroglobulinemia. Study of sixteen cases. N Engl J Med. 1970;283:123–9.
79. Gertz MA, Kyle RA, Noel P. Primary systemic amyloidosis: a rare complication of immunoglobulin M monoclonal gammopathies and Waldenström's macroglobulinemia. J Clin Oncol. 1993;11:914–20.
80. Moyner K, Sletten K, Husby G, Natvig JB. An unusually large (83 amino acid residues) amyloid fibril protein AA from a patient with Waldenström's macroglobulinaemia and amyloidosis. Scand J Immunol. 1980;11:549–54.
81. Gardyn J, Schwartz A, Gal R, Lewinski U, Kristt D, Cohen AM. Waldenström's macroglobulinemia associated with AA amyloidosis. Int J Hematol. 2001;74:76–8.
82. Dussol B, Kaplanski G, Daniel L, Brunet P, Pellissier JF, Berland Y. Simultaneous occurrence of fibrillary glomerulopathy and AL amyloid. Nephrol Dial Transplant. 1998;13:2630–2.
83. Rausch PG, Herion JC. Pulmonary manifestations of Waldenström macroglobulinemia. Am J Hematol. 1980;9:201–9.
84. Fadil A, Taylor DE. The lung and Waldenström's macroglobulinemia. South Med J. 1998;91:681–5.
85. Kyrtsonis MC, Angelopoulou MK, Kontopidou FN, Siakantaris MP, Dimopoulou MN, Mitropoulos F, et al. Primary lung involvement in Waldenström's macroglobulinaemia: report of two cases and review of the literature. Acta Haematol. 2001;105: 92–6.
86. Kaila VL. el Newihi HM, Dreiling BJ, Lynch CA, Mihas AA. Waldenström's macroglobulinemia of the stomach presenting with upper gastrointestinal hemorrhage. Gastrointest Endosc. 1996;44:73–5.
87. Yasui O, Tukamoto F, Sasaki N, Saito T, Yagisawa H, Uno A, et al. Malignant lymphoma of the transverse colon associated with macroglobulinemia. Am J Gastroenterol. 1997;92:2299–301.
88. Rosenthal JA, Curran Jr WJ, Schuster SJ. Waldenström's macroglobulinemia resulting from localized gastric lymphoplasmacytoid lymphoma. Am J Hematol. 1998;58:244–5.
89. Recine MA, Perez MT, Cabello-Inchausti B, Lilenbaum RC, Robinson MJ. Extranodal lymphoplasmacytoid lymphoma (immunocytoma) presenting as small intestinal obstruction. Arch Pathol Lab Med. 2001;125:677–9.
90. Veltman GA, van Veen S, Kluin-Nelemans JC, Bruijn JA, van Es LA. Renal disease in Waldenström's macroglobulinaemia. Nephrol Dial Transplant. 1997;12:1256–9.
91. Moore Jr DF, Moulopoulos LA, Dimopoulos MA. Waldenström macroglobulinemia presenting as a renal or perirenal mass: clinical and radiographic features. Leuk Lymphoma. 1995;17:331–4.
92. Mascaro JM, Montserrat E, Estrach T, Feliu E, Ferrando J, Castel T, et al. Specific cutaneous manifestations of Waldenström's macroglobulinaemia. A report of two cases. Br J Dermatol. 1982;106:17–22.
93. Schnitzler L, Schubert B, Boasson M, Gardais J, Tourmen A. Urticaire chronique, lésions osseuses, macroglobulinémie IgM: Maladie de Waldenström? Bull Soc Fr Dermatol Syphiligr. 1974;81:363–8.
94. Roux S, Fermand JP, Brechignac S, Mariette X, Kahn MF, Brouet JC. Tumoral joint involvement in multiple myeloma and Waldenström's macroglobulinemia—report of 4 cases. J Rheumatol. 1996;23: 2175–8.
95. Orellana J, Friedman AH. Ocular manifestations of multiple myeloma, Waldenström's macroglobulinemia and benign monoclonal gammopathy. Surv Ophthalmol. 1981;26:157–69.
96. Ettl AR, Birbamer GG, Philipp W. Orbital involvement in Waldenström's macroglobulinemia: ultrasound, computed tomography and magnetic resonance findings. Ophthalmologica. 1992;205:40–5.
97. Civit T, Coulbois S, Baylac F, Taillandier L, Auque J. Waldenström's macroglobulinemia and cerebral lymphoplasmocytic proliferation: Bing and Neel syndrome. Apropos of a new case. Neurochirurgie. 1997;43:245–9.
98. McMullin MF, Wilkin HJ, Elder E. Inaccurate haemoglobin estimation in Waldenström's macroglobulinaemia. J Clin Pathol. 1995;48:787.
99. Hunter ZR, Manning RJ, Hanzis C, et al. IgA and IgG hypogammaglobulinemia in Waldenstrom's macroglobulinemia. Haematologica. 2010;95:470–5.
100. Treon SP, Hunter Z, Ciccarelli BT, et al. IgA and IgG hypogammaglobulinemia is a constitutive feature in most Waldenstrom's macroglobulinemia patients and may be related to mutations associated with common variable immunodeficiency disorder (CVID). Blood. 2008;112:3749.
101. Dutcher TF, Fahey JL. The histopathology of macroglobulinemia of Waldenström. J Natl Cancer Inst. 1959;22:887–917.
102. Moulopoulos LA, Dimopoulos MA, Varma DG, Manning JT, Johnston DA, Leeds NE, et al. Waldenström macroglobulinemia: MR imaging of the spine and CT of the abdomen and pelvis. Radiology. 1993;188:669–73.

103. Gobbi PG, Bettini R, Montecucco C, Cavanna L, Morandi S, Pieresca C, et al. Study of prognosis in Waldenström's macroglobulinemia: a proposal for a simple binary classification with clinical and investigational utility. Blood. 1994;83:2939–45.
104. Morel P, Monconduit M, Jacomy D, Lenain P, Grosbois B, Bateli C, et al. Prognostic factors in Waldenström macroglobulinemia: a report on 232 patients with the description of a new scoring system and its validation on 253 other patients. Blood. 2000;96:852–8.
105. Dhodapkar MV, Jacobson JL, Gertz MA, Rivkin SE, Roodman GD, Tuscano JM, et al. Prognostic factors and response to fludarabine therapy in patients with Waldenström macroglobulinemia: results of United States intergroup trial (Southwest Oncology Group S9003). Blood. 2001;98:41–8.
106. Kyle RA, Treon SP, Alexanian R, Barlogie B, Bjorkholm M, Dhodapkar M, et al. Prognostic markers and criteria to initiate therapy in Waldenström's macroglobulinemia: consensus panel recommendations from the Second International Workshop on Waldenström's macroglobulinemia. Semin Oncol. 2003;30:116–20.
107. Dimopoulos M, Gika D, Zervas K, et al. The international staging system for multiple myeloma is applicable in symptomatic Waldenstrom's macroglobulinemia. Leuk Lymph. 2004;45: 1809–13.
108. Anagnostopoulos A, Zervas K, Kyrtsonis M, et al. Prognostic value of serum beta 2-microglobulin in patients with Waldenstrom's macroglobulinemia requiring therapy. Clin Lymph Myeloma. 2006;7: 205–9.
109. Morel P, Duhamel A, Gobbi P, Dimopoulos MA, Dhodapkar MV, McCoy J, et al. International prognostic scoring system for waldenstrom macroglobulinemia. Blood. 2009;113:4163–70.
110. Treon SP. Treatment with a bortezomib-containing regimen is associated with better therapeutic outcomes in patients with Waldenstrom's macroglobulinemia who have familial disease predisposition. Blood. 2011;118: Abstract 1643.
111. Treon SP, How I. Treat Waldenstrom's macroglobulinemia. Blood. 2009;114:419–31.
112. Dimopoulos MA, Gertz MA, Kastritis E, et al. Update on treatment recommendations from the Fourth International Workshop on Waldenstrom's macroglobulinemia. J Clin Oncol. 2009;27:120–6.
113. Leleu XP, Manning R, Soumerai JD, et al. Increased incidence of transformation and myelodysplasia/ acute leukemia in patients with Waldenström macroglobulinemia treated with nucleoside analogs. J Clin Oncol. 2009;27:250–5.
114. Kyle RA, Greipp PR, Gertz MA, Witzig TE, Lust JA, Lacy MQ, et al. Waldenström's macroglobulinaemia: a prospective study comparing daily with intermittent oral chlorambucil. Br J Haematol. 2000;108:737–42.
115. Dimopoulos MA, Alexanian R. Waldenstrom's macroglobulinemia. Blood. 1994;83:1452–9.
116. Petrucci MT, Avvisati G, Tribalto M, Giovangrossi P, Mandelli F. Waldenström's macroglobulinaemia: results of a combined oral treatment in 34 newly diagnosed patients. J Intern Med. 1989;226: 443–7.
117. Case Jr DC, Ervin TJ, Boyd MA, Redfield DL. Waldenström's macroglobulinemia: long-term results with the M-2 protocol. Cancer Invest. 1991;9:1–7.
118. Facon T, Brouillard M, Duhamel A, Morel P, Simon M, Jouet JP, et al. Prognostic factors in Waldenström's macroglobulinemia: a report of 167 cases. J Clin Oncol. 1993;11:1553–8.
119. Dimopoulos MA, Kantarjian H, Weber D, O'Brien S, Estey E, Delasalle K, et al. Primary therapy of Waldenström's macroglobulinemia with 2-chlorodeoxyadenosine. J Clin Oncol. 1994;12:2694–8.
120. Delannoy A, Ferrant A, Martiat P, Bosly A, Zenebergh A, Michaux JL. 2-Chlorodeoxyadenosine therapy in Waldenström's macroglobulinaemia. Nouv Rev Fr Hematol. 1994;36:317–20.
121. Fridrik MA, Jager G, Baldinger C, Krieger O, Chott A, Bettelheim P. First-line treatment of Waldenström's disease with cladribine. Arbeitsgemeinschaft Medikamentose Tumortherapie. Ann Hematol. 1997;74:7–10.
122. Liu ES, Burian C, Miller WE, Saven A. Bolus administration of cladribine in the treatment of Waldenström macroglobulinaemia. Br J Haematol. 1998;103:690–5.
123. Hellmann A, Lewandowski K, Zaucha JM, Bieniaszewska M, Halaburda K, Robak T. Effect of a 2-hour infusion of 2-chlorodeoxyadenosine in the treatment of refractory or previously untreated Waldenström's macroglobulinemia. Eur J Haematol. 1999;63:35–41.
124. Betticher DC, Hsu Schmitz SF, Ratschiller D, von Rohr A, Egger T, Pugin P, et al. Cladribine (2-CDA) given as subcutaneous bolus injections is active in pretreated Waldenström's macroglobulinaemia. Swiss Group for Clinical Cancer Research (SAKK). Br J Haematol. 1997;99:358–63.
125. Dimopoulos MA, Weber D, Delasalle KB, Keating M, Alexanian R. Treatment of Waldenström's macroglobulinemia resistant to standard therapy with 2-chlorodeoxyadenosine: identification of prognostic factors. Ann Oncol. 1995;6:49–52.
126. Dimopoulos MA, O'Brien S, Kantarjian H, Pierce S, Delasalle K, Barlogie B, et al. Fludarabine therapy in Waldenström's macroglobulinemia. Am J Med. 1993;95:49–52.
127. Foran JM, Rohatiner AZ, Coiffier B, Barbui T, Johnson SA, Hiddemann W, et al. Multicenter phase II study of fludarabine phosphate for patients with newly diagnosed lymphoplasmacytoid lymphoma, Waldenström's macroglobulinemia, and mantle-cell lymphoma. J Clin Oncol. 1999;17:546–53.

128. Thalhammer-Scherrer R, Geissler K, Schwarzinger I, Chott A, Gisslinger H, Knobl P, et al. Fludarabine therapy in Waldenström's macroglobulinemia. Ann Hematol. 2000;79:556–9.
129. Zinzani PL, Gherlinzoni F, Bendandi M, Zaccaria A, Aitini E, Salvucci M, et al. Fludarabine treatment in resistant Waldenström's macroglobulinemia. Eur J Haematol. 1995;54:120–3.
130. Leblond V, Ben Othman T, Deconinck E, Taksin AL, Harousseau JL, Delgado MA, et al. Activity of fludarabine in previously treated Waldenström's macroglobulinemia: a report of 71 cases. Groupe Cooperatif Macroglobulinemie. J Clin Oncol. 1998;16:2060–4.
131. Dimopoulos MA, Weber DM, Kantarjian H, Keating M, Alexanian R. 2-Chlorodeoxyadenosine therapy of patients with Waldenström macroglobulinemia previously treated with fludarabine. Ann Oncol. 1994;5:288–9.
132. Lewandowski K, Halaburda K, Hellmann A. Fludarabine therapy in Waldenström's macroglobulinemia patients treated previously with 2-chlorodeoxyadenosine. Leuk Lymphoma. 2002;43:361–3.
133. Thomas S, Hosing C, Delasalle KB, et al. Success rates of autologous stem cell collection in patients with Waldenstrom's macroglobulinemia. Proceedings of the fifth International Workshop on Waldenstrom's macroglobulinemia. 2008, Clinical Lymphoma and Meyloma.
134. Leleu X, Tamburini J, Roccaro A, et al. Balancing risk versus benefit in the treatment of Waldenstrom's macroglobulinemia patients with nucleoside analogue based therapy. Clin Lymphoma Myeloma. 2009;9:71–3.
135. Treon SP, Kelliher A, Keele B, et al. Expression of serotherapy target antigens in Waldenstrom's macroglobulinemia: therapeutic applications and considerations. Semin Oncol. 2003;30:248–52.
136. Treon SP, Agus DB, Link B, et al. CD20-Directed antibody-mediated immunotherapy induces responses and facilitates hematologic recovery in patients with Waldenstrom's macroglobulinemia. J Immunother. 2001;24:272–9.
137. Gertz MA, Rue M, Blood E, et al. Multicenter phase 2 trial of rituximab for Waldenstrom macroglobulinemia (WM): an Eastern Cooperative Oncology Group Study (E3A98). Leuk Lymphoma. 2004;45:2047–55.
138. Dimopoulos MA, Zervas C, Zomas A, et al. Treatment of Waldenstrom's macroglobulinemia with rituximab. J Clin Oncol. 2002;20:2327–33.
139. Treon SP, Emmanouilides C, Kimby E, Kelliher A, Preffer F, Branagan AR, et al. Extended rituximab therapy in Waldenström's macroglobulinemia. Ann Oncol. 2005;16:132–8.
140. Donnelly GB, Bober-Sorcinelli K, Jacobson R, Portlock CS. Abrupt IgM rise following treatment with rituximab in patients with Waldenstrom's macroglobulinemia. Blood. 2001;98:240b.
141. Treon SP, Branagan AR, Anderson KC. Paradoxical increases in serum IgM levels and serum viscosity following rituximab therapy in patients with Waldenstrom's macroglobulinemia. Ann Oncol. 2004;15:1481–3.
142. Ghobrial IM, Fonseca R, Greipp PR, et al. The initial "flare" of IgM level after rituximab therapy in patients diagnosed with Waldenstrom macroglobulinemia: an Eastern Cooperative Oncology Group Study. Cancer. 2004;101:2593–8.
143. Yang G, Xu L, Hunter ZR, Liu X, Cao Y, Jiang J, Tseng H, Zhou YS, Ioakimidis L, Hanzis C, Sheehy P, Patterson CJ, Treon SP. The rituximab and IVIG related IgM flare in Waldenstrom's macroglobulinemia is associated with monocytic activation of FCGR2A signaling, and triggering of IL-6 release by the PI3K/AKT and MAPK pathways. Blood. 2010;116: Abstract 2870.
144. Dimopoulos MA, Anagnostopoulos A, Zervas C, et al. Predictive factors for response to rituximab in Waldenstrom's macroglobulinemia. Clin Lymphoma. 2005;5:270–2.
145. Treon SP, Hansen M, Branagan AR, et al. Polymorphisms in Fcγ[gamma]RIIIA (CD16) receptor expression are associated with clinical responses to Rituximab in Waldenstrom's Macroglobulinemia. J Clin Oncol. 2005;23:474–81.
146. Treon SP, Yang G, Hanzis C, Ioakimidis L, Verselis SJ, Fox EA, et al. Attainment of complete/very good partial response following rituximab-based therapy is an important determinant to progression-free survival, and is impacted by polymorphisms in FCGR3A in Waldenstrom macroglobulinaemia. Br J Haematol. 2011;154:223–8.
147. Furman RR, Eradat H, Switzky JC, et al. A phase II trial of ofatumumab in subjects with Waldenstrom's macroglobulinemia. Blood. 2011;118: Abstract 3701.
148. Treon SP, Hanzis C, Tripsas C, Ioakimidis L, Patterson CJ, Manning RJ, et al. Bendamustine therapy in patients with relapsed or refractory Waldenström's macroglobulinemia. Clin Lymphoma Myeloma Leuk. 2011;11:133–5.
149. Treon SP, Soumerai JD, Hunter ZR, Patterson CJ, Ioakimidis L, Kahl B, et al. Long-term follow-up of symptomatic patients with lymphoplasmacytic lymphoma/Waldenstrom's macroglobulinemia treated with the anti-CD52 monoclonal antibody alemtuzumab. Blood. 2011;118:276–81.
150. Owen RG, Rawstron AC, Osterborg A, et al. Activity of alemtuzumab in relapsed/refractory Waldenstrom's macroglobulinemia. Blood. 2003;102:644a.
151. Treon SP, Hunter ZR, Matous J, et al. Multicenter clinical trial of bortezomib in relapsed/refractory Waldenstrom's macroglobulinemia: results of WMCTG trial 03–248. Clin Cancer Res. 2007;13:3320–5.
152. Chen CI, Kouroukis CT, White D, et al. Bortezomib is active in patients with untreated or relapsed Waldenstrom's macroglobulinemia: a phase II study of the National Cancer Institute of Canada Clinical Trials Group. J Clin Oncol. 2007;25:1570–5.

153. Dimopoulos MA, Anagnostopoulos A, Kyrtsonis MC, et al. Treatment of relapsed or refractory Waldenstrom's macroglobulinemia with bortezomib. Haematologica. 2005;90:1655–7.
154. Goy A, Younes A, McLaughlin P, et al. Phase II study of proteasome inhibitor bortezomib in relapsed or refractory B-cell non-Hodgkin's lymphoma. J Clin. 2005;23:657–8.
155. Dimopoulos MA, Zomas A, Viniou NA, Grigoraki V, Galani E, Matsouka C, et al. Treatment of Waldenström's macroglobulinemia with thalidomide. J Clin Oncol. 2001;19:3596–601.
156. Coleman C, Leonard J, Lyons L, Szelenyi H, Niesvizky R. Treatment of Waldenström's macroglobulinemia with clarithromycin, low-dose thalidomide and dexamethasone. Semin Oncol. 2003;30:270–4.
157. Dimopoulos MA, Zomas K, Tsatalas K, Hamilos G, Efstathiou E, Gika D, et al. Treatment of Waldenström's macroglobulinemia with single agent thalidomide or with combination of clarithromycin, thalidomide and dexamethasone. Semin Oncol. 2003;30:265–9.
158. Cheson BD, Rummel MJ. Bendamustine: rebirth of an old drug. J Clin Oncol. 2009;27:1492–501.
159. Hatjiharissi E, Mitsiades CS, Ciccarelli B, et al. Comprehensive molecular characterization of malignant and microenvironmental cells in Waldenstroms macroglobulinemia by gene expression profiling. Blood. 2007;110: Abstract 3174.
160. Leleu X, Jia X, Runnels J, et al. The Akt pathway regulates survival and homing in Waldenstrom macroglobulinemia. Blood. 2007;110:4417–26.
161. Ghobrial I, Gertz M, LaPlant B, et al. Phase II trial of the oral mammalian target of rapamycin inhibitor everolimus in relapsed or refractory Waldenström macroglobulinemia. J Clin Oncol. 2010;28:1408–14.
162. Treon SP, Tripsas C, Ioakimidis L, et al. Prospective, multicenter study of the MTOR inhibitor everolimus (RAD001) as primary therapy in Waldenstrom's macroglobulinemia. Blood. 2011;118: Abstract 2951.
163. Buske C, Hoster E, Dreyling MH, et al. The addition of rituximab to front-line therapy with CHOP (R-CHOP) results in a higher response rate and longer time to treatment failure in patients with lymphoplasmacytic lymphoma: results of a randomized trial of the German Low-Grade Lymphoma Study Group (GLSG). Leukemia. 2009;23:153–61.
164. Dimopoulos MA, Anagnostopoulos A, Kyrtsonis MC, et al. Primary treatment of Waldenstrom's macroglobulinemia with dexamethasone, rituximab and cyclophosphamide. J Clin Oncol. 2007;25:3344–9.
165. Treon SP, Hunter Z, Branagan A. CHOP plus rituximab therapy in Waldenström's macroglobulinemia. Clin Lymphoma Myeloma. 2005;5:273–7.
166. Ioakimidis L, Patterson CJ, Hunter ZR, Soumerai JD, Manning RJ, Sheehy P, et al. Comparative outcomes following CP-R, CVP-R and CHOP-R in Waldenstrom's macroglobulinemia. Clin Lymphoma Myeloma. 2009;9:62–6.
167. Weber DM, Dimopoulos MA, Delasalle K, et al. 2-Chlorodeoxyadenosine alone and in combination for previously untreated Waldenstrom's macroglobulinemia. Semin Oncol. 2003;30:243–7.
168. Laszlo D, Andreola G, Rigacci L, et al. Rituximab and subcutaneous 2-chloro-2′-deoxyadenosine combination treatment for patients with Waldenstrom macroglobulinemia: clinical and biologic results of a phase II multicenter study. J Clin Oncol. 2010;28:2233–8.
169. Treon SP, Branagan AR, Ioakimidis L, et al. Long term outcomes to fludarabine and rituximab in Waldenstrom's macroglobulinemia. Blood. 2009;113:3673–8.
170. Tam CS, Wolf MM, Westerman D, et al. Fludarabine combination therapy is highly effective in first-line and salvage treatment of patients with Waldenstrom's macroglobulinemia. Clin Lymphoma Myeloma. 2005;6:136–9.
171. Hensel M, Villalobos M, Kornacker M, et al. Pentostatin/cyclophosphamide with or without rituximab: an effective regimen for patients with Waldenstrom's macroglobulinemia/lymphoplasmacytic lymphoma. Clin Lymphoma Myeloma. 2005;6:131–5.
172. Dimopoulos MA, Hamilos G, Efstathiou E, et al. Treatment of Waldenstrom's macroglobulinemia with the combination of fludarabine and cyclophosphamide. Leuk Lymphoma. 2003;44:993–6.
173. Tamburini J, Levy V, Chateilex C, et al. Fludarabine plus cyclophosphamide in Waldenstrom's macroglobulinemia: results in 49 patients. Leukemia. 2005;19:1831–4.
174. Tedeschi A, Benevolo G, Varettoni M, et al. Fludarabine plus cyclophosphamide and rituximab in Waldenstrom macroglobulinemia: an effective but myelosuppressive regimen to be offered to patients with advanced disease. Cancer. 2012;118:434–43.
175. Treon SP, Ioakimidis L, Soumerai JD, Patterson CJ, Sheehy P, Nelson M, et al. Primary therapy of Waldenstrom's macroglobulinemia with bortezomib, dexamethasone and rituximab: results of WMCTG clinical trial 05–180. J Clin Oncol. 2009;27:3830–5.
176. Ghobrial IM, Xie W, Padmanabhan S, Badros A, et al. Phase II trial of weekly bortezomib in combination with rituximab in untreated patients with Waldenström macroglobulinemia. Am J Hematol. 2010;85:670–4.
177. Agathocleous A, Rohatiner A, Rule S, et al. Weekly versus twice weekly bortezomib given in conjunction with rituximab, in patients with recurrent follicular lymphoma, mantle cell lymphoma and Waldenström macroglobulinaemia. Br J Haematol. 2010;151:346–53.
178. Dimopoulos MA, García-Sanz R, Gavriatopoulou M, et al. Primary therapy of Waldenstrom's macroglobulinemia (WM) with weekly bortezomib, low-dose dexamethasone and rituximab (BDR): a phase II study of the European Myeloma Network. Blood. 2010;116: Abstract 1941.

179. Treon SP, Soumerai JD, Branagan AR, et al. Thalidomide and rituximab in Waldenstrom's macroglobulinemia. Blood. 2008;112:4452–7.
180. Treon SP, Soumerai JD, Branagan AR, et al. Lenalidomide and rituximab in Waldenström's macroglobulinemia. Clin Cancer Res. 2008;15:355–60.
181. Rummel MJ, von Gruenhagen U, Niederle N, et al. Bendamustine plus rituximab versus CHOP plus rituximab in the firstline treatment of patients with follicular, indolent and mantle cell lymphomas: Results of a randomized phase III study of the Study Group Indolent Lymphomas (StiL). Blood. 2008;112: Abstract 2596.
182. Treon SP, Hanzis C, Tripsas C, et al. Bendamustine therapy in patients with relapsed or refractory Waldenström's macroglobulinemia. Clin Lymph Myeloma Leuk. 2011;11:133–5.
183. Treon SP, Hanzis C, Manning RJ, et al. Maintenance rituximab is associated with improved clinical outcome in rituximab naïve patients with Waldenstrom's macroglobulinemia who respond to a rituximab containing regimen. Br J Haematol. 2011;154:357–62.
184. Desikan R, Dhodapkar M, Siegel D, Fassas A, Singh J, Singhal S, et al. High-dose therapy with autologous haemopoietic stem cell support for Waldenström's macroglobulinaemia. Br J Haematol. 1999;105:993–6.
185. Munshi NC, Barlogie B. Role for high dose therapy with autologous hematopoietic stem cell support in Waldenström's macroglobulinemia. Semin Oncol. 2003;30:282–5.
186. Dreger P, Glass B, Kuse R, Sonnen R, von Neuhoff N, Bolouri H, et al. Myeloablative radiochemotherapy followed by reinfusion of purged autologous stem cells for Waldenström's macroglobulinaemia. Br J Haematol. 1999;106:115–8.
187. Anagnostopoulos A, Dimopoulos MA, Aleman A, Weber D, Alexanian R, Champlin R, et al. High-dose chemotherapy followed by stem cell transplantation in patients with resistant Waldenström's macroglobulinemia. Bone Marrow Transplant. 2001;27:1027–9.
188. Tournilhac O, Leblond V, Tabrizi R, Gressin R, Colombat P, Milpied N, et al. Transplantation in Waldenström's macroglobulinemia—the French Experience. Semin Oncol. 2003;30:291–6.
189. Anagnostopoulos A, Hari PN, Perez WS, et al. Autologous or allogeneic stem cell transplantation in patients with Waldenstrom's macroglobulinemia. Biol Blood Marrow Transplant. 2006;12:845–54.
190. Kyriakou C, Canals C, Sibon D, et al. High-dose therapy and autologous stem-cell transplantation in Waldenstrom macroglobulinemia: the Lymphoma Working Party of the European Group for Blood and Marrow Transplantation. J Clin Oncol. 2010;28:2227–32.
191. Kyriakou C, Canals C, Cornelissen JJ, et al. Allogeneic stem-cell transplantation in patients with Waldenström macroglobulinemia: report from the Lymphoma Working Party of the European Group for Blood and Marrow Transplantation. J Clin Oncol. 2010;28:4926–34.
192. Weber D, Treon SP, Emmanouilides C, et al. Uniform response criteria in Waldenstrom's macroglobulinemia: consensus panel recommendations from the Second International Workshop on Waldenstrom's Macroglobulinemia. Semin Oncol. 2003;30:127–31.
193. Kimby E, Treon SP, Anagnostopoulos A, et al. Update on recommendations for assessing response from the Third International Workshop on Waldenstrom's macroglobulinemia. Clin Lymphoma Myeloma. 2006;6:380–3.
194. Treon SP, Merlini G, Morra E, et al. Report from the Sixth International Workshop on Waldenstrom's macroglobulinemia. Clin Lymph Myeloma Leukemia. 2011;11:69–73.
195. Nichols GL, Savage DG. Timing of rituximab/fludarabine in Waldenstrom's macroglobulinemia may avert hyperviscosity. Blood. 2004;104:237b.
196. Strauss SJ, Maharaj L, Hoare S, et al. Bortezomib therapy in patients with relapsed or refractory lymphoma: potential correlation of in vitro sensitivity and tumor necrosis factor alpha response with clinical activity. J Clin Oncol. 2006;24:2105–12.
197. Varghese AM, Rawstron AC, Ashcroft AJ, et al. Assessment of bone marrow response in Waldenström's macroglobulinemia. Clin Lymph Myeloma. 2009;9:53–5.
198. Ciccarelli BT, Yang G, Hatjiharissi E, et al. Soluble CD27 is a faithful marker of disease burden and is unaffected by the rituximab induced IgM flare, as well as plasmapheresis in patients with Waldenstrom's macroglobulinemia. Clin Lymphoma Myeloma. 2009;9:56–8.

MALT Lymphoma (Extranodal Marginal Zone B-Cell Lymphoma)

6

Emanuele Zucca, Francesco Bertoni, and Franco Cavalli

Abstract

Extranodal marginal zone lymphoma (MALT lymphoma) comprises approximately 8 % of non-Hodgkin's lymphomas. Recurrent karyotype abnormalities, despite involving different genes, appear to affect the same signalling pathway, resulting in the activation of nuclear factor-kappa B (NF-κB). The most common site of MALT lymphoma is the stomach, although involvement may occur at any other site. MALT lymphomas mostly arise at sites normally devoid of lymphoid tissue and are often preceded by chronic inflammatory conditions. There is a convincing evidence of the pathogenetic role of *Helicobacter pylori* in gastric lymphoma, and other infectious agents may also have a pathogenetic role in other anatomical sites. *H. pylori* eradication with antibiotics can lead to the regression of localized gastric MALT lymphoma in over 75 % of patients. Treatment of non-gastric localizations with antibiotics remains mainly investigational. Patients who do not respond to antibiotic therapy may be considered for localized radiotherapy. Chemotherapy and immunotherapy can be effective in patients with disseminated disease. Several active drugs have been tested in phase II trials. The efficacy of the combination of rituximab with chlorambucil in either non-gastric or gastric antibiotic-resistant MALT lymphoma has been shown in a randomized study. Aggressive anthracycline-containing regimens are not usually necessary and should be reserved for the few patients with high tumor burden and for those with diffuse large-cell

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infiltration. These latter, indeed, should be treated according to the recommendations for diffuse large-cell lymphoma.

Keywords
MALT lymphoma • *Helicobacter pylori* • Rituximab • Marginal zone lymphoma • Radiotherapy

Definition and Classification of Marginal Zone Lymphomas

The 2008 edition of the World Health Organization (WHO) classification of tumors of hematopoietic and lymphoid tissues incorporated the *extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue* (*MALT*), currently named *MALT lymphoma*, alongside two other distinct entities, namely, the *nodal marginal zone B-cell lymphoma* and the *splenic marginal zone B-cell lymphoma* [1, 2].

Epidemiology of Malt Lymphoma

Primary splenic and nodal marginal zone B-cell lymphomas (MZL) are quite rare, each comprising approximately 1–2 % of lymphomas, while MALT lymphomas are not uncommon, representing approximately 8 % of the total number of cases of non-Hodgkin's lymphoma [3].

The term MALT lymphoma was used for the first time by Isaacson and Wright in 1983 to describe indolent gastrointestinal (GI) lymphomas with common histological features consisting in the presence of an invasive epithelial lymphoid infiltrate and a dense noninvasive plasma cell infiltrate in the lamina propria, thus suggesting that MALT represents the tissue of origin of these lymphomas [4]. Different organs (stomach, thyroid, salivary glands, lung, and others) have then been identified where chronic antigenic stimuli, represented mainly by chronic infections (such as *Helicobacter pylori* in the stomach) or autoimmune disorders (such as Hashimoto's thyroiditis or Sjögren's syndrome), can induce an acquired lymphoid tissue (absent in normal conditions) that could then give origin to a MALT lymphoma [5].

There is convincing evidence of the pathogenetic role *Helicobacter pylori* in gastric lymphoma [6, 7]. A very high prevalence (70–90 % of cases) of *H. pylori* infection has been reported in gastric MALT lymphomas [8, 9]. The highest incidence of gastric MALT lymphoma has been reported in northeastern Italy (13.2 per 100,000 per year, 13 times higher than in corresponding communities in the United Kingdom), indicating important geographic variations [10]. In the United States, the incidence of gastric MALT lymphoma has been estimated as between 1:30,000 and 1:80,000 in the *H. pylori*-infected population [6]. Besides acting via a chronic stimulation, there are also data suggesting a possible direct role of *H. pylori* on B cells [11].

Other infectious agents have been linked to non-gastric MZL (*Borrelia burgdorferi* in cutaneous lymphomas, *Chlamydophila psittaci* in the lymphoma of the ocular adnexa, hepatitis C virus in splenic and nodal MZLs) [12–16], but the strength of these associations shows great and not completely explained geographic variations.

The risk of developing MALT lymphoma appears significantly increased in individuals affected by autoimmune disorders, especially Sjögren's syndrome and systemic lupus erythematosus [17].

The immunoproliferative small intestinal disease (IPSID) is a special variant of MALT lymphoma that occurs mainly in the Middle East, especially in the Mediterranean area where the disease is endemic, affecting young adults of both sexes, but predominantly the males [18]. It was known since the 1970s that durable remissions can be obtained in early phases of IPSID with antibiotic treatment, but only in 2004, Lecuit and coll. have demonstrated the presence of a specific pathogen, linking this lymphoma to *Campylobacter jejuni* [19].

Pathology

MALT lymphomas can present with evident tumorous masses, but most often, they are macroscopically indistinguishable from the underlying inflammatory lesion. MALT lymphomas are often multifocal with small clonally identical isolated foci of lymphoma [20].

MALT lymphoma is defined as an extranodal lymphoma composed of heterogeneous B cells including centrocyte-like (monocytoid) cells, small lymphocytes, and scattered large cells (immunoblasts and centroblast-like cells) [1]. A degree of plasma cell differentiation is a common feature. Tumor cells can appear as a monotonous cell population or a heterogenous population containing the different cytological types in various quantities. The number of scattered large B cells varies, but the latter usually represent only a small percentage of the whole neoplastic cell population. The prognostic significance of the number of the large cells is not fully understood. However, importantly, in the presence of solid or sheet-like proliferations of the blast cells, the diagnosis of an associated diffuse large B-cell lymphoma (DLBCL) must be made and not anymore of a "high-grade" MALT lymphoma [1]. Nonneoplastic T cells are frequently admixed with the lymphoma cells.

MALT lymphoma cells can infiltrate the mucosal crypts and glands forming lymphoepithelial lesions. These are constituted by aggregates of neoplastic lymphocytes inside glandular epithelium, typically determining disruption or necrosis of the epithelium. Stains for cytokeratin can help in the identification of lymphoepithelial lesions [1], which are highly characteristic of MALT lymphoma, especially gastric lymphoma, but not pathognomonic, and their presence is useful but not essential for the diagnosis, since they could be detected in other lymphoma subtypes [21, 22] and in some reactive conditions [23, 24].

There is currently no specific immunohistochemical marker for MALT lymphoma. The tumor cells typically express sIg (IgM, less often IgA or IgG) and are positive for CD20, CD79a, CD21, and CD35 and negative for CD5, CD23, CD10, and cyclin D1. The immunoglobulin light chain restriction is often difficult to be demonstrated in small bioptic samples.

Genetic Lesions

MALT lymphoma presents somatically mutated immunoglobulin heavy chain (*IGHV*) genes in all the cases. The pattern of somatic hypermutation and *IGHV* rearrangements strongly suggest that lymphoma cells have undergone antigen selection in germinal centers [25, 26]. Moreover, the presence of ongoing mutations within the IGHV (intraclonal variation) is suggestive of the fact that the lymphoma cells expansion might still be antigen driven. In addition, MALT lymphoma cells often express antibodies with specificity towards self-antigens [27–29]. Thus, it is assumed that, in the context of such a continual antigenic stimulation, abnormal B-cell clones acquiring subsequent genetic lesions would progressively replace the normal lymphocytes of the inflammatory tissue originating the lymphoma. Recognition of this antigenic drive has relevant therapeutic implications given the pathogenetic role possibly associated with some infections [6, 7, 12, 13].

A limited number of recurrent genomic lesions, including chromosomal translocations and unbalanced genomic aberrations, are present in MALT lymphomas [30–42] (Table 6.1).

The most common translocation is the t(11;18)(q21;q21), which determines the reciprocal fusion of *BIRC3*—previously named cellular inhibitor of apoptosis protein 2 (*cIAP2*)—on 11q21 with *MALT1* on 18q21 [30, 42, 43]. MALT lymphoma cases bearing the t(11;18) have a low probability of response to antibiotics, present with a more advanced disease, and, if with a primary gastric localization, are usually *H. pylori* negative [44–46]. On the other hand, in t(11;18)-positive cases, translocation may present a lower risk of transformation to DLBCL [47].

The t(14;18)(q32;q21) translocation juxtaposes the *MALT1* gene to the promoter region of the *IGHV* genes with subsequent *MALT1* deregulation [32]. Importantly, this translocation is cytogenetically virtually identical to the one targeting *BCL2* in follicular lymphoma or DLBCL,

Table 6.1 Main recurrent genetic abnormalities in MALT lymphomas [30–41]

Genetic aberration	Frequency (% of cases)	Involved genes	Pathogenetic mechanism	Main anatomical sites	MALT1 expression	BCL10 expression	NF-κB activation	Additional genomic alterations	Histologic transformation
t(11;18) (q21;q21)	15–40 %	*cIAP2-MALT1*	Fusion protein	Stomach, intestine, lung	Cytoplasmic, weak	Nuclear, strong	Yes	Infrequent	No
t(14;18) (q32;q21)	5–20 %	*IGHV-MALT1*	MALT 1 overexpression	Ocular adnexa, skin, liver, salivary glands	Cytoplasmic, strong	Cytoplasmic, strong	Yes	Yes	Yes
t(1;14) (p22;q32)	<5 %	*BCL10-IGHV*	BCL10 overexpression	Stomach, intestine, lung	Cytoplasmic, weak	Nuclear, strong	Yes	Yes	Yes
t(3;14) (p14;q32)	5–10 %	*FOXP1-IGHV*	FOXP1 overexpression	Skin, thyroid	Unknown	Unknown	Unknown	Yes	Yes
t(5;14) (q34;q32)	Unknown	*ODZ2-IGHV*	ODZ2 overexpression	Skin? Ocular adnexa?	Unknown	Unknown	Unknown	Unknown	Unknown
t(9;14) (p24;q32)	Unknown	*JMJD2C-IGHV*	JMJD2C overexpression	Salivary glands? Ocular adnexa?	Unknown	Unknown	Unknown	Unknown	Unknown
t(X;14) (p11;q32)	Unknown	*GPR34-IGHV*	GPR34 overexpression	Lung?	Unknown	Unknown	Unknown	Unknown	Unknown
Trisomy 3	20–50 %	*FOXP1*?	Unknown	Equal distribution	Unknown	Unknown	Unknown	Unknown	Unknown
Trisomy 18	20–50 %	*MALT1*?	Unknown	Equal distribution	Unknown	Unknown	Unknown	Unknown	Yes
del 6q23.3	20 %	*TNFAIP3*	Inactivation of TNFAIP3	Equal distribution	Unknown	Unknown	Yes	Unknown	Unknown

requiring fluorescence in situ (FISH) to distinguish them.

The t(1;14)(p22;q32) translocation causes a constitutively high level of expression of the *BCL10* gene due to the juxtaposition to the promoter region of the *IGHV* genes [31]. MALT lymphomas carrying this translocation have a high BCL10 nuclear expression, which is observed in t(11;18)-positive cases and in other patients as well [45, 46, 48, 49].

Differently from the t(11;18) and the t(14;18), the third translocation which has been described in MALT lymphomas, the t(3;14)(p13;q32), is not strictly specific for this lymphoma subtype, being observed also in DLBCL [33, 40]. The t(3;14) juxtaposes *FOXP1*, coding for a transcription factor, next to the enhancer region of the *IGHV* genes. A high expression of FOXP1 has been associated with a poor outcome in both DLBCL and in MALT lymphomas, and among the latter, it could be associated with a higher risk of transformation to an aggressive lymphoma [50, 51].

Additional less characterized translocations, such as the t(9;14)(9p24;q32), juxtaposing *JMJD2C*, coding for a histone demethylase recently shown to be target of recurrent DNA amplifications in primary mediastinal B-cell lymphoma and in Hodgkin's lymphoma [52], to the *IGHV* promoter regions, are still poorly characterized [53].

Similarly to nodal and splenic MZL, also MALT lymphomas present gains of the whole chromosomes 3 and 18 or of their long arms at a frequency higher than other B-cell tumors [35, 36, 54]. Also, a new recurrent 6q23.3 deletion has been described, which, together with somatic mutations, inactivates the *TNFAIP3/A20* gene [36, 55–57]. The high prevalence of gains affecting chromosomes 3 and 18 with the lack of other lesions such as deletions at 7q31 (common in splenic MZL), at 13q14.3 (common in chronic lymphocytic leukemia), or at 11q22 (common in chronic lymphocytic leukemia or mantle cell lymphoma) can help in the differential diagnosis of MALT lymphomas from other indolent lymphomas.

Of interest, at least four of the recurrent lesions observed in MALT lymphomas (*TNFAIP3* inactivation, *BIRC3-MALT1*, *IGHV-BCL10*, *IGHV-MALT1*) determine the activation of the nuclear factor kappa B (NF-kB) pathway, which can represent a therapeutic target [58]. The chromosomal translocations are mutually exclusive and differently from 3/3q and 18/18q gains and losses at 6q23, they present differences in their anatomical distribution [34, 38, 39].

Clinical Features

MALT lymphoma affects adults and the median age at presentation is around 60 years, with a slight preponderance among females. Pediatric cases are extraordinarily rare. Constitutional B symptoms are extremely uncommon, while the presenting symptoms are determined by the primary location of the disease. The stomach is the commonest localization, representing about one-third of the cases, but extranodal MZLs have been described in nearly all organs and tissues. Other typical presentation sites include the salivary glands, the orbit, the thyroid, and the lung; the frequency at different organs is shown in Table 6.2. Elevated [59–64] levels of lactate dehydrogenase (LDH) or β[beta]2 microglobulin are not usually detected.

As above-mentioned, MALT lymphoma is often multifocal, possibly explaining the reports of relapses in the gastric stump after the lymphoma surgical excision. MALT lymphoma tends to remain localized within the tissue of origin for a long period of time. However, dissemination to multiple sites has been reported in up to one-quarter of cases, with either synchronous or metachronous involvement of multiple mucosal sites or non-mucosal sites. Gastric MALT lymphoma can disseminate to the small intestine and to the splenic marginal zone. Concomitant gastrointestinal (GI) and non-GI localization occurs in about 10 % of cases. Disseminated disease appears to be more common in non-GI MALT lymphomas, in which it has been reported in up to 25 % of the patients. The disease can also involve regional lymph nodes. Bone marrow involvement is reported in approximately 10–20 % of cases [65, 66].

MALT lymphoma has a favorable outcome, with more than 85 % of overall survival at 5 years in most series. Patients with lymph node or bone marrow involvement at presentation, but not

Table 6.2 Main clinical features of MALT lymphomas at different anatomical sites [59–64]

Extranodal site	Frequency (%)	Nodal involvement	Bone marrow involvement (%)	Stage I (%)	Elevated LDH (%)	5-year overall survival
Stomach	33	4 %	8	88	2	82–88 %
Intestine	3–9	44	0	56	11	59–100 %
Ocular adnexa	10–12	10 %	13	84	26	90–94 %
Salivary glands	16	11 %	9	83	17	96–100 %
Lung	6–10	27 %	7	60	27	84–100 %
Upper airways	5–10	33 %	33	50	42	46–80 %
Breast	2–3	0 %	0	100	33	100 %
Thyroid	4	40 %	0	60	10	100 %
Skin	8–10	0	9	82	9	84–100 %
Multiple mucosal sites, with or without nodal and/or bone marrow involvement	10–30	45 %	33	Not applicable	26	77 % (43–93)

those with involvement of multiple mucosal sites, are associated with a worse prognosis [59]. Recurrences involve either extranodal or nodal sites. The median time to progression has been reported to be better for GI than for non-GI lymphomas, but without significant differences in overall survival. Indeed, despite presenting more often with stage IV disease, non-GI MALT lymphomas have usually a relatively indolent course, regardless of treatment type, but they relapse (most often at other mucosal sites) more commonly than primary gastric cases.

Localization may have prognostic relevance due to organ-specific clinical problems requiring specific management strategies but also because of different pathogenetic mechanisms, as suggested by the different frequency of chromosomal translocations at distinct anatomic locations. In a radiotherapy study from Toronto, gastric and thyroid MALT lymphomas had the best outcome [67, 68]. In a study of the International Extranodal Lymphoma Study Group (IELSG), patients with disease initially presenting in the upper airways presented a slightly poorer outcome, but no definitive conclusion could be made due to their small number [59].

Histological transformation to DLBCL occurs in about 10 % of the cases, apparently less than in other indolent lymphomas, sometimes late during the course of the disease and independently from dissemination [60, 69, 70]. It is unknown whether different anatomical sites have different incidence of transformation.

Recommended Staging Procedures

Different alternative staging systems have been proposed, but there is no general consensus on the best one to be used for extranodal lymphomas. Since patients presenting with lymphoma disseminated at multiple mucosal sites may have a favorable outcome not dissimilar from patients with localized disease, the traditional Ann Arbor staging system, which is mainly based on the extension of nodal areas, is not optimal.

Outside clinical trials, staging procedures should be tailored to the individual patient according to the clinical conditions (localization, age, intended treatment, performance status, symptoms). Staging procedures should always comprise a complete clinical history and physical examination with a careful evaluation of all lymph node regions, inspection of the upper airway and tonsils, thyroid examination and clinical evaluation of the size of liver and spleen [71]. Standard chest radiographs and a computed tomography (CT) scan of thorax, abdomen, and pelvis should be performed. Bone marrow biopsy should be performed at diagnosis, particularly in non-gastric cases [61, 64]. Laboratory tests should include complete blood counts with cytological examination, LDH and

Table 6.3 MALT lymphoma staging for non-gastric presentations

Extranodal site	Recommended additional investigations
Small intestine (IPSID)	Endoscopy
	Small bowel series (double-contrast X-ray examination of the small intestine)
	Campylobacter jejuni search in the tumor biopsy by PCR, immunohistochemistry, or in situ hybridization
Large intestine	Colonoscopy
Lung	Bronchoscopy + bronchoalveolar lavage
Salivary gland, tonsils, parotid	ENT examination and echography
Thyroid	Echography+/− CT scan of the neck
	Thyroid function tests
Ocular adnexa	MRI (or CT scan)
	Ophthalmologic examination
	Chlamydophila psittaci in the tumor biopsy and blood mononuclear cells by PCR
Breast	Mammography and MRI (or CT scan)
Skin	*Borrelia burgdorferi* in the tumor biopsy by PCR

PCR polymerase chain reaction, *ENT* ear, nose, throat, *CT* computed tomography, *MRI* magnetic resonance imaging

β[beta]2 microglobulin levels, evaluation of renal and liver function, and HCV and HIV serology. The utility of positron-emission tomography (PET) scanning remains unclear with conflicting reports on 18-FDG avidity of extranodal marginal zone lymphomas, and, thus, the exam is not currently recommended [71]. Then, depending upon the particular clinical presentation, the investigations should focus on the specific organs suspected of being involved (Table 6.3).

Diagnosis and Management of Gastric MALT Lymphoma

The most common symptoms of gastric MALT lymphoma are nonspecific upper GI complaints (dyspepsia, pain, nausea) or manifestations of occult chronic GI bleeding (anemia). The endoscopy frequently reveals nonspecific gastritis or peptic ulcer; mass lesions are unusual, and diagnosis is made on histological examination of gastric biopsies.

The best staging system is still controversial. We have largely used a modification of the Blackledge system known as the "Lugano" staging system [72] (Table 6.4) [72–74]. However, it does not accurately describe the depth of infiltration in the gastric wall, a parameter that is highly predictive for the MALT lymphoma response to anti-*Helicobacter* therapy [75].

Besides the above-presented procedures, there is a general consensus that initial staging for primary gastric MALT lymphoma should include a gastroduodenal endoscopy, with multiple biopsies from each region of the stomach, duodenum, and gastroesophageal junction, and from any abnormal-appearing site [71, 75]. Fresh biopsy and washing material should be available for cytogenetic studies in addition to routine histology and immunohistochemistry. FISH analysis or a molecular assay for the detection of t(11;18) can identify disease that is unlikely to respond to antibiotic therapy. The presence of active infection must be determined by histochemistry (Genta stain or Warthin-Starry stain) and breath test; serology studies are recommended when the results of histology are negative [75]. Endoscopic ultrasound is recommended in the initial follow-up for evaluation of depth of infiltration and presence of perigastric lymph nodes, parameters highly predictive of unresponsiveness to anti-*Helicobacter* therapy [71, 75].

H. pylori Eradication in Gastric MALT Lymphoma

Up to the early 1990s, the standard approach for gastric MALT lymphoma was represented by surgical resection, often with adjuvant radiotherapy or chemotherapy. With the identification of *H. pylori* as the

Table 6.4 Comparison of the Lugano and Paris staging systems for gastric lymphoma with the Ann Arbor stage

Lugano staging system [72]	Ann Arbor stage [74]	Paris staging system [73]	Lymphoma extension
Stage I = confined to the gastrointestinal tract (single primary or multiple, non-contiguous)	IE	T1m N0 M0	Mucosa
		T1sm N0 M0	Submucosa
		T2 N0 M0	Muscularis propria
		T3 N0 M0	Serosa
Stage II = extending into abdomen	IIE	T1–3 N1 M0	Perigastric lymph nodes
II_1 = local nodal involvement		T1–3 N2 M0	More distant regional nodes
II_2 = distant nodal involvement			
Stage II_E = penetration of serosa to involve adjacent organs or tissues	IE	T4 N0–2 M0	Invasion of adjacent structures with or without abdominal lymph nodes
Stage IV = concomitant supradiaphragmatic nodal involvement or disseminated extranodal involvement	IIIE	T1–4 N3 M0	Extra-abdominal lymph nodes and/or additional distant (noncontinuous) gastrointestinal sites or non-gastrointestinal sites
	IVE	T1–4 N0–3 M1	Bone marrow not assessed
		T1–4 N0–3 M2	Bone marrow not involved
		T1–4 N0–3 M0–2 BX	Bone marrow involvement
		T1–4 N0–3 M0–2 B0	
		T1–4 N0–3 M2 B1	

etiologic agent of most cases and the initial reports of lymphoma regression following antibiotics [76–78], a fundamental change took place in the management of patients with gastric MALT lymphoma, and eradication of *H. pylori* became the standard primary treatment procedure. Several groups have confirmed the achievement of durable lymphoma remissions in 60–100 % of patients with localized (i.e., confined to the gastric wall) *H. pylori*-positive gastric MALT lymphoma treated with antibiotics [70, 75, 79–87]. Differences in the response criteria adopted in the individual studies to evaluate the lymphoma eradication after antibiotics therapy may explain the wide range of reported remission rates. Indeed, there are no uniform criteria for the definition of histological remission, and the interpretation of residual lymphoid infiltrate in posttreatment gastric biopsies can be hard [88, 89]. A histological grading system has been developed to provide relevant information to the clinician [89, 90]. This system, summarized in Table 6.5 [90] and validated [91] in a series of patients form a multicenter clinical trial, might become a useful tool once its reproducibility will be confirmed on independent series [75].

Histological remission is sometimes achieved within 6 months from *H. pylori* eradication, but sometimes it can take up to more than 1 year. Due to the protracted process of lymphoma regression, it seems reasonable that asymptomatic patients with regressing or stable lymphoma may be observed safely. However, how long should these patients be expectantly observed (with repeat endoscopy) is still an unanswered practical question. Generally, if lymphoma is still present for over 1 year, many patients and clinicians would consider a different treatment [79, 92].

Several effective programs (Table 6.6 [93–95]) are available for the treatment of *H. pylori* infection, and the choice should be based on the epidemiology of the infection in the different countries, taking into account the locally expected antibiotic resistance [94, 95]. The most common regimen used for *H. pylori* eradication is triple therapy with a proton pump inhibitor (e.g., omeprazole, lansoprazole, pantoprazole, or esomeprazole) in association with clarithromycin and either amoxicillin or metronidazole. In case of failure, bismuth-based quadruple therapy is recommended. In areas where the incidence of clarithromycin resistance is known to be high, it is recommended either to avoid the drug or to test *H. pylori* sensitivity before using it [75, 93, 94]. The length of treatment is controversial, but treatment given for 10–14 days seems to allow better results compared to 7 days. The attainment of *H. pylori* eradication should be checked by breath test at least 6 weeks

Table 6.5 GELA grading system to define the histological response of gastric MALT lymphoma after *H. pylori* eradication [90]

Response (score)	Description	Histological characteristics
CR	Complete histological remission	Normal or empty LP and/or fibrosis with absent or scattered plasma cells and small lymphoid cells in the LP, no LEL
pMRD	Probable minimal residual disease	Empty LP and/or fibrosis with aggregates of lymphoid cells or lymphoid nodules in the LP/MM and/or SM, no LEL
rRD	Responding residual disease	Focal empty LP and/or fibrosis with dense, diffuse or nodular lymphoid infiltrate, extending around glands in the LP, focal LEL or absent
NC	No change	Dense, diffuse, or nodular lymphoid infiltrate, LEL usually present

LEL lymphoepithelial lesions, *LP* lamina propria, *MM* muscularis mucosa, *SM* submucosa

Table 6.6 Anti-*Helicobacter* treatments [93–95]

Triple therapy is the recommended first-choice treatment in populations with less than 15–20 % clarithromycin resistance (and in populations with less than 40 % metronidazole resistance, proton pump inhibitor-clarithromycin-metronidazole is preferable). The different proton pump inhibitors are equivalent when used in triple therapy, but double dosing is more effective than single dosing
Clarithromycin-based triple therapy
Proton pump inhibitor (standard dose twice daily)
Clarithromycin (500 mg twice daily)
Amoxicillin (1,000 mg twice daily) or metronidazole (400 or 500 mg twice daily) for 10–14 days
Quadruple therapy is an alternative first-choice treatment in areas with a high prevalence of clarithromycin resistance or in patients who have previously received a macrolide
Quadruple therapy
Proton pump inhibitor (standard dose twice daily)
Metronidazole 500 mg three times daily
Tetracycline 500 mg four times daily
Bismuth subcitrate 120 mg four times daily for 10–14 days
Bismuth-containing quadruple treatments remain the best second-choice treatment, if available. Proton pump inhibitor plus amoxicillin or tetracycline and metronidazole are recommended if bismuth is not available. If a third-choice therapy is needed, treatment should be based on antimicrobial susceptibility testing

after eradication therapy and at least 2 weeks after withdrawal of the proton pump inhibitor [75].

In a randomized study, chlorambucil conferred no benefit after antibiotics in terms of both progression-free survival and overall survival, although the statistical power of the study was limited from not having reached the planned accrual [86].

Predictors of Reduced Response to *H. pylori* Eradication

A clear prerequisite for a response to antibiotics is the presence of the *H. pylori* infection. In two series that included *H. pylori*-positive and negative patients, none of the *H. pylori*-negative lymphomas responded to antibiotic therapy [79, 80]. Hence, it is questionable whether patients with no evidence of active *H. pylori* infection will benefit from a therapeutic trial with antibiotics. On the other hand, there are reports of lymphoma regression following antibiotics also in *H. pylori*-negative patients [70, 96], and it might be worthwhile to consider an antibiotic treatment also in *H. pylori*-negative patients, at least in those without the t(11;18) translocation. The reasons are that *H. pylori* may have been missed by the diagnostic tests or that other microorganisms (e.g., the *H. heilmannii* group) may be involved [97, 98].

Several other factors have been reported to predict the likelihood of gastric MALT Lymphoma regression following antibiotic therapy.

The response rates of lymphomas restricted to the gastric mucosa are significantly different from those with less superficial lesions and are highest

for the mucosa-confined lymphomas (approximately 70–90 %) and then decreased for the tumors infiltrating the submucosa, the muscularis propria, and the serosa [79, 80, 99–101]. Also, the response is highly unlikely in cases with perigastric nodal involvement documented either by ultrasound endoscopy or CT [79, 80, 101].

Other clinical factors reported to be possibly associated with an inferior efficacy of antibiotics include a proximal location of the MALT lymphoma in the stomach [80, 85], the presence of a high-grade lymphoma component [102], and a history of autoimmune disease [103].

Nearly all gastric lymphomas with the t(11;18) translocation will not respond to *H. pylori* eradication therapy [44, 104]. The t(11;18) might also predict the resistance to chlorambucil or thalidomide as single agents [105, 106], but apparently not to rituximab [107], chlorambucil plus rituximab [108], or other therapeutic approaches [109, 110].

Also the *BCL10* rearrangements and a nuclear localization of BCL10 protein may predict a poor response *to H. pylori* eradication [46, 111].

Clinical and Molecular Follow-Up

Postantibiotic histological and endoscopic remission does not necessarily mean complete cure of the MALT lymphoma [71, 75, 82, 87, 112, 113]. The long-term persistence of monoclonal B cells after histological regression of the lymphoma has been reported in about half of the cases, suggesting that *H. pylori* eradication suppresses but does not eradicate the lymphoma clones. However, the clinical importance of the molecular detection of monoclonal B cells is still uncertain. Transient histological and molecular relapses can occur during long-term follow-up of antibiotic-treated patients but do not necessarily predict a clinical progression. In the long-term follow-up of cases with minimal residual disease, neither lymphoma clinical growth nor histological transformation was usually documented despite persistent clonality, suggesting that a watch-and-wait policy could be feasible and safe, at least for patients agreeable to frequent endoscopies, and these patients do not necessarily require additional treatment [70, 71, 75, 82, 113].

Several cases of synchronous or metachronous gastric adenocarcinomas in patients with gastric MALT lymphomas have been documented. A tumor-registry study has shown that gastric MALT lymphoma patients might have a six-times higher risk for gastric adenocarcinoma than the general population [114]. This supports a policy of strict follow-up with histological evaluation of repeated biopsies as the recommended follow-up procedure even in patients with lymphoma regression after *H. pylori* eradication [71, 75].

Management of Gastric *H. pylori*-Negative or Antibiotic-Resistant Cases and of Non-gastric Localizations

No definite guidelines are available for the management of *H. pylori*-negative or antibiotic-resistant cases and of patients with non-gastric lymphoma. The chosen approach should reflect the experience of each center and the patient preferences in terms of adverse effects.

In retrospective series, no significant difference was apparent in survival between patients who received different initial treatments (including chemotherapy alone, surgery alone, surgery with additional chemotherapy or radiation therapy, or antibiotics against *H. pylori*) [59–61, 115].

A limited dose of localized moderate-dose radiotherapy gives excellent disease control and might be the treatment of choice for patients with stage I–II gastric MALT lymphoma without evidence of *H. pylori* infection or with persistent lymphoma after antibiotics, as well as for most non-gastric localized presentations. Indeed, according to the 2011 guidelines of the National Comprehensive Cancer Network (NCCN), radiotherapy should be the preferred treatment for *H. pylori*-negative patients and for patients with persistent or relapsing lymphoma after *H. pylori* eradication [91]. Side effects of radiotherapy are mild and reversible. For patients with localized non-gastric MALT lymphoma, radiotherapy is often the first-line treatment of choice. Standard radiotherapy recommended doses for gastric MALT lymphomas and for most non-gastric sites is 30–35 Gy (in 20 fractions) [67, 91, 116]. The

emerging literature on localized MALT lymphomas confirms a high rate of local control in MALT lymphoma, with a high proportion of patients likely to be cured of the disease. The moderate doses of radiation required for cure are generally associated with a low risk of long-term toxicity, although special considerations are needed for particular localizations such as the orbital adnexa or the lung [67, 68, 117, 118].

Radiotherapy can also be an effective therapy in providing local disease control for some patients with stage III or IV disease, but the optimal management of disseminated MALT lymphomas is less clearly defined.

Because no curative treatment exists, a watchful waiting can be an adequate initial policy in most patients. Outside a clinical trial, the treatment should be "patient-tailored," taking into account the site, the stage, and the clinical characteristics of the individual patient. When systemic treatment is needed, enrollment in controlled clinical trials is recommended. In the presence of disseminated or advanced disease, chemotherapy and/or immunotherapy with anti-CD20 monoclonal antibodies is an obvious choice, despite the fact that only a limited number of drugs and regimens have been specifically tested in MALT lymphomas.

Oral alkylating agents (either cyclophosphamide or chlorambucil, with median treatment duration of 1 year) can result in a high rate of disease control. Phase II studies have demonstrated some antitumor activity of the purine analogs fludarabine [119] and cladribine, but there might be an increased risk of secondary MDS [120], and of a polychemotherapy regimen comprising chlorambucil/mitoxantrone/prednisone [121]. Aggressive anthracycline-containing chemotherapy should be reserved for patients with high tumor burden (bulky masses, unfavorable international prognostic index). The activity of the anti-CD20 monoclonal antibody rituximab has also been demonstrated in a phase II study (with a response rate of about 70 %), and this may represent an additional option for the treatment of systemic disease. The efficacy of the combination of rituximab with chlorambucil has been explored in a randomized study of the IELSG in a gastric (failing antibiotics) or non-gastric MALT lymphomas (IELSG19, NCT00210353). In comparison with chlorambucil alone, chlorambucil plus rituximab results in increased complete remission and event-free survival rates, but 5-year overall survival was identical in both groups [62]. Data on 13 patients suggest that the combination of chlorambucil and rituximab is also active in t(11;18)-positive cases [108]. The combination of rituximab and fludarabine has shown promising results in terms of responses in a phase II trial, but it had a far too high toxicity [122].

Antibiotic Treatment in Localized Non-gastric MALT Lymphomas

In principle, antibiotic treatment in non-gastric lymphomas should be regarded as investigational. Following the first demonstration that doxycycline treatment may cause tumor regression in patients with *C. psittaci*-associated lesions [16], subsequent reports on the antibiotic efficacy in ocular adnexal lymphoma showed conflicting data and apparent geographic variations [13, 123, 124]. A prospective phase II study was then launched by the IELSG (IELSG 27, NCT01010295), which has recently provided preliminary but encouraging results, showing lymphoma regression in more than 60 % of patients after front-line treatment with doxycycline [125]. Of interest, lymphoma regression after doxycycline treatment has been observed in some lymphomas with no evidence of *C. psittaci* as well as in cases in which the treatment had failed to eradicate the *C. psittaci* infection. These results are different from what is observed for gastric MALT lymphoma, where *H. pylori*-negative patients are generally unresponsive to the antibiotic treatment and seem to indicate other doxycycline-sensitive microorganisms might be linked with the lymphoma.

Anti-*Helicobacter* Therapy in Gastric Diffuse Large B-Cell Lymphoma

Cases of regression of gastric DLBCL after anti *Helicobacter* therapy have been reported [126, 127]. However, at present, we recommend treating

gastric large-cell lymphomas as localized DLBCL. Relying solely on antibiotic therapy for them must not be advised outside clinical trials until large-scale prospective studies have confirmed its use as an effective first-line therapy [128].

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References

1. Isaacson PI, Chott A, Nakamura H, Muller-Hermelink HK, Harris NL, Swerdlow SH, et al. Extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma). In: Swerdlow SH, Campo E, Harris NL, editors. World Health Organization Classification of Tumours. Pathology and genetics of tumours of haematopoietic and lymphoid tissues. Lyon: IARC Press; 2008. p. 214–7.
2. Campo E, Swerdlow SH, Harris NL, Pileri S, Stein H, Jaffe ES. The 2008 WHO classification of lymphoid neoplasms and beyond: evolving concepts and practical applications. Blood. 2011;117(19):5019–32.
3. A clinical evaluation of the International Lymphoma Study Group classification of non-Hodgkin's lymphoma. The Non-Hodgkin's Lymphoma Classification Project. Blood. 1997;89(11):3909–18.
4. Isaacson PG, Spencer J. Is gastric lymphoma an infectious disease? Hum Pathol. 1993;24(6):569–70.
5. Isaacson PG, Du MQ. MALT lymphoma: from morphology to molecules. Nat Rev Cancer. 2004;4(8):644–53.
6. Zucca E, Bertoni F, Roggero E, Cavalli F. The gastric marginal zone B-cell lymphoma of MALT type. Blood. 2000;96(2):410–9.
7. Du MQ, Isaacson PG. Gastric MALT lymphoma: from aetiology to treatment. Lancet Oncol. 2002;3:97–104.
8. Wotherspoon AC, Ortiz-Hidalgo C, Falzon MR, Isaacson PG. Helicobacter pylori-associated gastritis and primary B-cell gastric lymphoma. Lancet. 1991; 338(8776):1175–6.
9. Nakamura S, Yao T, Aoyagi K, Iida M, Fujishima M, Tsuneyoshi M. Helicobacter pylori and primary gastric lymphoma. A histopathologic and immunohistochemical analysis of 237 patients. Cancer. 1997;79(1):3–11.
10. Doglioni C, Wotherspoon AC, Moschini A, De Boni M, Isaacson PG. High incidence of primary gastric lymphoma in northeastern Italy. Lancet. 1992;339(8797): 834–5.
11. Lin WC, Tsai HF, Kuo SH, et al. Translocation of Helicobacter pylori CagA into Human B lymphocytes, the origin of mucosa-associated lymphoid tissue lymphoma. Cancer Res. 2010;70(14):5740–8.
12. Roggero E, Zucca E, Mainetti C, et al. Eradication of Borrelia burgdorferi infection in primary marginal zone B-cell lymphoma of the skin. Hum Pathol. 2000;31(2):263–8.
13. Ferreri AJ, Dolcetti R, Magnino S, Doglioni C, Ponzoni M. Chlamydial infection: the link with ocular adnexal lymphomas. Nat Rev Clin Oncol. 2009;6(11):658–69.
14. Arcaini L, Paulli M, Boveri E, et al. Splenic and nodal marginal zone lymphomas are indolent disorders at high hepatitis C virus seroprevalence with distinct presenting features but similar morphologic and phenotypic profiles. Cancer. 2004;100(1):107–15.
15. Hermine O, Lefrere F, Bronowicki JP, et al. Regression of splenic lymphoma with villous lymphocytes after treatment of hepatitis C virus infection. N Engl J Med. 2002;347(2):89–94.
16. Ferreri AJ, Guidoboni M, Ponzoni M, et al. Evidence for an association between Chlamydia psittaci and ocular adnexal lymphomas. J Natl Cancer Inst. 2004;96(8): 586–94.
17. Ekstrom Smedby K, Vajdic CM, Falster M, et al. Autoimmune disorders and risk of non-Hodgkin lymphoma subtypes: a pooled analysis within the InterLymph Consortium. Blood. 2008;111(8):4029–38.
18. Al-Saleem T, Al-Mondhiry H. Immunoproliferative small intestinal disease (IPSID): a model for mature B-cell neoplasms. Blood. 2005;105(6):2274–80.
19. Lecuit M, Abachin E, Martin A, et al. Immunoproliferative small intestinal disease associated with Campylobacter jejuni. N Engl J Med. 2004;350(3):239–48.
20. Du MQ, Diss TC, Dogan A, et al. Clone-specific PCR reveals wide dissemination of gastric MALT lymphoma to the gastric mucosa. J Pathol. 2000;192(4): 488–93.
21. Fraga M, Lloret E, Sanchez-Verde L, et al. Mucosal mantle cell (centrocytic) lymphomas. Histopathology. 1995;26(5):413–22.
22. Tzankov A, Hittmair A, Muller-Hermelink HK, Rudiger T, Dirnhofer S. Primary gastric follicular lymphoma with parafollicular monocytoid B-cells and lymphoepithelial lesions, mimicking extranodal marginal zone lymphoma of MALT. Virchows Arch. 2002;441(6):614–7.
23. Chan JK. Gastrointestinal lymphomas: an overview with emphasis on new findings and diagnostic problems. Semin Diagn Pathol. 1996;13(4):260–96.
24. Jonsson R, Bolstad AI, Brokstad KA, Brun JG. Sjogren's syndrome—a plethora of clinical and immunological phenotypes with a complex genetic background. Ann N Y Acad Sci. 2007;1108:433–47.
25. Bertoni F, Cazzaniga G, Bosshard G, et al. Immunoglobulin heavy chain diversity genes rearrangement pattern indicates that MALT-type gastric lymphoma B cells have undergone an antigen selection process. Br J Haematol. 1997;97(4):830–6.
26. Du M, Diss TC, Xu C, Peng H, Isaacson PG, Pan L. Ongoing mutation in MALT lymphoma immunoglobulin gene suggests that antigen stimulation plays a role in the clonal expansion. Leukemia. 1996;10(7):1190–7.
27. Craig VJ, Arnold I, Gerke C, et al. Gastric MALT lymphoma B cells express polyreactive, somatically mutated immunoglobulins. Blood. 2010;115(3):581–91.

28. Zucca E, Bertoni F, Roggero E, et al. Autoreactive B cell clones in marginal-zone B cell lymphoma (MALT lymphoma) of the stomach. Leukemia. 1998;12(2):247–9.
29. Hussell T, Isaacson PG, Crabtree JE, Dogan A, Spencer J. Immunoglobulin specificity of low grade B cell gastrointestinal lymphoma of mucosa-associated lymphoid tissue (MALT) type. Am J Pathol. 1993;142(1):285–92.
30. Dierlamm J, Baens M, Wlodarska I, et al. The apoptosis inhibitor gene API2 and a novel 18q gene, MLT, are recurrently rearranged in the t(11;18)(q21;q21) associated with mucosa- associated lymphoid tissue lymphomas. Blood. 1999;93(11):3601–9.
31. Willis TG, Jadayel DM, Du MQ, et al. Bcl10 is involved in t(1;14)(p22;q32) of MALT B cell lymphoma and mutated in multiple tumor types. Cell. 1999;96(1):35–45.
32. Streubel B, Lamprecht A, Dierlamm J, et al. T(14;18)(q32;q21) involving IGH and MALT1 is a frequent chromosomal aberration in MALT lymphoma. Blood. 2002;101(6):2335–9.
33. Streubel B, Vinatzer U, Lamprecht A, Raderer M, Chott A. T(3;14)(p14.1;q32) involving IGH and FOXP1 is a novel recurrent chromosomal aberration in MALT lymphoma. Leukemia. 2005;19(4):652–8.
34. Remstein ED, Dogan A, Einerson RR, et al. The incidence and anatomic site specificity of chromosomal translocations in primary extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma) in North America. Am J Surg Pathol. 2006;30(12):1546–53.
35. Dierlamm J, Pittaluga S, Wlodarska I, et al. Marginal zone B-cell lymphomas of different sites share similar cytogenetic and morphologic features. Blood. 1996; 87(1):299–307.
36. Rinaldi A, Mian M, Chigrinova E, et al. Genome-wide DNA profiling of marginal zone lymphomas identifies subtype-specific lesions with an impact on the clinical outcome. Blood. 2011;117(5):1595–604.
37. Murga Penas EM, Hinz K, Roser K, et al. Translocations t(11;18)(q21;q21) and t(14;18)(q32;q21) are the main chromosomal abnormalities involving MLT/MALT1 in MALT lymphomas. Leukemia. 2003;17(11):2225–9.
38. Kwee I, Rancoita PM, Rinaldi A, et al. Genomic profiles of MALT lymphomas: variability across anatomic sites. Haematologica. 2011;96(7):1064–6.
39. Ye H, Liu H, Attygalle A, et al. Variable frequencies of t(11;18)(q21;q21) in MALT lymphomas of different sites: significant association with CagA strains of H. pylori in gastric MALT lymphoma. Blood. 2003;102(3):1012–8.
40. Goatly A, Bacon CM, Nakamura S, et al. FOXP1 abnormalities in lymphoma: translocation breakpoint mapping reveals insights into deregulated transcriptional control. Mod Pathol. 2008;21(7):902–11.
41. Baens M, Finalet Ferreiro J, Tousseyn T, et al. t(X;14)(p11.4;q32.33) is recurrent in marginal zone lymphoma and upregulates GPR34. Haematologica. 2012;97:184–8.
42. Rosebeck S, Madden L, Jin X, et al. Cleavage of NIK by the API2-MALT1 fusion oncoprotein leads to noncanonical NF-kappaB activation. Science. 2011;331(6016): 468–72.
43. Hosokawa Y, Suzuki H, Suzuki Y, Takahashi R, Seto M. Antiapoptotic function of apoptosis inhibitor 2-MALT1 fusion protein involved in t(11;18)(q21;q21) mucosa-associated lymphoid tissue lymphoma. Cancer Res. 2004;64(10):3452–7.
44. Liu H, Ye H, Ruskone-Fourmestraux A, et al. T(11;18) is a marker for all stage gastric MALT lymphomas that will not respond to H. pylori eradication. Gastroenterology. 2002;122(5):1286–94.
45. Kuo SH, Chen LT, Yeh KH, et al. Nuclear expression of BCL10 or nuclear factor kappa B predicts Helicobacter pylori-independent status of early-stage, high-grade gastric mucosa-associated lymphoid tissue lymphomas. J Clin Oncol. 2004;22(17):3491–7.
46. Ye H, Gong L, Liu H, et al. Strong BCL10 nuclear expression identifies gastric MALT lymphomas that do not respond to H pylori eradication. Gut. 2006; 55(1):137–8.
47. Du MQ, Atherton JC. Molecular subtyping of gastric MALT lymphomas: implications for prognosis and management. Gut. 2006;55(6):886–93.
48. Ye H, Dogan A, Karran L, et al. BCL10 expression in normal and neoplastic lymphoid tissue: nuclear localization in MALT lymphoma. Am J Pathol. 2000;157(4):1147–54.
49. Liu H, Ye H, Dogan A, et al. T(11;18)(q21;q21) is associated with advanced mucosa-associated lymphoid tissue lymphoma that expresses nuclear BCL10. Blood. 2001;98(4):1182–7.
50. Sagaert X, de Paepe P, Libbrecht L, et al. Forkhead box protein P1 expression in mucosa-associated lymphoid tissue lymphomas predicts poor prognosis and transformation to diffuse large B-cell lymphoma. J Clin Oncol. 2006;24(16):2490–7.
51. Haralambieva E, Adam P, Ventura R, et al. Genetic rearrangement of FOXP1 is predominantly detected in a subset of diffuse large B-cell lymphomas with extranodal presentation. Leukemia. 2006;20(7):1300–3.
52. Rui L, Emre NC, Kruhlak MJ, et al. Cooperative epigenetic modulation by cancer amplicon genes. Cancer Cell. 2010;18(6):590–605.
53. Vinatzer U, Gollinger M, Mullauer L, Raderer M, Chott A, Streubel B. Mucosa-associated lymphoid tissue lymphoma: novel translocations including rearrangements of ODZ2, JMJD2C, and CNN3. Clin Cancer Res. 2008;14(20):6426–31.
54. Callet-Bauchu E, Baseggio L, Felman P, et al. Cytogenetic analysis delineates a spectrum of chromosomal changes that can distinguish non-MALT marginal zone B-cell lymphomas among mature B-cell entities: a description of 103 cases. Leukemia. 2005;19(10):1818–23.
55. Honma K, Tsuzuki S, Nakagawa M, et al. TNFAIP3/A20 functions as a novel tumor suppressor gene in several subtypes of non-Hodgkin lymphomas. Blood. 2009;114(12):2467–75.
56. Novak U, Rinaldi A, Kwee I, et al. The NF-K[KAPPA] B negative regulator TNFAIP3 (A20) is commonly inactivated by somatic mutations and genomic deletions in marginal zone B-cell lymphomas. Blood. 2009;113(20):4918–21.

57. Kato M, Sanada M, Kato I, et al. Frequent inactivation of A20 in B-cell lymphomas. Nature. 2009;459(7247): 712–6.
58. Conconi A, Martinelli G, Lopez-Guillermo A, et al. Clinical activity of bortezomib in relapsed/refractory MALT lymphomas: results of a phase II study of the International Extranodal Lymphoma Study Group (IELSG). Ann Oncol. 2011;22(3):689–95.
59. Zucca E, Conconi A, Pedrinis E, et al. Nongastric marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue. Blood. 2003;101(7):2489–95.
60. de Boer JP, Hiddink RF, Raderer M, et al. Dissemination patterns in non-gastric MALT lymphoma. Haematologica. 2008;93(2):201–6.
61. Thieblemont C, Berger F, Dumontet C, et al. Mucosa-associated lymphoid tissue lymphoma is a disseminated disease in one third of 158 patients analyzed. Blood. 2000;95(3):802–6.
62. Zucca E, Conconi A, Laszlo D, et al. Addition of rituximab to chlorambucil produces superior event-free survival in the treatment of patients with extranodal marginal-zone B-cell lymphoma: 5-year analysis of the IELSG-19 Randomized Study. J Clin Oncol. 2013;31:565–72.
63. Pinotti G, Zucca E, Roggero E, et al. Clinical features, treatment and outcome in a series of 93 patients with low-grade gastric MALT lymphoma. Leuk Lymphoma. 1997;26(5–6):527–37.
64. Mazloom A, Medeiros LJ, Mclaughlin PW, et al. Marginal zone lymphomas: factors that affect the final outcome. Cancer. 2010;116(18):4291–8.
65. Boveri E, Arcaini L, Merli M, et al. Bone marrow histology in marginal zone B-cell lymphomas: correlation with clinical parameters and flow cytometry in 120 patients. Ann Oncol. 2009;20(1):129–36.
66. Inamdar KV, Medeiros LJ, Jorgensen JL, Amin HM, Schlette EJ. Bone marrow involvement by marginal zone B-cell lymphomas of different types. Am J Clin Pathol. 2008;129(5):714–22.
67. Tsang RW, Gospodarowicz MK. Radiation therapy for localized low-grade non-Hodgkin's lymphomas. Hematol Oncol. 2005;23(1):10–7.
68. Tsang RW, Gospodarowicz MK, Pintilie M, et al. Localized mucosa-associated lymphoid tissue lymphoma treated with radiation therapy has excellent clinical outcome. J Clin Oncol. 2003;21(22):4157–64.
69. Thieblemont C. Clinical presentation and management of marginal zone lymphomas. Hematol Am Soc Hematol Educ Program. 2005;307–13.
70. Stathis A, Chini C, Bertoni F, et al. Long-term outcome following Helicobacter pylori eradication in a retrospective study of 105 patients with localized gastric marginal zone B-cell lymphoma of MALT type. Ann Oncol. 2009;20(6):1086–93.
71. Zucca E, Dreyling M. Gastric marginal zone lymphoma of MALT type: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol. 2010;21 Suppl 5Suppl 5:v175–6.
72. Rohatiner A, d'Amore F, Coiffier B, et al. Report on a workshop convened to discuss the pathological and staging classifications of gastrointestinal tract lymphoma. Ann Oncol. 1994;5(5):397–400.
73. Ruskone-Fourmestraux A, Dragosics B, Morgner A, Wotherspoon A, De Jong D. Paris staging system for primary gastrointestinal lymphomas. Gut. 2003;52(6): 912–3.
74. Carbone PP, Kaplan HS, Musshoff K, Smithers DW, Tubiana M. Report of the committee on Hodgkin's disease staging classification. Cancer Res. 1971;31(11): 1860–1.
75. Ruskone-Fourmestraux A, Fischbach W, Aleman BM, et al. EGILS consensus report. Gastric extranodal marginal zone B-cell lymphoma of MALT. Gut. 2011;60(6):747–58.
76. Wotherspoon AC, Doglioni C, Diss TC, et al. Regression of primary low-grade B-cell gastric lymphoma of mucosa- associated lymphoid tissue type after eradication of Helicobacter pylori. Lancet. 1993;342(8871):575–7.
77. Roggero E, Zucca E, Pinotti G, et al. Eradication of Helicobacter pylori infection in primary low-grade gastric lymphoma of mucosa-associated lymphoid tissue. Ann Intern Med. 1995;122(10):767–9.
78. Bayerdorffer E, Neubauer A, Rudolph B, et al. Regression of primary gastric lymphoma of mucosa-associated lymphoid tissue type after cure of Helicobacter pylori infection. MALT Lymphoma Study Group. Lancet. 1995;345(8965):1591–4.
79. Ruskone-Fourmestraux A, Lavergne A, Aegerter PH, et al. Predictive factors for regression of gastric MALT lymphoma after anti-Helicobacter pylori treatment. Gut. 2001;48(3):297–303.
80. Steinbach G, Ford R, Glober G, et al. Antibiotic treatment of gastric lymphoma of mucosa-associated lymphoid tissue. An uncontrolled trial. Ann Intern Med. 1999;131(2):88–95.
81. Thiede C, Wundisch T, Alpen B, et al. Persistence of monoclonal B cells after cure of Helicobacter pylori infection and complete histologic remission in gastric mucosa- associated lymphoid tissue B-cell lymphoma. J Clin Oncol. 2001;19(6):1600–9.
82. Wundisch T, Thiede C, Morgner A, et al. Long-term follow-up of gastric MALT lymphoma after Helicobacter pylori eradication. J Clin Oncol. 2005; 23(31):8018–24.
83. Fischbach W, Goebeler-Kolve ME, Dragosics B, Greiner A, Stolte M. Long term outcome of patients with gastric marginal zone B cell lymphoma of mucosa associated lymphoid tissue (MALT) following exclusive Helicobacter pylori eradication therapy: experience from a large prospective series. Gut. 2004; 53(1):34–7.
84. Montalban C, Santon A, Redondo C, et al. Long-term persistence of molecular disease after histological remission in low-grade gastric MALT lymphoma treated with H. pylori eradication. Lack of association with translocation t(11;18): a 10-year updated follow-up of a prospective study. Ann Oncol. 2005;16(9):1539–44.
85. Kim JS, Chung SJ, Choi YS, et al. Helicobacter pylori eradication for low-grade gastric mucosa-associated

lymphoid tissue lymphoma is more successful in inducing remission in distal compared to proximal disease. Br J Cancer. 2007;96(9):1324–8.
86. Hancock BW, Qian W, Linch D, et al. Chlorambucil versus observation after anti-Helicobacter therapy in gastric MALT lymphomas: results of the international randomised LY03 trial. Br J Haematol. 2009;144(3): 367–75.
87. Bertoni F, Conconi A, Capella C, et al. Molecular follow-up in gastric mucosa-associated lymphoid tissue lymphomas: early analysis of the LY03 cooperative trial. Blood. 2002;99(7):2541–4.
88. Bertoni F, Zucca E. State-of-the-art therapeutics: marginal-zone lymphoma. J Clin Oncol. 2005;23(26):6415–20.
89. Copie Bergman C, Wotherspoon A. MALT lymphoma pathology, initial diagnosis and post-treatment evaluation. In: Cavalli F, Stein H, Zucca E, editors. Extranodal lymphomas: pathology and management. London: Informa; 2008. p. 114–23.
90. Copie-Bergman C, Gaulard P, Lavergne-Slove A, et al. Proposal for a new histological grading system for post-treatment evaluation of gastric MALT lymphoma. Gut. 2003;52(11):1656.
91. Copie-Bergman C, Wotherspoon AC, Capella C, et al. Gela histological scoring system for post-treatment biopsies of patients with gastric MALT lymphoma is feasible and reliable in routine practice. Br J Haematol. 2013;160:47–52.
92. Yahalom J. Patients with H pylori-independent MALT lymphoma are curable with radiotherapy. Oncology (Williston Park). 2011;25(12):1147–9.
93. Fuccio L, Laterza L, Zagari RM, Cennamo V, Grilli D, Bazzoli F. Treatment of Helicobacter pylori infection. BMJ. 2008;337:a1454.
94. Malfertheiner P, Megraud F, O'Morain C, et al. Current concepts in the management of Helicobacter pylori infection: the Maastricht III Consensus Report. Gut. 2007;56(6):772–81.
95. Chey WD, Wong BC. American College of Gastroenterology guideline on the management of Helicobacter pylori infection. Am J Gastroenterol. 2007;102(8):1808–25.
96. Raderer M, Wohrer S, Streubel B, et al. Assessment of disease dissemination in gastric compared with extragastric mucosa-associated lymphoid tissue lymphoma using extensive staging: a single-center experience. J Clin Oncol. 2006;24(19):3136–41.
97. De Groote D, Van Doorn LJ, Van den Bulck K, et al. Detection of non-pylori Helicobacter species in "Helicobacter heilmannii"-infected humans. Helicobacter. 2005;10(5):398–406.
98. Morgner A, Lehn N, Andersen LP, et al. Helicobacter heilmannii-associated primary gastric low-grade MALT lymphoma: complete remission after curing the infection. Gastroenterology. 2000;118(5):821–8.
99. Sackmann M, Morgner A, Rudolph B, et al. Regression of gastric MALT lymphoma after eradication of Helicobacter pylori is predicted by endosonographic staging. MALT Lymphoma Study Group. Gastroenterology. 1997;113(4):1087–90.
100. Nakamura S, Matsumoto T, Suekane H, et al. Predictive value of endoscopic ultrasonography for regression of gastric low grade and high grade MALT lymphomas after eradication of Helicobacter pylori. Gut. 2001;48(4):454–60.
101. Caletti G, Zinzani PL, Fusaroli P, et al. The importance of endoscopic ultrasonography in the management of low-grade gastric mucosa-associated lymphoid tissue lymphoma. Aliment Pharmacol Ther. 2002;16(10):1715–22.
102. Neubauer A, Thiede C, Morgner A, et al. Cure of Helicobacter pylori infection and duration of remission of low- grade gastric mucosa-associated lymphoid tissue lymphoma. J Natl Cancer Inst. 1997;89(18):1350–5.
103. Raderer M, Osterreicher C, Machold K, et al. Impaired response of gastric MALT-lymphoma to Helicobacter pylori eradication in patients with autoimmune disease. Ann Oncol. 2001;12(7):937–9.
104. Alpen B, Neubauer A, Dierlamm J, et al. Translocation t(11;18) absent in early gastric marginal zone B-cell lymphoma of MALT type responding to eradication of Helicobacter pylori infection. Blood. 2000;95(12):4014–5.
105. Levy M, Copie-Bergman C, Gameiro C, et al. Prognostic value of translocation t(11;18) in tumoral response of low-grade gastric lymphoma of mucosa-associated lymphoid tissue type to oral chemotherapy. J Clin Oncol. 2005;23(22):5061–6.
106. Kuo SH, Cheng AL, Lin CW, et al. t(11;18)(q21;q21) translocation as predictive marker for non-responsiveness to salvage thalidomide therapy in patients with marginal zone B-cell lymphoma with gastric involvement. Cancer Chemother Pharmacol. 2011;68(6):1387–95.
107. Martinelli G, Laszlo D, Ferreri AJ, et al. Clinical activity of rituximab in gastric marginal zone non-Hodgkin's lymphoma resistant to or not eligible for anti-Helicobacter pylori therapy. J Clin Oncol. 2005; 23(9):1979–83.
108. Levy M, Copie-Bergman C, Molinier-Frenkel V, et al. Treatment of t(11;18)-positive gastric mucosa-associated lymphoid tissue lymphoma with rituximab and chlorambucil: clinical, histological, and molecular follow-up. Leuk Lymphoma. 2010;51(2): 284–90.
109. Streubel B, Ye H, Du MQ, Isaacson PG, Chott A, Raderer M. Translocation t(11;18)(q21;q21) is not predictive of response to chemotherapy with 2CdA in patients with gastric MALT lymphoma. Oncology. 2004;66(6):476–80.
110. Raderer M, Wöhrer S, Bartsch R, et al. Phase II study of oxaliplatin for treatment of patients with mucosa-associated lymphoid tissue lymphoma. J Clin Oncol. 2005;23(33):8442–6.
111. Du MQ, Peng H, Liu H, et al. BCL10 gene mutation in lymphoma. Blood. 2000;95(12):3885–90.
112. Fischbach W, Goebeler ME, Ruskone-Fourmestraux A, et al. Most patients with minimal histological residuals of gastric MALT lymphoma after successful eradication of Helicobacter pylori can be managed

safely by a watch and wait strategy: experience from a large international series. Gut. 2007;56(12):1685–7.
113. Nakamura S, Sugiyama T, Matsumoto T, et al. Long-term clinical outcome of gastric MALT lymphoma after eradication of Helicobacter pylori: a multicentre cohort follow-up study of 420 patients in Japan. Gut. 2012;61:507–13.
114. Capelle LG, de Vries AC, Looman CW, et al. Gastric MALT lymphoma: epidemiology and high adenocarcinoma risk in a nation-wide study. Eur J Cancer. 2008;44(16):2470–6.
115. Raderer M, Wohrer S, Streubel B, et al. Activity of rituximab plus cyclophosphamide, doxorubicin/mitoxantrone, vincristine and prednisone in patients with relapsed MALT lymphoma. Oncology. 2006;70(6):411–7.
116. Goda JS, Gospodarowicz M, Pintilie M, et al. Long-term outcome in localized extranodal mucosa-associated lymphoid tissue lymphomas treated with radiotherapy. Cancer. 2010;116(16):3815–24.
117. Yahalom J. MALT lymphomas: a radiation oncology viewpoint. Ann Hematol. 2001;80 Suppl 3Suppl 3:B100–5.
118. Koch P, Probst A, Berdel WE, et al. Treatment results in localized primary gastric lymphoma: data of patients registered within the German multicenter study (GIT NHL 02/96). J Clin Oncol. 2005;23(28):7050–9.
119. Zinzani PL, Stefoni V, Musuraca G, et al. Fludarabine-containing chemotherapy as frontline treatment of nongastrointestinal mucosa-associated lymphoid tissue lymphoma. Cancer. 2004;100(10):2190–4.
120. Jager G, Hofler G, Linkesch W, Neumeister P. Occurrence of a myelodysplastic syndrome (MDS) during first-line 2-chloro-deoxyadenosine (2-CDA) treatment of a low-grade gastrointestinal MALT lymphoma. Case report and review of the literature. Haematologica. 2004;89(4):ECR01.
121. Wohrer S, Drach J, Hejna M, et al. Treatment of extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma) with mitoxantrone, chlorambucil and prednisone (MCP). Ann Oncol. 2003;14(12):1758–61.
122. Brown JR, Friedberg JW, Feng Y, et al. A phase 2 study of concurrent fludarabine and rituximab for the treatment of marginal zone lymphomas. Br J Haematol. 2009;145(6):741–8.
123. Zucca E, Bertoni F. Chlamydia or not Chlamydia, that is the question: which is the microorganism associated with MALT lymphomas of the ocular adnexa? J Natl Cancer Inst. 2006;98(19):1348–9.
124. Ferreri AJ, Dolcetti R, Du MQ, et al. Ocular adnexal MALT lymphoma: an intriguing model for antigen-driven lymphomagenesis and microbial-targeted therapy. Ann Oncol. 2008;19(5):835–46.
125. Ferreri AJ, Govi S, Pasini E, et al. Chlamydophila psittaci eradication with doxycycline as first-line targeted therapy for ocular adnexae lymphoma: final results of an international phase II trial. J Clin Oncol. 2012;30:2988–94.
126. Chen LT, Lin JT, Tai JJ, et al. Long-term results of anti-Helicobacter pylori therapy in early-stage gastric high-grade transformed MALT lymphoma. J Natl Cancer Inst. 2005;97(18):1345–53.
127. Kuo SH, Yeh KH, Wu MS, et al. Helicobacter pylori eradication therapy is effective in the treatment of early-stage H pylori-positive gastric diffuse large B-cell lymphomas. Blood. 2012;119(21):4838–44; quiz 5057.
128. Möller P, Viardot A. Antibiotics as first-line therapy for Hp-associated gastric large B-cell lymphoma? Probably yes. Blood. 2012;119:4818–9; doi:10.1182/blood-2012-03-417345.

Splenic Marginal Zone Lymphoma

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Abstract

Splenic marginal zone lymphoma (SMZL) shares a common cell of origin from the "marginal zone" (MZ), with extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma) and nodal marginal zone lymphoma (NMZL). However, SMZL displays different clinical characteristics, reflecting probable biological variations according to the organ.

Within the past decade, new data regarding pathogenic mechanisms as well as therapeutic advances have been reported. Clinically, SMZL presents as an indolent disseminated disease at diagnosis with specific clinical presentation including predominant enlarged splenomegaly and autoimmune manifestations in 15 % of the patients. Diagnosis may be difficult among other small B-cell lymphomas, and the criteria for diagnosis have been recently improved. The therapeutic approaches comprise splenectomy or immunochemotherapy, but without consensus about the best treatment, except when associated with hepatitis C virus.

We are addressing here the current knowledge on the biological findings, clinical features, and therapeutic approaches for SMZL.

Keywords

Splenic marginal zone lymphoma (SMZL) • Diagnostic criteria • Splenectomy • Immunochemotherapy • Rituximab • Chemotherapy

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Introduction

Marginal zone lymphomas (MZL) represent a group of lymphomas that originate from memory B lymphocytes normally present in a distinct microanatomic compartment, the so-called marginal zone (MZ) of the secondary lymphoid follicles. The MZ is developed in those lymphoid organs where an abundant influx of antigens is known to occur. The MZ is especially developed in spleen and mucosa-associated lymphoid tissues, whereas it is rarely identifiable in lymph nodes [1]. According to the sites involved and characteristic molecular findings, the International Lymphoma Study Group distinguished three distinct subtypes of MZL: (1) extranodal MZL of mucosa-associated lymphoid tissue (MALT) type (MALT lymphoma), (2) splenic MZL (SMZL), and (3) nodal MZL (NMZL) [2]. Despite these advances in classification, patients with generalized disease at diagnosis are not easily ascribed to precise diagnostic groups. The relative rarity of these lymphomas, as well as the difficulties in distinguishing them from other low-grade lymphoma subtypes, poses crucial issues for the conduct of epidemiological surveys and for the accurate description of clinical features and outcomes. The present review will focus on the most recent data on the diagnostic and the treatment of SMZL.

Epidemiology: Role of Hepatitis C Virus

In adults, MZLs account for 5–17 % of all non-Hodgkin lymphoma (NHL) depending on the series. SMZL represents 20 % of MZL and accounts for less than 2 % of NHL [3]. The median age of occurrence is 65 years [4, 5]. Clear evidence indicates that MZLs in extranodal localizations as well as in spleen for SMZL and in lymph node for NMZL can be associated with chronic antigenic stimulation. Hepatitis C virus (HCV) has been highly correlated with SMZL [6], sometimes with the presence of cryoglobulin [7], lymphoplasmacytic immunocytoma, and NMZL, in some area such as in Italy [8]. A decrease in lymphoproliferation following antiviral treatments [9] reinforces the data suggesting this contribution of chronic antigenic stimulation to the physiopathologic process of HCV-related MZL. Interestingly, SMZL, here denominated as tropical splenic lymphoma, characterized by splenomegaly and circulating naive CD5-negative villous B lymphocytes, has been described in malaria-endemic areas, this supports the role of infectious agents on the pathogenesis of SMZL [10].

Clinical Features

Presentation

The hallmark of the clinical presentation of SMZL is massive splenomegaly. However, most of the patients seek medical attention because of an abnormal blood cell count, especially anemia and/or thrombocytopenia. These abnormalities are more related to splenic sequestration than to bone marrow infiltration and are consistently associated with lymphocytosis [11]. These patients are usually asymptomatic, but splenomegaly is detectable on clinical exam. In advanced cases of SMZL, patients present with a massive splenomegaly associated with asthenia and left upper quadrant pain. B symptoms are uncommon.

Autoimmune Manifestations

Serum lactate dehydrogenase (LDH) level is usually normal in SMZL, but the β[beta]2-microglobulin level is increased. A considerable proportion of patients (10–40 % of cases) have a serum monoclonal paraprotein (M-component), mainly of the μ[mu] subtype (IgM) [11, 12]. Autoimmune clinical phenomena are described in 10–15 % of patients including autoimmune hemolytic anemia, immune thrombocytopenia, cold agglutinin, circulating anticoagulant (lupus anticoagulant and/or anticardiolipin antibodies), acquired von Willebrand disease, and angioedema due to acquired C1-esterase inhibitor deficiency.

Revised Diagnostic Criteria for SMZL

Spleen histology is the gold standard approach to establish the diagnosis of SMZL (Table 7.1) [13], but most of the cases of SMZL do not require splenectomy as treatment. Moreover, spleen histology is not sufficient and must be completed by an immunophenotype of peripheral blood +/− marrow. According to the criteria proposed by the Splenic Lymphoma Group, minimum diagnosis criteria are based on either spleen histology plus immunophenotype of peripheral blood +/− marrow or typical peripheral blood and bone marrow morphology plus immunophenotype of peripheral blood +/− marrow. In addition, the recent revealing of recurring molecular abnormality is sometimes a precious help to the diagnosis.

Histology of the Spleen

Histology of the spleen shows a micronodular infiltration by a polymorphic population of B cells, including small cells, marginal cells, and scattered large-cell lymphomas involving the spleen, preferentially the white pulp but with a variable degree of red pulp involvement. The 2001 WHO classification [14] defines SMZL as a B-cell neoplasm composed of small lymphocytes that surround and replace the white pulp follicles and merge with a peripheral zone of larger MZ-like cell including scattered transformed blasts, giving the characteristic biphasic pattern. The 2008 WHO classification [15] expanded this definition to cases lacking a central core of smaller lymphocytes and having a monophasic pattern. For those cases, other criteria as peripheral blood cytology, bone marrow histology, immunophenotype, and/or presence of recurrent molecular or cytogenetic abnormalities are required.

In cases with not enough criteria for a definitive diagnosis, the choice of "unclassified splenic lymphoma" is better. This term applies to small B-cell clonal lymphoproliferations involving the spleen, but which do not fall into any of the other types of B-cell lymphoid neoplasms recognized in the WHO classification. The two best defined of these relatively rare provisional entities are splenic diffuse red pulp lymphoma [16] and hairy cell leukemia-variant. The relationship of those cases with SMZL needs to be clarified in the future. Other splenic small B-cell lymphomas not fulfilling the criteria for either of these provisional entities or for other better established B-cell lymphomas should be diagnosed as splenic B-cell lymphoma/leukemia, unclassifiable until more is known. Transformation to large B-cell lymphoma may also occur in SMZL as other indolent B-cell lymphomas [17].

Histology of the Bone Marrow

When the patient is not splenectomized, diagnosis may be required of bone marrow histology. The involvement can be intrasinusoidal, paratrabecular, nodular, or diffuse. The intrasinusoidal pattern initially described as a hallmark of SMZL is usual but cannot be considered as specific as it can be observed in other small B-cell lymphomas [18].

Immunophenotype

The immunophenotypic analysis of the tumor cells shows CD19+, CD20+, CD5−, CD10−, CD23−, CD43+/−, FMC7 +/−, CD103−, bcl-2+, and cyclin D1 cells. However, the expression of CD5 is found in 15–20 % of cases. The coexpression of IgM and IgD SIg is typical of SMZL. Matutes' international score (CD5, FMC7, CD22 or CD79b, CD23, surface Ig expression) is generally below 3 [13, 19].

Mutational Status of IGVH Genes

Initial analyses of the mutational status of the Ig heavy chain variable (IGHV) genes have reported the presence of somatic hypermutation in most of the cases in accordance with the origin of SMZL from a post-germinal MZ memory B cell [20]. However, more recent studies have found an

Table 7.1 Diagnostic and staging procedures for splenic marginal zone lymphoma

Procedures	Recommendations	Expected results
Full blood count	Mandatory	Presence or absence of anemia, thrombocytopenia, lymphocytosis: presence or absence
Blood cytology	Mandatory	Small lymphoid cells having a round nucleus with condensed chromatin and basophilic cytoplasm, with frequent short villi
Blood FCM	Mandatory	CD19+, CD20+, CD22+, CD79b+, CD5−, CD10−, CD23−, CD43−/+, CD24+, CD27++, FMC7++, CD76+/−, Sig+++ Score matutes <3 Moderate to strong intensity of IgM and IgD or Ig M alone; in rare cases, IgG or IgA Positive expression of CD5 in 15–20 %. Positive expression of CD23 in 30 % of case
Serology for hepatitis C	Mandatory	If HCV positive, RT-PCR for HCV RNA and virus genotyping
Cryoglobulins	Mandatory if HCV positive	
Serology for hepatitis B and HIV	Mandatory	
CT scan of thorax, abdomen, and pelvis	Mandatory	SMZL: massive splenomegaly
Bone marrow aspirate: cytology and FCM	Mandatory	Identical to blood
Reticulocyte, Coombs test	Recommended	Presence or absence of autoimmune hemolytic anemia
Bone marrow biopsy: morphology and IHC	Recommended	Involvement initially intra-sinusal, sometimes subtle, and then nodular. Cell morphology is monomorphic, with small-to-medium size, round to oval nucleus with regular contour and a small rim of cytoplasm. Plasmacytoid features can be observed CD20+, CD79a+, CD10−, BCL6−, CD5−, CD43+, CD23−, BCL2+, CCND1−, Sig+++ Moderate to strong intensity of IgM and IgD or IgM alone
Spleen/lymph node: morphology and IHC	In case of splenectomy or lymph node biopsy	Spleen: micronodular infiltration of the white pulp, with an inconstant marginal zone differentiation and a variable degree of red pulp involvement—cells are lymphoplasmacytoid and plasma cells CD20+, CD79a+, CD10−, BCL6−, CD5−, CD43+, CD23−, BCL2+, CCND1−, Sig+++
Autoimmune screen (ANA, anti-DNA, AMA, antithyroid, rheumatoid factor etc.)	Optional	In function of clinical symptoms or biological first screen abnormalities: immune thrombocytopenia, cold agglutinin, circulating anticoagulant (lupic or cardiolipidic), acquired von Willebrand disease, and angioedema due to acquired deficit in C1-esterase inhibitor
PET scan	Optional	Weak SUV signal in 50 % of the patients
FISH and cytogenetic analysis	Optional	SMZL: trisomy 3q (85 %) del or translocation of 7q32 (40 %) trisomy 18, 17q isochromosome, 13q14 deletion, and structural abnormalities of chr 1
IgVH status	Optional	Mutated in two-thirds of the cases Unmutated in one-third of the cases Biased usage: SMZL: V_H1.2, V_H1–2, V_H3–23, V_H4–34 genes

Modified from Matutes et al. [13]
IHC immunochemistry, *FCM* flow cytometry, *RT-PCR* reverse-transcriptase PCR, *FISH* fluorescent in situ hybridization, *Chr* chromosome

absence of somatic mutations in one-third of studied cases, possibly reflecting a relative degree of molecular heterogeneity of SMZL [20, 21]. In addition, SMZL B cells express a biased repertoire with preferential usage of certain IGHV genes such as IGHV1–2*04 (31 %), IGHV3–23 (8 %), and IGHV4–34 (13 %), and approximately 10 % of the cases express B cell (BCRs) with quasi-identical IGHV sequences including the antigen-binding site, strongly suggesting that antigen selection might contribute to the development of SMZL [15]. Antigen selection is particularly evident in HCV-associated SMZL, since a fraction of cases express a BCR having a rheumatoid factor activity with heavy and light chain encoded by the IGHV1–69 and IGKV3–20 genes, respectively.

It has been reported that SMZLs with Vh1–2 rearrangement produce polyreactive antibodies that react against self-antigens [22]. Those features argue for antigenic interactions through highly conserved residues, located throughout the VH domain, raising the possibility of a superantigen involved in lymphomagenesis and/or that lymphomas likely derive from polyreactive B cells [22]. In contrast, SMZL exhibit a low frequency of somatic mutation involving non-Ig genes such as BCL6 and or PAX5, PIM1, and RHO-H, suggesting a particular differentiation pathway of the cell of origin without transit through the germinal center [23].

Cytogenetics and Molecular Abnormalities

Cytogenetic analyses in SMZL demonstrate that complex chromosomal aberrations are common (72 % of cases with an abnormal karyotype 53 % complex). Deletion of chromosome 7q31 and complete or partial trisomy 3 are the most frequent cytogenetic abnormalities [24–29]. The minimal common deleted region in del7q is large, comprising over 4 Mb. No tumor suppressor genes have been found in this region of deletion 7q31, and evidence supports that the deletion of a cluster of miRNAs (MIR29A and MIR29B) [30] located in this region could contribute to the deregulation of some of the key oncogenes in this disorder, such as TCL1 [31]. Loss of sonic hedgehog gene (SHH) at 7q36.2 (four cases) and loss of protection of telomere 1 gene have been suggested [32]. A particular miRNA profile has been described [33].

Others cytogenetic alterations involving chromosomes 8, 9p34, 12q23–24, 18q, and 17p have been described [34]. More rare translocations involving CDK6 and cyclin D3 with IgH have been identified in small subsets of cases [35]. Del13q14.3 which constitutes the hallmark of chronic lymphocytic leukemia (CLL) when isolated can be observed in less than 10 % of cases. A translocation t(11;14)(q13;q32) combined with a rearrangement of bcl-1 and/or the expression of cyclin D1 was described as present in 15 % of cases diagnosed as SLVL, but these cases seem to harbor other morphological, phenotypic, and cytogenetic features suggesting a diagnosis of mantle cell lymphoma [36–38].

Recent genome-wide DNA profiling confirmed these cytogenetic data in a large series of 218 MZLs. Common abnormalities found in all subtypes (extranodal, splenic, and nodal) include gains of 3q and 18q which appeared to affect BCR signaling and Wnt-signaling, cell cycle, and apoptosis [34]. More specific abnormalities were described in SMZL such as del(7q31) and del(8p). In terms of prognostic impact, only the association with del(17p) and del(8p) was found to be associated with a significant negative impact on the outcome of SMZLs.

Overall, the only cytogenetic abnormality to be considered as typical in SMZL is the 7q32 deletion. Cytogenetic may help the diagnosis particularly for differential diagnosis with CLL, hairy cell leukemia, mantle cell lymphoma, follicular lymphoma, or lymphoplasmacytic lymphoma, but should not be taken in isolation.

Gene Expression Profiling

Although it cannot be applied to routine diagnosis yet, gene expression analysis clearly shows that SMZLs have a specific transcriptional profile compared with other lymphomas,

especially small B-cell lymphomas, such as follicular lymphomas, lymphocytic lymphomas, and mantle cell lymphomas [31, 39]. This specific molecular signature includes genes involved in the signaling cascade of the AKT1 pathway [39] but also the BCR signaling pathway, tumor necrosis factor (TNF), and NF-κ[kappa]B targets [31]. The importance of the latter pathway in the pathogenesis of SMZL has been strongly underlined by the detection of mutually exclusive somatic mutations of NF-κ[kappa]B genes such as TNFAIP3, IKBKB, MAP3K14, TRAF3, and BIRC3 in over one-third of the cases [40].

Biological and Clinical Prognostic Factors in SMZL

The median overall survival in SMZL ranges between 5 and 10 years, but in case of aggressive disease, seen in approximately one-third of patients, median survival is less than 4 years [41]. Clinical and biological prognostic factors have been identified by several investigators (Table 7.2) [5, 31, 42]. The Italian Intergroup of Lymphomas (IIL) have developed a prognostic model in 309 patients based on three factors (hemoglobin level less than 12 g/dL, LDH level greater than normal, and albumin level less than 3.5 g/dL) leading to a prognostic index [42]. This index allows one to separate patients into three groups displaying different 5-year survival rates: 88 % in the low-risk group (no risk factor), 73 % in the intermediate-risk group (one risk factor), and 50 % in the high-risk group (more than one factor). However, this index has not yet been demonstrated to have any therapeutic implications. In this analysis, IPI was found to predict survival, although the multivariate analysis selected the three indicated parameters. Other biological prognostic factors have been described, such as expression of CD38, unmutated IGHV gene status, and expression of NF-κ[kappa]B-activated genes based on gene expression analysis [31].

Histological transformation to large-cell lymphoma remains uncommon, occurring in 10–20 % of patients. Diffuse large B-cell lymphoma, when involving the spleen, usually is characterized by one or several large nodules, very rarely involving the bone marrow. Transformation occurs within a median interval ranging from 12 to 85 months [43]. This presents clinically with the appearance of general symptoms, increase in LDH level, and disseminated lymphoma involvement. After histological progression, the median survival time was shortened to 26 months [5].

Table 7.2 Clinical and biological adverse prognostic factors in splenic marginal zone lymphoma

Authors	*n*	PFS	OS
SMZL			
Thieblemont et al. [5]	81	Presence of M-component	Beta2 microglobulin ≥ 3 mg/L
		Presence of an immunological event	Leukocytes ≥ 20×10^9/L
			Lymphocytosis ≥ 9×10^9/L
			Presence of M-component
			Presence of an immunological event
Ruiz-Ballesteros et al. [31]	44	–	Expression of CD38
			Unmutated Ig-VH gene status
			Expression of NF-κ[kappa]B-activated genes by GEP
Arcaini et al. [42]	309	–	Hemoglobin <12 g/dL
			Elevated LDH
			Albumin >3.5 g/dL

New Therapeutic Strategies in SMZL

Treatment is required only in symptomatic patients with painful splenomegaly, with or without associated cytopenia due to hypersplenism. Asymptomatic patients, which represent a large percentage of the patients, can be appropriately managed with watchful waiting for several years. Withholding treatment does not influence the course of disease, and these patients often have stable disease for at least 10 years [7]. The only exception to this management approach is in the setting of SMZL associated with active HCV infection. Antiviral therapy with pegylated interferon-α[alpha] and ribavirin will lead to clearance of HCV RNA in 75 % of the patients and in concomitant clinical remission of the lymphoma [44].

When patients become symptomatic because of anemia (<10 g/dL), abdominal pain, and thrombocytopenia ($<80\times10^9$/L) [42], several treatment options may be proposed to the patient. Splenectomy will rapidly improve performance status and correct anemia, thrombocytopenia, and neutropenia within 6 months after splenectomy [11]. This improvement is maintained for years with a median period of freedom from treatment of 8 years, even if bone marrow and blood lymphocytosis persist, suggesting a partial response. Following splenectomy, adjuvant chemotherapy provides an increased remission rate without modifying relapse-free and overall survival [5]. For patients who are unfit for splenectomy or unwilling to undergo surgery, systemic therapy may be effective (Table 7.3) [6, 11, 45–50]. Rituximab alone is reported to afford excellent response rate with a shorter PFS than that observed when rituximab is combined with cladribine or fludarabine for polychemotherapy [6, 11, 47–52]. Recently, bendamustine has emerged as a highly effective drug for NHL, including marginal zone lymphomas [53]. A European trial for the evaluation of combined rituximab and bendamustine for these patients is scheduled to begin in the near future (EudraCT number 2011–000880–28). For clinical trials

Table 7.3 Response to treatment in splenic marginal zone lymphoma

Authors	*n*	Schedule	Status of disease	Response rate	CR/CRu	PR	PFS (At *n* years)	OS (At *n* years)
Splenectomy alone								
Chacon et al. 2002 [45]	29	–	First line	100 %	0 %	100 %		
Thieblemont et al. [11]	25	–	First line	100 %	0 %	100 %	71 % (2)	81 % (5)
Chemotherapy alone								
Lefrere et al. [46]	10	Fludarabine	Relapsed	100 %	70 %	30 %	42 % (4.7).	50 % (5)
Cervetti et al. [50]	50	2-Cda, 5 mg/m^2, once a week×6	First line or relapsed	63 %	62 %	–	83 % (2)	NA
Rituximab alone								
Tsimberidou et al. [47]	26	R once a week×4 or 8	First line	88 %	43 %	46 %	86 % (3)	95 % (3)
Kalpadakis et al. [48]	16	R once a week×6	First line	100 %	79 %	11 %	92 % (2.1)	100 % (3)
Bennett et al. [49]	14	R once a week×4	First line	78 %	57 %	21 %	60 % (6)	80 % (6)
Rituximab and chemotherapy								
Tsimberidou et al. [47]	6	R-FMD or RFC	First line	83 %	34 %	50 %	100 % (3)	100 % (3)
Arcaini et al. [6]	3	R-CVP	First line	100 %	–	–	100 % (1.3)	100 % (1.3)

Only survivals of the whole series of patients ($n=60$) treated by splenectomy with or without adjuvant chemotherapy is provided by the authors

Table 7.4 Response criteria in splenic marginal zone lymphoma

Complete response	Resolution of organomegaly (spleen longitudinal diameter <13 cm)
	Hemoglobin >12 g/dL and platelets $>100\times10^9$/L and neutrophils $>1.5\times10^9$/L
	No evidence of circulating clonal B cells by FC (light chain-restricted B cells)
	No evidence of BM infiltration detected by IHC
	Negative DAT and normal PET scan (if positive at diagnosis)
Partial response	Regression of ≥50 % in all the measurable disease manifestations
	No new sites of disease
	Improvement of cytopenias
	Decrease of infiltration and improvement of hemopoietic reserve at BM biopsy
No response	Less than 10 % improvement on the disease manifestations
Progression	>50 % of measurable signs of the disease from nadir
Relapse	Reappearance of any measurable sign of the disease

Modified from Matutes et al. [13]

to be evaluated, it is necessary to develop consistent staging and response criteria for the disease. The recent workshop of the European MZL group has redefined these parameters (Table 7.4) [13, 17].

Conclusion

SMZL is considered as a distinct entity among NHLs, with definite clinical and morphological characteristics. Although this entity is characterized by very different clinical presentations, strong similarities in the epidemiology and the biology of the tumors cells support a common origin in the memory B cells of the marginal zone. In the past 5 years, a large collaborative effort by biologists, pathologists, and clinicians has resulted in agreement on more stringent criteria for the diagnosis of the disease and for the evaluation of clinical response. These efforts should support the design of further prospective clinical trials to define the optimal therapeutic approach to these diseases.

Key Points

- Diagnosis of splenic marginal zone lymphomas should be the subject of review by expert hematopathologists.
- Diagnosis of splenic marginal zone lymphoma can be made without spleen histology following expert review of a combination of blood and bone marrow morphology, bone marrow trephine histology, and flow cytometry.
- Therapeutic options for patients with SMZL are splenectomy, chemotherapy, and rituximab, alone or associated with chemotherapy. Rituximab therapy produces a quick but usually short response, with a high overall response rate and a significant rate of complete response with negligible toxicity. This treatment is a reasonable first-line therapy and may be an alternative to splenectomy. Immunochemotherapy is indicated for fit patients with disseminated disease, constitutional symptoms, and/or signs of high-grade transformation.

References

1. Weill JC, Weller S, Reynaud CA. Human marginal zone B cells. Annu Rev Immunol. 2009;27:267–85. ** Outstanding review of physiology of human marginal zone.
2. Swerdow SH, Campo E, Lee Harris N, Jaffe ES, Pileri SA, Stein H, et al. WHO classification of tumours of haematopoietic and lymphoid tissue. Lyon: IARC; 2008.
3. Berger F, Felman P, Thieblemont C, et al. Non-MALT marginal zone B-cell lymphomas: a description of clinical presentation and outcome in 124 patients. Blood. 2000;95:1950–6.
4. Oscier D, Owen R, Johnson S. Splenic marginal zone lymphoma. Blood Rev. 2005;19:39–51.

5. Thieblemont C, Felman P, Berger F, et al. Treatment of splenic marginal zone B-cell lymphoma: an analysis of 81 patients. Clin Lymphoma. 2002;3:41–7.
6. Arcaini L, Paulli M, Boveri E, et al. Splenic and nodal marginal zone lymphomas are indolent disorders at high hepatitis C virus seroprevalence with distinct presenting features but similar morphologic and phenotypic profiles. Cancer. 2004;100:107–15.
7. Saadoun D, Boyer O, Trebeden-Negre H, et al. Predominance of type 1 (Th1) cytokine production in the liver of patients with HCV-associated mixed cryoglobulinemia vasculitis. J Hepatol. 2004;41:1031–7.
8. Arcaini L, Burcheri S, Rossi A, et al. Prevalence of HCV infection in nongastric marginal zone B-cell lymphoma of MALT. Ann Oncol. 2007;18:346–50.
9. Hermine O, Lefrere F, Bronowicki J, et al. Regression of splenic lymphoma with villous lymphocytes after treatment of hepatitis C virus infection. N Engl J Med. 2002;11:89–94.
10. Bates I, Bedu-Addo G, Jarrett RF, et al. B-lymphotropic viruses in a novel tropical splenic lymphoma. Br J Haematol. 2001;112:161–6.
11. Thieblemont C, Felman P, Callet-Bauchu E, et al. Splenic marginal-zone lymphoma: a distinct clinical and pathological entity. Lancet Oncol. 2003;4: 95–103.
12. Parry-Jones N, Matutes E, Gruszka-Westwood AM, Swansbury GJ, Wotherspoon AC, Catovsky D. Prognostic features of splenic lymphoma with villous lymphocytes: a report on 129 patients. Br J Haematol. 2003;120:759–64.
13. Matutes E, Oscier D, Montalban C, Berger F, Callet-Bauchu E, Dogan A, et al. Splenic marginal zone lymphoma proposals for a revision of diagnostic, staging and therapeutic criteria. Leukemia. 2008;22:487–95. **First proposal of international criteria for diagnosis of SMZL.
14. Jaffe ES, Harris NL, Stein H, Vardiman J. World Health Organization Classification of Tumours: pathology and genetics of tumours of haematopoietic and lymphoid tissues. Lyon: IARC Press; 2001.
15. Swerdlow SH. Small B-cell lymphomas of the lymph nodes and spleen: practical insights to diagnosis and pathogenesis. Mod Pathol. 1999;12:125–40.
16. Traverse-Glehen A, Baseggio L, Bauchu EC, et al. Splenic red pulp lymphoma with numerous basophilic villous lymphocytes: a distinct clinicopathologic and molecular entity? Blood. 2008;111:2253–60.
17. Dungarwalla M, Appiah-Cubi S, Kulkarni S, et al. High-grade transformation in splenic marginal zone lymphoma with circulating villous lymphocytes: the site of transformation influences response to therapy and prognosis. Br J Haematol. 2008;143:71–4.
18. Boveri E, Arcaini L, Merli M, et al. Bone marrow histology in marginal zone B-cell lymphomas: correlation with clinical parameters and flow cytometry in 120 patients. Ann Oncol. 2009;20:129–36.
19. Matutes E, Morilla R, Owusu-Ankomah K, Houlihan A, Catovsky D. The immunophenotype of splenic lymphoma with villous lymphocytes and its relevance to the differential diagnosis with other B-cell disorders. Blood. 1994;83:1558–62.
20. Algara P, Mateo MS, Sanchez-Beato M, et al. Analysis of the IgV(H) somatic mutations in splenic marginal zone lymphoma defines a group of unmutated cases with frequent 7q deletion and adverse clinical course. Blood. 2002;99:1299–304.
21. Traverse-Glehen A, Davi F, Ben Simon E, et al. Analysis of VH genes in marginal zone lymphoma reveals marked heterogeneity between splenic and nodal tumors and suggests the existence of clonal selection. Haematologica. 2005;90:470–8.
22. Warsame AA, Aasheim HC, Nustad K, Trøen G, Tierens A, Wang V, et al. Splenic marginal zone lymphoma with VH1–02 gene rearrangement expresses poly- and self-reactive antibodies with similar reactivity. Blood. 2011;118:3331–9.
23. Traverse-Glehen A, Verney A, Baseggio L, et al. Analysis of BCL-6, CD95, PIM1, RHO/TTF and PAX5 mutations in splenic and nodal marginal zone B-cell lymphomas suggests a particular B-cell origin. Leukemia. 2007;21:1821–4.
24. Callet-Bauchu E, Baseggio L, Felman P, et al. Cytogenetic analysis delineates a spectrum of chromosomal changes that can distinguish non-MALT marginal zone B-cell lymphomas among mature B-cell entities: a description of 103 cases. Leukemia. 2005;19:1818–23.
25. Dierlamm J, Michaux L, Wlodarska I, et al. Trisomy 3 in marginal zone B-cell lymphoma: a study based on cytogenetic analysis and fluorescence in situ hybridization. Br J Haematol. 1996;93:242–9.
26. Dierlamm J, Pittaluga S, Wlodarska I, et al. Marginal zone B-cell lymphomas of different sites share similar cytogenetic and morphologic features. Blood. 1996;87:229–307.
27. Dierlamm J, Rosenberg C, Stul M, et al. Characteristic pattern of chromosomal gains and losses in marginal zone B cell lymphoma detected by comparative genomic hybridization. Leukemia. 1997;11:747–58.
28. Salido M, Baro C, Oscier D, et al. Cytogenetic aberrations and their prognostic value in a series of 330 splenic marginal zone B-cell lymphomas: a multicenter study of the Splenic B-Cell Lymphoma Group. Blood. 2010;116:1479–88. **Largest series of cytogenetics analysis in SMZL.
29. Sole F, Woessner S, Florensa L, et al. Frequent involvement of chromosomes 1, 3, 7 and 8 in splenic marginal zone B-cell lymphoma. Br J Haematol. 1997;98:446–9.
30. Ruiz-Ballesteros E, Mollejo M, Mateo M, Algara P, Martínez P, Piris M. MicroRNA losses in the frequently deleted region of 7q in SMZL. Leukemia. 2007;21:2547–9.
31. Ruiz-Ballesteros E, Mollejo M, Rodriguez A, et al. Splenic marginal zone lymphoma: proposal of new diagnostic and prognostic markers identified after tissue and cDNA microarray analysis. Blood. 2005;106:1831–8.
32. Vega F, Cho-Vega JH, Lennon PA, Luthra MG, Bailey J, Breeden M, et al. Splenic marginal zone lymphomas

are characterized by loss of interstitial regions of chromosome 7q, 7q31.32 and 7q36.2 that include the protection of telomere 1 (POT1) and sonic hedgehog (SHH) genes. Br J Haematol. 2008;142:216–26.
33. Bouteloup M, Verney A, Rachinel N, Callet-Bauchu E, Ffrench M, Coiffier B, et al. MicroRNA expression profile in splenic marginal zone lymphoma. Br J Haematol. 2012;156:279–81.
34. Rinaldi A, Mian M, Chigrinova E, et al. Genome-wide DNA profiling of marginal zone lymphomas identifies subtype-specific lesions with an impact on the clinical outcome. Blood. 2011;117:1595–604. **An insight of genomic abnormalities in SMZL.
35. Corcoran M, Mould S, Orchard J, et al. Dysregulation of cyclin dependent kinase 6 expression in splenic marginal zone lymphoma through chromosome 7q translocations. Oncogene. 1999;18:6271–7.
36. Cuneo A, Bardi A, Wlodarska I, et al. A novel recurrent translocation t(11;14)(p11;q32) in splenic marginal zone B cell lymphoma. Leukemia. 2001;15:1262–7.
37. Jadayel D, Matutes E, Dyer M, et al. Splenic lymphoma with villous lymphocytes: analysis of bcl-1 rearrangements and expression of the cyclin D1 gene. Blood. 1994;83:3664–71.
38. Oscier DG, Matutes E, Gardiner A, et al. Cytogenetic studies in splenic lymphoma with villous lymphocytes. Br J Haematol. 1993;85:487–91.
39. Thieblemont C, Nasser V, Felman P, et al. Small lymphocytic lymphoma, marginal zone B-cell lymphoma, and mantle cell lymphoma exhibit distinct gene-expression profiles allowing molecular diagnosis. Blood. 2004;103:2727–37. **Gene expression profiling in SMZL with the identification of specific signatures compared to small B cell lymphomas (MCL and SLL).
40. Rossi D, Deaglio S, Dominguez-Sola D, Rasi S, Vaisitti T, Agostinelli C, et al. Alteration of BIRC3 and multiple other NF-κB pathway genes in splenic marginal zone lymphoma. Blood. 2011;118:4930–4. **An insight in the physiopathology of SMZL.
41. Bertoni F, Zucca E. State-of-the-art therapeutics: marginal-zone lymphoma. J Clin Oncol. 2005;23:6415–20.
42. Arcaini L, Lazzarino M, Colombo N, et al. Splenic marginal zone lymphoma: a prognostic model for clinical use. Blood. 2006;107:4643–9.
43. Camacho FI, Mollejo M, Mateo MS, et al. Progression to large B-cell lymphoma in splenic marginal zone lymphoma—A description of a series of 12 cases. Am J Surg Pathol. 2001;25:1268–76.
44. Vallisa D, Bernuzzi P, Arcaini L, et al. Role of anti-hepatitis C virus (HCV) treatment in HCV-related, low-grade, B-cell, non-Hodgkin's lymphoma: a multicenter Italian experience. J Clin Oncol. 2005;23:468–73.
45. Chacon J, Mollejo M, Munoz E, et al. Splenic marginal zone lymphoma: clinical characteristics and prognostic factors in a series of 60 patients. Blood. 2002;100:1648–54.
46. Lefrère F, Lévy V, François S, Delarue R, Ifrah N, Tilly H, et al. Fludarabine therapy in patients with splenic lymphoma with villous lymphocytes: an update. Leukemia. 2004;18(11):1924–5.
47. Tsimberidou AM, Catovsky D, Schlette E, O'Brien S, Wierda WG, Kantarjian H, et al. Outcomes in patients with splenic marginal zone lymphoma and marginal zone lymphoma treated with rituximab with or without chemotherapy or chemotherapy alone. Cancer. 2006;107(1):125–35.
48. Kalpadakis C, Pangalis GA, Dimopoulou MN, et al. Rituximab monotherapy is highly effective in splenic marginal zone lymphoma. Hematol Oncol. 2007;25:127–31.
49. Bennett M, Sharma K, Yegena S, Gavish I, Dave HP, Schechter GP. Rituximab monotherapy for splenic marginal zone lymphoma. Haematologica. 2005;90:856–8.
50. Cervetti G, Galimberti S, Cecconi N, Caracciolo F, Petrini M. Role of low-dose 2-CdA in refractory or resistant lymphoplasmocytic lymphoma. J Chemother. 2004;16:388–91.
51. Lefrere F, Hermine O, Belanger C, et al. Fludarabine: an effective treatment in patients with splenic lymphoma with villous lymphocytes. Leukemia. 2000;14:573–5.
52. Tsimberidou AM, Catovsky D, Schlette E, et al. Outcomes in patients with splenic marginal zone lymphoma and marginal zone lymphoma treated with rituximab with or without chemotherapy or chemotherapy alone. Cancer. 2006;107:125–35.
53. Cheson BD, Friedberg JW, Kahl BS, Van der Jagt RH, Tremmel L. Bendamustine produces durable responses with an acceptable safety profile in patients with rituximab-refractory indolent non-Hodgkin lymphoma. Clin Lymphoma Myeloma Leuk. 2010;10:452–7.

Nodal Marginal Zone Lymphoma

8

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Abstract

Nodal marginal zone lymphoma (NMZL) is a primary nodal B-cell tumor originating in the marginal zone of the lymph node and without clinical evidence of extranodal or splenic disease. This lymphoma was initially named monocytoid B-cell lymphoma (MBCL) due to monocytoid features of the tumor cells. According to the World Health Organization (WHO) classification, NMZL is a distinctive disease entity but with histologic and genetic similarities with the other two clinicopathological subtypes of MZL, extranodal MZL of mucosa-associated lymphoid tissue (MALT) type and splenic MZL, but with different and more aggressive clinical findings and a shorter survival time. NMZL is clinically similar to other low-grade (indolent) nodal lymphomas, such as follicular or small lymphocytic lymphomas. A clinical, morphological, and immunoarchitectural spectrum is seen also within the NMZL entity; why a careful evaluation of the disease is important before clinical decisions. The choice of optimal therapy for patients with NMZL represents a dilemma, since only small retrospective series and few clinical studies have been published.

Keywords

Nodal marginal zone lymphoma • Diagnosis • Pathology • Prognosis • Therapy

Introduction

Nodal marginal zone B-cell lymphoma (NMZL) is an uncommon well-defined lymphoma entity according to the 2008 World Health Organization (WHO) classification [1]. This lymphoma was initially described in 1986 by Sheibani et al. [2], who recognized the similarity of the neoplastic cells with monocytoid B-cells seen in the lymph node in toxoplasma lymphadenitis and in other

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type of inflammations. The malignant monocytoid B-cell proliferation was named monocytoid B-cell lymphoma (MBCL). The term parafollicular lymphoma was used by Cousar et al. [3] to illustrate that the distribution of the lymphoma cells was mainly around hyperplastic follicles. Later monocytoid lymphoma cells were found to share features of marginal zone B-cells with expression of IgM and well-developed endoplasmic reticula in the electron microscopy and with a distinctive nodal architecture [4]. The marginal zone features of the tumor cells and the expansion of the marginal zone in affected lymphoid tissues made the name marginal zone lymphoma (MZL) logic. According to the 2008 World Health Organization (WHO) criteria [1], three entities of MZLs are defined: extranodal mucosa-associated lymphoid tissue (MALT), splenic, and nodal type. NMZL can mostly be recognized with the combination of clinical picture and morphological and immunophenotyping findings, but cytogenetic and molecular methods are sometimes of value. As NMZL is a recently characterized and uncommon lymphoma, large clinical series are lacking and few data from clinical trials are published.

Epidemiology

NMZL accounts for 1.5–1.8 % of all lymphoid tumors and represents 10 % of the MZLs [5, 6]. In the report on the clinical evaluation of the International Lymphoma Study Group classification of NHL, a total 25 of 1,378 cases (1.8 %) were classified as primary NMZL [7]. The median age at diagnosis is between 50 and 64 years depending on the described series, but NMZL is also described in children [8]. In adult patients, the incidence of the disease is equal in males and females, while males predominate in children. Like in other MZLs, NMZL is sometimes associated with chronic antigenic stimulation and with infections, as hepatitis C virus (HCV) [9], with an especially high incidence in Italy [10, 11]. Marasca et al. [12] found the presence of the immunoglobulin heavy chain variable region (IG*HV*) *1–69* segment in a subset of HCV-positive NMZLs and with similar CDR3 sequences, supporting the hypothesis that the HCV antigen epitope was involved in the clonal B-cell selection. The causative role of HCV in MZL has been further supported by the response to antiviral therapy. In HCV-negative NMZL, involvement of the *IGHV 4–34* segment was found with different characteristics of the CDR3 regions, and a role of unknown B-cell superantigen(s) was suggested [12].

Pathology

In NMZL, the cell of origin is a marginal zone memory B-cell with centrocyte-, monocytoid-, and sometimes plasmacyte-like features. The marginal zone is an anatomically distinct compartment of the B-follicle, well developed in lymphoid organs with high influx of antigens as mesenteric lymph nodes, MALT, and spleen. Normally, monocytoid B-cells are found in lymph nodes in different types of inflammation (lymphadenitis), often in clusters within and around sinuses and in the interfollicular areas. Sometimes, these nonmalignant cells surround the follicles in a marginal-zone-like pattern. Monocytoid B-cells are medium sized with abundant pale to clear cytoplasm, and the nuclei are irregular with inconspicuous nucleoli. Malignant monocytoid B-cells are also recognized by their abundant pale cytoplasm, and proliferation of these cells in lymph nodes gave rise to the name monocytoid B-cell lymphoma (MBCL) [2]. Later the marginal zone memory B-cell features of the tumor cells were recognized, and the term nodal marginal zone B-cell lymphoma (NMZL) is now used in the WHO classification [1]. The morphological features of the NMZL tumor cells are heterogeneous, mostly marginal-zone-centrocyte like, and pure monocytoid B-cell lymphomas are rare, while a minor component of monocytoid B-cells is observed frequently. In one series [13], a monocytoid component was noted in 71 % of the cases, an admixture of large cells in 47 % and plasma cells in 39 %, respectively. In another study of NMZL, plasmacytoid or plasmacytic differentiation was a very common feature (61 %) [14]. Thus, nodal MZL can show varying degrees of overlap with nodal lymphoplasmacytic lymphoma (LPL).

The histology of affected lymph nodes shows different patterns of infiltration, marginal-zone-like/perifollicular, nodular, and diffuse with tumor cells

extending into the interfollicular area sometimes invading into the follicles in a pattern known as "follicular colonization." In the early phase of a NMZL, mostly with perifollicular growth, the lymph node shows an expanded marginal zone surrounding the mantle zone, but in advanced disease, the lymph node architecture is diffuse. Residual reactive germinal centers are often surrounded by the tumor cells. Salama et al. [13] evaluated the immunoarchitectural features of 51 NMZLs and described four different growth patterns: diffuse in 75 % of the cases, interfollicular in 14 %, well-formed nodular/follicular in 10 %, and perifollicular in only 2 %. A stromal sclerosis was found in 25 % and prominent blood vessel sclerosis in 20 %.

With immunophenotyping methods, all MZL cells show positivity for the bcl2 protein, for pan B-cell markers (CD20, CD19, CD79a), and for surface immunoglobulin (sIg) (sIgM > sIgA, sIgG), but sIgD expression is lacking in most NMZLs. Complement receptors (CD21 and CD35) are expressed in MZLs and CD21 was of value to highlight a disrupted follicular dendritic cell meshwork in 35 of 49 cases (71 %) [13].

MZL cells show negativity for CD5, CD10, and CD23, which markers are useful in distinction from mantle cell, follicular, and small lymphocytic lymphomas. Also the negativity in MZL for cyclin D1 and Bcl 6 is of value for differentiation. Younes et al. [15] used two germinal center B-cell markers, human germinal center-associated lymphoma (HGAL) and LIM-only transcription factor 2 (LMO2), to separate lymphomas derived from small B-cells, particularly follicular lymphoma (FL) and marginal zone lymphoma. HGAL and LMO2 were sensitive and specific for detecting FL in nodal and extranodal sites but were negative in NMZL.

The above data highlights the cytologic, histologic, and immunoarchitectural spectrum of NMZL and the need of immunohistochemical analysis to differentiate from other disease entities.

Genetic Aberrations

No unique cytogenetic abnormality has been documented in NMZL. Trisomy of chromosome 3, either complete or partial, represents the most frequent numerical chromosomal abnormality in MZL. In the series of Dierlamm et al. [16], trisomy 3 was detected with a similar frequency in nodal, extranodal, and splenic MZL. The gain of several regions of chromosome 3 has been reported on chromosome-based data [17]. The gain of chromosome 3 has been described to affect FOX p1 and bcl6. A high prevalence of trisomy 3 has also been noted in interphase fluorescence in situ hybridization (FISH) studies in all MZL subtypes [18]. Rinaldi et al. [19] performed a comprehensive analysis of genomic DNA copy number changes in a series of 218 MZL cases including 25 nodal, 57 MALT, 134 splenic, and 2 unspecified MZLs. Gains of 3q and 18q were common in all three subtypes, and splenic MZLs was associated with del(7q31) and del(8p), while MALT lymphoma presented significantly more frequent gains at 3p, 6p, 18p, and del(6q23) (TNFAIP3/A20). Bcl 2 protein overexpression is seen in nearly all NMZL cases but is not related to t(14;18) translocation, which is the case in follicular lymphoma. Also other translocations, which are detected in extranodal MZLs, are lacking in NMZL.

Molecular Findings

Molecular techniques could support the diagnosis of an NMZL by identifying clonality of B-cells with all lymphoma cells having the same immunoglobulin gene rearrangement. Marginal zone B-cells are functionally heterogeneous and differ with respect to the pattern of somatic hypermutation of immunoglobulin genes [20]. Most NMZL tumors harbor mutated immunoglobulin heavy variable chain (IGHV) region genes. Traverse-Glehen et al. [21] analyzed IGHV gene usage and mutation patterns in 35 SMZL and 14 NMZL patients. A biased usage of IGHV gene was found with overrepresentation of IGHV 4 in NMZL cases (7/14), with a biased use of IGHV 4–34, while IGHV 1 was used in 13/35 SMZL cases. Evidence for antigen-driven mutations was identified in 8 SMZL and 4 NMZL cases. In this report, only 2 NMZL (14 %) cases were unmutated, but 11 (31 %) of the SMZL cases. Thus, the

pattern of somatic mutation and the IGHV gene segment usage differed between SMZL and NMZL.

Clinical Features and Diagnosis

The median age at diagnosis of NMZL is mostly between 50 and 65 years, and the gender distribution is equal. The patients present with no specific clinical diagnostic features, but peripheral and para-aortic non-bulky lymphadenopathy is common. The disease is often advanced at the time of diagnosis, but the majority of patients are asymptomatic, and B symptoms are reported in less than 15 %. Bone marrow involvement is reported in 30–45 % of the patients but without other extranodal manifestation (according to the WHO definition of NMZL). Peripheral blood involvement is uncommon. A monoclonal paraprotein of IgM-type is detected in around 10 % of cases, mostly small to moderate in size. In one report by Traverse-Glehen et al. [14], the bone marrow involvement was higher (62 %), with a peripheral blood involvement in 23 % and a serum M component detected in 33 % of the patients.

There are no specific diagnostic markers for NMZL, but this lymphoma can mostly be recognized with the combination of clinical picture and morphological and immunophenotyping methods. The diagnosis of NMZL is always made on a surgical biopsy from a lymph node; fine-needle aspiration (FNA) is not sufficient, and requires according to the WHO definition "a primary nodal B-cell neoplasm that morphologically resembles lymph nodes involved by marginal zone lymphomas of extranodal or splenic types, but without evidence of extranodal or splenic disease." The presence of characteristic clear cells recognized as marginal/monocytoid B-cell positive for CD20 and CD79a and negative for CD5 antigens is required. Distinguishing NMZL from small B-cell lymphoma with plasmacytic differentiation, mainly LPL, and from mantle cell, follicular, and small lymphocytic lymphomas is sometimes challenging. The diagnostic workup, like in other indolent lymphomas, includes the following:

- Complete history and physical examination
- Laboratory evaluation
  - Complete blood count
  - Serum electrolytes
  - Kidney and liver function tests
  - Lactate dehydrogenase
  - Beta-2 microglobulin
  - Serum electrophoresis with immunoglobulin evaluation
  - Hepatitis B and C serology
- Bone marrow aspiration and biopsy
- CT scans of chest/abdomen/pelvis

The following immunological markers (for the diagnosis of NMZL in lymph node and bone marrow) are recommended:

- CD20, CD21, CD23, CD5, CD3, CD43, CD10, Ki-67, BCL1, BCL2, and BCL6
- HGAL and LMO2 might be of value

Cytogenetic findings are of not diagnostic, although the chromosomal aberrations, trisomies 3 and 18, as well as gain of part of chromosome 3 may support the diagnosis of NMZL.

Prognostic Factors

Nodal MZL is clinically more aggressive than the other two low-grade MZLs of MALT and splenic type. Patients with nodal MZL show a significantly higher incidence of advanced-stage disease, including peripheral and para-aortic lymphadenopathy, than those with MALT-type lymphoma with nodal localization [6]. Moreover, in this report, 20 patients with nodal MZL had shorter 5-year overall survival and failure-free survival than 73 patients with MALT-type lymphoma. When analysis was restricted to patients with 0–3 International Prognostic Index (IPI) adverse risk factors, patients with nodal MZL still had a significantly lower overall and failure-free survival at 5 years than patients with MALT-type lymphoma. Mostly, IPI will detect only a minority of NMZLs with high risk and is of low value for prognostication. Few data are published on any prognostic value of cytogenetic aberrations. In a small report from Norway, 4/5 patients with

NMZL had chromosome 3 abnormalities and patients with NMZL had a shorter median survival than patients in other morphological subgroups of MZL ($P < 0.003$) [17]. Some biological markers, as loss of survivin and active caspase 3, have been shown to be prognostic factors for short event-free survival and overexpression of cyclin E for overall survival [22].

Transformation to diffuse large B-cell lymphoma can occur like in most other indolent lymphomas and is a bad prognostic sign. In one series, 20 of 124 patients (16 %) transformed at a median time of 4.5 years from diagnosis [5]. Transformation to large-cell lymphoma has also been noted at the time of diagnosis in five of 25 (20 %) cases of nodal MZL, and in 32 of 105 (30 %) cases of MALT-type lymphoma [7]. However, there is no consensus of the definition of transformation in NMZL, and often, an admixture of large cells is seen at the time of diagnosis but without sheets and with no impact on the clinical course.

Thus, nodal MZL is a distinctive disease entity with a prognosis similar to other low-grade nodal lymphomas, such as the follicular or small lymphocytic lymphomas, but less favorable than MALT-type and splenic-type MZL.

Treatment

Nodal MZL still represents a therapeutic dilemma, since no studies of large prospective series have been published. Therapy is not different from that in other indolent lymphomas, and today mostly immunochemotherapy including the monoclonal anti-CD20 antibody rituximab or rituximab alone is recommended. In a report by Traverse-Glehen et al., patients receiving chemotherapy had a good initial response but relapses were frequent. The 5-year failure-free survival in the report from Arcaini et al. [11] was only 28 % vs. 65 % for extranodal MZL. Concise data on long-term results with rituximab-containing therapy is lacking. Still today, the disease does not appear to be curable, and there is a continuing pattern of relapse.

Some patients have a long median survival, approaching 10 years [5]. The median 5-year overall survival of patients with nodal MZL has been found to be lower than that of patients with extranodal MZL (56 % vs. 81 %, respectively) [12], and in several published series on NMZL, a 5-year survival is around 60–70 %. The role of different therapies for survival has not been evaluated.

In a phase II trial testing the new monoclonal antibody veltuzumab in relapsing indolent lymphoma, the response seemed to be high in marginal zone lymphoma with an overall response rate in five of six patients (83 %), two with CRs/CRus (33 %) [23]. In MZL, a deregulation of the nuclear factor-κ[kappa]B (NF-κ[kappa]B) pathway has been suggested, and bortezomib is a drug with activity in NMZL [24–26].

In young patients, high-dose chemotherapy with autologous transplantation is an alternative especially if early relapse [27].

In patients with hepatitis C-positive NMZL, an antiviral therapy should be discussed before the institution of any immunochemotherapy, as interferon and ribavirin have been successful with regress of the MZL [28].

References

1. Jaffe ES, Harris N, Stein H, Vardiman J. World Health Organisation Classification of Tumours. Pathology and genetics: tumours of haemopoietic and lymphoid tissues. Lyon: IAR Press; 2008.
2. Sheibani K, Sohn CC, Burke JS, Winberg CD, Wu AM, Rappaport H. Monocytoid B-cell lymphoma. A novel B-cell neoplasm. Am J Pathol. 1986;124(2):310–8.
3. Cousar JB, McGinn DL, Glick AD, List AF, Collins RD. Report of an unusual lymphoma arising from parafollicular B-lymphocytes (PBLs) or so-called "monocytoid" lymphocytes. Am J Clin Pathol. 1987;87(1):121–8.
4. Piris MA, Rivas C, Morente M, Cruz MA, Rubio C, Oliva H. Monocytoid B-cell lymphoma, a tumour related to the marginal zone. Histopathology. 1988;12(4):383–92.
5. Berger F, Felman P, Thieblemont C, et al. Non-MALT marginal zone B-cell lymphomas: a description of clinical presentation and outcome in 124 patients. Blood. 2000;95:1950–6.
6. Nathwani BN, Anderson JR, Armitage JO, et al. Marginal zone B-cell lymphoma: a clinical comparison of nodal and mucosa-associated lymphoid tissue types. Non-Hodgkin's Lymphoma Classification Project. J Clin Oncol. 1999;17:2486–92.

7. A clinical evaluation of the International Lymphoma Study Group classification of non-Hodgkin's lymphoma. The Non-Hodgkin's Lymphoma Classification Project. Blood. 1997;89(11):3909–18.
8. Taddesse-Heath L, Pittaluga S, Sorbara L, Bussey M, Raffeld M, Jaffe ES. Marginal zone B-cell lymphoma in children and young adults. Am J Surg Pathol. 2003;27(4):522–31.
9. Zuckerman E, Zuckerman T, Levine AM, et al. Hepatitis C virus infection in patients with B-cell non-Hodgkin lymphoma. Ann Intern Med. 1997;127(6):423–8.
10. Arcaini L, Paulli M, Boveri E, et al. Splenic and nodal marginal zone lymphomas are indolent disorders at high hepatitis C virus seroprevalence with distinct presenting features but similar morphologic and phenotypic profiles. Cancer. 2004;100(1):107–15.
11. Arcaini L, Paulli M, Burcheri S, et al. Primary nodal marginal zone B-cell lymphoma: clinical features and prognostic assessment of a rare disease. Br J Haematol. 2007;136(2):301–4.
12. Marasca R, Vaccari P, Luppi M, et al. Immunoglobulin gene mutations and frequent use of VH1–69 and VH4–34 segments in hepatitis C virus-positive and hepatitis C virus-negative nodal marginal zone B-cell lymphoma. Am J Pathol. 2001;159:253–61.
13. Salama ME, Lossos IS, Warnke RA, Natkunam Y. Immunoarchitectural patterns in nodal marginal zone B-cell lymphoma: a study of 51 cases. Am J Clin Pathol. 2009;132(1):39–49.
14. Traverse-Glehen A, Felman P, Callet-Bauchu E, et al. A clinicopathological study of nodal marginal zone B-cell lymphoma. A report on 21 cases. Histopathology. 2006;48:162–73. http://www.ncbi.nlm.nih.gov/pubmed/164056652006.
15. Younes SF, Beck AH, Ohgami RS, Lossos IS, Levy R, Warnke RA, et al. The efficacy of HGAL and LMO2 in the separation of lymphomas derived from small B cells in nodal and extranodal sites, including the bone marrow. Am J Clin Pathol. 2011;135(5):697–708.
16. Dierlamm J, Pittaluga S, Wlodarska I, Stul M, Thomas J, Boogaerts M, et al. Marginal zone B-cell lymphomas of different sites share similar cytogenetic and morphological features. Blood. 1996;87:299–307.
17. Aamot HV, Micci F, Holte H, Delabie J, Heim S. Q-banding and molecular cytogenetic analyses of marginal zone lymphoma. Br J Haematol. 2005;130(6):890–901.
18. Callet-Bauchu E, Baseggio L, Felman P, et al. Cytogenetic analysis delineates a spectrum of chromosomal changes that can distinguish non-MALT marginal zone B-cell lymphomas among mature B-cell entities: a description of 103 cases. Leukemia. 2005;19(10):1818–23.
19. Rinaldi A, Mian M, Chigrinova E, et al. Genome-wide DNA profiling of marginal zone lymphomas identifies subtype-specific lesions with an impact on the clinical outcome. Blood. 2011;117(5):1595–604.
20. Tierens A, Delabie J, Michiels L, Vandenberghe P, De Wolf-Peeters C. Marginal-zone B cells in the human lymph node and spleen show somatic hypermutations and display clonal expansion. Blood. 1999;93(1):226–34.
21. Traverse-Glehen A, Davi F, Ben Simon E, et al. Analysis of V-H genes in marginal zone lymphoma reveals marked heterogeneity between splenic and nodal tumors and suggests the existence of clonal selection. Haematologica. 2005;90(4):470–8.
22. Camacho FI, Algara P, Mollejo M, García JF, Montalbán C, Martínez N, et al. Nodal marginal zone lymphoma: a heterogeneous tumor: a comprehensive analysis of a series of 27 cases. Am J Surg Pathol. 2003;27(6):762–71.
23. Morschhauser F, Leonard JP, Fayad L, Coiffier B, et al. Humanized anti-CD20 antibody, veltuzumab, in refractory/recurrent non-Hodgkin's lymphoma: phase I/II results. J Clin Oncol. 2009;27(20):3346–53.
24. O'Connor OA, Wright J, Moskowitz C, et al. Phase II clinical experience with the novel proteasome inhibitor bortezomib in patients with indolent non-Hodgkin's lymphoma and mantle cell lymphoma. Clin Oncol. 2005;23(4):676–84.
25. Goy A, Younes A, McLaughlin P, Pro B, et al. Phase II study of proteasome inhibitor bortezomib in relapsed or refractory B-cell non-Hodgkin's lymphoma. Results of a phase 2 study of bortezomib in patients with relapsed or refractory indolent lymphoma. Blood. 2010;115(3):475–80.
26. Di Bella N, Taetle R, Kolibaba K, et al. Multicenter randomized phase II study of weekly or twice-weekly bortezomib plus rituximab in patients with relapsed or refractory follicular or marginal-zone B-cell lymphoma. Clin Oncol. 2009;27(30):5023–30.
27. Thieblemont C, Coiffier B. Management of marginal zone lymphomas. Curr Treat Options Oncol. 2006;7(3):213–22. Review.
28. Kelaidi C, Rollot F, Park S, Tulliez M, Christoforov B, Calmus Y, et al. Response to antiviral treatment in hepatitis C virus-associated marginal zone lymphomas. Leukemia. 2004;18(10):1711–6.

Follicular Lymphoma

9

Judith Trotman and Gilles Salles

Abstract

Follicular lymphoma is the most common and well-characterized low-grade lymphoma.

Gene expression profiling and biomarker development have improved our understanding of its biology, but there remains no robust biologic, immunohistochemical prognostic marker at diagnosis. Therefore, clinical criteria such as the Follicular Lymphoma International Prognostic Index (FLIPI) and the GELA/BNLI criteria for starting treatment remain the most useful tools to both assign prognosis and commence therapy.

Our better understanding of the heterogeneity of follicular lymphoma is paralleled by the development of a plethora of new first-line treatment options using monoclonal antibodies, either alone or in combination with chemotherapy or radio-conjugates. Emerging data supports the influence of depth of response to first-line therapy on long-term outcomes, and there is early evidence suggesting that rituximab maintenance therapy prolongs both progression-free and possibly overall survival. Improved patient understanding of this usually chronic and incurable disease is increasingly associated with a willingness to participate in treatment decision making. Thus, the selection of therapy at each phase of the disease, with subsequent impact on future therapeutic options, becomes a more sophisticated individualized process.

Keywords

Follicular lymphoma • FLIPI index • Tumor burden • Watch and wait strategy • Rituximab plus chemotherapy • Rituximab maintenance • Radioimmunotherapy • Autologous transplant • Patient participation to treatment decision

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Introduction

Follicular lymphoma, the second most common subtype of lymphoma, represents up to 25 % of non-Hodgkin lymphomas in Europe and the USA. The pathological diagnosis is robust and generally reproducible, noting the more recent exclusion of Grade 3b disease (diffuse areas containing >15 centroblasts per hpf without admixed centrocytes) from the common spectrum of follicular lymphoma. The disease course, typically indolent both at diagnosis and at relapse, is characterized by recurrent progression with shorter remissions. The appearance of a diffuse area of large cells in a new biopsy defines histological transformation, a feature usually associated with a poor outcome [1, 2] occurring in a variable number of patients.

Follicular lymphoma patients typically present with superficial lymphadenopathy, at times neglected by the patient for a prolonged period. In some patients, the first symptoms are related to the insidious growth of deep abdominal lymphadenopathy. Impaired performance status or B symptoms are uncommon. Nonetheless, the majority of patients, 70–85 %, have advanced-stage disease, with bone marrow involvement in 50–60 %.

Prognostic Factors

The FLIPI (Follicular Lymphoma International Prognostic Index [3]) is based on five simple independent risk factors (hemoglobin <12 g/dL, serum LDH > upper normal value, Ann Arbor stages III-IV, number of nodal sites >4, age >60 years). A robust prognostic indicator, the FLIPI separates newly diagnosed patients into three equal-sized groups with distinct survival probabilities (Table 9.1) [3]. The index is valid for both younger and older patients and retains its discriminating power in the context of combination chemotherapy plus rituximab [4–6] (Fig. 9.1). However, it does not identify a significant minority of patients with a really poor outcome for whom a more aggressive therapy may be considered. For instance, while 17 % of patients <60 years are categorized as "high-risk FLIPI," their predicted survival is still >50 % at 8 years. Finally, the FLIPI does not necessarily dictate a need for therapy. Young stage I or II patients with retroperitoneal tumor bulk, and elevated LDH, will be classified as low risk, yet most clinicians consider this presentation an indication for therapy. Conversely, a watch and wait approach is appropriate for many elderly patients with disseminated disease lacking systemic symptoms despite a high FLIPI. Most clinical trials assessing the role of frontline immunochemotherapy included 10–20 % of patients with a low FLIPI [4, 7, 8], while the same proportion of patients with a low tumor burden managed with watch and wait have a high-FLIPI score [9].

An interesting recent development has been that of the FLIPI2: a prognostic index developed for follicular lymphoma patients receiving immediate therapy using progression-free survival as the principal endpoint [10]. Again comprising five factors—$\beta_2$microglobulin > normal, longest diameter of the largest involved node >6 cm, bone marrow involvement, hemoglobin <12 g/dL, and age older than 60 years—the

Table 9.1 Prediction of follicular lymphoma patients' outcome based on the FLIPI

Number of risk factors[a]	FLIPI score	Proportion of patients (%)	Overall survival (%)	
			At 5 years	At 10 years
0 or 1	Low	36	91	71
2	Intermediate	37	78	51
3 to	High	27	53	36

Adapted from Solal-Celigny et al. [3]; used with permission

[a]Factors adversely affecting survival in the FLIPI include age greater than 60 years, Ann Arbor stages III–IV, number of nodal sites greater than 4, serum LDH level greater than the upper limit of normal, and hemoglobin level less than 12 g/dL

FLIPI2 identifies a 3-year PFS rate of 91, 69, and 51 % for patients at low, intermediate, and high risk, respectively ($p<0.0001$). This prospectively collected and externally validated series highlights the predictive power of β_2-microglobulin and a single lymph node measurement. However, in excluding >10 % of patients who underwent "watch and wait," it cannot be universally applied to all patients. Found to be equally valid in predicting PFS for the majority (59 %) of patients treated with rituximab-containing regimens, it will be interesting to chart the discriminating power of FLIPI2 for OS with more prolonged follow-up. To date, two additional comparisons between the FLIPI and FLIPI2 performed suggest that the FLIPI score may be more discriminatory [11, 12].

Gene expression profiling and immunohistochemical analyses of the malignant cells and tumor microenvironment, using the immune-response signatures referred to as IR-1 and IR-2, are promising prognostic markers [13–18]. With discordant results, however, they are not yet sufficiently robust nor available to replace the traditional clinical indices used to assess patients' prognosis and decide the optimal therapeutic strategy. The most commonly used international criteria for starting cytotoxic therapy are listed in Table 9.2 [30]. These indices include bulky disease (either masses >7 cm or >3 nodal areas measuring 3 cm), local symptoms or compromised organ function due to tumor, B symptoms, elevated LDH or β_2-microglobulin, and cytopenias due to marrow involvement.

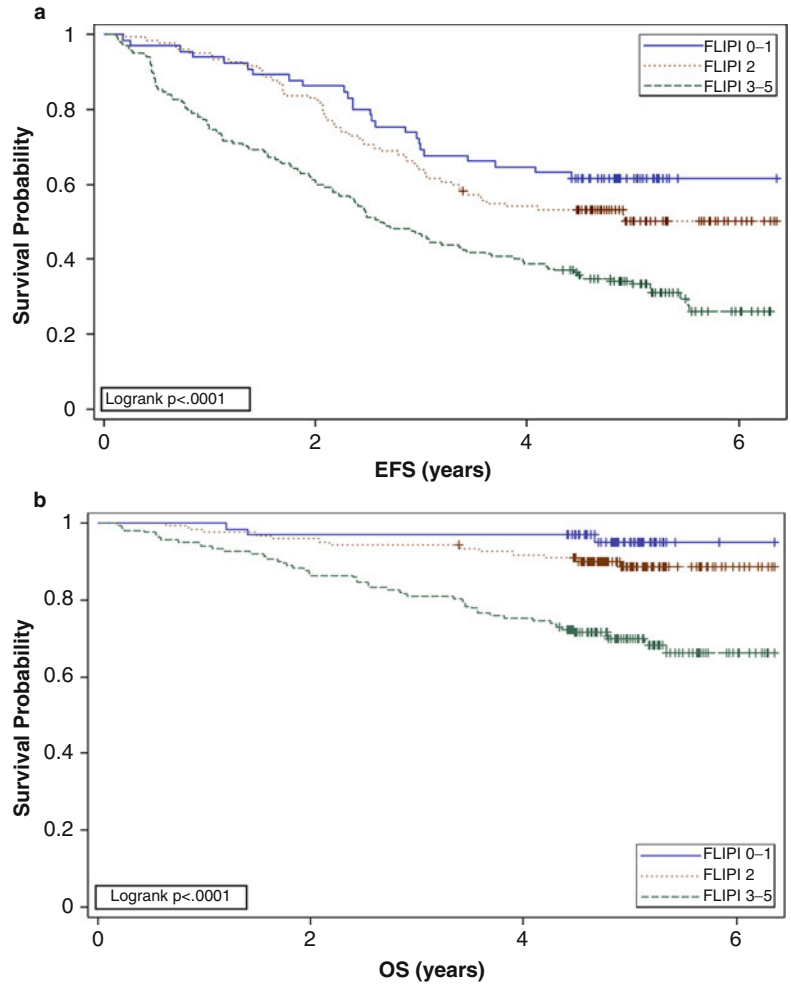

Fig. 9.1 Event-free survival (**a**) and overall survival (**b**) of patients receiving R-CHVP + interferon in the FL2000 study according to the FLIPI score

Table 9.2 Criteria for starting a cytotoxic treatment in follicular lymphoma patients

Adapted GELF criteria (FL2000 and PRIMA studies): any one of these criteria	BNLI criteria [30]: any one of these criteria
High tumor bulk defined by either: A tumor > 7 cm 3 nodes in 3 distinct areas each > 3 cm Symptomatic splenic enlargement Organ compression Ascites or pleural effusion	Rapid generalized disease progression in the preceding 3 months Life-threatening organ involvement Renal or macroscopic liver infiltration Bone lesions
Presence of systemic symptoms	Presence of systemic symptoms or pruritus
ECOG performance status > 1[a]	
Serum LDH or beta2-microglobulin above normal values	Hemoglobin < 10 g/dL or WBC < 3.0×10^9/L or platelet counts < 100×10^9/L, related to marrow involvement

[a]Used in the FL2000 but not in the PRIMA study, given the low percentage of patients with this sole criteria in the former studies (Salles G, personal communication 2012)

Table 9.3 Treatments used in newly diagnosed patients with follicular lymphoma (USA, 2004–2007) [27]

Treatment	Patients with all stages (n = 2,728) (%)	Patients with stage I (n = 474) (%)
Chemotherapy plus rituximab	51.9	30.4
Observation	17.7	28.7
Rituximab monotherapy	13.9	12.9
Radiation therapy	5.6	23.4
Clinical trial	6.1	–
Chemotherapy	3.2	2.5
Others	1.6	2.1

Initial Management of Early-Stage Disease

In the 10–15 % of patients with truly localized disease, the traditional treatment strategy had been radiation therapy (up to 36 Gy for bulky disease), given the radiosensitivity of FL, the prolonged OS in observational studies, and the alleged potential for cure [19, 20]. The FLIPI is of prognostic value in this patient group [21]. Despite the benefit of this strategy indicated in a large retrospective study [22] and published NCCN and ESMO guidelines [23, 24], the consensus on radiation therapy is not firm. Many clinicians adopt a watch and wait strategy [25], while others advocate combined modalities [26]. In a large prospective US cohort, radiotherapy was used as the sole treatment in only 23 % of patients with stage I disease and administered after chemotherapy in another 8 % [27] (Table 9.3). A systemic approach may indeed be appropriate for symptomatic patients with stage II disease when significant morbidity from radiotherapy could be expected based on tumor location. Prospective studies are lacking but there is merit in assessing whether a subset of high-risk patients with early-stage disease may benefit from a combined modality approach.

Advanced-Stage Disease: From Watch and Wait to Immunochemotherapy

Some Patients May Not Need Immediate Therapy

A period of observation has been a reasonable option for asymptomatic patients with low bulk disease to date. The median time to therapy with initial observation of asymptomatic patients was 2.6–3 years [28, 29]. Several retrospective and prospective studies demonstrate comparable overall survival using this approach compared

with initial chemotherapy treatment [25, 30, 31]. One study found no increased risk of histologic transformation [28], contrary to other reports [29, 32]. The rationale for observation is being challenged in an era of efficacious, minimally toxic immunotherapy such as rituximab. Furthermore, in this internet era, with broader patient understanding and participation in treatment decision making, given the absence of a survival detriment, many patients and their clinicians prefer the earlier introduction of therapy to the uncertainty of living with an untreated cancer. In a recent preliminary report of a Phase III study, rituximab monotherapy (4× weekly) followed by maintenance (every 2 months for 2 years) significantly improved the time to initiation of a new treatment and progression-free survival in patients with asymptomatic, non-bulky, advanced-stage disease when compared to watchful waiting [33]. Follow-up of this study is short and this outcome was anticipated, but both the "duration of rituximab benefit" (i.e., the potential impact of prior rituximab exposure on the response to first and second new treatments) and any overall survival difference have yet to be determined. Another caveat lies in the unknown long-term immune and infectious consequences of early and repeated rituximab exposure.

Options Available When Treatment Is Needed

Traditionally, therapeutic decision making for follicular lymphoma has been based on choosing between two goals: optimizing quality of life versus aiming for prolonged survival. However, rarely are patient priorities solely one or the other, but a relative balance of the two. Patient- and disease-related prognostic factors impact on the ability of the clinician to meet both priorities, after comprehensive discussion with the patient. Furthermore, patient priorities may change and clinicians need be mindful of the impact of first-line therapy on subsequent treatment options in this chronic "incurable" disease. The absence of consensus on the optimal first-line therapy for FL and the consequent plethora of individualized approaches are highlighted in a US prospective cohort study of patients treated between 2004 and 2007 [27] (Table 9.3).

Before the advent of monoclonal antibodies, several therapeutic approaches were studied. Institutional and epidemiologic data support improved outcomes, and important lessons can be learnt from this era [34–36]. In patients with symptomatic stage III–IV disease, past treatments included the combination of anthracycline with alkylating agents, interferon administration, the use of purine analogues, and high-dose therapy with autologous hematopoietic cell transplant. Although response duration was usually prolonged, leading to marginal survival improvements in subgroup analyses [36–39] until now, no approach has shown to be unequivocally superior with identified drawbacks to each. The significantly prolonged PFS after anthracycline use (most commonly in CHOP) incurs some additional morbidity and risk of cardiac toxicity without clear evidence of a reduction in risk of histologic transformation. The considerable morbidity from interferon despite its survival benefit has precluded common use of this agent, as has the stem cell toxicity and incidence of late infections after fludarabine. Likewise, while three studies demonstrate improved progression-free survival after autologous transplantation, the considerable morbidity, increased incidence of secondary neoplasia, and lack of overall survival benefit argue against its incorporation as a first-line consolidative approach [40–42].

It was also commonly believed that the initial treatment was unable to alter the ultimate prolonged course of this incurable disease, and therapies were used sequentially for disease progression. This classical paradigm has been strongly challenged with two observations. Firstly, it is now clear that overall survival can be improved by a combination of rituximab plus chemotherapy for patients needing therapy. Secondly, while most studies have been hampered by short-term follow-up, it is increasingly acknowledged that, even in this indolent histology, the depth of remission is correlated with both remission duration and prolonged overall survival. The very long-term follow-up (median 14.9 years) of patients in the GELF86 studies

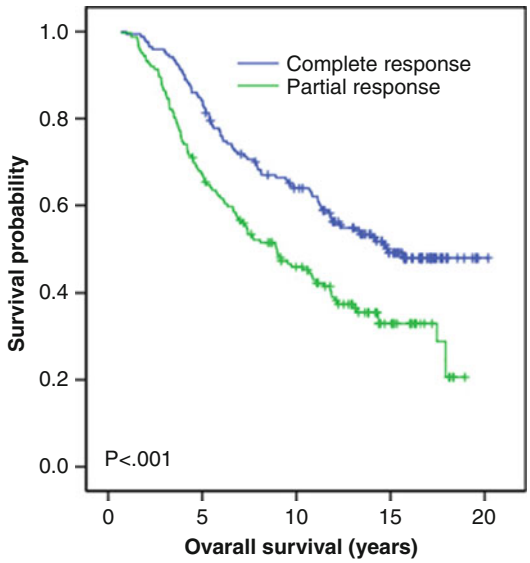

Fig. 9.2 Influence of response to first-line therapy in follicular lymphoma (excluding watch and wait patients) on overall survival ($p<.001$ in univariate and multivariate analysis)

recently demonstrated that patients achieving CR after first-line treatment had a significantly better OS than those reaching a PR (HR = 0.55, $p<0.001$) (Fig. 9.2) [43]. Furthermore, follicular lymphoma is universally [^{18F}]fluorodeoxyglucose (FDG) avid. A recent retrospective analysis demonstrated the markedly inferior outcome of a quarter of patients remaining PET positive after therapy with a significantly ($p<.0001$) inferior progression-free survival (PFS) at 42 months of 32.9 % compared to 70.7 % in those who became PET negative. The risk of death was also increased in PET-positive patients (hazard ratio 7.0; $p=.0011$) [44]. This data, if confirmed in prospective studies, strongly supports the benefit of achieving the best disease response in FL patients, but the definition of a true CR using PET will need to be clearly defined in this heterogeneously glucose avid histology.

First-Line Therapy with Rituximab Alone: As a Short Course or with Maintenance

Having decided that treatment is necessary, and where the principal priority is palliation of symptoms, there is a large body of literature using rituximab alone as a short course or with maintenance [45–48]. These studies mostly included patients with favorable disease characteristics (low tumor burden or low/intermediate FLIPI score). Such an approach is particularly relevant for elderly patients with multiple comorbidities and an otherwise shortened life expectancy. Approximately 75 % patients respond to 4 weekly doses of rituximab, with half of these responders achieving a complete response (CR). The median time to disease progression was reproducibly short: 18–24 months, but prolonged by maintenance rituximab. However, the "duration of rituximab benefit," defined as the time without need to start a cytotoxic regimen, was no different, suggesting rituximab retreatment at time of progression could be as effective as maintenance. Long-term follow-up of the ECOG 4402, RWW, and SAKK 35/03 studies will clarify this issue for these low tumor burden patients.

First-Line Therapy Combining Rituximab and Chemotherapy

Combination immunochemotherapy is appropriate when, most commonly, the treatment priority is to maximize depth of the response rate and progression-free and overall survival. There exists a plethora of therapeutic options often with attendant trade-offs between toxicity and depth of response. The addition of rituximab to conventional chemotherapy has demonstrated markedly improved response rates and progression-free and overall survival in several randomized studies (Table 9.4) [5, 8, 49–52]. The proportion of patients within each FLIPI score was similar, but control chemotherapy arms were different, hampering a straight comparison of these trials. A Phase III study of CVP chemotherapy with or without rituximab was performed in the first study [49]. Patients received consolidation with autologous stem cell transplant or interferon in the second study [50], or interferon alone in another [8], while in the final study (where patients also received interferon), the number of chemotherapy cycles was divided by 2 in the rituximab-containing arm [51] (Table 9.4). Sequential consolidation rituximab after chemotherapy was similarly shown to improve PFS in several studies [53–55].

Altogether, these studies demonstrate that first-line treatment combining rituximab with or after

Table 9.4 Randomized studies in follicular lymphoma patients using rituximab plus chemotherapy

Treatments	Patients within each FLIPI stratum (% with low/intermediate/high risk, respectively)	Median age (years)	Estimated PFS in the experimental arm	Progression-free survival (median)		Overall survival	
				Control arm	Experimental arm	Control arm	Experimental arm
CVP versus R-CVP [5, 49]	19/41/40	52	50 % (at 3 years)	15 months	34 months	77 % (at 4 years)	83 %[d] (at 4 years)
CHOP versus R-CHOP[a] [50, 52]	14/41/45	55	80 % (at 2 years)	31 months	Not reached	84 % (at 5 years)	90 %[d] (at 5 years)
MCP versus R-MCP[b] [8]	7/37/56	59	71 % (at 4 years)	26 months	Not reached	74 % (at 4 years)	87 %[d] (at 4 years)
CHVP+I versus R-CHVP+I[c] [51]	19/35/46	61	53 % (at 5 years)	35 months	Not reached	79 % (at 5 years)	84 % (at 5 years)

[a]CHOP or R-CHOP was followed by ASCT or IFN
[b]MCP and R-MCP were followed by IFN consolidation
[c]CHVP combined with interferon: 12 chemotherapy courses in the control arm versus 6 in the rituximab-containing arm
[d]*p* value for difference in overall survival significant

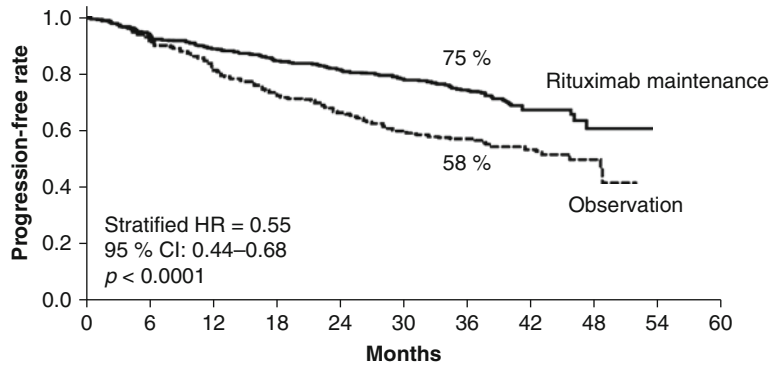

Fig. 9.3 Kaplan–Meier estimates of progression-free survival from randomization with rituximab maintenance versus observation

chemotherapy can improve outcomes. A meta-analysis (including studies for relapsing patients) estimated the benefit of this combination in terms of risk reduction (hazard ratio) for mortality to 0.63 (95 % confidence interval 0.51–0.79). The benefit in overall survival observed across the studies is noteworthy given that most patients not receiving rituximab as part of induction therapy likely received monoclonal antibodies at time of progression. Improved survival despite this crossover further endorses combined immunochemotherapy as a new standard in the first-line treatment of advanced follicular lymphoma.

Despite recent progress, the prognosis of the patient with high FLIPI remains unsatisfactory (median 5 year OS of 60 %) [51]. The preference for a first-line chemotherapy regimen containing or not an anthracycline remains debated, but when using R-CVP, median time to progression was only 26 months in one study [5]. Recent data, including a randomized study, indicate a significant improvement in progression-free survival with R-CHOP [56, 57]. Long-term follow-up of overall survival in randomized studies using anthracycline in the rituximab era may help to clarify this issue.

Maintenance Rituximab After Frontline Combination Therapy

Recently, the largest international study conducted in FL, the 1,200 patient PRIMA study, demonstrated the benefit of 2-year rituximab maintenance after first-line rituximab/chemotherapy [58]. Probability of achieving CR was significantly higher in patients receiving rituximab maintenance compared to those undergoing observation (72 vs. 52 %). After a median follow-up of 36 months, the PFS in patients receiving rituximab maintenance was 75 % compared to only 58 % in patients undergoing observation (hazard ratio 0.55; 95 % CI 0.44–0.68), Fig. 9.3. Maintenance therapy was well tolerated with Grade 3/4 adverse events occurring in 24 % compared to 17 % in the observation arm, and quality of life measures were comparable in both groups.

Frontline Therapy Using Radioimmunoconjugates, Alone or After Chemotherapy

Radioimmunoconjugates have also been studied in first-line treatment of follicular lymphoma, either as single agent or as consolidation therapy. Kaminski and colleagues reported the frontline use of ^{131}I-tositumomab in 76 patients [59] with a very high response rate (95 %) and 3 quarters of patients achieving CR. Toxicity was limited and the 5-year progression-free survival was 59 %. Although those patients were selected based on their limited marrow infiltration, these results are challenging in comparison with other trials including a substantial proportion of low tumor burden patients. Other studies demonstrated the potential of radioimmunotherapy to improve response rate and quality after either CHOP [60], fludarabine, [61] or rituximab followed by R-CHOP [62]. In the CHOP-^{131} I-tositumomab study, the estimated 5-year overall survival (OS) was 87 % and the progression-free survival (PFS) 67 % [60]. A large Phase III study [63] demonstrated a consistently high CR/CRu rate of 77 % with ^{90}Y-Ibritumomab tiuxetan used for remission consolidation after chemotherapy regardless of the initial chemotherapy used. Adjuvant ^{90}Y-ibritutomab also improved

progression-free survival ($p<0.0001$; HR 0.47) compared to observation. However, only a minority of patients in this study (13 %) received a rituximab-containing induction regimen [63]; hence, the role of radioimmunotherapy in the rituximab-chemotherapy era remains to be clarified. Prospective study of adjuvant radioimmunotherapy in patients failing to obtain CR may be of particular value. The current US intergroup trial is comparing R-CHOP versus CHOP followed by tositumomab. Despite its promise, access to radioimmunotherapy still remains limited internationally, and this will need to be addressed if the positive outcomes of clinical research are to be translated into the clinic.

Other Emerging Agents

Bendamustine, an agent with both alkylating agent and purine analogue properties, demonstrates excellent responses in patients refractory to rituximab and chemotherapy (ORR 77–92 % and CR 34–55 %) [64, 65]. Short-term toxicities are low, with an absence of alopecia or mucositis. A recently reported Phase III study compared first-line rituximab-bendamustine (90 mg/m^2 days1 + 2) with standard R-CHOP. Of 513 patients randomized, 54 % had follicular lymphoma. There was an improved tolerance in the R-bendamustine arm with a lower rate of neutropenia (11 % vs. 47 %, $p=0.0001$). There was a comparable 92 % overall response rate but notably an improved CR rate (39 vs. 30 %, $p=0.03$) and PFS (55 vs. 35 months, $p=0.00012$). While follow-up in this study is short (median 32 months) [66], and long-term toxicities remain unknown, this early data supports the possibility of using this agent first-line for follicular lymphoma patients. The validation of this data in other current trials is eagerly awaited.

Management of Patients in Second Line

Multiple options are available when the response to first-line therapy fails, and again it is not appropriate to define a "standard a care" to recommend for all patients. At diagnosis, the principal factors driving therapeutic decision making are patient age, fitness, and priorities. Additional important considerations are documentation of histological transformation (which would prompt strategies used in diffuse large B-cell lymphoma), the patient's tolerance of first-line therapy, and the depth and duration of previous response. Subsequent therapies include the ongoing observation of the asymptomatic patient with limited tumor bulk, the re-administration of single-agent rituximab, the use of multiple cytotoxic agents (alone, in combination, or with rituximab), as well as the use of autologous or allogeneic transplantation for remission consolidation. Multiple studies have been reported supporting the use of anthracycline when not incorporated in front line [67], fludarabine, [68–70] and bendamustine [65, 71–73], this last option being commonly used in recent years because of its favorable efficacy/toxicity ratio, including for patients failing previous rituximab-containing treatments. The few randomized studies available usually assessed the addition of another drug to a regimen commonly used, rather than comparing different strategies. For example, several studies have demonstrated the benefit of adding rituximab maintenance in patients responding to salvage therapy [67, 68] or the use of rituximab in the context of autologous transplant [74]. A recent trial indicated that bortezomib had little value when combined with rituximab [75].

The potential benefit of autologous stem cell transplantation as consolidation of second-line treatment is not firmly established [76]. Single center studies [77, 78] and retrospective cohorts [79] showed the efficacy of this approach, and one single randomized study, although underpowered, indicated a significant benefit in terms of event-free and overall survival [80]. Retrospective analyses of patients previously registered in first-line trials have also suggested a benefit of autologous transplant, even the rituximab era [81, 82]. Finally, in the European Bone Marrow Transplant study of rituximab for induction and maintenance in the context of autologous transplant [74], the median PFS after transplant exceeded 5 years in the rituximab-containing arms, a remarkable result generally not achieved with other strategies. For these reasons, many clinicians consider autologous transplant consolidation as a treatment of choice in eligible patients relapsing or progressing after first-line immunochemotherapy, particularly when the

disease tempo is rapid with an interval between first treatment and failure of only a few years. Allogeneic transplant approaches likewise lack prospective study, but registry data suggests allogeneic transplantation can be an effective therapy that may provide a plateau in progression-free survival curves [83]. Comparisons between autologous and allogeneic transplant from registry data demonstrate the predictably higher mortality (from infection and GVHD) over the first 5-year period with the latter approach, but a lower relapse rate thereafter [84]. Emerging single institution data also supports the role of reduced intensity conditioning in reducing this prohibitive mortality [85]. With the lure of a cure, allogeneic transplantation is an option that should be reserved only for very selected fit young patients with relapsed/resistant disease, usually after failure of autologous transplant.

Future Developments

New Agents in Follicular Lymphoma

There is encouraging preliminary phase II data on second-generation monoclonal antibodies, notably obinutuzumab (GA101) a fully humanized anti-CD20 [86], and on an immunomodulatory approach with combined rituximab and lenalidomide [87]. Other agents such as monoclonal antibodies directed against different antigens, drugs modulating apoptosis, or intracellular signaling are also worth investigating in FL patients, as long as they have a reasonable safety profile, given the prolonged life expectancy of these patients [88].

Current Risk-Adapted Therapeutic Strategies in Fl and Challenges for the Next Years

Since biologically derived prognostic factors are not yet available to identify patients with specific risks or deserving targeted therapeutic options, clinical criteria remain relevant for deciding on when to commence treatment for patients with FL (Table 9.2). These criteria, along with FLIPI 1/2 and patient individual priorities, assist clinicians in determining the appropriate first-line therapy for each patient. As an incurable disease, it remains important to consider the side effects and long-term risks of both first-line and subsequent therapies. Nonetheless, the development of highly efficient and tolerable strategies based around monoclonal antibody therapy has revised our therapeutic standards in FL. Combination immunochemotherapy strategies followed by maintenance rituximab aimed at durable complete remissions will likely lead to long-term survival improvement.

Finally, acknowledging the limitations of conventional CT response assessment, we need to better define remission status and our therapeutic goals. If prospective study of standardized post-therapy PET-CT response criteria confirms this imaging modality is highly predictive of both PFS and OS, then, as with other lymphomas, these criteria provide a meaningful clinical endpoint for study of response adapted strategies. The challenge will be in choosing from the plethora of promising consolidative therapies, beyond the now well-established program of maintenance rituximab to the study of alternative chemo- and antibody therapies, radioimmunoconjugates, and immunomodulatory agents, with or without autologous transplantation.

The near future promises to bring new standards of first-line therapy for follicular lymphoma. However, these are not likely to remain standard for long as, with each new research development prolonging survival, we may move closer to a cure.

References

1. Freedman AS. Biology and management of histologic transformation of indolent lymphoma. Hematol Am Soc Hematol Educ Program. 2005;2005:314–20.
2. Yuen AR, Kamel OW, Halpern J, Horning SJ. Long-term survival after histologic transformation of low-grade follicular lymphoma. J Clin Oncol. 1995;13(7):1726–33.
3. Solal-Celigny P, Roy P, Colombat P, et al. Follicular lymphoma international prognostic index. Blood. 2004; 104(5):1258–65.
4. Buske C, Hoster E, Dreyling M, Hasford J, Unterhalt M, Hiddemann W. The Follicular Lymphoma International Prognostic Index (FLIPI) separates high-risk from intermediate- or low-risk patients with advanced-stage

follicular lymphoma treated front-line with rituximab and the combination of cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP) with respect to treatment outcome. Blood. 2006;108(5):1504–8.
5. Marcus R, Imrie K, Solal-Celigny P, et al. Phase III study of R-CVP compared with cyclophosphamide, vincristine, and prednisone alone in patients with previously untreated advanced follicular lymphoma. J Clin Oncol. 2008;26(28):4579–86.
6. Hochster HS, Weller E, Gascoyne RD, et al. Maintenance rituximab after CVP results in superior clinical outcome in advanced follicular lymphoma (FL): results of the E1496 phase III trial from the Eastern Cooperative Oncology Group and the Cancer and Leukemia Group B. Blood. 2005;106:106a.
7. Marcus R, Solal-Celigny P, Imrie K, et al. Rituximab plus CVP improves survival in previously untreated patients with advanced follicular non-Hodgkin's lymphoma. Blood. 2006;108:481a.
8. Herold M, Haas A, Srock S, et al. Rituximab added to first-line mitoxantrone, chlorambucil, and prednisolone chemotherapy followed by interferon maintenance prolongs survival in patients with advanced follicular lymphoma: an East German Study Group Hematology and Oncology Study. J Clin Oncol. 2007;25(15):1986–92.
9. Solal-Celigny P, Salles G, Brousse N, et al. Single four-dose rituximab treatment for low-tumour burden follicular lymphoma: survival analyses with a follow-up of at least 5 years. Blood. 2004;104:169a.
10. Federico M, Bellei M, Marcheselli L, et al. Follicular Lymphoma International Prognostic Index 2: a new prognostic index for follicular lymphoma developed by the international follicular lymphoma prognostic factor project. J Clin Oncol. 2009;27(27):4555–62.
11. Terol MJ, Teruel AI, Amat P, Elaluf D, Tormo M, Solano C. The FLIPI2 score predicts progression free survival (PFS) and overall survival (OS) in an independent series of follicular lymphoma: a single institution experience. ASH Annu Meet Abstr. 2010;116(21):3128.
12. Numata A, Fujimaki K, Tomita N, et al. Retrospective study of the utility of Follicular Lymphoma International Prognostic Index (FLIPI) and FLIPI2 in patients with follicular lymphoma uniformly treated with rituximab, cyclophosphamide, doxorubicin, vincristin, and prednisone. ASH Annu Meet Abstr. 2010;116(21):3100.
13. Dave SS, Wright G, Tan B, et al. Prediction of survival in follicular lymphoma based on molecular features of tumor-infiltrating immune cells. N Engl J Med. 2004;351(21):2159–69.
14. Glas AM, Knoops L, Delahaye L, et al. Gene-expression and immunohistochemical study of specific T-cell subsets and accessory cell types in the transformation and prognosis of follicular lymphoma. J Clin Oncol. 2007;25(4):390–8.
15. Canioni D, Salles G, Mounier N, et al. High numbers of tumor-associated macrophages have an adverse prognostic value that can be circumvented by rituximab in patients with follicular lymphoma enrolled onto the GELA-GOELAMS FL-2000 trial. J Clin Oncol. 2008;26(3):440–6.
16. de Jong D, Koster A, Hagenbeek A, et al. Impact of the tumor microenvironment on prognosis in follicular lymphoma is dependent on specific treatment protocols. Haematologica. 2009;94(1):70–7 [see comment].
17. de Jong D, Rosenwald A, Chhanabhai M, et al. Immunohistochemical prognostic markers in diffuse large B-cell lymphoma: validation of tissue microarray as a prerequisite for broad clinical applications—a study from the Lunenburg Lymphoma Biomarker Consortium. J Clin Oncol. 2007;25(7):805–12.
18. Taskinen M, Karjalainen-Lindsberg ML, Nyman H, et al. A high tumor-associated macrophage content predicts favorable outcome in follicular lymphoma patients treated with rituximab and cyclophosphamide-doxorubicin-vincristine-prednisone. Clin Cancer Res. 2007;13(19):5784–9.
19. Tsang RW, Gospodarowicz MK. Radiation therapy for localized low-grade non-Hodgkin's lymphomas. Hematol Oncol. 2005;23(1):10–7.
20. Mauch P. Follicular non-Hodgkin's lymphoma: the role of radiation therapy. Ann Hematol. 2001;80 Suppl 3Suppl 3:B63–5.
21. Plancarte F, Lopez-Guillermo A, Arenillas L, et al. Follicular lymphoma in early stages: high risk of relapse and usefulness of the Follicular Lymphoma International Prognostic Index to predict the outcome of patients. Eur J Haematol. 2006;76(1):58–63.
22. Pugh T, Ballonoff A, Newman F, Rabinovitch R. Improved survival in patients with early stage low-grade follicular lymphoma treated with radiation: a surveillance, epidemiology, and end results database analysis. Cancer. 2010;116(16):3843–51.
23. Zelenetz AD, Advani RH, Buadi F, et al. Non-Hodgkin's lymphoma. Clinical practice guidelines in oncology. J Natl Compr Cancer Netw. 2006;4(3):258–310.
24. Hiddemann W, Dreyling M, Stahel RA, Force EGT. Minimum clinical recommendations for diagnosis, treatment and follow-up of newly diagnosed follicular lymphoma. Ann Oncol. 2005;16 Suppl 1Suppl 1:i56–7.
25. Advani R, Rosenberg SA, Horning SJ. Stage I and II follicular non-Hodgkin's lymphoma: long-term follow-up of no initial therapy. J Clin Oncol. 2004;22(8):1454–9.
26. Seymour JF, Pro B, Fuller LM, et al. Long-term follow-up of a prospective study of combined modality therapy for stage I-II indolent non-Hodgkin's lymphoma. J Clin Oncol. 2003;21(11):2115–22.
27. Friedberg JW, Taylor MD, Cerhan JR, et al. Follicular lymphoma in the United States: first report of the national LymphoCare study. J Clin Oncol. 2009;27(8):1202–8.
28. Horning SJ, Rosenberg SA. The natural history of initially untreated low-grade non-Hodgkin's lymphomas. N Engl J Med. 1984;311(23):1471–5.
29. Montoto S, Davies AJ, Matthews J, et al. Risk and clinical implications of transformation of follicular lymphoma to diffuse large B-cell lymphoma. J Clin Oncol. 2007;25(17):2426–33.

30. Ardeshna KM, Smith P, Norton A, et al. Long-term effect of a watch and wait policy versus immediate systemic treatment for asymptomatic advanced-stage non-Hodgkin lymphoma: a randomised controlled trial. Lancet. 2003;362:516–22.
31. Brice P, Bastion Y, Lepage E, et al. Comparison in low-tumor-burden follicular lymphomas between an initial no-treatment policy, prednimustine, or interferon alfa: a randomized study from the Groupe d'Etude des Lymphomes Folliculaires. Groupe d'Etude des Lymphomes de l'Adulte. J Clin Oncol. 1997;15(3): 1110–7.
32. Al-Tourah AJ, Gill KK, Chhanabhai M, et al. Population-based analysis of incidence and outcome of transformed non-Hodgkin's lymphoma. J Clin Oncol. 2008;26(32):5165–9.
33. Ardeshna KM, Qian W, Smith P, et al. An intergroup randomised trial of rituximab versus a watch and wait strategy in patients with stage II, III, IV, asymptomatic, non-bulky follicular lymphoma (grades 1, 2 and 3a). A preliminary analysis. ASH Annu Meet Abstr. 2010;116(21):6.
34. Fisher RI, LeBlanc M, Press OW, Maloney DG, Unger JM, Miller TP. New treatment options have changed the survival of patients with follicular lymphoma. J Clin Oncol. 2005;23(33):8447–52.
35. Sacchi S, Pozzi S, Marcheselli L, et al. Introduction of rituximab in front-line and salvage therapies has improved outcome of advanced-stage follicular lymphoma patients. Cancer. 2007;109(10):2077–82.
36. Swenson WT, Wooldridge JE, Lynch CF, Forman-Hoffman VL, Chrischilles E, Link BK. Improved survival of follicular lymphoma patients in the United States. J Clin Oncol. 2005;23(22):5019–26.
37. Rohatiner AZ, Gregory WM, Peterson B, et al. Meta-analysis to evaluate the role of interferon in follicular lymphoma. J Clin Oncol. 2005;23(10):2215–23.
38. Lister TA. Improved survival for patients with follicular lymphoma. J Clin Oncol. 2005;23(22):4830–1.
39. Peterson BA, Petroni GR, Frizzera G, et al. Prolonged single-agent versus combination chemotherapy in indolent follicular lymphomas: a study of the cancer and leukemia group B. J Clin Oncol. 2003;21(1):5–15.
40. Lenz G, Dreyling M, Schiegnitz E, Forstpointner R, Wandt H, et al. Myeloablative radiochemotherapy followed by autologous stem cell transplantation in first remission prolongs progression-free survival in follicular lymphoma: results of a prospective, randomized trial of the German Low-Grade Lymphoma Study Group. Blood. 2004;104(9):2667–74.
41. Sebban C, Mounier N, Brousse N, et al. Standard chemotherapy with interferon compared with CHOP followed by high-dose therapy with autologous stem cell transplantation in untreated patients with advanced follicular lymphoma: the GELF-94 randomized study from the Groupe d'Etude des Lymphomes de l'Adulte (GELA). Blood. 2006;108(8):2540–4.
42. Deconinck E, Foussard C, Milpied N, et al. High-dose therapy followed by autologous purged stem-cell transplantation and doxorubicin-based chemotherapy in patients with advanced follicular lymphoma: a randomized multicenter study by GOELAMS. Blood. 2005;105(10):3817–23.
43. Bachy E, Brice P, Delarue R, Brousse N, Haioun C, Le Gouill S, Delmer A, Bordessoulle D, Tilly H, Corront B, Allard C, Foussard C, Bosly A, Coiffier B, Gisselbrecht C, Solal-Celigny P, Salles G. Long term follow-up of 536 newly diagnosed follicular lymphoma patients in the pre-Rrituximab era: effect of pre-treatment prognostic variables and response quality on survival A study from the Groupe d'Etude des Lymphomes de l'Adulte (GELA). J Clin Oncol. 2009 (in press).
44. Trotman J, Fournier M, Lamy T, et al. Positron emission tomography-computed tomography (PET-CT) after induction therapy is highly predictive of patient outcome in follicular lymphoma: analysis of PET-CT in a subset of PRIMA trial participants. J Clin Oncol. 2011;29(23):3194–200.
45. Colombat P, Salles G, Brousse N, et al. Rituximab (anti-CD20 monoclonal antibody) as single first-line therapy for patients with follicular lymphoma with a low tumor burden: clinical and molecular evaluation. Blood. 2001;97(1):101–6.
46. Ghielmini M, Schmitz SF, Cogliatti SB, et al. Prolonged treatment with rituximab in patients with follicular lymphoma significantly increases event-free survival and response duration compared with the standard weekly x 4 schedule. Blood. 2004;103(12): 4416–23.
47. Hainsworth JD, Burris 3rd HA, Morrissey LH, et al. Rituximab monoclonal antibody as initial systemic therapy for patients with low-grade non-Hodgkin lymphoma. Blood. 2000;95(10):3052–6.
48. Witzig TE, Vukov AM, Habermann TM, et al. Rituximab therapy for patients with newly diagnosed, advanced-stage, follicular grade I non-Hodgkin's lymphoma: a phase II trial in the North Central Cancer Treatment Group. J Clin Oncol. 2005;23(6):1103–8.
49. Marcus R, Imrie K, Belch A, et al. CVP chemotherapy plus rituximab compared with CVP as first-line treatment for advanced follicular lymphoma. Blood. 2005;105:1417–23.
50. Hiddemann W, Kneba M, Dreyling M, et al. Frontline therapy with rituximab added to the combination of cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP) significantly improves the outcome for patients with advanced-stage follicular lymphoma compared with therapy with CHOP alone: results of a prospective randomized study of the German Low-Grade Lymphoma Study Group. Blood. 2005;106(12):3725–32.
51. Salles G, Mounier N, de Guibert S, et al. Rituximab combined with chemotherapy and interferon in follicular lymphoma patients: results of the GELA-GOELAMS FL2000 study. Blood. 2008;112(13): 4824–31.
52. Buske C, Hoster E, Dreyling M, et al. Rituximab in combination with CHOP in patients with follicular lymphoma: analysis of treatment outcome of 552

patients treated in a randomized trial of the German Low Grade Lymphoma Study Group (GLSG) after a follow up of 58 months. ASH Annu Meet Abstr. 2008;112(11):2599.
53. Rambaldi A, Lazzari M, Manzoni C, et al. Monitoring of minimal residual disease after CHOP and rituximab in previously untreated patients with follicular lymphoma. Blood. 2002;99(3):856–62.
54. Zinzani PL, Pulsoni A, Perrotti A, et al. Fludarabine plus mitoxantrone with and without rituximab versus CHOP with and without rituximab as front-line treatment for patients with follicular lymphoma. J Clin Oncol. 2004;22(13):2654–61.
55. Hochster H, Weller E, Gascoyne RD, Habermann TM, Gordon LI, Ryan T, et al. Maintenance rituximab after cyclophosphamide, vincristine, and prednisone prolongs progression-free survival in advanced indolent lymphoma: results of the randomized phase III ECOG1496 Study. J Clin Oncol. 2009;27(10):1607–14.
56. Federico M, Luminari S, Dondi A, et al. R-CVP vs R-CHOP vs R-FM for the initial treatment of patients with advanced stage follicular lymphoma. Preliminary results of FOLL05 ILL trial. Ann Oncol. 2011;22 Suppl 4Suppl 4:128.
57. Morschhauser F, Seymour JF, Feugier P, et al. Impact of induction chemotherapy regimen on response, safety and outcome in the PRIMA study. Ann Oncol. 2011;22 Suppl 4Suppl 4:89.
58. Salles G, Seymour J, Offner F, López-Guillermo A, Belada D, Xerri L. Rituximab maintenance for 2 years in patients with high tumour burden follicular lymphoma responding to rituximab plus chemotherapy (PRIMA): a phase 3, randomised controlled trial. Lancet. 2011;377:42–51.
59. Kaminski MS, Tuck M, Estes J, et al. 131I-tositumomab therapy as initial treatment for follicular lymphoma. N Engl J Med. 2005;352(5):441–9.
60. Press OW, Unger JM, Braziel RM, et al. Phase II trial of CHOP chemotherapy followed by tositumomab/iodine I-131 tositumomab for previously untreated follicular non-Hodgkin's lymphoma: five-year follow-up of Southwest Oncology Group Protocol S9911. J Clin Oncol. 2006;24(25):4143–9.
61. Leonard JP, Coleman M, Kostakoglu L, et al. Abbreviated chemotherapy with fludarabine followed by tositumomab and iodine I 131 tositumomab for untreated follicular lymphoma. J Clin Oncol. 2005;23(24):5696–704.
62. Shipley DL, Greco FA, Spigel DR. Rituximab with short duration chemotherapy followed by 90 Y ibritumomab tiuxetan as first-line treatment for patients with follicular lymphoma: update of a Minnie Pearl Cancer Research Network phase II trial. Proc Am Soc Clin Oncol. 2005;23:579s.
63. Morschhauser F, Radford J, Van Hoof A, et al. Phase III trial of consolidation therapy with yttrium-90-ibritumomab tiuxetan compared with no additional therapy after first remission in advanced follicular lymphoma. J Clin Oncol. 2008;26(32):5156–64.
64. Friedberg JW. The emerging role of bendamustine in follicular lymphoma. Leuk Lymphoma. 2009;50(3):317–8.
65. Robinson KS, Williams ME, van der Jagt RH, et al. Phase II multicenter study of bendamustine plus rituximab in patients with relapsed indolent B-cell and mantle cell non-Hodgkin's lymphoma. J Clin Oncol. 2008;26:4473–9.
66. Rummel MJ, Niederle N, Maschmeyer G, et al. Bendamustine plus rituximab is superior in respect of progression free survival and CR rate when compared to CHOP plus rituximab as first-line treatment of patients with advanced follicular, indolent, and mantle cell lymphomas: final results of a randomized phase III study of the StiL (Study Group Indolent Lymphomas, Germany). ASH Annu Meet Abstr. 2009;114(22):405.
67. van Oers MH, Klasa R, Marcus RE, et al. Rituximab maintenance improves clinical outcome of relapsed/resistant follicular non-Hodgkin lymphoma in patients both with and without rituximab during induction: results of a prospective randomized phase 3 intergroup trial. Blood. 2006;108(10):3295–301.
68. Forstpointner R, Unterhalt M, Dreyling M, et al. Maintenance therapy with rituximab leads to a significant prolongation of response duration after salvage therapy with a combination of rituximab, fludarabine, cyclophosphamide, and mitoxantrone (R-FCM) in patients with recurring and refractory follicular and mantle cell lymphomas: results of a prospective randomized study of the German Low Grade Lymphoma Study Group (GLSG). Blood. 2006;108(13):4003–8.
69. Sacchi S, Pozzi S, Marcheselli R, et al. Rituximab in combination with fludarabine and cyclophosphamide in the treatment of patients with recurrent follicular lymphoma. Cancer. 2007;110(1):121–8.
70. Morschhauser F, Mounier N, Sebban C, et al. Efficacy and safety of the combination of rituximab, fludarabine, and mitoxantrone for rituximab-naive, recurrent/refractory follicular non-Hodgkin lymphoma with high tumor burden: a multicenter phase 2 trial by the Groupe d'Etude des Lymphomes de l'Adulte (GELA) and Groupe Ouest Est des Leucemies et Autres Maladies du Sang (GOELAMS). Cancer. 2010;116(18):4299–308.
71. Friedberg JW, Cohen P, Chen L, et al. Bendamustine in patients with rituximab-refractory indolent and transformed non-Hodgkin's lymphoma: results from a phase II multicenter, single-agent study. J Clin Oncol. 2008;26(2):204–10.
72. Kahl BS, Bartlett NL, Leonard JP, et al. Bendamustine is effective therapy in patients with rituximab-refractory, indolent B-cell non-Hodgkin lymphoma: results from a Multicenter Study. Cancer. 2010;116(1):106–14.
73. Rummel MJ, Al-Batran SE, Kim SZ, et al. Bendamustine plus rituximab is effective and has a favorable toxicity profile in the treatment of mantle cell and low-grade non-Hodgkin's lymphoma. J Clin Oncol. 2005;23(15):3383–9.
74. Pettengell R, Schmitz N, Gisselbrecht C, et al. Randomized study of rituximab in patients with relapsed or resistant follicular lymphoma prior to

high-dose therapy as in vivo purging and to maintain remission following high-dose therapy. J Clin Oncol. 2010;28:15s(suppl; abstr 8005).
75. Coiffier B, Osmanov EA, Hong X, et al. Bortezomib plus rituximab versus rituximab alone in patients with relapsed, rituximab-naive or rituximab-sensitive, follicular lymphoma: a randomised phase 3 trial. Lancet Oncol. 2011;12(8):773–84.
76. Bachy E, Salles G. Marrow-ablative treatment and autologous stem cell transplantation in follicular NHL. Best Pract Res Clin Haematol. 2011;24(2):257–70. Netherlands.
77. Bastion Y, Brice P, Haioun C, et al. Intensive therapy with peripheral blood progenitor cell transplantation in 60 patients with poor-prognosis follicular lymphoma. Blood. 1995;86(8):3257–62.
78. Rohatiner AZ, Nadler L, Davies AJ, et al. Myeloablative therapy with autologous bone marrow transplantation for follicular lymphoma at the time of second or subsequent remission: long-term follow-up. J Clin Oncol. 2007;25(18):2554–9.
79. Montoto S, Canals C, Rohatiner AZ, et al. Long-term follow-up of high-dose treatment with autologous haematopoietic progenitor cell support in 693 patients with follicular lymphoma: an EBMT registry study. Leukemia. 2007;21(11):2324–31. England.
80. Schouten HC, Qian W, Kvaloy S, et al. High-dose therapy improves progression-free survival and survival in relapsed follicular non-Hodgkin's lymphoma: results from the randomized European CUP trial. J Clin Oncol. 2003;21(21):3918–27.
81. Sebban C, Brice P, Delarue R, et al. Impact of rituximab and/or high-dose therapy with autotransplant at time of relapse in patients with follicular lymphoma: a GELA study. J Clin Oncol. 2008;26(21):3614–20.
82. Le Gouill S, De Guibert S, Planche L, et al. Impact of the use of autologous stem cell transplantation at first relapse both in naive and previously rituximab exposed follicular lymphoma patients treated in the GELA/GOELAMS FL2000 study. Haematologica. 2011;96(8):1128–35.
83. van Besien K, Loberiza Jr FR, Bajorunaite R, et al. Comparison of autologous and allogeneic hematopoietic stem cell transplantation for follicular lymphoma. Blood. 2003;102(10):3521–9.
84. van Besien K. Allogeneic stem cell transplantation in follicular lymphoma: recent progress and controversy. Hematol Am Soc Hematol Educ Program. 2009; 2009:610–8. United States.
85. Khouri IF, McLaughlin P, Saliba RM, et al. Eight-year experience with allogeneic stem cell transplantation for relapsed follicular lymphoma after nonmyeloablative conditioning with fludarabine, cyclophosphamide, and rituximab. Blood. 2008;111(12):5530–6.
86. Salles GA, Morschhauser F, Thieblemont C, et al. Promising efficacy with the new anti-CD20 antibody GA101 in heavily pre-treated NHL patients—updated results with encouraging progression free survival (PFS) data from a phase II study in patients with relapsed/refractory indolent NHL (iNHL). ASH Annu Meet Abstr. 2010;116(21):2868.
87. Samaniego F, Hagemeister F, Mclaughlin P, et al. High response rates with lenalidomide plus rituximab for untreated indolent B-cell non-Hodgkin lymphoma, including those meeting GELF criteria. J Clin Oncol. 2011;29(suppl; abstr 8030).
88. Cheson BD. New agents in follicular lymphoma. Best Pract Res Clin Haematol. 2011;24(2):305–12, Netherlands.

Mantle Cell Lymphoma

10

Christian Schmidt and Martin H. Dreyling

Abstract

Mantle cell lymphoma is characterized clinically by an aggressive clinical course and is relatively resistant to conventional chemotherapies. When in its advanced stages, currently available immunochemotherapy regimens remain noncurative despite high initial response rates. In contrast, consolidating high-dose therapy with autologous stem cell retransfusion significantly extends progression-free survival of young patients. Currently, allogenic bone marrow transplantation represents the only therapy with the potential for a curative approach, although associated with a high rate of complications. New concepts of therapy are urgently warranted, including new molecular approaches, such as bortezomib, lenalidomide, and temsirolimus.

Keywords

Antineoplastic agents/therapeutic use • Bone marrow transplantation • Cyclin D1 • Drug resistance, neoplasm/drug effects • Humans • Lymphoma, mantle cell/pathology/therapy • Neoplasm staging/methods • Stem cell transplantation • Transplantation, autologous • Transplantation, allogeneic

Introduction

Mantle cell lymphoma represents a distinct histological subtype of malignant B-cell malignancies and accounts for 5–10 % of all lymphoid malignancies corresponding to an incidence of 2–3 new cases per 100.000 inhabitants. Mantle cell lymphoma is cytomorphologically marked by small- to medium-sized lymphoid cells with irregularly notched nuclei. The former Kiel classification described this entity in 1977 as centrocytical lymphoma. In contrast, it was subsumed under different histological entities. The term “mantle cell

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lymphoma" was first proposed by Banks et al. [1], based on the recognition of characteristic morphology, phenotype, and translocation t(11;14) which indicates the decent of mantle zone B cells. After introduction of the REAL and the current WHO classification, mantle cell lymphoma (short MCL) was recognized as a distinct lymphoma entity [2, 3]. The male to female ratio is 2–3:1, and the median age at first clinical manifestation is 65 years.

Most patients are diagnosed at an advanced stage of disease, often with extranodal involvement. The disease is clinically characterized by aggressive course and only short-term remissions after conventional chemotherapy. Except from allogeneic stem cell transplant, curative therapy is currently not available. Median survival is about 5 years. Immunochemotherapy with myeloablative consolidation and autologous stem cell transplantation in younger patients, an increasing number of effective therapies for sequential application, novel targeted agents, and maintenance therapy strategies are improving response durations and overall survival, so median survival in historic series is recently improving [4].

Etiology and Pathogenesis

Etiology and molecular pathogenesis that result in the clinical manifestation of mantle cell lymphoma are still an object of current research. Among first-degree relatives of MCL patients, there is an increased risk to develop other lymphoid malignancies, although familiar MCL is quite rare. Environmental toxins, e.g., longtime exposure to herbicides, are discussed as risk factors, although population-based data are limited so far.

Nevertheless, increasing insights into the underlying molecular pathogenesis could be achieved within the last decade [5]. On the genomic level, the chromosomal translocation t(11;14) represents the hallmark of MCL and can be detected in the vast majority of MCL cases [6, 7]. The resulting overexpression of cyclin D1, which is generally not expressed in B cells, has an important function in cell-cycle regulation at the G1/S-phase. It forms a complex with cyclin-dependent kinases 4 (CDK4) and 6 (CDK6), leading to an increasing level of such cyclin D1/CDK complexes. These complexes phosphorylate the retinoblastoma protein (Rb) and thus accelerate cell-cycle progression via promoting transition from G1- to S-phase. Alternative cell-cycle regulator $p27^{kip1}$ is simultaneously segregated from cyclin E/CDK2 complexes, thereby activating these complexes and additionally promoting S-phase entry. The level of cyclin D1 expression is directly associated with the proliferation rate of MCL cells and thus with the clinical course of this disease. Other genetic alterations have been reported in MCL cells. In a significant fraction of cases, reduced levels of CDK4 and -6-inhibitors (e.g., $p16I^{NK4a}$) could be detected. About 20 % of MCL display homogenous deletions of chromosomal band 9p21 which leads to deactivation of $p16I^{NK4a}$, which normally maintains the dephosphorylated, antiproliferative state of the Rb protein. Deactivation of $p16^{INK4a}$ may cooperate with overexpression of cyclin D1 and again augment cell-cycle progression. Additionally $p16^{ARF}$, the alternative reading frame gene of the same genomic locus which is involved in the DNA damage response via mdm2 and p53, is simultaneously deleted. Cases with 9p-deletions often show blastoid histology and a more aggressive clinical course.

In up to 75 % of MCL, the ataxia telangiectasia mutated (ATM) gene on chromosomal band 11p22–23 is mutated. ATM encodes a kinase that belongs to the PI-3 kinase-related superfamily and has an important role in p53-mediated response to DNA damage. Recent proteome analysis confirmed the central role of p53-triggered protein interactions in MCL [8]. In summary, the molecular pathogenesis of MCL is characterized by a dysregulation of cell-cycle control mechanism and disrupted response to DNA damage [7, 9] (Fig. 10.1 [112]).

Histopathology

Histologically, MCL presents a wide variation of morphological appearance.

Lymph node histology is usually characterized by a diffuse infiltration of monomorphic lymphoid cells, although the growth pattern can be nodal

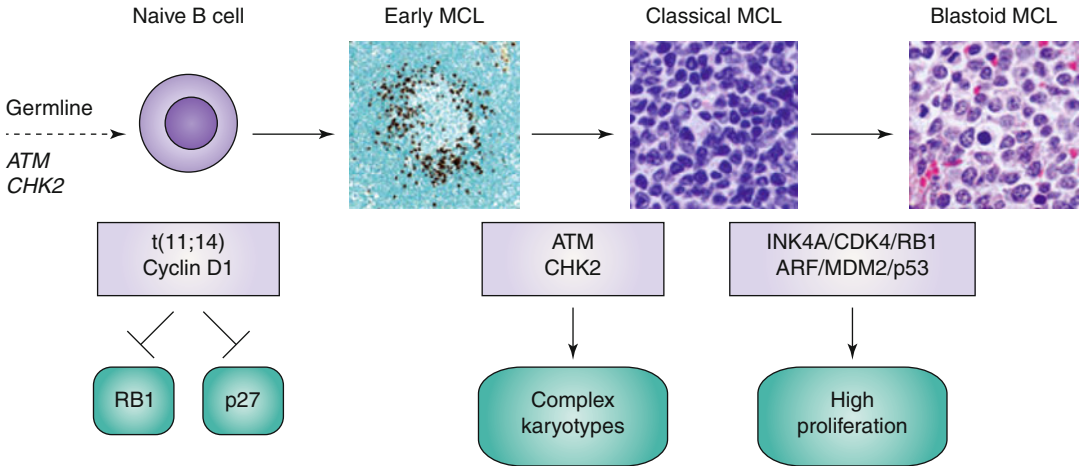

Fig. 10.1 MCL variants (From Jares et al. [119]. Used with permission)

or imitating a mantle zone pattern, with affected cells found around residual reactive germinal centers. Cells are small to medium sized with irregularly notched nuclei. Cytomorphologically, MCL can be differentiated between a classical versus a blastoid variant [3]. Mixtures of cell types (classical plus pleomorphic type) or transitions were identified, but more detailed classification was not of additional prognostic value [10, 11].

Immunophenotypically, MCL cells express IgM and often IgD and have a mature B-cell marker profile (CD 10–, CD19+, CD20+, CD22+, CD43+, CD79a+) with coexpression of CD5, CD43, and FMC7, although CD5 negativity is reported in up to 20 % in some series. In contrast to chronic lymphocytic leukemia (CLL), MCL cells do usually not express CD23. Additionally, MCL cells are negative for the germinal cell marker bcl-6 and CD10. MCL is more frequently associated with lambda rather than kappa light chain expression, which differs from other B-cell lymphoma entities. Proliferation marker expression is variable with ki67 values of 10–20 % in classical MCL and ≥ 40 % in blastoid variants.

The typical cyclin D1 overexpression can be detected either by immunohistochemistry or by detection of the underlying translocation t(11,14)(q13;q32) via fluorescence in situ hybridization (FISH), with most of the breakpoints occurring in the major translocation cluster. However, conventional genomic PCR is able to detect the translocation in only 30 % of cases due to the wide genomic range of breakpoints [12]. Recently, few cyclin D1-negative MCL cases were identified which show a similar gene expression and clinical course and are frequently driven by alternative cyclin D2 or D3 overexpression.

Clinical Presentation, Staging, and Prognostic Factors

Clinical Presentation

Generally, MCL is characterized by a rapidly progressing clinical course. The majority of patients are diagnosed in advanced clinical stages (Ann Arbor III–IV) with generalized lymphadenopathy. Extranodal manifestation emerge in up to 90 % of all cases, with bone marrow involvement most frequent (60–81 %), followed by liver (25 %) and GI tract manifestations (20–60 %); CNS manifestation is relatively frequent in relapsed disease (4–20 %) [13].

Recommendation for Diagnostic and Staging Procedures

The histological confirmation of diagnosis is essential. Lymph node biopsy is strongly recommended. In cases with only retroperitoneal lymph

node manifestations, diagnosis can be confirmed by CT-guided punch biopsy. Fine needle aspiration is not sufficient because of the restricted value of cytomorphology only, as the material often does not provide the diagnostic accuracy as immunohistochemistry. Bone marrow biopsy is mandatory and should be complemented by flow cytometry to quantify the percentage of infiltration. Because of the exceptional role of correct histological diagnosis, second opinion by an experienced hematopathology expert may be recommended.

Standard lymphoma staging procedures in MCL patients include a medical history and physical examination as well as CT scans of the neck, chest, abdomen and pelvic region, and bone marrow biopsy. Although colonoscopy and upper GI tract endoscopy generally are not routinely required, it is recommended if patients present gastrointestinal symptoms or history of GI bleeding. In case of neurological symptoms, cranial imaging with MRT and diagnostic lumbar puncture is recommended. The role of PET/PET-CT scanning in the initial diagnosis is not generally recommended even though MCL is typically PET avid [14, 15]. Under certain circumstances, PET can be useful for identification of extranodal disease because of the lower sensitivity of CT scans for these sites. A positive posttreatment PET is related with inferior progression-free survival but does not necessarily guide to additional treatment because of a noncurative approach. The laboratory workup should include differential blood, standard serum chemistry analysis, including LDH as one of the major risk markers. ß[beta]2-microglobuline may also be determined. Although leukemic disease can be detected by more sensitive methods like flow cytometry and molecular assays in nearly all patients, manifest lymphocytosis can be found in about 25 % only.

Prognostic Factors

The clinical course of MCL is characterized by a continuous progression with median survival of about 3–5 years, but recent reports observed an increased overall survival of 5–6 years and a subset of about 15 % long-term survivors with a rather indolent clinical course even after only conventional immunochemotherapy treatment [16–18]. This emphasizes the biological and clinical heterogeneity of this disease. The insights in the molecular pathogenesis of MCL during the last decade elucidate the underlying mechanism of this variability and may potentially guide therapeutic approaches according to the individual patients risk profile.

Risk Factors Based on Morphology

Cytomorphologically, MCL can be differentiated into a classical versus a blastoid variant [3]. A blastoid cell type, either present at diagnosis or developing during disease progression, has an inferior median survival of 1–2 years compared to 4–5 years in classical MCL. Additionally, the histological growth pattern has a prognostic value. Nodular and especially mantle zone growth patterns correlate with a more indolent disease progression.

Phenotypic and Molecular Risk Factors

The most important biological parameter is the rate of cell proliferation. Rosenwald et al. made a quantitative measurement of tumor cell proliferation available allowing the definition of prognostic subgroups that differ in their median survival by more than 5 years [12]. The rate of ki-67 expression identified by immunohistochemical staining is an important prognostic factor and correlates inversely with the clinical course [19] and was verified in a large European clinicopathological study [10]. Significant differences in overall survival were shown for MCL patients treated with CHOP or R-CHOP stratified by fewer than 10, 10–29 %, and over 30 % of ki-67-positive cells, so the central prognostic role of cell proliferation and its superiority to other histomorphological criteria was also confirmed for rituximab-containing regimens [20] (Fig. 10.2 [20]). Additional biomarkers like p53 mutations, 13q14 deletion, or microRNA aberrations allow new pathogenetic insights and indicate new approaches of prognostic stratification and targeted therapy [21–23] (Fig. 10.1).

Prognostic Index

The international prognostic index (IPI) established in diffuse large cell is only of minor relevance in MCL. In a multivariate analysis of more

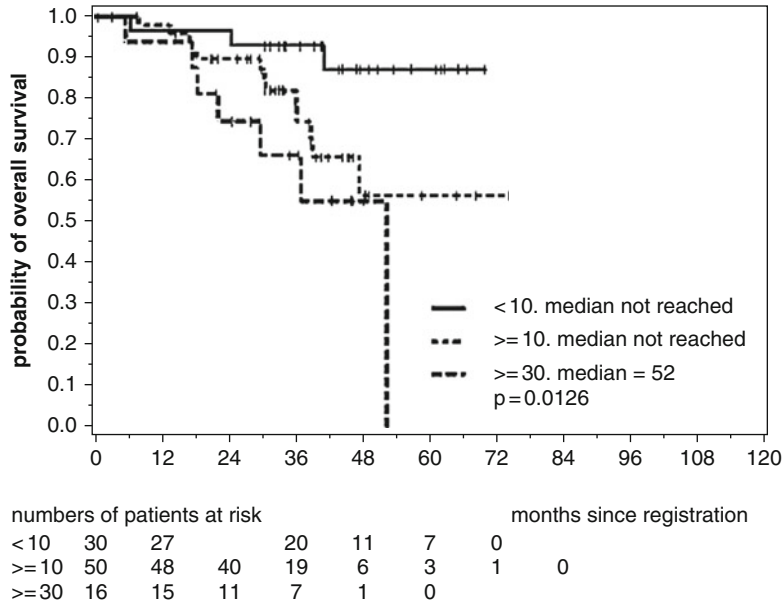

Fig. 10.2 Overall survival according to ki-67 (This research was originally published in *Blood*, Determann et al. [20]; © the American Society of Hematology)

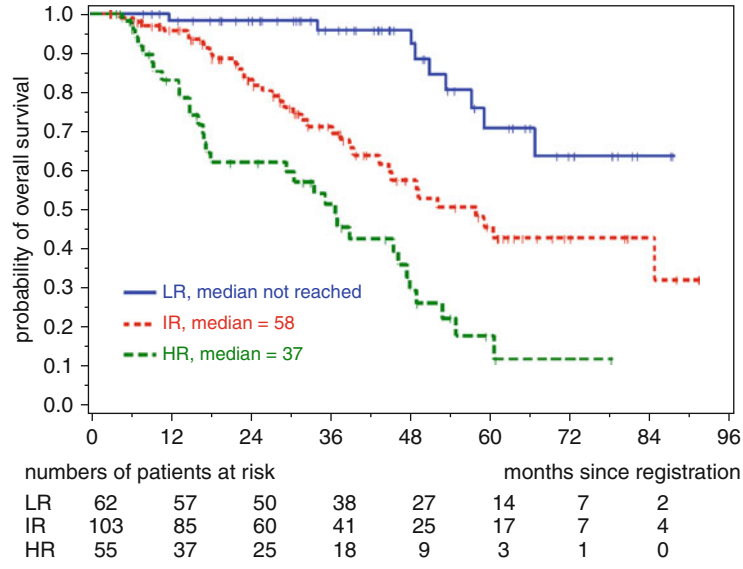

Fig. 10.3 Overall survival according to MIPI (This research was originally published in *Blood*, Determann et al. [20]; © the American Society of Hematology)

than 450 patients, age, ECOG (Eastern Cooperative Oncology Group) performance status, LDH, and leukocyte or lymphocyte count could be identified as independent prognostic factors, establishing an MCL-specific prognostic score (MIPI) [24]. Based on the calculated score, patients with advanced clinical stages (III, IV) can be separated into low-, intermediate-, and high-risk groups that directly correlate with overall survival following initial chemotherapy (Fig. 10.3 [20]). Additionally, in patients treated with combined immunochemotherapy and dose-intensive regimens including autologous stem cell transplant, MIPI could be also confirmed as a valid prognostic tool [25]. A further improvement of the MIPI could be achieved by the inclusion of the proliferation marker ki-67 (see above) to combine clinical and biological risk factors [24, 25].

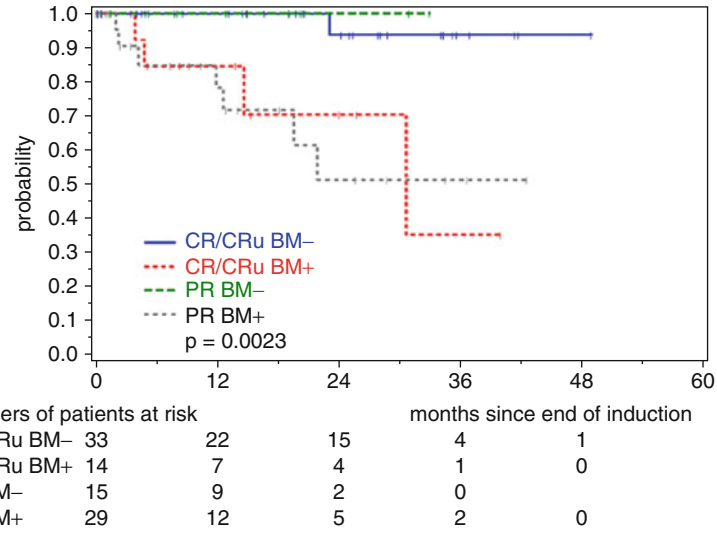

Fig. 10.4 MRD and remission duration after induction therapy (This research was originally published in *Blood*, Pott et al. [27]; © the American Society of Hematology)

Minimal Residual Disease

Although initial data after immunochemotherapy were contradictory [26], the achievement of molecular remission following induction therapy as reflected by MRD negativity was associated with prolonged clinical remission in two prospective randomized clinical trials in both younger patients (receiving intensive induction therapy including autologous stem cell transplantation) and elderly patients (receiving two lower intensity immunochemotherapy regimens) [27] (Fig. 10.4 [27]). Molecular marker as clonal igVH and t(11,14) breakpoints was determined in blood and bone marrow by sensitive PCR assays. Further standardization of this diagnostic tool will permit prospective assessment of molecular remission and enable MRD-based therapeutic intervention before a manifestation of clinical relapse.

Initial Therapy

Watch and Wait

The clinical course of MCL is usually aggressive with the worst longtime outcome of all B-cell lymphoma entities. A watch and wait strategy is therefore not generally recommended although conventional immunochemotherapy regimens are noncurative [28]. Nevertheless, a minor subgroup of 10–15 % displays a more indolent clinical course [18]. These asymptomatic patients with low tumor burden may be strictly monitored and treatment initiated immediately in case of progression or occurrence of symptoms. Gaining of more insights into the underlying biology of MCL, new prognostic tools could identify patients which benefit from a wait and see strategy [29]. Thus, a recent study of the Barcelona group suggested SOX11 negativity as a marker of this more indolent patient population [30]. However, so far, immunohistochemistry is not yet reliable enough for application in clinical routine.

Radiotherapy

Even though MCL is a radiation-sensitive disease [31], in the small number of patients which is diagnosed in early stages (I+II), extended- or involved-field radiotherapy achieves remissions with short duration only. In a retrospective analysis in 17 patients with limited-stage MCL, overall and progression-free survival were 71 and 68 %, respectively, after involved-field radiation either alone or in combination with conventional chemotherapy [32]. In contrast in advanced-stage disease, the benefit of radiotherapy is not proven and should be only considered in individual cases in which immunochemotherapy cannot be applied and local

tumor control is warranted as a palliative measure. In such a palliative setting, radiotherapy achieves an overall local response rate of 100 % with a median time to progression of 10 months [33].

Conventional Chemotherapy

In advanced stage, MCL conventional chemotherapy represents a noncurative approach. So far, the superiority of anthracycline-based regimens has not been confirmed in randomized trials. Overall response (89 % vs. 84 %), median progression-free, and overall survival were comparable for both CHOP protocol (cyclophosphamide, doxorubicin, vincristine, and prednisone) and alkylator-based combination (COP) [34]. In comparison with an anthrachinon-containing regimen (MCP), overall response rates after CHOP again were only slightly improved (87 % vs. 73 %, $p=0.08$) with a comparable time to treatment failure (21 months vs. 15 months, $p=0.14$) and overall survival rates (61 months vs. 48 months, $p=0.058$) [35]. In contrast, a retrospective study showed a significant longer overall survival after anthracycline-based regimens in patients with low- and low-intermediate-risk profile according to IPI [36]. Therefore, many clinicians favor CHOP-like induction therapy regimens at least in younger patients with MCL.

Purine analogs (e.g., fludarabine, cladribine) have been investigated in various studies. Fludarabine as single treatment achieved only moderate response rates in MCL (30–40 %) [37]. In contrast, combinations with either and idarubicin (FLU-ID) or cyclophosphamide achieved superior response rates (60 and 63 %, respectively) [38, 39]. Nevertheless, even in patients with molecular remissions, the median progression-free survival was 18.8 months only. However, hematological toxicity and stem cell toxicity have to be considered, especially for patients who are potential candidates for autologous stem cell harvest.

Promising data have been recently presented for the nitrogen mustard compound bendamustine, which is chemically related to the alkylating agents chlorambucil and cyclophosphamide [40]. Based on its molecular structure, it has been suggested that bendamustine may also act as a purine analog. A randomized phase III trial in patients with indolent non-Hodgkin's lymphoma and MCL demonstrated that bendamustine can efficaciously and safely replace cyclophosphamide in combination with vincristine and prednisone (BOP vs. COP) [41].

Rituximab and Other Monoclonal Antibodies

Rituximab is a chimerical IgG_1 anti-CD20 antibody that induces antibody-dependent cellular cytotoxicity and complement-dependent cytotoxicity. In addition, intracellular signaling may contribute to its efficacy; however, the exact in vivo function of CD20 is still unknown [42]. Despite high CD20 expression in MCL cells, rituximab monotherapy achieves only moderate response rates of 20–40 %. Data from the Swiss SAKK study group show an overall response rate of 27 % with a CR rate of 2 % in patients with newly diagnosed or relapsed MCL treated with a four weekly standard dose of rituximab. Median event-free survival was 6 months only [43]; thus, antibody monotherapy should be considered only in severe compromised patients with strict contraindications for systemic chemotherapy.

Other monoclonal antibodies targeting a variety of epitopes in addition to CD20 such as Cd22 [44, 45], CD74 [46], CD80 [47], and HLA-DR [48] are currently investigated in preclinical and clinical trials. Nevertheless, data for MCL are still rare. Modified antibodies (by bonding to either radioactive compounds or chemotoxins) promise better results in MCL. Inotuzumab ozogamicin (CMC-544), a calicheamicin-labeled murine anti-CD52 antibody, has shown activity in relapsed MCL. [49] Blinatumomab, a bi-specific anti-CD19/anti-CD3 antibody, has shown a high efficacy in an initial phase I/II trial [50, 51].

Immunochemotherapy

Rituximab monotherapy has only limited efficacy (see above), but based on an in vitro synergism, the effectiveness of rituximab in combination

Table 10.1 Immunochemotherapy in MCL

Author	*n*	Regimen	Disease status	OR (CR)	Median PFS/EFS	Median OS
Howard et al. [26]	40	R-CHOP	First line	96 % (48 %)	17 months	n.a.
Forstpointner et al. [56]	55	R-FCM	Relapse	62 % (33 %)	8 months	Median not reached
Herold et al. [55]	44	R-MCP	First line	71 % (32 %)	20 months	Median not reached
Lenz et al. [52], Hoster et al. [53]	123	R-CHOP	IFN vs. ASCT	94 % (34 %)	28 months (TTF)	59 % (5 years)
		CHOP	IFN vs. ASCT	75 % (7 %)	14 months (TTF)	46 % (5 years)
Rummel et al. [61]	48 45	R-CHOP BR	First line	95 % (35 %) 89 % (32 %)	22 months 33 months	Median not reached
Kluin-Nelemans et al. [58]	457	R-CHOP R-FC	First line	87 % 78 %	– –	64 months 40 months
Rule et al. [111]	370	FC R-FC	First line	79.8 % 90.6 %	16.1 months 30.6 months	37 months 45.7 months

with CHOP was explored in a phase III study. In the combination arm, 94 % of patients achieved a remission (CR 34 %) versus 75 % (CR 7 %) in the control arm with CHOP only ($p=0.0054$, CR $p=0.0002$) [52]. After longtime follow-up, progression-free survival was doubled in the combination arm (TTF; median 28 vs. 14 months, $p=0.0003$). Nevertheless, no differences were perceived in overall survival so far [53]. Other clinical trials confirmed the improved response rates after the addition of rituximab: in a combination with MCP (mitoxantrone, chlorambucil, and prednisone), response rates increased from 63 to 71 % (CR 32 % vs. 15 %), [54] but no significant improvement of progression-free survival was observed [55]. The combination of rituximab and FCM was also tested in relapsed mantle cell lymphoma in comparison to FCM chemotherapy only. The combined study arm R-FCM achieved significantly superior overall response rates (OR 58 % vs. 46 %, CR 29 % vs. 0 %) and even overall survival [56]. Recently a randomized study by the British study group confirmed significantly improved response rates and overall survival after R-FC (rituximab, fludarabine, cyclophosphamide) in comparison to chemotherapy only. This improvement of overall survival was also suggested by a meta-analysis [57], although this analysis showed considerable statistical heterogeneity. In a recent phase III study, two different regimens of immunochemotherapy were compared in 559 first-line patients. After R-FC regimen significantly lower, overall survival rates were observed in comparison to the R-CHOP regimen [58] (Table 10.1 [26, 52, 53, 55, 56, 58, 61, 111]).

In a phase II trial, bendamustine in combination with rituximab showed an overall response rate (OR) of 75 % with a complete response rate (CR) of 50 % in 16 patients with relapsed or refractory MCL [59]. Similarly, even in rituximab, pretreated patients with relapsed and refractory lymphoma including MCL, bendamustine in combination with mitoxantrone and rituximab was well tolerated and highly effective with an OR of 76 % and a CR of 38 % [60]. In a subset analysis of a phase III trial, rituximab in combination with bendamustine (BR) was compared to the standard R-CHOP regimen. BR displayed significantly less myelotoxicity with a 25 % reduction of infectious episodes, whereas response rates were only slightly lower, and progression-free survival was even prolonged in comparison with R-CHOP [61]. Based on these data, bendamustine may be considered especially in elderly patients not qualifying for dose-intensified regimen.

Dose-Intensified Regimen

Recent studies confirmed the benefit of dose-intensified approaches in younger patients even on overall survival (Fig. 10.5 [113]). Several studies show a high effectiveness of high-dose cytarabine in the therapy of mantle cell lymphomas (Table 10.2). Lefrere analyzed the sequential administration of CHOP and DHAP (dexamethasone, high-dose

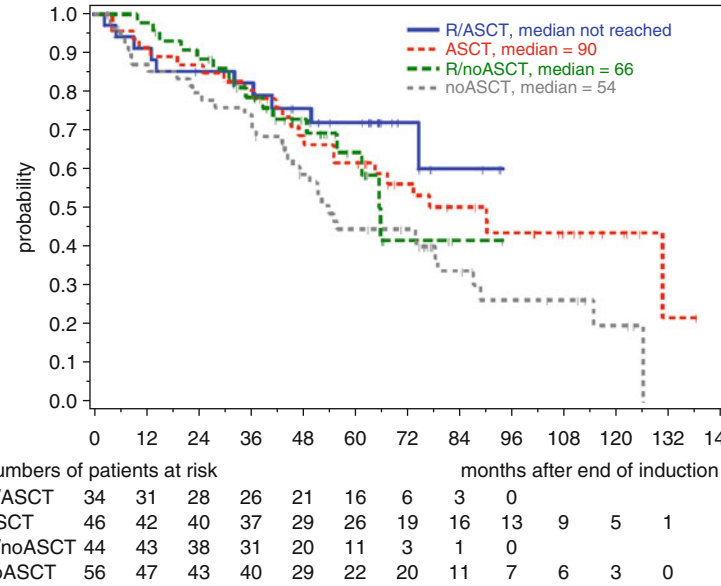

Fig. 10.5 Progression-free survival after a CHOP-like induction followed either by autologous stem cell transplantation (ASCT) or interferon (IFN) maintenance (From Dreyling et al. [120]. Used with permission)

AraC, cisplatine). After four cycles with CHOP, only 7 % of the patients reached complete remission. After four more cycles with DHAP, the remission rate rose to over 80 %. Another even more dose-intensified regimen of HyperCVAD/MA (fractionated cyclophosphamide, doxorubicin, vincristine, dexamethasone; alternated with high-dose methotrexate and cytarabine) was introduced by the MD Anderson group and demonstrated a CR of 38 % and a partial response of 55.5 % after four cycles in 45 patients with previously untreated as well as relapsed MCL [62]. In the initial trials, both DHAP and HyperCVAD protocols were applied as cytoreductive induction followed by consolidating myeloablative therapy and autologous HSCT. Meanwhile, even more impressive results have been reported when rituximab was combined with either the DHAP regimen [63] or the Hyper-CVAD/MA regimen [64, 65]. Unfortunately, these excellent results could not be replicated in a multicenter study evaluating R-Hyper-CVAD. ORR was 88 % with a 2-year PFS of 63 % only [66].

Based on the results of high-dose AraC-containing regimens, new trials have investigated the combination of these approaches. A French phase II study observed a median event-free survival of 83 months and an overall survival rate of 75 % at 5 years [67]. A study of the Nordic lymphoma study group reported an event-free survival of 63 % and an overall survival of 81 % at 4 years after high-dose AraC-containing induction therapy followed by autologous transplantation [68]. The European MCL Network recently confirmed the benefit of therapy intensification by addition of sequential AraC to conventional induction therapy with R-CHOP in a large international trial. In 497 patients in a randomized phase III study, a significant improvement of time to treatment failure had been observed for the AraC-containing regimen (76 % vs. 64 % after 3 years, $p=0.038$) with even a higher rate of molecular remissions [69, 70]. Thus, an R-AraC-containing induction therapy followed by ASCT represents the new standard for younger MCL patients.

Allogeneic Stem Cell Transplantation

Until today, allogeneic stem cell transplant is the only curative approach in advanced-stage MCL based on the induced graft versus lymphoma effect. Several phase II studies confirmed that even in multiple relapsed patients, long-lasting remissions can be achieved [71, 72]. Thirty-three patients with relapsed or refractory mantle cell

Table 10.2 Dose-intensified regimens in MCL treatment

Author	Study	*n*	Induction	Consolidation	Response rate (OR/CR)	Median PFS/EFS	Median OS
Dreyling et al. [112]	Phase III	75	Conventional (CHOP/MCP)	Intensive (ASCT)	78 % (42 %)	43 months	90 months
Dreger et al. [113]	Phase II	34	Conventional (CHOP/->R)	Intensive (ASCT)	88 % (24 %)	83 % (4 years)	87 % (4 years)
LeFrere et al.[114]	Phase II	28	Conventional (CHOP/DHAP)	Intensive (ASCT)	89 % (82 %)	51 months	81 months
de Guibert et al. [63]	Phase II	24	Conventional (R-DHAP)	Intensive (ASCT)	96 % (92 %)	65 % (3 years)	69 % (3 years)
Delarue et al. [67]	Phase II	60	Conventional (R-CHOP/R-DHAP)	Intensive (ASCT)	95 % (96 %)	83 months	75 % (5 years)
Dreyling et al. [112]	Phase III	390	Conventional (R-CHOP) Conventional (R-CHOP/ R-DHAP)	Intensive (ASCT) Intensive (ASCT)	91 % (51 %)	84 % (2 years)	77 % (2 years)
Romaguera et al. [64]	Phase II	97	Intensive (R-Hyper-CVAD/MA)	–	97 % (CR/CRu: 87 %)	54 months	82 % (3 years)
Epner et al. [66]	Phase II	97	Intensive (R-Hyper-CVAD/MA)	–	88 % (CR/CRu: 58 %)	64 % (2 years)	74 % (3 years)
Magni et al. [115]	Phase II	28	R-High-dose Cyclo, AraC, melphalan, mitoxantrone	Intensive (ASCT)	96 % (all CR)	48 % in low risk 34 % in high risk	76 % in low risk 68 % in high risk
Tam et al. [76]	Phase II	42 7	Intensive (R-Hyper-CVAD/MA) Conventional (R-CHOP)	Intensive (ASCT)	96 % (all CR/Cru)	42 months	93 months
Ritchie et al. [116]	Phase II	13	Intensive (R-Hyper-CVAD/MA)	Intensive (ASCT)	100 % (92 %)	92 % (3 years)	92 % (3 years)
Till et al. [117]	Phase II	21	Intensive (R-Hyper-CVAD/MA)	Intensive (ASCT)	100 % (CR/CRu: 81 %)	81 % (3 years)	94 % (3 years)
Vose et al. [118]	Phase II	32	Intensive (R-Hyper-CVAD/MA)	Intensive (ASCT)	100 % (CR/CRu: 81 %)	78 % (3 years)	97 % (3 years)
Geissler et al. [68]	Phase II	159	Intensive (R-CHOP-HA)	Intensive (ASCT)	96 % (55 %)	63 % (4 years)	81 % (4 years)
Hermine et al. [69]	Phase III	391	R-CHOP/R-DHAP following ASCT R-CHOP Following ASCT	Intensive (ASCT)	94 % (60 %) 90 % (41 %)		80 % (3 years) 79 % (3 years)

lymphoma were treated with non-myeloablative conditioning with fludarabine and 2-Gy total body irradiation followed by HCT. The disease-free and overall survival at 2 years were 60 and 65 %, respectively [73]. In another phase II study, patients with relapsed or refractory lymphoma including MCL received a conditioning therapy with alemtuzumab, fludarabine, and melphalan. Overall survival at 3 years was 60 % in the MCL subgroup [74]. Recently published phase II data about dose-reduced conditioning regimens report even more encouraging results. In 2008, a multicenter survey of 60 patients achieved a 3-year event-free survival with 69 % in CR and 45 % in PR [75]. After a reduced-intensity conditioning transplantation in relapsed MCL patients, Khouri et al. reported in 2009 a CR rate of 97 % with only three patients dead (9 %) after 1 year [76]. While acute graft versus host disease (GVHD) was moderate (grade I+II) and only observed in 37 % of patients, about 60 % suffered from chronic GVHD. After 56 months follow-up, estimated 6-year progression-free survival was 46 % with an overall survival of 53 % after 6 years. However, this allogeneic approach in relapsed disease was only superior to autologous transplantation. Thus, promising results in allogeneic transplantation should be applied only in relapsed disease or in selected high-risk patients not properly responding to dose-intensified first-line chemotherapy.

Rituximab Maintenance Therapy

As described above, the clinical course of MCL is characterized by only short-time remission after conventional chemotherapy; therefore, an effective consolidation therapy also in elderly patients is urgently warranted to prolong remission duration. Although in a Swiss SAKK trial rituximab maintenance showed no additional benefit compared to observation only after antibody monotherapy [43], a recent update of a randomized trial in relapsed malignant lymphoma could observe a major benefit of rituximab maintenance after a more effective induction regimen (FCM+/-R). The application of eight cycles, rituximab in standard dose of 375 mg/m^2 (four weekly doses after 6 and 9 months) improved 3-year progression-free survival from 9 to 45 % [77]. However, these data are based on a limited number of patients only ($n=50$). In a recent phase II trial, rituximab maintenance was applied after a modified hyperfractionated CVAD (cyclophosphamide, vincristine, doxorubicin, dexamethasone) regimen; time to progression was about 37 months in this setting [78]. Recently, the European MCL Network confirmed the benefit of regular post-induction antibody application in MCL in an international phase III trial. Duration of remission was almost doubled (51 vs. 24 months, $p=0.012$) in comparison to an interferon-based maintenance regimen [79], therefore representing the current standard approach in elderly patients (Fig. 10.6).

Radioimmunotherapy

Radioimmunotherapy is a novel therapeutic approach that combines the tumor-targeting attributes of lymphocyte-specific monoclonal antibodies with therapeutic radionucleotides and has been explored in various studies of mantle cell lymphoma, commonly considered to be inherently radiosensitive. Today there are two radioimmunoconjugates either approved in the USA or EU: 90Y-Ibritumomab tiuxetan and 131I-Tositumomab. Both are targeted against CD20, which is expressed on virtually all B-cell lymphomas. However, neither radioimmunoconjugate is currently approved for the treatment of MCL. In two phase II trials, the single-agent activity of 90Y-Ibritumomab tiuxetan in patients with relapsed or refractory mantle cell lymphoma has been investigated [80–82]. OR was of about 30–40 % with only short durations of response. In contrast, data on radioimmunotherapy integrated into a multimodal therapeutic approach, i.e., in segmental combination with chemotherapy as either induction or consolidation [83], seem to be more encouraging [84]. Another promising option seems to be the application of radioimmunoconjugates in a combination with high-dose chemotherapy followed by autologous or even allogeneic

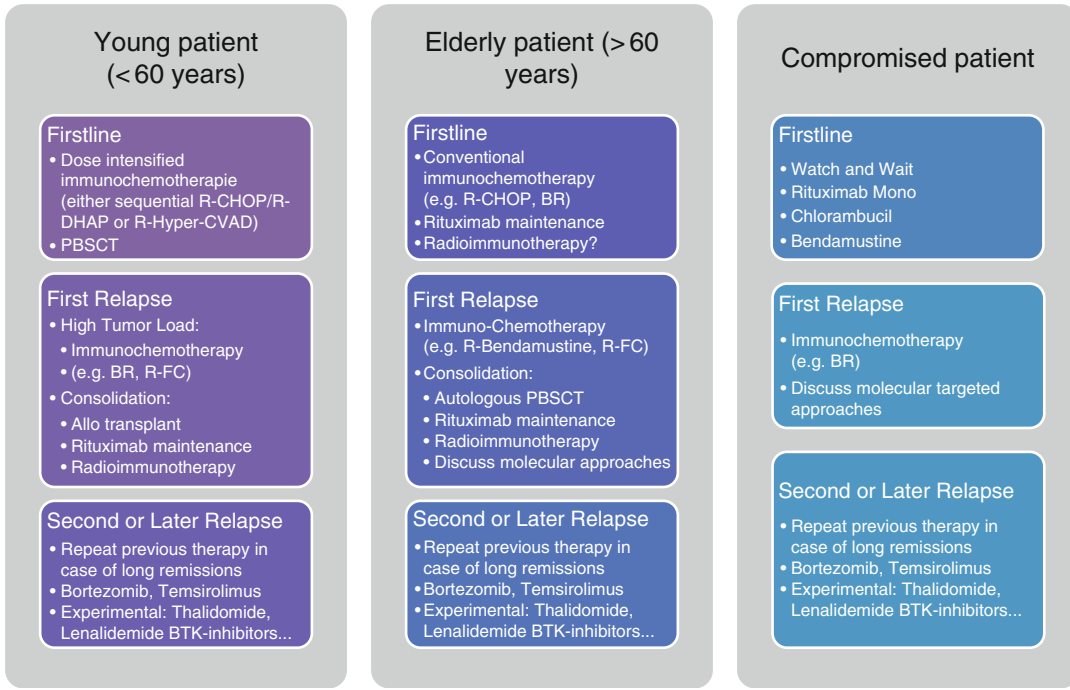

Fig. 10.6 Therapeutic approaches for MCL patients (advanced stage). *Note*: Since no standard therapy has been established for treatment of newly diagnosed or relapsed disease, treatment on clinical trial should be considered for all patients

stem cell transplantation. In a phase II study, 16 patients with relapsed or refracted MCL were enrolled to receive a high-dose radioimmunotherapy with 131I-Tositumomab followed by high-dose etoposide and cyclophosphamide as part of a myeloablative regimen before ASCT. OR was remarkable, (CR 91 %) with 3-year OS and PFS of 93 and 61 % [85].

Relapsed Disease

For relapsed MCL, only limited published data are available today. Therapeutic strategies depend on the previous applied regimens with consideration of patients' age, comorbidities, and clinical fitness. The addition of rituximab seems to be reasonable if a remission of at least 6 months has been achieved after a rituximab-containing regimen. In younger patients, allogeneic stem cell transplant should be considered after initial reduction of tumor load (Fig. 10.6). In elderly patients, non-cross-resistant conventional immunochemotherapy regimens are recommended. In a small US phase II trial ($n=12$), bendamustine plus rituximab (BR) achieved an overall response rate of 92 % (11 of 12) with about 50 % CR [86]. In a German phase II study ($n=16$), overall response rate was 75 % including 50 % CR with a median progression-free survival of 18 months [59]. Preliminary data of a phase III trial confirmed a significant better overall response and CR rate of BR in comparison to R-F [87].

Molecular Targeted Therapeutic Approaches

Although mantle cell lymphoma responses regularly to initial therapy and improved outcomes could be achieved over the last decade [4], the clinical course of this disease is still characterized by recurrent relapses. Most patients relapse within one till 5 years even after successful immunochemotherapy induction with autologous stem cell transplant consolidation and second-line chemotherapy, although highly effective

regarding response rates, achieving only short-term remissions, thus new therapeutic approaches are urgently warranted. During the last decade, more of the underlying biological pathways have been understood and new therapeutic targets identified. Such new approaches may target to deregulate cell-cycle characteristic for mantle cell lymphoma, or other proliferation or apoptosis pathways (Table 10.3) [23].

Proteasome Inhibitors

Bortezomib is a potent, selective, and reversible proteasome inhibitor registered for relapsed or refractory MCL in the USA. In two phase II trials, 141 and 40 relapsed or refractory MCL patients were enrolled. Objective response rates were only up to 44 % with a median progression-free survival of 5.3 and 6.7 months, respectively [88, 89], durable responses were observed among some CR patients. Bortezomib has surprisingly little toxicity considering the requirement of proteasome activity in every eukaryotic cell, so the combination with conventional chemotherapy is an interesting option. Preliminary data suggest synergistic effects of a combination with cytarabine; [90] thus, this approach has been investigated as well as the combination with other immunochemotherapy regimens in numerous phase II trials [91–94].

mTOR Inhibitors

The mammalian target of rapamycin (mTOR) is a downstream signaling molecule of the PI3K/Akt pathway that has a crucial role in the regulation of mRNA translation, including that of cyclin D1. Temsirolimus inhibits the translation of cyclin D1 messenger RNA by interfering with the mammalian target of rapamycin and so induce cell-cycle arrest. In a phase II trial, single-agent treatment yielded an OR of 38 %, while the CR was relatively low (3 %) with a short median time to progression and duration of response short (6.5 and 6.9 months, respectively) [95]. Considering high hematological toxicity, lower dose levels (25 mg) were evaluated and shown to be comparably efficient [96]. In a phase III multicenter study, two doses and schedules of temsirolimus were tested versus investigators choice of therapy. A schedule of 175 mg weekly for 3 weeks followed by 75 mg weekly displayed among 162 patients with relapsed or refractory disease to superiority in overall response rates (22 %) and progression-free survival (4,8 months) [97] which led to the registration in EU. Currently, temsirolimus is being investigated in combination with bendamustine with all of the nine patients responding [98]. RAD001 (Everolimus) has a similar mechanism of action with much higher in vitro efficacy [99]. In a phase II study, 19 patients with relapsed or refractory MCL had received 10 mg RAD001 daily as a flat oral dose. ORR was 32 % which seems to be comparable to the response rates in the phase II trials of temsirolimus [100].

Immunomodulatory Drugs (IMiDs)

Thalidomide is known to interfere with angiogenesis and the microenvironment. In a small phase II trial, the combination with rituximab yielded an OR of 81 % and a CR of 31 % [101]. The second-generation compound Lenalidomide achieved response rates up to 50 % in relapsed MCL [102–104].

Novel Therapeutic Approaches

PI3K and AKT are upstream of mTOR and frequently activated in MCL. CAL-101 and GDC-0941 are oral PI3K inhibitors that showed promising anti-lymphoma activity in preclinical trials [105–107] and is now being studied in a number of single-agent and combination trials. The Bruton's tyrosine kinase inhibitor PCI-32765 achieved a response rate of 67 % in relapsed MCL [108].

Flavopiridol downregulates cyclin D1 via inhibition of CDK4 and CDK-6. Although single-agent efficacy is seems to be limited in an Canadian trial with only 11 % ORR [109], combination with fludarabine and rituximab achieved responses in eight of ten patients with relapsed MCL [110]. Other interesting therapeutic approaches being investigated in clinical trials include bcl-2 inhibitors/BH3 mimetics as obatoclax (GX15–070) and vitoclax (ABT-263), protein kinase C inhibitors (Enzastaurin), or HDAC inhibitors (vorinostat). The upcoming trials will

Table 10.3 Novel Therapeutic Agents [23]

Drug class	Drugs	Mechanism of action and effects on MCL biology	Clinical development and results
Proteasome inhibitors	Bortezomib (CT-L), NPI-0052 (CT-L, T-L, C-L), PR-171 (CT-L), MLN9708 (CT-L)	Reversible or irreversible inhibition of ≥1 proteasome activities; cell-cycle arrest and induction of apoptosis through oxidative and ER stress-mediated upregulation of NOX	Bortezomib (phase 2): ORR 33 %–58 % in relapsed/refractory MCL; NPI-0052 and PR-171 (phase 1); MLN9708 (phase 1)
CDK inhibitors	Flavopiridol (pan-CDK), PD0332991 (CDK4/6)	Flavopiridol: pan-CDK inhibitor, decreased RNA stability; PD0332991: blocks cyclin D1/CDK4, leading to cell-cycle arrest	Flavopiridol (phase 1, 2): minimal responses as a single-agent, modified dosing schedules may be more effective; PD0332991(phase 1)
Serine/threonine and tyrosine kinase inhibitors	Enzastaurin (PKC-β[beta] II), fostamatinib (SYK), PCI-32765 (BTK)	Inhibition of BCR signal transduction cascade	Mostly stable disease for fostamatinib and enzastaurin; objective responses in phase 1 study of PCI-32765
PI3K/AKT inhibitors	CAL-101 (PI3Kδ[delta]), ON1910.Na (multikinase/PI3Kα[alpha]), perifosine (AKT)	Inactivation of AKT and mTOR; cell-cycle arrest, cyclin D1 downregulation; activation of p53 and BAD-mediated apoptosis	CAL-101 (oral agent, phase 1): PRs in MCL and CLL; ON-01910.Na (phase 1); Perifosine (phase 1)
mTOR inhibitors (rapalogs)	Rapamycin, temsirolimus (CCI-779), everolimus (RAD001), deforolimus (AP23573)	Partial allosteric TORC1 inhibition; cell-cycle arrest; inconsistent effects on expression of cell-cycle regulators (cyclin D1, p21, p27) may induce autophagy but not apoptosis	Temsirolimus (phase 2 and 3): ORR 30–40 % in relapsed patients, mostly PRs of rapid onset (median, 1 mo), median duration of response of 6.9 months; everolimus (phase 1 and 2): some objective responses; deforolimus (phase 2): 30 % PR and 40 % SD
BH3 mimetics	ABT-263, AT-101, obatoclax (GX15-070)	Inhibition of antiapoptotic members of BCL-2 family, BCL-2, BCL-X_L; GX 15-070 inhibits also MCL-1; mitochondrial depolarization through release of BH-3 only proteins and BAX/BAK activation	Obatoclax (phase 1 and 2) with or without bortezomib; AT-101 (phase 2); ABT-737 (phase 1 and 2)
IMIDs	Lenalidomide, thalidomide	Modulation of immune response; microenvironment or direct effect on tumor cells	Lenalidomide (phase 2): ORR 42–53 %, CR 20 %, PFS 5.6 months in relapsed MCL
HDAC inhibitor	Vorinostat (SAHA)	Cyclin D1 downregulation, p21 and p27 upregulation, and cell-cycle arrest; upregulation of the BH3 only proteins BIM and BMF	SAHA (phase 1 and 2) with or without bortezomib
HSP90 inhibitors	Ansamycins: 17-AAG, 17-DMAG: IPI-504; synthetic: SNX-5422, KW-2478	Degradation of client proteins, including cyclin D1, CDK4, IKKβ[beta], AKT, c-MYC, and c-RAF; cell-cycle arrest; activation of the mitochondrial apoptotic pathway	17-AAG (phase 2): mostly disease stabilization, with or without bortezomib; 17-DMAG clinical development stopped; IPI-504: water-soluble 17-AAG derivative; synthetic inhibitors: entering early stage testing

have to determine molecular markers allowing identification of patients especially prone to the different strategies.

References

1. Banks PM, Chan J, Cleary ML, et al. Mantle cell lymphoma. A proposal for unification of morphologic, immunologic, and molecular data. Am J Surg Pathol. 1992;16(7):637–40.
2. Harris NL, Jaffe ES, Diebold J, et al. World Health Organization classification of neoplastic diseases of the hematopoietic and lymphoid tissues: report of the Clinical Advisory Committee meeting-Airlie House, Virginia, November 1997. J Clin Oncol. 1999;17(12): 3835–49.
3. Harris NL, Jaffe ES, Stein H, et al. A revised European-American classification of lymphoid neoplasms: a proposal from the International Lymphoma Study Group. Blood. 1994;84(5):1361–92.
4. Herrmann A, Hoster E, Dreyling M, Unterhalt M, Brittinger G, Hiddemann W. Improvement of overall survival in mantle cell lymphoma during the last decades. Blood (ASH Annu Meet Abstr). 2006;108(11):2446.
5. Swerdlow SH, Williams ME. From centrocytic to mantle cell lymphoma: a clinicopathologic and molecular review of 3 decades. Hum Pathol. 2002;33(1):7–20.
6. Dreyling M, Hiddemann W. Current treatment standards and emerging strategies in mantle cell lymphoma. Hematol Am Soc Hematol Educ Program. 2009;2009:542–51.
7. Dreyling M, Bergsagel PL, Gordon LI, Cotter FE. The t(11;14) disorders: how biology can drive therapy. In: ASCO educational book. 2006. p. 476–84.
8. Weinkauf M, Christopeit M, Hiddemann W, Dreyling M. Proteome- and microarray-based expression analysis of lymphoma cell lines identifies a p53-centered cluster of differentially expressed proteins in mantle cell and follicular lymphoma. Electrophoresis. 2007;28(23):4416–26.
9. Fernandez V, Hartmann E, Ott G, Campo E, Rosenwald A. Pathogenesis of mantle-cell lymphoma: all oncogenic roads lead to dysregulation of cell cycle and DNA damage response pathways. J Clin Oncol. 2005; 23(26):6364–9.
10. Tiemann M, Schrader C, Klapper W, et al. Histopathology, cell proliferation indices and clinical outcome in 304 patients with mantle cell lymphoma (MCL): a clinicopathological study from the European MCL Network. Br J Haematol. 2005;131(1):29–38.
11. Swerdlow SH, Campo E, Harris NL. WHO classification of tumors of haematopoietic and lymphoid tissues. Lyon: IARC Press; 2008.
12. Rosenwald A, Wright G, Wiestner A, et al. The proliferation gene expression signature is a quantitative integrator of oncogenic events that predicts survival in mantle cell lymphoma. Cancer Cell. 2003;3(2):185–97.
13. Ferrer A, Bosch F, Villamor N, et al. Central nervous system involvement in mantle cell lymphoma. Ann Oncol. 2008;19(1):135–41.
14. Brepoels L, Stroobants S, De Wever W, et al. Positron emission tomography in mantle cell lymphoma. Leuk Lymphoma. 2008;49(9):1693–701.
15. Cheson BD, Pfistner B, Juweid ME, et al. Revised response criteria for malignant lymphoma. J Clin Oncol. 2007;25(5):579–86.
16. Herrmann A, Hoster E, Zwingers T, et al. Improvement of overall survival in advanced stage mantle cell lymphoma. J Clin Oncol. 2009;27(4):511–8.
17. Martin P, Chadburn A, Christos P, et al. Outcome of deferred initial therapy in mantle-cell lymphoma. J Clin Oncol. 2009;27(8):1209–13.
18. Martin P, Chadburn A, Christos P, et al. Intensive treatment strategies may not provide superior outcomes in mantle cell lymphoma: overall survival exceeding 7 years with standard therapies. Ann Oncol. 2008;19(7):1327–30.
19. Klapper W, Hoster E, Determann O, et al. Ki-67 as a prognostic marker in mantle cell lymphoma-consensus guidelines of the pathology panel of the European MCL Network. J Hematop. 2009;2(2):103–11.
20. Determann O, Hoster E, Ott G, et al. Ki-67 predicts outcome in advanced-stage mantle cell lymphoma patients treated with anti-CD20 immunochemotherapy: results from randomized trials of the European MCL Network and the German Low Grade Lymphoma Study Group. Blood. 2008;111(4):2385–7.
21. Navarro A, Bea S, Fernandez V, et al. MicroRNA expression, chromosomal alterations, and immunoglobulin variable heavy chain hypermutations in Mantle cell lymphomas. Cancer Res. 2009;69(17):7071–8.
22. Zhao JJ, Lin J, Lwin T, et al. microRNA expression profile and identification of miR-29 as a prognostic marker and pathogenetic factor by targeting CDK6 in mantle cell lymphoma. Blood. 2010;115(13):2630–9.
23. Perez-Galan P, Dreyling M, Wiestner A. Mantle cell lymphoma: biology, pathogenesis, and the molecular basis of treatment in the genomic era. Blood. 2011;117(1):26–38.
24. Hoster E, Dreyling M, Klapper W, et al. A new prognostic index (MIPI) for patients with advanced-stage mantle cell lymphoma. Blood. 2008;111(2):558–65.
25. Geisler CH, Kolstad A, Laurell A, et al. The Mantle Cell Lymphoma International Prognostic Index (MIPI) is superior to the International Prognostic Index (IPI) in predicting survival following intensive first-line immunochemotherapy and autologous stem cell transplantation (ASCT). Blood. 2010;115(8):1530–3.
26. Howard OM, Gribben JG, Neuberg DS, et al. Rituximab and CHOP induction therapy for newly diagnosed mantle-cell lymphoma: molecular complete responses are not predictive of progression-free survival. J Clin Oncol. 2002;20(5):1288–94.
27. Pott C, Hoster E, Delfau-Larue MH, et al. Molecular remission is an independent predictor of clinical outcome in patients with mantle cell lymphoma after combined immunochemotherapy: a European MCL intergroup study. Blood. 2010;115(16):3215–23.

28. Dreyling M. Therapy of mantle cell lymphoma: new treatment options in an old disease or vice versa? Semin Hematol. 2011;48(3):145–7.
29. Martin P, Leonard J. Is there a role for "watch and wait" in patients with mantle cell lymphoma? Semin Hematol. 2011;48(3):189–93.
30. Fernandez V, Salamero O, Espinet B, et al. Genomic and gene expression profiling defines indolent forms of mantle cell lymphoma. Cancer Res. 2010;70(4):1408–18.
31. M'Kacher R, Bennaceur A, Farace F, et al. Multiple molecular mechanisms contribute to radiation sensitivity in mantle cell lymphoma. Oncogene. 2003;22(39):7905–12.
32. Leitch HA, Gascoyne RD, Chhanabhai M, Voss NJ, Klasa R, Connors JM. Limited-stage mantle-cell lymphoma. Ann Oncol. 2003;14(10):1555–61.
33. Rosenbluth BD, Yahalom J. Highly effective local control and palliation of mantle cell lymphoma with involved-field radiation therapy (IFRT). Int J Radiat Oncol Biol Phys. 2006;65(4):1185–91.
34. Meusers P, Hense J, Brittinger G. Mantle cell lymphoma: diagnostic criteria, clinical aspects and therapeutic problems. Leukemia. 1997;11 Suppl 2Suppl 2:S60–4.
35. Nickenig C, Dreyling M, Hoster E, et al. Combined cyclophosphamide, vincristine, doxorubicin, and prednisone (CHOP) improves response rates but not survival and has lower hematologic toxicity compared with combined mitoxantrone, chlorambucil, and prednisone (MCP) in follicular and mantle cell lymphomas: results of a prospective randomized trial of the German Low-Grade Lymphoma Study Group. Cancer. 2006;107(5):1014–22.
36. Zucca E, Roggero E, Pinotti G, et al. Patterns of survival in mantle cell lymphoma. Ann Oncol. 1995;6(3): 257–62.
37. Foran JM, Rohatiner AZ, Coiffier B, et al. Multicenter phase II study of fludarabine phosphate for patients with newly diagnosed lymphoplasmacytoid lymphoma, Waldenstrom's macroglobulinemia, and mantle-cell lymphoma. J Clin Oncol. 1999;17(2):546–53.
38. Zinzani PL, Magagnoli M, Moretti L, et al. Randomized trial of fludarabine versus fludarabine and idarubicin as frontline treatment in patients with indolent or mantle-cell lymphoma. J Clin Oncol. 2000;18(4):773–9.
39. Cohen BJ, Moskowitz C, Straus D, Noy A, Hedrick E, Zelenetz A. Cyclophosphamide/fludarabine (CF) is active in the treatment of mantle cell lymphoma. Leuk Lymphoma. 2001;42(5):1015–22.
40. Cheson BD, Rummel MJ. Bendamustine: rebirth of an old drug. J Clin Oncol. 2009;27(9):1492–501.
41. Herold M, Schulze A, Niederwieser D, et al. Bendamustine, vincristine and prednisone (BOP) versus cyclophosphamide, vincristine and prednisone (COP) in advanced indolent non-Hodgkin's lymphoma and mantle cell lymphoma: results of a randomised phase III trial (OSHO# 19). J Cancer Res Clin Oncol. 2006;132(2):105–12.
42. Buske C, Weigert O, Dreyling M, Unterhalt M, Hiddemann W. Current status and perspective of antibody therapy in follicular lymphoma. Haematologica. 2006;91(1):104–12.
43. Ghielmini M, Schmitz SF, Cogliatti S, et al. Effect of single-agent rituximab given at the standard schedule or as prolonged treatment in patients with mantle cell lymphoma: a study of the Swiss Group for Clinical Cancer Research (SAKK). J Clin Oncol. 2005;23(4):705–11.
44. DiJoseph JF, Popplewell A, Tickle S, et al. Antibody-targeted chemotherapy of B-cell lymphoma using calicheamicin conjugated to murine or humanized antibody against CD22. Cancer Immunol Immunother. 2005;54(1):11–24.
45. Leonard JP, Goldenberg DM. Preclinical and clinical evaluation of epratuzumab (anti-CD22 IgG) in B-cell malignancies. Oncogene. 2007;26(25):3704–13.
46. Alinari L, Yu B, Christian BA, et al. Combination anti-CD74 (milatuzumab) and anti-CD20 (rituximab) monoclonal antibody therapy has in vitro and in vivo activity in mantle cell lymphoma. Blood. 2011;117(17):4530–41.
47. Leonard JP, Friedberg JW, Younes A, et al. A phase I/II study of galiximab (an anti-CD80 monoclonal antibody) in combination with rituximab for relapsed or refractory, follicular lymphoma. Ann Oncol. 2007;18(7):1216–23.
48. Rech J, Repp R, Rech D, et al. A humanized HLA-DR antibody (hu1D10, apolizumab) in combination with granulocyte colony-stimulating factor (filgrastim) for the treatment of non-Hodgkin's lymphoma: a pilot study. Leuk Lymphoma. 2006;47(10):2147–54.
49. Advani A, Coiffier B, Czuczman MS, et al. Safety, pharmacokinetics, and preliminary clinical activity of inotuzumab ozogamicin, a novel immunoconjugate for the treatment of B-cell non-Hodgkin's lymphoma: results of a phase I study. J Clin Oncol. 2010;28(12):2085–93.
50. Bargou R, Leo E, Zugmaier G, et al. Tumor regression in cancer patients by very low doses of a T cell-engaging antibody. Science. 2008;321(5891):974–7.
51. Nagorsen D, Baeuerle PA. Immunomodulatory therapy of cancer with T cell-engaging BiTE antibody blinatumomab. Exp Cell Res. 2011;317(9):1255–60.
52. Lenz G, Dreyling M, Hoster E, et al. Immunochemotherapy with rituximab and cyclophosphamide, doxorubicin, vincristine, and prednisone significantly improves response and time to treatment failure, but not long-term outcome in patients with previously untreated mantle cell lymphoma: results of a prospective randomized trial of the German Low Grade Lymphoma Study Group (GLSG). J Clin Oncol. 2005;23(9):1984–92.
53. Hoster E, Unterhalt M, Wormann B, et al. The addition of rituximab to first-line chemotherapy (R-CHOP) results in superior response rates, time to treatment failure and response duration in patients with advanced stage mantle cell lymphoma: long term results of a randomized GLSG trial. ASH Annu Meet Abstr. 2008;112(11):3049.
54. Herold M, Dolken G, Fiedler F, et al. Randomized phase III study for the treatment of advanced indolent non-Hodgkin's lymphomas (NHL) and mantle cell lymphoma: chemotherapy versus chemotherapy plus rituximab. Ann Hematol. 2003;82(2):77–9.
55. Herold M, Haas A, Doerken B. Immunochemotherapy (R-MCP) in advanced mantle cell lymphoma is not

superior to chemotherapy (MCP) alone - 50 months up date of the OSHO phase III study (abstract). Ann Oncol. 2008;19 Suppl 4Suppl 4:12.
56. Forstpointner R, Dreyling M, Repp R, et al. The addition of rituximab to a combination of fludarabine, cyclophosphamide, mitoxantrone (FCM) significantly increases the response rate and prolongs survival as compared with FCM alone in patients with relapsed and refractory follicular and mantle cell lymphomas: results of a prospective randomized study of the German Low-Grade Lymphoma Study Group. Blood. 2004;104(10):3064–71.
57. Schulz H, Bohlius JF, Trelle S, et al. Immunochemotherapy with rituximab and overall survival in patients with indolent or mantle cell lymphoma: a systematic review and meta-analysis. J Natl Cancer Inst. 2007;99(9):706–14.
58. Kluin-Nelemans JC, Hoster E, Walewski J, et al. R-CHOP versus R-FC followed by maintenance with rituximab versus interferon-alfa: outcome of the first randomized trial for elderly patients with mantle cell lymphoma. ASH Annu Meet Abstr. 2011;118(21): 439.
59. Rummel MJ, Al-Batran SE, Kim SZ, et al. Bendamustine plus rituximab is effective and has a favorable toxicity profile in the treatment of mantle cell and low-grade non-Hodgkin's lymphoma. J Clin Oncol. 2005;23(15):3383–9.
60. Weide R, Hess G, Koppler H, et al. High anti-lymphoma activity of bendamustine/mitoxantrone/rituximab in rituximab pretreated relapsed or refractory indolent lymphomas and mantle cell lymphomas. A multicenter phase II study of the German Low Grade Lymphoma Study Group (GLSG). Leuk Lymphoma. 2007;48(7):1299–306.
61. Rummel MJ, Niederle N, Maschmeyer G, et al. Bendamustine plus rituximab is superior in respect of progression free survival and CR rate when compared to CHOP plus rituximab as first-line treatment of patients with advanced follicular, indolent, and mantle cell lymphomas: final results of a randomized phase III study of the StiL (Study Group Indolent Lymphomas, Germany). Blood (ASH Annu Meet Abstr). 2009;114(22):405.
62. Khouri IF, Romaguera J, Kantarjian H, et al. Hyper-CVAD and high-dose methotrexate/cytarabine followed by stem-cell transplantation: an active regimen for aggressive mantle-cell lymphoma. J Clin Oncol. 1998;16(12):3803–9.
63. de Guibert S, Jaccard A, Bernard M, Turlure P, Bordessoule D, Lamy T. Rituximab and DHAP followed by intensive therapy with autologous stem-cell transplantation as first-line therapy for mantle cell lymphoma. Haematologica. 2006;91(3):425–6.
64. Romaguera JE, Fayad L, Rodriguez MA, et al. High rate of durable remissions after treatment of newly diagnosed aggressive mantle-cell lymphoma with rituximab plus hyper-CVAD alternating with rituximab plus high-dose methotrexate and cytarabine. J Clin Oncol. 2005;23(28):7013–23.
65. Romaguera JE, Fayad LE, Feng L, et al. Ten-year follow-up after intense chemoimmunotherapy with Rituximab-HyperCVAD alternating with Rituximab-high dose methotrexate/cytarabine (R-MA) and without stem cell transplantation in patients with untreated aggressive mantle cell lymphoma. Br J Haematol. 2010;150(2):200–8.
66. Epner EM, Unger J, Miller T, et al. A multi center trial of hyperCVAD+Rituxan in patients with newly diagnosed mantle cell lymphoma. Blood (ASH Annu Meet Abstr). 2007;110(11):387.
67. Delarue R, Haioun C, Ribrag V, et al. RCHOP and RDHAP followed by autologous stem cell transplantation (ASCT) in mantle cell lymphoma (MCL): final results of a phase II study from the GELA. ASH Annu Meet Abstr. 2008;112(11):581.
68. Geisler CH, Kolstad A, Laurell A, et al. Long-term progression-free survival of mantle cell lymphoma after intensive front-line immunochemotherapy with in vivo-purged stem cell rescue: a nonrandomized phase 2 multicenter study by the Nordic Lymphoma Group. Blood. 2008;112(7):2687–93.
69. Hermine O, Hoster E, Walewski J, et al. Alternating courses of 3x CHOP and 3x DHAP plus rituximab followed by a high dose ARA-C containing myeloablative regimen and autologous stem cell transplantation (ASCT) is superior to 6 courses CHOP plus rituximab followed by myeloablative radiochemotherapy and ASCT in mantle cell lymphoma: results of the MCL younger trial of the European mantle cell lymphoma network (MCL net). ASH Annu Meet Abstr. 2010;116(21):110.
70. Pott C, Hoster E, Beldjord K, et al. R-CHOP/R-DHAP compared to R-CHOP induction followed by high dose therapy with autologous stem cell transplantation induces higher rates of molecular remission in MCL: results of the MCL younger intergroup trial of the European MCL Network. ASH Annu Meet Abstr. 2010;116(21):965.
71. Khouri IF, Lee MS, Saliba RM, et al. Nonablative allogeneic stem-cell transplantation for advanced/recurrent mantle-cell lymphoma. J Clin Oncol. 2003; 21(23):4407–12.
72. Robinson SP, Goldstone AH, Mackinnon S, et al. Chemoresistant or aggressive lymphoma predicts for a poor outcome following reduced-intensity allogeneic progenitor cell transplantation: an analysis from the Lymphoma Working Party of the European Group for Blood and Bone Marrow Transplantation. Blood. 2002;100(13):4310–6.
73. Maris MB, Sandmaier BM, Storer BE, et al. Allogeneic hematopoietic cell transplantation after fludarabine and 2 Gy total body irradiation for relapsed and refractory mantle cell lymphoma. Blood. 2004;104(12): 3535–42.
74. Morris E, Thomson K, Craddock C, et al. Outcomes after alemtuzumab-containing reduced-intensity allogeneic transplantation regimen for relapsed and refractory non-Hodgkin lymphoma. Blood. 2004;104(13):3865–71.
75. Le Gouill S, Kroeger N, Dhedin N, et al. Interest of non-myeloablative allogeneic stem cell transplantation in

mantle cell lymphoma: a multicenter retrospective study. ASH Annu Meet Abstr. 2008;112(11):1965.
76. Tam CS, Bassett R, Ledesma C, et al. Mature results of the M. D. Anderson Cancer Center risk-adapted transplantation strategy in mantle cell lymphoma. Blood. 2009;113(18):4144–52.
77. Forstpointner R, Unterhalt M, Dreyling M, et al. Maintenance therapy with rituximab leads to a significant prolongation of response duration after salvage therapy with a combination of rituximab, fludarabine, cyclophosphamide, and mitoxantrone (R-FCM) in patients with recurring and refractory follicular and mantle cell lymphomas: results of a prospective randomized study of the German Low Grade Lymphoma Study Group (GLSG). Blood. 2006;108(13):4003–8.
78. Kahl BS, Longo WL, Eickhoff JC, et al. Maintenance rituximab following induction chemoimmunotherapy may prolong progression-free survival in mantle cell lymphoma: a pilot study from the Wisconsin Oncology Network. Ann Oncol. 2006;17(9):1418–23.
79. Kluin-Nelemans HC, Hoster E, Vehling-Kaiser U, Geisler C. Rituximab maintenance significantly prolongs duration of remission in elderly patients with mantle cell lymphoma. Results of a randomized trial of the European MCL Network. 16th EHA Congress, London.
80. Weigert O, von Schilling C, Rummel MJ, et al. Efficacy and safety of a single-course of yttrium-90 (90Y) ibritumomab tiuxetan (Zevalin(R)) in patients with relapsed or refractory mantle cell lymphoma (MCL) after/not appropriate for autologous stem cell transplantation (ASCT) - a phase II trial of the European MCL Network. Blood (ASH Annu Meet Abstr). 2005;106(11):4786.
81. Younes A, Pro B, Rodriguez MA, et al. Activity of yttrium 90 (90Y) ibritumomab tiuxetan (Zevalin(R)) in 22 patients with relapsed and refractory mantle cell lymphoma (MCL). Blood (ASH Annu Meet Abstr). 2005;106(11):2452.
82. Wang M, Oki Y, Pro B, et al. Phase II study of yttrium-90-ibritumomab tiuxetan in patients with relapsed or refractory mantle cell lymphoma. J Clin Oncol. 2009;27(31):5213–8.
83. Weigert O, Jurczak W, Von Schilling C, et al. Efficacy of radioimmunotherapy with (90Y) ibritumomab tiuxetan is superior as consolidation in relapsed or refractory mantle cell lymphoma: results of two phase II trials of the European MCL Network and the PLRG. ASCO Meet Abstr. 2006;24 Suppl 18Suppl 18:7533.
84. Smith MR, Zhang L, Gordon LI, et al. Phase II study of R-CHOP followed by 90Y-ibritumomab tiuxetan in untreated mantle cell lymphoma: Eastern Cooperative Oncology Group Study E1499. Blood (ASH Annu Meet Abstr). 2007;110(11):389.
85. Gopal AK, Rajendran JG, Petersdorf SH, et al. High-dose chemo-radioimmunotherapy with autologous stem cell support for relapsed mantle cell lymphoma. Blood. 2002;99(9):3158–62.
86. Robinson KS, Williams ME, van der Jagt RH, et al. Phase II multicenter study of bendamustine plus rituximab in patients with relapsed indolent B-cell and mantle cell non-Hodgkin's lymphoma. J Clin Oncol. 2008;26(27):4473–9.
87. Rummel MJ, Kaiser U, Balser C, et al. Bendamustine plus rituximab versus fludarabine plus rituximab in patients with relapsed follicular, indolent and mantle cell lymphomas - final results of the randomized phase III study NHL 2-2003 on behalf of the StiL (Study Group Indolent Lymphomas, Germany). ASH Annu Meet Abstr. 2010;116(21):856.
88. Goy A, Bernstein SH, Kahl BS, et al. Bortezomib in patients with relapsed or refractory mantle cell lymphoma: updated time-to-event analyses of the multicenter phase 2 PINNACLE study. Ann Oncol. 2009;20(3):520–5.
89. O'Connor OA, Moskowitz C, Portlock C, et al. Patients with chemotherapy-refractory mantle cell lymphoma experience high response rates and identical progression-free survivals compared with patients with relapsed disease following treatment with single agent bortezomib: results of a multicentre Phase 2 clinical trial. Br J Haematol. 2009;145(1):34–9.
90. Weigert O, Weidmann E, Mueck R, et al. A novel regimen combining high dose cytarabine and bortezomib has activity in multiply relapsed and refractory mantle cell lymphoma - long-term results of a multicenter observation study. Leuk Lymphoma. 2009;50(5):716–22.
91. Gressin R, Houot R, Uribe MO, et al. Final results of the RiPAD + C regimen including velcade in front line therapy for elderly patients with mantle cell lymphoma. A phase II prospective study of the GOELAMS group. ASH Annu Meet Abstr. 2010;116(21):2791.
92. Friedberg JW, Vose JM, Kelly JL, et al. The combination of bendamustine, bortezomib, and rituximab for patients with relapsed/refractory indolent and mantle cell non-Hodgkin lymphoma. Blood. 2011;117(10):2807–12.
93. Chiappella A, Pregno P, Zinzani PL, et al. Weekly infusion of bortezomib in combination with rituximab in relapsed/refractory indolent non-follicular and mantle cell lymphoma is safe and effective: two-years analysis of phase II trial BRIL06 of Intergruppo Italiano Linfomi (IIL). ASH Annu Meet Abstr. 2010;116(21):3965.
94. Ruan J, Martin P, Furman RR, et al. Bortezomib plus CHOP-rituximab for previously untreated diffuse large B-cell lymphoma and mantle cell lymphoma. J Clin Oncol. 2011;29(6):690–7.
95. Witzig TE, Geyer SM, Ghobrial I, et al. Phase II trial of single-agent temsirolimus (CCI-779) for relapsed mantle cell lymphoma. J Clin Oncol. 2005;23(23):5347–56.
96. Ansell SM, Inwards DJ, Rowland Jr KM, et al. Low-dose, single-agent temsirolimus for relapsed mantle cell lymphoma: a phase 2 trial in the North Central Cancer Treatment Group. Cancer. 2008;113(3):508–14.
97. Hess G, Herbrecht R, Romaguera J, et al. Phase III study to evaluate temsirolimus compared with investigator's choice therapy for the treatment of relapsed or refractory mantle cell lymphoma. J Clin Oncol. 2009;27(23):3822–9.

98. Hess G, Keller U, Atta J, et al. Temsirolimus in combination with bendamustine and rituximab for the treatment of relapsed mantle cell and follicular lymphoma: report on an ongoing phase I/II trial. ASH Annu Meet Abstr. 2011;118(21):2697.
99. Haritunians T, Mori A, O'Kelly J, Luong QT, Giles FJ, Koeffler HP. Antiproliferative activity of RAD001 (everolimus) as a single agent and combined with other agents in mantle cell lymphoma. Leukemia. 2007;21(2):333–9.
100. Witzig TE, Reeder CB, LaPlant BR, et al. A phase II trial of the oral mTOR inhibitor everolimus in relapsed aggressive lymphoma. Leukemia. 2011;25(2):341–7.
101. Kaufmann H, Raderer M, Wohrer S, et al. Antitumor activity of rituximab plus thalidomide in patients with relapsed/refractory mantle cell lymphoma. Blood. 2004;104(8):2269–71.
102. Habermann TM, Lossos IS, Justice G, et al. Lenalidomide oral monotherapy produces a high response rate in patients with relapsed or refractory mantle cell lymphoma. Br J Haematol. 2009;145(3): 344–9.
103. Zinzani PL, Witzig TE, Vose JM, et al. Confirmation of the efficacy and safety of lenalidomide oral monotherapy in patients with relapsed or refractory mantle-cell lymphoma: results of an international study (NHL-003). ASH Annu Meet Abstr. 2008;112(11): 262.
104. Witzig TE, Vose JM, Zinzani PL, et al. An international phase II trial of single-agent lenalidomide for relapsed or refractory aggressive B-cell non-Hodgkin's lymphoma. Ann Oncol. 2011;22(7):1622–7.
105. Lannutti BJ, Meadows SA, Herman SEM, et al. CAL-101, a p110δ selective phosphatidylinositol-3-kinase inhibitor for the treatment of B-cell malignancies, inhibits PI3K signaling and cellular viability. Blood. 2011;117(2):591–4.
106. Herman SEM, Gordon AL, Wagner AJ, et al. Phosphatidylinositol 3-kinase-δ inhibitor CAL-101 shows promising preclinical activity in chronic lymphocytic leukemia by antagonizing intrinsic and extrinsic cellular survival signals. Blood. 2010; 116(12):2078–88.
107. Iyengar S, Clear AJ, Owen A, et al. PI3K inhibition with GDC-0941 has greater efficacy compared to p110{delta}-selective inhibition with CAL-101 in mantle cell lymphoma and may be particularly advantageous in multiply relapsed patients. ASH Annu Meet Abstr. 2011;118(21):1654.
108. Wang L, Martin P, Blum KA, et al. The Bruton's tyrosine kinase inhibitor PCI-32765 is highly active as single-agent therapy in previously-treated mantle cell lymphoma (MCL): preliminary results of a phase II trial. ASH Annu Meet Abstr. 2011;118(21): 442.
109. Kouroukis CT, Belch A, Crump M, et al. Flavopiridol in untreated or relapsed mantle-cell lymphoma: results of a phase II study of the National Cancer Institute of Canada Clinical Trials Group. J Clin Oncol. 2003;21(9):1740–5.
110. Lin TS, Fischer B, Blum KA, et al. Flavopiridol, fludarabine and rituximab (FFR): an active regimen in indolent B-cell lymphoproliferative disorders and mantle cell lymphoma. ASH Annu Meet Abstr. 2008;112(11):1571.
111. Rule S, Smith P, Johnson PW, et al. The addition of rituximab to fludarabine and cyclophosphamide (FC) improves overall survival in newly diagnosed mantle cell lymphoma (MCL): results of the randomised UK National Cancer Research Institute (NCRI) trial. ASH Annu Meet Abstr. 2011;118(21): 440.
112. Dreyling MH, Hoster E, Van Hoof A, et al. Early consolidation with myeloablative radiochemotherapy followed by autologous stem cell transplantation in first remission in mantle cell lymphoma: long term follow up of a randomized trial. ASH Annu Meet Abstr. 2008;112(11):769.
113. Dreger P, Rieger M, Seyfarth B, et al. Rituximab-augmented myeloablation for first-line autologous stem cell transplantation for mantle cell lymphoma: effects on molecular response and clinical outcome. Haematologica. 2007;92(1):42–9.
114. Lefrere F, Delmer A, Levy V, Delarue R, Varet B, Hermine O. Sequential chemotherapy regimens followed by high-dose therapy with stem cell transplantation in mantle cell lymphoma: an update of a prospective study. Haematologica. 2004;89(10): 1275–6.
115. Magni M, Di Nicola M, Carlo-Stella C, et al. Efficacy and safety of high-dose chemotherapy with in vivo purged auto-SCT in relapsed follicular lymphoma: long-term follow-up. Bone Marrow Transplant. 2010;45(6):1119–20.
116. Ritchie DS, Seymour JF, Grigg AP, et al. The hyper-CVAD-rituximab chemotherapy programme followed by high-dose busulfan, melphalan and autologous stem cell transplantation produces excellent event-free survival in patients with previously untreated mantle cell lymphoma. Ann Hematol. 2007;86(2):101–5.
117. Till BG, Gooley TA, Crawford N, et al. Effect of remission status and induction chemotherapy regimen on outcome of autologous stem cell transplantation for mantle cell lymphoma. Leuk Lymphoma. 2008;49(6):1062–73.
118. Vose J, Loberiza F, Bierman P, Bociek G, Armitage J. Mantle cell lymphoma (MCL): induction therapy with HyperCVAD/high-dose methotrexate and cytarabine (M-C) ({+/-}rituximab) improves results of autologous stem cell transplant in first remission. ASCO Meet Abstr. 2006;24(18_suppl):7511.
119. Jares P, Colomer D, Campo E. Genetic and molecular pathogenesis of mantle cell lymphoma: perspectives for new targeted therapeutics. Nat Rev Cancer. 2007;7(10):750–62.
120. Dreyling M, Weigert O, Hiddemann W. Current treatment standards and future strategies in Mantle Cell Lymphoma. Ann Oncol. 2008;19 Suppl 4Suppl 4:iv41–4.

Diffuse Large B-Cell Lymphoma

11

Fabrice Jardin and Hervé Tilly

Abstract

The World Health Organization (WHO) classification defines diffuse large B-cell lymphoma (DLBCL) as a group of proliferations of large B-cell lymphoid cells with a diffuse growth pattern. It contains some specific entities and a large group of heterogeneous "not otherwise specified" diseases comprising morphologic variants and immunohistochemical, genetic, and molecular subgroups. DLBCL is the most common hematopoietic malignancy, accounting for one-third of mature B-cell neoplasms. Major advances have been observed in the knowledge and the management of DLBCL in the recent years. If the International Prognosis Index (IPI) is still the primary clinical tool used to predict outcome for patients with DLBCL and to guide therapeutic strategies, gene expression profiling and its related biomarkers delineate at least two major histologically indistinguishable molecular subtypes, the germinal center B-cell-like (GCB) subtype and the activated B-cell-like (ABC) subtype, that differ in cure rates and in responsiveness to targeted therapies, independently of the clinical variables. Functional imaging with fluorine-18 deoxyglucose (FDG) positron emission tomography (PET) has become an indispensable mean of assessing the extent of the disease and treatment response. The advent of rituximab has opened the era of targeted therapies in DLBCL and has markedly modified, in combination with chemotherapy, the outcomes in all DLBCL subgroups.

Keywords

Diffuse large B-cell lymphoma • Gene expression profiling • Germinal center B-cell-like (GCB) subtype and the activated B-cell-like (ABC) subtype • International Prognosis Index • Rituximab • PET scan imaging • Targeted therapy • Autologous stem-cell transplantation • Immunochemotherapy • Central nervous system prophylaxis

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Introduction

The World Health Organization (WHO) classification has defined diffuse large B-cell lymphoma (DLBCL) as a group of proliferations of large B-cell lymphoid cells that has a diffuse growth pattern [1]. It contains some specific entities and a large group of heterogeneous "not otherwise specified" diseases comprising morphologic variants and immunohistochemical, genetic, and molecular subgroups (Table 11.1).

DLBCL is the most common hematopoietic malignancy, accounting for 17 % of all these neoplasms and 28 % of mature B-cell neoplasms [2]. The incidence of DLBCL dramatically increased in the period 1970–1990 and appeared to stabilize from the 1990s [2, 3]. In the population, its incidence steadily increases with age and the median age is in the seventh decade [4].

Major advances have been observed in the knowledge and the management of DLBCL in the recent years. The advent of rituximab has opened the era of targeted therapies in DLBCL and has markedly modified the outcomes in all subgroups [5, 6]. Functional imaging with fluorine-18 deoxyglucose (FDG) positron emission tomography (PET) has become an indispensable means of assessing the extent of the disease and, more importantly, the evaluation of treatment response [7]. Although the studies of gene alterations and gene expression profile are not used at present in current practice, they are able to identify different subtypes of DLBCL and to reveal genetic events and molecular pathways involved in lymphomagenesis [8–11].

Table 11.1 Diffuse large B-cell classification according to the WHO 2008 classification

Diffuse large B-cell lymphoma (DLBCL), not otherwise specified (NOS)
Common morphologic variants
Centroblastic
Immunoblastic
Anaplastic
Molecular subgroups
Germinal center B-cell-like (GCB)
Activated B-cell-like (ABC)
Immunohistochemical subgroups
CD5-positive DLBCL
Germinal center B-cell-like (GCB)
Non-germinal center B-cell-like (non-GCB)
DLBCL subtypes
T-cell-/histiocyte-rich large B-cell lymphoma
Primary DLBCL of the CNS
Primary cutaneous DLBCL, leg type
Epstein-Barr virus-positive DLBCL of the elderly
Other lymphomas of large B cells
Primary mediastinal (thymic) large B-cell lymphoma
Intravascular large B-cell lymphoma
DLBCL associated with chronic inflammation
Lymphomatoid granulomatosis
ALK-positive DLBCL
Plasmablastic lymphoma
Large B-cell lymphoma arising in HHV8-associated multicentric Castleman disease
Primary effusion lymphoma
Borderline cases
B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and Burkitt lymphoma
B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and classical Hodgkin lymphoma

Clinical Presentation

As DLBCL could involve lymph nodes or virtually any extranodal sites, the clinical presentation is extremely variable. Up to 40 % of the cases could be initially confined to extranodal sites mimicking a solid tumor of the organ [1]. The most frequent sites are the gastrointestinal tract, Waldeyer ring, skin, and glands. Bone marrow is involved in 10–30 % of cases. Bone marrow could be infiltrated by small cells which could reflect the presence of previous indolent lymphoma and are not specifically associated with an adverse prognosis [12, 13]. Natural history of DLBCL is usually associated with a rapidly enlarging tumor mass or disseminating disease indicating the need of a precise diagnosis and staging in a short time.

Diagnosis

As for all lymphomas, a lymph node or extranodal tissue biopsy is mandatory. Sufficient material is required to conduct morphologic characterization

and immunophenotyping. Additional cytogenetic and molecular studies are advisable to insure complete characterization and biological prognostic features. Although image-guided needle biopsy is usually inadequate for primary diagnosis, it could be used when surgery should be avoided [14]. The diagnosis should be identified as a subtype of the WHO classification (Table 11.1) [1]. In case of difficulty, it should be confirmed by a hematopathologist expert in the field of lymphoma.

Staging

Personal and familial history should be documented. Evaluation of performance status and presence of B symptoms should be assessed, as physical examination. Blood tests include complete blood count, kidney and liver functions, LDH and β[beta]2 microglobulin levels, protein electrophoresis, and albumin level. Hepatitis B, hepatitis C, and HIV serologies are to be determined. Bone marrow biopsy and aspirate is mandatory. Bone marrow immunochemistry appears to increase sensitivity of morphologic examination [7, 15]. A possible discordance with lymph node subtype should be indicated [16]. Cytology of the fluid spinal should be examined, at least in patients at risk of central nervous system involvement, although this population is not definitively defined and could comprise an elevated LDH level, multiple extranodal sites, a bulky disease, an increased International Prognostic Index, or specific extranodal sites (bone marrow, testis, breast, Waldeyer ring, etc.) [17–21]. Flow cytometry could be a valuable tool to increase sensitivity of conventional cytology in the diagnosis of occult involvement [22–24]. Computed tomography with contrast of the neck, chest, abdomen, and pelvis is necessary to indicate bidimensional measurements of lymph nodes and extranodal lesions [16].

PET Scan Imaging

PET is highly recommended for staging and is considered as mandatory for evaluation of response at the end of treatment by the revised response criteria established by the International Working Group in 2007 [7]. Staging of non-Hodgkin lymphomas is determined according to the Ann Arbor classification initially developed for Hodgkin lymphoma [25]. PET/CT has currently replaced the independent PET scanner. PET/CT incorporates generally intravenous contrast, allowing a better delineation of lymph nodes, especially in the neck, in the mesenteric, or in the retroperitoneal regions, without any significant interference between the two imaging procedures [26, 27].

Initial Staging

Several studies demonstrated a better sensitivity of the PET/CT as compared to CT alone. Patients with DLBCL are upstaged in 20 % of cases, mostly those with stage I or II disease. Downstaging is obtained in fewer than 10 %, with a change in treatment in less than 15 % of patients. PET/CT with enhanced contrast is associated with a very low false-positive rate [26].

PET can detect focal or multifocal bone/bone marrow involvement in DLBCL with a negative bone marrow biopsy. In a recent meta-analysis, the sensibility and specificity of FDG PET for evaluation of bone marrow in aggressive NHL lymphoma showed a sensitivity of 74 % and a specificity of 84 %, indicating that PET scan may be an alternative approach to detect bone marrow involvement in DLBCL patients but cannot be substituted for bone marrow biopsy in DLBCL staging. Bone marrow biopsy remains critical to detect concordant or discordant histology with the primary tumor, PET scan displaying a lower sensibility to detect bone marrow involvement by a low-grade lymphoma [28, 29].

Standard uptake value (SUV) baseline as a prognostic predictor has shown conflicting results, but recent reports suggest that a high uptake value was related to an unfavorable prognosis [30, 31]. Phan et al., in a retrospective study involving 467 patients, suggested that a SUVmax > 13 correlated to an unfavorable outcome [32]. This prognostic value was not confirmed by univariate analysis. More recently, it was shown that a SUVmax > 30 was a significant poor prognostic factor, independent of IPI but related to Ki-67 expression and poor performance status [33].

Interim PET Prognosis Value

The interim PET has emerged as a powerful predictive tool in DLBCL [34–37]. In addition to final response criteria used at the end of the treatment, interim PET scan criteria have been proposed (Deauville criteria) and are currently evaluated prospectively [7, 38]. A recent prospective study was performed by the GELA group demonstrating the superiority of the quantitative assessment of interim PET (based on the decrease of the SUVmax during treatment), performed after two or four cycles of immunochemotherapy as compared to the simple visual assessment [39]. In a multicentric retrospective study involving 112 DLBCL patients, it was shown that an early PET scan after two cycles of R-CHOP or R-CHOP-like regimens can effectively predict the outcome using either a visual or quantitative approach [40]. Of note patients considered as fast metabolic responders but belonging to an unfavorable molecular subtype [activated B-cell-like (ABC) subgroup] may display a poor prognosis, similar to slow metabolic responders, indicating that other prognostic confounding factors could explain some discordant results regarding the interim PET prognosis value [31].

PET Scan Before Stem-Cell Transplantation

Several studies indicate that PET scan positivity before autologous stem-cell transplantation is highly predictive of the long-term success of the procedure [41–43]. In a meta-analysis including 12 studies with 630 patients displaying recurrent aggressive NHL, the combined use of clinical factors (including LDH, stage, performance status) and FDG PET response after 2 second-line chemotherapy courses defined a highly predictive prognostic method that adds to the predictability of the PET scan alone [44].

Surveillance PET Scans

PET has failed to show any utility in the detection of early relapse and cannot be recommended, clinical examination remaining the most useful tool in this context [26].

Prognostic Factors

Clinical Factors

The International Prognosis Index (IPI) is still the primary clinical tool used to predict outcome for patients with DLBCL [45]. This model includes patient age (>60 vs. ≤60 years), Ann Arbor stage (III–IV vs. I–II), LDH level (>1 vs. ≤1× normal upper value), the number of extranodal site (≥2 or <2), and performance status (Eastern Cooperative Oncology Group performance status ECOG 0–1 vs. 2–3). An age-adjusted model for patients younger than 60 (aaIPI) is also widely used. A revised IPI (R-IPI) has been proposed to discriminate more accurately prognostic groups in the rituximab era. The R-IPI is defined by the redistribution of the IPI factors allowing to identify three distinct prognostic groups with a very good (4-year progression-free survival [PFS] 94 %, overall survival [OS] 94 %), good (4-year PFS 80 %, OS 79 %), and poor (4-year PFS 53 %, OS 55 %) outcome, respectively [46]. R-IPI incorporating absolute lymphocyte count as additional clinical variable has been also proposed [47]. However, a recent retrospective analysis of 1,062 DLBCL patients treated by R-CHOP and R-CHOP-like regimens demonstrated that the IPI remains highly predictive of the EFS, PFS, and OS and showed that the relative risk estimates of single IPI factors and their order in patients treated with R-CHOP were similar to those found with CHOP [48].

In limited-stage (I–II) DLBCL, the presence of a bulky disease is associated to an unfavorable outcome and this point was confirmed in patients treated by CHOP-like regimens and rituximab [49]. A recent study had underscored the prognostic value of bone marrow positivity, showing in a retrospective series of 795 patients that involvement by large cell (8.4 % concordant), by contrast to small B-cell involvement (7.3 % discordant), was associated with a decrease of the

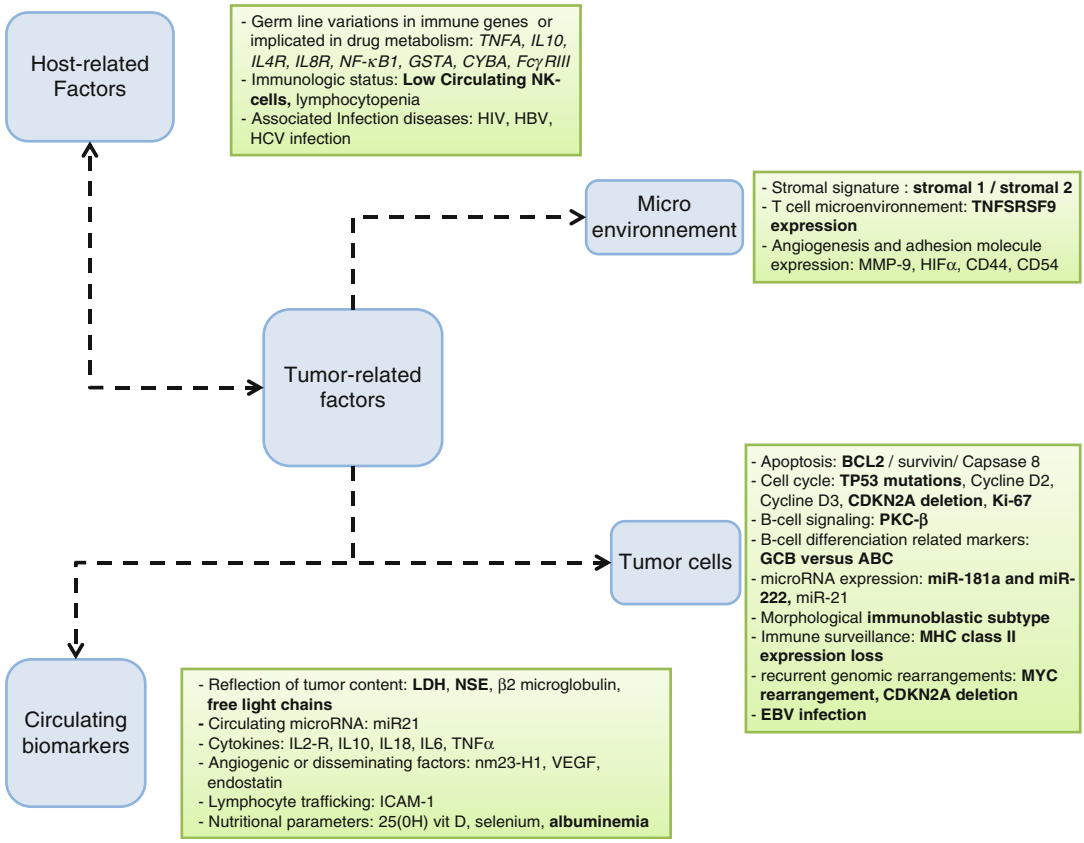

Fig. 11.1 Main biomarkers identified as prognostic factors in DLBCL. Biomarkers displaying a prognostic value in immunochemotherapy are indicated in *bold*

OS and the PFS. In a multivariate analysis controlling for the International Prognostic Index (IPI) score, concordant involvement remained an independent predictor of PFS and OS [29].

Biological Factors

Because patients with identical IPI still exhibit marked variability in survival, several biological and molecular markers independent of the clinical variables were identified and highlight the DLBCL heterogeneity. Biological prognostic factors can be classified as factors related to the characteristics of the host and those related to the tumor itself or its microenvironment. Circulating biomarkers has been also delineated, reflecting both biological features of the tumor and of the host (Fig. 11.1).

Host-Related Factors

To date most of the biological factors related to the host are directly or indirectly associated to the immune status or response to immunochemotherapy. Genetic determinants of the immune response including germline variations of *TNFα; IL10, IL4R,* or *IL8R;* or *NF-κB* genes have been identified as factors influencing DLBCL outcome, but their relevance in the context of immunochemotherapy remains to be confirmed [50, 51]. Some SNP located on *CYBA* and *GSTA1* genes, affecting doxorubicin pharmacodynamics and alkylator detoxification, were also identified as predictive of the outcome in R-CHOP-21-treated DLBCL [52]. By contrast, the 158 V/V RFCγIII genotype, known to be related to a more efficacy of rituximab, appears to play only a marginal prognostic impact in DLBCL patients treated by R-CHOP [53, 54]. The prognostic impact of the

immunologic status, assessed by either total lymphocyte count or by the determination of circulating NK-cell count, has been also recently underscored in the context of chemotherapy or immunochemotherapy [55, 56]. In keeping with these observations, coinfections with HIV, HBV, EBV, and HVC must be also considered as important prognostic factors, leading to specific clinical and biological features and adapted therapeutic strategies [57–61].

Biomarkers Related to the Tumor Cells and GCB/ABC Gene Expression Profile Surrogates to Predict the Outcome

Several individual biomarkers, mainly assessed by immunohistochemistry, have been identified, but regarding the retrospective nature of most studies and the lack of the definition of optimal and reproducible cut points, only a few of them are validated and to date none is widely used in a daily practice to tailor therapeutic strategies. Factors identified as related to the DLBCL outcome and determined by IHC included markers controlling apoptosis (BCL2/survivin/caspase 8), cell cycle (p53, cyclin D2, cyclin D3, Ki-67), B-cell signaling and immune response (PKC-β[beta], HLA-DR), and/or B-cell differentiation (GCB vs. non-GCB) [45, 62–68]. Of note, optimal cut points predicting overall survival were determined for CD5 and Ki67, whereas such cut points are not properly defined for BCL6, HLA-DR, or MUM1 markers in patients treated by immunochemotherapy [45]. Among the most constantly reported factor, BCL2 expression is one of the most robust in the rituximab era and remains predictive of the outcome for both GCB and non-GCB subtypes and can be used in combination with IPI and Ki-67 [45, 65, 69].

At the genomic level, deletions of the tumor suppressor gene *CDKN2A* and *MYC* rearrangements are the most constantly reported abnormalities associated with an unfavorable outcome [70–74]. MicroRNA expression, associated to regulation of several target genes, has been also identified as crucial and powerful biomarkers able to distinguish different physiopathological and prognostic subgroups [75–78].

In 2000, Alizadeh and colleagues identified two major subtypes with distinct outcomes: the germinal center B-cell-like (GCB) subtype, displaying a profile similar to normal germinal center B cell and related to a more favorable prognosis, and the activated B-cell-like subtype, mimicking activated peripheral blood B cells (ABC) and most likely issued from a more mature plasmablast cell [8, 79]. The relevance of this distinction was confirmed by subsequent studies and its prognosis value confirmed in the setting of immunochemotherapy [11, 71]. Considerable efforts in attempt to translate this molecular classification for a daily practice have been made. Different models are currently proposed (Fig. 11.2) using discriminant biomarkers and different technologies, but their clinical relevance is still a matter of debate, underscoring the need for standardization before their extensive usage in routine [45, 66, 67, 70, 73, 80–88]. The combination of a clinical index (IPI); a dynamic factor, such as metabolic response to chemotherapy; or FISH analysis with the GCB/ABC subclassification represents promising approaches to distinguish more accurately prognostic subgroups [31, 89, 90].

Biomarkers Related to Microenvironment

Despite its physiopathological and clinical relevance, the GCB and ABC molecular subclassification does not provide any information regarding the tumor microenvironment. Gene expression experiments defined recently a prognostically favorable stromal-1 signature reflecting extracellular matrix deposition and histiocytic infiltration and a prognostically unfavorable stromal-2 signature reflecting mainly tumor blood-vessel density. The stromal-1 and stromal-2 signatures are independent of the GCB/ABC subclassification and can be identified by IHC markers [11, 91]. These gene expression profile data are in accordance with previous studies that showed the unfavorable prognosis value of tumor angiogenesis or the expression of proangiogenic factors by the tumor or its microenvironment [92–94].

The combination of the expression of two genes, *LMO2*, a GCB marker, and *tumor necrosis*

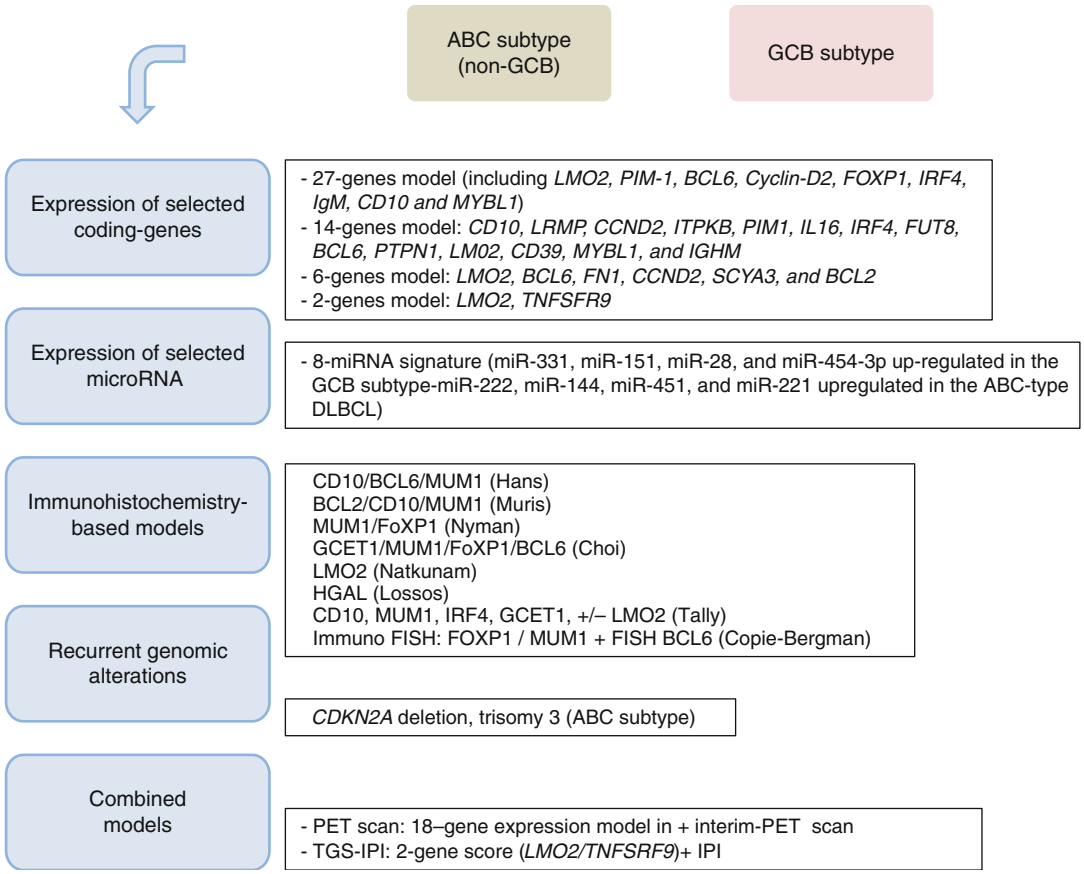

Fig. 11.2 The GCB/ABC molecular classification and potential surrogates with prognostic implication

factor receptor superfamily member 9 (TNFRSF9) by T cell of the microenvironment, has been recently proposed as a predictive score and validated in three independent cohorts of patients treated by immunochemotherapy [89].

Circulating Biomarkers

Some biomarkers evaluable in the serum of DLBCL patients have been identified as prognostic factors and can be routinely tested for some of them. These markers reflect the secretion or components of the tumor cells (NSE, LDH, clonal free light chains, β[beta]2 microglobulin); the tumor microenvironment (proangiogenic factors such as sVEGF or endostatin) production of miRNA by the tumor (miR21) can be related to secretion of proinflammatory or immunoregulatory cytokines (IL10, IL6, etc.) or classified as nutritional parameters (vitamin D insufficiency, selenium, albuminemia) [50, 95–104].

Response Evaluation

In 1999, an International Working Group (IWG) of clinicians, radiologists, and pathologists with expertise in the evaluation and management of patients with non-Hodgkin's lymphoma (NHL) published guidelines for response assessment and outcomes measurement. These criteria widely adopted in daily practice and clinical trial setting were reassessed and led to updated criteria that incorporate PET, IHC, and flow cytometry for definitions of response. These criteria are not specifically dedicated to DLBCL and are applicable at the end of the treatment. PET scan criteria

responses have been also recently proposed for interim or midterm treatment evaluation [7, 38].

Treatment

Principles

Immunochemotherapy, association of anti-CD20 monoclonal antibody and chemotherapy, is now the rule for every patient with DLBCL. Over the past 10 years, this association has both improved remission rates and survival outcomes of patients with DLBCL [105]. Pending the advent of newer targeted therapies, approaches of improvement could include modifications of the association with different chemotherapy schemes or variations in the administration of rituximab.

The principles laid down for over 20 years are still valid [106]. Specifically, cure must be obtained with frontline treatment and achieving complete remission is necessary for a prolonged survival. This explains the many attempts of intensification of chemotherapy and now of immunotherapy.

The International Prognostic Index helped to distinguish patient populations with very different clinical outcomes [107]. Many cooperative groups have stratified treatment approaches according to age and prognostic categories of the IPI. The IPI has been validated in the rituximab era and is a support to guidelines in DLBCL [48, 108].

First-Line Treatment

Low-Risk Patients, IPI = 0

These patients have a localized disease (stage I or II) and normal serum LDH level; a proportion of them have a bulky disease. In the 1990s, a study of the Southwest Oncology Group (SWOG) established the association of three cycles of CHOP followed by involved-field irradiation as a standard of treatment as compared with CHOP alone [109]. However, longer follow-up of this trial has shown a similar outcome in both treatment groups.

Thereafter, the Groupe d'Etudes des Lymphomes de l'Adulte (GELA) conducted a randomized study comparing four cycles of CHOP alone with four cycles of CHOP followed by involved-field radiotherapy in a population of 576 elderly patients with IPI = 0 [110]. The 5-year estimates of event-free (EFS) and overall survival (OS) did not differ between the two groups. With a follow-up of 7 years, the number of observed second cancers was twice in the chemoradiotherapy group.

In the same time, the GELA ran a study comparing three cycles of CHOP followed by radiotherapy and the intensified regimen ACVBP followed by sequential chemotherapy in 647 patients less than 60 years [111]. With a median follow-up of more than 7 years, EFS and OS were significantly longer in the group given ACVBP than in the group given CHOP plus radiotherapy. The 5-year estimates of event-free survival were 82 % for patients receiving ACVBP and 74 % for those receiving chemoradiotherapy. The 5-year estimates of OS were 90 % in the ACVBP group and 81 % in the CHOP plus radiotherapy group. These results were independent of the presence of a bulky disease. The rate of second cancer was the same in the two treatment groups. The German High-Grade Non-Hodgkin's Lymphoma Study Group (DSHNHL) investigated whether CHOP given every 2 weeks (CHOP-14) or the addition of etoposide (CHOEP-21, CHOEP-14) could improve results of six cycles of CHOP given every 3 weeks in patients younger than 60 years with normal LDH level [112]. Most patients had an adjusted IPI equal to 0. In this trial, patients received radiotherapy to sites of initial bulky disease and extranodal disease. In a 2×2 factorial analysis, the addition of etoposide was shown to improve the EFS, but the reduction of the interval was not associated with a longer EFS in this population.

In 2006, the Mint trial demonstrated the superiority of the association of rituximab and CHOP-like chemotherapy versus chemotherapy alone in patients less than 60 years with good-prognosis DLBCL [6]. Radiotherapy was given to sites of primary bulky disease or to extranodal sites. Of the 824 patients included, 352 had an age-adjusted IPI to 0. The subgroup of patients who had no bulky disease and IPI = 0 and who were treated with the association had a 3-year event-free survival of 89 % and an overall survival of 98 %. The association of etoposide did not benefit for patients

treated with rituximab (R-CHOP). Patients of the favorable subgroup (no bulky disease and IPI = 0) treated with CHOP and rituximab had a 3-year EFS of 97 % and an OS of 100 %. The excellent results of immunochemotherapy in this population have been confirmed with the association of rituximab and more intensive ACVBP [113] without the use of consolidation radiotherapy.

In the low-risk population, the European groups are now testing the possibility of treatment reduction. In the ongoing FLYER trial from the DSHNHL, the standard six cycles of CHOP and rituximab are compared to four cycles of CHOP and six infusions of rituximab. In the LNH09–1B study from the GELA, reduction of treatment is only proposed to patients who had a negative PET after two cycles of CHOP and rituximab.

Intermediate-Risk Patients <60 Years, Age-Adjusted IPI = 1

This risk category comprises either patients with localized-stage disease and elevated LDH level or patients with disseminated disease and normal LDH level. The outcome characteristics of both groups are usually similar.

In the Mint trial, patients with age-adjusted IPI to 1 were considered together with patients with age-adjusted IPI to 0 with bulky disease in a less favorable subgroup [6]. In this subgroup, 3-year EFS was 78 % for patients who received CHOP-21 and rituximab and was 76 % for those who received CHOEP-21 and rituximab. Three-year OS was 90 % for patients treated with CHOP-21 and rituximab and 93 % for patients treated with CHOEP-21 and rituximab. The association of a short treatment with three cycles of R-CHOP followed by involved-field radiotherapy has been investigated in a phase 2 study in 60 patients with limited-stage disease and at least one adverse risk factor (nonbulky stage II disease, age >60 years, performance status of 2, or elevated serum LDH) [114]. Although progression-free survival (PFS) was 88 % and OS was 92 % respectively at 4 years, the pattern of continuing late relapse remained a concern.

In the GELA study LNH03–2B, 380 patients with age adjusted equal to 1 were randomly assigned to R-ACVBP or R-CHOP [115]. R-ACVBP regimen contains a phase of intensified cycle induction with rituximab, doxorubicin, cyclophosphamide, vindesine, bleomycin, and prednisone followed by a consolidation phase with high-dose methotrexate, ifosfamide, etoposide, rituximab, and cytosine arabinoside. Complete remission rates were similar in the two groups. Grade 3–4 hematological toxicity was more frequent in the R-ACVBP group, resulting in increasing proportion of neutropenic episodes during treatment (39 % vs. 9 %). After a follow-up of 44 months, the 3-year PFS and OS of patients treated with R-ACVBP improved from 73 to 87 % ($p = 0.0015$) and 84 to 91 % ($p = 0.0071$), respectively.

High-Intermediate- and High-Risk Patients <60 Years, Age-Adjusted IPI = 2, 3

In this category of young patients with a 5-year survival of less than 50 % [107] in the 1990s, conventional chemotherapy was clearly considered as suboptimal. High-dose chemotherapy (HDC) with autologous stem-cell transplantation (ASCT) has been widely used as part of first-line treatment. Although several prospective trials have shown that this subgroup could benefit from intensive treatment, no trial has clearly demonstrated a prolongation of overall survival in this population [116–120]. A meta-analysis reporting 2,728 patients included in comparative trials yielded conflicting results for poor-risk population [121]. The recent results of the randomized SWOG S9704 comparing eight cycles of (R)CHOP and six cycles of (R)CHOP followed by ASCT have shown an improvement of PFS in patients treated with high-dose therapy who attained a partial or complete remission [122]. However, it appeared that the ASCT benefit was mainly observed in the high-IPI group.

In the rituximab era, several phase 2 studies have described intensive induction regimens followed by ASCT (Table 11.2) [123–126]. Results of these studies yielded very homogeneous results in this population despite different regimens. Historical comparisons suggested that the addition of rituximab to HDC followed by ASCT is effective in this poor-risk group where 4-year EFS is now above 70 % and 4-year OS reaches

Table 11.2 Phase 2 studies including intensive induction regimens followed by ASCT in DLBCL patients initially treated by rituximab

Author	*n*	Prognostic factors	Treatment	EFS at 4 years (%)	OS at 4 years (%)
Tarella C	112	aaIPI: 2–3	Sequential therapy and ASCT	73	76
Glass B	64	Elevated LDH	R-MegaCHOEP and ASCT	73 (at 3 years)	79 (at 3 years)
Vitolo U	94	aaIPI: 2–3	R-MegaCHOEP, R-MAD, and ASCT	73	80
Fitoussi O	209	aaIPI: 2–3	R-ACVBP and ASCT	76	78

80 %. However, recent results of a trial conducted by the DSHNHL appeared to indicate that an intensified conventional regimen (R-CHOEP-14) could be as effective and less toxic than HDC with ASCT (R-MegaCHOEP) [127]. In this study, the conventional immunochemotherapy yielded a 3-year EFS of 70 % and a 3-year OS of 85 %. In order to only propose HDC to patients with higher risk, the GELA conducted a trial where the intensity of consolidation was driven by the results of interim PET [128].

Intermediate- to High-Risk Patients from 60 to 80 Years Old

The beginning of the era of immunochemotherapy has been marked by the results of the GELA trial LNH98-5 published in 2002 [5]. This study compared CHOP alone versus R-CHOP in 399 patients aged from 60 to 80 and a DLBCL. Eight cycles were given with a 3-week interval. The complete remission rate was significantly higher in the R-CHOP group (76 % vs. 63 %, $p=0.0005$). The results from the 10-year analysis have confirmed the survival benefit and tolerability of the addition of rituximab to CHOP [129]. The 10-year PFS was 36.5 % in the R-CHOP group compared with 20 % in patients treated with CHOP alone. The 10-year OS was 43.5 % compared with 27.6 %. The risk of death due to concomitant diseases, secondary cancers, and late relapses was the same in both groups. These results were confirmed in the US Intergroup study E4494 in the same population [130]. Patients were randomized to receive R-CHOP or CHOP alone. Rituximab in the R-CHOP group was only given every two cycles. A second randomization to maintenance rituximab or observation was proposed to responding patients. The 3-year failure-free survival was 53 % in the R-CHOP group and 46 % in the CHOP group ($p=0.004$). However, OS difference did not reach significance at this analysis. Patients treated with CHOP alone experienced benefit from rituximab maintenance, but maintenance did not prolong failure-free survival in patients who received R-CHOP.

Several trials in the pre-rituximab era have questioned the possibility to give more intensive chemotherapy to these elderly patients. In a study comparing ACVBP to CHOP, the GELA demonstrated a benefit in EFS and OS for intensive treatment [131]. However, exploratory analyses indicated that the majority of the ACVBP benefit occurred in the group of patients aged from 60 to 65 years. Patients older than 65 years treated with ACVBP had excessive toxicity. In 2004, the DSHNHL have shown that patients with DLBCL aged from 60 to 75 have longer EFS and longer OS when treated with biweekly CHOP (CHOP-14) as compared with standard CHOP-21 [132]. This dose-dense regimen was later used in the RICOVER trial to address the question of the addition of rituximab to chemotherapy and the question of the number of cycles to deliver [133]. Patients were randomly assigned to receive six or eight cycles of chemotherapy with or without 8 biweekly dosings of rituximab. Again, this trial showed that the addition of rituximab improved EFS (66 % vs. 47 % at 3 years) and OS (78 % vs. 68 % at 3 years). Furthermore, the RICOVER trial showed that survival was not further improved by eight cycles of R-CHOP-14 as compared with six cycles. Given the favorable results by R-CHOP-14 regimen, a direct comparison with standard R-CHOP-21 was necessary. The

Table 11.3 Comparative incidence of CNS recurrence in patients treated with CHOP and R-CHOP

Author	*n*	Type of study	CHOP (%)	R-CHOP (%)	*p*
Feugier P	399	Prospective	4.6	5.4	NS
Villa D	435	Retrospective	9.7	6.4	NS (trend)
Boehme V	1,222	Prospective	6.9	4.1	0.046
Yamamoto W	375	Retrospective	2.9	3.9	NS
Chihara D	386	Retrospective	7.3	5.9	NS
Tai WM	499	Retrospective	5.1	6.0	NS

GELA conducted a randomized comparison between eight cycles of R-CHOP-21 and eight cycles of R-CHOP-14 in patients aged 60–80 [134]. The interim analysis did not show any significant difference in survival from both regimens. Hematological toxicity was more important in the R-CHOP-14 group. A similar trial has been conducted in the United Kingdom in 1,080 adult patients without age or stage restrictions [135]. Patients were randomly assigned to receive six cycles of R-CHOP-14 and two additional infusions of rituximab or eight cycles of R-CHOP-21. After a follow-up of 39 months, EFS and OS were not different between the two groups.

Central Nervous System (CNS) Prophylaxis

The incidence of CNS relapse in the evolution of patients treated for DLBCL ranges between 2 and 14 % according to initial risk factors and the treatment received [136]. An elevation of LDH, multiple extranodal sites, a bulky disease, a high-risk IPI, and specific extranodal sites as bone marrow, testis, breast, or Waldeyer ring are the main risk factors described [17–21]. Most recurrences occur during the first months of the course of the disease, often during treatment [131, 137]. This suggests the possibility of occult CNS disease at the time of diagnosis. The outcome of CNS relapse is particularly poor with a median PFS less than 3 months and only few patients alive at 2 years [18, 137].

Intrathecal injections of methotrexate have been the most widely used means of prevention. However, their efficacy has not been firmly established, even in high-risk patients [137, 138]. Demonstration of efficacy of a combined prophylaxis has been offered in a GELA trial comparing ACVBP, which contained four intrathecal injections of methotrexate and two intravenous infusions of high-dose methotrexate with leucovorin rescue, and CHOP which contained no CNS prevention [131]. ACVBP was associated with a reduced incidence of CNS relapses (3 % vs. 8 %, $p = 0.002$). Other series have shown a low incidence of CNS relapse in high-risk patients treated with high-dose intravenous methotrexate [17, 139]. Several studies have compared the incidence of CNS relapse in cohorts of patients treated with CHOP or R-CHOP (Table 11.3) [18–21, 140, 141]. Only the DSHNHL study gave strong arguments in favor of a decreased risk of relapse after treatment with rituximab [19].

Second-Line Treatment

First-line regimens associated with chemotherapy and rituximab have greatly improved complete remission rates and PFS of patients with DLBCL, but now the possibilities of salvage therapy became more difficult.

Since the results of the PARMA trial in 1995, treatment of relapsed or refractory patients with salvage therapy followed with HDT with ASCT has been considered as the standard [142]. Several chemotherapy combinations with different drugs than those used in first line have been proposed as DHAP (dexamethasone, cytarabine, and cisplatin) [143], ESHAP (etoposide, cytarabine, cisplatin, and prednisone) [144], IVAM (ifosfamide, etoposide, cytarabine, and methotrexate) [145], and ICE (ifosfamide, carboplatin, and etoposide) [146]. In the year 2000, rituximab was

incorporated to these salvage regimens with a substantial improving of response rates [147]. The HOVON group, in the Netherlands, conducted a phase 3 randomized trial where patients were assigned to receive DHAP or DHAP associated to rituximab (R-DHAP) [148]. Responsive patients received HDT and ASCT. The R-DHAP yielded better response rate (75 % vs. 54 %; $p = 0.01$) and improved PFS at 2 years (52 % vs. 31 %; $p = 0.002$).

The Collaborative Trial in Relapsed Aggressive Lymphoma (CORAL) was a collaborative study conducted in 396 patients in first relapse or who were refractory after first-line therapy [149]. It gave important information on salvage regimens, prognostic factors, and efficacy of maintenance treatment with rituximab after ASCT. First, it showed no significant difference between R-ICE and R-DHAP for complete response rate (63 % both), EFS, and overall survival. In a recent subgroup analysis, the GCB-like DLBCL as assessed by histochemistry according to the algorithm by Hans was significantly associated with a better PFS in the R-DHAP arm [150]. Second, three factors was associated with an unfavorable prognostic: an IPI more than 1 at relapse, an occurrence of relapse less than 12 months after initial diagnosis, and the administration of rituximab during first-line treatment. It is to underline that the 3-year EFS of the larger cohort of patients ($n = 187$) who had received previously rituximab and relapsed during the first year was less than 15 %. Third, patients who received the ASCT were randomized between observation and maintenance with rituximab every 2 months for 1 year. There was no difference in EFS, PFS, and OS between rituximab and observation groups [151].

A proportion of patients is not candidate for intensive salvage therapy and HDT plus ASCT; several attenuated regimens containing rituximab as R-GEMOX (rituximab, gemcitabine, and oxaliplatin) [152] or R-GIFOX (rituximab, ifosfamide, and oxaliplatin) [153] have been proposed [154]. For patients who relapsed after first-line or salvage ASCT, allogeneic transplantation could be a second salvage possibility [155].

Treatment According to Molecular Subtypes

Clinical heterogeneity, despite a similar morphologic appearance, is partially explained by distinct gene expression profiles and distinct related genetic molecular basis. These molecular bases become important information to guide new therapeutic strategies [156]. Of note, in addition to the two well-recognized molecular subtypes, namely, the GCB and the ABC subtypes, gene expression profile studies refined additional and interconnected pathways that may represent relevant targets.

Targeting the ABC Subtype-Related Pathways

Pathways predominantly involved in this molecular subtype include constitutive activation of the NF-κB pathway and chronic activation of the BCR signaling.

NF-KB Pathway

Constitutive activation of the NF-KB pathway is observed in the ABC subtype and several agents able to block this pathway are in preclinical or clinical development. Bortezomib, a proteasome inhibitor that blocks the degradation of the phosphorylated form of IκBα, had shown promising results in combination with immunochemotherapy for the treatment of ABC DLBCL, with acceptable toxicity [157–159]. Bortezomib as a single agent has no or low activity in relapsed/refractory DLBCL but shown synergy with DA-EPOCH chemotherapy, leading to significant different response rates (83 % ORR with 41. 5 % CR for ABC vs. 13 % for GCB) and overall survival (10.8 vs. 3.4 months) in ABC compared with GCB [2]. Discordant results were obtained in a phase II trial, where 40 DLBCL were treated by R-CHOP-21 plus bortezomib at escalading dosage, showing that non-GCB and GCB subtypes had similar outcomes [158]. Of note, the mechanism of action of the bortezomib is unclear and may also implicate inhibition of the aggressome and the activation of the unfolded protein stress response, explaining its activity in combination with chemotherapy in the GCB subtype [160, 161]. Other agents can be used to potentiate the

proteasome inhibitors, such as BCL2 antagonists or HDAC inhibitors, improving results obtained in the ABC subtype [162–164]. Another promising approach to block NF-κB activity has been recently described, based on the pharmacological inhibition of MALT1, a proteolytic molecule which is constitutively activated in the ABC subtype [165].

In a retrospective analysis of 40 DLBCL in relapse treated by lenalidomide, acting as a pleiotropic immunomodulatory drug, the response rate appears significantly better in the non-GCB subtype (53 %) as compared to the rate seen in the GCB subtype (9 %) [166]. Its precise mechanism of action remains to be determined in this DLBCL setting.

BCR Signaling

Chronic ABC signaling is a feature of the ABC subtype than can be interrupted by several inhibitors currently in clinical development. The signaling can be interrupted by inhibiting the SRC family kinase BTK, SYK, and PKC-β[beta] or the PI-3 K-mTOR pathway [167, 168]. The SYK inhibitor fostamatinib disodium (R406) is an orally available SYK inhibitor under development for rheumatoid arthritis but also evaluated in a phase I/II trial in a variety of lymphoma subtypes, leading to objective responses in 22 % of heavily pretreated DLBCL lymphomas [169]. Another approach to interrupting BCR signaling includes the use of BTK inhibitors. BTK is a component of the BCR signaling pathway and is downstream of Syk. It is expressed in B cells, mast cells, and monocytes and has an important function in B-cell activation. PCI-32765 is an oral BTK inhibitor that is in phase I for NHL and phase II studies are ongoing [170]. Dasatinib is a multikinase inhibitor that has especially high activity against SRC -family tyrosine kinases, leading to inactivation of the NF-κB, AKT, and ERK-MAP kinases pathway and the death of ABC DLBCL with chronic active BCR [171]. PKC-β[beta] is highly expressed in refractory DLBCL and considered as an adverse prognostic factor. Enzastaurin, a small-molecule inhibitor of PKC-β[beta], had shown clinical responses in a small fraction of DLBCL and is currently evaluated in maintenance therapy in DLBCL [62, 172].

Additional targets involved directly or indirectly the BCR signaling are currently investigated in clinical trials for DLBCL, including inhibitor of the MAP kinase pathway, antibodies targeting CD19, inhibitor of PI3K, and inhibitor of mTOR (see Fig. 11.3).

Targeting the GCB Subtype-Related Pathways

At the molecular and genomic level, the GCB DLBCL subtype is characterized by the more frequent occurrence of the t(14;18) translocation, the deletion of the *PTEN* gene, mutations of *TP53* and amplification of *c-REL,* and a high expression of *BCL6*, delineating potential targets for new agents. Molecules inhibiting BCL6 constitute promising agents in preclinical development for BCL6-positive lymphomas by modulating its level of acetylation (niacinamide or cambinol), by interacting with its dimerization domain, or by modifying its functional interaction with chaperone heat shock protein 90 (Hsp90) [173–176].

Combination of conventional chemotherapy agents has shown different efficacies according to the molecular subtype. The DA-EPOCH regimen displayed an apparent better efficacy in GCB DLBCL and BCL6-positive DLBCL than in ABC DLBCL. To explain such difference, it has been hypothesized that the GCB molecular subtype, mainly under the dependence of the BCL6 transcriptional program (characterized by the repression of p21/TP53/ATR and p27kip1), may be more sensitive to DNA damage and therefore to prolonged chemotherapy exposure [177]. Different effects of chemotherapy combination have been also recently suggested by the CORAL trial, showing that the GCB/ABC subclassification remains a major and independent factor in relapsed/refractory DLBCL, with a better response to R-DHAP in GCB DLBCL, as compared to ABC DLBCL patients [150].

Of note, a minority of GB DLBC may activate NF-κB, displaying mutations of CARD11 (in less than 4 % of cases), and can also display a chronic active BCR with mutations of the CD79B ITAM motifs, suggesting an overlap between the two molecular subgroups with common therapeutic opportunities [178].

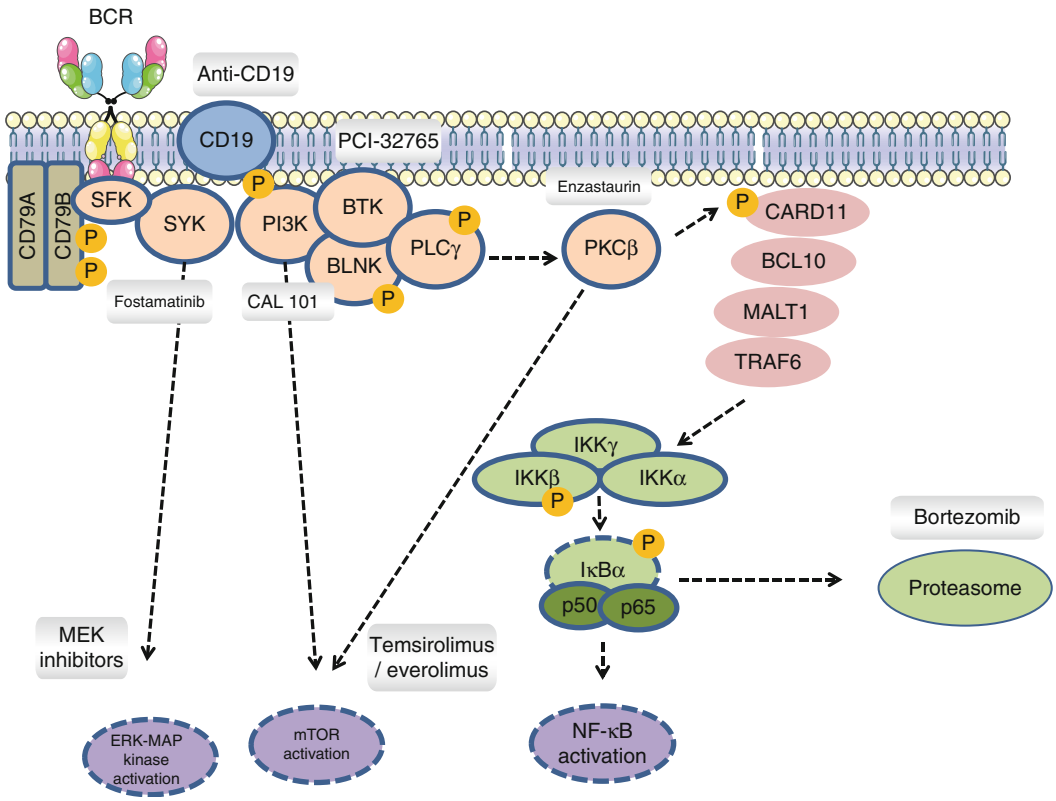

Fig. 11.3 B-cell receptor (BCR) and nuclear factor-κB (NF-κB) signaling pathways and potential targets for therapeutic agents in clinical development

Angiogenesis and Stromal Signature

The microenvironment has been also highlighted by recent gene expression profile studies. The stromal-2 signature is characterized by an increase of angiogenesis and an unfavorable prognosis, as compared to DLBCL expressing the stromal-1 signature. Expression of proangiogenic factors in serum or detected on the tumor by IHC is also related to impairment of the prognosis, giving a rationale to target angiogenesis in the treatment of DLBCL. Furthermore, angiogenesis appears more prominent in the ABC subtype, suggesting that targeting vessels will also be a benefit in this molecular subtype [179]. Based on phase I, bevacizumab has been used in combination with R-CHOP but was associated with a high toxicity rate leading to the premature discontinuation of the phase III trial [180, 181]. Of note, no information regarding the molecular microenvironment signature (viz., stromal 1/stromal 2) was provided by these studies, and to date the benefice of such strategy remains to be determined. The encouraging results obtained with lenalidomide in the non-GCB subgroup may be related to its antiangiogenic action but also to several additional targets and molecular pathways. To date the stromal-1 signature, reflecting extracellular matrix deposition and infiltration of the tumors with macrophage, has not been selectively targeted in lymphoma, but some antifibrotic agents may be useful in this setting [11].

Specificities for Patients Older than 80 Years

DLBCL is the most common lymphoma and its incidence is strongly related to increasing age. Population-based studies have reported that about 20 % of DLBCL patients are older than 80 years.

After 75 years, the rate of DLBCL increases to 1.4 % per year [2]. It is usually assumed that elderly patients are too frail to receive optimal chemotherapy regarding their frequent comorbidities and physiological organ function impairment, leading to non-manageable treatment toxicity. However, clinical trials dedicated to elderly patients provided a more comprehensive view of the geriatric DLBCL specificities and demonstrated that a curative approach is currently realistic in patients older than 80 years.

Biological Specificity

Despite the lack of well-conducted comparative studies, it appears that DLBCL in elderly patients are characterized by some specific histopathological and biological features as compared to young patients. Three histological DLBCL subtypes are clearly more frequently or almost exclusively observed in a geriatric context. (1) EBV-positive diffuse large B-cell lymphoma (DLBCL) of the elderly is an entity recently included in the WHO classification and mainly described in the Japanese population with a high geographic disparity. The median age is 75 years and 25 % of EBV + DLBCL are older than 90 years with a male predominance, advanced stage and B symptoms in most of cases, high IPI score, frequent extranodal localization (lung, skin), and a non-GCB phenotype [182–184]. (2) Primary cutaneous diffuse large B-cell lymphoma, leg type, is characterized by a predilection for the leg and an advanced age at onset (mean age, 76 years) [185]. (3) The pyothorax-related lymphoma, belonging to the spectrum of chronic inflammation-associated lymphomas, developing in the setting of long-standing chronic inflammation and typically associated with Epstein-Barr virus, is observed most exclusively in the historical context of elderly patients previously treated for tuberculosis by therapeutic pneumothorax [186, 187].

To date, no clear specific genomic characteristics in the geriatric setting have been described, but comparative studies with younger patients are still lacking. Recent studies have been demonstrated that the proportion of activated B-cell-like subtype among de novo diffuse large B-cell lymphoma increases with age. Two independent large series have shown that the percentage of ABC DLBCL increases with age, with an average increase of the ABC DLBCL proportion of 7–13 % per 10 years of aging after 50 years [11, 188]. Attempts to explain such an ABC skewing distribution during aging remain speculative and may be related to EBV infection or change in the normal B-cell repertoire during ageing [188].

Clinical Features in Elderly DLBCL Patients

By comparison to younger patients, no clear clinical or biological specificities in older patients have been reported [189–191]. The prevalence of B symptoms is observed in one-third of patients older than 80 years, an increase of LDH level in 43.4–68 %, and a stage III–IV disease in approximately two-thirds of cases [189–191].

Prognostic Factors, Staging, and Organ Functional Assessment

In addition to a more frequent unfavorable ABC gene expression profile, some prognostic factors have been identified as more relevant in a geriatric context than the IPI score. Nutritional parameters may play a crucial in the geriatric setting. Albuminemia has been the only prognostic factor retained in multivariate analysis in a large phase II trial involving patients uniformly treated by dose reduced intensity CHOP plus rituximab [189]. Vitamin D deficiency has been associated with an unfavorable prognosis in a large cohort and may be integrated in the usual poor nutritional status observed in elderly patients [97]. Of note, if the different components of the IPI score remain pertinent, IPI and age, the prognostic discrimination provided by the E-IPI for low and low-intermediate elderly DLBCL patients appears better than the R-IPI or the aaIPI [191, 192].

18-FDG PET scan displays a very high rate of sensitivity and specificity, but its relevance in a geriatric setting has not been specifically addressed. In a practical aspect, PET scan is recommended in the staging of DLBCL but can be performed only if the patient is able to stay immobilized during at

least 30 min. Oral antidiabetics (metformin) also constitute some limitations during this examination in a geriatric context [26].

Bone marrow biopsy in patients higher than 80 years is recommended for initial staging, especially in case of cytopenia to avoid concomitant associated myelodysplasia. In a recent meta-analysis, the sensibility and specificity of 18-FDG PET for evaluation of bone marrow in aggressive NHL lymphoma showed a sensitivity of 74 % and a specificity of 84 %, indicating that PET scan may be an alternative approach to detect bone marrow involvement in elderly DLBCL patients [26, 28]. Lumbar puncture, to detect meningeal involvement, is generally dedicated to patients with a high risk of CNS disease, including extranodal involvement (especially testicular), elevated LDH, performance status > 1, or an age-adjusted IPI > 1 [18, 193]. Cardiac assessment by clinical examination, either echocardiography or isotopic measure of the ejection fraction, is mandatory before any decision to use anthracycline-based regimens. Lung function tests are also recommended and influence therapeutic choices [193].

Geriatric Assessment

The rationale to incorporate geriatric assessment in the care of elderly patients in oncology is based on the high degree of heterogeneity of this population in terms of life expectancy, tolerance to chemotherapy, physiological changes, organ dysfunction, comorbidities, and social/environmental life-specific conditions, indicating that age or performance status only does not represent sufficient parameters to guide physicians and therapeutic strategies [194, 195]. The role of such geriatric assessments in the reduction of mortality was summarized in a meta-analysis of 28 control trials [196]. The comprehensive geriatric assessment (CGA) is a multidimensional method used by geriatricians and oncologists to detect and evaluate multiple age-related problems but is time-consuming and often not performed in daily practice [197]. Using assessment of comorbidity, socioeconomic conditions, functional dependence, frailty, emotional and cognitive conditions, nutrition status, and estimated life expectancy, Balducci proposed to define three groups which imply distinct therapeutic strategies: (1) functionally independent patients, without comorbidity, candidates for any form of standard cancer treatment, with the possible exception of bone marrow transplant; (2) frail patients (dependence in one or more activities of daily living, three or more comorbid conditions, one or more geriatric syndromes), candidates only for palliative treatment; and (3) intermediate patients who may benefit from some specific pharmacological approach, such as reduction in the initial dose of chemotherapy with subsequent dose escalation [197]. A simplified method of CGA appears more effective than clinical judgment to identify elderly DLBCL who could benefit from aggressive therapy [198].

In a daily practice, simple and validated tests can be proposed in the aim to detect a frail phenotype. The functional status is adequately assessed by the IADL (instrumental activities of daily living) scale, measuring the abilities of the patient for daily activities, autonomy, and dependence. This 8-criterion scale was associated in a univariate analysis with an unfavorable outcome in DLBCL treated by R-miniCHOP and appears more pertinent than the performance status [189, 199]. Tests, based on the assessment of weight loss, general feeling of exhaustion, weakness (as measured by grip strength), slow walking speed (gait speed or get-up-and-go test), or physical activity, are feasible by most of elderly patients with cancer [199–201]. To assess the comorbid medical status, the Charlson scale can be easily performed and is a prognostic factor independent of the IPI in patients older than 65 years [202]. More specifically, risk factors of chemotherapy toxicity and especially hematological toxicities warrant caution and can be predicted by a geriatric assessment. Its relevance in a lymphoma context remains to be determined [203, 204].

Treatment of Fit Patients in Frontline

Several reports highlighted the high rate of therapy-associated deaths in a geriatric setting after the first cycle of chemotherapy. This "first-cycle effect" has been successfully prevented by a prephase treatment

based on the use of a single injection of 1 mg of vincristine and prednisone, leading in some cases to improve significantly performance status, organ dysfunction and allowing delivering subsequently a more aggressive chemotherapy [189, 193]. Similar to younger patients, the improvement of the outcome for very elderly patients with DLBCL in the immunochemotherapy era has been recently shown in a historical comparison showing an increase of the estimated 3-year progression-free (PFS) and overall survival (OS) of 30 % following the usage of rituximab [205].

In most of cases, rituximab in combination with reduced-intensity anthracycline-based regimen can be proposed in fit patients and represents the current standard strategy in frontline [189, 206, 207]. In a large phase II study of the GELA group involving patients older than 80 years, the efficacy and safety of a decreased dose of CHOP (doxorubicin, cyclophosphamide, vincristine, and prednisone) chemotherapy with a conventional dose of rituximab (R-miniCHOP) was recently investigated. Analysis by intention to treat of the 149 included patients demonstrated a 2-year overall survival of 59 %, a 2-year PFS of 47 %, and a median progression-free survival of 21 months. The most frequent side effect was hematological toxicity (grade >/=3 neutropenia in 40 % of cases) but with infrequent febrile neutropenia (7 %) indicating that R-miniCHOP displays a good compromise between efficacy and safety in this population [189]. Similar results were obtained in a 70 % CHOP reduction regimen for patients older than 70, displaying a 3-year PFS of 72 % and a 3-year OS of 58 % [207]. Activity and safety of dose-adjusted infusional cyclophosphamide, doxorubicin, vincristine, and prednisone chemotherapy (DA-EPOCH) with rituximab in very elderly patients with poor-prognostic untreated DLBCL has been also proposed in frail patients older than 70 years [206].

Alternative Treatments in Frail and Unfit Patients or After Relapse

Patients with frailty phenotype, cardiac dysfunction (ejection fraction <40 %), or relapse following anthracycline-based regimens warrant alternative treatments. Liposomal doxorubicin has been proposed as a possible alternative in combination with rituximab, cyclophosphamide, vincristine, and prednisone (COMP-14) [208, 209]. Phase II trials indicate that non-anthracycline-based regimens may lead to a significant efficacy with an acceptable tolerance profile. Oral lenalidomide in combination with rituximab has shown promising results in patients older than 65 years with a high percentage of patients achieving a continuous CR after maintenance therapy. Of note, DLBCL with a non-GCB phenotype may be particularly sensitive to lenalidomide [166, 210]. Bendamustine in combination with rituximab has been also evaluated in a phase II study, including unfit patients with a median age of 85 years, giving an overall response rate of 69 % [211]. Oxaliplatin-based regimens or a combination of ifosfamide and etoposide have been also proposed as alternative strategies in frontline or in relapse but with unsatisfactory long-term results [152, 212]. Chemotherapy following autologous stem-cell transplantation has been retrospectively evaluated in patients between 60 and 75 years but remains an unrealistic approach in patients older than 80 years [213].

Supportive Care

The use of G-CSF is proposed according to recommendations, based on a risk of febrile neutropenia higher than 20 %, which is the case in this setting [214]. Pegfilgrastim given at day 4 of the R-CHOP regimen appears as the more efficient preventive strategy but has not been specifically evaluated with dose reduced intensity CHOP [215]. Corticoid-related side effects are particularly intense in elderly patients and implicate a careful metabolic follow-up, hyperglycemia being associated with an increase of chemotherapy toxicity [216]. By contrast to patients treated by dose-dense R-CHOP, patients older than 80 years treated by reduced dose intensity CHOP are not at a particular risk to develop *Pneumocystis* pneumonia, and systematic prophylaxis by cotrimoxazole in a geriatric setting cannot be recommended [189]. It has been suggested that the rate of fungal infection could be higher in patients

older than 80 years and treated by chemotherapy plus rituximab. However, to date no clear recommendation regarding the use of antifungal prophylaxis in this setting can be proposed [217].

References

1. Stein H, Chan JKC, Warnke RA, et al. Diffuse large B-cell lymphoma not otherwise specified. In: Swerdlow SH, Campo E, Harris NL, et al., editors. WHO classification of tumours of haematopoietic and lymphoid tissues. Lyon: IARC; 2008. p. 233–7.
2. Morton LM, Wang SS, Devesa SS, Hartge P, Weisenburger DD, Linet MS. Lymphoma incidence patterns by WHO subtype in the United States, 1992–2001. Blood. 2006;107:265–76.
3. Jemal A, Siegel R, Xu J, Ward E. Cancer statistics, 2010. CA Cancer J Clin. 2010;60:277–300.
4. d'Amore F, Brincker H, Christensen BE, et al. Non-Hodgkin's lymphoma in the elderly. A study of 602 patients aged 70 or older from a Danish population-based registry. The Danish LYEO-Study Group. Ann Oncol. 1992;3:379–86.
5. Coiffier B, Lepage E, Briere J, et al. CHOP chemotherapy plus rituximab compared with CHOP alone in elderly patients with diffuse large-B-cell lymphoma. N Engl J Med. 2002;346:235–42.
6. Pfreundschuh M, Trumper L, Osterborg A, et al. CHOP-like chemotherapy plus rituximab versus CHOP-like chemotherapy alone in young patients with good-prognosis diffuse large-B-cell lymphoma: a randomised controlled trial by the MabThera International Trial (MInT) Group. Lancet Oncol. 2006;7:379–91.
7. Cheson BD, Pfistner B, Juweid ME, et al. Revised response criteria for malignant lymphoma. J Clin Oncol (Off J Am Soc Clin Oncol). 2007;25:579–86.
8. Alizadeh AA, Eisen MB, Davis RE, et al. Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. Nature. 2000;403:503–11.
9. Rosenwald A, Wright G, Chan WC, et al. The use of molecular profiling to predict survival after chemotherapy for diffuse large-B-cell lymphoma. N Engl J Med. 2002;346:1937–47.
10. Shipp MA, Ross KN, Tamayo P, et al. Diffuse large B-cell lymphoma outcome prediction by gene-expression profiling and supervised machine learning. Nat Med. 2002;8:68–74.
11. Lenz G, Wright G, Dave SS, et al. Stromal gene signatures in large-B-cell lymphomas. N Engl J Med. 2008;359:2313–23.
12. Conlan MG, Bast M, Armitage JO, Weisenburger DD. Bone marrow involvement by non-Hodgkin's lymphoma: the clinical significance of morphologic discordance between the lymph node and bone marrow. Nebraska Lymphoma Study Group. J Clin Oncol. 1990;8:1163–72.
13. Chung R, Lai R, Wei P, et al. Concordant but not discordant bone marrow involvement in diffuse large B-cell lymphoma predicts a poor clinical outcome independent of the International Prognostic Index. Blood. 2007;110:1278–82.
14. de Kerviler E, de Bazelaire C, Mounier N, et al. Image-guided core-needle biopsy of peripheral lymph nodes allows the diagnosis of lymphomas. Eur Radiol. 2007;17:843–9.
15. Arber DA, George TI. Bone marrow biopsy involvement by non-Hodgkin's lymphoma: frequency of lymphoma types, patterns, blood involvement, and discordance with other sites in 450 specimens. Am J Surg Pathol. 2005;29:1549–57.
16. Cheson BD, Horning SJ, Coiffier B, et al. Report of an international workshop to standardize response criteria for non-Hodgkin's lymphomas. NCI Sponsored International Working Group. J Clin Oncol (Off J Am Soc Clin Oncol). 1999;17:1244.
17. Haioun C, Besson C, Lepage E, et al. Incidence and risk factors of central nervous system relapse in histologically aggressive non-Hodgkin's lymphoma uniformly treated and receiving intrathecal central nervous system prophylaxis: a GELA study on 974 patients. Groupe d'Etudes des Lymphomes de l'Adulte. Ann Oncol. 2000;11:685–90.
18. Feugier P, Virion JM, Tilly H, et al. Incidence and risk factors for central nervous system occurrence in elderly patients with diffuse large-B-cell lymphoma: influence of rituximab. Ann Oncol (Off J Eur Soc Medl Oncol/ESMO). 2004;15:129–33.
19. Boehme V, Schmitz N, Zeynalova S, Loeffler M, Pfreundschuh M. CNS events in elderly patients with aggressive lymphoma treated with modern chemotherapy (CHOP-14) with or without rituximab: an analysis of patients treated in the RICOVER-60 trial of the German High-Grade Non-Hodgkin Lymphoma Study Group (DSHNHL). Blood. 2009;113:3896–902.
20. Villa D, Connors JM, Shenkier TN, Gascoyne RD, Sehn LH, Savage KJ. Incidence and risk factors for central nervous system relapse in patients with diffuse large B-cell lymphoma: the impact of the addition of rituximab to CHOP chemotherapy. Ann Oncol. 2010;21:1046–52.
21. Chihara D, Oki Y, Matsuo K, et al. Incidence and risk factors for central nervous system relapse in patients with diffuse large B-cell lymphoma: analyses with competing risk regression model. Leuk Lymphoma. 2011;52(12):2270–5.
22. Hegde U, Filie A, Little RF, et al. High incidence of occult leptomeningeal disease detected by flow cytometry in newly diagnosed aggressive B-cell lymphomas at risk for central nervous system involvement: the role of flow cytometry versus cytology. Blood. 2005;105:496–502.
23. Quijano S, Lopez A, Manuel Sancho J, et al. Identification of leptomeningeal disease in aggressive B-cell non-Hodgkin's lymphoma: improved sensitivity of flow cytometry. J Clin Oncol. 2009;27:1462–9.
24. Sancho JM, Orfao A, Quijano S, et al. Clinical significance of occult cerebrospinal fluid involvement

assessed by flow cytometry in non-Hodgkin's lymphoma patients at high risk of central nervous system disease in the rituximab era. Eur J Haematol. 2010; 85:321–8.
25. Rosenberg SA. Validity of the Ann Arbor staging classification for the non-Hodgkin's lymphomas. Cancer Treat Rep. 1977;61:1023–7.
26. Cheson BD. Role of functional imaging in the management of lymphoma. J Clin Oncol (Off J Am Soc Clin Oncol). 2011;29:1844–54.
27. Ouvrier MJ, Diologent B, Edet-Sanson A, et al. Influence of PET/CT on radiologists and contrast-enhanced CT on nuclear medicine physicians in patients with lymphoma. Q J Nucl Med Mol Imaging. 2011;55:324–33.
28. Chen YK, Yeh CL, Tsui CC, Liang JA, Chen JH, Kao CH. F-18 FDG PET for evaluation of bone marrow involvement in non-Hodgkin lymphoma: a meta-analysis. Clin Nucl Med. 2011;36:553–9.
29. Sehn LH, Scott DW, Chhanabhai M, et al. Impact of concordant and discordant bone marrow involvement on outcome in diffuse large B-cell lymphoma treated with R-CHOP. J Clin Oncol (Off J Am Soc Clin Oncol). 2011;29:1452–7.
30. Herrmann K, Buck AK, Schuster T, et al. Predictive value of initial 18F-FLT uptake in patients with aggressive non-Hodgkin lymphoma receiving R-CHOP treatment. J Nucl Med (Off Publ Soc Nucl Med). 2011;52:690–6.
31. Lanic H, Mareschal S, Mechken F, et al. Interim positron emission tomography scan associated with international prognostic index and germinal center B cell-like signature as prognostic index in diffuse large B-cell lymphoma. Leuk Lymphoma. 2011;53(1): 34–42.
32. Phan J, Mazloom A, Jeffrey Medeiros L, et al. Benefit of consolidative radiation therapy in patients with diffuse large B-cell lymphoma treated with R-CHOP chemotherapy. J Clin Oncol (Off J Am Soc Clin Oncol). 2010;28:4170–6.
33. Chihara D, Oki Y, Onoda H, et al. High maximum standard uptake value (SUVmax) on PET scan is associated with shorter survival in patients with diffuse large B cell lymphoma. Int J Hematol. 2011; 93:502–8.
34. Meignan M, Itti E, Gallamini A, Haioun C. Interim 18 F-fluorodeoxyglucose positron emission tomography in diffuse large B-cell lymphoma: qualitative or quantitative interpretation—where do we stand? Leuk Lymphoma. 2009;50:1753–6.
35. Lin C, Itti E, Haioun C, et al. Early 18F-FDG PET for prediction of prognosis in patients with diffuse large B-cell lymphoma: SUV-based assessment versus visual analysis. J Nucl Med (Off Publ Soc Nucl Med). 2007;48:1626–32.
36. Haioun C, Itti E, Rahmouni A, et al. [18F]fluoro-2-deoxy-D-glucose positron emission tomography (FDG-PET) in aggressive lymphoma: an early prognostic tool for predicting patient outcome. Blood. 2005;106:1376–81.
37. Spaepen K, Stroobants S, Dupont P, et al. Early restaging positron emission tomography with (18) F-fluorodeoxyglucose predicts outcome in patients with aggressive non-Hodgkin's lymphoma. Ann Oncol (Off J Eur Soc Med Oncol/ESMO). 2002;13:1356–63.
38. Meignan M, Gallamini A, Haioun C. Report on the first international workshop on interim-PET-scan in lymphoma. Leuk Lymphoma. 2009;50:1257–60.
39. Casasnovas RO, Meignan M, Berriolo-Riedinger A, et al. SUVmax reduction improves early prognosis value of interim positron emission tomography scans in diffuse large B-cell lymphoma. Blood. 2011;118(1):37–43.
40. Safar V, Dupuis J, Itti E, Jardin F, Fruchart C, Bardet S, Véra P, Copie-Bergman C, Rahmouni A, Tilly H, Meignan M, Haioun C. Interim [18F]fluorodeoxyglucose positron emission tomography scan in diffuse large B-cell lymphoma treated with anthracycline-based chemotherapy plus rituximab. J Clin Oncol. 2012; 30:184–90.
41. Spaepen K, Stroobants S, Dupont P, et al. Prognostic value of pretransplantation positron emission tomography using fluorine 18-fluorodeoxyglucose in patients with aggressive lymphoma treated with high-dose chemotherapy and stem cell transplantation. Blood. 2003;102:53–9.
42. Cremerius U, Fabry U, Wildberger JE, et al. Pre-transplant positron emission tomography (PET) using fluorine-18-fluoro-deoxyglucose (FDG) predicts outcome in patients treated with high-dose chemotherapy and autologous stem cell transplantation for non-Hodgkin's lymphoma. Bone Marrow Transplant. 2002;30:103–11.
43. Schot BW, Pruim J, van Imhoff GW, Sluiter WJ, Vaalburg W, Vellenga E. The role of serial pre-transplantation positron emission tomography in predicting progressive disease in relapsed lymphoma. Haematologica. 2006;91:490–5.
44. Schot BW, Zijlstra JM, Sluiter WJ, et al. Early FDG-PET assessment in combination with clinical risk scores determines prognosis in recurring lymphoma. Blood. 2007;109:486–91.
45. Salles G, de Jong D, Xie W, et al. Prognostic significance of immunohistochemical biomarkers in diffuse large B-cell lymphoma: a study from the Lunenburg Lymphoma Biomarker Consortium. Blood. 2011;117:7070–8.
46. Sehn LH, Berry B, Chhanabhai M, et al. The revised International Prognostic Index (R-IPI) is a better predictor of outcome than the standard IPI for patients with diffuse large B-cell lymphoma treated with R-CHOP. Blood. 2007;109:1857–61.
47. Bari A, Marcheselli L, Sacchi S, et al. Prognostic models for diffuse large B-cell lymphoma in the rituximab era: a never-ending story. Ann Oncol (Off J Eur Soc Med Oncol/ESMO). 2010;21:1486–91.
48. Ziepert M, Hasenclever D, Kuhnt E, et al. Standard International prognostic index remains a valid predictor of outcome for patients with aggressive CD20+ B-cell lymphoma in the rituximab era. J Clin Oncol (Off J Am Soc Clin Oncol). 2010;28:2373–80.

49. Pfreundschuh M, Kuhnt E, Trumper L, et al. CHOP-like chemotherapy with or without rituximab in young patients with good-prognosis diffuse large-B-cell lymphoma: 6-year results of an open-label randomised study of the MabThera International Trial (MInT) Group. Lancet Oncol. 2011;12:1013–22.
50. Giachelia M, Voso MT, Tisi MC, et al. Interleukin-6 plasma levels are modulated by a polymorphism in the NF-κB1 gene and are associated with outcome following rituximab-combined chemotherapy in diffuse large B-cell non-Hodgkin lymphoma. Leuk Lymphoma. 2011;53(3):411–6.
51. Habermann TM, Wang SS, Maurer MJ, et al. Host immune gene polymorphisms in combination with clinical and demographic factors predict late survival in diffuse large B-cell lymphoma patients in the pre-rituximab era. Blood. 2008;112:2694–702.
52. Rossi D, Rasi S, Franceschetti S, et al. Analysis of the host pharmacogenetic background for prediction of outcome and toxicity in diffuse large B-cell lymphoma treated with R-CHOP21. Leuk (Off J Leuk Soc Am Leuk Res Fund UK). 2009;23:1118–26.
53. Kim DH, Jung HD, Kim JG, et al. FCGR3A gene polymorphisms may correlate with response to frontline R-CHOP therapy for diffuse large B-cell lymphoma. Blood. 2006;108:2720–5.
54. Ahlgrimm M, Pfreundschuh M, Kreuz M, Regitz E, Preuss KD, Bittenbring J. Impact of Fc-gamma receptor polymorphisms in elderly patients with diffuse large B-cell lymphoma treated with CHOP with or without rituximab. Blood. 2011;118(17):4657–62.
55. Oki Y, Yamamoto K, Kato H, et al. Low absolute lymphocyte count is a poor prognostic marker in patients with diffuse large B-cell lymphoma and suggests patients' survival benefit from rituximab. Eur J Haematol. 2008;81:448–53.
56. Plonquet A, Haioun C, Jais JP, et al. Peripheral blood natural killer cell count is associated with clinical outcome in patients with aaIPI 2–3 diffuse large B-cell lymphoma. Ann Oncol (Off J Eur Soc Med Oncol/ESMO). 2007;18:1209–15.
57. Wang F, Xu RH, Luo HY, et al. Clinical and prognostic analysis of hepatitis B virus infection in diffuse large B-cell lymphoma. BMC Cancer. 2008;8:115.
58. Besson C, Canioni D, Lepage E, et al. Characteristics and outcome of diffuse large B-cell lymphoma in hepatitis C virus-positive patients in LNH 93 and LNH 98 Groupe d'Etude des Lymphomes de l'Adulte programs. J Clin Oncol (Off J Am Soc Clin Oncol). 2006;24:953–60.
59. Morales D, Beltran B, De Mendoza FH, et al. Epstein-Barr virus as a prognostic factor in de novo nodal diffuse large B-cell lymphoma. Leuk Lymphoma. 2010;51:66–72.
60. Carbone A, Cesarman E, Spina M, Gloghini A, Schulz TF. HIV-associated lymphomas and gamma-herpesviruses. Blood. 2009;113:1213–24.
61. Park S, Lee J, Ko YH, et al. The impact of Epstein-Barr virus status on clinical outcome in diffuse large B-cell lymphoma. Blood. 2007;110:972–8.
62. Riihijarvi S, Koivula S, Nyman H, Rydstrom K, Jerkeman M, Leppa S. Prognostic impact of protein kinase C beta II expression in R-CHOP-treated diffuse large B-cell lymphoma patients. Mod Pathol (Off J US Can Acad Pathol Inc). 2010;23:686–93.
63. Adida C, Haioun C, Gaulard P, et al. Prognostic significance of survivin expression in diffuse large B-cell lymphomas. Blood. 2000;96:1921–5.
64. Fridberg M, Servin A, Anagnostaki L, et al. Protein expression and cellular localization in two prognostic subgroups of diffuse large B-cell lymphoma: higher expression of ZAP70 and PKC-beta II in the non-germinal center group and poor survival in patients deficient in nuclear PTEN. Leuk Lymphoma. 2007;48:2221–32.
65. Iqbal J, Meyer PN, Smith L, et al. BCL2 predicts survival in Germinal center B-cell-like diffuse large B-cell lymphoma treated with CHOP-like therapy and rituximab. Clin Cancer Res (Off J Am Assoc Cancer Res). 2011;17(24):7785–95.
66. Choi WW, Weisenburger DD, Greiner TC, et al. A new immunostain algorithm classifies diffuse large B-cell lymphoma into molecular subtypes with high accuracy. Clin Cancer Res (Off J Am Assoc Cancer Res). 2009;15:5494–502.
67. Meyer PN, Fu K, Greiner TC, et al. Immunohistochemical methods for predicting cell of origin and survival in patients with diffuse large B-cell lymphoma treated with rituximab. J Clin Oncol (Off J Am Soc Clin Oncol). 2011;29:200–7.
68. Young KH, Leroy K, Moller MB, et al. Structural profiles of TP53 gene mutations predict clinical outcome in diffuse large B-cell lymphoma: an international collaborative study. Blood. 2008;112:3088–98.
69. Iqbal J, Neppalli VT, Wright G, et al. BCL2 expression is a prognostic marker for the activated B-cell-like type of diffuse large B-cell lymphoma. J Clin Oncol (Off J Am Soc Clin Oncol). 2006;24:961–8.
70. Lossos IS. Diffuse large B cell lymphoma: from gene expression profiling to prediction of outcome. Biol Blood Marrow Transplant (J Am Soc Blood Marrow Transplant). 2008;14:108–11.
71. Lenz G, Wright GW, Emre NC, et al. Molecular subtypes of diffuse large B-cell lymphoma arise by distinct genetic pathways. Proc Natl Acad Sci U S A. 2008;105:13520–5.
72. Barrans S, Crouch S, Smith A, et al. Rearrangement of MYC is associated with poor prognosis in patients with diffuse large B-cell lymphoma treated in the era of rituximab. J Clin Oncol (Off J Am Soc Clin Oncol). 2010;28:3360–5.
73. Jardin F, Ruminy P, Kerckaert JP, et al. Detection of somatic quantitative genetic alterations by multiplex polymerase chain reaction for the prediction of outcome in diffuse large B-cell lymphomas. Haematologica. 2008;93:543–50.
74. Jardin F, Jais JP, Molina TJ, et al. Diffuse large B-cell lymphomas with CDKN2A deletion have a distinct gene expression signature and a poor prognosis under R-CHOP treatment: a GELA study. Blood. 2010;116:1092–104.
75. Culpin RE, Proctor SJ, Angus B, Crosier S, Anderson JJ, Mainou-Fowler T. A 9 series microRNA signature

differentiates between germinal centre and activated B-cell-like diffuse large B-cell lymphoma cell lines. Int J Oncol. 2010;37:367–76.
76. Lawrie CH, Soneji S, Marafioti T, et al. MicroRNA expression distinguishes between germinal center B cell-like and activated B cell-like subtypes of diffuse large B cell lymphoma. Int J Cancer J Int Cancer. 2007;121:1156–61.
77. Montes-Moreno S, Martinez N, Sanchez-Espiridion B, et al. miRNA expression in diffuse large B-cell lymphoma treated with chemoimmunotherapy. Blood. 2011;118:1034–40.
78. Alencar AJ, Malumbres R, Kozloski GA, et al. MicroRNAs are independent predictors of outcome in diffuse large B-cell lymphoma patients treated with R-CHOP. Clin Cancer Res (Off J Am Assoc Cancer Res). 2011;17:4125–35.
79. Lenz G, Staudt LM. Aggressive lymphomas. N Engl J Med. 2010;362:1417–29.
80. Rayman N, Lam KH, van der Holt B, et al. Prognostic relevance of immunohistochemical subclassification of diffuse large B-cell lymphoma in two prospective phase III clinical trials. Clin Lymphoma Myeloma Leuk. 2011;11:23–32.
81. Hans CP, Weisenburger DD, Greiner TC, et al. Confirmation of the molecular classification of diffuse large B-cell lymphoma by immunohistochemistry using a tissue microarray. Blood. 2004;103:275–82.
82. Nyman H, Adde M, Karjalainen-Lindsberg ML, et al. Prognostic impact of immunohistochemically defined germinal center phenotype in diffuse large B-cell lymphoma patients treated with immunochemotherapy. Blood. 2007;109:4930–5.
83. Muris JJ, Meijer CJ, Vos W, et al. Immunohistochemical profiling based on Bcl-2, CD10 and MUM1 expression improves risk stratification in patients with primary nodal diffuse large B cell lymphoma. J Pathol. 2006;208:714–23.
84. Natkunam Y, Farinha P, Hsi ED, et al. LMO2 protein expression predicts survival in patients with diffuse large B-cell lymphoma treated with anthracycline-based chemotherapy with and without rituximab. J Clin Oncol (Off J Am Soc Clin Oncol). 2008;26:447–54.
85. Lossos IS, Alizadeh AA, Rajapaksa R, Tibshirani R, Levy R. HGAL is a novel interleukin-4-inducible gene that strongly predicts survival in diffuse large B-cell lymphoma. Blood. 2003;101:433–40.
86. Lossos IS, Czerwinski DK, Alizadeh AA, et al. Prediction of survival in diffuse large-B-cell lymphoma based on the expression of six genes. N Engl J Med. 2004;350:1828–37.
87. Wright G, Tan B, Rosenwald A, Hurt EH, Wiestner A, Staudt LM. A gene expression-based method to diagnose clinically distinct subgroups of diffuse large B cell lymphoma. Proc Natl Acad Sci U S A. 2003;100:9991–6.
88. Rimsza LM, Wright G, Schwartz M, et al. Accurate classification of diffuse large B-cell lymphoma into germinal center and activated B-cell subtypes using a nuclease protection assay on formalin-fixed, paraffin-embedded tissues. Clin Cancer Res (Off J Am Assoc Cancer Res). 2011;17:3727–32.
89. Alizadeh AA, Gentles AJ, Alencar AJ, et al. Prediction of survival in diffuse large B-cell lymphoma based on the expression of 2 genes reflecting tumor and microenvironment. Blood. 2011;118:1350–8.
90. Copie-Bergman C, Gaulard P, Leroy K, et al. Immunofluorescence in situ hybridization index predicts survival in patients with diffuse large B-cell lymphoma treated with R-CHOP: a GELA study. J Clin Oncol (Off J Am Soc Clin Oncol). 2009;27:5573–9.
91. Meyer PN, Fu K, Greiner T, et al. The stromal cell marker SPARC predicts for survival in patients with diffuse large B-cell lymphoma treated with rituximab. Am J Clin Pathol. 2011;135:54–61.
92. Gratzinger D, Zhao S, Tibshirani RJ, et al. Prognostic significance of VEGF, VEGF receptors, and microvessel density in diffuse large B cell lymphoma treated with anthracycline-based chemotherapy. Lab Investig (J Tech Methods Pathol). 2008;88:38–47.
93. Evens AM, Sehn LH, Farinha P, et al. Hypoxia-inducible factor-1α expression predicts superior survival in patients with diffuse large B-cell lymphoma treated with R-CHOP. J Clin Oncol (Off J Am Soc Clin Oncol). 2010;28:1017–24.
94. Sakata K, Satoh M, Someya M, et al. Expression of matrix metalloproteinase 9 is a prognostic factor in patients with non-Hodgkin lymphoma. Cancer. 2004;100:356–65.
95. Wang L, Liu P, Chen X, Geng Q, Lu Y. Serum neuron-specific enolase is correlated with clinical outcome of patients with non-germinal center B cell-like subtype of diffuse large B-cell lymphoma treated with rituximab-based immunochemotherapy. Med Oncol. 2011;29(3):2153–8.
96. Maurer MJ, Micallef IN, Cerhan JR, et al. Elevated serum free light chains are associated with event-free and overall survival in two independent cohorts of patients with diffuse large B-cell lymphoma. J Clin Oncol (Off J Am Soc Clin Oncol). 2011;29:1620–6.
97. Drake MT, Maurer MJ, Link BK, et al. Vitamin D insufficiency and prognosis in non-Hodgkin's lymphoma. J Clin Oncol (Off J Am Soc Clin Oncol). 2010;28:4191–8.
98. Last KW, Cornelius V, Delves T, et al. Presentation serum selenium predicts for overall survival, dose delivery, and first treatment response in aggressive non-Hodgkin's lymphoma. J Clin Oncol (Off J Am Soc Clin Oncol). 2003;21:2335–41.
99. Lawrie CH, Gal S, Dunlop HM, et al. Detection of elevated levels of tumour-associated microRNAs in serum of patients with diffuse large B-cell lymphoma. Br J Haematol. 2008;141:672–5.
100. Lech-Maranda E, Bienvenu J, Michallet AS, et al. Elevated IL-10 plasma levels correlate with poor prognosis in diffuse large B-cell lymphoma. Eur Cytokine Netw. 2006;17:60–6.
101. Lopez-Guillermo A, Colomo L, Jimenez M, et al. Diffuse large B-cell lymphoma: clinical and biological characterization and outcome according to the nodal or extranodal primary origin. J Clin Oncol (Off J Am Soc Clin Oncol). 2005;23:2797–804.

102. Niitsu N, Okamoto M, Okabe-Kado J, et al. Serum nm23-H1 protein as a prognostic factor for indolent non-Hodgkin's lymphoma. Leuk (Off J Leuk Soc Am Leuk Res Fund UK). 2001;15:832–9.
103. Goto N, Tsurumi H, Kasahara S, et al. Serum interleukin-18 level is associated with the outcome of patients with diffuse large B-cell lymphoma treated with CHOP or R-CHOP regimens. Eur J Haematol. 2011;87:217–27.
104. Bono P, Teerenhovi L, Joensuu H. Elevated serum endostatin is associated with poor outcome in patients with non-Hodgkin lymphoma. Cancer. 2003;97:2767–75.
105. Molina A. A decade of rituximab: improving survival outcomes in non-Hodgkin's lymphoma. Annu Rev Med. 2008;59:237–50.
106. Armitage JO, Cheson BD. Interpretation of clinical trials in diffuse large-cell lymphoma. J Clin Oncol. 1988;6:1335–47.
107. A predictive model for aggressive non-Hodgkin's lymphoma. The international Non-Hodgkin's lymphoma prognostic factors project. N Engl J Med. 1993;329: 987–94.
108. Tilly H, Dreyling M. Diffuse large B-cell non-Hodgkin's lymphoma: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol. 2010;21 Suppl 5Suppl 5:v172–4.
109. Miller TP, Dahlberg S, Cassady JR, et al. Chemotherapy alone compared with chemotherapy plus radiotherapy for localized intermediate- and high-grade non-Hodgkin's lymphoma. N Engl J Med. 1998;339:21–6.
110. Bonnet C, Fillet G, Mounier N, et al. CHOP alone compared with CHOP plus radiotherapy for localized aggressive lymphoma in elderly patients: a study by the Groupe d'Etude des Lymphomes de l'Adulte. J Clin Oncol. 2007;25:787–92.
111. Reyes F, Lepage E, Ganem G, et al. ACVBP versus CHOP plus radiotherapy for localized aggressive lymphoma. N Engl J Med. 2005;352:1197–205.
112. Pfreundschuh M, Trumper L, Kloess M, et al. Two-weekly or 3-weekly CHOP chemotherapy with or without etoposide for the treatment of young patients with good-prognosis (normal LDH) aggressive lymphomas: results of the NHL-B1 trial of the DSHNHL. Blood. 2004;104:626–33.
113. Ketterer N, Coiffier B, Thieblemont C, et al. ACVBP versus ACVBP plus rituximab for young patients with localized low-risk diffuse large B-cell lymphoma. In: International conference on malignant lymphoma, Ann Oncol, Lugano; 2011. p. 91.
114. Persky DO, Unger JM, Spier CM, et al. Phase II study of rituximab plus three cycles of CHOP and involved-field radiotherapy for patients with limited-stage aggressive B-cell lymphoma: Southwest Oncology Group study 0014. J Clin Oncol (Off J Am Soc Clin Oncol). 2008;26:2258–63.
115. Recher C, Coiffier B, Haioun C, et al. Randomized phase 3 study (LNH03–2B) of intensified chemotherapy ACVBP plus rituximab versus standard CHOP plus rituximab for young patients with diffuse large B-cell lymphoma. 2011;378(9806):1858–67.
116. Gianni AM, Bregni M, Siena S, et al. High-dose chemotherapy and autologous bone marrow transplantation compared with MACOP-B in aggressive B-cell lymphoma. N Engl J Med. 1997;336: 1290–7.
117. Haioun C, Lepage E, Gisselbrecht C, et al. Survival benefit of high-dose therapy in poor-risk aggressive non-Hodgkin's lymphoma: final analysis of the prospective LNH87–2 protocol—a groupe d'Etude des lymphomes de l'Adulte study. J Clin Oncol. 2000;18:3025–30.
118. Santini G, Salvagno L, Leoni P, et al. VACOP-B versus VACOP-B plus autologous bone marrow transplantation for advanced diffuse non-Hodgkin's lymphoma: results of a prospective randomized trial by the non-Hodgkin's Lymphoma Cooperative Study Group. J Clin Oncol. 1998;16:2796–802.
119. Gisselbrecht C, Lepage E, Molina T, et al. Shortened first-line high-dose chemotherapy for patients with poor-prognosis aggressive lymphoma. J Clin Oncol. 2002;20:2472–9.
120. Milpied N, Deconinck E, Gaillard F, et al. Initial treatment of aggressive lymphoma with high-dose chemotherapy and autologous stem-cell support. N Engl J Med. 2004;350:1287–95.
121. Greb A, Bohlius J, Trelle S, et al. High-dose chemotherapy with autologous stem cell support in first-line treatment of aggressive non-Hodgkin lymphoma—results of a comprehensive meta-analysis. Cancer Treat Rev. 2007;33:338–46.
122. Stiff PJ, Unger JM, Cook J, et al. Randomized phase III U.S./Canadian intergroup trial (SWOG S9704) comparing CHOP ± R for eight cycles to CHOP ± R for six cycles followed by autotransplant for patients with high-intermediate (H-Int) or high IPI grade diffuse aggressive non-Hodgkin lymphoma (NHL). J Clin Oncol. 2011;29. Abstract 8001.
123. Tarella C, Zanni M, Di Nicola M, et al. Prolonged survival in poor-risk diffuse large B-cell lymphoma following front-line treatment with rituximab-supplemented, early-intensified chemotherapy with multiple autologous hematopoietic stem cell support: a multicenter study by GITIL (Gruppo Italiano Terapie Innovative nei Linfomi). Leukemia. 2007;21:1802–11.
124. Glass B, Ziepert M, Reiser M, et al. High-dose therapy followed by autologous stem-cell transplantation with and without rituximab for primary treatment of high-risk diffuse large B-cell lymphoma. Ann Oncol (Off J Eur Soc Med Oncol/ESMO). 2010; 21:2255–61.
125. Vitolo U, Chiappella A, Angelucci E, et al. Dose-dense and high-dose chemotherapy plus rituximab with autologous stem cell transplantation for primary treatment of diffuse large B-cell lymphoma with a poor prognosis: a phase II multicenter study. Haematologica. 2009;94:1250–8.
126. Fitoussi O, Belhadj K, Mounier N, et al. Survival impact of rituximab combined with ACVBP and upfront consolidation autotransplantation in high-risk diffuse large B-cell lymphoma for GELA. Haematologica. 2011;96:1136–43.

127. Schmitz N, Nickelsen M, Ziepert M, et al. Conventional chemoimmunotherapy (R-CHOEP-14) or high-dose therapy (R-Mega-CHOEP) for young, high-risk patients with aggressive B-cell lymphoma: final results of the randomized Mega-CHOEP trial of the German High-Grade Non-Hodgkin Lymphoma Study Group (DSHNHL). J Clin Oncol. 2012;13(12):1250–9.
128. Casasnovas RO, Meignan M, Berriolo-Riedinger A, et al. SUVmax reduction improves early prognosis value of interim positron emission tomography scans in diffuse large B-cell lymphoma. Blood. 2011;118:37–43.
129. Coiffier B, Thieblemont C, Van Den Neste E, et al. Long-term outcome of patients in the LNH-98.5 trial, the first randomized study comparing rituximab-CHOP to standard CHOP chemotherapy in DLBCL patients: a study by the Groupe d'Etudes des Lymphomes de l'Adulte. Blood. 2010;116(12):2040–5.
130. Habermann TM, Weller EA, Morrison VA, et al. Rituximab-CHOP versus CHOP alone or with maintenance rituximab in older patients with diffuse large B-cell lymphoma. J Clin Oncol. 2006;24:3121–7.
131. Tilly H, Lepage E, Coiffier B, et al. Intensive conventional chemotherapy (ACVBP regimen) compared with standard CHOP for poor-prognosis aggressive non-Hodgkin lymphoma. Blood. 2003;102:4284–9.
132. Pfreundschuh M, Trumper L, Kloess M, et al. Two-weekly or 3-weekly CHOP chemotherapy with or without etoposide for the treatment of elderly patients with aggressive lymphomas: results of the NHL-B2 trial of the DSHNHL. Blood. 2004;104:634–41.
133. Pfreundschuh M, Schubert J, Ziepert M, et al. Six versus eight cycles of bi-weekly CHOP-14 with or without rituximab in elderly patients with aggressive CD20+ B-cell lymphomas: a randomised controlled trial (RICOVER-60). Lancet Oncol. 2008;9:105–16.
134. Delarue R, Tilly H, Salles A, et al. R-CHOP14 compared to R-CHOP21 in elderly patients with diffuse large B-cell lymphoma: results of the interim analysis of the LNH03–6B GELA study. Blood. 2009;114. Abstract 406.
135. Cunningham D, Smith P, Mouncey P, et al. R-CHOP14 versus R-CHOP21: result of a randomized phase III trial for the treatment of patients with newly diagnosed diffuse large B-cell non-Hodgkin lymphoma. J Clin Oncol. 2011;29. Abstract 8000.
136. Ferreri AJ, Assanelli A, Crocchiolo R, Ciceri F. Central nervous system dissemination in immunocompetent patients with aggressive lymphomas: incidence, risk factors and therapeutic options. Hematol Oncol. 2009;27:61–70.
137. Bernstein SH, Unger JM, Leblanc M, Friedberg J, Miller TP, Fisher RI. Natural history of CNS relapse in patients with aggressive non-Hodgkin's lymphoma: a 20-year follow-up analysis of SWOG 8516—the Southwest Oncology Group. J Clin Oncol. 2009;27:114–9.
138. Kridel R, Dietrich PY. Prevention of CNS relapse in diffuse large B-cell lymphoma. Lancet Oncol. 2011;12(13):1258–66.
139. Abramson JS, Hochberg EP. Intravenous methotrexate as central nervous system (CNS) prophylaxis is associated with a low risk of CNS recurrence in high-risk patients with diffuse large B-cell lymphoma. Cancer. 2010;116(18):4283–90.
140. Yamamoto W, Tomita N, Watanabe R, et al. Central nervous system involvement in diffuse large B-cell lymphoma. Eur J Haematol. 2010;85:6–10.
141. Tai WM, Chung J, Tang PL, et al. Central nervous system (CNS) relapse in diffuse large B cell lymphoma (DLBCL): pre- and post-rituximab. Ann Hematol. 2011;90:809–18.
142. Philip T, Guglielmi C, Hagenbeek A, et al. Autologous bone marrow transplantation as compared with salvage chemotherapy in relapses of chemotherapy-sensitive non-Hodgkin's lymphoma. N Engl J Med. 1995;333:1540–5.
143. Velasquez WS, Cabanillas F, Salvador P, et al. Effective salvage therapy for lymphoma with cisplatin in combination with high-dose Ara-C and dexamethasone (DHAP). Blood. 1988;71:117–22.
144. Cabanillas F, Velasquez WS, McLaughlin P, et al. Results of recent salvage chemotherapy regimens for lymphoma and Hodgkin's disease. Semin Hematol. 1988;25:47–50.
145. Stamatoullas A, Fruchart C, Bastit D, et al. Ifosfamide, etoposide, cytarabine, and methotrexate as salvage chemotherapy in relapsed or refractory aggressive non-Hodgkin's lymphoma. Cancer. 1996;77:2302–7.
146. Moskowitz CH, Bertino JR, Glassman JR, et al. Ifosfamide, carboplatin, and etoposide: a highly effective cytoreduction and peripheral-blood progenitor-cell mobilization regimen for transplant-eligible patients with non-Hodgkin's lymphoma. J Clin Oncol. 1999;17:3776–85.
147. Gisselbrecht C, Mounier N. Rituximab: enhancing outcome of autologous stem cell transplantation in non-Hodgkin's lymphoma. Semin Oncol. 2003;30:28–33.
148. Vellenga E, van Putten WL, van't Veer MB, et al. Rituximab improves the treatment results of DHAP-VIM-DHAP and ASCT in relapsed/progressive aggressive CD20+ NHL: a prospective randomized HOVON trial. Blood. 2008;111:537–43.
149. Gisselbrecht C, Glass B, Mounier N, et al. Salvage regimens with autologous transplantation for relapsed large B-cell lymphoma in the rituximab era. J Clin Oncol. 2010;28(27):4184–90.
150. Thieblemont C, Briere J, Mounier N, et al. The Germinal center/activated B-cell subclassification has a prognostic impact for response to salvage therapy in relapsed/refractory diffuse large B-cell lymphoma: a bio-CORAL study. J Clin Oncol. 2011; 29(31):4079–87.
151. Gisselbrecht C, Glass B, Laurent G, et al. Maintenance with rituximab after autologous stem cell transplantation in relapsed patients with CD20 diffuse large B-cell lymphoma (DLBCL): CORAL final analysis. J Clin Oncol. 2011;29:8004.
152. El Gnaoui T, Dupuis J, Belhadj K, et al. Rituximab, gemcitabine and oxaliplatin: an effective salvage regimen for patients with relapsed or refractory B-cell lymphoma not candidates for high-dose

therapy. Ann Oncol (Off J Eur Soc Med Oncol/ESMO). 2007;18:1363–8.
153. Corazzelli G, Russo F, Capobianco G, Marcacci G, Della Cioppa P, Pinto A. Gemcitabine, ifosfamide, oxaliplatin and rituximab (R-GIFOX), a new effective cytoreductive/mobilizing salvage regimen for relapsed and refractory aggressive non-Hodgkin's lymphoma: results of a pilot study. Ann Oncol (Off J Eur Soc Med Oncol/ESMO). 2006;17 Suppl 4Suppl 4:iv18–24.
154. Gisselbrecht C. Use of rituximab in diffuse large B-cell lymphoma in the salvage setting. Br J Haematol. 2008;143:607–21.
155. van Kampen RJ, Canals C, Schouten HC, et al. Allogeneic stem-cell transplantation as salvage therapy for patients with diffuse large B-cell non-Hodgkin's lymphoma relapsing after an autologous stem-cell transplantation: an analysis of the European Group for Blood and Marrow Transplantation Registry. J Clin Oncol. 2011;29:1342–8.
156. Friedberg JW. New strategies in diffuse large B-cell lymphoma: translating findings from gene expression analyses into clinical practice. Clin Cancer Res (Off J Am Assoc Cancer Res). 2011;17:6112–7.
157. Dunleavy K, Wilson WH. Role of molecular subtype in predicting outcome of AIDS-related diffuse large B-cell lymphoma. J Clin Oncol (Off J Am Soc Clin Oncol). 2010;28:e260. Author reply e1–2.
158. Ruan J, Martin P, Furman RR, et al. Bortezomib plus CHOP-rituximab for previously untreated diffuse large B-cell lymphoma and mantle cell lymphoma. J Clin Oncol (Off J Am Soc Clin Oncol). 2011;29:690–7.
159. Furman RR, Martin P, Ruan J, et al. Phase 1 trial of bortezomib plus R-CHOP in previously untreated patients with aggressive non-Hodgkin lymphoma. Cancer. 2010;116:5432–9.
160. Mozos A, Roue G, Lopez-Guillermo A, et al. The expression of the endoplasmic reticulum stress sensor BiP/GRP78 predicts response to chemotherapy and determines the efficacy of proteasome inhibitors in diffuse large B-cell lymphoma. Am J Pathol. 2011;179(5):2601–10.
161. Hideshima T, Ikeda H, Chauhan D, et al. Bortezomib induces canonical nuclear factor-kappaB activation in multiple myeloma cells. Blood. 2009;114:1046–52.
162. Dasmahapatra G, Lembersky D, Rahmani M, et al. Bcl-2 antagonists interact synergistically with bortezomib in DLBCL cells in association with JNK activation and induction of ER stress. Cancer Biol Ther. 2009;8:808–19.
163. Dasmahapatra G, Lembersky D, Kramer L, et al. The pan-HDAC inhibitor vorinostat potentiates the activity of the proteasome inhibitor carfilzomib in human DLBCL cells in vitro and in vivo. Blood. 2010;115:4478–87.
164. Paoluzzi L, Gonen M, Bhagat G, et al. The BH3-only mimetic ABT-737 synergizes the antineoplastic activity of proteasome inhibitors in lymphoid malignancies. Blood. 2008;112:2906–16.
165. Ferch U, Kloo B, Gewies A, et al. Inhibition of MALT1 protease activity is selectively toxic for activated B cell-like diffuse large B cell lymphoma cells. J Exp Med. 2009;206:2313–20.
166. Hernandez-Ilizaliturri FJ, Deeb G, Zinzani PL, et al. Higher response to lenalidomide in relapsed/refractory diffuse large b-cell lymphoma in nongerminal center b-cell-like than in germinal center b-cell-like phenotype. Cancer. 2011;117(22):5058–66.
167. Leonard JP, Martin P, Barrientos J, Elstrom R. Targeted treatment and new agents in diffuse large B-cell lymphoma. Semin Hematol. 2008;45:S11–6.
168. Witzig TE, Gupta M. Signal transduction inhibitor therapy for lymphoma. Hematol Educ Program Am Soc Hematol Am Soc Hematol Educ Program. 2010;2010:265–70.
169. Friedberg JW, Sharman J, Sweetenham J, et al. Inhibition of Syk with fostamatinib disodium has significant clinical activity in non-Hodgkin lymphoma and chronic lymphocytic leukemia. Blood. 2010;115:2578–85.
170. Honigberg LA, Smith AM, Sirisawad M, et al. The Bruton tyrosine kinase inhibitor PCI-32765 blocks B-cell activation and is efficacious in models of autoimmune disease and B-cell malignancy. Proc Natl Acad Sci U S A. 2010;107:13075–80.
171. Davis RE, Ngo VN, Lenz G, et al. Chronic active B-cell-receptor signalling in diffuse large B-cell lymphoma. Nature. 2010;463:88–92.
172. Robertson MJ, Kahl BS, Vose JM, et al. Phase II study of enzastaurin, a protein kinase C beta inhibitor, in patients with relapsed or refractory diffuse large B-cell lymphoma. J Clin Oncol (Off J Am Soc Clin Oncol). 2007;25:1741–6.
173. Cerchietti LC, Ghetu AF, Zhu X, et al. A small-molecule inhibitor of BCL6 kills DLBCL cells in vitro and in vivo. Cancer Cell. 2010;17:400–11.
174. Briones J. Targeted therapy of BCL6-dependent diffuse large B-cell lymphomas by heat-shock protein 90 inhibition. Expert Rev Hematol. 2010;3:157–9.
175. Cerchietti LC, Hatzi K, Caldas-Lopes E, et al. BCL6 repression of EP300 in human diffuse large B cell lymphoma cells provides a basis for rational combinatorial therapy. J Clin Investig. 2010;pii:42869.
176. Cerchietti LC, Lopes EC, Yang SN, et al. A purine scaffold Hsp90 inhibitor destabilizes BCL-6 and has specific antitumor activity in BCL-6-dependent B cell lymphomas. Nat Med. 2009;15:1369–76.
177. Wilson WH, Dunleavy K, Pittaluga S, et al. Phase II study of dose-adjusted EPOCH and rituximab in untreated diffuse large B-cell lymphoma with analysis of germinal center and post-germinal center biomarkers. J Clin Oncol (Off J Am Soc Clin Oncol). 2008;26:2717–24.
178. Lenz G, Davis RE, Ngo VN, et al. Oncogenic CARD11 mutations in human diffuse large B cell lymphoma. Science. 2008;319:1676–9.
179. Cardesa-Salzmann TM, Colomo L, Gutierrez G, et al. High microvessel density determines a poor outcome in patients with diffuse large B-cell lymphoma treated with rituximab plus chemotherapy. Haematologica. 2011;96:996–1001.

180. Stopeck AT, Unger JM, Rimsza LM, et al. A phase II trial of single agent bevacizumab in patients with relapsed, aggressive non-Hodgkin lymphoma: southwest oncology group study S0108. Leuk Lymphoma. 2009;50:728–35.
181. Ganjoo KN, An CS, Robertson MJ, et al. Rituximab, bevacizumab and CHOP (RA-CHOP) in untreated diffuse large B-cell lymphoma: safety, biomarker and pharmacokinetic analysis. Leuk Lymphoma. 2006;47:998–1005.
182. Beltran BE, Castillo JJ, Morales D, et al. EBV-positive diffuse large B-cell lymphoma of the elderly: a case series from Peru. Am J Hematol. 2011;86:663–7.
183. Castillo JJ, Beltran BE, Miranda RN, Paydas S, Winer ES, Butera JN. Epstein-barr virus-positive diffuse large B-cell lymphoma of the elderly: what we know so far. Oncologist. 2011;16:87–96.
184. Hofscheier A, Ponciano A, Bonzheim I, et al. Geographic variation in the prevalence of Epstein-Barr virus-positive diffuse large B-cell lymphoma of the elderly: a comparative analysis of a Mexican and a German population. Mod Pathol (Off J US Can Acad Pathol Inc). 2011;24:1046–54.
185. Grange F, Beylot-Barry M, Courville P, et al. Primary cutaneous diffuse large B-cell lymphoma, leg type: clinicopathologic features and prognostic analysis in 60 cases. Arch Dermatol. 2007;143:1144–50.
186. Petitjean B, Jardin F, Joly B, et al. Pyothorax-associated lymphoma: a peculiar clinicopathologic entity derived from B cells at late stage of differentiation and with occasional aberrant dual B- and T-cell phenotype. Am J Surg Pathol. 2002;26:724–32.
187. Campo E, Swerdlow SH, Harris NL, Pileri S, Stein H, Jaffe ES. The 2008 WHO classification of lymphoid neoplasms and beyond: evolving concepts and practical applications. Blood. 2011;117:5019–32.
188. Mareschal S, Lanic H, Ruminy P, Bastard C, Tilly H, Jardin F. The proportion of activated B-cell like subtype among de novo diffuse large B-cell lymphoma increases with age. Haematologica. 2011;96(12):1888–90.
189. Peyrade F, Jardin F, Thieblemont C, et al. Attenuated immunochemotherapy regimen (R-miniCHOP) in elderly patients older than 80 years with diffuse large B-cell lymphoma: a multicentre, single-arm, phase 2 trial. Lancet Oncol. 2011;12:460–8.
190. Thieblemont C, Coiffier B. Lymphoma in older patients. J Clin Oncol (Off J Am Soc Clin Oncol). 2007;25:1916–23.
191. Thieblemont C, Grossoeuvre A, Houot R, et al. Non-Hodgkin's lymphoma in very elderly patients over 80 years. A descriptive analysis of clinical presentation and outcome. Ann Oncol (Off J Eur Soc Med Oncol/ESMO). 2008;19:774–9.
192. Advani RH, Chen H, Habermann TM, et al. Comparison of conventional prognostic indices in patients older than 60 years with diffuse large B-cell lymphoma treated with R-CHOP in the US Intergroup Study (ECOG 4494, CALGB 9793): consideration of age greater than 70 years in an elderly prognostic index (E-IPI). Br J Haematol. 2010;151:143–51.
193. Pfreundschuh M. How I, treat elderly patients with diffuse large B-cell lymphoma. Blood. 2010;116:5103–10.
194. Walter LC, Covinsky KE. Cancer screening in elderly patients: a framework for individualized decision making. J Am Med Assoc. 2001;285:2750–6.
195. Extermann M, Hurria A. Comprehensive geriatric assessment for older patients with cancer. J Clin Oncol (Off J Am Soc Clin Oncol). 2007;25:1824–31.
196. Stuck AE, Siu AL, Wieland GD, Adams J, Rubenstein LZ. Comprehensive geriatric assessment: a meta-analysis of controlled trials. Lancet. 1993;342:1032–6.
197. Balducci L, Extermann M. Management of cancer in the older person: a practical approach. Oncologist. 2000;5:224–37.
198. Tucci A, Ferrari S, Bottelli C, Borlenghi E, Drera M, Rossi G. A comprehensive geriatric assessment is more effective than clinical judgment to identify elderly diffuse large cell lymphoma patients who benefit from aggressive therapy. Cancer. 2009;115:4547–53.
199. Siegel AB, Lachs M, Coleman M, Leonard JP. Lymphoma in elderly patients: novel functional assessment techniques provide better discrimination among patients than traditional performance status measures. Clin Lymphoma Myeloma. 2006;7:65–9.
200. Hurria A, Gupta S, Zauderer M, et al. Developing a cancer-specific geriatric assessment: a feasibility study. Cancer. 2005;104:1998–2005.
201. Luciani A, Ascione G, Bertuzzi C, et al. Detecting disabilities in older patients with cancer: comparison between comprehensive geriatric assessment and vulnerable elders survey-13. J Clin Oncol (Off J Am Soc Clin Oncol). 2010;28:2046–50.
202. Kobayashi Y, Miura K, Hojo A, et al. Charlson Comorbidity Index is an independent prognostic factor among elderly patients with diffuse large B-cell lymphoma. J Cancer Res Clin Oncol. 2011;137:1079–84.
203. Hurria A, Togawa K, Mohile SG, et al. Predicting chemotherapy toxicity in older adults with cancer: a prospective multicenter study. J Clin Oncol (Off J Am Soc Clin Oncol). 2011;29:3457–65.
204. Janssen-Heijnen ML, Extermann M, Boler IE. Can first cycle CBCs predict older patients at very low risk of neutropenia during further chemotherapy? Crit Rev Oncol/Hematol. 2011;79:43–50.
205. Hasselblom S, Stenson M, Werlenius O, et al. Improved outcome for very elderly patients with diffuse large B-cell lymphoma in the immunochemotherapy era. Leuk Lymphoma. 2011;53(3):394–9.
206. Musolino A, Boggiani D, Panebianco M, et al. Activity and safety of dose-adjusted infusional cyclophosphamide, doxorubicin, vincristine, and prednisone chemotherapy with rituximab in very elderly patients with poor-prognostic untreated

diffuse large B-cell non-Hodgkin lymphoma. Cancer. 2011;117:964–73.
207. Meguro A, Ozaki K, Sato K, et al. Rituximab plus 70% cyclophosphamide, doxorubicin, vincristine and prednisone for Japanese patients with diffuse large B-cell lymphoma aged 70 years and older. Leuk Lymphoma. 2011;53(1):43–9.
208. Visani G, Ferrara F, Alesiani F, et al. R-COMP 21 for frail elderly patients with aggressive B-cell non-Hodgkin lymphoma: a pilot study. Leuk Lymphoma. 2008;49:1081–6.
209. Corazzelli G, Frigeri F, Arcamone M, et al. Biweekly rituximab, cyclophosphamide, vincristine, non-pegylated liposome-encapsulated doxorubicin and prednisone (R-COMP-14) in elderly patients with poor-risk diffuse large B-cell lymphoma and moderate to high 'life threat' impact cardiopathy. Br J Haematol. 2011;154:579–89.
210. Zinzani PL, Pellegrini C, Gandolfi L, et al. Combination of lenalidomide and rituximab in elderly patients with relapsed or refractory diffuse large B-cell lymphoma: a phase 2 trial. Clin Lymphoma Myeloma Leuk. 2011; 11(6):462–6.
211. Weidmann E, Neumann A, Fauth F, et al. Phase II study of bendamustine in combination with rituximab as first-line treatment in patients 80 years or older with aggressive B-cell lymphomas. Ann Oncol (Off J Eur Soc Med Oncol/ESMO). 2011;22:1839–44.
212. Rigacci L, Fabbri A, Puccini B, et al. Oxaliplatin-based chemotherapy (dexamethasone, high-dose cytarabine, and oxaliplatin)+/−rituximab is an effective salvage regimen in patients with relapsed or refractory lymphoma. Cancer. 2010;116:4573–9.
213. Jantunen E, Canals C, Rambaldi A, et al. Autologous stem cell transplantation in elderly patients (> or =60 years) with diffuse large B-cell lymphoma: an analysis based on data in the European Blood and Marrow Transplantation registry. Haematologica. 2008;93: 1837–42.
214. Aapro MS, Bohlius J, Cameron DA, et al. 2010 update of EORTC guidelines for the use of granulocyte-colony stimulating factor to reduce the incidence of chemotherapy-induced febrile neutropenia in adult patients with lymphoproliferative disorders and solid tumours. Eur J Cancer. 2011;47: 8–32.
215. Zwick C, Hartmann F, Zeynalova S, et al. Randomized comparison of pegfilgrastim day 4 versus day 2 for the prevention of chemotherapy-induced leukocytopenia. Ann Oncol (Off J Eur Soc Med Oncol/ ESMO). 2011;22:1872–7.
216. Brunello A, Kapoor R, Extermann M. Hyperglycemia during chemotherapy for hematologic and solid tumors is correlated with increased toxicity. Am J Clin Oncol. 2011;34:292–6.
217. Lin PC, Hsiao LT, Poh SB, et al. Higher fungal infection rate in elderly patients (more than 80 years old) suffering from diffuse large B cell lymphoma and treated with rituximab plus CHOP. Ann Hematol. 2007;86:95–100.

Primary Mediastinal B-Cell Lymphoma

12

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Abstract

Primary mediastinal B-cell lymphoma (PMBL) is a distinct subtype of diffuse large B-cell lymphoma (DLBCL) that arises in the mediastinum and is putatively of thymic B-cell origin. It was first recognized in 1980, from a review of 184 adult non-Hodgkin lymphoma (NHL) cases, and is now included as a distinct entity in the 4th edition of the World Health Organization (WHO) Classification of Tumours of Haematopoietic and Lymphoid Tissues. It is rare in that it only accounts for 2–4 % of all NHL, and this has been an obstacle to carrying out large-scale clinical trials to define optimal therapy for this disease entity. Intriguingly, it shares many biologic features with classical Hodgkin lymphoma (CHL), and its molecular profile more closely resembles that of CHL than other subtypes of DLBCL.

Keywords

Mediastinal • Gray zone • Thymic B cell • Genome sequencing • Obviating radiation • Janus kinase

Introduction

Primary mediastinal B-cell lymphoma (PMBL) has a propensity to affect females and is typically diagnosed in the third and fourth decades of life compared to other variants of DLBCL that peak in incidence in the seventh decade. It most likely arises from a thymic B cell and typically presents with an anterior mediastinal mass. Symptoms at diagnosis are related to the mediastinal mass and patients frequently present with superior vena cava (SVC) syndrome. Mediastinal masses of greater than 10 cm are common at diagnosis, and local infiltration into adjacent structures such as the

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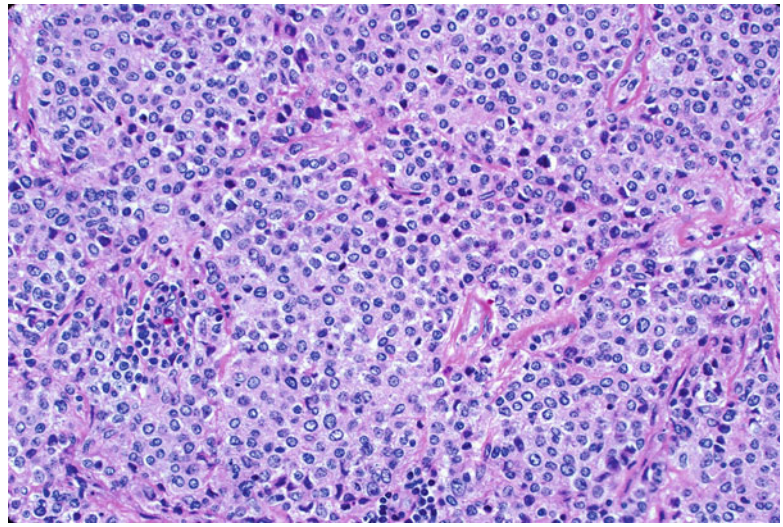

Fig. 12.1 Primary mediastinal large b-cell lymphoma; tumor cells are associated with fine trabecular fibrosis, but no well-formed fibrous bands. The cells have a rim of pale eosinophilic cytoplasm. An inflammatory background is absent (H&E, 400×) (Courtesy Dr. Elaine Jaffe and Dr. Stefania Pittaluga)

lungs and chest wall is frequently observed. The disease tends to be confined to the mediastinum at diagnosis, but at progression, it is not uncommon to have involvement of extranodal sites such as the kidneys, liver, adrenal glands, and central nervous system. It is interesting that the clinical characteristics of classical Hodgkin lymphoma of the nodular sclerosis subtype (CHL-NS) are very similar to PMBL with a female preponderance, young age at diagnosis, and mediastinal presentation—CHL-NS is also likely to be of thymic B-cell origin and shares many molecular features with PMBL. In that respect, it is intriguing that there exist mediastinal lymphomas with clinical and morphological features transitional between PMBL and CHL-NS, and these are recognized in the most recent WHO classification and have been termed "gray"-zone lymphomas (GZL) [1]. In contrast to PMBL and CHL-NS, GZLs predominantly affect males, and their morphological and biologic features and clinical outcome are also discussed in the subsequent sections [2].

Pathology

Histologically, PMBL is characterized by an infiltrate of large cells with round or lobulated nuclei and abundant clear cytoplasm. There is a background of fine, compartmentalizing sclerosis. Occasionally, Hodgkin/Reed-Sternberg cells can be seen, and although the architecture is typically diffuse, a minority of cases may show focal nodularity (Fig. 12.1). Necrosis, a characteristic feature of CHL, is seen in approximately 25 % of cases [3]. The immunophenotype of PMBL resembles that of a mature B cell expressing CD20 and pan B-cell markers such as CD79a, but tumor cells lack surface immunoglobulin expression, unlike most B-cell neoplasms [4, 5]. The B-cell-associated transcription factors PAX5, OCT2, and BOB1 are strongly expressed, and CD30 is expressed in most cases but with variable intensity [3–5]. The cells are variably positive for the germinal center markers CD10 and BCL6, and CD23 is positive in over 85 % of cases, suggesting a thymic B-cell origin [6, 7]. As well as sharing many clinical characteristics with PMBL, CHL-NS has morphological overlap with PMBL but also distinct features that make these two entities morphologically distinguishable. CHL is typified by a nodular growth pattern with broad bands of fibrosis and lacunar variants of HRS cells that have a characteristic immunophenotype. The cells are positive for CD30, negative for CD45, and positive for CD15 in 85 % of cases. Expression of B-cell-associated antigens is weak and heterogeneous in the neoplastic cells of CHL, with often, negative CD79a, weak or variable CD20 expression, and weak expression of PAX5 [8, 9]. Tumor cells are frequently positive for IRF4/MUM1. As with

PMBL, immunoglobulin expression is absent, and transcription factors that govern immunoglobulin production like OCT2 and BOB1 are frequently negative [10, 11].

The morphological features of GZL are transitional between PMBL and CHL. Frequently, pleomorphic tumor cells sheet out and grow in a diffusely fibrotic stroma. Tumor cells can be similar to PMBL or CHL, with a broad spectrum of cytologic appearance in different areas of the tumor. Clusters of cells similar to lacunar cells or even HRS cells may be seen in a background resembling PMBL. The inflammatory infiltrate is typically sparse, but scattered eosinophils, lymphocytes, and histiocytes may be present. The immunophenotypic features of GZL are also intermediate between PMBL and CHL [2, 12]. The tumor cells typically express CD45, CD20, and CD79a. CD30 is also positive and CD15 may be expressed as well. Immunoglobulin expression is absent, resembling both CHL and PMBL. The transcription factors PAX5, OCT2, and BOB1 are positive in most cases, but tumor cells can present a pattern with transitional features between CHL and PMBL, particularly with asynchrony between morphology and immunophenotype. GZL can present with a Hodgkin-like morphology and a phenotypic pattern of PMBL (CD20++, CD15−). Alternatively, the lymphoma can present with a PMBL-like morphology and a Hodgkin phenotype, with expression of CD30 and CD15 and loss of CD20 and CD79a. The transforming events that lead to the divergent transformation of a thymic B cell into either PMBL or CHL-NS are poorly understood, but the fact that PMBL can recur as CHL and vice versa suggests that there is plasticity in these events [2].

Biologic Characteristics

Gene expression profiling studies have interestingly demonstrated that there is extensive biologic overlap between PMBL and CHL and the molecular profile of PMBL is much closer to that of CHL than it is to other subtypes of DLBCL (i.e., germinal center B-cell like (GCB) and activated B-cell like (ABC)) [13, 14]. PMBL and CHL, in fact, share approximately a third of their genes [13]. PMBL shows frequent gains of gene regions on chromosome 9p (up to 75 %) and 2p (approximately 50 %)—these have also been described in CHL but rarely in other subtypes of DLBCL [1]. The 9p region encodes JAK2, a tyrosine kinase that phosphorylates/activates the transcription factor STAT 6 [13, 15]. SOCS1 suppresses JAK signaling and is regularly deleted in both PMBL and CHL [16]. Other genes that may be involved at 9p are PDL1 and PDL2, while c-REL may be involved at 2p [13]. Gains in chromosome X are also found in a third of cases, while rearrangements of BCL2, BCL6, and MYC genes are usually absent in PMBL [1]. A recent study of chromosomal aberrations for 2p16.1, 9p24.1, and 8q24, in children with PMBL, found that the frequencies of investigated loci were similar to those found in adults [17]. PMBL and CHL also have constitutively activated nuclear factor kappa-B (NF-κ[kappa]B). At this point in time, the molecular signature of GZL has not been elucidated, but a recent large-scale methylation analysis of PMBL, CHL, and GZL demonstrated a close epigenetic relationship between these entities and a unique epigenetic signature for GZL, validating its inclusion in the WHO classification as a separate disease [18].

Diagnostic Workup and Prognostic Features

The staging workup for PMBL should include a complete history and physical examination as well as measurement of serum hematological and biochemical parameters including lactate dehydrogenase (LDH) level. Computerized tomography of the chest, abdomen, and pelvis should be performed, and the CNS should be evaluated if indicated. A bone marrow aspirate and biopsy should also be performed. As pericardial effusions are not uncommonly detected, it is useful to perform echocardiography. While the international prognostic index (IPI) is useful in DLBCL, its utility in PMBL is limited by the age distribution of the disease and its typical confinement to the mediastinum [19]. One study that evaluated

the age-adjusted IPI in PMBL did not find it to be useful in prognosticating [20]. While retrospective studies have suggested that factors like LDH level, male sex, and performance status may be useful predictors of survival, these have not been validated in prospective studies [21, 22]. As we discuss subsequently, albeit based on limited experience, patients with GZL appear to have a much worse outcome than those with PMBL.

Management

Due to the rarity of these diseases and the paucity of prospective data, the optimal therapeutic approach and choice of regimen for PMBL are controversial. As these diseases are very curable and typically affect young females, the long-term effects of therapy are an important consideration, especially with respect to the administration of mediastinal radiation, which is a frequently used treatment modality in this disease. There are now several sobering reports detailing high incidences of, particularly, breast cancer and ischemic heart disease, several years after radiation therapy, in patients with mediastinal lymphomas [23–26]. Novel strategies that maintain high cure rates but obviate the need for mediastinal radiation are therefore needed.

Early studies suggested that PMBL had a poor outcome with CHOP (cyclophosphamide, hydroxydaunorubicin, vincristine, prednisone) chemotherapy alone, and several studies evaluated dose-intensified regimens in this patient population. One such study, in 50 untreated patients with PMBL, demonstrated high efficacy of MACOP-B (methotrexate, leucovorin, doxorubicin, cyclophosphamide, vincristine, prednisone, and bleomycin) followed by consolidation radiation treatment, but 66 % had a persistently positive gallium scan at the end of chemotherapy, suggesting active disease [27]. Following consolidation radiation therapy, however, only 19 % had a positive gallium scan and 80 % were event-free at 39 months median follow-up time [27]. Later, MACOP-B and VACOP-B (etoposide, doxorubicin, cyclophosphamide, vincristine, prednisone, bleomycin) were compared to CHOP in a retrospective analysis, and the outcome with the latter regimen was inferior, suggesting that dose intensity is important in this disease [28]. In another retrospective analysis, the International Extranodal Lymphoma Study Group (IELSG) compared outcomes of 426 patients with PMBL across 20 institutions where patients received MACOP-B, VACOP-B, ProMACECytaBom, or CHOP, and they found an inferior outcome in the CHOP group [22]. However, there have been no prospective comparisons of these regimens. At the time when the Southwest Oncology Group (SWOG) compared CHOP to second- and third-generation regimens in DLBCL, PMBL was not recognized as a distinct entity, and the outcome of these patients with first- versus second- and third-generation regimens was not assessed [29]. A retrospective study that looked at the outcome of 141 consecutive PMBL patients who were treated with CHOP-like therapy or approaches that included high-dose chemotherapy suggested that dose-dense chemotherapy was superior to CHOP in these patients [20].

While the addition of rituximab to CHOP chemotherapy in DLBCL has been shown to improve survival in several different studies, this has not been well studied in PMBL due to the rarity of the disease [30]. In a retrospective study carried out in British Columbia in the pre- and post-rituximab period, there was no survival advantage in patients when rituximab was added to CHOP (the number of patients in the R-CHOP arm was small, however, and the follow-up time relatively short) [21]. A recent subgroup analysis of the prospective, randomized, phase III MabThera International Trial (Mint) evaluated the role of rituximab in combination with CHOP-like regimens in PMBL [31]. The rituximab arm was clearly superior in terms of 3-year event-free survival (78 % versus 52 % in the chemotherapy arm alone), but no statistically significant difference in overall survival (OS) was detected due to small numbers. Of importance in interpreting these data, however, is the fact that preplanned radiotherapy was administered to 67 % of patients in the chemotherapy-alone arm and 73 % in the immunochemotherapy arm, and the addition of radiation improved remission rates. This

study suggested that adding rituximab to CHOP may be beneficial in PMBL, but it did not demonstrate that this regimen could obviate the need for radiation. Recently, a retrospective analysis of R-CHOP (with consolidation mediastinal radiation in the majority) in 58 patients with PMBL demonstrated a high rate of initial treatment failure and a progression-free survival (PFS) of 68 % at 5 years [32].

Based on observations that dose intensity appeared to be important in this disease historically, we investigated the dose-adjusted EPOCH-R (etoposide, prednisone, vincristine, cyclophosphamide, doxorubicin, and rituximab) regimen in PMBL, based on its efficacy in DLBCL [33–35]. This is a pharmacodynamically dose-adjusted regimen, and this strategy may especially benefit younger patients who likely require higher doses of drugs to achieve similar serum drug levels to older patients [35]. We set out to investigate if the regimen could obviate the need for mediastinal radiation treatment and, thus, eliminate the risk of long-term consequences such as secondary cancers and ischemic heart disease. In a recent update of 40 patients with untreated PMBL who received the regimen without consolidative radiation, the event-free survival and overall survival were 95 and 100 %, respectively, with only two patients requiring radiation treatment at a median follow-up time of 4 years [36]. In terms of assessing the additive benefit of rituximab with this regimen, when these results were compared to a historical PMBL group who received DA-EPOCH alone (no radiation), the addition of rituximab significantly improved EFS (95 % versus 65 %; $p=0.0012$) and OS (100 % versus 77 %; $p=0.013$) [36].

Outcome of Mediastinal Gray-Zone Lymphoma

Due to their rarity and relatively recent recognition, only one prospective study has reported the outcome of these mediastinal lymphomas, which have features intermediate between PMBL and CHL-NS. Historically, these diseases (often included under the diagnosis of "anaplastic large-cell lymphoma Hodgkin-like") had a poor clinical outcome, and short median survivals were reported with standard therapy [37]. In one retrospective study, in which patients received either a CHL or NHL regimen, the 5-year event-free survival was inferior to that reported by the International Database on Hodgkin's Disease for CHL, suggesting that they had more chemoresistant biology [37]. In one updated report, 16 patients with GZL treated prospectively with the DA-EPOCH-R regimen—despite clinical characteristics similar to those of the PMBL cohort—had a significantly inferior event-free survival (45 %) and overall survival (75 %) at a median follow-up time of 4 years, and 37 % required consolidation mediastinal radiation [36]. Studies are under way to explore the biologic basis for their poor clinical outcome; factors may include bulky disease and poor vascularization of these large tumors, which often have extensive areas of necrosis. It is hoped that novel biologic insights such as the elucidation of their epigenetic and other molecular biologic features may lead to the development of more effective therapies.

Assessment of Residual Mediastinal Masses After Primary Therapy

In patients with PMBL, it is very common to have a large residual mediastinal mass at the completion of therapy due to the fibrotic component of these tumors and their large size at initial diagnosis. Indeed, scar tissue can persist in the mediastinum for several months after the completion of therapy, and this needs to be considered in the follow-up of patients. Therefore, computed tomography scanning (CT) alone is not a very effective modality at assessing if there is residual disease present at the end of therapy as it gives no information about the activity of the mass. In the past, gallium scanning was a helpful imaging modality to assess activity, but it is a cumbersome test and is rarely used today. FDG-PET imaging has been found to be helpful for response assessment in DLBCL but has not been well studied in PMBL specifically [38]. Recently, the role of FDG-PET in assessing residual masses when using the DA-EPOCH-R

regimen was investigated, and it was found to have a very high negative but low positive predictive value for relapse in PMBL [36]. With this regimen in PMBL, if the end of therapy FDG-PET scan is negative, no further FDG-PET scans are performed and patients are followed with routine CT scanning. Patients with suspicious FDG-PET scans—with low-level abnormal activity—generally undergo a repeat FDG-PET scan 4–6 weeks later and, if specific uptake values (SUVs) are increasing, undergo a biopsy ideally to confirm residual disease. Patients with positive post-therapy FDG-PET scans ideally undergo a biopsy also. Newer imaging modalities for the assessment of residual mediastinal masses are needed and are under investigation at this time.

Treatment of Relapsed or Refractory Disease

Relapses in PMBL tend to occur within the first year or 18 months following the completion of therapy, and optimal or standard therapy for patients with relapsed and refractory disease has not yet been defined. How best to approach relapsed disease depends on the pattern of relapse, and for localized relapses confined to the mediastinum, localized radiation treatment may be a curative treatment option, particularly in patients who did not receive radiation therapy initially. Otherwise, approaches such as using salvage chemotherapy and autologous transplantation have, for the most part, been disappointing, but patient numbers have been small. Allogeneic transplantation in patients with resistant and refractory disease is another experimental treatment option.

Conclusions

PMBL is a distinct entity that is clinically and molecularly different to other subtypes of DLBCL and has a gene expression profile that more closely resembles that of classical Hodgkin lymphoma, nodular sclerosis type. One of the major downsides of "standard" therapy for this disease historically has been the use of consolidation mediastinal radiotherapy, which has been associated with a high rate of secondary complications. Newer treatment approaches, however, successfully obviate the need for radiotherapy while maintaining high cure rates. Mediastinal "gray-zone" lymphoma is a recently recognized entity with features in between PMBL and classical Hodgkin lymphoma and appears to be more immunochemotherapy resistant. Future directions in these diseases should include the continuation of strategies that obviate the need for radiation and the exploration of selective targeting of pathways such as Janus kinase 2 (JAK2).

References

1. Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, et al. WHO classification of tumours of haematopoietic and lymphoid tissues. Lyon: IARC; 2008.
2. Traverse-Glehen A, Pittaluga S, Gaulard P, Sorbara L, Alonso MA, Raffeld M, et al. Mediastinal gray zone lymphoma: the missing link between classic Hodgkin's lymphoma and mediastinal large B-cell lymphoma. Am J Surg Pathol. 2005;29(11):1411–21.
3. Pileri SA, Zinzani PL, Gaidano G, Falini B, Gaulard P, Zucca E, et al. Pathobiology of primary mediastinal B-cell lymphoma. Leuk Lymphoma. 2003;44 Suppl 3:S21–6.
4. Moller P, Moldenhauer G, Momburg F, Lammler B, Eberlein-Gonska M, Kiesel S, et al. Mediastinal lymphoma of clear cell type is a tumor corresponding to terminal steps of B cell differentiation. Blood. 1987;69(4):1087–95.
5. Pileri SA, Gaidano G, Zinzani PL, Falini B, Gaulard P, Zucca E, et al. Primary mediastinal B-cell lymphoma: high frequency of BCL-6 mutations and consistent expression of the transcription factors OCT-2, BOB.1, and PU.1 in the absence of immunoglobulins. Am J Pathol. 2003;162(1):243–53.
6. Calaminici M, Piper K, Lee AM, Norton AJ. CD23 expression in mediastinal large B-cell lymphomas. Histopathology. 2004;45(6):619–24.
7. Salama ME, Rajan Mariappan M, Inamdar K, Tripp SR, Perkins SL. The value of CD23 expression as an additional marker in distinguishing mediastinal (thymic) large B-cell lymphoma from Hodgkin lymphoma. Int J Surg Pathol. 2010;18(2):121–8.
8. Schmid C, Pan L, Diss T, Isaacson PG. Expression of B-cell antigens by Hodgkin's and Reed-Sternberg cells. Am J Pathol. 1991;139(4):701–7.
9. Zukerberg LR, Collins AB, Ferry JA, Harris NL. Coexpression of CD15 and CD20 by Reed-Sternberg cells in Hodgkin's disease. Am J Pathol. 1991;139(3):475–83.

10. Foss HD, Reusch R, Demel G, Lenz G, Anagnostopoulos I, Hummel M, et al. Frequent expression of the B-cell-specific activator protein in Reed-Sternberg cells of classical Hodgkin's disease provides further evidence for its B-cell origin. Blood. 1999;94(9): 3108–13.
11. Loddenkemper C, Anagnostopoulos I, Hummel M, Johrens-Leder K, Foss HD, Jundt F, et al. Differential Emu enhancer activity and expression of BOB.1/ OBF.1, Oct2, PU.1, and immunoglobulin in reactive B-cell populations, B-cell non-Hodgkin lymphomas, and Hodgkin lymphomas. J Pathol. 2004;202(1):60–9.
12. Garcia JF, Mollejo M, Fraga M, Forteza J, Muniesa JA, Perez-Guillermo M, et al. Large B-cell lymphoma with Hodgkin's features. Histopathology. 2005;47(1): 101–10.
13. Rosenwald A, Wright G, Leroy K, Yu X, Gaulard P, Gascoyne RD, et al. Molecular diagnosis of primary mediastinal B cell lymphoma identifies a clinically favorable subgroup of diffuse large B cell lymphoma related to Hodgkin lymphoma. J Exp Med. 2003; 198(6):851–62.
14. Savage KJ, Monti S, Kutok JL, Cattoretti G, Neuberg D, de Leval L, et al. The molecular signature of mediastinal large B-cell lymphoma differs from that of other diffuse large B-cell lymphomas and shares features with classical Hodgkin's lymphoma. Blood 2003;102(12):3871–9.
15. Guiter C, Dusanter-Fourt I, Copie-Bergman C, Boulland ML, Le Gouvello S, Gaulard P, et al. Constitutive STAT6 activation in primary mediastinal large B-cell lymphoma. Blood. 2004;104(2):543–9.
16. Melzner I, Bucur AJ, Bruderlein S, Dorsch K, Hasel C, Barth TF, et al. Biallelic mutation of SOCS-1 impairs JAK2 degradation and sustains phospho-JAK2 action in the MedB-1 mediastinal lymphoma line. Blood. 2005;105(6):2535–42.
17. Oschlies I, Burkhardt B, Salaverria I, Rosenwald A, d'Amore ES, Szczepanowski M, et al. Clinical, pathological and genetic features of primary mediastinal large B-cell lymphomas and mediastinal gray zone lymphomas in children. Haematologica. 2011; 96(2):262–8.
18. Eberle FC, Rodriguez-Canales J, Wei L, Hanson JC, Killian JK, Sun HW, et al. Methylation profiling of mediastinal gray zone lymphoma reveals a distinctive signature with elements shared by classical Hodgkin's lymphoma and primary mediastinal large B-cell lymphoma. Haematologica. 2011;96(4): 558–66.
19. A predictive model for aggressive non-Hodgkin's lymphoma. The International Non-Hodgkin's Lymphoma Prognostic Factors Project. N Engl J Med. 1993;329(14):987–94.
20. Hamlin PA, Portlock CS, Straus DJ, Noy A, Singer A, Horwitz SM, et al. Primary mediastinal large B-cell lymphoma: optimal therapy and prognostic factor analysis in 141 consecutive patients treated at Memorial Sloan Kettering from 1980 to 1999. Br J Haematol. 2005;130(5):691–9.
21. Savage KJ, Al-Rajhi N, Voss N, Paltiel C, Klasa R, Gascoyne RD, et al. Favorable outcome of primary mediastinal large B-cell lymphoma in a single institution: the British Columbia experience. Ann Oncol. 2006;17(1):123–30.
22. Zinzani PL, Martelli M, Bertini M, Gianni AM, Devizzi L, Federico M, et al. Induction chemotherapy strategies for primary mediastinal large B-cell lymphoma with sclerosis: a retrospective multinational study on 426 previously untreated patients. Haematologica. 2002;87(12):1258–64.
23. Bassal M, Mertens AC, Taylor L, Neglia JP, Greffe BS, Hammond S, et al. Risk of selected subsequent carcinomas in survivors of childhood cancer: a report from the Childhood Cancer Survivor Study. J Clin Oncol. 2006;24(3):476–83.
24. Castellino SM, Geiger AM, Mertens AC, Leisenring WM, Tooze JA, Goodman P, et al. Morbidity and mortality in long-term survivors of Hodgkin lymphoma: a report from the Childhood Cancer Survivor Study. Blood. 2011;117(6):1806–16.
25. Dunleavy K, Bollard CM. Sobering realities of surviving Hodgkin lymphoma. Blood. 2011;117(6): 1772–3.
26. Tukenova M, Guibout C, Oberlin O, Doyon F, Mousannif A, Haddy N, et al. Role of cancer treatment in long-term overall and cardiovascular mortality after childhood cancer. J Clin Oncol. 2010;28(8): 1308–15.
27. Zinzani PL, Martelli M, Magagnoli M, Pescarmona E, Scaramucci L, Palombi F, et al. Treatment and clinical management of primary mediastinal large B-cell lymphoma with sclerosis: MACOP-B regimen and mediastinal radiotherapy monitored by (67)Gallium scan in 50 patients. Blood. 1999;94(10):3289–93.
28. Todeschini G, Secchi S, Morra E, Vitolo U, Orlandi E, Pasini F, et al. Primary mediastinal large B-cell lymphoma (PMLBCL): long-term results from a retrospective multicentre Italian experience in 138 patients treated with CHOP or MACOP-B/VACOP-B. Br J Cancer. 2004;90(2):372–6.
29. Fisher RI, Gaynor ER, Dahlberg S, Oken MM, Grogan TM, Mize EM, et al. Comparison of a standard regimen (CHOP) with three intensive chemotherapy regimens for advanced non-Hodgkin's lymphoma. N Engl J Med. 1993;328(14):1002–6.
30. Coiffier B, Lepage E, Briere J, Herbrecht R, Tilly H, Bouabdallah R, et al. CHOP chemotherapy plus rituximab compared with CHOP alone in elderly patients with diffuse large-B-cell lymphoma. N Engl J Med. 2002;346(4):235–42.
31. Rieger M, Osterborg A, Pettengell R, White D, Gill D, Walewski J, et al. Primary mediastinal B-cell lymphoma treated with CHOP-like chemotherapy with or without rituximab: results of the Mabthera International Trial Group study. Ann Oncol. 2011; 22(3):664–70.
32. Abramson J, Hellmann M, Feng Y, Barnes J, Takvorian T, Toomey C, et al. High rate of initial treatment failure in patients with primary mediastinal B-cell

lymphoma treated with R-CHOP. Blood ASH Annu Meet Abstr. 2011;118:1601.
33. Wilson WH, Bryant G, Bates S, Fojo A, Wittes RE, Steinberg SM, et al. EPOCH chemotherapy: toxicity and efficacy in relapsed and refractory non-Hodgkin's lymphoma. J Clin Oncol. 1993;11(8): 1573–82.
34. Wilson WH, Dunleavy K, Pittaluga S, Hegde U, Grant N, Steinberg SM, et al. Phase II study of dose-adjusted EPOCH and rituximab in untreated diffuse large B-cell lymphoma with analysis of germinal center and post-germinal center biomarkers. J Clin Oncol. 2008; 26(16):2717–24.
35. Wilson WH, Jung SH, et al. A Cancer and Leukemia Group B multi-center study of DA-EPOCH-rituximab in untreated diffuse large B-cell lymphoma with analysis of outcome by molecular subtype. Haematologica. 2012:97(5);758–65.
36. Dunleavy K, Pittaluga S, Shovlin M, Grant N, Grant C, Chen C, et al. Untreated primary mediastinal B-cell (PMBL) and mediastinal grey zone (MGZL) lymphomas: comparison of biological features and clinical outcome following DA-EPOCH-R without radiation. Ann Oncol. 2011; 22(4):abstract 149.
37. Cazals-Hatem D, Andre M, Mounier N, Copin MC, Divine M, Berger F, et al. Pathologic and clinical features of 77 Hodgkin's lymphoma patients treated in a lymphoma protocol (LNH87): a GELA study. Am J Surg Pathol. 2001;25(3):297–306.
38. Dunleavy K, Mikhaeel G, Sehn LH, Hicks RJ, Wilson WH. The value of positron emission tomography in prognosis and response assessment in non-Hodgkin lymphoma. Leuk Lymphoma. 2010;51 Suppl 1:28–33.

T-Cell Lymphomas

13

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Abstract

T-cell lymphomas account for 10–15 % of all adult non-Hodgkin lymphomas. Progress in understanding the biology of these lymphomas leads to improvement in their molecular classifications. However, the management of patients with T-cell lymphoma remains challenging, as these patients continue to have worse treatment outcome compared with B-cell lymphomas. In this chapter, we review the recent advances in T-cell lymphoma biology and emerging therapeutic strategies.

Keywords

Non-Hodgkin lymphoma • T-cell lymphoma • Hematopoietic cell transplantation

Introduction

T-cell non-Hodgkin lymphomas (NHL) are uncommon malignancies accounting for only 10–15 % of all NHL [1]. T-cell NHLs are classified, by the WHO, as precursor T-cell acute lymphoblastic leukemia/lymphoma (T-ALL/LBL) and mature (peripheral) T-cell lymphomas (PTCL). The designation "peripheral" describes that these lymphomas arise at peripheral lymphoid tissues from mature T cells that had already undergone maturation, and acquisition of function, in the "central" lymphoid tissues: the bone marrow and thymus gland. Because natural killer (NK) cells are closely related and share some immunophenotypic and functional properties with T cells, NK and T-cell neoplasms are considered together under PTCL designation [2]. The WHO system classifies T-cell NHLs into 16 major subtypes in adults (Table 13.1). PTCLs, like B-cell NHL, appear to recapitulate stages of normal T-cell differentiation by expressing a certain set of surface antigens analogous to their normal counterparts. However, immune profiling seems less helpful in the subclassification of PTCL due to

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significant variation in expression of these antigens. Reactive T-cell infiltrations of lymph nodes, extranodal tissues, or B-cell lymphomas pose a diagnostic problem in differentiating if the nature of the infiltration is inflammatory (reactive) or neoplastic. Determining the clonality of T cells is helpful and, frequently, required to establish the diagnosis utilizing rearrangements of the T-cell receptor (TCR). There are two classes of T cell based on specific sequence of their TCR: α[alpha]β[beta] T cells and γ[gamma]δ[delta] T cells. Clonal rearrangements in each of the four chains are possible [2]. NK cells do not rearrange the T-cell receptor genes. Analysis of clonality in NK-cell proliferations can utilize antibodies to various killer inhibitory receptors (KIRs). Granzyme M, a novel member of a family of cytolytic molecules unique to T cells, is expressed in hepatosplenic γ[gamma]δ[delta] lymphoma, cutaneous γ[gamma]δ[delta] lymphomas, and most intestinal T-cell lymphomas tested and may aid in the proper identification of these uncommon lymphomas [3].

Epidemiology and Etiology

Geographic variations have been well described and reflect exposure to specific infectious agents, such as the Epstein-Barr virus (EBV) and the human T-cell leukemia virus-1 (HTLV-1) [4, 5]. Exposure to these agents explains the higher prevalence of PTCL in Asian countries, 18 % of all NHL in Hong Kong compared to only 1.5 % in Vancouver [6]. Recent date from the SEER database suggests that the incidence of T-cell neoplasms in the USA had modestly increased to 2.6 cases per 100,000 persons per year [7]. In a large international study [8] that evaluated lymphoma cases from the United States, Europe, Asia, and South Africa, PTCL accounted for only 12 % of all NHL. The commonest subtypes of PTCL were PTCL not otherwise specified (PTCL-NOS) (25.9 %) and angioimmunoblastic T-cell lymphoma (AITL) (18.5 %), respectively (Table 13.2) [8]. Each of the major subtypes has unique clinical and biologic characteristics that are addressed separately in this chapter. Cutaneous T-cell lymphomas (CTCL) are also classified under mature T-cell lymphomas but will be discussed separately.

Table 13.1 2008 WHO classification of peripheral NK/T-cell neoplasms

Classification	NK/T-cell neoplasm
Leukemic or disseminated	T-cell prolymphocytic leukemia (T-PLL)
	T-cell granular lymphocytic leukemia (T-LGL)
	Aggressive NK leukemia
	Adult T-cell lymphoma/leukemia (ATLL)
Extranodal	Extranodal NK/T-cell lymphoma (nasal type and extranasal types)
	Enteropathy-type T-cell lymphoma
	Hepatosplenic T-cell lymphoma (HTCL)
	Subcutaneous panniculitis-like T-cell lymphoma
Cutaneous	Mycosis fungoides
	Sezary syndrome
	Primary cutaneous CD30+ lymphoma
	Primary cutaneous T-cell lymphoma, rare subtypes
Nodal	Peripheral T-cell lymphoma, unspecified
	Angioimmunoblastic T-cell lymphoma (AILD)
	Anaplastic large-cell lymphoma (ALK+)
	Anaplastic large-cell lymphoma (ALK-)
EBV-positive lymphoproliferative disorder of childhood	Systemic EBV-positive lymphoproliferative disorder of childhood
	Hydroa vacciniforme-like lymphoma

Peripheral T-Cell Lymphoma: Not Otherwise Specified

Peripheral T-cell lymphoma (PTCL), not otherwise specified (NOS), is the most common type of PTCL and is a heterogeneous group of mature T-cell neoplasms that do not meet the specific diagnostic criteria for other specific T-cell lymphomas listed in Table 13.1 (above). Most

patients present with nodal disease but extranodal disease is common. Most patients have advanced disease at presentation (60 %) and bone marrow involvement occurs in 20 % of cases. Extranodal presentation is common and the commonest sites involved are the skin and gastrointestinal tract [6, 9]. PTCL was considered the diffuse large-cell equivalent of B-cell NHL but the histologic picture of PTCL is extremely broad, from highly polymorphous to monomorphous [10]. Three histologic variants were recognized: lymphoepitheliod or Lennert's [11], follicular [12], and T-zone lymphomas [13]. Median age at diagnosis is 61 years with a male to female ratio of 1.5:1. Majority of patients have lymphadenopathy and unfavorable characteristics, at presentation, including B symptoms, elevated lactate dehydrogenase (LDH), bulky disease, poor performance status (PFS), and extranodal disease. As such, at least 50 % of patients fall into the unfavorable International Prognostic Index (IPI) category of 3–5 [6, 9]. Paraneoplastic features such as eosinophilia, pruritis, or rarely hemophagocytic syndrome may be also seen at presentation [14]. The prognostic index for PTCL-NOS (PIT) was proposed by the Intergruppo Italiano Linfomi and incorporates age, PFS, LDH, and bone marrow involvement to predict prognosis in PTCL [15]. Positivity for Epstein-Barr virus (EBV), CD15 staining, and high Ki-67 expression, identified by immunohistochemistry, were associated with worse prognosis [16]. The Bologna score, incorporating patient (age >60 years, PFS, LDH) and tumor (Ki-67 staining ≥80 %) characteristics, seemed to be able to predict PTCL outcomes better than IPI and PIT [17]. Based on recent gene expression profiling (GEP) studies, high expression of NFkB [18] and proliferation markers [19] were associated with worse outcomes for PTCL. Also, based on GEP analyses, PTCL-NOS can be subclassified into at least two distinct groups based on the cell of origin: activated helper and cytotoxic subtypes [20, 21]. There is preliminary evidence that the cytotoxic subtype confers a worse prognosis [21].

Treatment of PTCL-NOS with standard NHL chemotherapy regimens, like cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP), produces a 50–60 % response rate, but the long-term disease-free survival is poor with long-term survival rates between 10 and 30 % [22]. Alternative, more intense, induction regimens were proposed, such as HyperCAVD or regimens incorporating platinum compounds. None of these alternative regimens were better than the standard CHOP regimen [23, 24]. There are multiple chemo- and immunotherapy agents, including stem cell transplants, that have activity and are frequently used in the relapsed setting; these are discussed separately under treatment of relapsed/refractory disease. Limited-stage PTCL is a well-recognized clinical entity, albeit not recognized by the WHO classification, which has a favorable clinical outcome. Most cases of limited-stage PTCL are PTCL-NOS (81 %) and are typically treated with an abbreviated course of CHOP followed by involved-field radiotherapy with an OS of 92 % in one series [25].

Angioimmunoblastic T-Cell Lymphoma

Angioimmunoblastic T-cell lymphoma (AITL), previously known as angioimmunoblastic lymphadenopathy with dysproteinemia, was the second most common PTCL in the international T-cell lymphoma classification project accounting for 18.5 % of all PTCL [8]. Histologically, AITL is characterized by a polymorphous infiltrate of small lymphocytes, immunoblasts, plasma cells, eosinophils, histiocytes, and epitheloid cells with neovascularization with arborizing high endothelial venules (HEV), many of which show thickened or hyalinized walls [26]. T-cell receptor genes are rearranged in 75–90 %; immunoglobulin heavy chains may be rearranged in 25 %, corresponding to the secondarily expanded B-cell clones [27, 28]. Epstein-Barr virus (EBV) and human herpes virus-6 (HHV6) genomes are detected in many cases and may be present in either T or B cells, although EBV is far more common in the B cells [29]. B immunoblasts that are EBV positive are sometimes very prominent and can give rise to a secondary EBV positive diffuse large B-cell

lymphomas [30, 31]. Also, albeit rare, CD20 expression was reported on malignant T cells [32]. Most patients present with advanced stage and isolated extranodal disease at presentation is rare. Bone marrow is involved in 50–60 % of cases [33]. The median age was 64 years with slight male predominance, and the majority of patients present with an acute systemic illness with B symptoms and generalized lymphadenopathy [9]. Other commonly associated manifestations are rash, hepatosplenomegaly, anemia, ascitis, pleural effusion, polyarthritis, and a myriad of autoimmune manifestations including hemolytic anemia, rheumatoid arthritis, vasculitis, and thyroid diseases [34, 35]. The rash is usually pruritic and may demonstrate lymphohistiocytic vasculitis on biopsy [36]. Multiple laboratory findings were reported including elevated LDH, erythrocyte sedimentation rate (ESR), polyclonal hypergammaglobulinemia which can occasionally be monoclonal, hypoalbuminemia, elevated β-2 microglobulin, positive Coombs test or cold agglutinins, cryoglobulinemia, eosinophilia, anemia, lymphopenia, and thrombocytopenia [34, 35]. Serum interleukin-6 (IL-6) level is frequently elevated and can be used to monitor disease activity and response to treatment [37, 38]. The clinical course of AITL is moderately aggressive yet spontaneous remissions have been reported [39]. Most patients fall into the high-risk category, when assessed by IPI or PIT models, yet these prediction models have limited applicability to patients with AITL. In some studies, high doses of prednisone were used upfront followed by standard chemotherapy if patients progress or relapse. The standard treatment for medically fit patients with AITL usually involved a conventional anthracycline-based regimen, like CHOP, with complete response rates of 50–70 %, yet only 10–30 % of patients survive long term [40, 41]. Factors predictive of poor survival in AITL, in a recent analysis by the Groupe d'Etudes des Lymphomes de L'Adulte (GELA), were male sex and mediastinal adenopathy. Only 30 % 7-year survival rates were reported in the GELA studies [39]. A favorable result was observed with adding rituximab to CHOP in patients with AITL who have an expanded B-cell clone [42], yet a recent phase II trial conducted by GELA failed to show any benefit [43]. There are anecdotal reports of patients with AITL who responded to immunosuppressive therapy, such as low-dose methotrexate/prednisone, cyclosporine, or purine analogs [44, 45]. There are also anecdotal reports, in few patients, of CRs after treatment with bevacizumab, an antibody against vascular endothelial growth factor [46, 47]. The results of stem cell transplantation for AITL are similar to PTCL-NOS and will be discussed separately at the end of this chapter.

Anaplastic Large-Cell Lymphoma: Systemic Type

Anaplastic large-cell lymphoma (ALCL), primary systemic type, accounts for 2–3 % of all NHL [1], 10.2 % of all T-cell lymphomas in adults [8], and up to 20 % of all childhood lymphomas [48]. Primary cutaneous ALCL will be discussed separately in Chap. 19. This type of lymphoma usually involves both nodal and extranodal sites, commonest being skin, bone, soft tissues, lung, and liver. ALCL shows a broad morphologic spectrum, yet all cases contain a variable proportion of cells with eccentric kidney-shaped nuclei and an eosinophilic region near the nucleus. These cells have been referred to as hallmark cells and they show a strong positive staining for CD30. Bone marrow is involved in 10–30 % of cases [49]. There are two major subtypes of ALCL based on expression of the anaplastic lymphoma kinase (ALK) protein on chromosome 2p23: ALK+ and ALK− ALCL. The commonest translocation observed in ALK+ ALCL cases is t(2;5)(p23;q35) which results in the fusion protein NPM-ALK; this is present in 84 % of cases and is considered pathognomonic for ALK+ ALCL [50]. The fusion protein TPM3-ALK results from t(1;2)(q25;p23) and occurs in 13 % of cases of ALK+ ALCL. Other rare translocation partners of ALK gene have been reported [51]. ALK+ ALCL is usually diagnosed in younger patients (median age 34 years) and ALK- ALCL in older patients

(median age 58 years), although this is not an exclusive cutoff. Slight male predominance 1.5:1 has been reported for both subtypes [8, 52]. Majority of patients presents with advanced disease, peripheral and abdominal adenopathy, and B symptoms with high fevers. The US Food and Drug Administration (FDA) has recently expressed concern about silicone breast implants and ALCL developing in the scar capsule adjacent to the implant based on 34 unique cases reported in the literature [53]. Therapy for systemic ALCL typically includes an anthracycline-based regimen (CHOP being most popular). The ALK status of the patient with ALCL is very important as patients with ALK+ ALCL have a 5-year survival of 70 % compared with 49 % for ALK- ALCL when treated with an anthracycline-based regimen [8, 52, 54, 55]. Relapsed patients with ALCL benefit from salvage chemotherapy followed by an autologous stem cell transplant (ASCT); the outcomes of salvage therapy and ASCT remain inferior for ALK- ALCL [56].

Extranodal NK/T-Cell Lymphoma

There are two major subtypes of extranodal NK/T-cell lymphomas: nasal and extranasal. These are predominantly extranodal lymphomas characterized by vascular invasion, hence the historic term "angiocenteric lymphoma." Most cases appear to express the NK phenotype ($CD2^+$ $CD56^+$ surface $CD3^-$ cytoplasmic CD3ε[epsilon]$^+$); however, some cases may express a cytotoxic T-cell phenotype, hence the designation "NK/T." TCR is clonally rearranged in T cell but germline in NK-cell lymphomas [57]. The presence of EBV, most accurately determined by in situ hybridization (ISH) for EBV-encoded RNA (EBER) in tumor cells, is universal [58], required by the WHO scheme to make the diagnosis, and useful to exclude bone marrow involvement on staging biopsies [59]. The two types of extranodal NK/T-cell lymphomas are more common in Asians and Native Americans. EBV plays a central role in the pathogenesis of these lymphomas irrespective of the ethnic origin of the patient, and disease activity may be monitored by measuring circulating EBV DNA [60]. The male to female ratio is 3:1 with disease peaking in the fifth decade of life. Nasal NK/T-lymphoma is the commonest nasal lymphoma in Asian patients and presents as destructive mass lesion involving the nasal cavity, nasopharynx, paranasal sinuses, tonsils, hypopharynx, and larynx. Destruction of the hard palate leads to the characteristic midline perforation, from which the historic term "lethal midline granuloma" was originally derived [57, 61]. Nonnasal NK/T-lymphoma may involve any anatomic site, commonest being the skin, gastrointestinal tract, salivary glands, spleen, and testis. These are the same sites that nasal NK/T-lymphoma disseminates to; hence, imaging studies and a comprehensive ENT examination are indicated to exclude an occult nasal primary. In contrast to nonnasal type, nasal NK/T-lymphoma is locally malignant with distant organ dissemination, including bone marrow involvement, occurring in only 10 % of cases. Peripheral blood cytopenias occur in 10–15 % of cases of nasal and nonnasal NK/T-cell lymphomas are usually secondary to active hemophagocytosis in the marrow, not necessarily associated with direct involvement of the bone marrow with lymphoma [57]. Aggressive NK/T-cell leukemia/lymphoma is a rare and catastrophic disorder usually presenting in the third decade of life with men and women equally affected. Clinical features include high fevers, significant weight loss, jaundice, skin infiltration, lymphadenopathy, hepatosplenomegaly, circulating leukemia cells, and marrow hemophagocytosis leading to severe cytopenias. Liver failure and disseminated intravascular coagulopathy (DIC) appear progressively. This disorder is lethal in few weeks with few treatment successes reported [62]. Disseminated NK/T-lymphoma usually presents in the same way yet the clinical course is usually less aggressive. Different prognostic models were developed for extranodal NK/T-lymphoma but the IPI remains predictive of prognosis [63]. The 5-year survival for localized and disseminated extranodal NK/T-cell lymphomas is 42 and 9 %, respectively [8]. Localized (stage I/II) nasal NK/T-cell lymphoma should be treated by combined chemotherapy concurrent with, or followed by, radiotherapy with an expected cure rate of 70–80 % [63–65].

Systemic relapse was reported in 30 % of patients treated with radiotherapy alone, and inferior outcomes were reported with radiotherapy lower than 50 Gy. Combination chemotherapy is the mainstay for treatment of advanced NK/T-cell lymphoma. Historically, conventional CHOP or CHOP-like regimens were associated with poor outcomes with CR achieved in less than 20 % of cases [57]. This could be secondary to the expression of multidrug-resistant 1 (MDR-1) gene leading to the high level of P-glycoprotein-mediated efflux of many chemotherapeutic agents including anthracyclines [66]. A novel regimen SMILE, comprising dexamethasone, methotrexate, ifosfamide, L-asparaginase, and etoposide, resulted in ORR of 74 % and CR rate of 30–50 % in patients with relapsed/refractory NK/T-cell lymphomas [67]. This regimen is based on agents exported by the P-glycoprotein together with reported single-agent activity of L-asparaginase against these lymphomas [68]. Results of autologous or allogenic SCT in patients with disseminated or relapsed/refractory disease are disappointing owing to the fact that it is difficult to get these patients into remission prior to the transplant [57]. A marginal benefit for consolidation with ASCT in patients with localized nasal NK/T-cell, in a retrospective analysis, in terms of decreased risk of relapse however there was no survival benefit [69].

Hepatosplenic T-Cell Lymphoma

Hepatosplenic T-cell lymphoma (HSTL) is an extranodal and systemic neoplasm derived from cytotoxic/memory T cells of the innate immune system and is characterized by marked sinusoidal infiltration of the liver, spleen, and bone marrow by medium-sized lymphocytes ($CD3^+$, $CD4^-$, $CD5^-$, $CD8^{+/-}$, $CD56^{+/-}$, TCRα[alpha]/β[beta]$^-$, TCR γ[gamma]/δ[delta]$^+$, $EBER^-$, granzyme M^+, granzyme B^+). Most cases have clonal rearrangements of γ[gamma]/δ[delta] TCR and few have a/b rearrangements. Isochromosome 7q is present in most cases and copy numbers seem to increase with disease progression [70]. HSTL is a rare lymphoma (1.4 % of all T-cell NHL) and usually presents in young patients (median age is 35) with male predominance [8]. Up to 20 % of HSTL arise in chronically immune-suppressed patients, as in solid organ transplant recipients or after chronic antigenic stimulation [71]. HTSL was reported in patients with Crohn's disease especially after treatment with azathioprine and infliximab [72]. Most patients present with B symptoms, marked hepatosplenomegaly, and cytopenias secondary to bone marrow involvement. Bone marrow is usually involved and hemophagocytosis may be evident on presentation. The clinical course is typically aggressive despite treatment with intensive anthracycline-based chemotherapy regimens [71]. Pentostatin- [73] and platinum/cytarabine-based [71] regimen also has some activity. In a recent series of 15 patients, 50 % of patients achieved CR but the medical overall survival was 1 year [74]. Autologous and allogeneic SCT may be in option in fit patients who sustained a CR with induction chemotherapy.

Enteropathy-Associated T-Cell Lymphoma

Enteropathy-associated T-cell lymphoma (EATL) is a rare disorder than accounts for less than 1 % of all NHL. More than 90 % of cases of EATL arise on a background of active celiac disease and involves the proximal jejunum or less commonly the ileum [75]. Many patients have a DQA1*0501 or DQB1*0201 HLA haplotype that is commonly associated with celiac disease [76]. EATL usually presents as multiple ulcerated mucosal lesions invading into the intestinal wall and leading to bowel perforation. Less commonly, the tumor may invade other organs. EATL is usually characterized by a pleomorphic cellular infiltrate. Tumor cells have a characteristic phenotype ($CD3^+$, $CD5^-$, $CD7^+$, $CD8^-$, $CD56^-$, $CD103^+$), and most cases have rearranged TCRβ[beta]. An even rarer type II (monomorphic) EATL represents only 10–20 % of all cases of EATL. Type II EATL is characterized by small/medium monomorphic cells (that have the same phenotype as the classic type except being

CD8+, CD56+) and is not associated with celiac disease [77]. Most cases of EATL (58–70 %) harbor complex segmental amplifications of the 9q31 chromosomal region, which are typically absent in other forms of PTCL [78]. Most patients have adult-onset celiac disease and present with abdominal pain associated with bowel perforation. EATL can be effectively prevented with control of celiac disease with a gluten-free diet, as other celiac-disease-associated NHLs. In a proportion of patients, there is a prodromal period of refractory celiac disease associated with ulcerative jejunitis [75]. EATL is an aggressive disorder with a reported 5-year survival rate of only 20 % [8]. Treatment is complicated with intestinal perforations, fistula formation, and malnutrition. Treatment of EATL typically involves an anthracycline-based regimen along with gluten-free diet and support with total parenteral nutrition (TPN). A retrospective study of 26 patients with EATL treated with an intensive chemotherapy regimen (ifosfamide, vincristine, etoposide, and methotrexate) followed by ASCT reported rates of PFS and OS at 5 years of 52 and 60 %, respectively [79]. Although a randomized trial is needed for a direct comparison, these rates are much higher than those expected from treatment with chemotherapy alone.

Subcutaneous Panniculitis-Like T-Cell Lymphoma

Subcutaneous panniculitis-like T-cell lymphoma (SPTCL) is a rare lymphoma that infiltrates the subcutaneous fat without epidermal or dermal involvement. The neoplastic cells vary in size and infiltrate the fat lobules usually sparing the septa [80]. The neoplastic cells are usually CD8+ CD56− granzyme B+ EBER− and have clonally rearranged TCRα[alpha]/β[beta] genes. Visceral dissemination is rare with this disease [80, 81]. In contrast to the 3rd edition of the WHO classification, cases expressing the γ[gamma]δ[delta] TCR are excluded from SPTCL designation and are reclassified as primary cutaneous γ[gamma]δ[delta] T-cell lymphoma. Primary cutaneous γ[gamma]δ[delta] T-cell lymphomas may show panniculitis-like features but commonly involve the epidermis and dermis and typically run a more aggressive course than SPTCL [82]. Some SPTCL lesions have overlapping features with lupus profundus panniculitis, and the diagnosis of systemic lupus erythematosus (SLE) has been documented in 20 % of patients. The median age of presentation is 35 years but 20 % of patients are under the age of 20 [83]. Patients usually present with skin plaques or violaceous nodules, hepatosplenomegaly (which is almost always reactive with no evidence of lymphomatous involvement), lung infiltrates, and fever. Lymphadenopathy is rare. Systemic symptoms are present in at least 50 % of patients. Liver function abnormalities and cytopenias are common [80, 81]. A frank hemophagocytic syndrome occurs in 10–20 % of patients and confers a poor prognosis [83, 84]. Otherwise, the prognosis of SPTCL is usually good with a 5-year median OS of 80 % [83]. Traditional anthracycline-based combination chemotherapy has been used but one study suggests that more conservative regimens may be as effective, including chlorambucil, prednisone, and cyclosporine [85]. A distinction from cutaneous γ[gamma]δ[delta] T-cell lymphomas is important as SPTCL carries a much better prognosis [83].

Adult T-Cell Leukemia/Lymphoma

Adult T-cell leukemia lymphoma (ATLL) accounted for 9.6 % of all PTCL diagnoses in the International Peripheral T-cell Lymphoma (IPTCL) project [8]. ATLL is causally related to HTLV-1 and is more common in regions of the world where HTLV-1 infection is endemic: Southwestern Japan, the Caribbean basin, and parts of Central Africa [86, 87]. In the United States, the incidence of ATLL is 0.05 cases per 100,000 and is more common in African-Americans [88]. HTLV-1 infection alone is not sufficient to cause the disease and the disease had a long latency; the cumulative incidence of ATLL is 2.5 % among all HTLV-1 carries in Japan. HTLV-1 is transmitted by blood products and through breast milk [86]. A widespread screening program for HTLV-1 was commenced

in Nagasaki where carrier mothers were identified prior to starting breast-feeding. The Nagasaki ATLL Prevention Program resulted in a decrease of mother-to-child transmission of HTLV-1 from 20.3 to 2.5 % by substituting bottle-feeding for carrier mothers [89]. Most ATLL patients present with disseminated disease widespread lymph node and peripheral blood (PB) involvement. The skin is involved in more than 50 % of cases. Other organs that may be involved are the spleen, liver, lung, gastrointestinal tract (GI), and the central nervous system (CNS). ATLL is characterized by a broad spectrum of cytologic features; small, large, anaplastic, and AITL-like forms were described [86]. Lymph nodes in some patients with early form of ATLL show an expanded EBV^+ B-cell clone and may exhibit a Hodgkin-like histology [90]. In the PB, typically polylobated "flower" cells are seen but small cells with convoluted nuclei may be seen in the chronic variant (see below). The typical phenotype of neoplastic cells is $CD2^+$, $CD3^+$, $CD5^+$, $CD7^-$, $CD4^{+/-}$, and $CD8^{+/-}$. CD25 is strongly positive in most cases and CD30 may be positive in large transformed cells [86]. Cells are typically positive for the chemokine receptor CCR4 and FOXP3, markers of regulatory (Treg) T-cells. Hence, ATLL is believed to arise from Treg cells which would explain the immune deficiency state observed with this disorder [91]. TCR genes are clonally rearranged in ATLL and the most common karyotypic changes involve trisomy or partial trisomy of 3q, 6q, 14q, and inv(14) [92].

The median age of presentation varies based on geographic distribution; the mean age of presentation was 62 years, in the IPTCL project, with slight male predominance [8]. Several clinical variants of ATLL have been identified: acute, lymphomatous, chronic, and smoldering ATLL [93]. The acute variant is the most common and is characterized by a leukemic phase (with typically very high white count), generalized lymphadenopathy, hypercalcemia (with or without lytic bone lesions), hepatosplenomegaly, fever, skin rash (simulating mycosis fungoides), elevated LDH, and eosinophilia. Most patients have an associated T-cell immunodeficiency with frequent opportunistic infections with Pneumocystis jirovecii, strongyloidiasis, and fungal infections. The lymphomatous variant is similar to the acute variant except for PB involvement. The chronic variant is associated with exfoliative skin rash, subtle or absent lymphocytosis in the PB, and no hypercalcemia. In the smoldering variant, the PB white cell count is normal with a small morphologically normal ATLL clone (<5 %), no hypercalcemia, and frequent skin and pulmonary lesions. Progression from the chronic and the smoldering forms to the acute forms occurs in 25 % of cases usually after a long duration [93].

The prognosis of the acute variants of ATLL is poor with median survival of 6–12 months and a 5-year OS of only 14 % [8, 93]. The indolent variants of ATLL have favorable prognosis with a median survival of 2–5 years [94]. Watchful waiting is an acceptable option in selected patients with chronic/smoldering ATLL who are asymptomatic and have no high-risk features [94]. In addition to imaging, bone marrow biopsy, upper GI endoscopy with biopsy, and lumbar puncture should be considered for staging evaluation. Patients had been traditionally treated with standard anthracycline-based chemotherapy regimens, but the results have been disappointing because of frequent relapses [95]. A novel regimen, VCAP-AMP-VECP (also known as LSG15), which includes treatment with vincristine, cyclophosphamide, doxorubicin, prednisone, ranimustine, vindesine, etoposide, and carboplatin, was proposed by the Japan Clinical Oncology Group (JCOG) and resulted in a higher 3-year OS rate of 24 % when compared to 13 % with every 2 weeks CHOP. This was at the expense of more toxicity and treatment-related mortality [96, 97]. Intrathecal chemotherapy is recommended for all patients who are actively treated because of the high rate of CNS involvement (10–25 % at diagnosis or relapse) [98, 99]. Results of ASCT had been disappointing because of the extensive involvement of PB in most patients and frequent early relapses [100]. Allogeneic SCT may be considered for selected patients who are

medically fit, who have an available donor, and whose disease is in remission. Retrospective data are available from Japan regarding 386 patients with ATL who underwent allogeneic SCT from an HLA-matched related donor (154 patients), HLA-mismatched related donor (43 patients), unrelated marrow donor (99 patients), or unrelated cord blood donor (90 patients). After a median follow-up of 41 months, the estimated 3-year survival rate was 33 %. Four factors were significantly associated with worse outcomes: age >50 years, male sex, disease not in complete remission at the time of HCT, and unrelated donor source [101].

Treatment of Relapsed/Refractory Disease

Unfortunately, most patients with PTCL will not achieve remission or will relapse [22]. Patients with PTCL who relapse, or fail to attain a CR, after frontline chemotherapy have poor prognosis overall and rare long-term survival. Traditional salvage chemotherapy regimens including ICE (ifosfamide, carboplatin, etoposide), DHAP (dexamethasone, high-dose cytarabine, and cisplatin), ESHAP (etoposide, methylprednisolone, cytarabine, and cisplatin), single-agent gemcitabine, gemcitabine/cisplatin/dexamethasone, and gemcitabine/oxaliplatin had been used with mixed results. Published experience is largely limited to case reports of patients with PTCL and phase II trials in a heterogeneous population of patients with aggressive NHLs. Patients with PTCL have comprised a minority of the patients included in trials of these regimens for aggressive NHL. With these regimens, overall response rates for patients with PTCL are approximately 40–50 % [102]. A novel regimen of dose-dense CHOP along with etoposide and bleomycin (CyclOBEAP) had a promising activity in 84 previously untreated patients with PTCL with a 5-year PFS and OS of 69 and 72 %. However, this study included patients with low- and intermediate-risk IPI, and the 5-year OS for patients with PTCL-NOS who has a high-risk IPI score was still inferior at 25 % [103]. The results of the pivotal PROPEL (Pralatrexate in Patients with Relapsed or Refractory Peripheral T-Cell Lymphoma) trial has recently been published. Pralatrexate is a novel antifolate agent that showed a significant preclinical activity in models of T-cell NHLs. This was multinational phase II trial that enrolled a total of 115 patients with PTCL. These were heavily pretreated patients; median number of prior treatment was 3 and 16 % had prior ASCT. The majority of these patients had PTCL-NOS (53 %). The ORR to pralatrexate 30 mg/m^2 weekly for six of every 7 weeks along with folic acid 1 mg by mouth daily and vitamin B12 injections monthly was 27 % (with 10 % CRs), and the mean duration of response was 10.1 month. The median PFS was 3.5 month and the median OS was 14.5 months. Pralatrexate was generally well tolerated; the most common grade 3/4 adverse events were thrombocytopenia (32 %), mucositis (22 %), neutropenia (22 %), and anemia (18 %) [104].

Several trials examined the role of alemtuzumab, a monoclonal antibody against CD52 (a surface antigen that is expressed on 50 % of PTCL), in patients with PTCL either alone or in combination with chemotherapy [105]. In 2 small pilot studies, alemtuzumab, as a single-agent, had an ORR of 36–60 % [106, 107]. Three small trials examined the addition of alemtuzumab to standard CHOP (or CHOP every 2 weeks), and a significant efficacy was observed with ORR as high as 90 % with a highest median OS of 23 months. The cost was an unacceptably high rate of infectious complications, with occasional mortalities, despite the use of growth factors. The rate of cytomegalovirus (CMV) reactivation was as high as 35 % with frequent invasive CMV disease, invasive fungal disease, bacterial sepsis, and EBV$^+$ lymphoproliferative disorders [108– 110]. The role of CHOP plus alemtuzumab in PTCL is undefined. The regimen has significant activity but also worrisome infectious toxicity. There are additional reports of alemtuzumab being used in combination with other agents, such as pentostatin, gemcitabine, and cladribine, but a paucity of systematized data precludes any meaningful

comment about the future of such combinations [102]. A phase II trial of single-agent denileukin diftitox (DD), a recombinant interleukin-2 (IL-2)-diphtheria toxin fusion protein that is approved by the United States FDA for patients with persistent or recurrent cutaneous T-cell lymphoma (CTCL) expressing the CD25 component of the IL-2 receptor, in patients with relapsed or refractory PTCL reported an ORR of 61 %. Toxicities included hypoalbuminemia, transaminitis, flu-like symptoms and/or an infusion reaction, and vascular leak syndrome [111]. A recently reported multi-institutional phase II trial of CHOP plus DD in 49 newly diagnosed patients with PTCL showed an ORR of 68 % with 57 % CRs. Median PFS for the 49 patients was 12 months and 2-year estimated OS was 60 %. Median response duration for 32 responders was 29 months. The combination of CHOP plus DD was generally well tolerated; the most frequent grade 3/4 adverse events were leukopenia (20 %), thrombocytopenia (12 %), and febrile neutropenia (12 %). DD-associated toxicities included infusion-related rigors, hypoalbuminemia, and acute hypersensitivity reactions [112]. Based on these encouraging results, a multicenter randomized trial comparing CHOP to DD with CHOP is being initiated.

The efficacy of bortezomib, a proteasome inhibitor used in the treatment of multiple myeloma, in PTCL was observed in a phase I clinical trial that enrolled 13 patients with aggressive NK/T-cell lymphomas where the CR rate was 62 % [113]. The NFκ[kappa]B pathway, whose activity is downregulated by bortezomib, is critical for the proliferation and survival of normal T cells, and a differential expression of NFκ[kappa]B pathway genes has been observed in most subtypes of PTCL [18]. A phase II trial of bortezomib (1.3 mg/m^2 on days 1, 4, 8, and 11 of a 21-day cycle) in 15 patients with relapsed/refractory PTCL (2 patients) or CTCL reported an ORR of 67 % with 2 CRs. Of the two patients with PTCL, one attained a CR. The responses were durable, lasting on average 7–14 months [114]. The primary toxicity of bortezomib in this setting appears to be similar to that seen in patients with multiple myeloma and mainly consists of neuropathy and thrombocytopenia. The GELA group had examined the addition of bortezomib to an intensified CHOP-like regimen ACVBP (dose-intensified doxorubicin, cyclophosphamide, vindesine, bleomycin, and prednisone) in 57 previously untreated patients, mostly of the AITL and PTCL-NOS subtypes in attempt to enrich for NFκ[kappa]B-overexpressing PTCLs. In this trial, the CR rates were 45 % after induction and 45 % after consolidation cycles. The ORR and survival was not different than the old historic cohort treated with ACVBP [115]. A phase II study of lenalidomide, another anti-myeloma agent, at a dose of 25 mg/m^2 daily for 21 days of a 28-day cycle was conducted in 24 relapsed PTCL patients. The ORR was 30 % with a PFS of 95 days. Toxicities included neutropenia and thrombocytopenia in 20 and 33 % of patients, respectively [116].

Histone deacetylase (HDAC) inhibitors are potent inducers of histone acetylation, which results in the expression of tumor suppressor genes that had been previously silenced by deacetylation. This gene expression leads to cell cycle arrest and apoptosis. There are a number of HDAC inhibitors being used or studied in T-cell lymphoma, including vorinostat, romidepsin (also known as depsipeptide), panobinostat, and belinostat. Both romidepsin and vorinostat are approved by the US FDA for the treatment of CTCL that failed one prior therapy [102]. The efficacy of romidepsin, as single agent, in relapsed/refractory PTCL was confirmed in a recent multi-institutional phase II trial of 45 heavily pretreated patients (median number of prior treatments = 3). In this trial, the ORR was 38 % with 18 % CRs. The median duration of response was 8 months for all patients who responded and 29.7 months in patients who achieved a CR [117]. The results of a pivotal multinational phase II trial of romidepsin in 130 relapsed/refractory PTCL had been recently presented. These were also heavily pretreated patients with at least two prior treatments and 16 % have failed an ASCT. In that trial, the ORR was 26 % with 15 % CRs with a median duration of response of 12 months [118]. Romidepsin was generally well tolerated in both trials, and the major toxicities reported

were constitutional, gastrointestinal, and thrombocytopenia. Based on the results of these two trials, romidepsin has very recently received an accelerated approval by the US FDA for the treatment of PTCL in patients who have received at least one prior therapy. Belinostat, given as an IV infusion for 5 days every 3 weeks, has shown efficacy in a recent multinational phase II trial in 53 patients including 19 with refractory PTCL and 29 with refractory CTCL. The ORR in patients with PTCL was 32 % with 2 CRs and median response duration of 8.9 months. Severe (grade 3/4) adverse events reported included peripheral edema, apraxia, adynamic ileus, pruritus, rash, thrombocytopenia, and infection. One patient died of ventricular fibrillation 6 days after discontinuing treatment. There have been concerns regarding the potential for HDAC inhibitors to prolong the QT interval and result in cardiac arrhythmias [119]. A multicenter phase II registration trial of belinostat in relapsed PTCL patients is underway, and a cohort dose escalation study of oral belinostat is ongoing in patients with relapsed lymphoma.

Novel Therapies

Because many of the standard chemotherapeutic agents don't work well for PTCL, new alternatives are currently explored. Many agents, including monoclonal antibodies (MAb) and immunoconjugates, are in various phases of development from preclinical to phase I/II studies (Table 13.2). In a recent interim analysis of a multicenter phase II trial of brentuximab vedotin (SGN-35), an antibody-drug conjugate that delivers the highly potent antimicrotubule agent monomethyl auristatin E (MMAE) to $CD30^+$ malignant cells, an impressive overall response rate of 87 % (with a CR of 57 %) was observed. In this study, 75 % of patients were ALK^-, 27 % had prior ASCT, and the mean number of prior treatments was two [120]. These are preliminary yet very exciting data and will likely change the landscape for treatment of ALCL specially patients with relapsed/refractory disease or ALK^- patients where there aren't much options currently available to offer these patients. Siplizumab is an anti-CD2 MAb. CD2 is an adhesion molecule highly expressed on activated T cells and NK cells and on the majority of cells from patients with T-cell lymphoma and leukemia. In a phase I trial in patients with $CD2^+$ lymphoproliferative disease, siplizumab showed clinical activity, inducing CRs in two patients with large granular lymphocyte leukemia (LGL), 3 PRs in patients with ATLL, and 1 PR in a patient with CTCL [121]. However, siplizumab also predisposes patients to the development of lymphoproliferative syndrome though it may be possible to prevent that with prophylactic rituximab [122]. CD4 is expressed in half of all T cells and by most CTCL and nodal PTCL cells. Zanolimumab, an anti-CD4 MAb, is being used in both disease types, though clinical development for CTCL is farther along. Zanolimumab was shown to be active and well tolerated in a study of 21 PTCL patients, with an ORR in 24 % of patients [123]. Clinical studies of zanolimumab in combination with CHOP are ongoing and include a phase I/II dose escalation trial in patients with noncutaneous $CD4^+$ PTCL. Antiviral agents were examined in ATLL given the direct causal link between HTLV-1 and the disease. The use of interferon and zidovudine has been shown to induce responses in up to 50 % of patients with acute or lymphomatous ATLL [124]. In a recent meta-analysis of 245 patients with ATLL, the 5-year OS rates were 46 % for 75 patients who received first-line antiviral therapy, 20 % for 77 patients who received first-line chemotherapy, and 12 % for 55 patients who received first-line chemotherapy followed by antiviral therapy. In a subset analysis, the patients with acute, chronic, and smoldering ATLL significantly benefited from first-line antiviral therapy, whereas patients with lymphomatous ATLL experienced a better outcome with chemotherapy. Multivariate analysis showed that first-line antiviral therapy significantly improved overall survival (hazard ratio = 0.47) [125]. Despite that the difference between the treated groups was highly statistically significant, considering the potential selection bias in a retrospective design, future prospective studies are needed to further define

Table 13.2 Novel agents currently in clinical trials for PTCL

Type of agent	Name	Description	Disease(s)	Status
Antifolates	Pralatrexate	10-deazaaminopterin	PTCL, CTCL	Approved for PTCL
Conjugates	LMB-2	Anti-Tac (anti-CD25 fused to Pseudomonas toxin)	CTCL, PTCL (esp ATL)	Phase II
	Denileukin diftitox	IL-2 targeting domain fused with diphtheria toxin	CTCL, PTCL	Approved for CTCL
	Brentuximab vedotin	CD30 antibody conjugated to monomethyl auristatin E	CD30+ T-cell lymphoma	Phase II
HDAC inhibitors	Belinostat	PXD101	CTCL, PTCL	Phase II
	Panobinostat	LBH589	CTCL, ATL	Phase II
	Romidepsin	Depsipeptide	CTCL, PTCL	Approved for CTCL
	Vorinostat	Suberoylanilide hydroxamic acid (SAHA)	CTCL	Approved for CTCL
Immunomodulatory agents	Lenalidomide	Derivative of thalidomide	PTCL, CTCL	Phase II
Immunosuppressive agents	Cyclosporine	Inhibitor of the NF-AT transcription complex	A1TL	Phase II
Monoclonal antibodies	Alemtuzumab	Anti-CD52	PTCL	Phase III
	Bevacizumab	Anti-VEGF	PTCL (esp AITL), NK cell	Phase II
	Iratumumab	Anti-CD 30	CD30+ ALCL	Phase I/II
	KW-0761	Anti-CCR4	ATL, PTCL	Phase II
	SGN-30	Anti-CD30	CD30+, ALCL	Phase II
	Siplizumab	Anti-CD2	PTCL, NK cell, ATL	Phase I
	Zanolimumab	Anti-CD4	CTCL, PTCL	Phase II
Nucleoside analogs	Cladribine	Purine nucleoside analog	PTCL	Phase IV
	Clofarabine	Purine nucleoside analog	PTCL, NK cell	Phase I/II
	Fludarabine	Purine nucleoside analog	PTCL, CTCL	Phase II
	Forodesine	Metabolic enzyme inhibitor	PTCK, CTCL	Phase II
	Gemcitabine	Pyrimidine nucleoside analog	PTCL	Phase II
	Nelarabine	Purine nucleoside analog	T-ALL, T-NHL	Phase II
	Pentostatin	Metabolic enzyme inhibitor	PTCL	Phase II
Proteasome inhibitors	Bortezomib	Proteasome inhibitor	CTCL	Phase II
Signaling inhibitors	Enzastaurin	Selective inhibitor of protein kinase C	PTCL, CTCL	Phase II
	R788	Syk inhibitor	PTCL	Phase II

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the role of antiviral agents in patients with ATLL. KW-0761 is a defucosylated, humanized, monoclonal antibody with enhanced antibody-dependent cellular cytotoxicity (ADCC) that binds to CC chemokine receptor 4 (CCR4). CCR4 is expressed in ATLL, PTCL-NOS, and CTCL. A phase I study of KW-0761 in patients with CCR4-positive ATLL and PTCL showed an ORR of 31.3 % [126]. An interim analysis of a multicenter phase II trial of KW-0761 in 27 patients with relapsed ATLL showed an ORR of 54 % with 27 % CRs. The drug was well tolerated and the major toxicities were hematologic, cutaneous, and infusion/hypersensitivity reactions including Stevens-Johnson syndrome [127]. These are early yet quite encouraging results given that historic ORR to salvage therapy in ATLL had been in single digits. Plitidepsin, a

naturally occurring cyclic depsipeptide originally isolated from the Mediterranean tunicate *Aplidium albicans*, had a significant preclinical activity against human lymphoma and leukemia cells through induction of CD95-mediated apoptosis [128]. In a recent interim analysis of a multinational phase II clinical trial of 29 patients with relapsed/refractory PTCL, plitidepsin had a modest activity with an ORR of 20 % with 6.8 % CRs and median OS of 10 months. The interesting part is that hematological toxicity was very mild in this study, and the authors proposed that plitidepsin may be an attractive option in patients with PTCL who have severe cytopenias or extensive bone marrow involvement [129]. We have recently reported an interim analysis of a phase II trial of 27 patients with relapsed/refractory NHL treated with dasatinib, a potent, broad-spectrum inhibitor of five critical oncogenic tyrosine kinase families: BCR-ABL, SRC, c-KIT, PDGF receptors (α[alpha] and β[beta]), and ephrin (EPH) receptor kinases that are currently approved by the US FDA for the treatment of chronic myeloid leukemia (CML). Dasatinib had a modest activity with an ORR of 32 % in 19 patients who were evaluable at the time of analysis. The interesting part is that the two patients who sustained a CR had PTCL; both patients remained alive, and disease-free, for over 3 years since start of treatment [130].

Role of Stem Cell Transplantation

High-dose chemotherapy and autologous stem cell transplantation (ASCT) have been proposed to improve the inferior results obtained with conventional chemotherapy for PTCL. Patients with chemotherapy-sensitive response to salvage therapy have long-term disease-free survivals of 35–45 % with ASCT [131, 132]. Because of the poor results with standard therapy and the difficulty of attaining a second CR with standard therapy, some centers are using high-dose therapy and ASCT in CR1 for all high-risk patients with PTCL [133, 134]. In small studies, this strategy demonstrated a high disease-free survival yet data from large randomized clinical trials are lacking. The use of allogeneic SCT transplantation had been proposed as an alternative harnessing a potential graft-versus-lymphoma (GVL) effect. The morbidity and mortality remains high in PTCL undergoing an allogeneic SCT, yet a 5-year OS of 63 % was reported [135]. A recent retrospective analysis from the center of blood and marrow transplantation (CIBMTR) registry of 101 patients with PTCL showed inferior adjusted 3-year PFS of allogeneic versus autologous SCT, 36 % versus 47 % which didn't reach statistical significance. The 3-year OS was significantly inferior with allogeneic versus autologous SCT, 47 % versus 59 % probably because of higher risk of treatment-related mortality, TRM (hazard ratio = 3.03). The relapse risk after allogeneic SCT was lower though (hazard ratio = 0.5). The authors concluded that patients undergoing ASCT for PTCL appear to be selected for less advanced disease and greater chemosensitivity, making direct outcome comparisons with allogeneic SCT difficult. Allogeneic SCT is an effective strategy for high-risk patients yet TRM is problematic. Higher numbers of chemotherapy regimens prior to transplant adversely impacted both TRM and survival, suggesting that SCT should be considered earlier in the disease course [136]. A recent report from a single institution reported higher 3-year PFS in patients with PTCL with nodal histologies compared to extranodal disease, 45 % versus 6 % [137]. Many questions remain unanswered about the role of SCT in PTCL: should all patients with high-risk PTCL undergo SCT, what is the proper induction regimen, and does ASCT in CR1 alter the natural history of the disease? Multicenter trials are needed to answer these important questions. More recently, reduced-intensity conditioning (RIC) has emerged as an attractive alternative, in an attempt to reduced excessive TRM seen with traditional myeloablative allogeneic SCTs. A small pilot study of RIC in PTCL demonstrated a 3-year OS and PFS of 81 and 64 %, respectively [138]. This approach appears promising in highly selected patients.

References

1. A clinical evaluation of the International Lymphoma Study Group classification of non-Hodgkin's lymphoma. The Non-Hodgkin's Lymphoma Classification Project. Blood. 1997;89(11):3909–18.
2. Jaffe ES, Harris NL, Stein H, Campo E, Pileri SA, Swerdlow SH. Introduction and overview of the classification of the lymphoid neoplasms. In: Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, et al., editors. WHO classification of tumours of haematopoietic and lymphoid tissues. 4th ed. Lyon: IARC; 2008.
3. Krenacs L, Smyth MJ, Bagdi E, Krenacs T, Kopper L, Rudiger T, et al. The serine protease granzyme M is preferentially expressed in NK-cell, gamma delta T-cell, and intestinal T-cell lymphomas: evidence of origin from lymphocytes involved in innate immunity. Blood. 2003;101(9):3590–3.
4. Quintanilla-Martinez L, Kumar S, Fend F, Reyes E, Teruya-Feldstein J, Kingma DW, et al. Fulminant EBV(+) T-cell lymphoproliferative disorder following acute/chronic EBV infection: a distinct clinicopathologic syndrome. Blood. 2000;96(2):443–51.
5. Takatsuki K, Matsuoka M, Yamaguchi K. Adult T-cell leukemia in Japan. J Acquir Immune Defic Syndr Hum Retrovirol. 1996;13 Suppl 1:S15–9.
6. Rudiger T, Weisenburger DD, Anderson JR, Armitage JO, Diebold J, MacLennan KA, et al. Peripheral T-cell lymphoma (excluding anaplastic large-cell lymphoma): results from the Non-Hodgkin's Lymphoma Classification Project. Ann Oncol. 2002;13(1):140–9.
7. Morton LM, Wang SS, Devesa SS, Hartge P, Weisenburger DD, Linet MS. Lymphoma incidence patterns by WHO subtype in the United States, 1992–2001. Blood. 2006;107(1):265–76.
8. Vose J, Armitage J, Weisenburger D. International peripheral T-cell and natural killer/T-cell lymphoma study: pathology findings and clinical outcomes. J Clin Oncol. 2008;26(25):4124–30.
9. Vose JM. Peripheral T-cell non-Hodgkin's lymphoma. Hematol Oncol Clin North Am. 2008;22(5):997–1005, x.
10. Harris NL, Jaffe ES, Stein H, Banks PM, Chan JK, Cleary ML, et al. A revised European-American classification of lymphoid neoplasms: a proposal from the International Lymphoma Study Group. Blood. 1994;84(5):1361–92.
11. Geissinger E, Odenwald T, Lee SS, Bonzheim I, Roth S, Reimer P, et al. Nodal peripheral T-cell lymphomas and, in particular, their lymphoepithelioid (Lennert's) variant are often derived from CD8(+) cytotoxic T-cells. Virchows Arch. 2004;445(4):334–43.
12. de Leval L, Savilo E, Longtine J, Ferry JA, Harris NL. Peripheral T-cell lymphoma with follicular involvement and a CD4+/bcl-6+ phenotype. Am J Surg Pathol. 2001;25(3):395–400.
13. Rudiger T, Ichinohasama R, Ott MM, Muller-Deubert S, Miura I, Ott G, et al. Peripheral T-cell lymphoma with distinct perifollicular growth pattern: a distinct subtype of T-cell lymphoma? Am J Surg Pathol. 2000;24(1):117–22.
14. Rizvi MA, Evens AM, Tallman MS, Nelson BP, Rosen ST. T-cell non-Hodgkin lymphoma. Blood. 2006;107(4):1255–64.
15. Gallamini A, Stelitano C, Calvi R, Bellei M, Mattei D, Vitolo U, et al. Peripheral T-cell lymphoma unspecified (PTCL-U): a new prognostic model from a retrospective multicentric clinical study. Blood. 2004;103(7):2474–9.
16. Went P, Agostinelli C, Gallamini A, Piccaluga PP, Ascani S, Sabattini E, et al. Marker expression in peripheral T-cell lymphoma: a proposed clinical-pathologic prognostic score. J Clin Oncol. 2006;24(16):2472–9.
17. Piccaluga PP, Agostinelli C, Gazzola A, Mannu C, Bacci F, Sabattini E, et al. Prognostic markers in peripheral T-cell lymphoma. Curr Hematol Malig Rep. 2010;5(4):222–8.
18. Martinez-Delgado B, Cuadros M, Honrado E, Ruiz de la Parte A, Roncador G, Alves J, et al. Differential expression of NF-kappaB pathway genes among peripheral T-cell lymphomas. Leukemia. 2005;19(12):2254–63.
19. Cuadros M, Dave SS, Jaffe ES, Honrado E, Milne R, Alves J, et al. Identification of a proliferation signature related to survival in nodal peripheral T-cell lymphomas. J Clin Oncol. 2007;25(22):3321–9.
20. Piccaluga PP, Agostinelli C, Califano A, Rossi M, Basso K, Zupo S, et al. Gene expression analysis of peripheral T cell lymphoma, unspecified, reveals distinct profiles and new potential therapeutic targets. J Clin Invest. 2007;117(3):823–34.
21. Iqbal J, Weisenburger DD, Greiner TC, Vose JM, McKeithan T, Kucuk C, et al. Molecular signatures to improve diagnosis in peripheral T-cell lymphoma and prognostication in angioimmunoblastic T-cell lymphoma. Blood. 2010;115(5):1026–36.
22. Savage KJ, Chhanabhai M, Gascoyne RD, Connors JM. Characterization of peripheral T-cell lymphomas in a single North American institution by the WHO classification. Ann Oncol. 2004;15(10):1467–75.
23. Escalon MP, Liu NS, Yang Y, Hess M, Walker PL, Smith TL, et al. Prognostic factors and treatment of patients with T-cell non-Hodgkin lymphoma: the M. D. Anderson Cancer Center experience. Cancer. 2005;103(10):2091–8.
24. Gisselbrecht C, Gaulard P, Lepage E, Coiffier B, Briere J, Haioun C, et al. Prognostic significance of T-cell phenotype in aggressive non-Hodgkin's lymphomas. Groupe d'Etudes des Lymphomes de l'Adulte (GELA). Blood. 1998;92(1):76–82.
25. Savage KJ, O'Leary HM, Connors JM, Chhanabhai M, Campbell B, Sehn LH, et al. The prognosis of limited stage peripheral T-cell lymphoma (PTCL): a population-based analysis and comparison to diffuse large B-cell lymphoma (DLBCL). ASH Ann Meet Abstr. 2010;116(21):4129.
26. Dogan A, Gaulard P, Jaffe ES, Ralfkiaer E, Muller-Hermelink HK. Angioimmunoblastic T-cell lymphoma. In: Swerdlow SH, Campo E, Harris NL, Jaffe

ES, Pileri SA, Stein H, et al., editors. WHO classification of tumours of hematopoietic and lymphoid tissues. 4th ed. Lyon: IARC; 2008.
27. Feller AC, Griesser H, Schilling CV, Wacker HH, Dallenbach F, Bartels H, et al. Clonal gene rearrangement patterns correlate with immunophenotype and clinical parameters in patients with angioimmunoblastic lymphadenopathy. Am J Pathol. 1988;133(3): 549–56.
28. Weiss LM, Strickler JG, Dorfman RF, Horning SJ, Warnke RA, Sklar J. Clonal T-cell populations in angioimmunoblastic lymphadenopathy and angioimmunoblastic lymphadenopathy-like lymphoma. Am J Pathol. 1986;122(3):392–7.
29. Zhou Y, Attygalle AD, Chuang SS, Diss T, Ye H, Liu H, et al. Angioimmunoblastic T-cell lymphoma: histological progression associates with EBV and HHV6B viral load. Br J Haematol. 2007;138(1):44–53.
30. HawleyRC,CankovicM,ZarboRJ.Angioimmunoblastic T-cell lymphoma with supervening Epstein-Barr virus-associated large B-cell lymphoma. Arch Pathol Lab Med. 2006;130(11):1707–11.
31. Xu Y, McKenna RW, Hoang MP, Collins RH, Kroft SH. Composite angioimmunoblastic T-cell lymphoma and diffuse large B-cell lymphoma: a case report and review of the literature. Am J Clin Pathol. 2002;118(6):848–54.
32. Tachibana T, Tomita N, Furuya M, Yamanaka S, Takeuchi K, Nakamura N, et al. Aberrant CD20 expression in angioimmunoblastic T-cell lymphoma. Intern Med. 2011;50(5):495–9.
33. Grogg KL, Morice WG, Macon WR. Spectrum of bone marrow findings in patients with angioimmunoblastic T-cell lymphoma. Br J Haematol. 2007;137(5):416–22.
34. Park BB, Ryoo BY, Lee JH, Kwon HC, Yang SH, Kang HJ, et al. Clinical features and treatment outcomes of angioimmunoblastic T-cell lymphoma. Leuk Lymphoma. 2007;48(4):716–22.
35. Pautier P, Devidas A, Delmer A, Dombret H, Sutton L, Zini JM, et al. Angioimmunoblastic-like T-cell non Hodgkin's lymphoma: outcome after chemotherapy in 33 patients and review of the literature. Leuk Lymphoma. 1999;32(5–6):545–52.
36. Seehafer JR, Goldberg NC, Dicken CH, Su WP. Cutaneous manifestations of angioimmunoblastic lymphadenopathy. Arch Dermatol. 1980;116(1): 41–5.
37. Hsu SM, Waldron Jr JA, Fink L, King C, Xie SS, Mansouri A, et al. Pathogenic significance of interleukin-6 in angioimmunoblastic lymphadenopathy-type T-cell lymphoma. Hum Pathol. 1993;24(2):126–31.
38. Hsu SM, Waldron JA, Xie SS, Barlogie B. Expression of interleukin-6 in Castleman's disease. Hum Pathol. 1993;24(8):833–9.
39. Mourad N, Mounier N, Briere J, Raffoux E, Delmer A, Feller A, et al. Clinical, biologic, and pathologic features in 157 patients with angioimmunoblastic T-cell lymphoma treated within the Groupe d'Etude des Lymphomes de l'Adulte (GELA) trials. Blood. 2008;111(9):4463–70.
40. Vose JM. The international PP. International peripheral T-cell lymphoma (PTCL) clinical and pathologic review project: poor outcome by prognostic indices and lack of efficacy with anthracyclines. ASH Annu Meet Abstr. 2005;106(11):811.
41. Reiser M, Josting A, Soltani M, Staib P, Salzberger B, Diehl V, et al. T-cell non-Hodgkin's lymphoma in adults: clinicopathological characteristics, response to treatment and prognostic factors. Leuk Lymphoma. 2002;43(4):805–11.
42. Joly B, Frenkel V, Belhadj K, El Gnaoui T, Rahmouni A, Gaulard P, et al. Rituximab in combination with CHOP regimen in T-cell angioimmunoblastic lymphoma (AILD-TL) rich in large B cells. Favourable results in four patients. ASCO Meet Abstr. 2004;22(14 suppl):6694.
43. Joly B, Plonquet A, Grare M, Delfau-Larue M, Delarue R, Delmer A, et al. Rituximab in combination with CHOP regimen in angioimmunoblastic T-cell lymphoma: results of the phase II RAIL trial—A prospective study of the Groupe d'Etude des Lymphomes de l'Adulte (GELA). ASCO Meet Abstr. 2010;28(15 suppl):8049.
44. Quintini G, Iannitto E, Barbera V, Turri D, Franco V, Florena AM, et al. Response to low-dose oral methotrexate and prednisone in two patients with angio-immunoblastic lymphadenopathy-type T-cell lymphoma. Hematol J. 2001;2(6):393–5.
45. Advani R, Horwitz S, Zelenetz A, Horning SJ. Angioimmunoblastic T cell lymphoma: treatment experience with cyclosporine. Leuk Lymphoma. 2007;48(3):521–5.
46. Aguiar Bujanda D. Complete response of relapsed angioimmunoblastic T-cell lymphoma following therapy with bevacizumab. Ann Oncol. 2008;19(2):396–7.
47. Bruns I, Fox F, Reinecke P, Kobbe G, Kronenwett R, Jung G, et al. Complete remission in a patient with relapsed angioimmunoblastic T-cell lymphoma following treatment with bevacizumab. Leukemia. 2005;19(11):1993–5.
48. Stein H, Foss HD, Durkop H, Marafioti T, Delsol G, Pulford K, et al. CD30(+) anaplastic large cell lymphoma: a review of its histopathologic, genetic, and clinical features. Blood. 2000;96(12):3681–95.
49. Delsol G, Falini B, Muller-Hermelink HK, Campo E, Jaffe ES, Gascoyne RD, et al. Anaplastic large cell lymphoma (ALCL), ALK-positive. In: Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, et al., editors. WHO classification of tumours of hematopoietic and lymphoid tissues. 4th ed. Lyon: IARC; 2008.
50. Lamant L, Meggetto F, al Saati T, Brugieres L, de Paillerets BB, Dastugue N, et al. High incidence of the t(2;5)(p23;q35) translocation in anaplastic large cell lymphoma and its lack of detection in Hodgkin's disease. Comparison of cytogenetic analysis, reverse transcriptase-polymerase chain reaction, and P-80 immunostaining. Blood. 1996;87(1):284–91.
51. Cools J, Wlodarska I, Somers R, Mentens N, Pedeutour F, Maes B, et al. Identification of novel fusion partners

of ALK, the anaplastic lymphoma kinase, in anaplastic large-cell lymphoma and inflammatory myofibroblastic tumor. Genes Chromosom Cancer. 2002;34(4):354–62.
52. Falini B, Pileri S, Zinzani PL, Carbone A, Zagonel V, Wolf-Peeters C, et al. ALK+ lymphoma: clinico-pathological findings and outcome. Blood. 1999;93(8): 2697–706.
53. U. S. Food and Drug Administration: Reports of Anaplastic large cell lymphoma (ALCL) in women with breast implants. Medical device safety communications. 2011. Available from: www.fda.gov. Updated 2011; cited.
54. Gascoyne RD, Aoun P, Wu D, Chhanabhai M, Skinnider BF, Greiner TC, et al. Prognostic significance of anaplastic lymphoma kinase (ALK) protein expression in adults with anaplastic large cell lymphoma. Blood. 1999;93(11):3913–21.
55. Savage KJ, Harris NL, Vose JM, Ullrich F, Jaffe ES, Connors JM, et al. ALK- anaplastic large-cell lymphoma is clinically and immunophenotypically different from both ALK+ ALCL and peripheral T-cell lymphoma, not otherwise specified: report from the International Peripheral T-Cell Lymphoma Project. Blood. 2008;111(12):5496–504.
56. Jantunen E, Wiklund T, Juvonen E, Putkonen M, Lehtinen T, Kuittinen O, et al. Autologous stem cell transplantation in adult patients with peripheral T-cell lymphoma: a nation-wide survey. Bone Marrow Transplant. 2004;33(4):405–10.
57. Yok-Lam K. The diagnosis and management of extranodal NK/T-cell lymphoma, nasal-type and aggressive NK-cell leukemia. J Clin Exp Hematop. 2011;51(1):21–8.
58. Chan JK, Yip TT, Tsang WY, Ng CS, Lau WH, Poon YF, et al. Detection of Epstein-Barr viral RNA in malignant lymphomas of the upper aerodigestive tract. Am J Surg Pathol. 1994;18(9):938–46.
59. Lee J, Suh C, Huh J, Jun HJ, Kim K, Jung C, et al. Effect of positive bone marrow EBV in situ hybridization in staging and survival of localized extranodal natural killer/T-cell lymphoma, nasal-type. Clin Cancer Res. 2007;13(11):3250–4.
60. Au WY, Pang A, Choy C, Chim CS, Kwong YL. Quantification of circulating Epstein-Barr virus (EBV) DNA in the diagnosis and monitoring of natural killer cell and EBV-positive lymphomas in immunocompetent patients. Blood. 2004;104(1):243–9.
61. Cheung MM, Chan JK, Lau WH, Foo W, Chan PT, Ng CS, et al. Primary non-Hodgkin's lymphoma of the nose and nasopharynx: clinical features, tumor immunophenotype, and treatment outcome in 113 patients. J Clin Oncol. 1998;16(1):70–7.
62. Suzuki R, Suzumiya J, Nakamura S, Aoki S, Notoya A, Ozaki S, et al. Aggressive natural killer-cell leukemia revisited: large granular lymphocyte leukemia of cytotoxic NK cells. Leukemia. 2004;18(4):763–70.
63. Chim CS, Ma SY, Au WY, Choy C, Lie AK, Liang R, et al. Primary nasal natural killer cell lymphoma: long-term treatment outcome and relationship with the International Prognostic Index. Blood. 2004;103(1):216–21.
64. Kim SJ, Kim K, Kim BS, Kim CY, Suh C, Huh J, et al. Phase II trial of concurrent radiation and weekly cisplatin followed by VIPD chemotherapy in newly diagnosed, stage IE to IIE, nasal, extranodal NK/T-Cell Lymphoma: Consortium for Improving Survival of Lymphoma study. J Clin Oncol. 2009;27(35):6027–32.
65. Yamaguchi M, Tobinai K, Oguchi M, Ishizuka N, Kobayashi Y, Isobe Y, et al. Phase I/II study of concurrent chemoradiotherapy for localized nasal natural killer/T-cell lymphoma: Japan Clinical Oncology Group Study JCOG0211. J Clin Oncol. 2009;27(33):5594–600.
66. Yamaguchi M, Kita K, Miwa H, Nishii K, Oka K, Ohno T, et al. Frequent expression of P-glycoprotein/MDR1 by nasal T-cell lymphoma cells. Cancer. 1995;76(11):2351–6.
67. Kwong YL, Yamaguchi M, Maeda Y, Hashimoto C, Kim WS, Suh CW, et al. Phase II Study of SMILE chemotherapy for newly-diagnosed stage IV, relapsed or refractory extranodal NK/T-cell lymphoma, nasal type: NKTSG study. Haematologica. 2010;95(suppl 2):a0299.
68. Yong W, Zheng W, Zhu J, Zhang Y, Wang X, Xie Y, et al. L-asparaginase in the treatment of refractory and relapsed extranodal NK/T-cell lymphoma, nasal type. Ann Hematol. 2009;88(7):647–52.
69. Lee J, Au WY, Park MJ, Suzumiya J, Nakamura S, Kameoka J, et al. Autologous hematopoietic stem cell transplantation in extranodal natural killer/T cell lymphoma: a multinational, multicenter, matched controlled study. Biol Blood Marrow Transplant. 2008;14(12):1356–64.
70. Gaulard P, Jaffe ES, Krenacs L, Macon WR. Hepatosplenic T-cell lymphoma. In: Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, et al., editors. WHO classification of tumours of hematopoietic and lymphoid tissues. 4th ed. Lyon: IARC; 2008.
71. Belhadj K, Reyes F, Farcet JP, Tilly H, Bastard C, Angonin R, et al. Hepatosplenic gammadelta T-cell lymphoma is a rare clinicopathologic entity with poor outcome: report on a series of 21 patients. Blood. 2003;102(13):4261–9.
72. Mackey AC, Green L, Liang LC, Dinndorf P, Avigan M. Hepatosplenic T cell lymphoma associated with infliximab use in young patients treated for inflammatory bowel disease. J Pediatr Gastroenterol Nutr. 2007;44(2):265–7.
73. Corazzelli G, Capobianco G, Russo F, Frigeri F, Aldinucci D, Pinto A. Pentostatin (2′-deoxycoformycin) for the treatment of hepatosplenic gammadelta T-cell lymphomas. Haematologica. 2005;90(3):ECR14.
74. Falchook GS, Vega F, Dang NH, Samaniego F, Rodriguez MA, Champlin RE, et al. Hepatosplenic gamma-delta T-cell lymphoma: clinicopathological features and treatment. Ann Oncol. 2009;20(6):1080–5.
75. Zettl A, deLeeuw R, Haralambieva E, Mueller-Hermelink HK. Enteropathy-type T-cell lymphoma. Am J Clin Pathol. 2007;127(5):701–6.
76. Howell WM, Leung ST, Jones DB, Nakshabendi I, Hall MA, Lanchbury JS, et al. HLA-DRB, -DQA, and

-DQB polymorphism in celiac disease and enteropathy-associated T-cell lymphoma. Common features and additional risk factors for malignancy. Hum Immunol. 1995;43(1):29–37.
77. Isaacson PG, Chott A, Ott G, Stein H. Enteropathy-associated T-cell lymphoma. In: Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, et al., editors. WHO classification of tumours of hematopoietic and lymphoid tissues. 4th ed. Lyon: IARC; 2008.
78. Zettl A, Ott G, Makulik A, Katzenberger T, Starostik P, Eichler T, et al. Chromosomal gains at 9q characterize enteropathy-type T-cell lymphoma. Am J Pathol. 2002;161(5):1635–45.
79. Sieniawski M, Angamuthu N, Boyd K, Chasty R, Davies J, Forsyth P, et al. Evaluation of enteropathy-associated T-cell lymphoma comparing standard therapies with a novel regimen including autologous stem cell transplantation. Blood. 2010;115(18):3664–70.
80. Kumar S, Krenacs L, Medeiros J, Elenitoba-Johnson KS, Greiner TC, Sorbara L, et al. Subcutaneous panniculitic T-cell lymphoma is a tumor of cytotoxic T lymphocytes. Hum Pathol. 1998;29(4):397–403.
81. Salhany KE, Macon WR, Choi JK, Elenitsas R, Lessin SR, Felgar RE, et al. Subcutaneous panniculitis-like T-cell lymphoma: clinicopathologic, immunophenotypic, and genotypic analysis of alpha/beta and gamma/delta subtypes. Am J Surg Pathol. 1998;22(7):881–93.
82. Jaffe ES, Gaulard P, Ralfkiaer E, Cerroni L, Meijer CJ. Subcutaneous panniculitis-like T-cell lymphoma. In: Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, et al., editors. WHO classification of tumours of hematopoietic and lymphoid tissues. 4th ed. Lyon: IARC; 2008.
83. Willemze R, Jansen PM, Cerroni L, Berti E, Santucci M, Assaf C, et al. Subcutaneous panniculitis-like T-cell lymphoma: definition, classification, and prognostic factors: an EORTC Cutaneous Lymphoma Group Study of 83 cases. Blood. 2008;111(2):838–45.
84. Gonzalez CL, Medeiros LJ, Braziel RM, Jaffe ES. T-cell lymphoma involving subcutaneous tissue. A clinicopathologic entity commonly associated with hemophagocytic syndrome. Am J Surg Pathol. 1991;15(1):17–27.
85. Tsukamoto Y, Katsunobu Y, Omura Y, Maeda I, Hirai M, Teshima H, et al. Subcutaneous panniculitis-like T-cell lymphoma: successful initial treatment with prednisolone and cyclosporin A. Intern Med. 2006;45(1):21–4.
86. Oshima K, Jaffe ES, Kikuchi M. Adult T-cell leukemia/lymphoma. In: Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, et al., editors. WHO classification of tumours of hematopoietic and lymphoid tissues. 4th ed. Lyon: IARC; 2008.
87. Chen YC, Wang CH, Su IJ, Hu CY, Chou MJ, Lee TH, et al. Infection of human T-cell leukemia virus type I and development of human T-cell leukemia lymphoma in patients with hematologic neoplasms: a possible linkage to blood transfusion. Blood. 1989;74(1):388–94.
88. Yamamoto JF, Goodman MT. Patterns of leukemia incidence in the United States by subtype and demographic characteristics, 1997–2002. Cancer Causes Control. 2008;19(4):379–90.
89. Hino S. Establishment of the milk-borne transmission as a key factor for the peculiar endemicity of human T-lymphotropic virus type 1 (HTLV-1): the ATL Prevention Program Nagasaki. Proc Jpn Acad Ser B Phys Biol Sci. 2011;87(4):152–66.
90. Ohshima K, Suzumiya J, Kato A, Tashiro K, Kikuchi M. Clonal HTLV-I-infected CD4+ T-lymphocytes and non-clonal non-HTLV-I-infected giant cells in incipient ATLL with Hodgkin-like histologic features. Int J Cancer. 1997;72(4):592–8.
91. Karube K, Ohshima K, Tsuchiya T, Yamaguchi T, Kawano R, Suzumiya J, et al. Expression of FoxP3, a key molecule in CD4CD25 regulatory T cells, in adult T-cell leukaemia/lymphoma cells. Br J Haematol. 2004;126(1):81–4.
92. Sanada I, Tanaka R, Kumagai E, Tsuda H, Nishimura H, Yamaguchi K, et al. Chromosomal aberrations in adult T cell leukemia: relationship to the clinical severity. Blood. 1985;65(3):649–54.
93. Shimoyama M. Diagnostic criteria and classification of clinical subtypes of adult T-cell leukaemia-lymphoma. A report from the Lymphoma Study Group (1984–87). Br J Haematol. 1991;79(3):428–37.
94. Takasaki Y, Iwanaga M, Imaizumi Y, Tawara M, Joh T, Kohno T, et al. Long-term study of indolent adult T-cell leukemia-lymphoma. Blood. 2010;115(22):4337–43.
95. Yamada Y, Kamihira S, Amagasaki T, Kinoshita K, Kusano M, Chiyoda S, et al. Adult T cell leukemia with atypical surface phenotypes: clinical correlation. J Clin Oncol. 1985;3(6):782–8.
96. Tsukasaki K, Utsunomiya A, Fukuda H, Shibata T, Fukushima T, Takatsuka Y, et al. VCAP-AMP-VECP compared with biweekly CHOP for adult T-cell leukemia-lymphoma: Japan Clinical Oncology Group Study JCOG9801. J Clin Oncol. 2007;25(34):5458–64.
97. Yamada Y, Tomonaga M, Fukuda H, Hanada S, Utsunomiya A, Tara M, et al. A new G-CSF-supported combination chemotherapy, LSG15, for adult T-cell leukaemia-lymphoma: Japan Clinical Oncology Group Study 9303. Br J Haematol. 2001;113(2):375–82.
98. Dungerwalla M, Osuji N, Waldman AD, Al Jehani F, Mehta A, Tailor R, et al. Isolated central nervous system involvement in adult T-cell lymphoma/leukaemia. Br J Haematol. 2005;130(4):511–5.
99. Teshima T, Akashi K, Shibuya T, Taniguchi S, Okamura T, Harada M, et al. Central nervous system involvement in adult T-cell leukemia/lymphoma. Cancer. 1990;65(2):327–32.
100. Tsukasaki K, Maeda T, Arimura K, Taguchi J, Fukushima T, Miyazaki Y, et al. Poor outcome of autologous stem cell transplantation for adult T cell leukemia/lymphoma: a case report and review of the literature. Bone Marrow Transplant. 1999;23(1):87–9.

101. Hishizawa M, Kanda J, Utsunomiya A, Taniguchi S, Eto T, Moriuchi Y, et al. Transplantation of allogeneic hematopoietic stem cells for adult T-cell leukemia: a nationwide retrospective study. Blood. 2010;116(8):1369–76.
102. Jacobsen E, Freedman AS. Treatment of relapsed or refractory peripheral T cell lymphoma. In: Basow DS, editor. UpToDate, Waltham; 2011.
103. Niitsu N, Hayama M, Hagiwara Y, Tanae K, Kohri M, Takahashi N, et al. Multicenter phase II study of the CyclOBEAP regimen for patients with peripheral T-cell lymphoma with analysis of biomakers. ASH Annu Meet Abstr. 2010;116(21): 2795.
104. O'Connor OA, Pro B, Pinter-Brown L, Bartlett N, Popplewell L, Coiffier B, et al. Pralatrexate in patients with relapsed or refractory peripheral T-cell lymphoma: results from the pivotal PROPEL study. J Clin Oncol. 2011;29(9):1182–9.
105. Rodig SJ, Abramson JS, Pinkus GS, Treon SP, Dorfman DM, Dong HY, et al. Heterogeneous CD52 expression among hematologic neoplasms: implications for the use of alemtuzumab (CAMPATH-1H). Clin Cancer Res. 2006;12(23):7174–9.
106. Enblad G, Hagberg H, Erlanson M, Lundin J, MacDonald AP, Repp R, et al. A pilot study of alemtuzumab (anti-CD52 monoclonal antibody) therapy for patients with relapsed or chemotherapy-refractory peripheral T-cell lymphomas. Blood. 2004;103(8):2920–4.
107. Zinzani PL, Alinari L, Tani M, Fina M, Pileri S, Baccarani M. Preliminary observations of a phase II study of reduced-dose alemtuzumab treatment in patients with pretreated T-cell lymphoma. Haematologica. 2005;90(5):702–3.
108. Gallamini A, Zaja F, Patti C, Billio A, Specchia MR, Tucci A, et al. Alemtuzumab (Campath-1H) and CHOP chemotherapy as first-line treatment of peripheral T-cell lymphoma: results of a GITIL (Gruppo Italiano Terapie Innovative nei Linfomi) prospective multicenter trial. Blood. 2007;110(7):2316–23.
109. Kim JG, Sohn SK, Chae YS, Cho YY, Yang DH, Lee JJ, et al. Alemtuzumab plus CHOP as front-line chemotherapy for patients with peripheral T-cell lymphomas: a phase II study. Cancer Chemother Pharmacol. 2007;60(1):129–34.
110. Kluin-Nelemans HC, van Marwijk Kooy M, Lugtenburg PJ, van Putten WL, Luten M, Oudejans J, et al. Intensified alemtuzumab-CHOP therapy for peripheral T-cell lymphoma. Ann Oncol. 2011;22(7): 1595–1600.
111. Dang NH, Pro B, Hagemeister FB, Samaniego F, Jones D, Samuels BI, et al. Phase II trial of denileukin diftitox for relapsed/refractory T-cell non-Hodgkin lymphoma. Br J Haematol. 2007;136(3): 439–47.
112. Foss FM, Sjak-Shie NN, Goy A, Advani R, Jacobsen ED. Phase II study of denileukin diftitox with CHOP chemotherapy in newly-diagnosed PTCL: CONCEPT trial. ASCO Meet Abstr. 2010;28(15 suppl):8045.
113. Lee J, Suh C, Kang HJ, Ryoo BY, Huh J, Ko YH, et al. Phase I study of proteasome inhibitor bortezomib plus CHOP in patients with advanced, aggressive T-cell or NK/T-cell lymphoma. Ann Oncol. 2008;19(12):2079–83.
114. Zinzani PL, Musuraca G, Tani M, Stefoni V, Marchi E, Fina M, et al. Phase II trial of proteasome inhibitor bortezomib in patients with relapsed or refractory cutaneous T-cell lymphoma. J Clin Oncol. 2007;25(27):4293–7.
115. Delmer A, Fitoussi O, Gaulard P, Laurent G, Bordessoule D, Morschhauser F, et al. A phase II study of bortezomib in combination with intensified CHOP-like regimen (ACVBP) in patients with previously untreated T-cell lymphoma: Results of the GELA LNH05–1T trial. ASCO Meet Abstr. 2009;27(15S):8554.
116. Dueck GS, Chua N, Prasad A, Stewart D, White D, vander Jagt R, et al. Activity of lenalidomide in a phase II trial for T-cell lymphoma: report on the first 24 cases. ASCO Meet Abstr. 2009;27(15S):8524.
117. Piekarz RL, Frye R, Prince HM, Kirschbaum MH, Zain J, Allen SL, et al. Phase 2 trial of romidepsin in patients with peripheral T-cell lymphoma. Blood. 2011;117(22):5827–34.
118. Coiffier B, Pro B, Prince HM, Foss FM, Sokol L, Greenwood M, et al. Final results from a pivotal, multicenter, international, open-label, phase 2 study of romidepsin in progressive or relapsed peripheral T-cell lymphoma (PTCL) following prior systemic therapy. ASH Annu Meet Abstr. 2010;116(21):114.
119. Pohlman B, Advani R, Duvic M, Hymes KB, Intragumtornchai T, Lekhakula A, et al. Final results of a phase II trial of belinostat (PXD101) in patients with recurrent or refractory peripheral or cutaneous T-cell lymphoma. ASH Annu Meet Abstr. 2009;114(22):920.
120. Shustov AR, Advani R, Brice P, Bartlett NL, Rosenblatt JD, Illidge T, et al. Complete remissions with brentuximab vedotin (SGN-35) in patients with relapsed or refractory systemic anaplastic large cell lymphoma. ASH Annu Meet Abstr. 2010;116(21):961.
121. O'Mahony D, Morris JC, Moses L, O'Hagan D, Gao W, Stetler-Stevenson M, et al. Phase I trial of siplizumab in CD2-positive lymphoproliferative disease. ASH Annu Meet Abstr. 2005;106(11):3353.
122. O'Mahony D, Morris JC, Stetler-Stevenson M, Matthews H, Brown MR, Fleisher T, et al. EBV-related lymphoproliferative disease complicating therapy with the anti-CD2 monoclonal antibody, siplizumab, in patients with T-cell malignancies. Clin Cancer Res. 2009;15(7):2514–22.
123. d'Amore F, Radford J, Jerkeman M, Relander T, Tilly H, Osterborg A, et al. Zanolimumab (HuMax-CD4TM), a fully human monoclonal antibody: efficacy and safety in patients with relapsed or treatment-refractory non-cutaneous CD4+ T-cell lymphoma. ASH Annu Meet Abstr. 2007;110(11):3409.
124. Gill PS, Harrington Jr W, Kaplan MH, Ribeiro RC, Bennett JM, Liebman HA, et al. Treatment of adult

T-cell leukemia-lymphoma with a combination of interferon alfa and zidovudine. N Engl J Med. 1995;332(26):1744–8.
125. Bazarbachi A, Plumelle Y, Carlos Ramos J, Tortevoye P, Otrock Z, Taylor G, et al. Meta-analysis on the use of zidovudine and interferon-alfa in adult T-cell leukemia/lymphoma showing improved survival in the leukemic subtypes. J Clin Oncol. 2010;28(27):4177–83.
126. Yamamoto K, Utsunomiya A, Tobinai K, Tsukasaki K, Uike N, Uozumi K, et al. Phase I study of KW-0761, a defucosylated humanized anti-CCR4 antibody, in relapsed patients with adult T-cell leukemia-lymphoma and peripheral T-cell lymphoma. J Clin Oncol. 2010;28(9):1591–8.
127. Ishida T, Joh T, Uike N, Yamamoto K, Utsunomiya A, Yoshida S, et al. Multicenter phase II study of KW-0761, a defucosylated Anti-CCR4 antibody, in relapsed patients with adult T-cell leukemia-lymphoma (ATL). ASH Annu Meet Abstr. 2010;116(21):285.
128. Gajate C, An F, Mollinedo F. Rapid and selective apoptosis in human leukemic cells induced by Aplidine through a Fas/CD95- and mitochondrial-mediated mechanism. Clin Cancer Res. 2003;9(4):1535–45.
129. Ferme C, Mateos MV, Szyldergemajn S, Corrado CS, Zucca E, Extremera S, et al. Aplidin(R) (Plitidepsin) activity in peripheral T-cell lymphoma (PTCL): final results. ASH Annu Meet Abstr. 2010;116(21):1767.
130. William BM, Hohenstein M, Loberiza Jr FR, Caponetti GC, Bociek RG, Bierman P, et al. Phase I/II study of dasatinib in relapsed or refractory non-Hodgkin's lymphoma (NHL). ASH Annu Meet Abstr. 2010;116(21):288.
131. Chen AI, McMillan A, Negrin RS, Horning SJ, Laport GG. Long-term results of autologous hematopoietic cell transplantation for peripheral T cell lymphoma: the Stanford experience. Biol Blood Marrow Transplant. 2008;14(7):741–7.
132. Kewalramani T, Zelenetz AD, Teruya-Feldstein J, Hamlin P, Yahalom J, Horwitz S, et al. Autologous transplantation for relapsed or primary refractory peripheral T-cell lymphoma. Br J Haematol. 2006;134(2):202–7.
133. Reimer P, Schertlin T, Rudiger T, Geissinger E, Roth S, Kunzmann V, et al. Myeloablative radiochemotherapy followed by autologous peripheral blood stem cell transplantation as first-line therapy in peripheral T-cell lymphomas: first results of a prospective multicenter study. Hematol J. 2004;5(4):304–11.
134. Rodriguez J, Conde E, Gutierrez A, Arranz R, Leon A, Marin J, et al. Frontline autologous stem cell transplantation in high-risk peripheral T-cell lymphoma: a prospective study from The Gel-Tamo Study Group. Eur J Haematol. 2007;79(1):32–8.
135. Le Gouill S, Milpied N, Buzyn A, De Latour RP, Vernant JP, Mohty M, et al. Graft-versus-lymphoma effect for aggressive T-cell lymphomas in adults: a study by the Societe Francaise de Greffe de Moelle et de Therapie Cellulaire. J Clin Oncol. 2008;26(14):2264–71.
136. Smith S, Burns LJ, van Besien K, LeRademacher J, He W, Laport GG, et al. Autologous (auto) versus allogeneic (allo) hematopoietic cell transplantation (HCT) for T-NHL: a CIBMTR analysis. ASH Annu Meet Abstr. 2010;116(21):689.
137. Jacobsen ED, Kim HT, Ho VT, Cutler CS, Koreth J, Fisher DC, et al. A large single-center experience with allogeneic stem-cell transplantation for peripheral T-cell non-Hodgkin lymphoma and advanced mycosis fungoides/Sezary syndrome. Ann Oncol. 2011;22(7):1608–1613.
138. Corradini P, Dodero A, Zallio F, Caracciolo D, Casini M, Bregni M, et al. Graft-versus-lymphoma effect in relapsed peripheral T-cell non-Hodgkin's lymphomas after reduced-intensity conditioning followed by allogeneic transplantation of hematopoietic cells. J Clin Oncol. 2004;22(11):2172–6.
139. Foss FM, Zinzani PL, Vose JM, Gascoyne RD, Rosen ST, Tobinai K. Peripheral T-cell lymphoma. Blood. 2011;117(25):6756–67.

Burkitt Lymphoma

14

Dieter Hoelzer and Thomas Burmeister

Abstract

Diagnostic criteria for Burkitt lymphoma (BL) are a strong expression of mature B-cell antigens, chromosomal translocations involving *MYC* t(8;14) in 80 % and more rarely t(8;22) or t(2;8), and a very high proliferation index. Clinically, 80 % of patients with a sporadic form of BL show extranodal manifestations, most often an abdominal mass. Owing to fast progression, tumor lysis syndrome at diagnosis or after initiation of therapy warrants immediate therapeutic intervention. Treatment regimens consist of four to eight cycles of short intensive sequential therapy blocks with high-dose (HD) fractionated alkylating agents, HD methotrexate, and HD cytarabine. Complete remission is achieved in about 80 % of adults and overall survival is 60 %. Stem cell transplantation is not superior and therefore has no place in first-line treatment. The addition of the anti-CD20 antibody rituximab with sequential high-dose chemotherapy has improved CR rates to >80–100 % and survival rates to 70 to <90 %.

Keywords

Deregulation of *MYC* oncogene • Sequential high-dose therapy • Toxicities and supportive care • Stem cell transplantation • Immunochemotherapy with rituximab • Salvage therapies

Diagnostic Approaches to Burkitt Lymphoma

Morphology and Immunophenotype

Historically, the diagnosis of Burkitt lymphoma (BL) was based solely on histomorphological findings. The lymphoma tissue has a characteristic "starry sky" appearance due to a repetitive pattern of medium-sized lymphoblasts interspersed with macrophages. Cytomorphological

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Table 14.1 Diagnostic features of Burkitt lymphoma

	Findings typical for Burkitt lymphoma	Atypical findings	Differential diagnosis to be considered
Histology	Monomorphous medium-sized blasts, "starry sky"	No starry sky, heterogeneous blasts	Other high-grade lymphomas
Immunohisto-chemistry and flow cytometry	CD19+, CD22+, CD79a+, BCL6+, CCND1−, BCL2−, CD5−, Ki-67>95 %, CD10+, sIg+, TdT−, CD44−	CCND1+, CD5+, BCL2+, Ki-67<95 %, CD10−, sIg−, TdT+, CD44+	Mantle cell lymphoma, follicular lymphoma, DLBCL, aggressive lymphoma (NOS), BCP ALL
Cytomorphology	FAB L3	No FAB L3 morphology	BCP ALL, other high-grade lymphomas
Cytogenetics	Chromosomal translocations involving *MYC* on 8q24: t(8;14), t(2;8), t(8;22)	No MYC translocation, additional translocations	DLBCL, double-hit lymphoma/ multiple-hit lymphoma

NOS not otherwise specified, *BCP* B-cell precursor

features of BL lymphoblasts include deeply basophilic cytoplasm, round nuclei with one or more nucleoli, and frequent cytoplasmic lipid vacuoles. This cytomorphological appearance is referred to as "L3" in the French–American–British (FAB) classification of acute leukemias [1]. Immunological methods like immunohistochemistry or flow cytometry (for cell suspensions such as bone marrow or minced lymph node tissue) detect the expression of mature B-cell antigens: surface IgM (sIg), usually with monotypical light chain (κ[kappa] or λ[lambda]) restriction, and CD19, CD20, CD22, CD79a, and the germinal center antigens BCL6 and CD10 are positive, while CD5, BCL2, and TdT are typically negative. Recent work has indicated that CD44 is typically negative [2, 3]. BL cells show an extraordinarily high mitotic division rate, as visualized by immunostaining for the MKI67 protein (Ki-67 index nearly 100 % positive). This makes BL the most rapidly proliferating neoplasm of hematological origin.

It became apparent quite early, however, that these morphological and immunological criteria do not always readily enable a clear separation from other high-grade lymphomas, and thus, the terms "variant Burkitt lymphoma" and "Burkitt-like lymphoma" were coined [4, 5]. Burkitt-like lymphoma cells have been described in the 2001 World Health Organization (WHO) classification as exhibiting a greater pleomorphism in nuclear size and shape with fewer but more prominent nucleoli [5]. In addition, several case reports have described aberrant antigen expressions in BL (Table 14.1).

Endemic, Sporadic, and HIV-Associated Burkitt Lymphoma and the Role of Epstein–Barr Virus

Apart from being classified on the basis of its morphological, immunological, and genetic features, BL has been further subdivided according to its geographic origin and its association with human immunodeficiency virus (HIV) infection. Three types are distinguished: endemic BL (eBL), which is found mainly in tropical Africa where it was first described in 1958 by Denis Burkitt [6]; sporadic BL (sBL) which occurs in temperate climates; and HIV-associated BL (HIV-BL) which develops in patients infected with HIV type 1 or 2. The three types are histologically indistinguishable but differ in their clinical presentation. While eBL occurs almost exclusively in children and involves the jaw or facial bones in around 50 %, sBL can be found in all age groups and rarely affects the facial bones. The most striking difference between the three types is their association with Epstein–Barr virus (EBV). EBV, which was actually first discovered in cell lines derived from endemic BL [7], is found in more than 90 % of endemic cases but in only up to 15 % of sporadic and about 40 % of HIV-associated BL cases. Despite years of intense research, the role of this herpes virus in the etiology of BL still remains

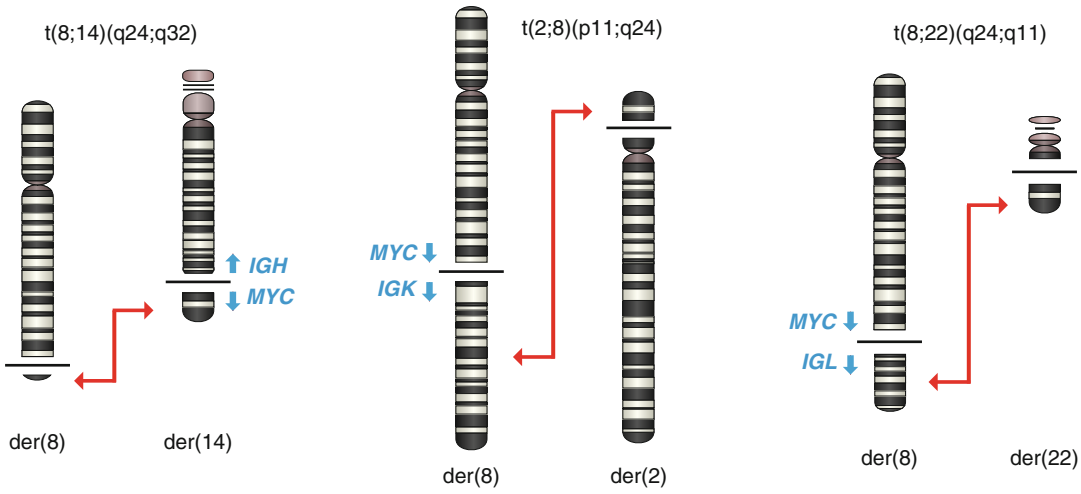

Fig. 14.1 *MYC* translocations are a typical cytogenetic hallmark of Burkitt lymphoma. In around 85 % of cases *MYC* is juxtaposed to the immunoglobulin heavy chain (*IGH*) locus, in around 10 % to the light chain lambda (*IGL*) locus and in 5 % to the light chain kappa (*IGK*) locus

largely abstruse [8]. It has been suggested that the rare EBV-negative BL cases in tropical Africa might actually represent African "sporadic type" BLs [9] and that the terms "endemic" and "sporadic" are misleading because they should denote different etiologies rather than different geographic origins [10]. A higher degree of somatic hypermutation was found in EBV-positive BL, leading to the hypothesis that EBV-positive and -negative BL might arise from different cells of origin, e.g., the former from memory B cells or late germinal center lymphoblasts and the latter from more immature early centroblasts [9].

Genetics: The Central Role of MYC

The key genetic hallmark of BL is the deregulation of the *MYC* oncogene. *MYC* is an evolutionarily conserved gene and apparently plays a key role not only in B-cell lymphopoiesis but also in the development of a large number of different tumors of both hematopoietic and non-hematopoietic origin. Gene expression data suggest that hundreds to thousands of genes are modulated by *MYC* [11], whose expression in BL is typically dysregulated by chromosomal translocations involving immunoglobulin (IG) gene loci (Fig. 14.1). *MYC* is found to be translocated to the heavy chain (*IGH*) gene locus on chromosome 14q32 in around 85 % of cases, to the light chain lambda (*IGL*) locus on chromosome 22q11 in around 10 %, and to the light chain kappa (*IGK*) locus on chromosome 2p11 in 5 % of cases [12]. Rare translocations to non-IG loci have also been described [13]. However, *MYC* translocations are not specific for BL but are also found in a subset of patients with diffuse large B-cell lymphoma (DLBCL) and occasionally in patients with other B-cell neoplasms such as multiple myeloma [12]. Since DLBCL is much more common than BL, a significant percentage of lymphoma patients with *MYC* translocation are diagnosed with DLBCL.

Different diagnostic procedures are used for assessing *MYC* alterations in BL. Classical cytogenetic analysis requires vital and proliferating cells and generates a low-resolution whole-genome overview (a karyogram). It can provide valuable information not only on the type of *MYC* translocation (e.g., t(8;14)) as well as on other aberrations present (e.g., t(14;18) with *BCL2-IGH* fusion) but may sometimes fail to detect existing aberrations owing to a low percentage of blasts or insufficient cell viability. Interphase fluorescence in situ hybridization (FISH) requires no vital cells but only intact cell nuclei and can visualize the presence of a *MYC* translocation

with a higher sensitivity than metaphase cytogenetics. However, other concurrent chromosomal alterations will not be detected without additional separate FISH analyses. Long-distance PCR can be used to detect the t(8;14)/*MYC-IGH* translocation with relatively high sensitivity and requires no cells but only intact (i.e., non-fixed) genomic DNA. This special PCR technique is more complex than standard FISH and provides additional molecular insight into the t(8;14) chromosomal translocation [14].

Thus, BL can only be diagnosed correctly by taking into account histo-/cytomorphological, immunophenotypic, and genetic features. At present, no single diagnostic criterion differentiates BL from other related high-grade lymphomas. The 2008 WHO classification avoided the term "Burkitt-like" or "atypical" Burkitt lymphoma used in the 1994 REAL and 2001 WHO classifications and introduced a number of new lymphoma entities to better distinguish BL from other lymphoma subtypes [15]. Most importantly, the term "double-hit" lymphoma was coined [16]. Double-hit lymphomas show a *MYC*/8q24 translocation in combination with another recurrent translocation, mainly t(14;18) with *BCL2-IGH* fusion. Some cases present with complex translocations involving multiple loci besides *MYC* (most frequently *BCL6*/3q27, *BCL2*/18q21, *BCL3*/19q13), resulting in "triple-hit" or even "quadruple-hit" lymphomas. "Double-hit" or "multiple-hit" lymphomas are aggressive neoplasms with an unfavorable prognosis. In the past, they were often classified as BL-like, DLBCL, or BL. This lymphoma category is still evolving, but it underscores the necessity for thorough genetic characterization at diagnosis, including cytogenetics and FISH.

Gene Expression Profiling in Burkitt Lymphoma

Attempts have been made to define BL by its gene expression profile [17, 18]. A Burkitt "gene signature" was derived from a set of conventionally defined typical BL cases that had been carefully reviewed by an expert panel of hematopathologists. The use of this molecular classifier to analyze large sets of cases conventionally diagnosed as BL and DLBCL resulted in reclassification of several samples as either "molecular BL" or "molecular DLBCL." The two groups showed marked differences in their gene expression profiles, the most prominent being a higher expression of *MYC* target genes and a lower expression of genes involved in the nuclear factor kappa B pathway (e.g., CD44, MUM1) in the group with the BL gene signature. Subsequent analyses further refined the BL gene signature and disclosed differences between sBL and HIV-BL on the one hand and eBL on the other [19]. The microRNA expression profile of BL was found to differ from that of DLBCL but no significant differences were detected between sBL, eBL, and HIV-BL [20]. Although some retrospective analyses have suggested that such a molecularly defined BL signature might be useful in guiding therapeutic decisions, prospective trials are still needed to validate its clinical relevance. Gene expression profiling of BL is not yet used in the routine clinical setting.

Clinical Characteristics

The median age of adult patients is 25–35 years. Burkitt lymphoma shows a rapid tumor growth. Patients often present with bulky disease, e.g., abdominal masses. Mesenteric and retroperitoneal lymph nodes are often involved, while peripheral lymphadenopathy is less prominent. Extranodal disease is seen in 70–80 %, including the tonsils, ovaries, mammary glands, kidney, pleural effusions, central nervous system (CNS), and rarely liver and spleen. The high tumor burden is often accompanied by a tumor lysis syndrome (TLS) which needs immediate treatment intervention. The serum LDH is elevated in more than half of the patients owing to the high cell turnover. High white blood cell count (WBC) is seen in only very few patients, and low platelet counts (≤25/nl), hemoglobin <80 g/dl, and granulocytopenia (<500/μ[mu]l) are rare.

Occasionally BL infiltrates also the bone marrow. If the bone marrow shows more than 25 % BL blasts or if BL cells are detectable in the peripheral blood smear, this condition is designated "Burkitt leukemia." Other terms that were used earlier are

Table 14.2 Treatment results in adult Burkitt lymphoma/leukemia with short intensive sequential chemotherapy

Regimen	Author	Year	Disease	Age	*N*	CR (%)	EFS	OS (%)
Codox-M/ IVAC	Magrath et al. [26]	1996	BL	25 (18–59)	41 20	95	92 % 2 years	
	Adde et al. [27]	1998	BL		26	92		
	Mead et al. [28]	2002	BL	35 (15–60)	52	77		73
	Lacasce et. al. [29]	2004	BL	47 (18–65)	14			71
	Moleti et al. [30]	2007	BL	<21	35	91		83
	Mead et al. [31]	2008	BL	37 (17–76)	58			68
B-NHL GMALL	Hoelzer et al. [32]	1996	L3	34 (15–65)	24	63		49
			L3		35	74		51
	Hoelzer et al. [40]	2002	BL, L3		118	83		70
			B-ALL		89	75		38
	Rizzieri et al. [41]	2004	BL, L3	47 (17–78)	92	74		50–54
Hyper-CVAD	Thomas et al. [35]	1999	BL, L3	58 (17–79)	26	81		49
			<60 years		*14*	*93*		*77*
			>60 years		*12*			*17*
	Thomas et al. [36]	2006	BL, L3	48 (16–79)	48	85		53
CMVP-16/ Ara-C/CDDP	Di Nicola et al. [37]	2004	BL, L3	36	22	77		77
LMB	Divine et al. [42]	2005	BL	33 (18–76)	72	72	65	70
	Choi et al. [39]	2009	BL, L3	48 (34–63)	11	90		82
Weighted mean						83		62

"L3 ALL" or "(mature) B-ALL." Burkitt leukemia often shows an increased WBC and a higher frequency (up to 25 %) of anemia, thrombocyto-, or neutropenia. Almost all cases show LDH levels that are at least twice the norm. Clinically, there is a high rate of CNS involvement, up to 20–30 % with a wide variation in the different reports, requiring CNS focussed therapeutic measures.

Staging is usually performed according to the St Jude/Murphy and the Ann Arbor system, complemented by the International Prognostic Index (IPI) or the age-adjusted IPI (aaIPI). When elderly patients are included, the ECOG performance status should also be taken into account.

Current Chemotherapy: Treatment Principles

Since the doubling time of blast cells in Burkitt lymphoma/leukemia is very short (~25 h) [21], it was postulated that each cell should enter the cell cycle once, which requires a prolonged duration of drug concentration. Thus, high-dose fractionated drugs were given in repeated treatment cycles of 4–5 days. The intervals between the cell cycles are short to prevent recovery of the malignant clone.

The treatment strategies for Burkitt lymphoma/leukemia were pioneered in pediatric studies. Murphy et al. [22] introduced fractionated high doses of cyclophosphamide (HDC) and high-dose methotrexate (HDMTX), in addition to vincristine, doxorubicin, and cytarabine. The German BFM (Berlin–Frankfurt–Münster) group initiated a B-ALL protocol for children using six cycles of HDMTX and fractionated doses of cyclophosphamide or ifosfamide, in addition to cytarabine, teniposide, doxorubicin, and steroids [23, 24]. The *Société française d'oncologie pédiatrique* (SFOP) used high-dose cytarabine (HDAC) in addition to HDC and HDMTX, vincristine, prednisone, and doxorubicin [25]. With these protocols the outcome in children improved substantially to CR rates from 89 to 92 % and event-free survival (EFS) from 50 to 87 %. These successful pediatric approaches have been adapted for adults with Burkitt lymphoma/leukemia.

The CODOX-M/IVAC regimen for BL was developed at the NCI by Magrath for children as well as adults [26]. This regimen includes four cycles of alternating CODOX-M/IVAC for high-risk patients. It combines fractionated cyclophosphamide (1,600 mg/m^2), doxorubicin, vincristine, HDMTX (6,720 mg/m^2 over 24 h) alternating with fractionated ifosfamide (7,500 mg/m^2), etoposide, and HDAC (8,000 mg/m^2) together with intrathecal methotrexate and cytarabine.

In a cohort of 41 patients including 20 adults, the CR rate was 95 % and the 2-year EFS 92 %. The toxicity in this protocol consisted of an infection rate of 50 % and severe stomatitis in up to 60 % of CODOX-M cycles. This regimen was used by several other study groups treating older patients up to 76 years (see Table 14.2) [26–32, 35–37, 39–42]. It may be noted from these studies that the CR rates are high in all age groups but that the EFS/OS in the three studies with a median age of 35–37 years decreased to 68–73 %.

The German Multicenter Study Group for Adult Acute Lymphoblastic Leukemia (GMALL) conducted several studies for Burkitt lymphoma/leukemia (B-ALL/NHL83, B-ALL/NHL86, B-NHL90) based on the pediatric BFM protocols. In these GMALL B-NHL studies, the patients received a gentle cytoreductive pre-phase with cyclophosphamide (200 mg/m^2/day) and prednisone (60 mg/m^2/day) on days 1–5. This pre-phase therapy was aimed at stabilizing the clinical condition of the patient and reducing the risk of TLS.

The B-NHL 83 protocol was primarily designed to treat patients with Burkitt leukemia, who had obtained a very poor outcome with the conventional ALL protocols [32]. The regimen consisted of six cycles of 5-day alternating courses including fractionated cyclophosphamide and methotrexate 500 mg/m^2, in addition to conventional-dose cytarabine, teniposide, and prednisone or doxorubicin. CNS prophylaxis consisted of intrathecal methotrexate, cytarabine, and dexamethasone along with CNS irradiation of 24 Gy after the second cycle. The median age was 34 (15–65) years. In 24 patients the response rate was 63 % and the OS 49 % (Table 14.2). In the B-NHL86 protocol, methotrexate was increased to 1,500 mg/m^2 and cyclophosphamide was alternated with ifosfamide. Twenty-six (74 %) out of 35 patients achieved a CR and the OS was 51 %. In the B-NHL90 treatment protocol, CNS irradiation after cycle 2 was omitted since it aggravated cytopenias, leading to a delay in further treatment. It was compensated by increasing methotrexate from 1,500 to 3,000 mg/m^2 and shortening the interval between each cycle to 14 days. The response rate in 118 patients with Burkitt leukemia, B-NHL increased to 83 % and the OS was 70 %. In 89 patients with Burkitt leukemia, CR rate was 75 % and OS 38 %. The toxicity mostly consisted of severe but reversible WHO grade III and IV mucositis.

The pediatric LMB 95 protocol was adapted for adults in the French lymphoma cooperative groups (GELA and GOELAMS). After a cytoreductive pretreatment the protocol consisted of two induction and two consolidation cycles followed by up to four maintenance cycles. Important drugs were high-dose methotrexate with a dose range of 1,000–8,000 mg/m^2 depending on age and CNS disease and high-dose cytarabine with 1,000–3,000 mg/m^2 if the CNS was involved and intensifying therapy was necessary. Seventy-two adults with a median age of 33 (range: 18–76) years were reported. At 2 years the CR rate was 72 % and the 2-year EFS and OS were 65 and 70 % (Table 14.2). Risk factors were age above 33 years and an elevated LDH.

The M.D. Anderson Cancer Center has explored the hyper-CVAD scheme, based on the Murphy regimen to treat adult Burkitt lymphoma/leukemia patients. The regimen consists of four cycles with hyperfractionated cyclophosphamide 1,800 mg/m^2 alternating with four cycles of methotrexate 1,000 mg/m^2 for 24 h and cytarabine 3,000 mg/m^2 every 12 h for four doses. In an early report by Thomas [35], 26 patients were included with a median age of 58 (17–79) years. The overall CR rate was 81 and 93 % for the patients <60 years. An overall survival of 49 % was achieved which greatly differed between the patient group aged <60 years with 77 % compared to 12 patients 60 years or older of 17 %. The rate of induction death was 19 %. In a larger study [36] comprising 48 patients, the high CR

rate of 85 % and the overall survival of 53 % were confirmed (Table 14.2).

In a somewhat different regimen, the CMVP-16/Ara-C/CDDP scheme, cisplatin was included, also based on a pediatric approach successfully used in children with advanced BL. For 22 adult patients the CR rate was 77 % and the OS 77 % confirming the results obtained with other regimens but was not superior [37]. In a very small cohort of 11 patients with a median age of 48 years (34–63), a high CR rate of 90 % and an OS of 82 % were obtained [39].

Taken together, the treatment results in adult Burkitt lymphoma/leukemia with short intensive sequential therapy showed a substantial improvement. The weighted mean for the CR rate is ~80 % and for OS is ~60 %. All regimens obtained similar results, probably somewhat inferior in some studies patients with Burkitt leukemia compared to lymphoma and in the older patient cohorts.

Toxicities and Supportive Care

Treatment of Burkitt lymphoma/leukemia requires immediate and careful supportive management of the patients since they are at highest risk of a tumor lysis syndrome (TLS) [43], either present already at diagnosis or developing under subsequent chemotherapy. Risk factors for a TLS are bulky disease, elevated LDH, elevated white blood cell count, and preexisting renal insufficiency. The clinical monitoring includes renal function with creatinine clearance, fluid balancing, and serum electrolytes, particularly calcium, phosphate, and uric acid. Immediate hyperfiltration with 150–250 ml/h is required. The treatment with allopurinol, which blocks the conversion of xanthine and hypoxanthine to uric acid, is recommended. In more severe cases of TLS, recombinant uric oxidase, Rasburicase, is immediately effective [43]. Carboxypeptidase G2 might be the drug of choice if MTX elimination is delayed after high-dose methotrexate. In a study with 43 patients (CNS 16, ALL 13, NHL 12), it was also effective in the patients with CNS involvement [44].

During the intensive chemotherapy cycles, oral mucositis mainly caused by high-dose methotrexate is the major obstacle for the patients. In the GMALL B-NHL studies, WHO grade 3–4 mucositis occurs in 30–40 % which is painful and limits life quality. A new approach is the use of the mucosal growth factor palifermin, which has proven successful in the prevention of severe mucositis after stem cell transplantation and is currently explored in Burkitt lymphoma/leukemia in the ongoing GMALL-B-ALL/NHL 2002 study. There is also a risk of infections, particularly owing to the combination of mucositis and neutropenia which is aggravated by the short 2-week cycle intervals. If granulocytes are below 500/μ[mu]l, G-CSF is recommended in several studies and in some is given prophylactically to all patients. Since time/dose intensity is so important for the overall outcome of Burkitt lymphoma/leukemia patients, rigorous prophylaxis and supportive treatment is mandatory to avoid or reduce treatment delays.

High frequency of neurologic complication, severe sensory problems, or cortical dysfunction is now largely avoided by reduction of high doses of cyclophosphamide, HD-C, or HDMTX and omission of CNS irradiation.

Hematopoietic Stem Cell Transplantation

Stem cell transplantation, either autologous or allogeneic, has been explored in BL in several studies with contradictory results (Table 14.3) [45–52]. The largest series of patients is a EBMT registry matched study comprising lymphoma patients who had received either an allogeneic or an autologous stem cell transplantation [47]. There were 284 Burkitt NHLs comprising 213 out of 14,687 patients with autologous and 71 out of 1,185 patients with allogeneic transplants registered between 1982 and 1998. In the autologous cohort, 77 (36 %) relapsed at 2.5 years compared to 19 (27 %) in the allogeneic cohort. However, the treatment-related mortality (TRM) in the allogeneic transplant group was 31 %. There was a wide age range from young children up to

Table 14.3 SCT in Burkitt lymphoma

SCT	Age	Disease stage	No. pts	PFS/EFS	OS
Autologous					
Chopra et al. [51]	22 (1–44)	All stages	8	38 %	
Sweetenham et al. [46]	31 (16–57)	CR1	70	84 %	72 % 3 years
		>CR1, res.	47	15 %	37 % 3 years
Peniket et al. [47]	35 (0–84)	All	213		37 % 2.5 years
Gada et al. [49]	16 (4–65)		25	21 % 10 years	23 % 10 years
van Imhoff et al. [48]	36 (15–64)	At least PR	27		81 % 5 years
Song et al. [45]	36 (16–62)	All	21 auto 6 allo	51 % 3 years	
Majhail et al. [50]	47 (7–72)	All	69		85 % 10 years
Gross et al. [52]	<18		17	27 % 5 years	
Allogeneic					
Chopra et al. [51]	23 (1–40)	All	8	25 %	
Peniket et al. [47]	29 (2–74)	All	71		27 % 2.5 years
Gada et al. [49]	13 (2–62)		13	31 % 10 years	31 % 10 years
Gross et al. [52]	<18		24	31 % 5 years	

patients 84 years old. The overall survival for autologous SCT was with 37 % superior to the OS after allogeneic SCT of 27 % at 2.5 years. Thus, the lower relapse rate was outweighted by the higher TRM rate in the allogeneic setting, resulting in a similar overall survival.

The European Group for Blood and Marrow Transplantation reported 70 patients treated with high-dose therapy and autologous stem cell transplantation in first remission (CR) or at relapse [46]. The median age was 31 (16–57) years; the overall survival rate for the entire group was 53 % at 3 years and 72 % for the patients transplanted in first CR compared to 37 % for patients with chemosensitive relapse. In chemoresistant patients the overall survival was 7 %.

The Dutch–Belgian Cooperative Trial Group for Hemato-Oncology (HOVON) initiated a prospective study to evaluate a short intensive sequential therapy followed by autologous stem cell transplantation in adult Burkitt, Burkitt-like, and lymphoblastic lymphoma [48]. Twenty-seven patients with a median age of 36 (15–64) years were included (Table 14.3); the majority (81 %) had a CR at the time of transplant, while the remaining patients were at least in partial remission. The overall response rate was 93 %, the OS at 5 years was 81 % for Burkitt/Burkitt-like lymphoma, and the event-free survival was 73 %. However, patients with CNS or extensive bone marrow involvement were excluded. Grade III–IV mucositis was seen in 39 % of patients after induction and after BEAM.

In a Canadian study in British Columbia between 1987 and 2003, patients received cyclophosphamide, HDMTX 3,000 mg/m^2, a CHOP-like regimen, or CODOX-M [45]. Twenty-seven out of 43 patients proceeded to hematopoietic stem cell transplantation (HCT), while the remaining patients had refractory disease or other contraindications. The EFS for the 21 pts receiving an autologous HCT and the six allografted was 51 % at 3 years (Table 14.3).

In a single-center transplant study of the University of Minnesota, 25 patients with a median age of 16 (range, 4–65) years received an auto-HCT and 13 patients with a median age of 13 (2–62) years received an allo-SCT [49]. In this study with a long follow-up of 10 years, the progression-free survival (PFS) was 21 %, and the overall survival 23 % after auto-HCT compared to 31 % for allo-HCT (Table 14.3).

In a further recent study with a long follow-up, a cohort of 69 patients with Burkitt-lymphoblastic lymphoma was included. The median age was 47 (7–72) years [50] and all stages were included. The OS for lymphoblastic/Burkitt NHL was reported as 85 %. For patients surviving in remis-

sion for at least 2 years after autologous SCT, the EFS was about 60 % at 15 years.

Two other reports compared autologous versus allogeneic HCT. In a small study published 1992 [51], the PFS for eight patients with autologous SCT was 38 and 25 % for the allogeneic patients (Table 14.3). Also in a somewhat larger study published in 2010 [52], 70 patients with an autograft had a 5-year EFS of 27 %, and for 24 patients who underwent allogeneic SCT, the PFS was 31 %. In a small series of six Burkitt leukemia patients with a median age of 24 years (range 24–27), the survival rate was 50 % at >10 years. This may also support the assumption that there is a graft-versus-leukemia/lymphoma effect [32].

In conclusion there is a great variation in the EFS and OS in autologous SCT, mostly in the order of 30 %, but also up to 80 %. This might be due to different selection criteria, e.g., if only CR1 patients were included or if patients with CNS involvement or bone marrow infiltration were excluded. Prognostic factors differ in nearly all studies. The overall results are not superior to those achieved with short intensive sequential chemotherapy.

Chemoimmunotherapy

In adult Burkitt lymphoma/leukemia further treatment, intensification of chemotherapy is limited owing to toxicity, particularly in older patients. A new option emerged with the availability of antibody therapy. Based on the encouraging experience with the anti-CD20 monoclonal antibody rituximab in other NHLs, rituximab was combined with the established short intensive chemotherapy regimen.

The GMALL study group initiated the protocol B-ALL/NHL-2002 which uses rituximab in combination with chemotherapy [32]. Rituximab was administered at a dose of 375 mg/m^2 on day 1 before each chemotherapy cycle and thereafter twice for consolidation at monthly intervals for a total of eight applications. Two alternating cycles with 4 doses of high-dose cytarabine (HDAC) at 2,000 mg/m^2 every 12 h were added for patients below age 55. Patients older than 55 received methotrexate 500 mg/m^2, but no HDAC to avoid toxicity. The CR rate in 115 BL patients was 90 %. Three-year OS was 91 % in patients of age 15–55 and 84 % in patients >55 years. Among 70 Burkitt leukemia patients, the CR rate was 83 %; 3-year OS in younger versus older patients was 79 and 39 %, respectively. CNS relapses occurred in 3 of 22 older CR patients which were most likely related to the exclusion of HDAC. Therefore, HDAC in a lower dose of 1,000 mg/m^2 is included in the current protocol for Burkitt leukemia patients. In contrast to the Burkitt lymphoma pts >55 years, leukemic patients did equally as well as the younger patients.

In a pilot study 2003, 82 patients from 39 centers entered the protocol. Out of 53 evaluable patients, the CR rate was 91 % in mature B-ALL and 96 % in Burkitt NHL; the overall survival was 70 and 80 %, respectively. Rituximab administration did not result in excessive toxicity. In the meantime more than 227 patients have been included. The overall survival is 88 % for Burkitt NHL and 70 % for mature B-ALL, which is a considerable improvement compared to the previous trial [53]. When the GMALL immunochemotherapeutic approach was applied in HIV-positive Burkitt NHL, combined with antiretroviral HAART therapy, the survival rate improved to 77 % [54].

In a trial by the Cancer and Leukemia Group B (CALGB 9251), adult patients with Burkitt or Burkitt-like leukemia/lymphoma received a similar high-intensity chemotherapy and rituximab, intensified in cycle 2 and given for a total of eight times [55]. The intended 7-therapy courses could be completed in 75 out of 105 enrolled patients. Eighty-two percent achieved a CR and 87 % of those remained in CR. There was a clear difference in outcome based on IPI score with a 2-year EFS and OS for low-risk patients of 98 and 92 % versus 55 and 55 % for high-risk patients, respectively.

In the approach at the M.D. Anderson Cancer Center, rituximab was added to the hyper-CVAD regimen (cyclophosphamide/vincristine/adriamycin/dexamethasone HDMTX/HDAC) [36]. Rituximab was given at the beginning and end of the first four chemotherapy cycles, for a total of

eight doses. In 31 patients with newly diagnosed Burkitt NHL or mature B-ALL, 86 % complete responses were observed, and the 3-year overall survival was 89 %. The authors observed a significant reduction in relapse rate and an improvement in outcome, particularly in elderly patients. This study revealed no additional toxicity compared to the previous protocol with chemotherapy only.

Rituximab (R) was also added to the CODOX-M/IVAC regimen for a total of 4 doses. In the 40 R+ patient cohort, the overall response/CR rate was 90 %/90 % compared to 85 %/81 % in the 47 R− patients. The PFS and overall survival in the R+ arm was 70 and 73 % compared to 61 and 68 % in the R− arm. The outcome for the R+ patients was not statistically significantly superior to the R− patients, and it was discussed whether more frequent dosing of rituximab might provide the optimal benefit.

In a recent U.S. NCI study, dose-adjusted infusional chemotherapy was combined with rituximab [33]. Four patients in the small series of 17 patients with a median age of 27 (18–66) were HIV-positive. All patients responded with CR and the OS was 100 % and EFS 92 %, respectively. This single study needs confirmation with more patients, a longer follow-up, and hopefully more institutions.

In conclusion it appears that rituximab added to short intensive chemotherapy regimen has substantially increased the survival rate of adult patients with mature B-ALL/Burkitt NHL by 20–30 %.

Salvage Therapy and Treatment of Refractory/Relapsed Burkitt Lymphoma

In most reports salvage therapy for patients after induction or consolidation is not reported in detail. In the GMALL B-NHL studies, patients with initial CNS involvement received a cranial irradiation of 24 Gy, and those with remaining bulky disease but otherwise in CR received a bulk irradiation with 36 Gy.

The outcome in refractory/relapsed patients with different chemotherapy regimens was dismal. From a variety of new and innovative drugs considered [34], 57 so far have not been transferred to BL. The OS was also poor in patients with refractory disease, receiving SCT, most likely because of the fast progression and the difficulty to find a donor in due time.

What could be further concepts? Early detection of relapse by evaluation of minimal residual disease (MRD) as in pediatric Burkitt leukemia [38] might be an option also for adults. In Burkitt lymphoma confirmation of a CR by fludeoxyglucose positron emission tomography (FDG-PET) and by follow-up for earlier detection of relapse is currently under investigation.

However, the overall cure rate of >80 % of BL patients is extremely promising and ways to deescalate therapy—as already done for low disease stages—are now considered.

References

1. Bene MC, Castoldi G, Knapp W, Ludwig WD, Matutes E, Orfao A, et al. Proposals for the immunological classification of acute leukemias. European Group for the Immunological Characterization of Leukemias (EGIL). Leukemia. 1995;9:1783–6.
2. Attarbaschi A, Mann G, Schumich A, König M, Pickl WF, Haas OA, et al. CD44 deficiency is a consistent finding in childhood Burkitt's lymphoma and leukemia. Leukemia. 2007;21:1110–3.
3. Schniederjan SD, Li S, Saxe DF, Lechowicz MJ, Lee KL, Terry PD, et al. A novel flow cytometric antibody panel for distinguishing Burkitt lymphoma from CD10+ diffuse large B-cell lymphoma. Am J Clin Pathol. 2010;133:718–26.
4. Harris NL, Jaffe ES, Stein H, Banks PM, Chan JK, Cleary ML, et al. A revised European-American classification of lymphoid neoplasms: a proposal from the International Lymphoma Study Group. Blood. 1994;84:1361–92.
5. Diebold J, Jaffe ES, Raphael M, Warnke RA. Burkitt lymphoma. In: Jaffe ES, Harris NL, Stein H, Vardiman JW, editors. Pathology and genetics of tumours of haematopoietic and lymphoid tissues. Lyon: IARC Press; 2001. p. 181–5.
6. Burkitt D. A sarcoma involving the jaws in African children. Br J Surg. 1958;46:218–23.
7. Epstein MA, Achong BG, Barr YM. Virus particles in cultured lymphoblasts from Burkitt's lymphoma. Lancet. 1964;1:702–3.
8. Bornkamm GW. Epstein-Barr virus and the pathogenesis of Burkitt's lymphoma: more questions than answers. Int J Cancer. 2009;124:1745–55.

9. Bellan C, Lazzi S, Hummel M, Palummo N, de Santi M, Amato T, et al. Immunoglobulin gene analysis reveals 2 distinct cells of origin for EBV-positive and EBV-negative Burkitt lymphomas. Blood. 2005;106: 1031–6.
10. Wright DH. What is Burkitt's lymphoma and when is it endemic? Blood. 1999;93(2):758.
11. Eilers M, Eisenman RN. Myc's broad reach. Genes Dev. 2008;22:2755–66.
12. Janz S. Myc translocations in B cell and plasma cell neoplasms. DNA Repair. 2006;5:1213–24.
13. Bertrand P, Bastard C, Maingonnat C, Jardin F, Maisonneuve C, Courel MN, et al. Mapping of MYC breakpoints in 8q24 rearrangements involving non-immunoglobulin partners in B-cell lymphomas. Leukemia. 2007;21:515–23.
14. Burmeister T, Schwartz S, Horst HA, Rieder H, Gökbuget N, Hoelzer D, et al. Molecular heterogeneity of sporadic adult Burkitt-type leukemia/lymphoma as revealed by PCR and cytogenetics: correlation with morphology, immunology and clinical features. Leukemia. 2005;19:1391–8.
15. Leoncini L, Raphael M, Stein H, Harris NL, Jaffe ES, Kluin PM, et al. Burkitt lymphoma. In: Swerdlow SH, Campo E, Harris NL, editors. WHO classification of tumours of haematopoietic and lymphoid tissues. Lyon: IARC; 2008. p. 262–4.
16. Aukema SM, Siebert R, Schuuring E, van Imhoff GW, Kluin-Nelemans HC, Boerma EJ, et al. Double-hit B-cell lymphomas. Blood. 2011;117:2319–31.
17. Hummel M, Bentink S, Berger H, Klapper W, Wessendorf S, Barth TF, et al. A biologic definition of Burkitt's lymphoma from transcriptional and genomic profiling. N Engl J Med. 2006;354:2419–30.
18. Dave SS, Fu K, Wright GW, Lam LT, Kluin P, Boerma EJ, et al. Molecular diagnosis of Burkitt's lymphoma. N Engl J Med. 2006;354:2431–42.
19. Piccaluga PP, De Falco G, Kustagi M, Gazzola A, Agostinelli C, Tripodo C, et al. Gene expression analysis uncovers similarity and differences among Burkitt lymphoma subtypes. Blood. 2011;117: 3596–608.
20. Lenze D, Leoncini L, Hummel M, Volinia S, Liu CG, Amato T, et al. The different epidemiologic subtypes of Burkitt lymphoma share a homogenous micro RNA profile distinct from diffuse large B-cell lymphoma. Leukemia. 2011;25(12):1869–76.
21. Iversen OH, Iversen U, Ziegler JL, Bluming AZ. Cell kinetics in Burkitt lymphoma. Eur J Cancer. 1974;10: 155–63.
22. Murphy SB, Bowman WP, Abromowitch M, Mirro J, Ochs J, Rivera G, et al. Results of treatment of advanced-stage Burkitt's lymphoma and B cell (SIg+) acute lymphoblastic leukemia with high-dose fractionated cyclophosphamide and coordinated high-dose methotrexate and cytarabine. J Clin Oncol. 1986; 4:1732–9.
23. Müller-Weihrich S, Henze G, Langermann HJ, Odenwald E, Riehm H. Childhood B-cell lymphomas and leukemias. Improvement of prognosis by a therapy developed for B-neoplasms by the BFM study group. Onkologie. 1984;7:205–8.
24. Reiter A, Schrappe M, Ludwig WD, Lampert F, Harbott J, Henze G, et al. Favorable outcome of B-cell acute lymphoblastic leukemia in childhood: a report of three consecutive studies of the BFM group. Blood. 1992;80:2471–8.
25. Patte C, Philip T, Rodary C, Bernard A, Zucker JM, Bernard JL, et al. Improved survival rate in children with stage III and IV B cell non-Hodgkin's lymphoma and leukemia using multi-agent chemotherapy: results of a study of 114 children from the French Pediatric Oncology Society. J Clin Oncol. 1986;4:1219–26.
26. Magrath I, Adde M, Shad A, Venzon D, Seibel N, Gootenberg J, et al. Adults and children with small non-cleaved-cell lymphoma have a similar excellent outcome when treated with the same chemotherapy regimen. J Clin Oncol. 1996;14:925–34.
27. Adde M, Shad A, Venzon D, Arndt C, Gootenberg J, Neely J, et al. Additional chemotherapy agents improve treatment outcome for children and adults with advanced B-cell lymphomas. Semin Oncol. 1998; 25:33–9; discussion 45–8.
28. Mead GM, Sydes MR, Walewski J, Grigg A, Hatton CS, Pescosta N, et al. An international evaluation of CODOX-M and CODOX-M alternating with IVAC in adult Burkitt's lymphoma: results of United Kingdom Lymphoma Group LY06 Study. Ann Oncol. 2002;13: 1264–74.
29. Lacasce A, Howard O, Lib S, Fisher D, Weng A, Neuberg D, et al. Modified magrath regimens for adults with Burkitt and Burkitt-like lymphomas: preserved efficacy with decreased toxicity. Leuk Lymphoma. 2004;45:761–7.
30. Moleti ML, Testi AM, Giona F, Malandruccolo L, Pescarmona E, Martino P, et al. CODOX-M/IVAC (NCI 89-C-41) in children and adolescents with Burkitt's leukemia/lymphoma and large B-cell lymphomas: a 15-year monocentric experience. Leuk Lymphoma. 2007;48:551–9.
31. Mead GM, Barrans SL, Qian W, Walewski J, Radford JA, Wolf M, et al. A prospective clinicopathologic study of dose-modified CODOX-M/IVAC in patients with sporadic Burkitt lymphoma defined using cytogenetic and immunophenotypic criteria (MRC/NCRI LY10 trial). Blood. 2008;112:2248–60.
32. Hoelzer D, Ludwig WD, Thiel E, Gassmann W, Löffler H, Fonatsch C, et al. Improved outcome in adult B-cell acute lymphoblastic leukemia. Blood. 1996;87:495–508.
33. Dunleavy K, Pittaluga S, Janik J, et al. Novel treatment of Burkitt lymphoma with dose-adjusted EPOCH-rituximab: preliminary results showing excellent outcome. Blood. 2006;108:abstract 2736.
34. Blum KA, Lozanski G, Byrd JC. Adult Burkitt leukemia and lymphoma. Blood. 2004;104:3009–20.
35. Thomas DA, Cortes J, O'Brien S, Pierce S, Faderl S, Albitar M, et al. Hyper-CVAD program in Burkitt's-

type adult acute lymphoblastic leukemia. J Clin Oncol. 1999;17:2461–70.
36. Thomas DA, Faderl S, O'Brien S, Bueso-Ramos C, Cortes J, Garcia-Manero G, et al. Chemoimmunotherapy with hyper-CVAD plus rituximab for the treatment of adult Burkitt and Burkitt-type lymphoma or acute lymphoblastic leukemia. Cancer. 2006;106:1569–80.
37. Di Nicola M, Carlo-Stella C, Mariotti J, Devizzi L, Massimino M, Cabras A, et al. High response rate and manageable toxicity with an intensive, short-term chemotherapy programme for Burkitt's lymphoma in adults. Br J Haematol. 2004;126:815–20.
38. Mussolin L, Pillon M, Conter V, Piglione M, Lo Nigro L, Pierani P, et al. Prognostic role of minimal residual disease in mature B-cell acute lymphoblastic leukemia of childhood. J Clin Oncol. 2007;25:5254–61.
39. Choi MK, Jun HJ, Lee SY, Kim KH, Lim do H, Kim K, et al. Treatment outcome of adult patients with Burkitt lymphoma: results using the LMB protocol in Korea. Ann Hematol. 2009;88:1099–106.
40. Hoelzer D, Arnold R, Diedrich H, et al. Successful treatment of Burkitt's NHL and other high-grade NHL according to a protocol for mature B-ALL. Blood. 2002;100:abstract#595.
41. Rizzieri DA, Johnson JL, Niedzwiecki D, Lee EJ, Vardiman JW, Powell BL, et al. Intensive chemotherapy with and without cranial radiation for Burkitt leukemia and lymphoma: final results of Cancer and Leukemia Group B Study 9251. Cancer. 2004;100: 1438–48.
42. Divine M, Casassus P, Koscielny S, Bosq J, Sebban C, Le Maignan C, et al. Burkitt lymphoma in adults: a prospective study of 72 patients treated with an adapted pediatric LMB protocol. Ann Oncol. 2005;16: 1928–35.
43. Coiffier B, Altman A, Pui CH, Younes A, Cairo MS. Guidelines for the management of pediatric and adult tumor lysis syndrome: an evidence-based review. J Clin Oncol. 2008;26:2767–78.
44. Schwartz S, Borner K, Müller K, Martus P, Fischer L, Korfel A, et al. Glucarpidase (carboxypeptidase g2) intervention in adult and elderly cancer patients with renal dysfunction and delayed methotrexate elimination after high-dose methotrexate therapy. Oncologist. 2007;12:1299–308.
45. Song KW, Barnett MJ, Gascoyne RD, Horsman DE, Forrest DL, Hogge DE, et al. Haematopoietic stem cell transplantation as primary therapy of sporadic adult Burkitt lymphoma. Br J Haematol. 2006;133: 634–7.
46. Sweetenham JW, Pearce R, Taghipour G, Blaise D, Gisselbrecht C, Goldstone AH. Adult Burkitt's and Burkitt-like non-Hodgkin's lymphoma—outcome for patients treated with high-dose therapy and autologous stem-cell transplantation in first remission or at relapse: results from the European Group for Blood and Marrow Transplantation. J Clin Oncol. 1996;14: 2465–72.
47. Peniket AJ, Ruiz de Elvira MC, Taghipour G, Cordonnier C, Gluckman E, de Witte T, et al. An EBMT registry matched study of allogeneic stem cell transplants for lymphoma: allogeneic transplantation is associated with a lower relapse rate but a higher procedure-related mortality rate than autologous transplantation. Bone Marrow Transplant. 2003;31: 667–78.
48. van Imhoff GW, van der Holt B, MacKenzie MA, Ossenkoppele GJ, Wijermans PW, Kramer MH, et al. Short intensive sequential therapy followed by autologous stem cell transplantation in adult Burkitt, Burkitt-like and lymphoblastic lymphoma. Leukemia. 2005; 19:945–52.
49. Gada P, Defor T, Weisdorf DJ, Miller JS, Orchard PJ, MacMillan ML, et al. Prolonged remission with autologous and allogeneic stem cell transplantation for Burkitt's lymphoma: long term follow-up at a single institution. Blood. 2005;106:abstract #1133.
50. Majhail NS, Bajorunaite R, Lazarus HM, Wang Z, Klein JP, Zhang MJ, et al. Long-term survival and late relapse in 2-year survivors of autologous haematopoietic cell transplantation for Hodgkin and non-Hodgkin lymphoma. Br J Haematol. 2009;147: 129–39.
51. Chopra R, Goldstone AH, Pearce R, Philip T, Petersen F, Appelbaum F, et al. Autologous versus allogeneic bone marrow transplantation for non-Hodgkin's lymphoma: a case-controlled analysis of the European Bone Marrow Transplant Group Registry data. J Clin Oncol. 1992;10:1690–5.
52. Gross TG, Hale GA, He W, Camitta BM, Sanders JE, Cairo MS, et al. Hematopoietic stem cell transplantation for refractory or recurrent non-Hodgkin lymphoma in children and adolescents. Biol Blood Marrow Transplant. 2010;16:223–30.
53. Hoelzer D, Goekbuget N, Beck J, et al. Subtype adjusted therapy improves outcome of elderly patients with Acute Lymphoblastic Leukemia (ALL). Blood. 2004;104:abstract#2732.
54. Oriol A, Ribera JM, Bergua J, Gimenez Mesa E, Grande C, Esteve J, et al. High-dose chemotherapy and immunotherapy in adult Burkitt lymphoma: comparison of results in human immunodeficiency virus-infected and noninfected patients. Cancer. 2008;113: 117–25.
55. Rizzieri D, Johnson J, Byrd J, et al. Efficacy and toxicity of rituximab and brief duration, high intensity chemotherapy with filgrastim support for Burkitt or Burkitt—like leukemia/lymphoma: Cancer and Leukemia Group B (CALGB) Study 10002. Blood. 2010;116:abstract#858.

Lymphoblastic Lymphoma

15

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Abstract

Lymphoblastic lymphoma (LBL) and acute lymphoblastic leukemia (ALL) are largely overlapping entities based on morphology, immunophenotype, and molecular aberrations. Arbitrary distinctions are made between these entities by the extent of marrow involvement. Juxtaposition of genes encoding oncogenic transcription factor to T-cell receptor genes, activating Notch mutations, and loss of tumor suppressors are common events in T-LBL. Adoption of ALL-like therapy and pediatric regimens has improved outcome in LBL. Treatments targeting Notch, mTOR/Akt pathways, etc. and newer nucleoside analogs are expected to improve outcome in LBL.

Keywords

Lymphoma • Lymphoblastic • Acute lymphoblastic leukemia

Introduction

Lymphoblastic lymphoma is a malignancy of immature T or B cells. The distinction between lymphoblastic lymphoma (LBL) and acute lymphoblastic leukemia (ALL) appears to be arbitrary. Morphologically and immunophenotypically both entities seem indistinguishable. Bone marrow involvement of ≥25 % is taken to be indicative of a diagnosis of ALL. Bulky mediastinal mass without overt spillover of "lymphoma" cells to bone marrow or blood is considered to be indicative of "lymphoma" diagnosis. The World Health Organization classification combines both entities as precursor lymphoma/leukemia.

Among children with NHL, the incidence of LBL is 30 % but LBL is a rare entity among adults with NHL, comprising <2 %. On the other hand, among adults with ALL, the frequency of LBL is about 25 %, while among children with ALL, it is 12–15 %. The incidence among adults is bimodal with one peak at age <20 years and another at >50 years. The male to female ratio is 2.5:1 and the median age is younger in males [1]. B-cell LBL comprises about 10 % of all LBLs.

Symptomatic supradiaphragmatic lymphadenopathy associated with cough, wheezing, shortness of breath, and B symptoms in an adolescent or young adult male is the "classical" presentation. Acute respiratory distress can develop due to

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mediastinal mass; pleuro–pericardial effusions are common and can rarely lead to tamponade. Other extramedullary sites of involvement can include skin, CNS, liver, spleen, and gonads. Most patients present with bone marrow involvement. For patients with bone marrow involvement, a spinal tap with CSF cytology is imperative as the chance of CNS involvement is high among these patients even though the overall frequency of CNS involvement is 5–10 % among patients with LBL.

Mediastinal and marrow involvement are uncommon in B-LBL, while extranodal involvement is frequent [2, 3]. Peripheral blood counts may be normal in LBL in contrast to patients with ALL indicating preserved bone marrow function.

An arbitrary cutoff of 25 % marrow involvement has been used to distinguish between ALL and LBL. Gene expression profiling followed by unsupervised hierarchical clustering can identify non-overlapping signatures between T-ALL and T-LBL [4]. Adhesion molecules, extracellular protein, and CARD10 (a member of the caspase recruitment domain family) encoding genes were overexpressed in T-LBL, while CD47, a regulator of cell proliferation and apoptosis, was overexpressed in the T-ALL cohort. Findings from comparative expressed sequence hybridization (CESH) indicated that T-LBL is derived from thymocytes, while T-ALL is derived from T-cell progenitors in the bone marrow [5].

Suggested Initial Workup

The Ann Arbor staging system is the commonly followed staging system for adult LBL. Suggested initial work up includes:

- Physical examination with lymph node survey
- Complete blood count with differential and platelet count
- Electrolytes including phosphorus, uric acid, lactate dehydrogenase
- Chest radiograph
- CT scan of chest, abdomen, and pelvis
- Bone marrow aspiration/biopsy with immunohistochemistry, flow cytometry, cytogenetic, T-cell receptor, and immunoglobulin gene rearrangement studies
- Mediastinal or extramedullary disease site biopsy if bone marrow not involved
- Spinal tap with spinal fluid cytology
- Cytologic assessment of pleural fluid or other effusions
- Positron emission tomography (optional)

Apart from blood counts and electrolytes, the workup should include parameters like phosphorus, potassium, uric acid, and lactate dehydrogenase that can indicate tumor lysis. Imaging studies can help to assess extramedullary involvement particularly mediastinal involvement. Positron emission tomography (PET) scan can be considered at baseline as persistence of PET-avid disease on follow-up scans after completion of therapy may indicate areas of viable disease that can possibly be irradiated. Bone marrow aspiration and biopsy studies should be accompanied by cytogenetic studies, immunophenotyping studies, and molecular studies for T-cell receptor and immunoglobulin heavy chain gene rearrangement. Apart from disease characterization, these studies can help minimal residual disease (MRD) evaluation at follow-up. In patients with minimal or no bone marrow involvement, mediastinal biopsies may be needed to establish diagnosis. Central nervous system involvement incidence is higher in patients with mediastinal involvement, and spinal tap with intrathecal administration of chemotherapy should be part of initial workup. Tapping of effusions with cytologic, biochemical, and flow cytometric examination can help to determine disease involvement.

Pathology

Morphologically LBL blasts are indistinguishable from ALL blasts. The cells diffusely infiltrate lymph node effacing the nodal architecture; a "pseudo-follicular" pattern may be seen due to restrictions imposed by tissue planes. A starry-sky pattern caused by the presence of tangible body macrophages can be seen and mitoses are frequent. B-LBL may have pro-B (CD19+, cytoplasmic 79a+, cytoplasmic CD22+, nuclear TdT+), common (CD10+), or pre-B immunophenotype (CD20+, cytoplasmic mu heavy chain+).

Table 15.1 Immunophenotype of adult T-lymphoblastic lymphoma [8, 9]

	T-cell phenotype			Frequency (%)	Prognosis
CD marker	Early	Cortical or thymic	Mature		
CD1a	–	+	–	20	Poor
cCD3	+	+	+	60	Good
sCD3	–	±	+	20	Poor

In Marks et al. [9] both lack of CD1a expression and presence of CD13 expression were associated with poor outcome

They also commonly express CD24 and PAX5. The presence of surface immunoglobulin does not rule out B-LBL.

T-ALL/LBL cells are usually positive for TdT, CD7, and cytoplasmic CD3. Aberrant expression of myeloid antigens like CD13 or 33 is not uncommon [6]. Cytoplasmic CD3 (cCD3) in the absence of surface CD3 (sCd3) is a fairly specific finding for T-LBL/ALL. The distinction between cytoplasmic and surface CD3 should be made by flow cytometry and not by immunohistochemistry. CD1a, when present, also favors the diagnosis of T-LBL/ALL. The European Group for the Immunologic Classification of Leukemia (EGIL) [7] divides T-ALL/LBL in to four groups: pro(cCD3+, CD7+, CD2−, CD5−, CD8−, sCD3−,CD1a−), pre (cCD3+, CD7+, CD2+ and/or CD5+ and/or CD8+, sCD3−, CD1a−), cortical/thymic (CD1a+), and mature (sCD3+, CD1a−). From a prognostic point of view, this can be simplified in to three groups: early – sCD3−/CD1a−, cortical or thymic – sCD3−/CD1a+, and mature – sCD3+/CD1a− [8], with the cortical or thymic group having better prognosis (Table 15.1). A recent report from the UKALLXII/ECOG 2993 study also confirmed the better prognosis in adult T-ALL with CD1a+ blasts [9]. In this study CD13 positivity in T-ALL was associated with poor outcome.

Karyotypic Abnormalities

Specific karyotypic abnormalities beyond that in B-ALL have not been described for B-LBL. The literature describing karyotype abnormalities in B-LBL is scant. Similarly, karyotypic abnormalities specific to T-LBL have not been adequately studied and most large reports include patients with T-ALL. On the other hand, as extent of bone marrow involvement may vary in T-LBL, cytogenetic studies from bone marrow samples may not be representative. This may be reflected in the fact that in a small cohort of 13 pediatric patients, cytogenetic abnormalities were detected in 85 % [10], while a report from MD Anderson Cancer Center (MDACC) involving 33 patients (karyotyping done in 73 % of patients) reported cytogenetic abnormalities in only two patients [11] and these two patients did not carry a karyotypic abnormality typical of T-ALL/LBL. In the pediatric report, translocations at 14q11.2 likely involving the T-cell receptor alpha/delta locus (TCR A/D) occurred in 4 (31 %). In another report including 50 adult patients [12] (33 T-ALL and 17 T-LBL), no significant differences were found between the two groups for the frequency of translocations involving 14q11-13, 7q32-36, or 7p15, where T-cell receptor alpha and delta, beta, and gamma subunit genes reside. On the basis of karyotype, patients could be classified into three groups: group A, 14q11, 7q32-36, or 7p15 translocations; group B, other translocations, and/or deletions; and group C, diploid.

T(9;17)(q34;q23) abnormality, however, appears to occur exclusively in LBL, perhaps pointing to the existence of subsets of LBLs that are distinct from T-ALL [12]. A myeloproliferative disorder, now collectively termed 8p11 myeloproliferative disorder, is associated with eosinophilia, T-LBL, development of AML, and cytogenetic abnormalities including t(8;13)(p11-12;q11-12), t(8;9)(p11;q32-34), or t(6;8)(q27;p12) [13]. In the t(8;13) abnormality, fibroblastic growth factor receptor 1 (FGFR1) is fused with a zinc-finger gene, ZNF198 (also called FIM), while the t(6;8) translocation results in the fusion of FGFR1 and FOP [14].

Immunoglobulin heavy chain (IgH) as well as T-cell receptor gene rearrangements can occur in LBL irrespective of the immunophenotype [15, 16]. This feature can be used to monitor MRD by QPCR after therapy, and ideally QPCR tests for at least two gene rearrangements should be included in the MRD monitoring as clonal rearrangements may change or disappear at relapse or on therapy [17].

Molecular Pathogenesis

Translocations or deletions in T-ALL/LBL typically result in the juxtaposition of oncogenic transcription factors next to strong regulatory elements related to TCR β[beta] (TCRB) or α[alpha]–δ[delta] (TCRAD) genes. Such T-ALL-specific transcription factors include basic helix-loop-helix (bHLH) family members (TAL1 [18, 19], TAL2 [20], LYL1 [21], BHLB1 [22] etc.), LIM-only domain factors (LMO1 [23, 24], LMO2 [25, 26]), and homeobox genes (TLX1/HOX11 [27, 28], TLX3/HOX11L2 [29], HOXA [30, 31]), MYC [32, 33] and MYB [34], TAN1 [35], etc. Non-TCR-associated chromosomal abnormalities also can activate some of the same oncogenes [36–38]. T–ALL/LBL is a multistep process and involves multiple genetic events beyond these translocations. Losses of p16/INK4A [39, 40] and p14/ARF [39] tumor suppressor genes are the most common genetic events in T-ALL. In addition inactivation of tumor suppressors PTEN [41] and NF1 [42] and activation of genes driving proliferation like NRAS [43], LCK [44], and JAK1 [45] contribute to development of T-ALL. Activating mutations in NOTCH1 are encountered in over 50 % of T-ALL [46], which provides a potential therapeutic target.

NOTCH1 Signaling

NOTCH1 is a class 1 membrane receptor that is activated by delta-like and jagged ligands (reviewed in [47]). The extracellular subunit of NOTCH1 (N_{EC}) contains a negative regulatory region (NRR) composed of three Lin12/NOTCH repeats (LNR). The LNR domains fold over the heterodimerization (HD) domain and prevent spontaneous activation of NOTCH1 receptor in the absence of ligand. Upon ligation of the ligand, a conformational change in the NRR region allows the cleavage of the HD domain by ADAM10 and ADAM17 metalloproteases. This is followed by a second proteolytic cleavage in the transmembrane region of NOTCH1 catalyzed by γ-secretase. This releases the intracellular domain of NOTCH1, allowing its translocation to the nucleus. In the nucleus it binds to the RBPJ-CSL DNA-binding protein leading to recruitment of the mastermind family of coactivators and p300. Eventually this leads to gene expression. RNA polymerase II holoenzyme forms a complex with NOTCH1-RBPJ-CSL-Mastermind-like transcriptional complex to trigger phosphorylation of NOTCH1 at the PEST domain. This phosphorylation targets NOTCH1 for degradation by the FBXW7/SCF ubiquitin ligase complex and proteasomal pathway.

NOTCH1 activation in T-ALL can occur through two mechanisms. One is through loss of inhibitory regulation exerted by NRR and the other through disruption of its proteasomal degradation. Loss of inhibitory regulation by NRR leads to ligand-independent activation or constitutive proteolysis of NOTCH1. Mutations in the c-terminal PEST domain of NOTCH1 lead to deletion of sequences that are necessary for targeting NOTCH1 for proteasomal degradation. The FBXW7/SCF ubiquitin ligase complex degrades NOTCH1 and mutations in arginine residues in FBXW7 that recognize phosphorylation sites on NOTCH1 also lead to impaired proteasomal degradation of NOTCH1. Dual mutations in the HD and PEST domains of NOTCH1 or coexistence of a HD domain mutation in NOTCH1 with a mutation in FBXW7 is present in approximately 20 % of patients with T-ALL.

MYC oncogene is a direct downstream target of NOTCH1. NOTCH1-MYC activation leads to increased expression of genes implicated in anabolic growth, ribosomal biogenesis, protein translation, and nucleic acid and amino acid metabolism. NOTCH1-MYC also upregulates the PI3K-AKT-

mTOR pathway. The activation of PI3K-AKT-mTOR pathway downstream of NOTCH1 is essential for T-cell development. NOTCH1 can also increase expression of the transcriptional downregulator HES1 that inhibits PTEN. PTEN is a negative regulator of PI3K and downregulation of its expression by HES1 leads to activation of the PI3K pathway. Activation of HES1 by NOTCH1 signaling also leads to suppression of CYLD, a negative IKK regulator, and this in turn activates the NFκ[kappa]B pathway in T-ALL.

Prognostic Factors in Lymphoblastic Lymphoma

Based on immunophenotype, B-LBL may have better outcome than T-LBL [3, 48] but this has not been uniformly reported [11, 49]. GMALL studies in adult T-ALL have identified early and mature immunophenotypes to be associated with poor outcomes [8] (Table 15.1) and advocated a risk-adapted approach to therapy. CD1a positivity and absence of CD13 expression were associated with better outcomes in the UKALLXII/ECOG 2993 study [9]. Immunophenotypic risk stratification has not been specifically reported for T-LBL.

No specific cytogenetic abnormality has been linked to prognosis except that t(9;17)(q34;q23), translocation restricted to patients with LBL, is associated with a poor prognosis [50]. In adults, early-stage disease, younger age (<30 or <40 years), low LDH levels, the absence of a leukemic phase at diagnosis, and, in particular, the attainment of CR have been associated with a good prognosis [51, 52]. Similarly, advanced stage disease, CNS disease at presentation, and bone marrow involvement have been associated with a poor prognosis [1, 53]. With more effective therapy attainment of CR has been the most important determinant of outcome [11, 54]. The size of the mediastinal mass and response to steroids used in the prophase have also been shown to be strong prognostic factors [55]. While MRD at various time points after therapy has been linked to poor outcome in adult ALL, [56–60] systematic assessment of the role of MRD in prognosis in LBL has not been carried out.

Treatment of Adult Lymphoblastic Lymphoma

Treatment of adult LL has evolved by drawing from the pediatric experience. Approaches that have contributed towards improving treatment results include adoption of ALL-like therapy, CNS and extramedullary disease directed treatment, and maintenance therapy. The role of radiation in CNS prophylaxis and control of mediastinal disease is still evolving.

While outcomes with radiotherapy alone were dismal in the pediatric population, in 1971 Aur et al. [61] reported improved outcomes with the combination of chemotherapy and radiotherapy. Non-randomized comparison of ALL-like LSA2-L2 regimen indicated that DFS and OS were clearly superior with LSAL2 therapy [62]. The patients treated on the LSA2-L2 protocol had a DFS of 73 % at a median follow-up time of 70+ months [63]. Randomized comparison of a four-drug regimen of COMP and a ten-drug regimen of LSA2-L2 demonstrated the superiority of the LSA2-L2 regimen in children with LL (2-year failure-free survival rate, 76 % vs. 26 %, respectively; $P = 0.0002$) [64]. Long-term follow-up confirmed that patients treated on the LSA2-L2 regimen had a better EFS than those treated with COMP (5-year EFS of 64 % vs. 35 % for LSA2-L2 and COMP, respectively) [65]. CNS prophylaxis was limited to intrathecal methotrexate and sites of bulky disease were radiated in both arms. While treatment durations were similar between COMP and LSA2-L2 arms, anthracycline, asparaginase, cytarabine, thioguanine, hydroxyurea, and carmustine were incorporated in the LSA2-L2 regimen. The Pediatric Oncology Group compared results of a six-drug A-COP+ (Adriamycin, vincristine, prednisone, cyclophosphamide, methotrexate, and hydrocortisone) regimen to LSA2-L2 regimen in pediatric patients with LL. The A-COP arm included cranial radiation as well as mediastinal radiation, while the LSA2-L2 arm provided radiation to mediastinal

tumor only. Though this study confirmed the effectiveness of the LSA2-L2 regimen, 3-year survival and disease-free survival were not significantly different, (62 % vs. 72 % and 53 % vs. 58 %, respectively, for A-COP+ and LSA2-L2) [66]. While the lack of difference may be due to sample size, incorporation of effective therapies like anthracycline might have contributed positively to the A-COP+ regimen.

The LSA2-L2 regimen was modified in the LMT81 regimen to add ten courses of high-dose systemic methotrexate (in addition to intrathecal methotrexate) to improve CNS prophylaxis [67]. Radiation was limited to patients with initial testicular or CNS involvement and with residual mediastinal mass. The CR rate was 96 % and only one among 77 patients without CNS involvement at presentation had isolated CNS relapse indicating the effectiveness of CNS prophylaxis with systemic and intrathecal methotrexate. Three-year EFS and OS were 75 and 76 %, respectively. Magrath et al. [68] also reported effective CNS prophylaxis and 3-year OS of 81 % with a regimen that combined CHOP-like therapy with high-dose methotrexate infusion followed by leucovorin rescue. Finally the Berlin-Frankfurt-Munster (BFM) group [69] reported a 5-year EFS of 90 % among children with T-LL with a regimen of eight-drug induction over 9 weeks followed by an 8-week consolidation that included methotrexate 5 g/m^2 × 4. Patients with stage III/IV disease (the majority of patients) received an additional 7-week reinduction/intensification and cranial radiotherapy. The 8-week consolidation containing high-dose methotrexate was termed extra-compartment M phase and was directed to better control extramedullary disease. Similar to the BFM report, among 119 patients with T-LL enrolled in a Children's Leukemia Group trial and treated with a regimen based on the BFM regimen, the EFS at 6 years was 77.5 % and OS was 81 % [55]. Cranial irradiation was omitted even for patients with CNS involvement at diagnosis. These reports indicate that ALL-type chemotherapies improve outcome of LL among children and young adults and may reduce/eliminate the need for mediastinal and cranial radiation.

Initial approaches to treatment of adult LL were based on NHL-like therapies. Voakes et al. [70] reported a CR rate of 53 % in a cohort of 32 patients mostly treated with a CHOP-like regimen. CNS relapse was frequent among patients who did not receive CNS prophylaxis. The addition of agents like asparaginase and high-dose methotrexate improved upon results that can be achieved with CHOP-like regimen. The Stanford/North California Oncology Group (Stanford/NCOG) reported an overall response rate of 100 % (95 % CR) and 3-year actuarial freedom from relapse (FFR) of 56 % in a cohort of 44 adults with LL with a regimen that added asparaginase to CHOP-like induction, included maintenance with methotrexate and 6-mercaptopurine, and incorporated CNS prophylaxis [53] (Table 15.2). Within this cohort, patients who received early CNS prophylaxis with IT methotrexate and cranial radiation had a CNS relapse rate of 3 % versus 30 % in the group that received later CNS prophylaxis with IT methotrexate and high-dose methotrexate. The LSA2-L2 regimen designed to treat childhood lymphoma was modified to treat 15 adult patients with T-LL (median age 25 year, range 16–73) [71]. CNS prophylaxis and mediastinal radiation were included and the maintenance phase continued for a total treatment period of 3 years. Eleven patients (73 %) achieved CR and median survival of complete responders was in excess of 71 months. The improved outcomes with more aggressive NHL-like or ALL-like regimens have been confirmed in reports from various groups [49, 51, 72]. Hoelzer et al. [54] reported a 90 % CR rate among 45 patients with T-LBL treated on one of two German Multicenter Study Group for Adult Acute Lymphoblastic Leukemia (GMALL) protocols designed for ALL with OS and DFS at 7 years of 51 and 62 %, respectively. Prophylactic cranial radiation and mediastinal radiation (24 Gy) were included but there was no extended maintenance beyond 1 year of therapy. Despite inclusion of prophylactic mediastinal radiation, most relapses occurred in the mediastinum. In contrast to the pediatric BFM regimen [69], these adult GMALL studies did not include high-dose methotrexate "extra-compartment M" therapy. The MD Anderson group treated 33

Table 15.2 Treatment outcome in adult patients with lymphoblastic lymphoma without stem cell transplant in first remission

Author	No. of patients	Age, median Years (range)	Chemotherapy	XRT	IT	CR	DFS (median or % at no. of years)	OS
Levine	15	25 (16–73)	Modified LSA2-L2	Mediastinum, cranial	Yes	73	21 months Median	28.3 months
Coleman	44		Cy, Dox, VCR,Pred,L-asp	Cranial	Yes	95	56 % at 3 years	56 % at 3 years
Morel	Total = 80[a]	34				82	53 % at 2 years	30 months
	21		CHOP > COP	±Cranial	±	71		
	30		LNH-84	No	Yes	83		
	22		FRALLE	Cranial	Yes	91		
	7		LALA	Cranial	Yes	86		
Colgan	39	26 (18–62)	CHOP + L-asp > 6-TG + cytarabine	Cranial	Yes	79	49 % at 6 years	≈50 % at 6 years
Hoelzer	45	25 (15–65)	GMALL 04/89 GMALL 05/93	Cranial, mediastinal	Yes	93	60 % at 41 months	51 % at 7 years
Thomas	33		Hyper-CVAD	Mediastinal	yes	91	66 % at 3 years	70 % at 3 years

[a]Five patients underwent SCT in first CR

CHOP cyclophosphamide, vincristine, doxorubicin, prednisone, *COP* cyclophosphamide, vincristine, prednisone, *Cy* cyclophosphamide, *Dox* doxorubicin, *VCR* vincristine, *Pred* prednisone, *L-asp* L-asparaginase, *6-TG* 6-thioguanine, *CR* complete remission, *XRT* radiation, *IT* intrathecal, *DFS* disease-free survival, *OS* overall survival, *Hyper-CVAD* cyclophosphamide, vincristine, doxorubicin, dexamethasone alternating with methotrexate and cytarabine, *LSA2-L2* Induction: cyclophosphamide, vincristine, prednisone, doxorubicin; Consolidation: cytarabine, 6-TG, L-asp, CCNU, *FRALLE* Induction: cyclophosphamide, daunorubicin, prednisolone, asparaginase; Consolidation: risk based, *LNH-84* Induction: cyclophosphamide, doxorubicin, vindesine, bleomycin, prednisolone; Consolidation: methotrexate, ifosfamide, etoposide, asparaginase, cytarabine, *LALA* Induction: cyclophosphamide, prednisolone, vincristine, daunorubicin?; Consolidation: daunorubicin, cytarabine, and asparaginase, *GMALL 04/89 and 5/93* Induction: daunorubicin, L-asparaginase, vincristine, prednisone, cyclophosphamide, cytarabine, 6-mercaptopurine; Consolidation: high-dose cytarabine, mitoxantrone, methotrexate, L-asparaginase, 6-mercaptopurine, etoposide, cyclophosphamide

patients LBL (80 % T-LBL, 70 % stage III/IV) with Hyper-CVAD or the modified Hyper-CVAD regimen(fractionated cyclophosphamide, vincristine, Adriamycin, and dexamethasone alternating with methotrexate and cytarabine) along with IT chemotherapy for CNS prophylaxis and mediastinal radiation to patients with mediastinal disease at presentation [11]. The CR rate was 91 % and the estimated 3-year PFS and OS rates were 66 and 70 %, respectively. Slower achievement of CR was not associated with worse outcome. Recent updates of results confirm the effectiveness of the Hyper-CVAD regimen or its variants in patients with LBL [73]. Thus, ALL-like regimens have improved CR rates and survival outcomes among adult patients with LBL as in pediatric patients.

Intensification of induction/consolidation chemotherapy of LBL is expected to improve results. Incorporation of asparaginase, repeated cycles of systemic methotrexate, and nucleoside analogs like nelarabine may be expected to improve response and survival among patients with LBL provided regimen related toxicities do not limit escalation of therapy.

Autologous Stem Cell Transplantation

Autologous stem cell transplant (auto-SCT) has been pursued as part of induction/consolidation chemotherapy or as salvage for relapsed disease. A retrospective analysis of 214 patients with LBL who underwent auto-SCT and registered in the Lymphoma registry of the European Group for Bone Marrow Transplantation (EBMT) showed that patients transplanted in first CR had a 6-year actuarial overall survival of 63 % compared with 15 % for those with resistant disease at the time of transplantation and 31 % for transplantation in second CR [74]. About half the patients included in this report received auto-SCT in first CR. Though the survival data from this study appears to be better compared to historical data with chemotherapy for patients beyond first CR, the role of auto-SCT at first CR could not be confirmed. The EBMT and United Kingdom Lymphoma Group undertook a randomized trial of auto-SCT versus conventional-dose consolidation and maintenance chemotherapy as postremission therapy in adults with lymphoblastic lymphoma in first CR [75] (Table 15.3). One hundred nineteen patients were entered onto this prospective randomized trial; 98 patients were eligible for randomization and only 65 were randomized. The use of auto-SCT in adults with lymphoblastic lymphoma in first remission produced a trend for improved relapse-free survival ($P = .065$) but did not improve overall survival ($P = .71$) compared with conventional-dose therapy.

The T-LBL/ALL-GOELAL02 study randomized patients with T-LBL to reinduction chemotherapy or intensified conditioning followed by auto-SCT, after an induction regimen of the type used for ALL [76]. While good-risk patients were randomized, patients with poor-risk disease (bone marrow involvement and age over 35 years old or leukocytosis $>30 \times 10^9$/L or failure to achieve marrow remission after one induction course)received a second induction course and auto-SCT. No differences in OS were observed between good-risk and high-risk groups; among the good-risk group, no differences in OS were noted between chemotherapy and auto-SCT.

Allogeneic Stem Cell Transplantation

A retrospective review of 62 patients (30 underwent SCT, auto-SCT = 18, allo-SCT = 12) treated with ALL or NHL-like regimens suggested that patients undergoing allogeneic stem cell transplantation (allo-SCT) had a trend to better OS [77]. Levine et al. [78] carried out a retrospective review of 204 patients who underwent autologous (auto, $n = 128$) or HLA-identical sibling (allo, $n = 76$) SC transplantations from 1989 to 1998 and were reported to the International Bone Marrow Transplant Registry (IBMTR) or Autologous Blood and Marrow Transplant Registry (ABMTR). According to this analysis, allo-SCT did not provide any survival benefit even though relapses were less frequent in the all-SCT group. As reported in other studies, SCT in first CR was associated with better survival.

Table 15.3 Treatment outcome in adult patients with lymphoblastic lymphoma in studies incorporating stem cell transplant

				DFS	OS
Author	No. of patients (allo/auto/total)	Randomized (yes/no) comparators	CR	*P* value	*P* value
Bouabdallah	(12/18/62)	No SCT vs. No SCT	74 %	SCT vs. No SCT 66 % vs. 33 % at 5 years *P* = .01	SCT vs. No SCT 66 % vs. 33 % at 5 years *P* = .09
Sweetenham	(0/31/65)	Auto SCT vs. CC	62 %	SCT vs. No SCT 55 % vs. 24 % at 3 years *P* = .065	SCT vs. No SCT 57 % vs. 53 % at 2 years *P* = .71
Levine	(128/76/204)	No Auto vs. allo-SCT	NA	Auto vs. allo 39 % vs. 36 % at 5 years P = .82	Auto vs. allo 44 % vs. 39 % at 5years *P* = .47
Hunault	(10/0/27)	Auto vs. CC	CR patients only	64 % at 7 year for all patients Auto vs. CC *P* = not significant	69 % at 7 year for all patients Auto vs. CC *P* = not significant

allo allogeneic stem cell transplant, *auto* autologous stem cell transplant, *SCT* stem cell transplant, *DFS* disease-free survival, *OS* verall survival, *CR* complete remission, *CC* consolidation chemotherapy

In summary, the role of auto or allo-SCT in patients with LBL in first CR is not defined. Patients with high-risk disease may benefit from SCT in first CR, but this needs to be confirmed. Among patients with refractory or relapsed disease, SCT may offer better outcome than conventional chemotherapy alone.

Radiation Therapy

Cranial Radiation

The non-Hodgkin's Lymphoma-Berlin-Frankfurt-Munster (NHL-BFM) 95 trial tested the need for prophylactic cranial radiation therapy (PCRT) by excluding PCRT among patients with CNS-negative stage III/IV LBL who respond well to induction therapy [79]. The historical control group was comprised of the patients enrolled in combined trials NHL-BFM90 and NHL-BFM86; both trials included PCRT and treatment regimens were identical to NHL-BFM 95 except for the amount of l-asparaginase and daunorubicin during induction. Among patients with stage III/IV LBL and good response to induction, exclusion of PCRT did not result in inferior DFS.

Among trials in adult patients incorporating ALL-like regimens [11, 54], CNS prophylaxis in the GMALL trial included PCRT and IT chemotherapy, while the MD Anderson Cancer Center trial included IT chemotherapy alone. Isolated CNS relapse rates were 2–3 % in these trials. It appears that early institution of CNS prophylaxis with IT chemotherapy may be sufficient particularly if an ALL-like therapy is pursued. Incorporation of high-dose methotrexate and cytarabine in induction/consolidation regimen also potentially obviates the need for PCRT.

Mediastinal Radiation

Mediastinal radiation is used as part of LBL therapy in two settings: all patients presenting with mediastinal disease or patients with residual mass after induction/consolidation. The need for mediastinal radiation among children with LBL is questionable. Among children treated with an ALL-like BFM regimen [69], mediastinal relapse was only 7 % despite the fact that mediastinal radiation was not administered, even to patients with mediastinal involvement at presentation. Residual mediastinal masses that were resected were all necrotic. The use of high-dose methotrexate as part of "extra-compartment M phase" therapy as used in pediatric BFM regimens, repeated doses of anthracycline, and use of cyclophosphamide in reinduction therapy for stage III/IV disease likely contributed to the low incidence of mediastinal relapse.

Among adults, the mediastinum is one of the most frequent sites of relapse. Both in the GMALL study [54] and the MD Anderson study [11], approximately 10–15 % of patients relapsed in the mediastinum (about 50 % of all relapses) despite the use of mediastinal radiation as part of initial therapy. A summary of the MD Anderson experience among patients treated with Hyper-CVAD or a CVAD regimen suggested benefit from using 26–39 Gy of mediastinal radiation [80]. The use of high-dose methotrexate, particularly at the doses used in the pediatric protocols, is uncommon in adults due to potential nephrotoxicity. On the other hand incorporation of asparaginase and nelarabine into induction/consolidation may reduce the incidence of mediastinal relapse.

Minimal Residual Disease

Minimal residual disease (MRD) detection in ALL can be carried out by three methods: (1) multiparameter flow cytometric immunophenotyping, (2) real-time quantitative polymerase chain reaction (RQ-PCR)-based detection of fusion gene transcripts or breakpoints, and (3) RQ-PCR-based detection of clonal immunoglobulin (Ig) and T-cell receptor (TCR) gene rearrangements. With immunophenotyping and RQ-PCR-based analysis of clonal gene rearrangements, MRD detection can be extended to 80–90 % of patients with ALL and with sensitivities approaching (10^{-3}–10^{-4} to 10^{-4}–10^{-5}).

As MRD positivity at end of induction is higher in adults and presence of MRD predicts

for relapse or a high-risk disease [57, 59, 60, 81, 82], it is important to incorporate MRD evaluation in treatment of adult ALL. Prospective MRD monitoring of adult ALL has been carried out in a limited number of clinical trials, but a GMALL trial [58] and a Northern Italy Leukemia Group (NILG) trial [56] clearly show that risk stratification based on MRD is clinically important and may be used for therapeutic decisions.

No MRD evaluation of LBL in adults has been prospectively carried out. Moreover, as some patients with LBL will not have bone marrow or blood involvement, the value of MRD monitoring from bone marrow or blood samples may be questioned. On the other hand among children with T-LBL, flow cytometric immunophenotyping using CD3+/TdT+T-LBL can detect the presence of T-LBL cells in the bone marrow from more than 2/3 of cases including those with stage II/III disease, indicating the presence of disease dissemination at diagnosis [83]. Detection of T-LBL cells in marrow by multiparameter flow cytometry allows for MRD monitoring during therapy.

Salvage Therapy

A Cancer and Leukemia Group B study (CALGB 19801) [84] demonstrated single-agent activity of nelarabine, a purine nucleoside analog, in adult patients with relapsed/refractory T-ALL/LBL (T-ALL = 26 patients, T-LBL = 13 patients). The ORR was 41 % (CR 31 %) and grade 3/4 neurotoxicities were encountered in 18 %. In a larger study of 126 patients [85], the CR rate was 36 % with grade 3/4 neurotoxicity in 7 %. Autologous [74] or allogeneic SCT following successful salvage therapy can produce durable responses. Though front-line therapies are very successful in children, salvage outcomes are quite poor [86, 87] and SCT offers better long-term outcomes in this setting.

Conclusion

The issue of LBL being a separate entity from ALL is debatable. Nevertheless, the use of ALL-like therapy or intensification of NHL-like therapies has improved outcomes in adult LBL. The role of radiation in cranial prophylaxis or mediastinal disease control is still evolving. Addition of nucleoside analogs, monoclonal antibodies, PI3k/Akt inhibitors, drugs targeting NOTCH1 activation, etc. is expected to improve outcome in LBL.

References

1. Nathwani BN, Diamond LW, Winberg CD, et al. Lymphoblastic lymphoma: a clinicopathologic study of 95 patients. Cancer. 1981;48:2347–57.
2. Lin P, Jones D, Dorfman DM, Medeiros LJ. Precursor B-cell lymphoblastic lymphoma: a predominantly extranodal tumor with low propensity for leukemic involvement. Am J Surg Pathol. 2000;24:1480–90.
3. Soslow RA, Baergen RN, Warnke RA. B-lineage lymphoblastic lymphoma is a clinicopathologic entity distinct from other histologically similar aggressive lymphomas with blastic morphology. Cancer. 1999;85:2648–54.
4. Raetz EA, Perkins SL, Bhojwani D, et al. Gene expression profiling reveals intrinsic differences between T-cell acute lymphoblastic leukemia and T-cell lymphoblastic lymphoma. Pediatr Blood Cancer. 2006;47:130–40.
5. Uyttebroeck A, Vanhentenrijk V, Hagemeijer A, et al. Is there a difference in childhood T-cell acute lymphoblastic leukaemia and T-cell lymphoblastic lymphoma? Leuk Lymphoma. 2007;48:1745–54.
6. Wiersma SR, Ortega J, Sobel E, Weinberg KI. Clinical importance of myeloid-antigen expression in acute lymphoblastic leukemia of childhood. N Engl J Med. 1991;324:800–8.
7. Bene MC, Castoldi G, Knapp W, et al. Proposals for the immunological classification of acute leukemias. European Group for the Immunological Characterization of Leukemias (EGIL). Leukemia. 1995;9:1783–6.
8. Hoelzer D, Thiel E, Arnold R, et al. Successful subtype oriented treatment strategies in adult T-All; results of 744 patients treated in three consecutive GMALL studies. Blood. 2009;114:324.
9. Marks DI, Paietta EM, Moorman AV, et al. T-cell acute lymphoblastic leukemia in adults: clinical features, immunophenotype, cytogenetics, and outcome from the large randomized prospective trial (UKALL XII/ECOG 2993). Blood. 2009;114:5136–45.
10. Lones MA, Heerema NA, Le Beau MM, et al. Chromosome abnormalities in advanced stage lymphoblastic lymphoma of children and adolescents: a report from CCG-E08. Cancer Genet Cytogenet. 2007;172:1–11.
11. Thomas DA, O'Brien S, Cortes J, et al. Outcome with the hyper-CVAD regimens in lymphoblastic lymphoma. Blood. 2004;104:1624–30.
12. Kaneko Y, Frizzera G, Shikano T, Kobayashi H, Maseki N, Sakurai M. Chromosomal and immunophenotypic patterns in T cell acute lymphoblastic

leukemia (T ALL) and lymphoblastic lymphoma (LBL). Leukemia. 1989;3:886–92.
13. Macdonald D, Aguiar RC, Mason PJ, Goldman JM, Cross NC. A new myeloproliferative disorder associated with chromosomal translocations involving 8p11: a review. Leukemia. 1995;9:1628–30.
14. Popovici C, Zhang B, Gregoire MJ, et al. The t(6;8)(q27;p11) translocation in a stem cell myeloproliferative disorder fuses a novel gene, FOP, to fibroblast growth factor receptor 1. Blood. 1999;93:1381–9.
15. Kimura N, Takihara Y, Akiyoshi T, et al. Rearrangement of T-cell receptor delta chain gene as a marker of lineage and clonality in T-cell lymphoproliferative disorders. Cancer Res. 1989;49:4488–92.
16. Jacobs JC, Katz RL, Shabb N, el-Naggar A, Ordonez NG, Pugh W. Fine needle aspiration of lymphoblastic lymphoma. A multiparameter diagnostic approach. Acta Cytol. 1992;36:887–94.
17. Szczepanski T, Willemse MJ, Brinkhof B, van Wering ER, van der Burg M, van Dongen JJ. Comparative analysis of Ig and TCR gene rearrangements at diagnosis and at relapse of childhood precursor-B-ALL provides improved strategies for selection of stable PCR targets for monitoring of minimal residual disease. Blood. 2002;99:2315–23.
18. Brown L, Cheng JT, Chen Q, et al. Site-specific recombination of the tal-1 gene is a common occurrence in human T cell leukemia. EMBO J. 1990;9:3343–51.
19. Jonsson OG, Kitchens RL, Baer RJ, Buchanan GR, Smith RG. Rearrangements of the tal-1 locus as clonal markers for T cell acute lymphoblastic leukemia. J Clin Invest. 1991;87:2029–35.
20. Xia Y, Brown L, Yang CY, et al. TAL2, a helix-loop-helix gene activated by the (7;9)(q34;q32) translocation in human T-cell leukemia. Proc Natl Acad Sci U S A. 1991;88:11416–20.
21. Mellentin JD, Smith SD, Cleary ML. lyl-1, a novel gene altered by chromosomal translocation in T cell leukemia, codes for a protein with a helix-loop-helix DNA binding motif. Cell. 1989;58:77–83.
22. Wang J, Jani-Sait SN, Escalon EA, et al. The t(14;21)(q11.2;q22) chromosomal translocation associated with T-cell acute lymphoblastic leukemia activates the BHLHB1 gene. Proc Natl Acad Sci U S A. 2000;97:3497–502.
23. Boehm T, Foroni L, Kaneko Y, Perutz MF, Rabbitts TH. The rhombotin family of cysteine-rich LIM-domain oncogenes: distinct members are involved in T-cell translocations to human chromosomes 11p15 and 11p13. Proc Natl Acad Sci U S A. 1991;88:4367–71.
24. Valge-Archer V, Forster A, Rabbitts TH. The LMO1 and LDB1 proteins interact in human T cell acute leukaemia with the chromosomal translocation t(11;14)(p15;q11). Oncogene. 1998;17:3199–202.
25. Royer-Pokora B, Rogers M, Zhu TH, Schneider S, Loos U, Bolitz U. The TTG-2/RBTN2 T cell oncogene encodes two alternative transcripts from two promoters: the distal promoter is removed by most 11p13 translocations in acute T cell leukaemia's (T-ALL). Oncogene. 1995;10:1353–60.
26. Rabbitts TH, Axelson H, Forster A, et al. Chromosomal translocations and leukaemia: a role for LMO2 in T cell acute leukaemia, in transcription and in erythropoiesis. Leukemia. 1997;11 Suppl 3Suppl 3:271–2.
27. Hatano M, Roberts CW, Minden M, Crist WM, Korsmeyer SJ. Deregulation of a homeobox gene, HOX11, by the t(10;14) in T cell leukemia. Science. 1991;253:79–82.
28. Kennedy MA, Gonzalez-Sarmiento R, Kees UR, et al. HOX11, a homeobox-containing T-cell oncogene on human chromosome 10q24. Proc Natl Acad Sci U S A. 1991;88:8900–4.
29. Hansen-Hagge TE, Schafer M, Kiyoi H, et al. Disruption of the RanBP17/Hox11L2 region by recombination with the TCRdelta locus in acute lymphoblastic leukemias with t(5;14)(q34;q11). Leukemia. 2002;16:2205–12.
30. Soulier J, Clappier E, Cayuela JM, et al. HOXA genes are included in genetic and biologic networks defining human acute T-cell leukemia (T-ALL). Blood. 2005;106:274–86.
31. Speleman F, Cauwelier B, Dastugue N, et al. A new recurrent inversion, inv(7)(p15q34), leads to transcriptional activation of HOXA10 and HOXA11 in a subset of T-cell acute lymphoblastic leukemias. Leukemia. 2005;19:358–66.
32. Erikson J, Finger L, Sun L, et al. Deregulation of c-myc by translocation of the alpha-locus of the T-cell receptor in T-cell leukemias. Science. 1986;232:884–6.
33. Inaba T, Murakami S, Oku N, et al. Translocation between chromosomes 8q24 and 14q11 in T-cell acute lymphoblastic leukemia. Cancer Genet Cytogenet. 1990;49:69–74.
34. Clappier E, Cuccuini W, Kalota A, et al. The C-MYB locus is involved in chromosomal translocation and genomic duplications in human T-cell acute leukemia (T-ALL), the translocation defining a new T-ALL subtype in very young children. Blood. 2007;110:1251–61.
35. Ellisen LW, Bird J, West DC, et al. TAN-1, the human homolog of the Drosophila notch gene, is broken by chromosomal translocations in T lymphoblastic neoplasms. Cell. 1991;66:649–61.
36. Lahortiga I, De Keersmaecker K, Van Vlierberghe P, et al. Duplication of the MYB oncogene in T cell acute lymphoblastic leukemia. Nat Genet. 2007;39:593–5.
37. Van Vlierberghe P, van Grotel M, Beverloo HB, et al. The cryptic chromosomal deletion del(11)(p12p13) as a new activation mechanism of LMO2 in pediatric T-cell acute lymphoblastic leukemia. Blood. 2006;108:3520–9.
38. O'Neil J, Tchinda J, Gutierrez A, et al. Alu elements mediate MYB gene tandem duplication in human T-ALL. J Exp Med. 2007;204:3059–66.
39. Hebert J, Cayuela JM, Berkeley J, Sigaux F. Candidate tumor-suppressor genes MTS1 (p16INK4A) and MTS2 (p15INK4B) display frequent homozygous deletions in primary cells from T- but not from B-cell lineage acute lymphoblastic leukemias [see comments]. Blood. 1994;84:4038–44.
40. Kawamura M, Ohnishi H, Guo SX, et al. Alterations of the p53, p21, p16, p15 and RAS genes in childhood

T-cell acute lymphoblastic leukemia. Leuk Res. 1999;23:115–26.
41. Palomero T, Sulis ML, Cortina M, et al. Mutational loss of PTEN induces resistance to NOTCH1 inhibition in T-cell leukemia. Nat Med. 2007;13:1203–10.
42. Balgobind BV, Van Vlierberghe P, van den Ouweland AM, et al. Leukemia-associated NF1 inactivation in patients with pediatric T-ALL and AML lacking evidence for neurofibromatosis. Blood. 2008;111:4322–8.
43. Bar-Eli M, Ahuja H, Foti A, Cline MJ. N-RAS mutations in T-cell acute lymphocytic leukaemia: analysis by direct sequencing detects a novel mutation. Br J Haematol. 1989;72:36–9.
44. Tycko B, Smith SD, Sklar J. Chromosomal translocations joining LCK and TCRB loci in human T cell leukemia. J Exp Med. 1991;174:867–73.
45. Flex E, Petrangeli V, Stella L, et al. Somatically acquired JAK1 mutations in adult acute lymphoblastic leukemia. J Exp Med. 2008;205:751–8.
46. Weng AP, Ferrando AA, Lee W, et al. Activating mutations of NOTCH1 in human T cell acute lymphoblastic leukemia. Science. 2004;306:269–71.
47. Ferrando AA. The role of NOTCH1 signaling in T-ALL. Hematology. 2009;2009:353–61.
48. Yeh KH, Cheng AL, Su IJ, et al. Prognostic significance of immunophenotypes in adult lymphoblastic lymphomas. Anticancer Res. 1997;17:2269–72.
49. Slater DE, Mertelsmann R, Koziner B, et al. Lymphoblastic lymphoma in adults. J Clin Oncol. 1986;4:57–67.
50. Kaneko Y, Frizzera G, Maseki N, et al. A novel translocation, t(9;17)(q34;q23), in aggressive childhood lymphoblastic lymphoma. Leukemia. 1988;2:745–8.
51. Zinzani PL, Bendandi M, Visani G, et al. Adult lymphoblastic lymphoma: clinical features and prognostic factors in 53 patients. Leuk Lymphoma. 1996;23:577–82.
52. Morel P, Lepage E, Brice P, et al. Prognosis and treatment of lymphoblastic lymphoma in adults: a report on 80 patients. J Clin Oncol. 1992;10:1078–85.
53. Coleman CN, Picozzi Jr VJ, Cox RS, et al. Treatment of lymphoblastic lymphoma in adults. J Clin Oncol. 1986;4:1628–37.
54. Hoelzer D, Gokbuget N, Digel W, et al. Outcome of adult patients with T-lymphoblastic lymphoma treated according to protocols for acute lymphoblastic leukemia. Blood. 2002;99:4379–85.
55. Uyttebroeck A, Suciu S, Laureys G, et al. Treatment of childhood T-cell lymphoblastic lymphoma according to the strategy for acute lymphoblastic leukaemia, without radiotherapy: long term results of the EORTC CLG 58881 trial. Eur J Cancer. 2008;44:840–6.
56. Bassan R, Spinelli O, Oldani E, et al. Improved risk classification for risk-specific therapy based on the molecular study of minimal residual disease (MRD) in adult acute lymphoblastic leukemia (ALL). Blood. 2009;113:4153–62.
57. Brisco J, Hughes E, Neoh SH, et al. Relationship between minimal residual disease and outcome in adult acute lymphoblastic leukemia. Blood. 1996;87: 5251–6.
58. Bruggemann M, Raff T, Flohr T, et al. Clinical significance of minimal residual disease quantification in adult patients with standard-risk acute lymphoblastic leukemia. Blood. 2006;107:1116–23.
59. Mortuza FY, Papaioannou M, Moreira IM, et al. Minimal residual disease tests provide an independent predictor of clinical outcome in adult acute lymphoblastic leukemia. J Clin Oncol. 2002;20:1094–104.
60. Vidriales MB, Perez JJ, Lopez-Berges MC, et al. Minimal residual disease in adolescent (older than 14 years) and adult acute lymphoblastic leukemias: early immunophenotypic evaluation has high clinical value. Blood. 2003;101:4695–700.
61. Aur RJ, Hustu HO, Simone JV, Pratt CB, Pinkel D. Therapy of localized and regional lymphosarcoma of childhood. Cancer. 1971;27:1328–31.
62. Wollner N, Burchenal JH, Lieberman PH, Exelby P, D'Angio G, Murphy ML. Non-Hodgkin's lymphoma in children. A comparative study of two modalities of therapy. Cancer. 1976;37:123–34.
63. Wollner N, Exelby PR, Lieberman PH. Non-Hodgkin's lymphoma in children: a progress report on the original patients treated with the LSA2-L2 protocol. Cancer. 1979;44:1990–9.
64. Anderson JR, Wilson JF, Jenkin DT, et al. Childhood non-Hodgkin's lymphoma. The results of a randomized therapeutic trial comparing a 4-drug regimen (COMP) with a 10-drug regimen (LSA2-L2). N Engl J Med. 1983;308:559–65.
65. Anderson JR, Jenkin RD, Wilson JF, et al. Long-term follow-up of patients treated with COMP or LSA2L2 therapy for childhood non-Hodgkin's lymphoma: a report of CCG-551 from the Childrens Cancer Group. J Clin Oncol. 1993;11:1024–32.
66. Hvizdala EV, Berard C, Callihan T, et al. Lymphoblastic lymphoma in children—a randomized trial comparing LSA2-L2 with the A-COP+ therapeutic regimen: a Pediatric Oncology Group Study. J Clin Oncol. 1988;6:26–33.
67. Patte C, Kalifa C, Flamant F, et al. Results of the LMT81 protocol, a modified LSA2L2 protocol with high dose methotrexate, on 84 children with non-B-cell (lymphoblastic) lymphoma. Med Pediatr Oncol. 1992;20:105–13.
68. Magrath IT, Janus C, Edwards BK, et al. An effective therapy for both undifferentiated (including Burkitt's) lymphomas and lymphoblastic lymphomas in children and young adults. Blood. 1984;63:1102–11.
69. Reiter A, Schrappe M, Ludwig WD, et al. Intensive ALL-type therapy without local radiotherapy provides a 90 % event-free survival for children with T-cell lymphoblastic lymphoma: a BFM group report. Blood. 2000;95:416–21.
70. Voakes JB, Jones SE, McKelvey EM. The chemotherapy of lymphoblastic lymphoma. Blood. 1981;57:186–8.
71. Levine AM, Forman SJ, Meyer PR, et al. Successful therapy of convoluted T-lymphoblastic lymphoma in the adult. Blood. 1983;61:92–8.
72. Bernasconi C, Brusamolino E, Lazzarino M, Morra E, Pagnucco G, Orlandi E. Lymphoblastic lymphoma in

adult patients: clinicopathological features and response to intensive multiagent chemotherapy analogous to that used in acute lymphoblastic leukemia. Ann Oncol. 1990;1:141–6.
73. Thomas DA, Faderl S, O'Brien S, et al. Long-term outcome for De Novo lymphoblastic lymphoma (LL) after frontline therapy with hyper-CVAD regimen and variants. ASH annual meeting abstracts. Blood. 2010;116:2831.
74. Sweetenham JW, Liberti G, Pearce R, Taghipour G, Santini G, Goldstone AH. High-dose therapy and autologous bone marrow transplantation for adult patients with lymphoblastic lymphoma: results of the European Group for Bone Marrow Transplantation. J Clin Oncol. 1994;12:1358–65.
75. Sweetenham JW, Santini G, Qian W, et al. High-dose therapy and autologous stem-cell transplantation versus conventional-dose consolidation/maintenance therapy as postremission therapy for adult patients with lymphoblastic lymphoma: results of a randomized trial of the European Group for Blood and Marrow Transplantation and the United Kingdom Lymphoma Group. J Clin Oncol. 2001;19:2927–36.
76. Hunault M, Truchan-Graczyk M, Caillot D, et al. Outcome of adult T-lymphoblastic lymphoma after acute lymphoblastic leukemia-type treatment: a GOELAMS trial. Haematologica. 2007;92:1623–30.
77. Bouabdallah R, Xerri L, Bardou VJ, et al. Role of induction chemotherapy and bone marrow transplantation in adult lymphoblastic lymphoma: a report on 62 patients from a single center. Ann Oncol. 1998;9:619–25.
78. Levine JE, Harris RE, Loberiza Jr FR, et al. A comparison of allogeneic and autologous bone marrow transplantation for lymphoblastic lymphoma. Blood. 2003;101:2476–82.
79. Burkhardt B, Woessmann W, Zimmermann M, et al. Impact of cranial radiotherapy on central nervous system prophylaxis in children and adolescents with central nervous system-negative stage III or IV lymphoblastic lymphoma. J Clin Oncol. 2006;24:491–9.
80. Dabaja BS, Ha CS, Thomas DA, et al. The role of local radiation therapy for mediastinal disease in adults with T-cell lymphoblastic lymphoma. Cancer. 2002;94:2738–44.
81. Ciudad J, San Miguel JF, Lopez-Berges MC, et al. Prognostic value of immunophenotypic detection of minimal residual disease in acute lymphoblastic leukemia. J Clin Oncol. 1998;16:3774–81.
82. Foroni L, Coyle LA, Papaioannou M, et al. Molecular detection of minimal residual disease in adult and childhood acute lymphoblastic leukaemia reveals differences in treatment response. Leukemia. 1997;11:1732–41.
83. Coustan-Smith E, Sandlund JT, Perkins SL, et al. Minimal disseminated disease in childhood T-cell lymphoblastic lymphoma: a report from the children's oncology group. J Clin Oncol. 2009;27:3533–9.
84. DeAngelo DJ, Yu D, Johnson JL, et al. Nelarabine induces complete remissions in adults with relapsed or refractory T-lineage acute lymphoblastic leukemia or lymphoblastic lymphoma: Cancer and Leukemia Group B study 19801. Blood. 2007;109:5136–42.
85. Gokbuget N, Basara N, Baurmann H, et al. High single drug activity of nelarabine in relapsed T-lymphoblastic leukemia/lymphoma offers curative option with subsequent stem cell transplantation. Blood. 2011;118(13):3504–11.
86. Burkhardt B, Reiter A, Landmann E, et al. Poor outcome for children and adolescents with progressive disease or relapse of lymphoblastic lymphoma: a report from the Berlin–Frankfurt–Muenster Group. J Clin Oncol. 2009;27:3363–9.
87. Mitsui T, Mori T, Fujita N, Inada H, Horibe K, Tsurusawa M. Retrospective analysis of relapsed or primary refractory childhood lymphoblastic lymphoma in Japan. Pediatr Blood Cancer. 2009;52:591–5.

AIDS-Related Lymphoma

16

Nicolas Mounier and Michele Spina

Abstract

Patients infected with human immunodeficiency virus (HIV) are at greater risk of developing non-Hodgkin lymphoma (NHL) than the general population. As highly active antiretroviral therapy became available, the survival of many NHL patients has become comparable to that of HIV-negative patients. In addition, Hodgkin lymphoma (HL) has become one of the most common cancers in this population. HIV-HL is a different entity from HL in HIV-negative subjects with a poorer prognosis that is associated with tumor subtype, Epstein-Barr virus (EBV) infection, and "B" symptoms.

This review considers the prognostic factors and new approaches to the treatment of patients with AIDS-related NHL and HL. Both developments can also be attributed to new treatment strategies, such as the use of effective infusional regimens, rituximab combinations, and also high-dose therapy with autologous stem cell transplantation. Functional imaging such as positron emission tomography and computed tomography (FDG-PET) may help guide treatment strategy and minimize long-term toxicity. However, unresolved issues persist, such as the optimal therapy for patients with Burkitt ARL or central nervous system involvement.

Keywords

AIDS-related lymphoma • Highly active antiretroviral • Therapy • Lymphoma • Hodgkin • Rituximab • Peripheral blood stem cell transplantation • Prognosis

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Introduction

The risk of developing non-Hodgkin lymphoma (NHL) and Hodgkin lymphoma (HL) in human immunodeficiency virus (HIV)-positive individuals has been estimated to be increased about respectively 200-fold and 20-fold compared to

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the general population. Whereas the decline of the incidence of AIDS-related lymphomas (ARL) has been noted since the development of highly active antiretroviral therapy (HAART), the incidence of HL seems to remain relatively stable [1]. The aggressive presentation of HIV-related lymphomas in immunosuppressed patients raises the problem of the best therapeutic attitude: using more aggressive chemotherapies to reach complete remission (CR) without increasing the risk of opportunist infections.

HIV Non-Hodgkin Lymphoma

Epidemiology and Pathology

According to the World Health Organization, ARL are divided into three categories: firstly, lymphomas also occurring in immunocompetent patients, such as diffuse large B-cell lymphoma (DLBCL) (including centroblastic, immunoblastic, and anaplastic variants) and Burkitt's lymphoma (BL); secondly, lymphomas occurring more specifically in HIV-infected patients, such as primary effusion lymphoma (PEL) and plasmablastic lymphoma (PBL); and thirdly, lymphomas also occurring in other immunodeficiency states, such as the polymorphic or posttransplant lymphoproliferative disorders associated with HIV infection like B-cell lymphoma [2].

Guiget et al. investigated the incidence of AIDS-defining cancers by systematically testing 78 models for each cancer [3]. The only model retained for non-Hodgkin lymphoma included current immunodeficiency, current viral replication, and antiretroviral therapy. The relative risk (RR) of non-Hodgkin lymphoma increased as the CD4 cell count fell (RR = 4.9 for CD4 <200 cell/mm^3; RR = 11.6 for CD4 <100 cell/mm^3) and viral replication rose (RR = 1.5 for viral load >10^4; RR = 2.9 for viral load >10^5). HAART during at least 6 months decreased that risk with a RR at 0.8. Coinfection with HCV did not increase the risk of non-Hodgkin lymphoma, and the associations of non-Hodgkin lymphoma with the current CD4 cell count, viral load, and HAART were not modified.

Despite effective treatment of HIV infection, some patients still develop ARL. Gerard et al. analyzed 128 patients with HIV-associated NHL and undetectable plasma HIV-RNA, according to the duration of HIV suppression [4]. They found that NHL occurred mainly within the first 18 months following HIV suppression. In the 83 patients developing NHL after long-term HIV suppression, the level of CD4 cell count was higher (359 vs. 270 cell/mm^3, $p < 0.02$), but the association with EBV and the prognosis were similar to that observed in the remaining patients with recent HIV suppression. Recently, Landgren et al. found that the presence of elevated free light chain (FLC) levels, a marker of polyclonal B-cell activation, is a strong risk factor for ARL [5]. After matching 66 NHL patients with 225 lymphoma-free controls, they showed a dose–response pattern up to 2–5 years before diagnosis (e.g., NHL risk 8.13-fold higher with FLC concentration at least 2.00 times the upper limit of normal compared with normal levels). In contrast, IgG, IgM, and IgA levels were similar in NHL patients and controls.

Prognostic Factors

In the post-HAART era, the median overall survival (OS) increased from 6 months to 4 years, similarly to HIV-negative patients with aggressive lymphoma. From now on, lymphoma-related factors like the achievement of complete remission or a high International Prognostic Index (IPI) score have a stronger impact on survival than factors associated with the underlying HIV infection. Consequently the IPI score (age, disease stage, extranodal involvement, performance status, LDH levels) is the most discriminating negative prognostic factor in patients with ARL, together with Burkitt subtype [6–8].

At the present time, large-scale gene expression profiling (GEP), however, has led to the recognition of new subtypes of DLBCL [9]. Though still retaining the histological description of a neoplasm of large B lymphoid cells with a diffuse growth pattern, DLBCL can now be subdivided into diseases that arise from B cells at

different stages of differentiation with distinctive molecular characteristics, so-called the germinal center B-cell-like (GCB) and activated B-cell-like (ABC) molecular subgroups. Genes associated with GCB DLBCL included known markers of germinal center differentiation such as CD10 and the bcl-6 gene. In contrast, most genes that defined ABC DLBCL were not expressed by normal germinal center B cells, but instead were induced during in vitro activation of peripheral B cells such as cyclin D2 and CD44. These results suggested that GCB DLBCL appears to arise from germinal center B cells, whereas ABC DLBCL likely arises from post-germinal center B cells that are blocked during plasmacytic differentiation. Moreover, Dunleavy et al. recently showed that, among ARL, only the tumor histogenesis was associated with lymphoma-specific outcome with 95 % of germinal center B-cell (GCB) versus 44 % of non-GCB DLBCL progression-free survival (PFS) at 5 years [10].

For lymphomas occurring more specifically in HIV-infected, such as primary effusion lymphoma and plasmablastic lymphoma, data are very scarce.

PEL is a rare high-grade B-cell non-Hodgkin's lymphoma associated with Kaposi sarcoma-associated herpesvirus/human herpesvirus 8 (KSHV/HHV-8) infections. The prognosis is poor, with reported median OS shorter than 1 year. Based on a retrospective series of 28 patients, Boulanger et al. identified two prognostic factors as being independently associated with impaired clinical outcome: poor performance status and the absence of HAART before PEL diagnosis [11].

PBL is a distinct variant of diffuse large B-cell lymphoma (DLBCL). Pathologically, PBL lacks expression of CD20 but, because of its plasmacytic differentiation, expresses plasma cell markers such as CD38, CD138, or MUM1 (multiple myeloma oncogene 1). The clinical course of PBL is characteristically aggressive, with a reported median OS around 1 year. Recent literature review has been provided by Castillo et al. [12]. They reported that advanced stage and failure to achieve remission were independent adverse prognostic factors.

Table 16.1 Chemotherapy combined with rituximab for non-Hodgkin lymphoma

Authors	Chemotherapy	Sample size	CR (%)	2-year OS (%)
Boue et al. [15]	R-CHOP	61	77	75
Ribera et al. [16]	R-CHOP	81	69	56
Kaplan et al. [17]	R-CHOP	99	55	55
Spina et al. [8]	R-CDE	74	70	64
Sparano et al. [18]	R-EPOCH	106	73	70

R-CHOP Rituximab, Cyclophosphamide, Adriamycin, Vincristine, Prednisone, *R-CDE* Rituximab, Cyclophosphamide, Doxorubicine, Etoposide, *R-EPOCH* Rituximab, Etoposide, Prednisolone, Vincristine, Cyclophosphamide, Doxorubicin

Finally, HIV infection has also been associated with an increased risk of developing various types of malignancies, including low-grade lymphomas or aggressive peripheral T-cell lymphomas (PTCL) [12, 13]. However, this is a rare occurrence with no more than a 100 cases reported in the literature. Recently, Castillo et al. reported on 51 PTCL patients: the most common subtypes were PTCL unspecified (61 %) and anaplastic large cell lymphoma (ALCL, 22 %) [12]. None of the ALCL patients tested expressed ALK. The median OS was 12 months. In the multivariate survival analysis, the use of HAART and patients' performance status were independent adverse prognostic factors.

Chemotherapy for DLBCL

In recent years, the introduction of rituximab has significantly improved the survival of people with DLBCL in the general population as compared with patients receiving CHOP alone [14]. Based on these data, several authors have explored the feasibility and effectiveness of rituximab in combination with chemotherapy in patients with ARL (Table 16.1) [8, 15–18]. The first trial took place in France and used rituximab + CHOP in combination to treat 61 patients. A 77 % CR rate was reported. After a median follow-up of 33 months, 2-year OS was 75 % and PFS was 69 % [15].

A phase II study was performed in Spain using the same treatment regimen. Out of 60 patients the following rates were achieved: CR 69 % and 3-year OS 56 % [16]. Severe (grade 3–4) neutropenia is a common complication of rituximab chemotherapy, occurring in 33–78 % of patients [8, 17]. Consequently, the use of G-CSF support and opportunistic infection prophylaxis are mandatory during rituximab-based chemotherapy. Presently, caution in the use of rituximab, especially in patients with CD4 counts <50/μ[mu]L, is advocated [17]. However, the benefit of rituximab for tumor control should not be underestimated [19].

Continuous infusional chemotherapy is an alternative regimen in ARL. Little et al. performed a study with the new continuous infusional "dose-modified" EPOCH (etoposide, prednisolone, vincristine, cyclophosphamide, doxorubicin) regimen to treat good-prognosis patients (median CD4 >200 cell/mm^3) [20]. Results are very satisfactory both in terms of CR (74 %) and 4-year OS (60 %). Recently Sparano et al. showed the increase efficacy of EPOCH combined to rituximab with a CR rate of 73 % and 2-year OS and PFS rates of 70 and 66 %, respectively [18].

The Italian Cooperative Group on AIDS and Tumors (GICAT) performed a study on the administration of rituximab and infusional CDE every 4 weeks for a total of 6 cycles with concomitant HAART. Totally 74 patients were enrolled and 70 % reached CR. Non-opportunistic infections developed in 23 % of the patients during neutropenia; 14 % of the patients were diagnosed with AIDS-defining opportunistic infections during chemotherapy or in the first 3 months after conclusion of the treatment plan. Again, results are very satisfactory in term of 2-year OS (62 %) and PFS (86 %) [8].

Chemotherapy for Other Subtypes of ARL

Burkitt lymphoma (BL) is a full-fledged entity which has been considered as a more aggressive tumor than DLBCL. Galicier et al. evaluated an intensive chemotherapy regimen (LMB86) for 63 patients with St Jude stage IV AIDS-related Burkitt [21]. The estimate 2-year OS and DFS were 47 and 68 %, respectively. Two poor-prognosis factors were identified: low CD4 count (<200 cell/mm^3) and ECOG performance status more than 2. Patients with 0 or 1 factor had good outcome (2-year OS at 60 %) contrasting with patients with 2 factors (2-year OS at 12 %). Consequently some studies performed the feasibility of intensive aggressive chemotherapy regimens (i.e., CODOX-M/IVAC or PETHEMA-LAL3/97), which are usually used in the treatment of BL in the general population [22]. The results of investigations reported a 63–68 % CR rate, a 46–60 % PFS at 2 years, and the same toxicity as in the general population, which confirms the feasibility of the above regimens also in HIV setting [23]. Very recent results with combination of CODOX-M/IVAC to rituximab are encouraging (2-year OS 73 %). In patients not candidate to such intensive chemotherapy, the R-EPOCH seems to be a good alternative [18].

Turning to lymphomas which occurred more specifically in HIV-infected (such as PEL or PBL), no specific treatment regimen has been recommended. Apart from exceptional reports of antiretroviral therapy-induced response, only few patients achieved CR. Impaired clinical condition and severe immunodeficiency enhanced the chemotherapy toxicity and increased the risk of treatment-related mortality. For PEL treatment, some cases of CHOP-induced remission have been reported in patients simultaneously treated with HAART [11]. In addition, several precautions are then required to avoid severe toxicities, especially in a context of severe hypoalbuminemia and abundant effusions. When clinical condition or visceral failures hamper chemotherapy use, interferon alfa might represent an alternative. For PBL the prognosis is strongly associated with achieving a complete clinical response to CHOP or CHOP-like chemotherapy [24]. The role of more intensive regimens is currently unclear. Further research is needed to improve responses using novel therapeutic agents and strategies.

Lymphoma progression is the leading cause of death in 35–55 % of the patients with HIV-NHL receiving chemotherapy, of whom around half

need second-line chemotherapy following progression or relapse of the disease. As ARL patients can benefit from the same first-line treatment than non-HIV, the high-dose chemotherapy followed by autologous stem cell transplantation (ASCT) was also investigated within the relapse/refractory framework [25]. Recently, Diez-Martin et al. performed a retrospective study to compare the survival between HIV-positive and HIV-negative lymphomas for patients who undergo ASCT [26]. They showed similar rates of relapse, OS, and PFS in both cohorts. Consequently, since the HAART era, HIV patients should be considered within the same criteria rate for ASCT as HIV-negative lymphomas.

HIV Hodgkin Lymphoma

Epidemiology and Pathology

The relative risk of HIV patients to develop a HL is higher than that of the general population ranging from 5 to 25-fold, with an increase in incidence of this disease in the post-HAART era [27, 28]. According to Powles et al., HAART is associated with an increased risk of disease (standardized incidence ratios (SIR) 2.67) [29]. This might be explained because the risk of HL peaks when CD4 counts range from 150 to 199 CD4 cells/μ[mu]L, as reported by Biggar et al. [30]. As the overall effect of HAART is to increase the CD4 count level, it paradoxically increases HL incidence, leading to speculate that, with severe immunosuppression, the cellular background surrounding the Reed-Sternberg tumoral cells may be altered. However, this pattern disappeared in a sensitivity analysis censoring follow-up when a serious AIDS-defining event was diagnosed, and then, the relation between CD4 cell count and incidence of Hodgkin's lymphoma was linear. This finding might indicate that serious AIDS-defining events and Hodgkin's lymphoma are competing risks at very low CD4 cell counts [3]. In contrast to HL of the general population, a high frequency of EBV association has been shown in HL (80–100 %) tissues from HIV-infected people [31, 32]. The elevated frequency of EBV association with HIV-HL indicates that EBV probably does represent a relevant factor involved in the pathogenesis of HIV-HL.

The Italian series demonstrated that [33] HIV-infected patients are more likely to present with an unfavorable histologic subtype than non-HIV-infected patients (mixed cellularity (MC) and lymphocyte-depleted (LD) were generally observed in the second peak of incidence in older HIV-negative patients, whereas the nodular sclerosis (NS) subtype predominates in young adults without HIV), "B" symptoms (i.e., fever, night sweats, and/or weight loss more than 10 % of the normal body weight), advanced-stage disease, or extranodal disease. Bone marrow involvement can be found in more than 50 % of patients in certain series and may be the initial feature at diagnosis in 20 % of cases, so that bone marrow biopsy is mandatory [34].

Prognostic Factors

The classical prognostic criteria of the general population, such as stage, bone marrow involvement, bulky disease, B symptoms, and high erythrocyte sedimentation rate, are applicable in the HIV setting [35, 36]. Recently, Spina et al. reanalyzed a European series on 596 patients [37]. In comparison, patients which have never been treated by HAART, patients in HAART before the onset of HL are older, have less extranodal involvement (in particular liver and spleen), less B symptoms, a higher leukocyte, neutrophil count, and hemoglobin level, higher CD4 cell count, and fewer patients with detectable HIV viral load. It demonstrated the positive impact of the use of HAART (hazard ratio (HR) 2.27, $p=0.01$). Moreover, multivariate analysis confirmed that the so-called International Prognostic Score (IPS) (HR 1.57, $p=0.02$) and a number of CD4 cell count higher than 200/μ[mu]L (HR 1.43, $p=0.04$) were predictive for treatment failure. A similar study, carried out within the Spanish group GESIDA, observed that the median OS was not reached in HAART group and was 39 months in no-HAART group ($p=0.0089$); the median disease-free survival (DFS) was not

reached in HAART group and was 85 months in no-HAART group ($p=0.129$). Factors independently associated with CR were a CD4 cell count >100/μ[mu]L and the use of HAART.

In addition, Hoffman et al. reported that the response to HAART is a key point [38]. In fact, whereas the median OS in patients who did not respond to HAART was comparable with those reported in previous cohorts in the pre-HAART era (18.6 months), the median OS in patients that responded to HAART was not reached.

Chemotherapy

Even though HAART combined with conventional chemotherapy regimens has yielded a strong effect on outcome in HIV-HL patients, treatment strategy still remains to be improved in order to increase OS (Table 16.2) [39–42]. The ABVD regimen (i.e., doxorubicin, bleomycin, vinblastine, dacarbazine, considered as the standard therapy for HL in the general population), plus HAART and G-CSF, produced encouraging results in terms of mortality rate (only 10 %), CR (87 %), and relapse (11 %), as reported by Xicoy et al. [39]. However, due to the aggressiveness of HIV-HL, two other regimens, BEACOPP (i.e., bleomycin, etoposide, doxorubicin, cyclophosphamide, vincristine, procarbazine, and prednisone) and Stanford V (i.e., mechlorethamione, doxorubicine, vinblastine, vincristine, bleomycin, etoposide, and prednisone), were developed as alternative to ABVD. In the post-HAART era, they were tested in HIV-HL patients. Stanford V regimen plus G-CSF and concomitant HAART was reported by Spina et al. [40]. Of 59 patients with HIV-HL, 53 had an objective response: 48 (81 %) achieved CR and 5 (8 %) achieved partial remission (PR) and 6 patients (10 %) progressed. With a median follow-up time of 17 months, 33 (56 %) patients were alive and disease-free. Both have similar hematological toxicity (grade 3–4 neutropenia in 78 and 75 % of patients, respectively). BEACOPP regimen with concomitant HAART, reported by Hartman et al., showed that the CR rate was 100 % [41]. Overall, these studies suggest that more intensive regimen may be as effective in HIV-HL as it is in the HIV-negative population with HL, but, due to the treatment-related toxicity, the question remains to de-escalate or to escalate.

Table 16.2 Chemotherapy for Hodgkin lymphoma

Authors	Chemotherapy	Sample size	CR (%)	OS (%)
Spina et al. [40]	Standford V	56	81	51 (3 years)
Hartmann et al. [41]	BEACOPP	12	100	75 (3 years)
Spina et al. [42]	VEBEP	28	75	82 (2 years)
Xicoy et al. [39]	ABVD	52	87	76 (5 years)

Standford V Doxorubicin, Mechloretamine, Etoposide, Vincristine, Bleomycin, Prednisone, *BEACOPP21* "baseline" Cyclophosphamide, Doxorubicin, Etoposide, Procarbazine, Prednisone, Bleomycin, Vincristine, *VEBEP* Epirubicin, Cyclophosphamide, Vinorelbine, Bleomycin, Prednisone, *ABVD* Doxorubicin, Bleomycin, Vinblastine, Dacarbazine

Supportive Therapy and Follow-Up

Interactions Between Antineoplastic and Antiretroviral Therapies

The use of concomitant antineoplastic chemotherapy and HAART has proved to be feasible and effective in patients with HIV-related malignancies; however, some interactions, pharmacokinetic or pharmacodynamic, between HAART and chemotherapy have to be considered. It can involve cumulated toxicity on the same organ (e.g., myelotoxicity such as severe anemia occurring in patients treated by zidovudine, neurotoxicity of stavudine associated with vincristine or vinblastine) or toxicity by increase of the plasmatic rates of cytotoxic. Indeed, many drugs used in HAART regimens have the potential of causing drug interactions as a result of their ability to either inhibit or induce the cytochrome P450 enzyme system. Since many antineoplastic drugs are also metabolized by the CYP system, coadministration with HAART could result in either drug accumulation and possible toxicity or rapid drug metabolism and decreased efficacy. Unfortunately, very limited prospective interaction data are available to

Table 16.3 Antineoplastic agents active in ARL and interaction with antiviral drugs

Anticancer therapy	Primary isoforms that mediate biotransformation	Interaction with NNRTI drugs (CYP inducers)	Interaction with PI drugs (CYP inhibitors)
Alkylating agents			
Cyclophosphamide	3A4,2B6, 2D6	↑	_
Ifosfamide	3A4	↑	↓
Procarbazine	2B	_	↓
Dacarbazine	1A	↑	↓
Mechlorethamine	Chemical transformation	_	_
Anthracyclines			
Doxorubicin	3A4	_	↓
Mitoxantrone	3A4	_	↓
Epipodophyllotoxins			
Etoposide	3A4	↓	↑
Bleomycin	Intracellular aminopeptidase	_	_
Vinca alkaloids			
Vinblastine	3A4	↓	↑
Vincristine	3A4	↓	↑

NNRTI non-nucleoside reverse transcriptase inhibitors, *PI* protease inhibitors, ↑ interaction increases concentration of active metabolite, ↓ interaction decreases concentration of active metabolite; _ potential for interaction appears minimal

safely guide the combined use of HAART and chemotherapy. Table 16.3 lists the potential drug interactions and therapeutic considerations of the antiretroviral agents used to treat ARL and the most common anticancer agents used in the treatment of malignancies found in patients with HIV infection. For complete review, see the paper of Mounier et al. [43].

The issue of timing of the administration of antiretroviral therapy in combination with chemotherapy (concurrent with chemotherapy vs. after treatment) is still unsolved. To avoid possible pharmacokinetic interactions between HAART and chemotherapy, Sparano et al. omitted HAART during the administration of EPOCH regimen fin patients who were never previously treated with antiretroviral agents. However, a particular attention should be paid when deferring HAART therapy in all cases where the patient has a severe immune deficiency and/or the chemotherapy regimen used is very immunosuppressive. Clinicians must be vigilant about implementing infection prophylaxis and promptly recognizing, diagnosing, and treating bacterial, parasitic, fungal, and viral infections that may occur as consequence of therapy. Infection prophylaxis should be instituted in all NHL patients receiving chemotherapy, irrespective of their CD4 counts.

The Role of PET Scanning

Positron emission tomography using [18F]-fluoro-2-deoxy-D-glucose (FDG-PET) is now recognized as an important tool for staging and treatment response assessment in Hodgkin and non-Hodgkin lymphomas. Within the HIV framework, some preliminary reports suggested FDG activity may also correlate with detectable lymphoma [10, 44, 45]. Although initial staging may not alter the treatment plan, it can provide additional information, assess possible involvement of critical location, and help to foresee and to avoid possible further complications. However, experience with PET scanning in the HIV-HL needs to be further studied. A baseline study is strongly mandatory, since early PET interpretation is based on a site-to-site comparison of FDG uptake both before and after chemotherapy.

PET imaging requires cautious reading and pertinent clinical correlation to avoid diagnosing benign disease as malignant, such as hypermetabolic foci seen in lung or esophagus, which

Table 16.4 Criteria for PET interpretation after two cycles of chemotherapy [46]

Negative	
0	No uptake
1	Uptake ≤ mediastinum
2	Uptake > mediastinum but ≤ liver
Positive	
3	Uptake > liver in some sites even if uptake ≤ liver or mediastinum at other sites
4	Uptake > liver in over 90 % of sites or development of new uptake consistent with progressive disease

are common sites of HIV- or chemotherapy-promoted infections. Nodal FDG uptake can be observed in lymphoma, various infections (e.g., Mycobacterium avium-intracellulare, Mycobacterium tuberculosis, Herpes simplex virus), and AIDS-related malignancies such as Kaposi sarcoma. In addition, stimulation of bone marrow following treatment with G-CSF induces a striking increase in FDG uptake in bone marrow. Moreover, to take into account the possibility of minimal residual uptake, a semi-quantitative approach has recently been proposed for interim PET interpretation in the context of an international protocol for advanced-stage HL (Table 16.4) [46].

Finally, PET/CT is useful for an accurate initial staging. On the other hand, we also recommend PET/CT to monitor treatment response, because PET/CT appears to have a prognostic value, since a negative scan always seem associated with a favorable outcome. Significance of residual uptake at sites of disease, however, needs further evaluation (e.g., biopsy). Nevertheless, the use of FDG-PET in the follow-up of HL patients who achieved CR cannot routinely recommend and further studies are warranted prior to any definite conclusion.

Conclusion

HIV lymphoma is a singular entity within lymphomas. The various studies demonstrated that these lymphomas could be treated by the standard protocols used in non-HIV lymphomas (i.e., R-CHOP for non-Hodgkin lymphomas and ABVD for Hodgkin lymphomas) provided that HAART and adequate supportive therapy and anti-infectious prophylaxis are given concomitantly. Improvement in the response to HAART is essential to achieve maximum benefits from the chemotherapy. Finally, due to the aggressiveness of the disease, more effective antineoplastic regimens, such as high-dose chemotherapy with ASCT, should be considered in therapeutic trials to improve the CR rate and OS of these patients.

References

1. Bohlius J, Schmidlin K, Boue F, et al. HIV-1-related Hodgkin lymphoma in the era of combination antiretroviral therapy: incidence and evolution of CD4+ T-cell lymphocytes. Blood. 2011;117(23):6100–8.
2. Jaffe ES, Harris NL, Stein H, Isaacson PG. Classification of lymphoid neoplasms: the microscope as a tool for disease discovery. Blood. 2008;112:4384–99.
3. Guiguet M, Boue F, Cadranel J, Lang JM, Rosenthal E, Costagliola D. Effect of immunodeficiency, HIV viral load, and antiretroviral therapy on the risk of individual malignancies (FHDH-ANRS CO4): a prospective cohort study. Lancet Oncol. 2009;10:1152–9.
4. Gerard L, Meignin V, Galicier L, et al. Characteristics of non-Hodgkin lymphoma arising in HIV-infected patients with suppressed HIV replication. AIDS. 2009;23:2301–8.
5. Landgren O, Goedert JJ, Rabkin CS, et al. Circulating serum free light chains as predictive markers of AIDS-related lymphoma. J Clin Oncol. 2010;28:773–9.
6. Lim ST, Karim R, Nathwani BN, Tulpule A, Espina B, Levine AM. AIDS-related Burkitt's lymphoma versus diffuse large-cell lymphoma in the pre-highly active antiretroviral therapy (HAART) and HAART eras: significant differences in survival with standard chemotherapy. J Clin Oncol. 2005;23:4430–8.
7. Mounier N, Spina M, Gabarre J, et al. AIDS-related non-Hodgkin lymphoma: final analysis of 485 patients treated with risk-adapted intensive chemotherapy. Blood. 2006;107:3832–40.
8. Spina M, Jaeger U, Sparano JA, et al. Rituximab plus infusional cyclophosphamide, doxorubicin, and etoposide in HIV-associated non-Hodgkin lymphoma: pooled results from 3 phase 2 trials. Blood. 2005;105:1891–7.
9. Rosenwald A, Wright G, Chan WC, et al. The use of molecular profiling to predict survival after chemotherapy for diffuse large-B-cell lymphoma. N Engl J Med. 2002;346:1937–47.

10. Dunleavy K, Little RF, Pittaluga S, et al. The role of tumor histogenesis, FDG-PET, and short-course EPOCH with dose-dense rituximab (SC-EPOCH-RR) in HIV-associated diffuse large B-cell lymphoma. Blood. 2010;115:3017–24.
11. Boulanger E, Gerard L, Gabarre J, et al. Prognostic factors and outcome of human herpesvirus 8-associated primary effusion lymphoma in patients with AIDS. J Clin Oncol. 2005;23:4372–80.
12. Castillo JJ, Beltran BE, Bibas M, et al. Prognostic factors in patients with HIV-associated peripheral T-cell lymphoma: a multicenter study. Am J Hematol. 2011; 86:256–61.
13. Girard T, Luquet-Besson I, Baran-Marszak F, Raphael M, Boue F. HIV+ MALT lymphoma remission induced by highly active antiretroviral therapy alone. Eur J Haematol. 2005;74:70–2.
14. Coiffier B, Lepage E, Briere J, et al. CHOP chemotherapy plus rituximab compared with CHOP alone in elderly patients with diffuse large-B-cell lymphoma. N Engl J Med. 2002;346:235–42.
15. Boue F, Gabarre J, Gisselbrecht C, et al. Phase II trial of CHOP plus rituximab in patients with HIV-associated non-Hodgkin's lymphoma. J Clin Oncol. 2006;24:4123–8.
16. Ribera JM, Oriol A, Morgades M, et al. Safety and efficacy of cyclophosphamide, adriamycin, vincristine, prednisone and rituximab in patients with human immunodeficiency virus-associated diffuse large B-cell lymphoma: results of a phase II trial. Br J Haematol. 2008;140:411–9.
17. Kaplan LD, Lee JY, Ambinder RF, et al. Rituximab does not improve clinical outcome in a randomized phase 3 trial of CHOP with or without rituximab in patients with HIV-associated non-Hodgkin lymphoma: AIDS-Malignancies Consortium Trial 010. Blood. 2005;106:1538–43.
18. Sparano JA, Lee JY, Kaplan LD, et al. Rituximab plus concurrent infusional EPOCH chemotherapy is highly effective in HIV-associated B-cell non-Hodgkin lymphoma. Blood. 2010;115:3008–16.
19. Mounier N, Spina M, Gisselbrecht C. Modern management of non-Hodgkin lymphoma in HIV-infected patients. Br J Haematol. 2007;136:685–98.
20. Little RF, Pittaluga S, Grant N, et al. Highly effective treatment of acquired immunodeficiency syndrome-related lymphoma with dose-adjusted EPOCH: impact of antiretroviral therapy suspension and tumor biology. Blood. 2003;101:4653–9.
21. Galicier L, Fieschi C, Borie R, et al. Intensive chemotherapy regimen (LMB86) for St Jude stage IV AIDS-related Burkitt lymphoma/leukemia: a prospective study. Blood. 2007;110:2846–54.
22. Montoto S, Wilson J, Shaw K, et al. Excellent immunological recovery following CODOX-M/IVAC, an effective intensive chemotherapy for HIV-associated Burkitt's lymphoma. AIDS. 2010;24:851–6.
23. Oriol A, Ribera JM, Bergua J, et al. High-dose chemotherapy and immunotherapy in adult Burkitt lymphoma: comparison of results in human immunodeficiency virus-infected and noninfected patients. Cancer. 2008; 113:117–25.
24. Castillo JJ, Winer ES, Stachurski D, et al. Clinical and pathological differences between human immunodeficiency virus-positive and human immunodeficiency virus-negative patients with plasmablastic lymphoma. Leuk Lymphoma. 2010;51:2047–53.
25. Re A, Cattaneo C, Michieli M, et al. High-dose therapy and autologous peripheral-blood stem-cell transplantation as salvage treatment for HIV-associated lymphoma in patients receiving highly active antiretroviral therapy. J Clin Oncol. 2003;21:4423–7.
26. Diez-Martin JL, Balsalobre P, Re A, et al. Comparable survival between HIV+ and HIV- non-Hodgkin and Hodgkin lymphoma patients undergoing autologous peripheral blood stem cell transplantation. Blood. 2009;113:6011–4.
27. Herida M, Mary-Krause M, Kaphan R, et al. Incidence of non-AIDS-defining cancers before and during the highly active antiretroviral therapy era in a cohort of human immunodeficiency virus-infected patients. J Clin Oncol. 2003;21:3447–53.
28. Engels EA, Pfeiffer RM, Goedert JJ, et al. Trends in cancer risk among people with AIDS in the United States 1980–2002. AIDS. 2006;20:1645–54.
29. Powles T, Imami N, Nelson M, Gazzard BG, Bower M. Effects of combination chemotherapy and highly active antiretroviral therapy on immune parameters in HIV-1 associated lymphoma. AIDS. 2002;16:531–6.
30. Biggar RJ, Jaffe ES, Goedert JJ, Chaturvedi A, Pfeiffer R, Engels EA. Hodgkin lymphoma and immunodeficiency in persons with HIV/AIDS. Blood. 2006; 108:3786–91.
31. Carbone A, Gloghini A, Larocca LM, et al. Human immunodeficiency virus-associated Hodgkin's disease derives from post-germinal center B cells. Blood. 1999;93:2319–26.
32. Rezk SA, Weiss LM. Epstein-Barr virus-associated lymphoproliferative disorders. Hum Pathol. 2007;38: 1293–304.
33. Vaccher E, Spina M, Tirelli U. Clinical aspects and management of Hodgkin's disease and other tumours in HIV-infected individuals. Eur J Cancer. 2001;37: 1306–15.
34. Spina M, Berretta M, Tirelli U. Hodgkin's disease in HIV. Hematol Oncol Clin North Am. 2003;17: 843–58.
35. Gisselbrecht C, Mounier N, Andre M, et al. How to define intermediate stage in Hodgkin's lymphoma? Eur J Haematol Suppl. 2005;66:111–4.
36. Hasenclever D, Diehl V. A prognostic score for advanced Hodgkin's disease. International prognostic factors project on advanced Hodgkin's disease. N Engl J Med. 1998;339:1506–14.
37. Spina M, Ribera J, Gabarre J, et al. Hodgkin's disease and HIV infection: prognostic factors in 596 patients within the European group for the study of HIV and tumours (GECAT). 8th international symposium on

Hodgkin Lymphoma. Cologne 23–26 Oct 2010. Hematologica. 2010;95:23C060.
38. Hoffmann C, Chow KU, Wolf E, et al. Strong impact of highly active antiretroviral therapy on survival in patients with human immunodeficiency virus-associated Hodgkin's disease. Br J Haematol. 2004;125: 455–62.
39. Xicoy B, Ribera JM, Miralles P, et al. Results of treatment with doxorubicin, bleomycin, vinblastine and dacarbazine and highly active antiretroviral therapy in advanced stage, human immunodeficiency virus-related Hodgkin's lymphoma. Haematologica. 2007;92: 191–8.
40. Spina M, Gabarre J, Rossi G, et al. Stanford V regimen and concomitant HAART in 59 patients with Hodgkin disease and HIV infection. Blood. 2002;100: 1984–8.
41. Hartmann P, Rehwald U, Salzberger B, et al. BEACOPP therapeutic regimen for patients with Hodgkin's disease and HIV infection. Ann Oncol. 2003;14:1562–9.
42. Spina M, Rossi G, Antinori A, et al. VEBEP regimen and highly active antiretroviral therapy (HAART) in patients (pts) with HD and HIV infection (HD-HIV) Abst 227. Ann Oncol. 2008;19:152.
43. Mounier N, Katlama C, Costagliola D, Chichmanian RM, Spano JP. Drug interactions between antineoplastic and antiretroviral therapies: implications and management for clinical practice. Crit Rev Oncol Hematol. 2009;72:10–20.
44. Just PA, Fieschi C, Baillet G, Galicier L, Oksenhendler E, Moretti JL. 18F-fluorodeoxyglucose positron emission tomography/computed tomography in AIDS-related Burkitt lymphoma. AIDS Patient Care STDS. 2008;22:695–700.
45. Goshen E, Davidson T, Avigdor A, Zwas TS, Levy I. PET/CT in the evaluation of lymphoma in patients with HIV-1 with suppressed viral loads. Clin Nucl Med. 2008;33:610–4.
46. Meignan M, Gallamini A, Haioun C. Report on the first international workshop on interim-PET scan in lymphoma. Leuk Lymphoma. 2009;50:1–4.

Posttransplant Lymphomas

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Abstract

PTLDs are a heterogeneous group of diseases ranging from reactive hyperplasia to malignant lymphoma that occur in patients after solid organ transplantation (SOT) and hematopoietic stem cell transplantation (HSCT). PTLD can occur anywhere from weeks to decades after transplantation. Most are Epstein-Barr virus (EBV)-positive. In at least some instances, EBV gene expression appears to drive the lymphoproliferation in an environment of decreased cell-mediated immunity. Treatment may include reducing or changing immunosuppression, rituximab, adoptive T-cell therapy, or cytotoxic chemotherapy. Judicious selection of immunosuppressive agents, early intervention with rituximab, or utilization of EBV-targeted therapies is a promising prevention strategy.

Keywords

Posttransplantation lymphoproliferative disorders (PTLDs) • Epstein-Barr virus (EBV) • Immune suppression • Solid organ transplantation (SOT) • Hematopoietic stem cell transplantation (HSCT)

Introduction

The incidence of PTLD is approximately 1 % but varies widely depending on the type of transplant, the type of immunosuppression, and host factors [1–3]. PTLD is more common in pediatric than adult SOT recipients, which mirrors the increased risk of PTLD in EBV-seronegative transplant recipients. The highest incidences are seen in small bowel transplant recipients, followed by dual-organ, lung, and heart SOT patients, with renal transplant patients having the lowest rates of PTLD [1]. In HSCT, the incidence varies as a function of the kinds of graft manipulation and

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graft-versus-host disease (GVHD) prophylaxis undertaken.

Most PTLDs are of B-cell origin [1]. Greater than 75 % of the tumors that arise within the first year posttransplantation are EBV positive, while the PTLDs occurring many years after transplantation are typically EBV negative [4]. Extranodal involvement occurs in 70–90 % of patients and one third will have involvement of the transplanted organ [1, 5–7]. A broad spectrum of viral latency genes is often expressed including those that drive proliferation. Present evidence suggests that lymphomagenesis reflects a failure of cellular immune responses against the virus-infected cells, at least in part as a consequence of immunosuppressive drugs [8–11]. The occurrence of EBV-negative PTLD suggests that other less well-characterized factors also contribute to PTLD pathogenesis [12, 13].

The World Health Organization (WHO) has divided PTLDs pathologically into the four subcategories: early lesions, polymorphic PTLD, monomorphic PTLD, and classical Hodgkin lymphoma-type PTLD [14]. Indolent lymphomas such as follicular lymphoma are not classified as PTLD tumors.

Primary central nervous system PTLD (PCNS-PTLD) accounted for approximately 10 % of PTLD in early reports [15] but is likely <1 % based on more recent reports [16]. Over 90 % of PCNS-PTLDs are EBV positive [17] and they tend to present with parenchymal rather than leptomeningeal involvement [16].

PTLD Subtypes

Early lesions, including plasmacytic hyperplasia and infectious mononucleosis-like PTLD, are seen within weeks to months of transplantation and present as a mononucleosis-like illness with lymphadenopathy or oropharyngeal involvement, typically in EBV-seronegative patients who received organs from EBV-seropositive donors [9]. These lesions have preserved architecture with little cellular atypia. They are polyclonal, lack cytogenetic abnormalities, and are always EBV-associated [18].

Polymorphic PTLD tumors are comprised of a heterogeneous population of cells, including plasma cells, immunoblasts, and histiocytes. Cellular atypia with a post-germinal center phenotype is seen [19]. Clonal immunoglobulin gene rearrangements and EBV association are usual; cytogenetic abnormalities are present in 20 % of polymorphic tumors [12, 20].

Monomorphic PTLD tumors are comprised of a homogenous, monoclonal population of lymphocytes and are further classified based on the pathological criteria for non-Hodgkin lymphomas (NHL). Most commonly, monomorphic PTLDs resemble diffuse large B-cell lymphoma, although lesions meeting pathological criteria for Burkitt lymphoma, plasma cell myeloma, or plasmacytoma can also occur [20]. Cytogenetic abnormalities are seen in over 70 % of monomorphic tumors and genetic changes associated with aberrant somatic hypermutation are also common [12, 13, 18]. Most EBV-negative PTLDs are of the monomorphic subtype. NK/T-cell lesions are rare and are always monomorphic, monoclonal, and of late onset [21]. Hodgkin lymphoma-type PTLD typically occurs many years after transplantation, exhibits the standard features of classic Hodgkin lymphoma, and is nearly always EBV positive [20].

Pathogenesis

Early and polymorphic lesions typically develop within days to months of transplantation, while monomorphic lymphoma and particularly Hodgkin lymphoma to develop late, occurring months to years after transplantation. PTLDs occurring within months of transplantation are almost always EBV positive, while late lesions are more variably EBV-associated [7, 19]. EBV negative comprises 20–40 % of all PTLDs, with most of these occurring years after transplantation [12, 22]. The majority of NK/T-cell PTLDs are EBV negative, although 20–30 % are EBV-associated [21, 23]. The pathogenesis of EBV-negative PTLD is very poorly understood. Some have suggested that unidentified viruses may play a role, while others believe that chronic inflammation and immune dysregulation are likely to explain the pathogenesis.

Risk Factors for the Development of PTLD

In SOT, one of the greatest risk factors for developing PTLD is EBV seronegativity of the recipient at the time of transplantation, as these patients are at risk for developing primary EBV infection while immunosuppressed posttransplantation [24]. The incidence of PTLD is higher in pediatric transplantation patients likely for this reason. EBV-seronegative recipients of organs from EBV-seropositive donors are at the highest risk [6, 25]. After SOT, tumors predominantly arise in host B lymphocytes.

In HSCT patients, the risk factors for developing PTLD are somewhat different. The type of graft manipulation and the approach to GVHD prophylaxis are both important factors, highlighting the role of T-cell depletion in PTLD pathogenesis [26]. HSCT patients that receive unmanipulated marrow or stem cells from HLA-matched related donors have rates of PTLD of 1 % or less [9], while patients with T-cell-depleted grafts have rates of PTLD 15-fold higher. Of note, patients who receive grafts that undergo nonselective T- and B-cell depletion have a risk of PTLD similar to unmanipulated grafts [27–29]. The use of anti-thymocyte globulin (ATG), either for GVHD prophylaxis or treatment, is also associated with increased risk for PTLD in HSCT patients [27]. PTLD is typically an early complication of HSCT due to the unopposed donor B-cell proliferation in a T-cell-depleted environment and arises in donor B lymphocytes [9]. However, late PTLD arising in patients with chronic GVHD is well described [29].

Immunomodulatory drugs play a key role in PTLD pathogenesis. With the introduction of the calcineurin inhibitor cyclosporine, the incidence of PTLD in SOT recipients increased [30]. With therapeutic drug level monitoring and implementation of low-dose cyclosporine protocols, the rates of PTLD fell [31]. The immunosuppressive effects of tacrolimus are quite similar to those of cyclosporine and whether one agent is more likely to lead to PTLD remains controversial [2, 6, 32]. Comparison of regimens that do or do not include MMF has not identified any clear change in risk of PTLD [33].

In contrast to the immunosuppressive agents above, the effects of rapamycin are much less well defined. The mammalian target of rapamycin (mTOR) pathway is important in lymphocyte proliferation in a variety of settings, including EBV-associated tumors [34]. Rapamycin and other mTOR inhibitors have antineoplastic activity in several tumors, including Hodgkin and NHL, in clinical trials. It is worth noting that Kaposi's sarcoma (KS) in the posttransplantation setting resembles PTLD in many regards. Similar to PTLD, KS is associated with a gammaherpesvirus (Kaposi's sarcoma herpesvirus also known as HHV-8) and will sometimes regress in response to reduction in immunosuppression. In a provocative report from Italy, 15 patients with posttransplantation KS had their immunosuppression changed from a calcineurin inhibitor-based regimen to rapamycin and, in every case, the tumor regressed [35]. The use of mTOR inhibitors in PTLD patients remains an area of active research and interest.

Monoclonal antibodies that selectively deplete T cells are associated with increased risk of PTLD. These include muromonab-CD3 (OKT3), ATG, and others [36, 37]. In contrast, alemtuzumab is associated with a much lower risk of PTLD or none at all, perhaps because it depletes B cells in parallel with T cells [38].

Evaluation

The evaluation of a transplant patient with new lymphadenopathy or mass should include a prompt biopsy. When the tissue involved is the transplanted organ, PTLD must be histologically differentiated from graft rejection. EBER in situ hybridization and CD20 staining should be performed on tissue specimens. The patient's immunosuppressive regimen, performance status, and organ function should be assessed. Positive EBV viral load assays by PCR may increase the index of suspicion for PTLD, but are often difficult to interpret and should not replace diagnostic biopsy. With the diagnosis of PTLD, PET/CT of the chest, abdomen, and pelvis should be performed, as well as brain imaging (CT or MRI), diagnostic lumbar puncture, and bone marrow biopsy in some cases.

Prognostic Factors

The prognosis for patients with PTLD has steadily improved, with overall survival approximating 70 % in some series [4, 39]. As with most cancers, poor performance status predicts inferior outcomes [40], as does advanced stage disease and higher International Prognosis Index scores [4, 6, 41]. CNS involvement consistently portends inferior survival [16, 40–42]. T-cell PTLDs tend to have poorer outcomes compared to those of B-cell origin [40].

EBV Monitoring

EBV establishes lifelong infection in resting memory B cells. With sensitive PCR techniques, viral DNA is readily detected in the lymphocytes of most healthy seropositive people. There is considerable interindividual variation in the copy numbers of viral DNA in individual infected cells and variation in the frequency of infected cells. In immunocompromised populations, there are often higher proportions of infected cells and, in some cases, increased copy numbers of viral DNA per cell [43]. Therapies such as rituximab often eliminate most circulating memory B cells, including EBV-infected B cells. Following such therapy, the viral copy number in peripheral blood mononuclear cells (PBMCs) may therefore not be valuable for monitoring PTLD. Certainly, PTLD may progress in patients with no detectable EBV DNA in PBMCs.

Monitoring viral DNA in plasma is an alternative, although one must understand the sources of plasma viral DNA. In EBV-associated Hodgkin lymphoma (HL) and in nasopharyngeal carcinoma (NPC), EBV copy number in plasma has proven useful as a tumor marker. The approach works well in NPC and is promising in HL because the viral DNA detected seems to be mainly tumor-derived [44, 45]. In contrast, in immunocompromised patients, the EBV DNA detected in plasma is commonly virion-packaged. While virion DNA may reflect immunocompromise, it does not necessarily reflect PTLD tumor burden. In fact, patients can have high EBV copy number in plasma without any EBV-associated tumor [46]. A common misconception is that high EBV copy number in plasma indicates a role for acyclovir or ganciclovir. When the DNA detected is tumor-derived rather than virion-packaged, these antiviral nucleoside analogues are not expected to impact copy number in any direct way. Plasma EBV copy number can be a function of tumor cells releasing cellular DNA in association with apoptosis and does not reflect the viral DNA polymerase-driven synthesis that is targeted by these agents.

Monitoring any of the measures of EBV DNA may be useful in raising the index of suspicion for PTLD. Such measurements in transplant patients may be useful as triggers for adjustment in immunomodulatory drugs, preemptive treatment with rituximab, or initiation of T-cell therapy (discussed below). The particulars of the laboratory test being used (PBMCs, plasma, whole blood) should be considered in interpreting the results and cytotoxic chemotherapy should never be initiated solely on the basis of these measurements.

Treatment

Treatment paradigms for PTLD need to consider both the risk of graft rejection and its consequences, as well as the pace of tumor progression. Many have suggested a stepwise risk-stratified treatment approach if the biology and location of the PTLD allows the time to do so [5, 47, 48].

In general, the treatment of PTLD is focused on several maneuvers, including reduced immunosuppression (RI), rituximab, EBV-specific cytotoxic T-cell therapy, and systemic chemotherapy. There are select cases where surgery or involved field radiation have been successful. Table 17.1 outlines interventions that have been previously tried but that have little utility or unnecessary associated risks based on our current understanding of PTLD. Table 17.2 discusses innovative strategies in PTLD treatment and the possible biologic factors behind their efficacy.

Whereas there may be a rationale for antiviral agents such as ganciclovir in the prevention of

Table 17.1 Interventions that may not be justified

Intervention	Arguments against	Associated drawbacks
CMV IVIG	Only anecdotal evidence. Randomized trials have failed to show benefit. May delay primary infection in EBV-seronegative recipients or reduce CMV-related immune dysfunction	Renal impairment Risk of hematologic complications – DVT, hemolysis Risk of aseptic meningitis High cost Limited supply Volume load Infusion reactions
Reduced immunosuppression		Graft rejection GVHD (HSCT) Some immunosuppressants have antitumor activity (steroids, mTOR inhibitors, cyclophosphamide)
Ganciclovir	Requires EBV lytic viral gene expression (not active in most PTLD tumor cells). Only relevant to EBV+ tumors	Marrow suppression
Reduction of steroids	Steroids may be beneficial due to lympholytic activity. Steroids are components of most lymphoma chemotherapy regimens. No evidence that steroids are the component of immunosuppression that leads to PTLD	Risk of organ rejection Adrenal insufficiency
Surgery	Ineffective for systemic disease Patients with localized disease may do better for reasons other than the surgery itself	Surgery-related morbidity Lesions may be inoperable or result in significant loss of organ function Loss of allograft (nephrectomy)

PTLD [25], there is no established role in its treatment. In vitro, EBV-tumor cell lines are not inhibited in their growth by ganciclovir, and as a sole agent, ganciclovir is not active in the treatment of EBV-associated malignancy.

Reduced Immunosuppression (RI)

The decision to reduce immunosuppression, as well as the degree to which to do so, must be balanced against the risk of graft rejection, as well as the risk of GVHD in HSCT. Aggressive PTLD tumors can progress in the days to weeks it takes for RI to have any effect. Early lesions or polyclonal tumors occurring shortly after transplantation may respond to RI alone, but PTLDs occurring years after transplantation rarely respond to this approach [9].

Some series report complete remission rates with RI alone approaching 75 % [49], while others report that such successes are rare [5, 6, 17, 47]. It is clear that RI is effective in some circumstances, such as with early PTLD. However, RI carries with it the risk of graft rejection, which greatly impacts which patients are candidates for this approach. In making decisions about RI as a therapeutic maneuver, we believe it is worthwhile to consider immunosuppressive agents individually.

There are some immunosuppressive agents for which there is little or no evidence that tapering or withdrawal is associated with tumor regression. Steroids, sirolimus, and cyclophosphamide are in this category. Steroids and cyclophosphamide are components of most chemotherapy regimens used in PTLD and are clearly active in a broad range of B-cell malignancies. If these agents are already part of the patient's immunosuppressive regimen, there is little rationale for tapering them for PTLD management. Sirolimus is not a "standard" anti-lymphoma agent, but closely related drugs are now being studied for the treatment of lymphoma. Thus, we would also be reluctant to taper sirolimus.

Table 17.2 Interventions that have biologic rationale

Intervention	Biologic rationale and potential benefits
Combination chemotherapy	Cytotoxic therapy effective against clonally proliferating tumor cells
	Systemic treatment for a systemic disease
	Can be used in EBV-positive and EBV-negative PTLDs
Rituximab	Targets B cells, the reservoir for latent EBV. May improve ratio of EBV-specific cytotoxic T cell to immortalized, proliferating EBV+ B cells
	Known efficacy in other CD20+ B-cell malignancies
	No direct threat to transplanted organ
	Minimal risk of infection or marrow suppression
	Readily available
	Generally well tolerated
	Can be used in EBV-positive and EBV-negative PTLDs
Use of mTOR inhibitors	Can serve as immunosuppressant to prevent allograft rejection
	Has antiproliferative properties that may target PTLD – mTOR pathways are activated in PTLD tumors.
	Can be used in EBV-positive and EBV-negative PTLDs
	Efficacy as treatment for PTLD remains speculative
EBV-specific cytotoxic T cells	Addresses the immune dysregulation (lack of T-cell control of EBV+ B-cell proliferation) that lies at the root of PTLD pathogenesis
	Targeted therapy may limit need for other interventions with greater toxicities
	No threat to transplanted organ

Rather, in patients whose immunosuppressive regimens do not include sirolimus, we would consider substituting the calcineurin inhibitor for sirolimus [50]. For renal transplant patients without history of PTLD, a randomized controlled trial demonstrated that a change from a calcineurin inhibitor-based regimen to a sirolimus-based regimen was associated with a lower rate of malignancy at 2 years [51].

In contrast, the withdrawal of agents such as calcineurin inhibitors, methotrexate, mycophenolate mofetil, and azathioprine has been associated with PTLD tumor regression in retrospective reports. Tapering or stopping these agents should be considered, first weighing the risks of graft rejection and estimating the likelihood of response to RI.

Role of Surgery and Involved Field Radiation Therapy (IFRT)

For localized PTLD, successes have been seen with RI combined with IFRT or surgical resection [5, 6, 52]. In renal transplant patients, nephrectomy, and thus withdrawal of immunosuppression, has been curative, although this is not a decision made lightly [53]. For PCNS-PTLD, cranial radiation has been standard; experience with high-dose methotrexate, rituximab, or other chemotherapy is limited albeit encouraging [16, 17, 41]. IFRT and RI may be of utility in patients with lesions such as localized, plasmacytoma-like PTLD [54].

Rituximab

The advent of rituximab has changed the approach to PTLD, since most are CD20+ B-cell malignancies. Rituximab spares some PTLD patients from more toxic systemic chemotherapy and may allow immunosuppressive drugs that prevent graft rejection to be continued [48]. Rituximab is well tolerated, although there are concerns about hepatitis B and C reactivation and decreased B-cell-mediated immune function. Prospective studies have shown the first-line use of weekly rituximab with RI to be effective in obtaining complete remissions in 30–60 % of PTLD patients [39, 55, 56]. There is some evidence that EBV-negative tumors occurring late after transplantation are less likely to respond to rituximab [56], as well as evidence that

EBV positivity in the tumor predicts rituximab response [57]. Given the role of T-cell depletion and B-cell proliferation in PTLD pathogenesis, selectively targeting the B-cell compartment with rituximab and altering the T- to B-cell ratio makes biologic sense. Rituximab has likely contributed to the decrease in morbidity and mortality that has been seen in PTLD over recent years.

Combination Chemotherapy

Chemotherapy-related toxicities are of concern when choosing regimens for transplant patients with PTLD. Hematologic toxicities, such as neutropenia, can result in serious complications in this population that is already quite vulnerable to infection. The potential toxicities to the transplanted organ are also factors to be considered when choosing a chemotherapy regimen. For example, there is some evidence that allografted hearts may be more sensitive to anthracyclines than native hearts, showing signs of cardiac toxicity well below the generally accepted threshold [52]. However, it is difficult to parse out the degree to which continuing the patient's immunosuppressive regimen, or even a fraction of the regimen, contributes to the infectious, hematologic, and end-organ complications seen when chemotherapy is used in PTLD.

Several chemotherapy regimens have been effective in obtaining complete remissions in PTLD patients. ProMACE/CytaBOM has been used owing to its low anthracycline dose, particularly for cardiac transplantation patients or those with reduced cardiac ejection fractions [47, 52]. R-CHOP has proven to be effective in large series of PTLD patients [6]. Gentler regimens such as low doses of cyclophosphamide and prednisone have been successful in pediatric PTLD patients [58].

Donor Lymphocyte Infusion (DLI)

For allogeneic HSCT recipients with PTLD, DLI has been used with success, even in aggressive subtypes [59]. DLI is now most typically used in combination with rituximab [60]. The major complication of DLI is GVHD.

EBV-Specific T-Lymphocyte Infusion

Donor-derived EBV-specific T cells are effective in preventing PTLD in high-risk HSCT settings and are often effective in treating PTLD [61]. In SOT patients, autologous EBV-specific cytotoxic T cells have been used similarly, although continued immunosuppression to maintain solid organ grafts has limited the long-term persistence and expansion of these T cells. EBV-specific cytotoxic T-cell lines engineered to be resistant to calcineurin inhibitors are being investigated [62, 63]. Because over 90 % of the population has been exposed to EBV and mounted an immune response to the virus, healthy blood donors are a readily available source of EBV-specific T cells. These healthy donor EBV-specific T cells have been successfully used in SOT patients who developed PTLD, with HLA matching for donor selection [64]. Methods are being optimized to eliminate delays and reduce costs associated with the production of such cell lines, as well as to tailor the products to the tumor being treated [65]. We anticipate that such approaches will be more widely available in the near future.

HDAC Inhibitors and Antiviral Therapies

In a phase I/2 study, the combination of arginine butyrate, a HDAC inhibitor, and ganciclovir in patients with EBV-positive tumors, including patients with PTLD, led to regression of these tumors, which had been resistant to other therapies [66]. Whether arginine butyrate was functioning to upregulate viral kinases and sensitize tumor cells to ganciclovir or was exerting a direct anti-lymphoma effect is not clear. The role of HDAC inhibitors in PTLD treatment remains to be determined.

Prevention

Because EBV-seronegative patients who receive organs from EBV-seropositive donors are at the highest risk for PTLD, it would be attractive to select EBV-negative organ donors. However,

given the ubiquitous nature of EBV, this is not practical. An alternative approach is to vaccinate the seronegative recipient. Vaccines consisting of the epitopes of several immunodominant viral latency proteins ("polytope") appear promising but have not yet been clinically vetted. Also of interest are innovative approaches to transplantation, such as the coupling of SOT with HSCT from the same donor to reduce the need for long-term immunosuppression, which might dramatically change the incidence of PTLD overall [67].

Optimization of immunosuppressive regimens may prevent PTLD. Shortening the duration of immunosuppression may also reduce the incidence of PTLD. HLA-matched related donor allogeneic HSCT recipients can receive high-dose cyclophosphamide treatment at days 3 and 4 after transplant as their sole immunosuppression, thus decreasing the duration of posttransplantation immunosuppression from months to days [68].

Many centers have monitored blood EBV DNA levels in patients after transplantation in attempts to better define which patients are at high risk for developing PTLD [69–71]. When faced with rising EBV DNA levels, some centers reduce the patient's immunosuppression and initiate antiviral therapy [70, 71]. Preemptive rituximab infusions in the setting of rising EBV DNA levels have also been studied in HSCT patients, with promising results [72, 73]. Other centers administer EBV-specific cytotoxic T cells in the setting of rising EBV viral loads, which appear to be remarkably effective in HSCT recipients [74].

The role for antiviral prophylaxis with acyclovir, ganciclovir, or related antiviral nucleoside analogues in preventing EBV-associated PTLD is controversial. The argument in favor of antiviral prophylaxis presumes that by eliminating the production of infectious virions and thus new rounds of infection, it is possible to reduce the incidence of PTLD. The proposition has never been prospectively tested but has biologic plausibility, particularly in seronegative SOT recipients, and is supported by retrospective data [25].

Future Directions

Many unanswered questions related to PTLD remain, with current research attempting to address many of these topics. More sophisticated approaches to EBV monitoring that distinguish virion DNA from infected cell DNA may prove beneficial as a guide to therapy. However, the optimal utilization to EBV monitoring (prophylaxis, vs. "preemptive" therapy based on rising EBV viral load in blood, vs. therapy for established tumor) remains to be defined. EBV-specific T-cell infusions are clearly effective in HSCT patients and sometimes in SOT patients, although this approach does not yet have a clear place in PTLD treatment algorithms. Still undefined but promising is the role of mTOR inhibitors as antiproliferative agents, both in PTLD prevention and treatment. Similarly, HDAC inhibitors may be important for direct antitumor properties or as inducers of viral enzymes that render these EBV-associated tumors susceptible to antiviral nucleosides such as ganciclovir.

References

1. Domingo-Domenech E, de Sanjose S, Gonzalez-Barca E, et al. Post-transplant lymphomas: a 20-year epidemiologic, clinical and pathologic study in a single center. Haematologica. 2001;86(7):715–21.
2. Caillard S, Dharnidharka V, Agodoa L, Bohen E, Abbott K. Posttransplant lymphoproliferative disorders after renal transplantation in the United States in era of modern immunosuppression. Transplantation. 2005;80(9):1233–43.
3. Opelz G, Henderson R. Incidence of non-Hodgkin lymphoma in kidney and heart transplant recipients. Lancet. 1993;342(8886–8887):1514–6.
4. Evens AM, David KA, Helenowski I, et al. Multicenter analysis of 80 solid organ transplantation recipients with post-transplantation lymphoproliferative disease: outcomes and prognostic factors in the modern era. J Clin Oncol. 2010;28(6):1038–46.
5. Davis CL, Wood BL, Sabath DE, Joseph JS, Stehman-Breen C, Broudy VC. Interferon-alpha treatment of posttransplant lymphoproliferative disorder in recipients of solid organ transplants. Transplantation. 1998;66(12):1770–9.
6. Knight JS, Tsodikov A, Cibrik DM, Ross CW, Kaminski MS, Blayney DW. Lymphoma after solid organ transplantation: risk, response to therapy, and survival at a transplantation center. J Clin Oncol. 2009;27(20):3354–62.

7. Ghobrial IM, Habermann TM, Macon WR, et al. Differences between early and late posttransplant lymphoproliferative disorders in solid organ transplant patients: are they two different diseases? Transplantation. 2005;79(2):244–7.
8. Tanner JE, Alfieri C. The Epstein-Barr virus and post-transplant lymphoproliferative disease: interplay of immunosuppression, EBV, and the immune system in disease pathogenesis. Transpl Infect Dis. 2001;3(2):60–9.
9. Hopwood P, Crawford DH. The role of EBV in post-transplant malignancies: a review. J Clin Pathol. 2000; 53(4):248–54.
10. Krams SM, Martinez OM. Epstein-Barr virus, rapamycin, and host immune responses. Curr Opin Organ Transplant. 2008;13(6):563–8.
11. Murray PG, Swinnen LJ, Constandinou CM, et al. BCL-2 but not its Epstein-Barr virus-encoded homologue, BHRF1, is commonly expressed in posttransplantation lymphoproliferative disorders. Blood. 1996;87(2):706–11.
12. Vakiani E, Basso K, Klein U, et al. Genetic and phenotypic analysis of B-cell post-transplant lymphoproliferative disorders provides insights into disease biology. Hematol Oncol. 2008;26(4):199–211.
13. Capello D, Gaidano G. Post-transplant lymphoproliferative disorders: role of viral infection, genetic lesions and antigen stimulation in the pathogenesis of the disease. Mediterr J Hematol Infect Dis. 2009;1(2):e2009018.
14. Campo E, Swerdlow SH, Harris NL, Pileri S, Stein H, Jaffe ES. The 2008 WHO classification of lymphoid neoplasms and beyond: evolving concepts and practical applications. Blood. 2011;117(19):5019–32.
15. Penn I, Porat G. Central nervous system lymphomas in organ allograft recipients. Transplantation. 1995;59(2):240–4.
16. Cavaliere R, Petroni G, Lopes MB, Schiff D. Primary central nervous system post-transplantation lymphoproliferative disorder: an International Primary Central Nervous System Lymphoma Collaborative Group Report. Cancer. 2010;116(4):863–70.
17. Snanoudj R, Durrbach A, Leblond V, et al. Primary brain lymphomas after kidney transplantation: presentation and outcome. Transplantation. 2003;76(6): 930–7.
18. Knowles DM, Cesarman E, Chadburn A, et al. Correlative morphologic and molecular genetic analysis demonstrates three distinct categories of posttransplantation lymphoproliferative disorders. Blood. 1995;85(2):552–65.
19. Capello D, Rossi D, Gaidano G. Post-transplant lymphoproliferative disorders: molecular basis of disease histogenesis and pathogenesis. Hematol Oncol. 2005;23(2):61–7.
20. Evens AM, Roy R, Sterrenberg D, Moll MZ, Chadburn A, Gordon LI. Post-transplantation lymphoproliferative disorders: diagnosis, prognosis, and current approaches to therapy. Curr Oncol Rep. 2010;12(6):383–94.
21. Swerdlow SH. T-cell and NK-cell posttransplantation lymphoproliferative disorders. Am J Clin Pathol. 2007;127(6):887–95.
22. Nelson BP, Nalesnik MA, Bahler DW, Locker J, Fung JJ, Swerdlow SH. Epstein-Barr virus-negative post-transplant lymphoproliferative disorders: a distinct entity? Am J Surg Pathol. 2000;24(3):375–85.
23. Sivaraman P, Lye WC. Epstein-Barr virus-associated T-cell lymphoma in solid organ transplant recipients. Biomed Pharmacother. 2001;55(7):366–8.
24. Cockfield SM. Identifying the patient at risk for post-transplant lymphoproliferative disorder. Transpl Infect Dis. 2001;3(2):70–8.
25. Funch DP, Walker AM, Schneider G, Ziyadeh NJ, Pescovitz MD. Ganciclovir and acyclovir reduce the risk of post-transplant lymphoproliferative disorder in renal transplant recipients. Am J Transplant. 2005;5(12):2894–900.
26. Wagner HJ, Wessel M, Jabs W, et al. Patients at risk for development of posttransplant lymphoproliferative disorder: plasma versus peripheral blood mononuclear cells as material for quantification of Epstein-Barr viral load by using real-time quantitative polymerase chain reaction. Transplantation. 2001;72(6):1012–9.
27. Landgren O, Gilbert ES, Rizzo JD, et al. Risk factors for lymphoproliferative disorders after allogeneic hematopoietic cell transplantation. Blood. 2009; 113(20):4992–5001.
28. Meijer E, Slaper-Cortenbach IC, Thijsen SF, Dekker AW, Verdonck LF. Increased incidence of EBV-associated lymphoproliferative disorders after allogeneic stem cell transplantation from matched unrelated donors due to a change of T cell depletion technique. Bone Marrow Transplant. 2002;29(4):335–9.
29. Curtis RE, Travis LB, Rowlings PA, et al. Risk of lymphoproliferative disorders after bone marrow transplantation: a multi-institutional study. Blood. 1999;94(7):2208–16.
30. Beveridge T, Krupp P, McKibbin C. Lymphomas and lymphoproliferative lesions developing under cyclosporin therapy. Lancet. 1984;1(8380):788.
31. Ganschow R, Schulz T, Meyer T, Broering DC, Burdelski M. Low-dose immunosuppression reduces the incidence of post-transplant lymphoproliferative disease in pediatric liver graft recipients. J Pediatr Gastroenterol Nutr. 2004;38(2):198–203.
32. Dayton JD, Richmond ME, Weintraub RG, Shipp AT, Orjuela M, Addonizio LJ. Role of immunosuppression regimen in post-transplant lymphoproliferative disorder in pediatric heart transplant patients. J Heart Lung Transplant. 2011;30(4):420–5.
33. Robson R, Cecka JM, Opelz G, Budde M, Sacks S. Prospective registry-based observational cohort study of the long-term risk of malignancies in renal transplant patients treated with mycophenolate mofetil. Am J Transplant. 2005;5(12):2954–60.
34. Cen O, Longnecker R. Rapamycin reverses splenomegaly and inhibits tumor development in a transgenic model of Epstein-Barr virus-related Burkitt's lymphoma. Mol Cancer Ther. 2011;10(4):679–86.
35. Stallone G, Schena A, Infante B, et al. Sirolimus for Kaposi's sarcoma in renal-transplant recipients. N Engl J Med. 2005;352(13):1317–23.

36. Martin PJ, Hansen JA, Anasetti C, et al. Treatment of acute graft-versus-host disease with anti-CD3 monoclonal antibodies. Am J Kidney Dis. 1988;11(2):149–52.
37. Swinnen LJ, Costanzo-Nordin MR, Fisher SG, et al. Increased incidence of lymphoproliferative disorder after immunosuppression with the monoclonal antibody OKT3 in cardiac-transplant recipients. N Engl J Med. 1990;323(25):1723–8.
38. Kirk AD, Cherikh WS, Ring M, et al. Dissociation of depletional induction and posttransplant lymphoproliferative disease in kidney recipients treated with alemtuzumab. Am J Transplant. 2007;7(11):2619–25.
39. Choquet S, Leblond V, Herbrecht R, et al. Efficacy and safety of rituximab in B-cell post-transplantation lymphoproliferative disorders: results of a prospective multicenter phase 2 study. Blood. 2006;107(8):3053–7.
40. Leblond V, Dhedin N, Mamzer Bruneel MF, et al. Identification of prognostic factors in 61 patients with posttransplantation lymphoproliferative disorders. J Clin Oncol. 2001;19(3):772–8.
41. Buell JF, Gross TG, Hanaway MJ, et al. Posttransplant lymphoproliferative disorder: significance of central nervous system involvement. Transplant Proc. 2005;37(2):954–5.
42. Phan TG, O'Neill BP, Kurtin PJ. Posttransplant primary CNS lymphoma. Neuro Oncol. 2000;2(4):229–38.
43. Yang J, Tao Q, Flinn IW, et al. Characterization of Epstein-Barr virus-infected B cells in patients with posttransplantation lymphoproliferative disease: disappearance after rituximab therapy does not predict clinical response. Blood. 2000;96(13):4055–63.
44. Gandhi MK, Lambley E, Burrows J, et al. Plasma Epstein-Barr virus (EBV) DNA is a biomarker for EBV-positive Hodgkin's lymphoma. Clin Cancer Res. 2006;12(2):460–4.
45. Chan AT, Lo YM, Zee B, et al. Plasma Epstein-Barr virus DNA and residual disease after radiotherapy for undifferentiated nasopharyngeal carcinoma. J Natl Cancer Inst. 2002;94(21):1614–9.
46. Stevens SJ, Verschuuren EA, Verkuujlen SA, Van Den Brule AJ, Meijer CJ, Middeldorp JM. Role of Epstein-Barr virus DNA load monitoring in prevention and early detection of post-transplant lymphoproliferative disease. Leuk Lymphoma. 2002;43(4):831–40.
47. Swinnen LJ, LeBlanc M, Grogan TM, et al. Prospective study of sequential reduction in immunosuppression, interferon alpha-2B, and chemotherapy for posttransplantation lymphoproliferative disorder. Transplantation. 2008;86(2):215–22.
48. Trappe R, Oertel S, Leblond V, et al. Sequential treatment with rituximab followed by CHOP chemotherapy in adult B-cell post-transplant lymphoproliferative disorder (PTLD): the prospective international multicentre phase 2 PTLD-1 trial. Lancet Oncology. 2012;13(2):196-206.
49. Tsai DE, Hardy CL, Tomaszewski JE, et al. Reduction in immunosuppression as initial therapy for posttransplant lymphoproliferative disorder: analysis of prognostic variables and long-term follow-up of 42 adult patients. Transplantation. 2001;71(8):1076–88.
50. Boratynska M, Smolska D. Inhibition of mTOR by sirolimus induces remission of post-transplant lymphoproliferative disorders. Transpl Int. 2008;21(6):605–8.
51. Alberu J, Pascoe MD, Campistol JM, et al. Lower malignancy rates in renal allograft recipients converted to sirolimus-based, calcineurin inhibitor-free immunotherapy: 24-month results from the CONVERT trial. Transplantation. 2011;92(3):303–10.
52. Swinnen LJ, Mullen GM, Carr TJ, Costanzo MR, Fisher RI. Aggressive treatment for postcardiac transplant lymphoproliferation. Blood. 1995;86(9):3333–40.
53. Kew 2nd CE, Lopez-Ben R, Smith JK, et al. Postransplant lymphoproliferative disorder localized near the allograft in renal transplantation. Transplantation. 2000;69(5):809–14.
54. Trappe R, Zimmermann H, Fink S, et al. Plasmacytoma-like post-transplant lymphoproliferative disorder, a rare subtype of monomorphic B-cell post-transplant lymphoproliferation, is associated with a favorable outcome in localized as well as in advanced disease: a prospective analysis of 8 cases. Haematologica. 2011;96(7):1067–71.
55. Gonzalez-Barca E, Domingo-Domenech E, Capote FJ, et al. Prospective phase II trial of extended treatment with rituximab in patients with B-cell post-transplant lymphoproliferative disease. Haematologica. 2007;92(11):1489–94.
56. Oertel SH, Verschuuren E, Reinke P, et al. Effect of anti-CD 20 antibody rituximab in patients with post-transplant lymphoproliferative disorder (PTLD). Am J Transplant. 2005;5(12):2901–6.
57. Elstrom RL, Andreadis C, Aqui NA, et al. Treatment of PTLD with rituximab or chemotherapy. Am J Transplant. 2006;6(3):569–76.
58. Gross TG, Bucuvalas JC, Park JR, et al. Low-dose chemotherapy for Epstein-Barr virus-positive post-transplantation lymphoproliferative disease in children after solid organ transplantation. J Clin Oncol. 2005;23(27):6481–8.
59. Papadopoulos EB, Ladanyi M, Emanuel D, et al. Infusions of donor leukocytes to treat Epstein-Barr virus-associated lymphoproliferative disorders after allogeneic bone marrow transplantation. N Engl J Med. 1994;330(17):1185–91.
60. Styczynski J, Einsele H, Gil L, Ljungman P. Outcome of treatment of Epstein-Barr virus-related post-transplant lymphoproliferative disorder in hematopoietic stem cell recipients: a comprehensive review of reported cases. Transpl Infect Dis. 2009;11(5):383–92.
61. Rooney CM, Smith CA, Ng CY, et al. Infusion of cytotoxic T cells for the prevention and treatment of Epstein-Barr virus-induced lymphoma in allogeneic transplant recipients. Blood. 1998;92(5):1549–55.
62. De Angelis B, Dotti G, Quintarelli C, et al. Generation of Epstein-Barr virus-specific cytotoxic T lymphocytes resistant to the immunosuppressive drug tacrolimus (FK506). Blood. 2009;114(23):4784–91.
63. Brewin J, Mancao C, Straathof K, et al. Generation of EBV-specific cytotoxic T cells that are resistant to calcineurin inhibitors for the treatment of

posttransplantation lymphoproliferative disease. Blood. 2009;114(23):4792–803.
64. Haque T, Wilkie GM, Jones MM, et al. Allogeneic cytotoxic T-cell therapy for EBV-positive posttransplantation lymphoproliferative disease: results of a phase 2 multicenter clinical trial. Blood. 2007;110(4):1123–31.
65. Moosmann A, Bigalke I, Tischer J, et al. Effective and long-term control of EBV PTLD after transfer of peptide-selected T cells. Blood. 2010;115(14):2960–70.
66. Perrine SP, Hermine O, Small T, et al. A phase 1/2 trial of arginine butyrate and ganciclovir in patients with Epstein-Barr virus-associated lymphoid malignancies. Blood. 2007;109(6):2571–8.
67. Chen F, Yamane M, Inoue M, et al. Less maintenance immunosuppression in lung transplantation following hematopoietic stem cell transplantation from the same living donor. Am J Transplant. 2011;11(7):1509–16.
68. Luznik L, Bolanos-Meade J, Zahurak M, et al. High-dose cyclophosphamide as single-agent, short-course prophylaxis of graft-versus-host disease. Blood. 2010;115(16):3224–30.
69. Holmes MV, Caplin B, Atkinson C, et al. Prospective monitoring of Epstein-Barr virus DNA in adult renal transplant recipients during the early posttransplant period: role of mycophenolate mofetil. Transplantation. 2009;87(6):852–6.
70. Holmes RD, Orban-Eller K, Karrer FR, Rowe DT, Narkewicz MR, Sokol RJ. Response of elevated Epstein-Barr virus DNA levels to therapeutic changes in pediatric liver transplant patients: 56-month follow up and outcome. Transplantation. 2002;74(3):367–72.
71. Bakker NA, Verschuuren EA, Erasmus ME, et al. Epstein-Barr virus-DNA load monitoring late after lung transplantation: a surrogate marker of the degree of immunosuppression and a safe guide to reduce immunosuppression. Transplantation. 2007;83(4):433–8.
72. Ahmad I, Cau NV, Kwan J, et al. Preemptive management of Epstein-Barr virus reactivation after hematopoietic stem-cell transplantation. Transplantation. 2009;87(8):1240–5.
73. van Esser JW, Niesters HG, van der Holt B, et al. Prevention of Epstein-Barr virus-lymphoproliferative disease by molecular monitoring and preemptive rituximab in high-risk patients after allogeneic stem cell transplantation. Blood. 2002;99(12):4364–9.
74. Heslop HE, Slobod KS, Pule MA, et al. Long-term outcome of EBV-specific T-cell infusions to prevent or treat EBV-related lymphoproliferative disease in transplant recipients. Blood. 2010;115(5):925–35.

Cutaneous B-Cell Lymphoma

18

Bouthaina S. Dabaja

Abstract

Primary cutaneous B-cell lymphomas are relatively rare types of non-Hodgkin's lymphoma with unique clinical presentation. Based on the WHO/EORTC classification, PCBCL are grouped in five major categories: marginal zone, follicular, diffuse large B cell of the leg type or non-leg type, and intravascular large B-cell lymphoma. In this chapter, we will review the clinical pathological characteristics of these categories and current treatment recommendations.

Keywords

B-cell lymphoma • Cutaneous lymphoma • Radiation • Marginal zone lymphoma • Follicular lymphoma • Leg type

Introduction

Primary cutaneous B-cell lymphomas (PCBCL) often present with a different clinical behavior and prognosis from their nodal equivalents, for that reason they are included as a separate entity in the classification systems for non-Hodgkin's lymphomas. PCBCL are much less common than primary cutaneous T-cell lymphomas and represent approximately 20–15 % of all primary cutaneous lymphomas [1]. Following two consensus meetings in Lyon, France (2003), and Zurich, Switzerland (2004), a consensus classification grouped PCBCL into five categories [1–3]:

1. Primary cutaneous marginal zone B-cell lymphoma (PCMZL)
2. Primary cutaneous follicle center lymphoma (PCFCL)
3. Primary cutaneous diffuse large B-cell lymphoma, leg type (PCLBCL, LT)
4. Primary cutaneous diffuse large B-cell lymphoma, other (PCLBCL, O)
5. Intravascular large B-cell lymphoma (IVLBCL)

This classification was adopted by the updated version of the World Health Organization (WHO) classification in 2008 [4]. Recent clinical, histologic, immunohistochemical, and molecular genetic studies have resulted in establishing and validating the different entities adopted by the WHO/EORTC classification [5–8]; therefore, we will use the categories defined by the joint effort

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of WHO and EORTC as the backbone of this chapter.

Staging workup includes a complete history and physical exam, photography of the skin lesion, and laboratory studies, such as a complete blood cell count with differential and comprehensive blood chemistry measurement, including lactate dehydrogenase, and, in selected cases, serum electrophoresis to exclude a monoclonal gammopathy and/or flow cytometry on peripheral blood, bone marrow aspirate, and biopsy. Adequate imaging studies, including contrast-enhanced computed tomography (CT) scan with and without positron emission tomography (PET)/CT, and getting the correct pathological diagnosis are primordial to design therapy. It is generally accepted to consider PCMZL and PCFCL as indolent types for local treatment as opposed to the PCBCL (LT) and PCBCL (O) that are considered more aggressive and need a systemic multiple chemotherapy treatment.

Primary Cutaneous Marginal Zone B-Cell Lymphoma

Histopathology, Immunophenotype, and Genetic Features

This category of lymphoma includes cases previously designated as primary cutaneous immunocytoma and cases of cutaneous follicular lymphoid hyperplasia with monotypic plasma cells. Primary cutaneous marginal zone lymphomas (PCMZL) show nodular to diffuse infiltrates that spares the epidermis; they are composed of small to medium lymphocytes with abundant cytoplasm, marginal zone B cells. In addition lymphoplasmacytoid cells, and plasma cells, admixed with numbers of centroblasts and many reactive T cells [9, 10]. The infiltrates are frequently surrounded with monotypic plasma cells at the periphery and in the superficial dermis. Reactive germinal centers are frequently seen. The marginal zone B cells express CD20, CD79, and bcl-2; they are typically negative for CD5, CD10, and bcl-6 [11]. Unlike marginal B cell of the stomach, PCMZL will rarely show translocation t (18:180)(q21; q21); rather, it shows immunoglobulin heavy chain (IgH) rearrangement, including t (14; 18)(q32; q21) and t (3; 14)(p14.1; q32) observed in a minority of cases [12, 13]. A link between PCMZL and Borrelia burgdorferi infection has been suggested in European patients [14–16] but was not seen in patients from Asia or the United States [17, 18]. Other investigators reported PCMZL developing in preexisting areas of autoimmune disease, acrodermatitis chronica atrophicans, or previous vaccination sites.

Clinical, Presentation, and Therapy

This type of lymphoma represents 2–16 % of all cutaneous lymphomas [5]. Most patients present with multiple lesions (72 %) [19]. Lesions are non-painful red to violaceous papules, plaques, or nodules preferentially localized on the trunk or upper extremities. Ulceration is uncommon. Median age at presentation is 50 years and the duration of symptoms before diagnosis range from 1 to 180 months. There is a reported tendency of recurrence in the skin (46 %) [20] but rarely in extracutaneous sites. Interestingly spontaneous resolution of these lesions has been observed [9, 10, 17, 21].

Patients with solitary or few lesions are usually treated with local radiation therapy with a dose ranging from 10 to 45 Gy; the reported CR reaches 99 % [20]. Some patients are also treated with local excision especially if done for diagnostic purposes. In patients with associated B. burgdorferi infection, systemic antibiotics should be the first line of therapy. Other treatments reported include intralesional steroids, interferon, rituximab, and single-agent chlorambucil [22]. Patients, who present with multifocal skin lesions that cannot be contained within a safe radiation field, are usually treated with systemic therapy. Systemic therapy can be single agent like chlorambucil or rituximab or multiple agents like CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone) or COP-omitting doxorubicin.

Outcome and Prognostic Factors

The majority of patients will achieve a complete remission to any modality of therapy used. The lymphoma-specific survival is close to 100 %. Patients who present with solitary lesions enjoys a 5-year relapse-free survival of 77 % versus 39 % for those who presents with multifocal skin lesions [9, 19, 23, 24]. Attempt to achieve complete remission should be aimed at with single or multiple lesions on first presentation. On the other hand with multiple recurrences, since cutaneous relapses do not signify a worse prognosis, treatment should be aimed at palliation rather than sustained complete remission, the adverse effects of therapy have to be weighed against the long survival and the low death-related lymphoma in these patients.

Primary Cutaneous Follicle Center Lymphoma

Histopathology, Immunophenotype, and Genetic Features

Primary cutaneous follicle center lymphoma (PCFCL) is defined as a tumor of neoplastic follicle center cells, with nodular or diffuse infiltrates sparing the epidermis. It is well defined as an entity now in the WHO/EORTC classification to clarify the original doubt of its presence and its attribution to be a variant of PCMZL or pseudolymphoma. Location and growth rate affects the pathological diagnosis. Lesions arising on the scalp and/or early small lesions show a clear-cut follicular growth pattern than lesions on the trunk and/or older lesions [25, 26]. The growth over time will efface the follicular pattern; at an early stage the abnormal follicles are composed of malignant bcl6+ bcl2- cells in a network of CD21+ or CD35+ follicular dendritic cells; the neoplastic B cells with progression to tumors will increase, while the reactive T cells decrease; therefore, the follicular pattern is no more visible [27]. In addition, in the later stage the cells are more of a monotonous population of large follicle center cells. Immunophenotypically the neoplastic cells express CD20 and CD79a; at the early stage pre-tumor surface immunoglobulins can be present. PCGCL consistently express bcl6, while CD10 expression is mainly seen in follicular pattern. In most cases t (14; 18) and bcl 2-protein expression is extremely rare, confirming the difference between nodal and cutaneous follicular lymphoma [25, 28].

Clinical Presentation and Therapy

PCFCL is the most common PCBCL; it can present with solitary or multiple sites of plaques and tumors that can be tender but not ulcerative. Scalp, forehead, and trunk are the most common locations. The median age is 61 years [8, 25]. The rate of growth from papules to a tumor might take several years. Local radiation therapy is the preferred therapy for single and cluster but multiple skin lesions. The rate of achieving complete remission (CR) with radiation therapy is near 100 %. The typical dose is 20–54 Gy. The reported relapse rate is 30 % [29]. It is worth to mention that currently most patients are treated with a dose of ≤30 Gy with an electron field that would cover around 2 cm all around the edge of the skin lesion to prevent marginal miss of the radiation field [30, 31]. Other modalities of therapy include local excision, intralesional interferon, and rituximab [20]. Anthracycline-based chemotherapy has been rarely used; cases with very extensive disease or extracutaneous relapse might justify its use.

Outcome and Prognostic Factors

Patients with PCFCL have an excellent outcome with a 5-year overall survival of more than 95 % [25, 26, 30, 31]. Patients treated with radiation therapy achieve a complete remission and an overall survival of 100 % with 73–89 % relapse-free survival according to different studies. These results are independent of the number of lesions at presentation or the pattern of growth (follicular or diffuse). Clinicians should noticed that the presence of diffuse large centrocytes although

makes it look like primary cutaneous large cell but the clinical behavior still fits PCFCL. Rijlaarsdam et al. reported a high rate of recurrence for lesions located to the lower extremities. In addition, recent studies report that the presence of bcl2 expression by more than 50 % of neoplastic B cells in PCFCL with a diffuse proliferation of large centrocytes is associated with an unfavorable prognosis [32]; this very same finding is considered by the WHO/EORTC classification as a reason to raise suspicion about a systemic lymphoma involving the skin secondarily.

In conclusion, radiation therapy is the preferred treatment for solitary or localized multiple skin lesions. Patients with extensive or recurrent disease multiagent chemotherapy should be rarely considered; these patients should be treated with palliative approach to symptomatic lesions and the risk of therapy should be carefully considered in such disease with an indolent behavior.

Primary Cutaneous Diffuse Large B-Cell Lymphoma, Leg Type

Histopathology, Immunophenotype, and Genetic Features

The term "leg type" was proposed by Vermeer et al. in 1996 [33] and adopted by the WHO/EORTC classification. These lymphomas show diffuse monotonous infiltrates of centroblasts and immunoblasts, which extend to the subcutaneous tissue and, usually, destroy the adnexal structures [33, 34]. As opposed to other cutaneous types described above, the leg type frequently shows mitotic figures and scarce centrocytes, reactive T cells, or stromal reaction. Neoplastic cells typically express CD20 and CD79a and are negative for CD5 and CD10. An important feature of this lymphoma is the strong positivity for bcl 2-protein and MUM-1/IRF4 [11, 32, 35, 36]. It is worth to mention that bcl 2 can be negative in 19 % of leg type and positive in 28 % of non-leg type; therefore, its presence does not strongly correlate to the anatomic site [37, 38]. Although bcl 2 expression is strong, t (14; 18) is not found in the leg type. Chromosomal translocations involving c-MYC, bcl6, and IgH genes are frequent in leg type compared to PCFCL [39].

Chromosome imbalance with gain of 18q and 7q and loss of 6q occurs in up to 85 % of cases [36]. Recent studies suggested that the worse outcome of leg type compared to follicle center is related to an activated B-cell gene expression profile [40]. The etiology of cutaneous LBCL is unknown, recent studies looked at the association of this lymphoma type with infectious agents including HHV-8, EBV, and B. burgdorferi and could not identify any association [38].

Clinical Presentation and Therapy

Patients usually present with rapidly growing red to bluish tumors on the leg; the term leg type is better than "large B-cell lymphoma of the leg" since it reflects the predominant but not exclusive anatomic location of these tumors. This type of lymphoma affects elderly patients with a median age of 68 years. In contrast to the indolent cutaneous lymphoma discussed earlier, these lymphomas tend to relapse in extracutaneous sites, as shown by Grange et al., who reported extracutaneous relapse in 24 out 48 patients (50 %) [34]. In view of the aggressive nature of this disease, it is treated like a diffuse large B-cell lymphoma with anthracycline-based chemotherapy combined with rituximab [41, 42]. Very few studies reported on the use of anthracycline-based chemotherapy; the reported CR ranges from 81 to 92 %; the relapse rate range from 9 to 54 %. Longer follow-up is needed on the use of multiagent chemotherapy.

With a high rate of relapse of 58 % [20], local radiation therapy should be reserved as a consolidation or as a palliative modality [43].

Outcome and Prognostic Factors

Multivariate analysis of disease-specific survival of patients with leg type and as compared to their counterpart in other sites showed that round cell morphology, duration of skin lesions before diagnosis, and size and extent of skin lesions were independent adverse prognostic factors. Further analysis of the relation between number and extent of skin lesions and survival showed that only 1 out 11 (9 %) patients with solitary tumor compared to 12 of 23 (52 %) with multiple tumors on one leg,

and 7 out of 14 (52 %) with generalized skin lesions die of lymphoma. The 5-year disease-specific survival ranges from 55 to 63 % [44]. The number of lesions had no prognostic value in another study by Kodama et al. [38], while morphology, site, and the expression of bcl 2, MUM-1, and FOX-P1 were strongly linked to prognosis. In conclusion, it is established that PCBCL leg type is a separate entity with an inferior outcome and it should be treated with aggressive systemic therapy with and without radiation therapy.

Primary Cutaneous Diffuse Large B-Cell Lymphoma, Other

Histopathology, Immunophenotype, and Genetic Features

They present with diffuse growth pattern composed of large transformed B cells that lack the typical features of PCLBCL, LT, or the diffuse growth pattern of PCFCL. These tumors contain a monomorphic population of centroblast-like cells with a mixed inflammatory background. The large neoplastic cells express pan-B-cell antigens.

This type includes morphologic variants of diffuse large B-cell lymphoma, such as anaplastic or plasmablastic subtypes or T-cell/histiocyte-rich large B-cell lymphomas.

Clinical Presentation, Treatment, and Outcome

They are generally a skin manifestation of a systemic lymphoma, as in plasmablastic type in the setting of HIV infection or immune deficiency. They are clinically more indolent like PCFCL and PCMZL with an excellent prognosis [45].

Intravascular Large B-Cell Lymphoma

Histopathology, Immunophenotype, and Genetic Features

This is a highly malignant large cell lymphoma with systemic spread with presence of tumor cells in the lamina of the small vessels. The skin and nervous system are preferential sites. Tumors express B-cell markers in most cases with over expression of bcl2 protein [46, 47].

Clinical Presentation, Treatment, and Outcome

Patients have widely systemic disease in most cases; the skin lesions have a violaceous patches and plaques or telangiectatic lesions usually involving the trunk and legs. Patients with only skin presentations tend to have a better prognosis. In view of the aggressive presentation, multiagent chemotherapy is the treatment of choice with and without radiation therapy [48].

In summary, identification of the different subtypes of cutaneous B-cell lymphoma is of prime importance to classify patients into indolent versus aggressive pattern and to subsequently determine if a local therapy with minimal side effects such as radiation or multiagent chemotherapy such as anthracycline based (with rituximab) is the way to treat.

References

1. Willemze R, Jaffe ES, Burg G, Cerroni L, Berti E, Swerdlow SH, et al. WHO-EORTC classification for cutaneous lymphomas. Blood. 2005;105:3768–85.
2. Campo E, Swerdlow SH, Harris NL, Pileri S, Stein H, Jaffe ES. The WHO classification of lymphoid neoplasms and beyond: evolving concepts and practical applications. Blood. 2008;117:5019–32.
3. Jaffe ES, Harris NL, Stein H, Isaacson PG. Classification of lymphoid neoplasms: the microscope as a tool for disease discovery. Blood. 2008;112:4384–99.
4. Swerdlow SH, Campo E, Harris NL, et al. WHO classification of tumours of haematopoietic and lymphoid tissues. Lyon: IARC Press; 2008.
5. Fink-Puches R, Zenahlik P, Back B, Smolle J, Kerl H, Cerroni L. Primary cutaneous lymphomas: applicability of current classification schemes (European Organization for Research and Treatment of Cancer, World Health Organization) based on clinicopathologic features observed in a large group of patients. Blood. 2002;99:800–5.
6. Grange F, Hedelin G, Joly P, Beylot-Barry M, D'Incan M, Delaunay M, et al. Prognostic factors in primary cutaneous lymphomas other than mycosis fungoides and the Sezary syndrome. The French Study Group on Cutaneous Lymphomas. Blood. 1999;93:3637–42.
7. Willemze R, Meijer CJ. EORTC classification for primary cutaneous lymphomas: the best guide to good

clinical management. European Organization for Research and Treatment of Cancer. Am J Dermatopathol. 1999;21:265–73.
8. Willemze R, Kerl H, Sterry W, Berti E, Cerroni L, Chimenti S, et al. EORTC classification for primary cutaneous lymphomas: a proposal from the Cutaneous Lymphoma Study Group of the European Organization for Research and Treatment of Cancer. Blood. 1997; 90:354–71.
9. Bailey EM, Ferry JA, Harris NL, Mihm Jr MC, Jacobson JO, Duncan LM. Marginal zone lymphoma (low-grade B-cell lymphoma of mucosa-associated lymphoid tissue type) of skin and subcutaneous tissue: a study of 15 patients. Am J Surg Pathol. 1996; 20:1011–23.
10. Cerroni L, Signoretti S, Hofler G, Annessi G, Putz B, Lackinger E, et al. Primary cutaneous marginal zone B-cell lymphoma: a recently described entity of low-grade malignant cutaneous B-cell lymphoma. Am J Surg Pathol. 1997;21:1307–15.
11. Hoefnagel JJ, Vermeer MH, Jansen PM, Fleuren GJ, Meijer CJ, Willemze R. Bcl-2, Bcl-6 and CD10 expression in cutaneous B-cell lymphoma: further support for a follicle centre cell origin and differential diagnostic significance. Br J Dermatol. 2003;149: 1183–91.
12. Streubel B, Lamprecht A, Dierlamm J, Cerroni L, Stolte M, Ott G, et al. T(14;18)(q32;q21) involving IGH and MALT1 is a frequent chromosomal aberration in MALT lymphoma. Blood. 2003;101:2335–9.
13. Streubel B, Vinatzer U, Lamprecht A, Raderer M, Chott A. T(3;14)(p14.1;q32) involving IGH and FOXP1 is a novel recurrent chromosomal aberration in MALT lymphoma. Leukemia. 2005;19:652–8.
14. Goodlad JR, Davidson MM, Hollowood K, Ling C, MacKenzie C, Christie I, et al. Primary cutaneous B-cell lymphoma and Borrelia burgdorferi infection in patients from the Highlands of Scotland. Am J Surg Pathol. 2000;24:1279–85.
15. Roggero E, Zucca E, Mainetti C, Bertoni F, Valsangiacomo C, Pedrinis E, et al. Eradication of Borrelia burgdorferi infection in primary marginal zone B-cell lymphoma of the skin. Hum Pathol. 2000;31:263–8.
16. Cerroni L, Zochling N, Putz B, Kerl H. Infection by Borrelia burgdorferi and cutaneous B-cell lymphoma. J Cutan Pathol. 1997;24:457–61.
17. Li C, Inagaki H, Kuo TT, Hu S, Okabe M, Eimoto T. Primary cutaneous marginal zone B-cell lymphoma: a molecular and clinicopathologic study of 24 Asian cases. Am J Surg Pathol. 2003;27:1061–9.
18. Takino H, Li C, Hu S, Kuo TT, Geissinger E, Muller-Hermelink HK, et al. Primary cutaneous marginal zone B-cell lymphoma: a molecular and clinicopathological study of cases from Asia, Germany, and the United States. Mod Pathol. 2008;21:1517–26.
19. Hoefnagel JJ, Vermeer MH, Jansen PM, Heule F, van Voorst Vader PC, Sanders CJ, et al. Primary cutaneous marginal zone B-cell lymphoma: clinical and therapeutic features in 50 cases. Arch Dermatol. 2005;141:1139–45.
20. Senff NJ, Noordijk EM, Kim YH, Bagot M, Berti E, Cerroni L, et al. European Organization for Research and Treatment of Cancer and International Society for Cutaneous Lymphoma consensus recommendations for the management of cutaneous B-cell lymphomas. Blood. 2008;112:1600–9.
21. Schmid U, Eckert F, Griesser H, Steinke C, Cogliatti SB, Kaudewitz P, et al. Cutaneous follicular lymphoid hyperplasia with monotypic plasma cells. A clinicopathologic study of 18 patients. Am J Surg Pathol. 1995;19:12–20.
22. Zenahlik P, Fink-Puches R, Kapp KS, Kerl H, Cerroni L. Therapy of primary cutaneous B-cell lymphomas. Hautarzt. 2000;51:19–24.
23. Rijlaarsdam JU, van der Putte SC, Berti E, Kerl H, Rieger E, Toonstra J, et al. Cutaneous immunocytomas: a clinicopathologic study of 26 cases. Histopathology. 1993;23:117–25.
24. Schreuder MI, Hoefnagel JJ, Jansen PM, van Krieken JH, Willemze R, Hebeda KM. FISH analysis of MALT lymphoma-specific translocations and aneuploidy in primary cutaneous marginal zone lymphoma. J Pathol. 2005;205:302–10.
25. Cerroni L, Arzberger E, Putz B, Hofler G, Metze D, Sander CA, et al. Primary cutaneous follicle center cell lymphoma with follicular growth pattern. Blood. 2000;95:3922–8.
26. Goodlad JR, Krajewski AS, Batstone PJ, McKay P, White JM, Benton EC, et al. Primary cutaneous follicular lymphoma: a clinicopathologic and molecular study of 16 cases in support of a distinct entity. Am J Surg Pathol. 2002;26:733–41.
27. Santucci M, Pimpinelli N, Arganini L. Primary cutaneous B-cell lymphoma: a unique type of low-grade lymphoma. Clinicopathologic and immunologic study of 83 cases. Cancer. 1991;67:2311–26.
28. Triscott JA, Ritter JH, Swanson PE, Wick MR. Immunoreactivity for bcl-2 protein in cutaneous lymphomas and lymphoid hyperplasias. J Cutan Pathol. 1995;22:2–10.
29. Eich HT, Eich D, Micke O, Suttzer H, Casper C, Krieg T, et al. Long-term efficacy, curative potential, and prognostic factors of radiotherapy in primary cutaneous B-cell lymphoma. Int J Radiat Oncol Biol Phys. 2003;55:899–906.
30. Rijlaarsdam JU, Toonstra J, Meijer OW, Noordijk EM, Willemze R. Treatment of primary cutaneous B-cell lymphomas of follicle center cell origin: a clinical follow-up study of 55 patients treated with radiotherapy or polychemotherapy. J Clin Oncol. 1996; 14:549–55.
31. Smith BD, Glusac EJ, McNiff JM, Smith GL, Heald PW, Cooper DL, et al. Primary cutaneous B-cell lymphoma treated with radiotherapy: a comparison of the European Organization for Research and Treatment of Cancer and the WHO classification systems. J Clin Oncol. 2004;22:634–9.

32. Grange F, Petrella T, Beylot-Barry M, Joly P, D'Incan M, Delaunay M, et al. Bcl-2 protein expression is the strongest independent prognostic factor of survival in primary cutaneous large B-cell lymphomas. Blood. 2004;103:3662–8.
33. Vermeer MH, Geelen FA, van Haselen CW, van Voorst Vader PC, Geerts ML, van Vloten WA, et al. Primary cutaneous large B-cell lymphomas of the legs. A distinct type of cutaneous B-cell lymphoma with an intermediate prognosis. Dutch Cutaneous Lymphoma Working Group. Arch Dermatol. 1996;132:1304–8.
34. Grange F, Bekkenk MW, Wechsler J, Meijer CJ, Cerroni L, Bernengo M, et al. Prognostic factors in primary cutaneous large B-cell lymphomas: a European multicenter study. J Clin Oncol. 2001;19: 3602–10.
35. Goodlad JR, Krajewski AS, Batstone PJ, McKay P, White JM, Benton EC, et al. Primary cutaneous diffuse large B-cell lymphoma: prognostic significance of clinicopathological subtypes. Am J Surg Pathol. 2003;27:1538–45.
36. Paulli M, Viglio A, Vivenza D, Capello D, Rossi D, Riboni R, et al. Primary cutaneous large B-cell lymphoma of the leg: histogenetic analysis of a controversial clinicopathologic entity. Hum Pathol. 2002;33: 937–43.
37. Geelen FA, Vermeer MH, Meijer CJ, Van der Putte SC, Kerkhof E, Kluin PM, et al. bcl-2 protein expression in primary cutaneous large B-cell lymphoma is site-related. J Clin Oncol. 1998;16:2080–5.
38. Kodama K, Massone C, Chott A, Metze D, Kerl H, Cerroni L. Primary cutaneous large B-cell lymphomas: clinicopathologic features, classification, and prognostic factors in a large series of patients. Blood. 2005;106:2491–7.
39. Hallermann C, Kaune KM, Gesk S, Martin-Subero JI, Gunawan B, Griesinger F, et al. Molecular cytogenetic analysis of chromosomal breakpoints in the IGH, MYC, BCL6, and MALT1 gene loci in primary cutaneous B-cell lymphomas. J Invest Dermatol. 2004;123:213–9.
40. Hoefnagel JJ, Dijkman R, Basso K, Jansen PM, Hallermann C, Willemze R, et al. Distinct types of primary cutaneous large B-cell lymphoma identified by gene expression profiling. Blood. 2005;105: 3671–8.
41. Wobser M, Kneitz H, Brocker EB, Becker JC. Primary cutaneous diffuse large B-cell lymphoma, leg-type, treated with a modified R-CHOP immunochemotherapy—diagnostic and therapeutic challenges. J Dtsch Dermatol Ges. 2011;9:204–11.
42. Guyot A, Ortonne N, Valeyrie-Allanore L, Bagot M. Combined treatment with rituximab and anthracycline-containing chemotherapy for primary cutaneous large B-cell lymphomas, leg type, in elderly patients. Arch Dermatol. 2010;146:89–91.
43. Heinzerling LM, Urbanek M, Funk JO, Peker S, Bleck O, Neuber K, et al. Reduction of tumor burden and stabilization of disease by systemic therapy with anti-CD20 antibody (rituximab) in patients with primary cutaneous B-cell lymphoma. Cancer. 2000;89: 1835–44.
44. Hallermann C, Niermann C, Fischer RJ, Schulze HJ. Survival data for 299 patients with primary cutaneous lymphomas: a monocentre study. Acta Derm Venereol. 2011;91:521–5.
45. Li S, Griffin CA, Mann RB, Borowitz MJ. Primary cutaneous T-cell-rich B-cell lymphoma: clinically distinct from its nodal counterpart? Mod Pathol. 2001;14: 10–3.
46. Kanda M, Suzumiya J, Ohshima K, Haraoka S, Nakamura N, Abe M, et al. Analysis of the immunoglobulin heavy chain gene variable region of intravascular large B-cell lymphoma. Virchows Arch. 2001; 439:540–6.
47. Perniciaro C, Winkelmann RK, Daoud MS, Su WP. Malignant angioendotheliomatosis is an angiotropic intravascular lymphoma. Immunohistochemical, ultrastructural, and molecular genetics studies. Am J Dermatopathol. 1995;17:242–8.
48. Ferreri AJ, Campo E, Seymour JF, Willemze R, Ilariucci F, Ambrosetti A, et al. Intravascular lymphoma: clinical presentation, natural history, management and prognostic factors in a series of 38 cases, with special emphasis on the 'cutaneous variant'. Br J Haematol. 2004;127:173–83.

Cutaneous T-Cell Lymphomas: Mycosis Fungoides and Sézary Syndrome

19

Madeleine Duvic

Abstract

Although cutaneous lymphomas arising in skin are rare, they can be the cause of significant morbidity and mortality. The most common of the cutaneous T-cell lymphomas (CTCLs) are mycosis fungoides (MF) and its leukemic variant, Sézary syndrome (SS), followed by the CD30+ lymphoproliferative disorders. CTCLs are separated by T-cell markers and by clinical presentations and response to therapy. Early MF is treated with skin-directed therapies whereas refractory lesions need combination of skin therapy and biological response modifiers. Advanced MF responds to monotherapy with targeted therapies or chemotherapies, whereas Sezary patients often do well on photopheresis with skin therapy and biological response modifiers. In spite of new approved therapies for T-cell lymphomas of the skin, they remain challenging and uncurable at the current time.

Keywords

Mycosis fungoides • Sézary syndrome • Cd30+ lymphoproliferative disorders • Total skin electron beam • Histone deacetylase inhibitors

Introduction

Cutaneous T-cell lymphomas (CTCLs) are a heterogeneous group of non-Hodgkin's lymphomas. They are characterized by their unique clinical features, histology, and immunohistochemistry profiling of the neoplastic T lymphocytes infiltrating the epidermis, dermis, subcutaneous/fat, or adnexal structures. The term "cutaneous T-cell lymphoma (CTCL)" was formally adopted in 1979 at a conference sponsored by the National Cancer Institute to describe the group as a heterogeneous group of malignant T-cell lymphomas with primary manifestations in the skin [1]. An updated EORTC-WHO classification system is now widely used to subclassify these disorders based on clinical behavior and surface markers (Table 19.1) [2, 3].

Since mycosis fungoides (MF) is the most common form of CTCL, "CTCL" is often used synonymously with MF or its leukemic variant,

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Table 19.1 EORTC-WHO classification system for cutaneous t-cell lymphomas with primary cutaneous manifestations [2]

Cutaneous T-cell and NK-cell lymphomas
Mycosis fungoides
MF variants and subtypes
Folliculotropic MF
Pagetoid reticulosis
Granulomatous slack skin
Sézary syndrome
Adult T-cell leukemia/lymphoma
Primary cutaneous CD30 lymphoproliferative disorders
Primary cutaneous anaplastic large cell lymphoma
Lymphomatoid papulosis
Subcutaneous panniculitis-like T-cell lymphoma
Extranodal NK/T-cell lymphoma, nasal type
Primary cutaneous peripheral T-cell lymphoma, unspecified
Primary cutaneous aggressive epidermotropic CD8 T-cell lymphoma (provisional)
Cutaneous γ[gamma]/δ[delta] T-cell lymphoma (provisional)
Primary cutaneous CD4 small/medium-sized pleomorphic T-cell lymphoma (provisional)

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Sézary syndrome. However, CTCL also includes the $CD30^+$ lymphoproliferative disorders, peripheral T-cell lymphomas, gamma delta lymphomas, and other rare variants (Table 19.1). MF is characterized by pleomorphic skin lesions resembling eczema or psoriasis that contain atypical $CD4^+CD45RO^+$ helper/memory cells invading the epidermis (epidermotropism) or adnexa (folliculotropic MF). Alibert, in 1806, first described a patient with facial tumors, possibly the folliculotropic variant of mycosis fungoides (MF) or possibly transformed MF [4]. A leukemic variant of MF presenting with generalized erythroderma (as well as keratoderma and pruritus) is known as Sézary syndrome (SS). SS can present de novo with erythroderma, pruritus, and blood involvement or it may evolve from preexisting MF lesions [5]. Histologically, in de novo SS, there is an absence of epidermotropism with cells surrounding the dermal vessels and lack of epidermotropism. SS arising from MF will retain some of the epidermotropism or folliculotropism making the diagnosis easier [6].

Recently, two studies have provided evidence of molecular differences between MF and SS. Campbell et al., using flow cytometry, found that the leukemic T cells express CCR7 and L-selectin and the differentiation marker CD27, a phenotype consistent with central memory T cells [7]. On the other hand, cells extracted from MF lesions lacked CCR7/L-selectin and CD27 but strongly expressed CCR4 and CLA, which is a phenotype suggestive of skin resident effector memory T cells [7]. Molecular phenotyping of T-cell DNA also revealed differences in chromosomal number variants, suggesting that MF and SS differ [8]. Patients may also have aberrant T cells in their blood without meeting the other criteria for SS (stage IVA), and conversely, erythroderma without blood involvement (stage IIIA) can exist.

Epidemiology

The first epidemiologic study of CTCL in the United States was conducted by Weinstock et al. [9]. In a follow-up study of SEER databases from 1973 to 2002, Criscione and Weinstock reported that the overall age adjusted MF/SS of CTCL was 6.4/million persons, with approximately 1,500 new cases reported in the USA each year [10]. The peak incidence is in the sixth to seventh decade. The annual incidence of CTCL had increased by 2.9×10^{-6}/year over the duration of the study period. Higher incidences were reported for males 8.7×10^{-6} compared to females (vs. 4.6×10^{-6}) and African Americans (9×10^{-6}) compared to Caucasians (6.1×10^{-6}). Racial differences decreased with age. Our study showed that early-onset and more aggressive MF present in young African American females is more likely to progress [11]. Of interest, higher density of MF was associated with high density of physicians, higher education, and home values which might reflect increased change of diagnosis at an earlier stage [10].

Molecular and Immunologic Pathogenesis

The pathogenesis of MF and other forms of CTCL, with the exception of human T-lymphotropic virus-1/adult T-cell leukemia and lymphoma, is incompletely known. Clonal emergence of T cells appears to require a series of genetic mutations leading to the generation of clones with enhanced T-cell signaling, proliferation, and mobility. Clonal emergence of one or more dominant clones may be associated with loss of the remaining T-cell repertoire that could contribute to immunosuppression [12]. MF/SS is initiated when antigen presentation activates expansion of a T cell through IL-2. Secondly, mutations in Fas/Fas ligand promote T-cell survival instead of promoting activation-induced cell death (AICD) [13–15]. Mutations in the Fas gene may contribute to the development and progression of MF by allowing clonal expansion of activated T cells and reducing susceptibility to $CD8^+$ cytotoxic, antitumor immune responses. Additionally, $CD4^+$ T cells expressing Fas ligand and lacking Fas may induce apoptosis of $CD8^+$ tumor-infiltrating cytotoxic T cells and allow the accumulation of the malignant clone [15].

Since MF often starts as a chronic, indolent dermatitis, Tan first suggested in 1974 that it arises as a delayed hypersensitivity reaction to a chronic persistent antigen [16]. If this is the case, then exposure of genetically susceptible individuals to specific but private environment (e.g., viral, chemical, infectious) and/or endogenous (e.g., antigenic) factors may trigger the emergence of MF. Specific antigens or conditions proposed include chemicals and infectious agents including the HTLV-1 retrovirus [17], smoking, medications, atopy, and sun exposure [18]. Other epidemiologic case-control studies have failed to support the hypothesis that MF is initiated by a single chronic antigen stimulation [18]. In erythrodermic patients, we have found an association between colonization with *Staphylococcus aureus* expressing superantigens capable of stimulating the specific T-cell receptor V beta 2 which was clonally expanded in patients' blood [19]. In support of the superantigen hypothesis, eradication of staphylococcus colonization can result in marked clinical improvement or even complete responses [20].

Further evidence for Tan's hypothesis is that MF is associated with human leukocyte antigens (HLA) class II antigens DR5 and DQB1*O3 [21]. The association of HLA chains, the T-cell receptor chains, and accessory adhesion molecules that facilitate the immune response is known as the immunologic synapse. Class II molecules on antigen-presenting cells are able to present processed peptides to the antigen-specific T-cell receptor expressed on a naïve $CD4^+$ lymphocyte [22]. Antigen-presenting Langerhans cells located in the epidermis attract clusters of $CD4^+$ T cells and are known as Pautrier's microabscesses, a pathognomonic feature used to diagnose MF by skin histology. Engagement results in the clonal proliferation and activation of a $CD4^+$ T-lymphocyte subset defined by its T-cell beta or gamma receptor gene rearrangements [22]. $CD4^+$ helper/inducer T lymphocytes with a T-helper type 1 (Th_1) effector cell phenotype are a seminal component of cell-mediated immunity (CMI) and adaptive immune responses.

Cytokines secreted by Th_1 cells include interleukin (IL)-2, interferon-gamma, interleukin-12 and tumor necrosis factor (TNF), and boost cellular immunity; Th_1 cytokines are lost with progression from patch-stage MF to Sézary syndrome [23]. Histopathologic and immunopharmacologic studies indicate that skin-infiltrating and circulating malignant $CD4^+$ T lymphocytes in patients with MF and SS express a Th_2 cytokine profile of IL-4, -5, and -10 [23]. This contributes to depressed cellular immunity in patients who have advanced MF/Sézary syndrome. In patients with Sézary syndrome, the dominance of Th_2 clones results in eosinophilia, pruritus, and decreased Th_1 cutaneous delayed-type hypersensitivity reactions [24]. Thus, the restoration of T-lymphocyte subset homeostasis, or improvement in the Th_1/Th_2 lymphocyte ratio, is a rational goal for the development of targeted therapies and may underlie the success of allogeneic stem cell transplantation [25].

It is noteworthy that Th_1 lymphocytes, especially cytotoxic $CD8^+$ cells, are the principal effector cells of cell-mediated immunity against tumor cells. Loss of $CD8^+$ infiltrating lymphocytes has long been recognized as a poor prognostic factor in MF [26].

Cytogenetics

Various chromosomal deletions on chromosomes 1p, 17p, 10q, and 19 and gains on 4q, 18, and 17q have been detected on comparative genomic hybridization analysis [27, 28]. These copy number variant loci contain key genes influencing cell growth, such as the tumor suppressor, p53 on 17p, and stat transcription factors on 17q. In addition, microsatellite instability has been detected in patients with MF [29, 30]. Another group has implicated the c-Myc pathway in MF and SS proliferation [8]. These data implicate mutations in tumor-suppressor genes as well as oncogenes may underlie clonal expansion and disease progression.

To this end, a broad spectrum of new therapies for CTCLs, including biologic response modifiers, monoclonal antibodies (mAbs), histone deacetylase inhibitors, and purine nucleoside phosphorylase (PNP) inhibitors, has emerged as potential treatments that target malignant T lymphocytes [31, 32].

Staging and Diagnosis

MF most often evolves slowly from a chronic dermatitis with limited skin involvement—patches or plaques (T1) on <10 % of the body in areas that are shielded from sunlight exposure [5, 33]. Patients can stay as T1 indefinitely, but over time and without treatment, MF can progress to more extensive skin involvement with patches or plaques over more than 10 % of the body (T2) or to tumor formation (T3) or to generalized erythroderma (T4) with blood involvement (B2). Further progression to nodes or visceral areas may occur over time but is rare. Advanced stage of MF (>IIB) is more likely to progress to large-cell transformation that is defined by having >25 % of the malignant T cells having nuclei four times normal size [34, 35]. Individual patients may present de novo at any T stage along the spectrum of progression.

MF, unlike other peripheral T-cell lymphomas of the skin [36], is staged using the tumor, node, metastasis, blood (TNMB) staging system, grading skin involvement, the presence of lymph nodes, visceral disease, and blood involvement at the time of diagnosis (Tables 19.2 and 19.3) [3]. The stage of MF is classified by the extent of skin involvement: T_1 (patches/plaques covering <10 % of body surface), T_2 (patches/plaques covering ≥10 % of body surface), T_3 (tumors), or T_4 (erythroderma) (Table 19.3). At the time of diagnosis, these stages are reported in 42, 30, 15, and 12 % of patients, respectively [5].

The International Society for Cutaneous Lymphomas (ISCL)/European Organization for Research and Treatment of Cancer (EORTC) [3] revised the previous Mycosis Fungoides Cooperative Group (MFCG) classification and staging system for CTCL [37]. The update took into account the degree of peripheral blood involvement, which is a major prognostic factor for MF/SS patients but did not alter clinical stage in the previous classification system [3]. The B designation can be used for assessing blood involvement (Table 19.3), with B_0 signifying ≤5 % atypical (Sézary) cells, B_2 signifying positive clonal rearrangement of the T-cell receptor, *and* either ≥1,000/μL Sézary cells *or* one of the following: (1) increased $CD4^+$ or $CD3^+$ cells with CD4/CD8 ratio ≥10 or (2) increased $CD4^+$ cells with abnormal immunophenotype including loss of CD7 or CD26. B_1 is now defined as >5 % Sézary cells and less than B_1.

Under the ISCL/EORTC update, erythrodermic MF patients without overt lymph node involvement ($T_4N_{0-2}M_0$) are now differentiated into two subgroups based on the blood involvement: stage IIIA ($T_4N_{0-2}M_0\mathbf{B_0}$) or IIIB ($T_4N_{0-2}M_0\mathbf{B_1}$) [3]. B_2 is now comparable with lymph node involvement (N_3). Stage IVA represents either blood involvement (B_2), which is designated as IVA_1, or lymph node involvement (N_3), which is designated as IVA_2. Therefore, erythrodermic

Table 19.2 ISCL/EORTC revision to the staging of mycosis fungoides and Sézary syndrome [3]

Stage	*T*	*N*	*M*	*B*
IA	1	0	0	0.1
IB	2	0	0	0.1
IIA	1.2	1.2	0	0.1
IIB	3	0–2	0	0.1
III	4	0–2	0	0.1
IIIA	4	0–2	0	0
IIIB	4	0–2	0	1
IVA_1	1–4	0–2	0	2
IVA_2	1–4	3	0	0–2
IVB	1–4	0–3	1	0–2

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B blood, *M* metastasis, *N* node, *T* tumor

patients are stage III, and erythrodermic patients with SS would be at least stage IVA_1 or IVB if they also have bone marrow involvement. Scarisbrick et al. [38] and Vidulich et al. [39] found that worse prognosis in erythrodermic patients was associated with H4 or SS cell counts of >10,000 cells/ul compared to SS counts of 1,000–10,000 cells/ul.

We recommend that bone marrow biopsy be done when there is blood involvement as marrow involvement is rarely seen in its absence. There is not a specific independent rating system for bone marrow involvement. We propose that major bone marrow involvement should warrant stage IVB which is used for visceral involvement. If there is an aggregate or minimal bone marrow involvement, we propose to consider staging the patient as IVA. The ISCL/EORTC recommends bone marrow biopsy specifically for patients with B_2 blood involvement or unexplained hematologic abnormalities [3].

Another change reflected in the ISCL/EORTC classification system is the elimination of the T_0 category for "clinically and/or histopathologically suspicious lesions," since clinical staging should be performed only for patients who have a definitive diagnosis of MF/SS and/or algorithmic diagnosis of early MF [33]. The T_0 stage could be used in MF/SS patients whose lesions have clinically resolved or in rare cases of clinically invisible MF which have positive histology [40]. The term "parapsoriasis" is often used in the cases of suspicious clinical lesions in the absence of diagnostic histology.

The updated classification also eliminated the need for biopsy of lymph nodes that are not enlarged on physical examination or imaging for staging purposes. A clinically abnormal peripheral node is now defined as measuring ≥1.5 cm in the longest transverse diameter or any size of palpable peripheral node that is firm, irregular, clustered, or fixed on physical examination [3]. The revision also further specified histopathologic grading systems for lymph nodes. Table 19.4 [41–44] compares the histopathologic staging of lymph nodes in the updated ISCL/EORTC classification system with the Dutch system [41] and the National Cancer Institute-Veteran's Affairs (NCI-VA) classification system [42–44]. The new system also divided N_1 and N_2 rating to include a and b to represent T-cell clone negative and positive, respectively, defined by PCR or Southern blot analysis of the T-cell receptor gene.

The ISCL/EORTC revision also considers visceral involvement to include splenomegaly on physical examination and by imaging that shows either enlargement or focal defects that are not cystic or vascular, even without biopsy confirmation. On the other hand, liver disease should be confirmed with biopsy. However, hepatic enlargement or focal defects that are not cystic or vascular on at least two imaging techniques may be considered to show tumor involvement. Any abnormalities found on imaging of the lungs or visceral organs other than the above would still warrant pathological evaluation, since they could be secondary to another malignancy or infectious disease.

Clinical Presentation of Mycosis Fungoides

The clinical presentation of MF is extremely variable, and indeed heterogeneity of skin lesions is one of the diagnostic criteria for early disease (Fig. 19.1) [33]. The overlapping clinical features of CTCLs present a challenge to successful diagnosis and medical management. Classic MF presents as faint-pink patches which can be

Table 19.3 ISCL/EORTC revision to the classification of mycosis fungoides and Sézary syndrome [3]

TNMB stages	Description
Skin	
T_1	Limited patches,[a] papules, and/or plaques[b] covering <10 % of the skin surface. It may further stratify into T_{1a} (patch only) vs T_{1b} (plaque ± patch)
T_2	Patches, papules or plaques covering ≥10 % of the skin surface. It may further stratify into T2a (patch only) vs T2b (plaque ± patch)
T_3	One or more tumors[c] (≥1-cm diameter)
T_4	Confluence of erythema covering ≥80 % body surface area
Node	
N_0	No clinically abnormal peripheral nodes[d]; biopsy not required
N_1	Clinically abnormal peripheral lymph nodes; histopathology Dutch grade 1 or NCI $LN_{0–2}$
N_{1a}	Clone negative[e]
N_{1b}	Clone positive[e]
N_2	Clinically abnormal peripheral lymph nodes; histopathology Dutch grade 2 or NCI LN_3
N_{2a}	Clone negative[e]
N_{2b}	Clone positive[e]
N_3	Clinically abnormal peripheral lymph nodes; histopathology Dutch grades 3–4 or NCI LN_4; clone positive or negative
N_x	Clinically abnormal peripheral lymph nodes; no histologic confirmation
Visceral	
M_0	No visceral organ involvement
M_1	Visceral involvement (must have pathology confirmation[f] and organ involved should be specified)
Blood	
B_0	Absence of significant blood involvement: ≤5 % of peripheral blood lymphocytes are atypical (Sézary) cells[g]
B_{0a}	Clone negative[e]
B_{0b}	Clone positive[e]
B_1	Low blood tumor burden: >5 % of peripheral blood lymphocytes are atypical (Sézary) cells but does not meet the criteria of B_2
B_{1a}	Clone negative[e]
B_{1b}	Clone positive[e]
B_2	High blood tumor burden: ≥1,000/μL Sézary cells[g] with positive clone[e]

From Olsen et al. [3]; © American Society of Hematology. Used with permission.
B blood, *M* metastasis, *N* node, *NCI* National Cancer Institute, *T* tumor
[a]For skin, patch indicates any size skin lesion without significant elevation or induration. Presence/absence of hypo- or hyperpigmentation, scale, crusting, and/or poikiloderma should be noted
[b]For skin, plaque indicates any size skin lesion that is elevated or indurated. Presence or absence of scale, crusting, and/or poikiloderma should be noted. Histologic features such as folliculotropism or large-cell transformation (>25 % large cells), $CD30^+$ or $CD30^-$, and clinical features such as ulceration are important to document
[c]For skin, tumor indicates at least one 1-cm diameter solid or nodular lesion with evidence of depth and/or vertical growth. Note total number of lesions, total volume of lesions, largest size lesion, and region of body involved. Also note if histologic evidence of large-cell transformation has occurred. Phenotyping for CD30 is encouraged
[d]For node, abnormal peripheral lymph node(s) indicates any palpable peripheral node that on physical examination is firm, irregular, clustered, fixed or 1.5 cm or larger in diameter. Node groups examined on physical examination include cervical, supraclavicular, epitrochlear, axillary, and inguinal. Central nodes, which are not generally amenable to pathologic assessment, are not currently considered in the nodal classification unless used to establish N_3 histopathologically
[e]A T-cell clone is defined by PCR or Southern blot analysis of the T-cell receptor gene
[f]For viscera, spleen and liver may be diagnosed by imaging criteria
[g]For blood, Sézary cells are defined as lymphocytes with hyperconvoluted cerebriform nuclei. If Sézary cells are not able to be used to determine tumor burden for B_2, then one of the following modified ISCL criteria along with a positive clonal rearrangement of the TCR may be used instead: (1) expanded $CD4^+$ or $CD3^+$ cells with CD4/CD8 ratio of 10 or more or (2) expanded $CD4^+$ cells with abnormal immunophenotype including loss of CD7 or CD26

Table 19.4 Histopathologic staging of lymph nodes in mycosis fungoides and Sézary syndrome [3]

Updated ISCL/EORTC classification	Dutch system [41]	NCI-VA classification [42–44]
N_1	Grade 1: dermatopathic lymphadenopathy (DL)	LN_0: no atypical lymphocytes LN_1: occasional and isolated atypical lymphocytes (not arranged clusters) LN_2: many atypical lymphocytes or in three to sixcell clusters
N_2	Grade 2: DL; early involvement by MF (presence of cerebriform nuclei >7.5 μm)	LN_3: aggregates of atypical lymphocytes; nodal architecture preserved
N_3	Grade 3: partial effacement of LN architecture; many atypical cerebriform mononuclear cells (CMCs) Grade 4: complete effacement	LN_4: partial/complete effacement of nodal architecture by atypical lymphocytes or frankly neoplastic cells

From Olsen et al. [3]; © American Society of Hematology. Used with permission
CMCs cerebriform mononuclear cells, *DL* dermatopathic lymphadenopathy, *LN* lymph node, *MF* mycosis fungoides

discreet lesions or diffuse areas. In skin of color, early MF lesions are often hypopigmented or hyperpigmented or have multiple colors. Lesions can be purple, red, salmon colored, orange, brown, grey, or white in color. Use of a Wood's light can distinguish hypopigmentation from depigmentation seen with vitiligo. Early lesions of any shade may also have dryness or fine scaling present and may or may not have symptomatic itching. The most common sites of early lesions are in photo-protected areas (e.g., the buttocks, medial thighs, and breasts) which is another criteria used for early diagnosis. The lesions are insidious or vague in onset and may come and go unrecognized and be misdiagnosed for years being passed off as dermatitis, eczema, or dry skin. Lesions may be well demarcated or with diffuse borders becoming confluent. When the infiltrate involves follicles or adnexal structures, the clinical lesions are follicular papules or accentuation or areas of alopecia. The MF variant "poikiloderma vasculare atrophicans" is characterized by large patches of red, brown, and white colors with telangiectasias and atrophy. PVA most often involves the bathing trunk area, buttocks, or breasts and is difficult to clear due to melanophages and pigment alteration.

Over a variable time period, MF can evolve from flat patches (eczematous lesions) into more infiltrated and scaly plaques (psoriasiform lesions), and dermal infiltrates can give rise to tumors and ulceration (T3). Patients with MF may also have coexistent lymphomatoid papulosus (LyP) which are self-regressing papules with high expression of CD30. Since the latter receptor is also seen in anaplastic large T-cell lymphoma, LyP may be mistaken for transformation of MF. MF patients may present with or become erythrodermic (defined as >80 % pink or red color of their body surface) and may or may not have significant blood involvement. "Erythrodermic MF" is used when patients do not have significant blood involvement to be labeled as SS [39]. Since staphylococcal colonization is frequently associated with development of erythroderma with significant improvement when addressed, erythrodermic patients should have baseline cultures of skin performed routinely, and staph colonization should be eradicated since staph sepsis is the most common cause of death, usually as the result of central line placement [20].

The diagnosis of early MF requires a combination of specific clinical and histological findings which are detailed in a new algorithm proposed by the ISCL [33]. Early patch-stage MF is notoriously difficult to diagnose histologically as it evolves over time from chronic eczematous or psoriasiform dermatitis. Multiple skin biopsies are often performed before the diagnosis is assured. Biopsies should be taken from the oldest, established skin lesions or the thickest lesions following a 4-week washout from topical corticosteroid or other therapy. The International

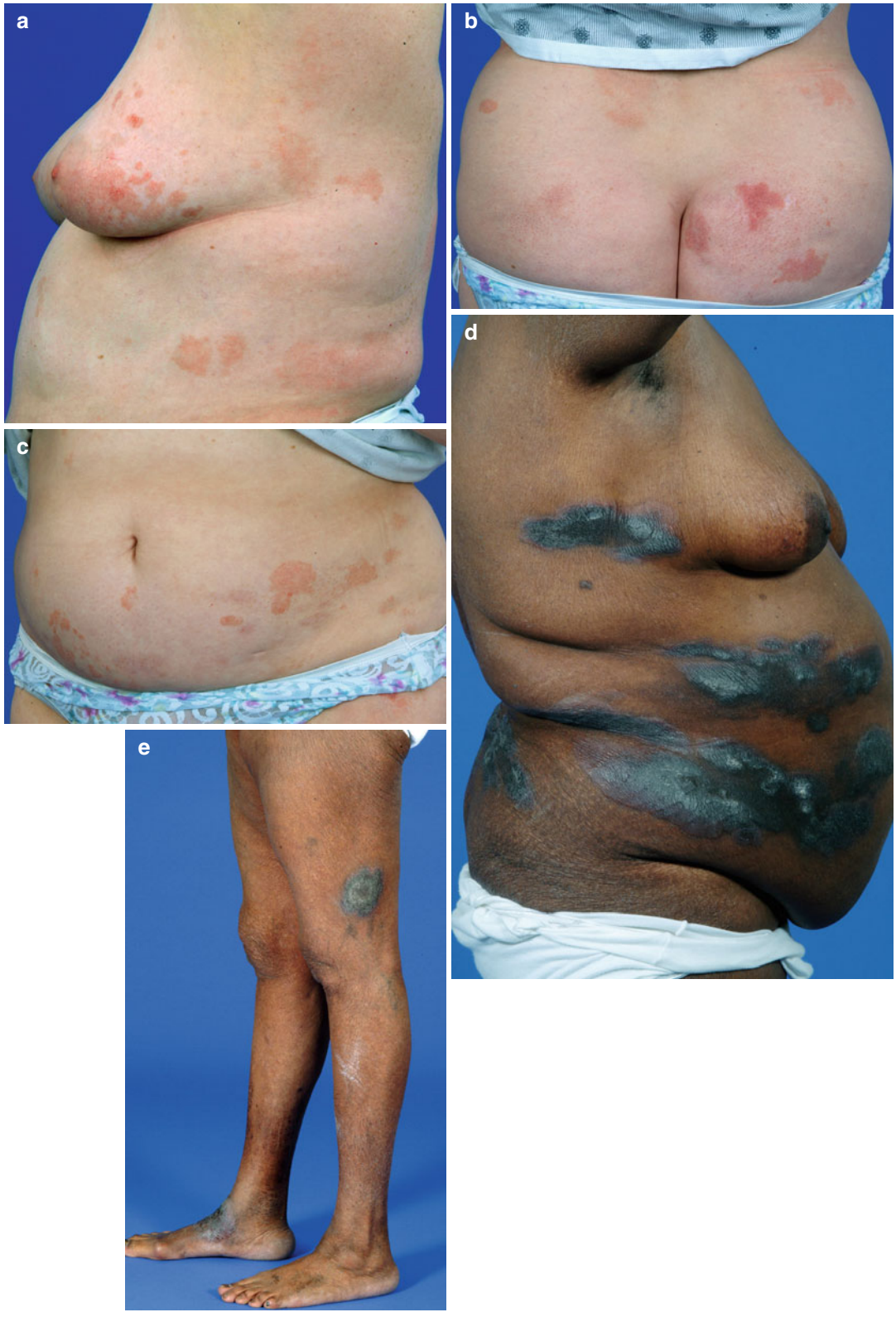


Fig. 19.1 Heterogeneous clinical lesions of patients with MF at all T stages. Pink patches (T1) on sun-shielded area of early MF Stage IA. (**a**-**c**); Hyperpigmented plaques on body and foot (T2) (**d**-**e**)

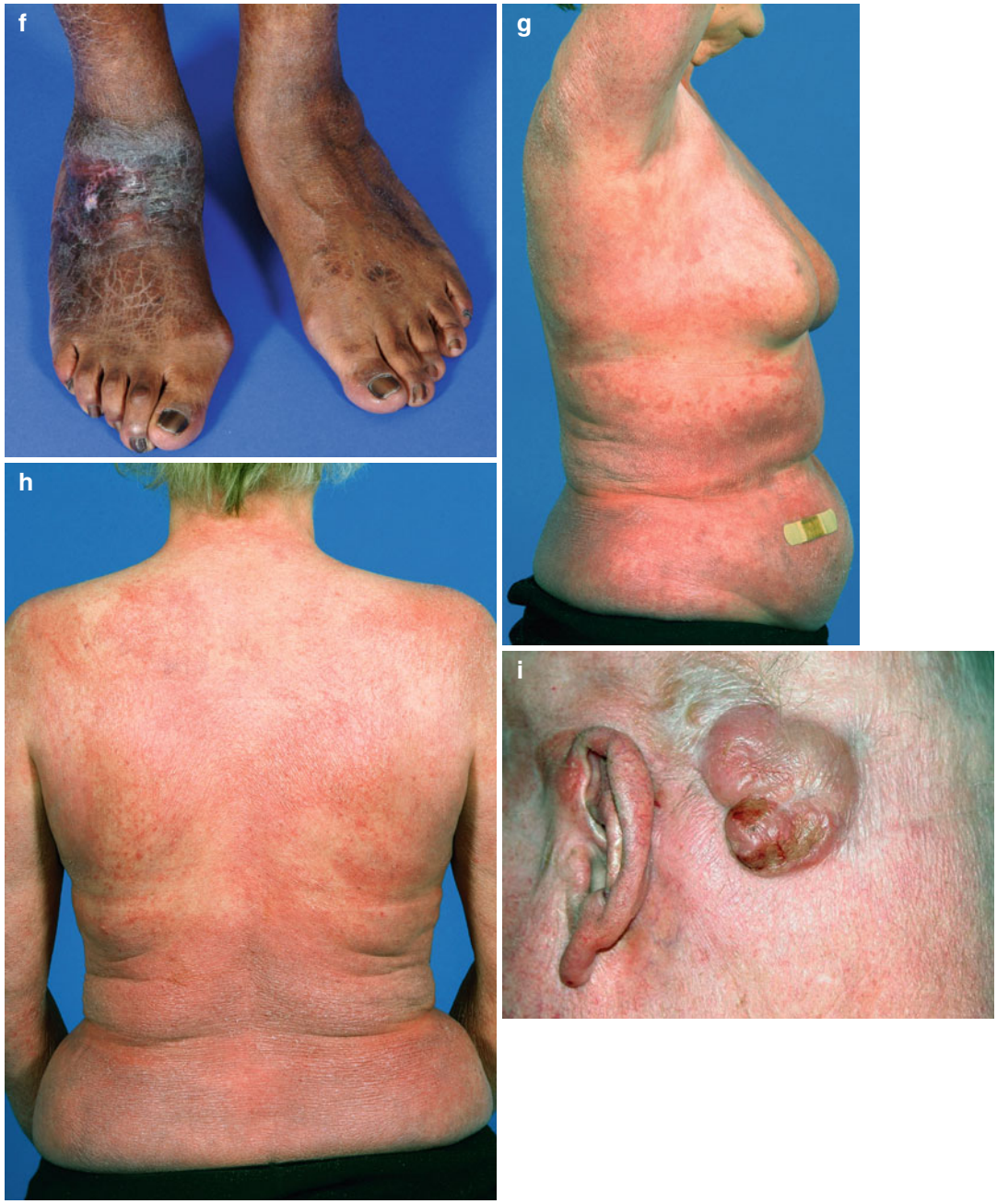


Fig. 19.1 (continued) Exfolative erythroderma (T4) with erythrodermic MF or Sezary Syndrome and staphylococcus colonization (**g**-**h**); MF Tumor T3 Stage IIB (**i**)

Society for Cutaneous Lymphomas (ISCL) [33] proposed a new diagnostic algorithm for the diagnosis of early MF, based on a point system evaluating clinical appearance, histopathologic diagnosis, molecular biology (clonal T-cell receptor gene rearrangement), and immunopathology (Table 19.5) [3, 33]. At least four points—two clinical and two histological—are required for the diagnosis. The clinical criteria include persistent dermatitis, heterogeneous lesions appearing on sun-shielded areas, or poikiloderma (Table 19.5). Figure 19.1 shows the spectrum of

Table 19.5 Algorithm for the diagnosis of early mycosis fungoides [3, 33]

Criteria	Major (2 points)	Minor (1 point)
Clinical		
Persistent and/or progressive patches and plaques plus	Any 2	Any 1
(1) Non-sun-exposed location		
(2) Size/shape variation		
(3) Poikiloderma		
Histopathologic		
Superficial lymphoid infiltrate plus	Both	Either
(1) Epidermotropism without spongiosis		
(2) Lymphoid atypia[a]		
Molecular/biological		
Clonal TCR gene rearrangement	NA[b]	Present
Immunopathologic		
(1) CD2,3,5 less than 50 % of T cells	NA[b]	Any 1
(2) CD7 less than 10 % of T cells		
(3) Epidermal discordance from expression of CD2,3,5, or CD 7 on dermal T cells		

This research From Olsen et al. [3]; © American Society of Hematology. Used with permission
Original material was reprinted from Pimpinelli et al. [33]; with permission from Elsevier
MF Mycosis fungoides, *NA* not applicable, *TCR* T-cell receptor
[a]Lymphoid atypia is defined as cells with enlarged hyperchromatic nuclei and irregular or cerebriform nuclear contours
[b]Not applicable since it cannot fulfill any major criteria

MF lesions including patch, plaque, tumor, and erythroderma.

Minimal diagnostic histologic criteria for early MF include a superficial perivascular infiltrate of lymphocytes with enlarged, hyperchromatic, cerebriform nuclei (*atypical lymphocytes*) and the presence of $CD4^+$ T-cell *epidermotropism*. The clustering of clonal T cells around epidermal Langerhans' cells (Pautrier's microabscesses) (Fig. 19.2a) shows the dependence of the T cells on antigen presentation by dendritic cells. Only a subset of cases (4–38 %) have Pautrier's microabscesses [45] and more commonly seen are single T cells lining up along the basal layer or single epidermal T cells. Skin biopsies from diffuse erythroderma in de novo Sézary syndrome will not show epidermotropism but rather perivascular atypical lymphocytic infiltrates (Fig. 19.2b) [6]. Furthermore, if an MF patient has nodal biopsy first without examination of the skin, the diagnosis will be that of "peripheral T-cell lymphoma," since MF cannot be diagnosed in a node. Patients presenting with nodal peripheral T-cell lymphoma should also have a careful history and skin exam to exclude the diagnosis of MF/SS which can be treated less aggressively.

Current Treatment Strategies

Based on the pathophysiology and absence of a cure for MF/SS, the application of a stage-focused skin-directed treatment approach is recommended for patients with early-stage MF and has recently been reviewed in detail [46, 47]. The EORTC has recently published consensus recommendations for the stage-dependent management of patients with MF/SS; however, more agents are likely available in the United States [48]. A treatment algorithm by stage is shown in Fig. 19.3.

Since the atypical T cells are skin-homing lymphocytes, skin-directed therapies can produce long-term remissions that can last for many years. Most of the agents available for use topically induce the apoptosis of T cells and may also alter epidermal differentiation that may provide growth factor support to the malignant cells. There is currently no evidence that aggressive

upfront chemotherapy is able to cure MF or impact overall survival [49]; hence, sequential skin-directed therapy for early-stage patients is recommended [50].

In early stages of MF, frontline therapy recommended first is topical steroids with low to higher potency [46]. For small and limited lesions, including coexisting lymphomatoid papulosis, clobetasol or targretin gel is very effective [51]. When larger areas need to be treated or for adjuvant therapy in SS, triamcinolone or hydrocortisone cream is recommended, sparing intertriginous areas. Retinoids are most effective for hypertrophic plaques, scalp alopecia, or acral lesions on palms or soles [52], and nitrogen mustard is used in steroid-resistant T1 or T2 patients for follicular lesions and as adjuvant therapy after stopping UVB or radiation. One generally reserves phototherapy alone or with biological response modifiers for more extensive skin disease (T2). Chemotherapy does not cure CTCL patients, and monotherapy agents with high response rates are preferred to combination therapies in

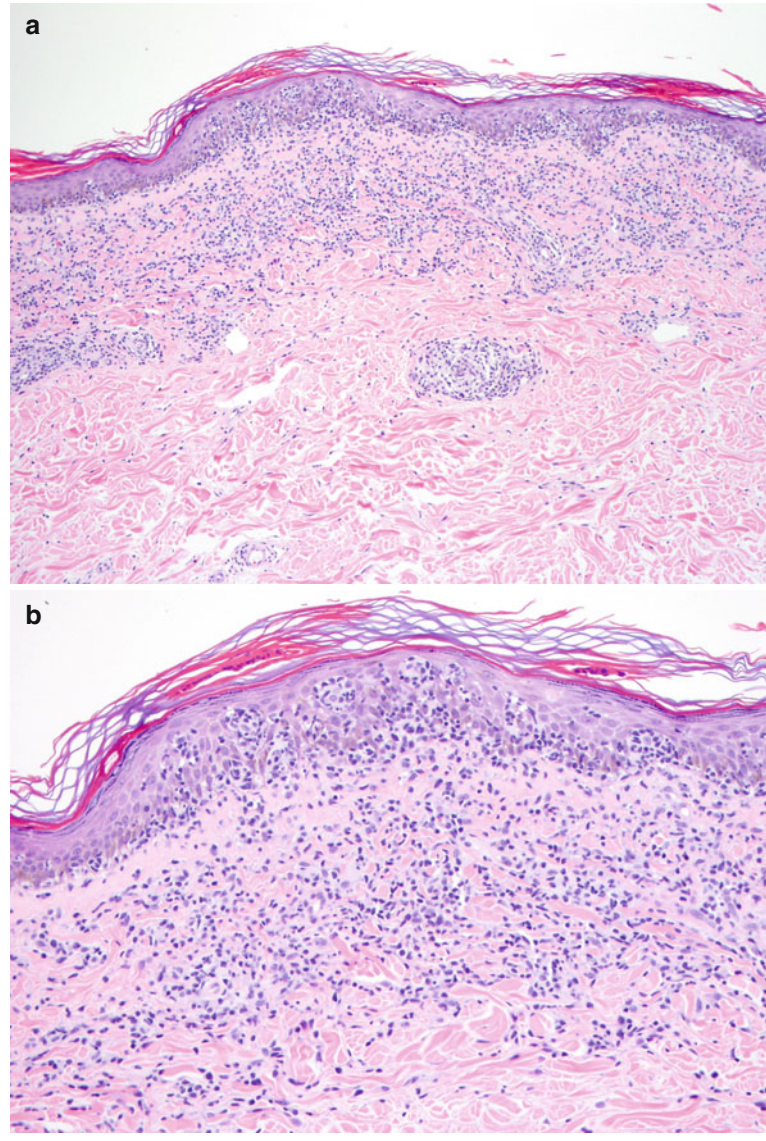


Fig. 19.2 Mycosis fungoides, patch-plaque lesion. (**a**–**c**) Routine H&E stained sections demonstrate skin (**a**, 10×) with a lichenoid lymphocytic infiltrate occupying the superficial dermis with marked epidermotropism (**b**, 20×). The cells exhibit cytologic atypia, including enlarged, hyperchromatic, hyperconvoluted nuclei, and they form numerous Pautrier microabscesses in the epidermis (**c**, 40×). Immunohistochemical studies demonstrate the infiltrate to be comprised predominantly of $CD3^+$ (**d**, 40×), $CD4^+$ (**e**, 40×) T cells. CD8 (**f**, 40×) highlights rare cells in the infiltrate (Courtesy of Michael Tetzlaff, MD, Assistant Professor, Department of Pathology, UT-MD Anderson Cancer Center, Houston, TX)

Fig.19.2 (continued)

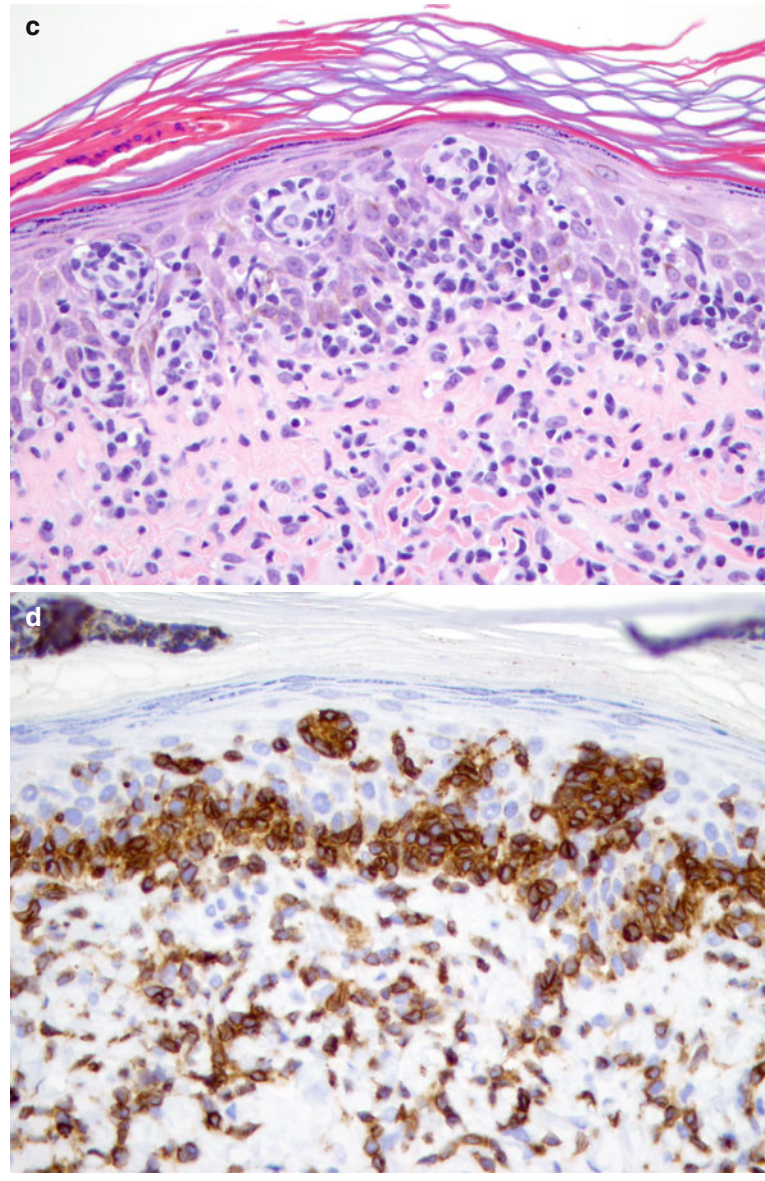
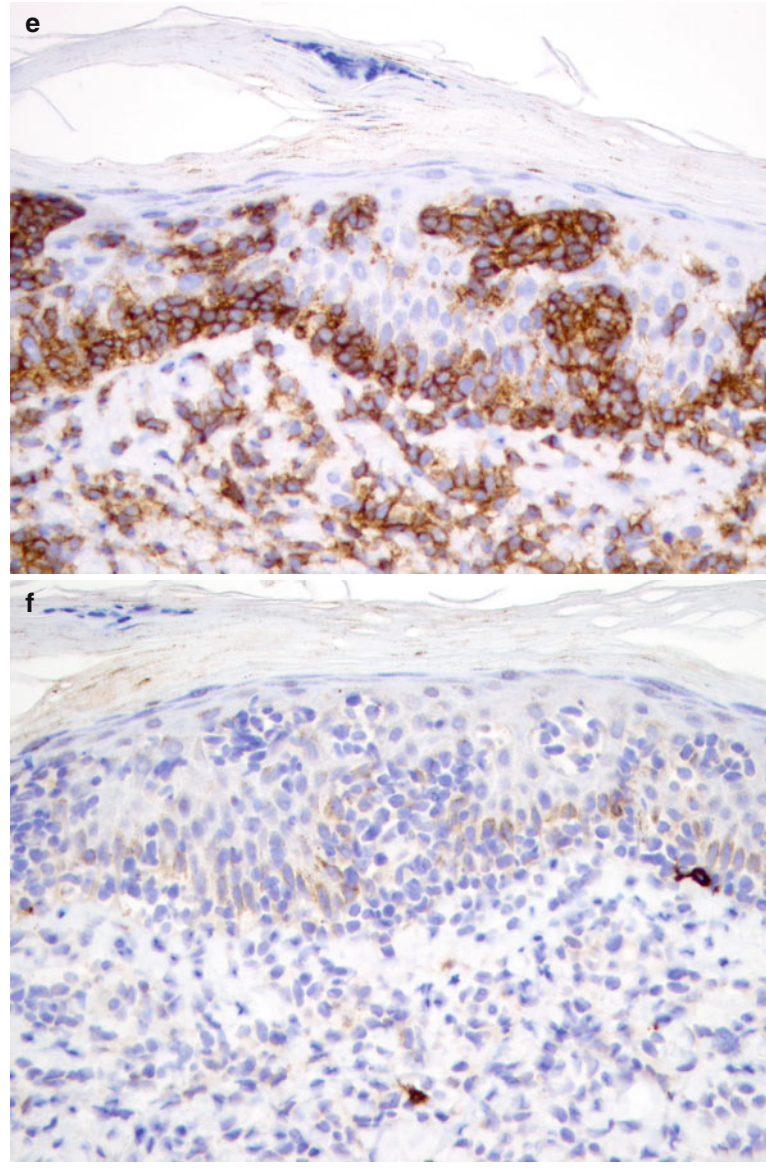


Fig.19.2 (continued)



these highly immunocompromised advanced patients. Non-ablative allogeneic transplantation following electron beam radiation may be successful in selected patients, but there are risks including mortality from infection, recurrent disease after transplant, and chronic graft-versus-host disease [25].

Skin-Directed Topical Therapies

Emollients

Use of skin emollients containing lipids or glycerin is very helpful as an adjunct therapy for patients with early MF, just as they are in

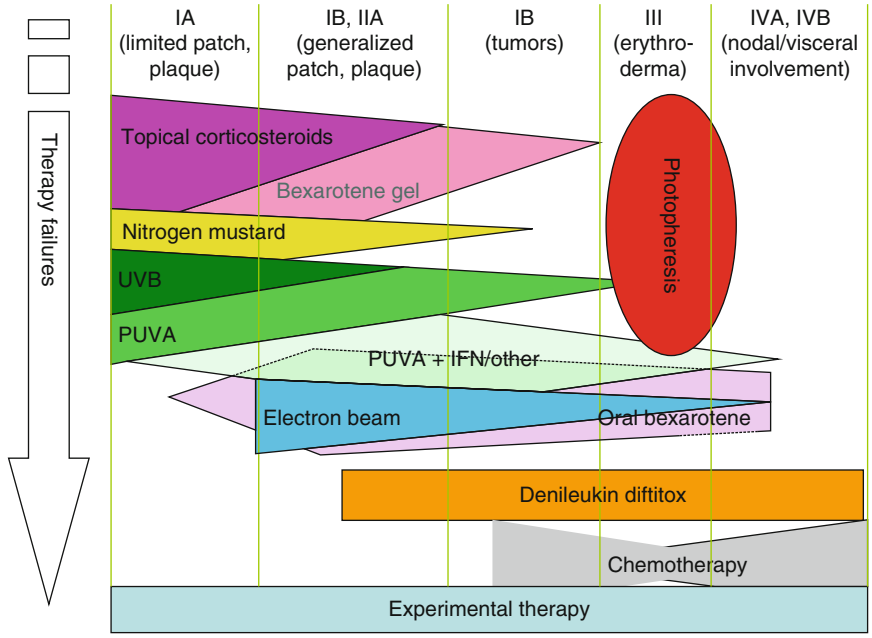


Fig. 19.3 Treatment of MF by stage. Stage is across the *top* of the diagram from T1 (*left*) to T4 (*right*)

patients with eczema, atopic dermatitis, and psoriasis. Hydration or repair of a compromised epidermal barrier helps decrease erythema, pruritus, and scaling within lesions and diminishes inflammatory cytokines. In the only randomized, blinded, placebo-controlled trial to date investigating topical treatments of MF, BCX-34 (peldesine) cream was evaluated against a placebo control vehicle of 100 % glycerin for its efficacy in treating patch and plaque MF. Although 28 % of the treated patients responded to the drug, 24 % of patients using the glycerin base placebo also responded to treatment (p-value = 0.677), including patients with complete response [53]. These data suggest an important adjunct role for emollients in the treatment of early MF patients. Glycerin-based moisturizers (e.g., in Cetaphil, intensive care lotions), Vaseline or Aquaphor, and the newer lipid barrier repair lotions (e.g., Ceravé, Restoraderm) can be purchased over the counter. Emollients should be applied generously to wet skin after bathing for maximal efficacy.

Topical Corticosteroids

Corticosteroids (CS) remain the most commonly used initial therapies for early-stage MF; their use often precedes a definitive diagnosis of MF in many patients. Topical steroids also have an adjunct role in combination with other skin-directed or systemic therapies for patients at all stages. Although no randomized placebo-controlled trials have been conducted, early anecdotal studies indicated total response rates of 80–90 % [54–56]. The largest prospective study was conducted by Zackheim et al. who evaluated 79 patients with patch-stage Tl and T2 MF [57]. Tl and T2 patients were treated with class I–III compounds, and the remainder of T2 patients applied either class II or III CS. The overall response rate (ORR) was higher for Tl (94 %) than for T2 (82 %) patients. Complete responses (CRs), as defined by complete clinical regression of all MF lesions for a minimum of 4 weeks, were achieved in 63 % of the T1 and 25 % of T2 patients. Posttreatment biopsy specimens were

obtained in seven patients who achieved CR, and all showed histological clearing. This study underscores the need to take patients off steroids before doing a biopsy to make the diagnosis of MF, since steroids will get rid of the epidermic T cells that are required for a diagnostic biopsy. Unfortunately, after discontinuation of topical steroids, responses are not sustained in all patients, and long-term use of potent steroids may result in atrophy. Only 37 and 18 % of T1 and T2 patients attained lasting CRs, and 43 and 50 % of T1 and T2 patients, respectively, attained partial remissions (PR) [57].

Triamcinolone 0.1 % cream with warm wet-wrap occlusion is very effective in all stages of MF, reducing redness, scaling, and pruritus, especially in erythrodermic patients [58]. It is advisable to use the lowest potency CS to achieve disease responsiveness while avoiding adverse effects. For example, hydrocortisone 2.5 % compounded in Eucerin can provide both steroid and lubrication following initial treatment with higher potency steroids and is especially useful in areas like face, groin, and axilla where atrophy is more likely. Advantages of topical corticosteroids are a response comparable to other topical MF treatments, low cost, availability, patient acceptance, product stability, minimal side effects, and familiarity with drug class. Further evaluation of short- and long-term efficacy and relapse rates with a larger study group would be helpful. Topical CS is often used in combination with other skin-directed and systemic therapies and may abrogate the irritancy of topical nitrogen mustard and topical retinoids.

Retinoids and Rexinoids

Retinoids are vitamin A derivatives that modulate proliferation and differentiation of both keratinocytes and lymphocytes. Retinoids are steroid-like ligands for RAR or RXR retinoid receptors belonging to the large superfamily of steroid hormone receptors. Other receptors in this family include glucocorticoid, thyroid hormone, and vitamin D_3 receptors that are DNA-binding proteins. Ligand-receptor dimers bind to specific retinoid response elements in the DNA of promoters forming nuclear transcription complexes that modulate gene transcription.

Given systemically, retinoids modulate pathways involved in inflammation, cellular differentiation, apoptosis, and sebaceous gland differentiation [59].

The first RXR receptor-selective retinoid, called a rexinoid, is oral bexarotene which was approved for skin manifestations of CTCL in 1999 [60–62]. RAR retinoids, including accutane, etretinate, and now acitretin, have been widely used in MF since the 1980s and were the first biological agents used for managing CTCL patients. Topical retinoids and rexinoids have fewer side effects than oral retinoids but are not used topically over wide areas due to cost and irritation. Bexarotene 1 % gel was approved by the United States Food and Drug Administration (FDA) for treatment of refractory IA and IB MF skin lesions [60, 63]. The response to topical bexarotene was found to be dependent upon dose and frequency of application, and local irritation was also dose related. In a phase I/II trial of 67 stage IA–IIA patients, CR (defined as clearing of all treated lesions) was reported in 21 % of 67 patients [64]. An additional 42 % of patients experienced partial responses (PR) and with disease progression in only 16 %. Improvements in lesional erythema, scaling, pruritus, and plaque elevation were reported, and overall severity improved in 51 % of patients. Relapse-free intervals averaged 11–21 weeks but have been reported up to 5 years with maintenance application [64]. In a phase III clinical trial of topical bexarotene therapy in 50 patients with refractory stage IA, IB, or IIA MF, the overall response rates by Physician's Global Assessment of Clinical Condition, Composite Assessment of Index Lesion Disease Severity, and primary end-point classification were 44, 46, and 54 %, respectively [63].

Adverse reactions are few and predominantly mild to moderate in severity and are reversible with a break in therapy. Local reactions included

pruritus, pain, and irritation where the gel is applied. Irritation is managed by decreasing frequency of application or using low- to mid-potency topical corticosteroids [65]. Severe dose-related and treatment-limiting toxic reactions reported in 19 % of patients include facial edema, pain, neuralgia, skin necrosis, rash, and ulceration; however, only 3 % withdrew from studies because of adverse reactions [63]. Bexarotene is considered a pregnancy category X drug. Although significant systemic absorption is low with topical use, pregnant women should not use this product, and women of childbearing age must use reliable methods of contraception.

Tazarotene is a synthetic retinoid with affinity for RAR-γ[gamma] and RAR-β[beta] receptors, not RAR-α[alpha] receptors or RXR receptors. RAR-γ[gamma] is the predominant receptor in the epidermis, and its selectivity eliminates many of the treatment-limiting irritation reactions associated with generalized activation of all RAR subtypes. Tazarotene gel and cream are FDA approved for the topical treatment of psoriasis and acne vulgaris [66, 67]. Tazarotene also can prevent or improve steroid-induced atrophy [65]. The beneficial effects of tazarotene for photoaging [68] and basal cell carcinoma formation [69, 70] make it an attractive alternative therapy to counteract side effects of prolonged phototherapy [71].

We conducted a small open-label pilot study assessing the efficacy and tolerability in 20 patients with MF involving <20 % body surface area (BSA) [52]. Sixteen MF patients with 99 index lesions were treated for at least 4 weeks with tarazotene 0.1 % gel applied once daily for 12–24 weeks. This regimen cleared 35 % of MF index lesions. Although no complete responses occurred, 63 % of all patients enrolled experienced partial responses with 58 % of patients experiencing at least a moderate (>50 %) global improvement of all index lesions. Histopathology and immunohistochemistry results showed reductions in lymphocytic infiltrates and percentage of CD45RO (+) lymphocytes and increases in the percentage of $CD8^+$ lymphocytes during the course of therapy.

The most common adverse reactions with the 0.1 % aqueous gel of tazarotene were mild to moderate erythema, pruritus, burning, dryness, desquamation, and irritation [52]. Fissuring, allergic dermatitis, and nausea are less common but have been reported. As with bexarotene, topical tazarotene has possible teratogenic potential as enough is absorbed and is not recommended for use during pregnancy.

Alitretinoin, or 9-cis-retinoic acid, is a naturally occurring pan-acting retinoid that activates both RARs and RXRs. The FDA approved the 0.1 % gel for treatment of cutaneous lesions of AIDS-related Kaposi's sarcoma [72]. A total of six MF patients were treated with alitretinoin gel in a phase I–II study and one case report [73]. After local application of 0.1 % alitretinoin gel twice daily, patients experienced significant improvement in scaling, plaque elevation, and erythema. One patient attained CR of a recalcitrant lesion on the sole. There is a higher incidence of local reactions in comparison with other retinoids.

In clinical practice, topical retinoids are most effective in thicker psoriasiform plaques and even tumors, plaques, for lymphomatoid papulosus [51], for facial or hand lesions, and scalp or follicular MF lesions [74]. Regrowth of hair in areas of alopecia is another beneficial effect [75]. Retinoids may counteract steroid atrophy but are too irritating to use in the intertriginous areas where atrophy is most likely to occur. Topical retinoids are helpful adjuvants with UVB phototherapy and an alternative to steroids or nitrogen mustard in children or young adults with MF.

Nitrogen Mustard (Mustargen)

Early-stage MF with disease limited to the skin that is refractory to topical steroids or retinoids can be managed successfully with topical nitrogen mustard (NM, Mustargen, mechlorethamine hydrochloride). This drug has been used for treating MF since 1959 and allows large areas of the body to be treated in contrast to the former agents [76]. NM is an alkylating agent that reacts with DNA, resulting in the donation of alkyl groups to DNA, disrupting DNA synthesis. The action of NM may be mediated by a combination

of cytological properties and immune stimulation. In the past, NM was mixed in water or, more recently, in petroleum ointment for clinical use but was not approved by the FDA. The compounding requirement as well as an exorbitant increase in price has made topical NM difficult to obtain for many patients. A large randomized non-inferiority trial has been completed, and the data has been submitted to the FDA for approval.

Remissions with the use of NM ointment may require 6–12 months or longer to achieve [76]. Complete response (CR) rates are reported from 63 to 75 % in stage IA and IB patients, making it as effective as corticosteroids, without unwanted atrophy or adrenal suppression [77–79]. There is a high median overall 5-year survival of 94 % for patients with stage IA (Tl) and 85 % for stage IB (T2) MF patients who have used NM [78, 80, 81]. An 11 % complete cure rate has been demonstrated, and complete responses (CRs) have also been reported with nitrogen mustard monotherapy in both T3 and T4 patients [79]. In general, the effectiveness of NM is predominately found among patients with patch- or plaque-stage MF because NM may not adequately penetrate far enough into the reticular dermis to clear tumors. After cessation of maintenance therapy, MF relapses at the same rate as in patients without maintenance therapy; therefore, after relapse, NM may be restarted with similar beneficial effect [81].

In one study, freedom-from-progression rates in Tl disease at 5 and 10 years were 92 and 85 % and in T2, 83 % at 5 and 10 years, respectively [5, 82]. One third of Tl patients enjoy long-term disease-free survival with CRs of up to 14 years, as reported by Vonderheid [79]. This indicates that long-standing remissions are possible with topical nitrogen mustard topical therapy. Nitrogen mustard has been helpful for maintenance to prolong remissions obtained with other methods such as total skin electron beam (TSEB) [83]. We have also found topical NM to be useful to treat patients with $CD30^+$ anaplastic large-cell lymphoma (ALCL) and in managing patients with lymphomatoid papulosis when other agents failed or could not be used.

NM is generally well tolerated without the toxicity caused by systemic administration. The most common side effects are irritant contact dermatitis or allergic contact dermatitis, occurring in 20–80 % patients treated with an aqueous solution. These side effects occur less frequently (<5 %) with the ointment formulation of 10 % in aquaphor [80]. Approximately 75 % of patients who develop intolerance to NM demonstrate positive to weakly positive patch tests. There may be the beneficial effect of developing contact dermatitis with subsequent clearing of MF lesions [84]. Other reactions include urticaria, pruritus, xerosis, and hyperpigmentation. Long-term, nonmelanoma skin cancers, especially squamous cell carcinoma, may occur with prolonged treatment with an incidence of approximately 11 %, and patients should be under skin cancer surveillance even if their MF is in remission [80].

Carmustine

Carmustine (bis-chlorethylnitrosuea, BCNU) is a nitrosourea alkylating agent used to treat early MF since the early 1970s but is rarely used today [85]. Carmustine undergoes spontaneous, nonenzymatic degradation to form electrophiles that alkylate DNA, leading to cross-linking of DNA. Like nitrogen mustard, it can be used in two formulations: a weak alcohol solution or ointment formulation. CR was achieved in 85 % of Tl patients and in approximately 50 % of T2 patients [86, 87]. Of individuals attaining CRs, relapse-free survival at 5 years was 37–60 % in Tl patients and 12–32 % in T2 patients. At 8 years, 26 % of Tl patients and 12 % T2 patients continued to be relapse-free [86, 87].

Adverse effects with carmustine are more common and more severe than for nitrogen mustard. The most common reactions are erythema and tenderness, often followed by persistent telangiectasias that are difficult to distinguish from MF lesions or radiation changes. Allergic contact dermatitis occurs less frequently than with nitrogen mustard and has been reported in less than 10 % patients [88]. The total percutaneous absorption or carmustine may be up to 28 % (detected by urinary excretion) with systemic side effects such as mild to moderate myelosuppression that occurs in

3–7 % of patients treated with either ointment or aqueous solution [89]. A complete blood count should be monitored during treatment. Although carmustine is an efficacious therapy for early MF and can be widely applied to involved areas, it is not used widely as a first-line agent due to its high frequency of side effects, and it remains a second- or third-line treatment for refractory skin-limited MF.

Topical Methotrexate

Methotrexate is a potent competitive inhibitor of dihydrofolate reductase, thereby selectively inhibiting DNA synthesis and preventing mitosis. Methotrexate also may block migration of activated T cells and decrease cutaneous lymphocytic-associated antigen (CLA) positive T-cell interactions with endothelial E-selectin [90]. Oral and parenteral forms are approved by the FDA for treatment of lymphoma [91]. Low-dose oral and intravenous methotrexate has been used for the treatment of advanced MF (Sézary syndrome) [92] and has demonstrated some efficacy in treating resistant patch, plaque, and tumor MF [93]. Oral methotrexate is also commonly used to suppress lymphomatoid papulosis [94, 95].

Topical formulations provide efficacious local concentrations while minimizing systemic exposure. One percent methotrexate has been compounded with Laurocapram (Azone®), a lipophilic compound with penetration-enhancing properties [96, 97]. Two pilot phase I/II studies of 14 patients total evaluated the efficacy of this compound in stage IA and IB MF [98]. Three patients attained partial remissions (PR) defined as greater than 50 % improvement. Four additional patients improved less than 50 %, and none had progressive disease (PD). Further studies are needed to establish proper concentration, frequency of application, long-term follow-up data, and duration of treatment needed to attain optimal efficacy. Its role as adjuvant therapy has not been investigated. Topical methotrexate may avoid immunosuppressive effects with systemic administration including emergence of B-cell lymphoma in patients with rheumatoid arthritis [99, 100].

Topical Calcineurin Inhibitors

The calcineurin inhibitors tacrolimus and pimecrolimus were approved by the FDA for the topical treatment of atopic dermatitis but have not been systematically studied in MF patients. Tacrolimus and pimecrolimus bind to the cellular protein, FK506-binding protein, and the complex binds to and inhibits the enzyme calcineurin's ability to dephosphorylate nuclear factor of activated T cells (NFAT), a key transcription factor [101]. Blocking NFAT prevents transcription of the cytokine T-cell growth factor IL-2 and prevents T-cell activation, decreases cytokine production, and downregulates IgE receptors [101, 102]. Tacrolimus also blocks superantigen-induced T-cell proliferation caused by *Staphylococcus aureus* in patients with atopic dermatitis. It may reduce staph colonization with staph which would be also desirable in MF patients [20, 103].

Topical tacrolimus potentially offers an anti-inflammatory effect, while avoiding the atrophy and acneiform eruptions commonly associated with the use of topical corticosteroids. An anecdotal case of a 29-year-old man with patch-stage MF was treated with tacrolimus ointment 0.1 % twice daily for 1 month and achieved complete remission [104].

While topical tacrolimus may be useful for facial lesions of early-stage MF, we found that pimecrolimus had been used in several patients preceding a diagnosis of folliculotropic MF (Duvic, submitted). Further studies are needed to evaluate the role of topical tacrolimus and pimecrolimus cream in MF patients, especially since they are immunosuppressive agents when given parentally with the risk of allowing lymphoma to emerge.

Imiquimod: Toll Receptor Agonist

Imiquimod is a novel topical immune response modifier belonging to the imidazoquinolone family of drugs [105]. It interacts with toll-like receptors, especially TLR-7 and has indirect antiviral and antitumor effects. Imiquimod is approved for the topical treatment of genital and perianal

warts/condylomata acuminate, actinic keratoses on the face and scalp, and superficial basal cell carcinomas. Imiquimod activates both the innate and acquired immune systems [106]. Imiquimod induces synthesis and release of the cytokines interferon (IFN)-α[alpha], tumor necrosis factor (TNF)-α[alpha], interleukin (IL)-6, and IL-12 that activate the adaptive immune response toward the TH-1 or cell-mediated pathway, while inhibiting the TH-2 pathway. Since progression of MF is characterized by an increasing tumor burden of TH-2 cytokine-producing cells, imiquimod is an obvious and attractive topical agent for MF. The same rationale underlies the use of CpGs (small pieces of DNA that mimic bacterial or viral genome, which stimulates the immune system through toll receptor) that also induce a TH-1 response [106].

The evidence for imiquimod in CTCL is based only on anecdotal case reports. A stage IA MF patient's lesions completely cleared after 4 months of treatment with imiquimod nightly [107]. In another case series reported by Deeths [108], six patients with stage IA to IIB mycosis fungoides treated their lesions with topical imiquimod 5 % cream three times per week for 12 weeks with both a histologic and clinical response rate of 50 %.

Data from isolated case reports and pilot studies indicates that imiquimod may be useful in the treatment of MF lesions. However, a randomized double-blind trial is needed to determine the safety and efficacy of this drug in the treatment of MF and to determine the most efficacious dosing regimen.

Phototherapy

Ultraviolet B Phototherapy

Ultraviolet light B (UVB) phototherapy is well tolerated and effective for the treatment of early-stage MF patients and as adjuvant therapy for patients who also need systemic therapy. Clinical studies of UVB therapy have demonstrated a compete response rate as high as 74 % in patients with stage I disease and a median time to remission of approximately 5 months [109, 110]. Generally, remissions induced by UVB phototherapy are long lasting with a median duration of 22–51 months. UVB in sunshine is most convenient, but UVB may be administered in a home light box or through the dermatology office. Although burning may occur with UVB phototherapy, the treatment is generally well tolerated [111].

Narrowband UVB (310 nm) has been shown to be effective for the treatment of mycosis fungoides in recent years. It is considered more effective than broadband UVB and nearly as effective as psoralen plus ultraviolet A (PUVA) phototherapy [112]. In a small study of eight patients with patch-stage MF, complete clearance of MF was achieved in six cases, and four patients had prolonged remissions. Mean duration of clinical improvement was 20 months. Narrowband UVB is preferable to PUVA because it does not require injection of oral psoralens which have side effects of nausea, headaches, and dizziness. Also protective eyewear is not required after treatment and UVB may be given during pregnancy. Narrowband and broadband UVB may have less photocarcinogenic risk than PUVA. Although potentially as effective as PUVA, it may be more difficult to wean a patient from narrowband UVB without relapse (Duvic, unpublished data).

Ultraviolet A-1 Phototherapy

Ultraviolet (UV) A-1 ranges between 340 and 400 nm and is thought to work mainly through induction of apoptosis in T-helper cells present in the dermal compartment [113] without reducing epidermal Langerhans cells [114, 115]. Continuation of UVA-1 phototherapy leads to a gradual increase in the number of apoptotic T-helper cells and a subsequent reduction of the inflammatory infiltrate and clinical improvement [116]. Early apoptosis appears to be highly specific for UVA-1 and is mediated through the generation of singlet oxygen species [113, 117]. Diseases treated with UVA-1 include cutaneous T-cell lymphoma, atopic dermatitis, cutaneous mastocytosis, scleroderma, morphea,

and graft-versus-host disease (GVHD) [113]. In a study of three patients with stage IA and IB MF, skin lesions began to resolve after only a few UVA-l exposures [117]. Complete clearing was observed between 16 and 20 exposures, regardless of whether the high- or medium-dose regimen had been employed. The main acute side effects of UVA-1 phototherapy are erythema, pigmentation, and reactivation of herpes simplex infection.

Photodynamic Therapy

Photodynamic therapy (PDT) involves the activation of a photosensitizer by light of the appropriate wavelength to induce a therapeutic effect. Activation of the photosensitizer leads to the formation of reactive oxygen species, especially singlet oxygen or free radicals, promoting apoptosis and tumor destruction. PDT has been approved by the FDA only for the treatment of actinic keratosis [118], with only anecdotal information for MF. Hypericin and D-aminolevulinic acid (ALA) topical formulations have each been investigated for their value as photodynamic compounds for the treatment of MF. In one study, seven lesions from four patients with cutaneous T-cell lymphomas (MF stage IB, $CD30^+$ anaplastic T-cell lymphoma, $CD8^+$ CTCL, and MF stage IIB) were treated with photodynamic therapy [119]. In another study, four patients with partial remissions from conventional therapies received ALA-PDT and achieved complete remissions [120]. In a third study, five MF tumors had complete responses following irradiation with a total light dose of 380 J/cm^2 [121].

Excimer Laser

Recently, several small case series have evaluated the efficacy of the monochromatic 308-nm excimer laser to treat individual lesions of early-stage MF and lymphomatoid papulosis [122, 123]. In five patients with stage IA MF, excimer laser was used twice weekly until clinical clearance or minimal residual activity was achieved [123]. A complete clinical response was obtained in four of the five patients' lesions, and minimal residual activity was observed in the fifth patient. Posttreatment biopsies of the treated areas showed a marked decrease of inflammatory infiltrates, with loss of epidermotropism and Pautrier's microabscesses [123]. Excimer laser may be a beneficial new treatment for early-stage MF lesions; however, it needs further study and clarification to determine its role as an adjunctive therapy for MF.

Total and Local Skin Electron Beam Therapy

Total skin electron beam (TSEB) is perhaps the most effective of the skin-directed therapies for patients with skin-limited disease, including tumors. Combined international data of 1,165 patients receiving TSEB therapy have shown complete response rates of close to 70 % [124–127]. Complete response rates are highest in patients with T1-limited disease, although given their already favorable prognosis TBSEB is usually reserved for patients with greater skin involvement. Five-year disease-free survival was shown to be 40–60 % for stage IA, 25 % for stage IB, 15 % for stage IIA, 2–20 % for stage IIB, and 10–25 % for stage III, patients [124].

Patients with disease stages greater than stage IA who achieve a complete response are prone to relapse. TBSEB is effective in patients with stage IIB tumor disease; however, complete responses may be short lived due to relapse with new lesions. The use of adjuvant nitrogen mustard after TBSEB remission can increase disease-free survival from approximately 15–55 % at 5 years in patients with T2 stage disease [128]. Patients with T4 stage erythrodermic MF may also have prolonged remissions when TBSEB is combined with extracorporeal photochemotherapy (ECP), and it is important for disease palliation [129].

Side effects of TSEB include erythema, swelling, exfoliation, tenderness, blister formation, alopecia, anhidrosis, and nail loss [130]. Effects are usually transient although hair and nail thinning may persist and skin aging is common. There is also an increased risk of developing

nonmelanoma skin cancers, which is higher in patients who receive adjuvant PUVA or topical nitrogen mustard. Due to the side effect profile of TBSEB, studies are under way to determine the efficacy of low-dose TSEB which has the advantage of shorter time course and potential for repeated use and reduced side effects.

Systemic Biological Response Modifiers

Biological response modifiers, such as interferon (IFN)-α[alpha] oral and oral retinoids, are usually reserved for second-line therapy in patients with extensive skin involvement, relapse, or refractory to topical therapy but is first-line therapy for patients with SS. As discussed above, as MF advances there is a shift from Th1 to Th2 cytokines leading to eosinophilia, atopy, and immunosuppression [131]. Biological response modifiers are important and very effective modulators of the immune response. The prototypic and most effective agent is alpha interferon that increases gamma interferon and TNF production, generating a cytotoxic T-cell response to the tumor that is mediated by $CD8^+$ cells. Interleukin 12 is also induced by gamma interferon and induces a $CD8^+$ T-cell response [132, 133]. Retinoids also cause T-cell apoptosis, modulate antigen-presenting cells, and modulate epidermal differentiation [134].

Interferons

Interferons comprise interferon-α[alpha], interferon-β[beta], and interferon-γ[gamma]. Interferons have antiproliferative, cytotoxic, and immunomodulating actions in CTCL. Administration of interferon-α[alpha] or Intron-A is usually initiated at a low dose of one to five million units subcutaneously given three times a week and gradually increased as tolerated. IFN-α[alpha] plus photopheresis is commonly administered as frontline combined immunomodulatory therapy to patients with SS or erythrodermic-CTCL [135]. Bexarotene is also added at lower doses.

The combination of PUVA plus IFN-α[alpha] is also widely used and appears to be synergistic. In a study of 39 patients with all stages of MF and SS, 36 of 39 patients achieved a complete response (62 %) or partial response (28 %) with median duration of 28 months, while on the combined regimen of IFN-α[alpha] and PUVA [136]. The response and response duration is superior with a combination of PUVA and IFN, to either treatment alone [137, 138]. However, patients may develop anti-interferon antibodies that may induce tolerance and resistance to response from drug. Combined therapy may suppress anti-interferon antibody formation [139]. In a study of 24 MF patients treated with combination of PUVA and IFN, none developed antibodies [140]. Interferon-alpha and PUVA are usually initiated concurrently, each given three times per week. PUVA administration is tapered gradually after the patients clear.

Interferon-α[alpha] has been studied in combination with other therapies. Surprisingly, a combination of IFN-α[alpha] plus retinoids has complete response rates similar to that observed with either therapy used alone [141–143]. Surprisingly, the combination of bexarotene plus IFN-α[alpha] induced a response rate of only 39 % [144] with 95 % confidence interval [CI] 17–64 %, compared to the higher response rate of bexarotene monotherapy for advanced and early MF (45–54 %) [58, 59]. However, in a case series of only 12 patients with refractory late-stage MF, an 83 % complete response rate was demonstrated using interferon and oral tretinoin together [145].

IFN-α[alpha] has also been used in combination with systemic chemotherapeutic agents and may be beneficial when combined with fludarabine or vinblastine compared to either chemotherapy alone [146, 147]. The response to IFN-α[alpha] plus pentostatin (overall response rate of 41 %) [148] was similar to response with pentostatin alone (overall response rate for CTCL ranging from 14 to 60 %, ≥35 % for majority of trials) [149–154].

Disease stage is predictive of response to IFN-α[alpha] therapy as a single agent, with more complete responses in patients with stage I (62.5 % complete remission) compared with

stage III–IV disease (16.5 % complete remission) [155]. Duration of disease is also a predictor of response, and patients who have had the disease longer may perform less well on IFN-α[alpha] [139, 156].

IFN-γ[gamma] and IFN-β[beta] have not demonstrated superiority to IFN-α[alpha] and are less well studied. In a phase II study of 16 patients who received intramuscular recombinant IFN-γ[gamma], 31 % of patients demonstrated partial responses, and none were in complete clinical remission [157]. There is one case of a patient achieving complete response after intravenous administration of IFN-γ[gamma] (14–16 MU/week) for 22 weeks [158]. Rook has reported responses to addition of interferon-gamma in SS patients who have failed to improve with interferon-alpha and photopheresis [159–163]. Interferon-β[beta] has been studied the least, and preliminary data shows poor efficacy in the treatment of CTCL [162].

Adverse effects of IFN-α[alpha] and IFN-γ[gamma] include flu-like symptoms consisting of fever, chills, myalgias, and malaise which may be reduced by premedication with acetaminophen [139]. Leukopenia, thrombocytopenia, hepatitis, mental status changes, fatigue, diarrhea, and anorexia are dose-related side effects. IFN-α[alpha] is associated with a 6 % incidence of thyroid dysfunction; hypothyroidism is more common than hyperthyroidism [163].

Interleukin-12

Cytokines are important in the pathogenesis of CTCL, and defects in IL-12 production may play a role in the cytokine profile shift from Thl to Th2 type that accompanies disease progression [131, 164]. IL-12 plays a significant role in the activation and differentiation of cytotoxic T lymphocytes [165]. Recombinant 1 L-12 normalizes 1FN production, enhances cell-mediated cytotoxicity, and augments natural killer cell cytotoxicity when added to peripheral blood mononuclear cells from advanced CTCL patients [133].

Three clinical trials with recombinant human IL-12 have shown its potency in treating CTCL patients. However, the drug has been removed from clinical development and is no longer available for investigation. IL-12 administered subcutaneously was associated with a 50 % response rate [166, 167]. Repeated administration of IL-12 induces a reversible suppression of IL-12-dependent responses, which may be secondary to decreased IL-12 receptor (IL-12R) expression and increased degradation of IL-12 signaling factors [166, 168]. IL-12 has also demonstrated efficacy as an intralesional agent in two patients with tumors [166]. A multicenter study of 23 MF patients treated with subcutaneous IL-12 at a dose of 300 ng/kg reported a partial response rate of 43 %. Adverse effects included low-grade fever, headache, depression, and one death from sudden onset upper respiratory infection with hemolytic anemia [132]. IL-2 following IL-12 was associated with synergistic enhancement reflecting a promising therapeutic benefit for CTCL [169]. IL-12 is also a powerful adjuvant therapy used for tumor vaccinations.

Isotretinoin, Etretinate, Acitretin

Oral retinoids, whose metabolites bind to retinoic acid receptors (RAR), have been widely used for CTCL for several decades. Isotretinoin, etretinate (which is no longer on the market), and acitretin have all been used. Most data centers around etretinate and isotretinoin, and cumulative data show an ORR of 50 % that is similar to that of bexarotene [170–173]. These agents are also often used in combination with PUVA [170], photopheresis [174, 175], or interferon [83, 176]. We reported long-term disease control and complete responses with isotretinoin in combination with interferon-alpha, which were used as the first arm of a combination approach (see combined modality protocol in section "Combination Chemotherapy"in Chap. 6, for details on this study) [83, 176]. Although response rates are similar with retinoids plus PUVA versus PUVA alone, the benefits of combination therapy included lower PUVA dose to clear and a longer remission on retinoid maintenance [170]. There has been no demonstrated improvement in the

response rates of chemotherapy plus retinoids versus chemotherapy alone [172]. Side effects of RAR retinoids include dryness, alopecia, arthritis, hepatitis, and bone spurs, and are less well tolerated than those seen with bexarotene.

Oral Bexarotene

Retinoids regulate multiple biologic pathways through two families of nuclear receptors: retinoic acid receptors (RARs) and retinoid X receptors (RXRs). Retinoid actions on tumor growth occur through binding of nuclear receptors, which subsequently function as transcription factors and mediate gene expression. Oral bexarotene was the first synthetic, RXR-selective retinoid or "rexinoid," to be studied in humans [177]. In December of 1999, bexarotene became the first and only retinoid approved by the FDA for the treatment of CTCL. Oral administration and lack of immune suppression are advantages treatment with bexarotene affords compared to chemotherapy alternatives. In vitro, bexarotene induced apoptosis of CTCL cell lines but required high doses, suggesting other effects may be important [134]. Richardson et al. reported that bexarotene alters adhesion molecules that govern T-cell trafficking resulting in a shift of T cells from the skin to the periphery [178].

Response to bexarotene monotherapy is dose dependent with an optimal dose of 300 mg/m^2/day. Two multicenter trials, one involving early-stage disease and the other advanced-stage patients, led to FDA approval of bexarotene [61, 62]. Higher doses of bexarotene led to higher response rates. The pivotal trial started at 650 mg/m^2/day, was reduced to 500 mg/m^2/day, and then to an optimal dose of 300 mg/m^2/day based on response and dose-limiting toxicity. The response rates for early-stage MF were 67 % at doses above 300 mg/m^2/day versus 54 % at 300 mg/m^2/day [159]. The response rates for advanced-stage disease patients were 55 % at >300 mg/m^2/day and 45 % at 300 mg/m^2/day [62]. Dose-limiting toxicity, hyperlipidemia with pancreatitis, occurred in a few patients at a dose of >300 mg/m^2/day. Lack of response at lower-dose levels was the rationale for 300 mg/m^2/day as the optimal dose for response and tolerability. At the optimal dose, overall response was 48 % in the combined group of early- and late-stage MF patients [61, 62].

We evaluated 70 patients with stage IA–IVB CTCL treated with oral bexarotene as a monotherapy or in combination with other agents and confirmed a response rate of 48 % for bexarotene monotherapy versus a response rate of 90 % when combined with two lipid-lowering agents [179]. Bexarotene may also reduce tumor and lymph node burden and has demonstrated efficacy in treating some patients with large-cell transformation and erythrodermic MF [179, 180]. Combinations of bexarotene with PUVA, interferon, ECP, and denileukin diftitox may lead to higher overall response rates of 90 % [179].

The most common adverse effects reported with bexarotene are dose dependent and include hypertriglyceridemia (82 %), hypercholesterolemia (30 %), central hypothyroidism (29 %), headache (20 %), asthenia (16 %), pruritus (13 %), and leukopenia (11 %) [62]. Hypertriglyceridemia can be prevented or reduced in severity by lipid-lowering agents and synthroid replacement, initiated with treatment. We prefer fenofibrate 145 mg with addition of atorvastatin if needed. Low-fat diet and omega 3 fatty acids are also helpful. Gemfibrozil should not be used to control bexarotene-induced hypertriglyceridemia as it is paradoxically associated with higher bexarotene levels, increased hypertriglyceridemia, and increased risk for pancreatitis.

Initially, thyroid function by free thyroxine levels should be monitored frequently initially. Thyroid replacement in the form of levothyroxine should be administered to keep the free T_4 normal. While the patient takes bexarotene, we generally give 25 μg of levothyroxine for each 75 mg capsule of bexarotene and increase the levothyroxine by 25 μg for each additional bexarotene tablet given. In a study at MD Anderson Cancer Center (MDACC), we first demonstrated that bexarotene suppresses thyrotropin secretion leading to reversible central hypothyroidism with low thyroid-stimulating hormone (TSH) and T_4 levels. Note that since bexarotene reduces

mRNA of TSH, TSH levels will always be low when patients are on bexarotene [181]. Normalization of thyroid function occurs as early as 8 days after cessation of therapy [181], and patients should be weaned off levothyroxine if bexarotene is discontinued. Bexarotene binds to RXR receptors, and they can bind to peroxisome proliferator-activated receptors (PPARs), increasing insulin sensitivity leading to hypoglycemia in diabetic patients on insulin. Thus, glucose levels should be monitored carefully, especially in diabetic patients.

HDAC Inhibitors

Histone deacetylases (HDAC) are enzymes which remove acetyl groups from core histone proteins [182]. Core histone proteins sterically control access of transcription factors to DNA and therefore modulate gene transcription. HDAC inhibitors are small molecules that bind to and block deacetylation by HDACs. Various HDACs with different selectivities appear to be effective in CTCL and are being investigated as antineoplastic agents in clinical trials. HDAC inhibitors repress deacetylation of tumor-suppressor genes and cell cycle regulatory genes, leading to the arrest of neoplastic cell growth and apoptosis [183]. HDAC inhibitors may also work as antiangiogenesis agents by decreasing VEGF expression [184, 185]. HDAC enzymes are divided into families (Class I–IV) based on homology to yeast HDAC proteins [186, 187].

HDAC inhibitors have demonstrated additive or synergistic effects with anthracyclines, tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), and all-trans retinoic acid [164, 183, 184, 188–190]. They are efficient radiation modifying agents and may be used as clinical radiation sensitizers/protectors [191]. A multicenter study combining low-dose electron beam radiation with vorinostat is in progress (Y. Kim, 2012 personal communication).

Vorinostat (Zolinza®; Merck, Whitehouse Station, USA), formerly suberoylanilide hydroxamic acid (SAHA), which was FDA approved for the treatment of relapsed or refractory CTCL in October of 2006, is an orally bioavailable inhibitor of class I and II HDACs [183, 185]. In addition to vorinostat, the HDAC inhibitors romidepsin (depsipeptide, FK-228), belinostat (PXD101), and LAQ824/LBH589 (panobinostat) have demonstrated therapeutic benefit as monotherapy in CTCL [192].

Vorinostat has demonstrated antineoplastic effects in leukemia, lymphoma, and solid tumor models in vivo [184, 190, 193, 194]. Preclinical studies have shown that vorinostat causes accumulation of acetylated histones in treated patients' tumors and blood cells and induces apoptosis in a broad range of cancer cell lines including Sézary cells [195]. Romidepsin also induces apoptosis of CTCL cells in vitro [196]. Vorinostat induces tumor cell apoptosis at concentrations to which normal cells are relatively resistant [197]. Both vorinostat and romidepsin down-modulate expression of the Th2 cytokine, IL-10 which is overexpressed in tumor cells [198].

Kelly et al. at Memorial Sloan-Kettering performed a dose-escalation study of 37 patients with advanced cancer given vorinostat/SAHA by 2-h intravenous infusions [189]. The starting dose of 75 mg/m^2/day was escalated to <900 mg/m^2/day with no dose-limiting toxicities. In part B of the trial, vorinostat was administered for 5 days every 1–3 weeks for solid tumor patients ($n=17$) and 5 days for 3 weeks for those patients with hematologic malignancies ($n=12$). The maximum-tolerated dose (MTD) in hematologic malignancies was 300 mg/m^2/day × 5 days for 3 weeks, and median duration of therapy was 6.4 weeks (range: 1.6–40 weeks) [189]. In their report of 73 patients with hematologic or solid tumors treated with oral vorinostat, the MTD was 400 mg/day and 200 mg twice daily for continuous daily dosing and 300 mg twice daily for 3 consecutive days per week. Thrombocytopenia was reported in 87 % of the patients with hematologic malignancies compared with 44 % of the patients with solid tumors.

In 35 patients with hematologic malignancies treated in phase I with either intravenous ($n=12$) or oral vorinostat ($n=23$) at continuous doses in the range of 400–600 mg/day or 200–400 mg twice daily, vorinostat demonstrated activity in

Hodgkin's disease, diffuse B-cell lymphomas, and CTCL (one MF patient with a >4-month PR) [199]. Observed dose-limiting toxicities were anorexia, dehydration, diarrhea, fatigue, neutropenia, and thrombocytopenia.

In a phase II dose-ranging trial, 33 patients with refractory or relapsed CTCL (stage IA–IVB) were treated with oral vorinostat in one of three sequential dosing cohorts [183]. Four of the 33 patients participated in two different dosing cohorts. Patients had a median of five prior therapies (range: 1–15), 85 % had advanced stage (≥ IIB) CTCL, and a third had SS. Twenty-four percent of patients achieved a documented PR, defined by ≥50 % decrease in severity-weighted assessment tool (SWAT) score, and one-third had pruritus relief, stable disease, or both. Responses were seen in a broad spectrum of patients: early-stage refractory MF, tumor stage with large-cell transformation, and in nodal and/or blood involvement. The median duration of response was 15.1 weeks (range 9.4–19.4 weeks) overall, which was lowest (9.4 weeks) in the intermittent dosing group of 300 mg twice daily 3 days out of 7 and highest (16.1 weeks) in the group treated with 400 mg/day. Grade 3/4 thrombocytopenia was most common (42 %) in the cohort treated with 300 mg twice daily continuously for 14 days and less common (8 %) in the other two cohorts. The most common toxicities were fatigue, diarrhea, altered taste, nausea, and dehydration. Overall, the 400 mg/day dose provided the most favorable risk-benefit profile and was selected for the pivotal registration trial [200].

In the phase IIB multicenter trial, oral vorinostat 400 mg/day was administered to 74 patients with stage IB–IVA MF/SS [200, 201]. The ORR was 29.7 %, 32 % patients had pruritus relief, and one patient with facial tumors had a near complete long-lasting response. Median time to progression (TTP) in all patients was 4.9 months. Eleven percent of patients had related serious adverse events, 11 patients required dose modifications, and there were three deaths in the study including one patient with hypertension and valvular heart disease. The most common drug-related adverse events were gastrointestinal symptoms (diarrhea [49 %], nausea [43 %], anorexia [26 %], dysgeusia, dry mouth, vomiting, constipation, and anorexia) or fatigue (46 %), thrombocytopenia, weight decrease, alopecia, muscle spasms, increase in creatinine, anemia, and chills [200]. Caution is indicated in patients with a history of deep-vein thrombosis or on warfarin therapy due to reported adverse events of pulmonary embolism and thrombocytopenia. ECG changes including ST-T wave changes, and QT prolongation was observed but were clinically insignificant [200].

Romidepsin is a cyclic pan-HDAC inhibitor that has been approved by the FDA in November 2009 for treatment of patients with CTCL who have received at least one prior systemic therapy [202]. Approval was based on two phase II studies, including a multicenter international study [203]. The overall response rate was 38 % with five complete responses, and median duration of response was 15 months. More recently, depsipeptide (romidepsin) also received approval for peripheral T-cell lymphoma based on a response rate of 38 % and median duration of response of 8.9 months (2–74 months) [204, 205]. It is administered intravenously at 14 mg/m^2 for 3 weeks out of 4 and has a slightly higher overall response rate than vorinostat. The adverse events are similar to other HDAC inhibitors. Both histone deacetylase inhibitors were associated with decrease in pruritus scores in patients who had baseline pruritus.

Photopheresis

Extracorporeal photochemotherapy (ECP, photopheresis) combines phototherapy with leukapheresis and is based on the DNA-damaging effect of light combined with photoactivated 8-methoxypsoralen (8-MOP) on pathogenic T lymphocytes [206–208]. Psoralens are furocumarins, a group of chemicals which strongly absorb UV light maximally in the UVA range [209]. The most extensively used therapeutic psoralen, 8-MOPP, intercalates between DNA base pairs. Upon exposure to UVA radiation, covalent cross-linking of DNA occurs resulting in proliferative arrest of treated cells. The

combination of photosensitizing agent 8-MOP and mononuclear cells collected by apheresis are irradiated by ultraviolet light A (UVA) ex vivo and reinfused into the patient.

The precise mechanism of action of ECP is not completely elucidated. Multiple mechanisms are thought to generate an immune response or vaccination against tumor cell antigens and generation of cytotoxic CD8 cells. Treatment effects include photodestruction of cells, induction of T-cell apoptosis, monocyte activation and maturation, stimulation of cytokines, and stimulation of cell-mediated immune response with changes in immune reactivity of the patient [159, 210–214].

In an animal model, ECP reverses GVHD by inducing donor regulatory T cells [215].

CTCL patients, especially those with erythrodermic MF and Sézary syndrome, have been treated with ECP for more than 20 years using the FDA-approved device for CTCL [216]. ECP has recently shown promising results for SS [217] and has been found to be effective for GVHD [216]. It is also effective to treat solid organ transplantation rejection and is being investigated as therapy for multiple autoimmune diseases. Whether the mechanism of action is the same in different conditions is unknown.

In 1987, Edelson et al. published the first multicenter trial suggesting the benefits of ECP in CTCL [218]. Twenty-seven of 37 patients (73 %) had greater than 25 % improvement, with an average 64 % decrease in cutaneous involvement after 22 weeks. Additionally, 88 % percent of lymphocytes in the treated cell concentrate were not viable after treatment. Infusion of the damaged cells led to a reduction in the $CD4^+/CD8^+$ ratio. Long-term follow-up demonstrated that erythrodermic patients treated with ECP had prolonged survival (median 60 months) compared to historic control groups (median 30 months) [218].

Follow-up studies involving ECP as a monotherapy have shown partial response rates from 20 to 88 % and complete response rates of 13–33 % of patients [219–233]. Studies demonstrating that ECP clinically improves and prolongs survival in patients with erythrodermic, and advanced-stage CTCL support the use of ECP as first line for stages III and IV patients [208, 225, 234–240]. There is controversy regarding the benefit of ECP in SS in prolonging survival [241]. Fraser-Andrews et al. found no significant difference in overall survival of 29 patients with SS who had received ECP (median 39 months) compared with 15 patients who did not receive ECP (median 22 and 27.5 months) [241]. Opponents argued that the study was limited by a small study population, patients were inadequately treated, ECP patients were heavily pretreated, and the ECP-treated patients may have had worse disease [234, 235]. We have studied overall survival in a cohort of 124 erythrodermic CTCL patients who were treated with ECP-combined immunotherapy, and their median survival overall was twice that reported by other centers [242]. Median survival of 2.5 years, reported previously, was limited only to leukemic SS patients whose count exceeded 10,000 SS cells. Prospective studies are needed to confirm the importance of ECP over immunomodulator therapy alone in patients with SS.

Certain features in patients with CTCL make ECP more likely to have a favorable therapeutic effect, and the presence of $CD8^+$ T cells is thought to be required [221, 222, 243–248]. ECP responders have been reported to have absence of bulky lymphadenopathy or visceral involvement, discrete lower numbers of Sézary cells (10–20 % of mononuclear cells), limited leukemia ($WBC < 20{,}000/mm^3$), short duration of disease (less than 2 years), normal numbers of cytotoxic T cells and normal natural killer cell activity, early response to treatment (within 5 months of treatment), and plaque stage less than 10–15 % of the skin surface [221, 222, 243–248]. ECP should always be given prior to chemotherapy as it requires an intact immune response.

The role of ECP for early-stage patients remains to be established, but some dramatic responses have occurred. In a recent review by Miller et al., 124 early-stage patients treated with ECP or ECP plus adjuvant therapy from 1987 to 2007 were identified in 16 different reports [174, 208, 219, 221, 225, 226, 232, 237, 240, 248–255]. Response rates for early-stage patients varied from 33 to 88 %. Most of these reports had

insufficient patient numbers to enable adequate statistical analysis within each cohort. We recently treated 19 patients with early-stage MF with photopheresis with favorable and durable responses noted [256]. Large-scale randomized prospective studies are needed to establish if ECP is beneficial in this patient population.

Combined Immunomodulatory Therapy

To improve response rates to ECP, interferon (IFN) and/or systemic retinoids have been added as a combined immuno-modality regimen [135]. Oral bexarotene is the most commonly used retinoid for combined modality therapy. Although the optimal dose is 300 mg/m^2, as a monotherapy, lower doses 75–225 mg are generally used with photopheresis to avoid lipemic plasma. Patients who were initially on ECP monotherapy experienced higher response rates when systemic retinoids were added to their regimen [174]. The addition of IFN-α[alpha]-2b, the first reported therapy used with ECP, may also have a synergistic effect with ECP [250, 257]. The dose of IFN used with ECP is also lower: one to five million units subcutaneously three times weekly and can be increased as tolerated. Anemia from the ECP, interferon, and bexarotene is often present after prolonged therapy. Zackheim criticized studies comparing ECP as a monotherapy versus ECP combined with interferon because other studies have documented that IFN-α[alpha]-2b maybe be as good or better when used as monotherapy [258]. Prospective, randomized studies are lacking to confirm these observations.

Maintenance with ECP following total skin electron beam therapy (TSEB) may improve overall survival [259]. Wilson et al. evaluated patients who achieved a PR or CR to TSEB who subsequently were treated with either adjuvant chemotherapy (doxorubicin/cyclophosphamide) or ECP. At 3 years, the group treated with ECP had improved overall survival which approached statistical significance ($p < 0.06$). They also evaluated erythrodermic (T4) patients treated with TSEB and concurrent ECP or TSEB only [129]. Patients with CR had a disease-free survival (DFS) of 63 %. Within this group, DFS was 49 % for patients who received TSEB alone versus 81 % for patients who had received TSEB and ECP.

There has been one report of a higher response rates in patients treated with ECP preceded by fludarabine versus fludarabine monotherapy, although no significant improvement in response duration or overall survival was observed [260]. Nineteen patients, including SS, erythrodermic MF, and MF with peripheral blood involvement, were studied. ECP was able to induce a response in six patients unresponsive to fludarabine alone and in three patients who relapsed after fludarabine. Although a low number of patients were treated and the patients were not randomized, the authors felt that preliminary results were positive and could be the basis for planning randomized multicenter trials on a larger scale.

Targeted Therapies

New targets for the treatment of CTCL include two types of agents: those which directly target the clonal tumor cells based on surface markers and those which modulate immunomodulatory cytokines favoring differentiation toward Th1 cells. Targeted therapy to the malignant clone is preferable to preserve the immune system of patients.

Denileukin Diftitox

Patients who fail interferon and oral bexarotene or who have tumors or nodal disease (stage IIB to IV MF) are good candidates to receive denileukin diftitox (ONTAK®) [261]. This is a recombinant IL-2-diphtheria toxin fusion protein targeted to the IL-2 receptor expressed on T cells, and it does not cause myelosuppression. Denileukin diftitox was approved by the FDA in 1998 for the treatment of cutaneous manifestations of relapsed CTCL. A phase III trial of denileukin diftitox in 73 patients with refractory CTCL who had received ≥3 prior therapies demonstrated a 30 % ORR, a 10 % complete response rate, and a median duration of response of 6.9 months from

time of first dose. Denileukin diftitox is quite effective in patients with stage IIB tumor disease, with a response rate of 50 %, and offers an attractive tumor burden debulking agent without causing neutropenia [262, 263]. Higher response rates of 60 % and fewer acute symptoms were seen in patients with the highest levels of CD25 expression in lesional skin biopsies using the fixed tissue assay. The need for 25 % CD25 expression for denileukin diftitox to work is controversial and did not show up in a recent larger study [264].

In a phase I study, denileukin diftitox was combined with bexarotene to increase expression of CD25 levels [265]. Fourteen patients with relapsed or refractory CTCL were treated with escalating doses of bexarotene (75–300 mg/day), and denileukin diftitox (18 μg/kg/day × 3 days every 21 days) had an overall response of 67 % (four complete responses, four partial responses) [265].

The results of a multicenter phase III double-blinded randomized trial of denileukin diftitox were recently published, showing significant response rates of both 9 and 18 μg/kg dose levels compared to placebo controls in patients who had received less than or equal to 3 prior therapies [266]. Unfortunately, due to manufacturing issues, denileukin diftitox had been unavailable as of fall 2011.

Side effects of denileukin diftitox include constitutional symptoms, hypersensitivity rash, and transient elevation of hepatic transaminases, thyroiditis with subsequent hypothyroidism, and vision changes [262, 267]. Capillary leak syndrome, which is defined as edema, hypoalbuminemia, and hypotension, may occur in 20–30 % of individuals and is maximal at about day 10. It can be severe in some patients secondary to pulmonary edema but is generally self-limited. Premedication with systemic corticosteroids has been shown to decrease the frequency of acute hypersensitivity reactions but does not prevent capillary leak syndrome [268]. Administering 500 cc of saline after each denileukin diftitox infusion may decrease the frequency of capillary leak syndrome but may also lead to increased peripheral edema [269]. It is important to carefully monitor the patient's weight before, during, and after therapy and administer low doses of furosemide.

Targeted Monoclonal Antibodies

Monoclonal antibodies targeting key activation determinants expressed on T lymphocytes have shown clinical efficacy in preliminary studies in CTCL. An antibody targeted to the malignant T cell specifically would be extremely useful for CTCLs administered alone or in combination with other agents.

Alemtuzumab

Alemtuzumab (Campath–H1; Genzyme Corporation, Cambridge, MA/Berlex Oncology, Wayne, NJ), is a humanized immunoglobulin that targets CD52, which is expressed on most T and B lymphocytes. A response rate of 50–70 % has been reported in CTCL patients treated with alemtuzumab [270]; however, prolonged depression of T, B, and NK cells is reported. Alemtuzumab has been associated with immunosuppression leading to reactivation of cytomegalovirus and opportunistic infections, and general infectious prophylaxis is recommended. Alternative dosing schedules with lower doses and subcutaneous administration are being investigated. Querfeld et al. reported favorable responses when IV was followed with lower-dose subcutaneous antibody [271].

Zanolimumab

CD4 is a molecule in the T-cell receptor complex that defines the helper T-cell lymphocyte membrane determinant. It is present on 90 % of all CTCLs and represents a specific target for therapy. Zanolimumab (HuMax-CD4® or HuMax®, or MDX-CD4; Genmab, Copenhagen, Denmark) is a humanized monoclonal antibody that binds to CD4, blocking receptor-mediated T-cell signaling and inducing antibody-dependent cell-mediated toxicity of malignant $CD4^+$ T lymphocytes without complement fixation or apoptosis. A dose-related response was seen in patients with MF/SS treated with zanolimumab. There was a 25 % response rate at a lower dose of 280 mg/m^2/week for 16 weeks compared to a 75 % response rate at a dose of 980 mg/m^2/week [272]. An ongoing phase II trial for registration comparing 8 and 12 mg IV weekly infusions accrued most of its subjects but was halted due to change in ownership.

SGN-30 (Anti-CD30 Monoclonal Antibody) and SGN-35 Brentuximab Vedotin

SGN-30 is a chimeric anti-CD30 monoclonal antibody targeting cells expressing CD30. CD30 is also known as tumor necrosis factor-receptor family member 8 and the Kiel-1 antigen. CD30 is expressed on Reed-Sternberg cells of Hodgkin's disease, in cutaneous anaplastic large-cell lymphoma (ALCL), and in lymphomatoid papulosis lesions. CD30 is also expressed on lesions of MF especially during transformation to large-cell lymphoma. CD30 also may be induced by viral infections as an activation marker.

A 20 % objective response rate in patients with systemic nodal CD30+ refractory ALCL has been observed in patients treated with antibody alone [273]. In a phase II multicenter trial of patients with one or more primary cutaneous CD30+ lymphoproliferative disorders (primary cutaneous ALCL (PC-ALCL), lymphomatoid papulosis, or CD30+ MF), responses were seen in 87 % of patients with ALCL, CD30+-transformed MF, and lymphomatoid papulosis [274]. Based on this study and high response rates seen in systemic relapsed ALCL, there are two investigator-initiated phase II trials of the tubulin inhibitor conjugated MMAE to CD30 antibody, brentuximab vedotin in patients with $CD30^+$ CTCL, ALCL, or lymphomatoid papulosus.

Forodesine

A novel molecular strategy for treating CTCLs is by targeting and inhibiting an enzyme, purine nucleoside phosphorylase (PNP). PNP regulates the purine salvage pathway that catalyzes the reversible phosphorolysis of ribonucleosides and 2-deoxyribonucleosides of guanine and hypoxanthine to the corresponding bases and ribose-1-phosphate or 2-deoxy-1-phoshate. Forodesine (formerly BCX-1777; BioCryst Pharmaceuticals, Birmingham, AL) is a small-molecule transition-state analog inhibitor of PNP with the structure of a nucleoside analog. It is not incorporated into DNA but requires presence of the cyclin-dependent kinase (CDK) found in cancer cells [275].

In vitro, forodesine inhibits the proliferation of activated human T lymphocytes and acute lymphoblastic leukemic T cells [276, 277]. In a phase I, open-label, multicenter, dose-ranging study in sequential cohorts of patients with refractory MF/SS, preliminary data shows that forodesine administration reduced body surface area affected by CTCL from baseline screening and produced a pronounced clinical improvement in erythroderma severity during and subsequent to forodesine therapy [277]. A phase II dose-ranging study of oral forodesine found that the optimal biological dose of 80 mg/m^2 gave an overall response rate of 37 % [275, 277].

Single-Agent Chemotherapy

In CTCL patients with tumors, nodal, or visceral disease, single-agent or combined chemotherapy is administered with hope of inducing partial remission. Methotrexate, pegylated liposomal doxorubicin (Doxil; Ortho Biotech Products LP, Bridgewater, NJ); gemcitabine (Gemzar; Eli Lilly and Company, Indianapolis, IN), and pentostatin (Nipent; SuperGen Inc., Des Plains, IL) have been used and studied for CTCL. Pralatrexate, a folic acid inhibitor, has shown efficacy in both MF and transformed MF in a phase I/II dose-escalation trial with optimal dose of 15 mg/kg given 3 weeks out of 4. It is currently under investigation in combination with bexarotene based on preclinical activity. The main side effects are mouth ulcers and myelosuppression.

Single-agent chemotherapy can be effective, but the duration of response may be short. Choice of therapy is based on stage, concomitant medical conditions, and prior treatments as each agent has a unique side effect and efficacy profile.

Gemcitabine

Gemcitabine hydrochloride (Gemzar®; Eli Lilly and Company, Indianapolis, IN), a nucleoside analog of deoxycytidine that inhibits DNA synthesis, has shown activity against solid tumors as well as hematologic malignancies [278]. In 1998, Zinzani et al. first documented one CR and four PRs to gemcitabine (1,200 mg/m^2) in eight

patients with cutaneous peripheral T-cell lymphoma (PTCL) and four of five patients with MF [279]. In 2001, Sallah et al. reported an overall response rate of 60 % with a median duration of response of 13.5–16.2 months in ten patients treated with gemcitabine (1,200 mg/m^2) [280]. The multicenter phase II clinical trial by Zinzani et al. of 44 patients (30 MF, 14 PTCLs) treated with gemcitabine (1,200 mg/m^2 administered for 3 of 4 weeks for three courses) reported an ORR of 70.5 % and median duration of response of 15 months [281]. Similar results were documented in the phase II study by Marchi et al. of 32 patients (26 MF, 1 SS, 5 PTCLs) treated with gemcitabine (1,200 mg/m^2 once per week for 3 of 4 weeks for six courses) reported an ORR of 75 % with a median duration of response of 10 months (4–22 months) [282].

We have demonstrated that a lower dose of gemcitabine (1,000 mg/m^2 once per week for 3-week cycles) produced an ORR of 68 % in 25 patients with advanced-stage and refractory CTCL [283]. It was especially active in patients with cutaneous tumors. Gemcitabine can be used in combination with a maintenance therapy of bexarotene to manage the plaques and patches of mycosis fungoides [31]. Adverse effects of gemcitabine have most frequently involved bone marrow suppression (leukopenia, anemia), mild alopecia, generalized hyperpigmentation, and elevation of hepatic transaminase and creatinine levels [283]. Three of 25 CTCL patients who each had SS developed hemolytic uremic syndrome, although the overall incidence previously reported was only 0.6 % [283]. Sapacitabine (also known as CYC682), a deoxycytidine analog like gemcitabine, was studied in a phase I/II trial but was not active at the doses tested.

Pentostatin

Pentostatin (2′-deoxycoformycin or dCF or Nipent—SuperGen Inc, Des Plaines, IL) is a potent inhibitor of adenosine deaminase and is selectively toxic to lymphocytes [149, 150, 284]. Griener et al. first documented an ORR of 39 % in 18 patients with stage I to IVB CTCL treated with 4–5 mg/m^2 of intravenous pentostatin every 1–4 weeks. Two patients had CRs with duration of response of 4 months to 6 years, and five patients had PRs lasting for 1.5–6 months [150]. Foss et al. reported a 40 % OR rate and 7 % CR rate in 94 CTCL patients treated with pentostatin studied in multicenter phase II trials. The median time to progression ranged from 1.3 to 8.3 months [285]. Kurzrock et al. reported a 71 % OR rate and 25 % CR rate in 14 patients with SS and 6 patients with tumor-stage disease treated with pentostatin [286]. In a phase II study combining pentostatin with intermittent high-dose interferon-α[alpha], Foss demonstrated median progression-free survival of responders of 13.1 months [148]. Although duration of response was longer, response rates (ORR 41 %) were similar to those seen with single-agent pentostatin [148]. Toxicities include hematologic, renal insufficiency, nausea, and conjunctivitis [285]. Pentostatin has also been associated with angina and myocardial infarction, heart failure, and acute arrhythmias in patients with predisposing conditions such as coronary artery disease, congestive heart failure, hypertension, and pulmonary metastases [287]. It is now recommended to correct anemia by transfusion if warranted, optimize cardiac medications, control nausea and vomiting, correct any hypercalcemia, reduce pentostatin dose for patients with impaired renal function, and monitor fluid balance to prevent fluid overload [287].

Pegylated Liposomal Doxorubicin

Doxorubicin (Doxil; Ortho Biotech Products LP, Bridgewater, NJ) is an anthracycline with antineoplastic effects in nodal lymphomas [288], solid tumors [288], myeloma [289], and acute leukemia [290]. The pegylated liposomal form of doxorubicin allows for reduced toxicity, improved efficacy, and a longer half-life [291]. Wollina et al. first published the efficacy and safety of liposomal doxorubicin in 2000 [288]. Ten patients with MF (stage IB to IVA) were treated with liposomal doxorubicin at a dose of 20 mg/m^2 with an OR rate of 80 % and a high CR rate of 60 %.

Table 19.6 Multi-chemotherapy for mycosis fungoides, selected trials [47]

Therapy	*n*	Response rate (%)	Duration (months)	Reference
Chlorambucil, prednisone	21	57	NR	[293]
CVP	17	76	16	[294, 300]
CVPB	12	92	11.5	[295]
CHOP	12	100	5	[295]
COPP/MOPP	21	70	14	[296]
CAVE	52	90	NR	[49]
BAM-M	10	80	41	[297]

Courtesy of Steven M. Horwitz, Sloan-Kettering Cancer Center
CVP cyclophosphamide, vincristine, prednisone, *CVPB* cyclophosphamide, vincristine, prednisone, and bleomycin, *CHOP* cyclophosphamide, adriamycin, vincristine, and prednisone, *COPP* mechlorethamine, vincristine, procarbazine, prednisone, *MOPP* cyclophosphamide, vincristine, procarbazine, prednisone, *CAVE* cyclophosphamide, doxorubicin, etoposide, and vincristine, *BAM-M* bleomycin, adriamycin, methotrexate, topical nitrogen mustard

Mean disease-free survival was 13.3 months [288]. In a retrospective multicenter study evaluating 34 CTCL patients treated with various doses and schedules of liposomal doxorubicin (20–40 mg/m^2 every 2–4 weeks), an OR rate of 88 % was reported [292]. Grade 3–4 toxicities included three patients with lymphopenia, three with anemia, and one with capillary leak syndrome. Side effects include nausea, vomiting, hand/foot syndrome, and myelosuppression. Cardiomyopathy is dose dependent and not generally seen in cumulative doses less than 450–500 mg/m^2. We studied liposomal doxorubicin at 30 mg/m^2 given every 3 weeks followed by bexarotene maintenance therapy in a small exploratory phase II trial [144]. The response rate of around 43 % was lower than expected based on the other reports in the literature. Although patients with transformed MF tumors had dramatic responses, their tumors relapsed on bexarotene. One patient with blood and node and erythroderma (SS) has had a durable complete response.

Combination Chemotherapy

Table 19.6 highlights multiple studies that reported results of combination chemotherapies alone or combined with topical nitrogen mustard or total skin electron beam radiation. With combination chemotherapy, the response rates are high, but duration of response may be short lived [49, 293–298].

In 1974, Winkelmann et al. first advocated the use of oral low-dose chlorambucil and prednisolone as a relatively nontoxic chemotherapeutic option for palliation of advanced Sézary syndrome. In 21 patients treated with the Winkelmann regimen, the OR rate was 57 % with three complete responders (14 %) [293]. CVP (cyclophosphamide, vincristine, prednisone), CVPB (cyclophosphamide, vincristine, prednisone, and bleomycin), and CHOP (cyclophosphamide, adriamycin, vincristine, and prednisone) have also been efficacious in MF (See Table 19.1) [294, 295]. In 1998, Hallahan et al. treated patients with T3 stage MF with TBSEB and MOPP (methotrexate, vincristine, procarbazine, prednisone) or COPP (cyclophosphamide, vincristine, procarbazine, prednisone) and reported a 70 % OR rate and 14 month duration of response [296]. In the first randomized trial for MF, Kaye et al. compared combined modality (TSE and chemotherapy) with topical sequential conservative therapy (mechlorethamine, PUVA, TSEB, methotrexate) and found no difference in disease-free survival and overall survival with either modality [49]. Zakem et al. treated ten patients with stage IIB–IVB MF with a combination chemotherapy program consisting of bleomycin and methotrexate weekly, doxorubicin every 3 weeks, and topical nitrogen mustard daily (BAM-M). The OR rate was 80 % with seven patients obtained histologically documented complete remissions lasting 4–105+ months [297].

Table 19.7 Response rates by stage for combined modality therapy [83]

Stage	% out of 94 patients evaluable for response	Partial response (%)	Complete response (%)	Overall response (%)	Median survival (years)
IA	13.8	8	77	85	[a]
IB	30.9	17	79	96	12.8
IIA	8.5	37	63	100	10
IIB	26.6	24	48	72	3.2
III	4.25	0	25	25	4.6
IVA	11.7	55	27	82	2.5
IVB	4.25	50	50	100	2.5
Overall	100	24	60	84	[b]

[a]Similar to age-matched control patients
[b]Ninety-five patients were evaluable for survival. The median overall survival time was 119 months. The median survival times were 145 months for patients with early-stage disease (IA-IIA) and 36 months for those with late-stage disease (IIB-IVB), $p<0.0001$

Although CTCL is often transiently responsive to combined regimen chemotherapy, their effect on increased survival or ability to induce durable remissions is limited. To improve treatment efficacy and outcome in CTCL, we reported a combined modality protocol using three to four consecutive phases of therapy, which was initiated in 1987 at M.D. Anderson Cancer Center [83]. Between 1987 and 2001, 95 patients with early-stage (IA–IIA, $n=50$) or late-stage (IIB–IVB, $n=45$) MF were treated initially with subcutaneous interferon-α[alpha] (IFN-α[alpha]) and oral isotretinoin for 4 months, followed by total body skin electron beam (TSEB), and long-term maintenance therapy with topical nitrogen mustard and IFN-α[alpha]. Patients with late-stage (IIB–IVB) disease also received six cycles of combination chemotherapy with cyclophosphamide, methotrexate, etoposide, and dexamethasone (CMED) before receiving electron beam radiation. Standard CMED was given as a 21-day cycle according to the following schedule: intravenous (IV) cyclophosphamide, 500 mg/m^2 on day 1; IV methotrexate, 1 g/m^2 on day 3; IV etoposide, 100 mg/m^2 daily on days 1–3; and oral dexamethasone, 40 mg daily for 5 days. Combined modality therapy yielded a response rate of 85 % with a 60 % complete response rate (Table 19.7). Thirty-eight patients (76 %) with early-stage disease and 18 of 45 (40 %) patients with late-stage MF and SS achieved complete response. Nine (24 %) patients with early-stage MF and three patients (17 %) with late-stage MF have achieved sustained remissions lasting more than 5 years. Median disease-free survival (DFS) for early and late stages of disease was 62 and 7 months, respectively, with 5-year Kaplan-Meier estimated rates of 50 and 27 %, respectively [83]. The multiphase combined modality regimen is well tolerated and may yield higher response rates and disease-free survival than TSEB therapy alone [83].

Allogeneic Stem Cell Transplant

Non-ablative, allogeneic hematopoietic stem cell transplant (HSCT) is now being considered for young, healthy patients with advanced CTCL (≥IIB) who have advanced stages at presentation affecting overall survival and fail to respond to first-line therapy. Patients need to have a related or unrelated matched donor and be physically and emotionally able to undergo the procedure. The existence of a graft-versus-T-cell lymphoma effect has been suggested in recent reports, particularly using nonmyeloablative conditioning [299]. Select patients have achieved long-term remissions and curative responses [299]. The timing of HSCT is controversial, and patients with rapidly progressing MF often become ineligible for treatment. Tumor debulking with chemotherapy for nodal disease or with TBSEB for skin involvement needs must be successful. Allogeneic stem cell transplant has superior

survival and event-free outcome over autologous HSCT in MF/SS [300]. The procedure remains high risk; thus, early-stage patients with good prognosis are not candidates for this procedure. Although this is not standard procedure at all centers, we found that pretreatment with TSEB reduces the rate of relapse, lengthens disease-free survival, and may reduce severity of acute GVHD.

Conclusions

Cutaneous T-cell lymphomas, of which mycosis fungoides and Sézary syndrome are the most commonly encountered, are currently uncurable. Patients with early, skin-limited disease do extremely well on skin-directed therapies and should not be subjected to therapy that will decrease their immune competency. Novel targeted therapy and combination therapies are producing higher response rates with more durable remissions. The key to finding better treatments is to better understand the disease pathogenesis and heterogeneity at a molecular level. The best clinical results are achieved when skin care and skin-directed therapy are combined with effective biological response modifiers or targeted therapy. New therapies under development are exciting prospects to improve the treatment of these diseases. Recent withdrawal or unavailability of active agents, including denileukin diftitox and liposomal doxyrubicin, has decreased access to the most highly effective therapies.

References

1. Lamberg SI, Bunn Jr PA. Proceeding of the workshop on cutaneous T-cell lymphomas (mycosis fungoides and Sezary syndromes). Cancer Treat Rep. 1979;63:561–4.
2. Burg G, Kempf W, Cozzio A, Feit J, Willemze RS, Jaffe E, et al. WHO/EORTC classification of cutaneous lymphomas, histological and molecular aspects. J Cutan Pathol. 2005;32(10):647–74.
3. Olsen E, Vonderheid E, Pimpinelli N, Willemze R, Kim Y, Knobler R, et al. ISCL/EORTC Revisions to the staging and classification of mycosis fungoides and Sezary syndrome: a proposal of the International Society for Cutaneous Lymphomas (ISCL) and the cutaneous lymphoma task force of the European Organization of Research and Treatment of Cancer (EORTC). Blood. 2007;110(6):1713–22.
4. Alibert JLM. Description des maladies de la peau observees a l'hopital St Louis *1991* 1806; Barrois L'aine et Fils(1). Paris, France, p. 413–46.
5. Kim YH, Liu HL, Mraz-Gernhard S, Verghese A, Hoppe RT. Long-term outcome of 525 patients with mycosis fungoides and Sézary syndrome: clinical prognostic factors and risk for disease progression. Arch Dermatol. 2003;139:857–66.
6. Diwan AH, Prieto VG, Herling M, Duvic M, Jones D. Primary Sézary syndrome commonly shows low-grade cytologic atypia and an absence of epidermotropism. Am J Clin Pathol. 2005;123(4):510–5.
7. Campbell JJ, Clark RA, Watanabe R, Kupper TS. Sézary syndrome and mycosis fungoides arise from distinct T-cell subsets: a biologic rationale for their distinct clinical behaviors. Blood. 2010;116:767–71.
8. Van Doorn R, Van Kester M, Dijkman R, Vermeer MH, Mulder AA, Szuhai K, et al. Oncogenomic analysis of mycosis fungoides reveals major differences with Sezary syndrome. Blood. 2009;113:127–36.
9. Weinstock MA. Mycosis fungoides in the United States increasing incidence and descriptive epidemiology. JAMA. 1988;260(1):42–6.
10. Criscione VD, Weinstock MA. Incidence of cutaneous T-cell lymphoma in the United States, 1973–2002. Arch Dermatol. 2007;143(7):854–9.
11. Sun G, Berthelot C, Li Y, Glass 2nd DA, George D, Pandya A, et al. Poor prognosis in non-Caucasian patients with early-onset mycosis fungoides. J Am Acad Dermatol. 2008;60(2):231–5.
12. Yawalkar N, Ferenczi K, Jones DA, Yaanaka K, Suh K-Y, Sadat S, et al. Profound loss of T-cell receptor repertoire complexity in cutaneous T-cell lymphoma. Blood. 2003;102(12):4059–66.
13. Zoi-Toli O, Vermeer MH, De Vries E, Van Beek P, Meijer CJ, Willemze R. Expression of Fas and Fas-ligand in primary cutaneous T-cell lymphoma (CTCL): association between lack of Fas expression and aggressive types of CTCL. Br J Dermatol. 2000;143(2):313–9.
14. Ni X, Hazarika P, Zhang C, Talpur R, Duvic M. Fas ligand expression by neoplastic T lymphocytes mediates elimination of $CD8^+$ cytotoxic T lymphocytes in mycosis fungoides: a potential mechanism of tumor immune escape? Clin Cancer Res. 2001;7(9):2682–92.
15. Ni X, Zhang C, Talpur R, Duvic M. Resistance to activation-induced cell death and bystander cytotoxicity via the Fas/Fas ligand pathway are implicated in the pathogenesis of cutaneous T cell lymphomas. J Invest Dermatol. 2005;124(4):741–50.
16. Tan RS, Butterworth CM, McLaughlin H, Malka S, Samman PD. Mycosis fungoides—a disease of antigen persistence. Br J Dermatol. 1974;91(6):607–16.
17. Kikuchi A, Ohata Y, Matsumoto H, Sugiura M, Nishikawa T. Anti-HTLV-1 antibody positive cutaneous T-cell lymphoma. Cancer. 1997;79:269–74.
18. Whittemore AS, Holly EA, Lee IM, Abel EA, Adams RM, Nickoloff BJ, et al. Mycosis fungoides in relation to

environmental exposures and immune response: a case–control study. J Natl Cancer Inst. 1989;81:1560–7.
19. Jackow CM, Cather JC, Hearne V, Asano AT, Musser JM, Duvic M. Association of erythrodermic cutaneous T-cell lymphoma, superantigen positive *Staphylococcus aureus* and oligoclonal T cell receptor VB gene expression. Blood. 1997;89:32–40.
20. Talpur R, Bassett R, Duvic M. Prevalence and treatment of *Staphylococcus aureus* colonization in patients with mycosis fungoides and Sézary syndrome. Br J Dermatol. 2008;159(1):105–12.
21. Jackow CM, Breuer McHam J, Friss A, Alvea J, Reveille JR, Duvic M. HLA-DR5 and DQB1*03 Class II alleles are associated with cutaneous T-cell lymphoma. J Invest Dermatol. 1996;107:373–6.
22. Bromley SK, Burack KG, Johnson K, et al. The immunological synapse. Annu Rev Immunol. 2001;19:375–96.
23. Kim EJ, Hess S, Richardson SK, Newton S, Showe LC, Benoit BM, et al. Immunopathogenesis and therapy of cutaneous T cell lymphoma. J Clin Invest. 2005;115(4):798–812.
24. Drummer R, Kohl O, Gillisson J, et al. Peripheral blood mononuclear cells in non-leukemic cutaneous-cell lymphoma patients: reduced proliferation and preferential secretion of a T helper 2 like cytokine pattern, on stimulation. Arch Dermatol. 1993;129:433–6.
25. Duvic M, Donato M, Dabaja B, Richmond H, Singh L, Wei W, et al. Total skin electron beam and non-myeloablative allogeneic hematopoietic stem-cell transplantation in advanced mycosis fungoides and Sezary syndrome. J Clin Oncol. 2010;28(14):2365–72.
26. Wood GS, Greenberg HL. Diagnosis, staging, and monitoring of cutaneous T-cell lymphoma. Dermatol Ther. 2003;16:269–75.
27. Mao X, Lillington D, Scarsbrick JJ, Mitchell T, Czepulkowsyi B, Russell-Jones RR, et al. Molecular cytogenetic analysis of cutaneous T-cell lymphomas: identification of common genetic alterations in Sézary syndrome and mycosis fungoides. Br J Dermatol. 2002;147:464–75.
28. Scarisbrick J, Woolford A, Russell Jones R, Whittaker S. Allelotyping in mycosis fungoides and Sezary syndrome common regions of allelic loss identified on 1p, 9p, 10q, and 17p. J Invest Dermatol. 2001;117:663–70.
29. Scarisbrick JJ, Woolford AJ, Russell-Jones R, Whittaker SJ. Loss of heterozygosity on 10q and microsatellite instability in advanced stages of primary cutaneous T-cell lymphoma and possible association with homozygous deletion of PTEN. Blood. 2000;95:2937–42.
30. Scarisbrick JJ, Mitchell TJ, Calonje E, Orchard G, Russell-Jones R, Whittaker SJ. Microsatellite instability is associated with hypermethylation of the hMLH1 gene and reduced gene expression in mycosis fungoides. J Invest Dermatol. 2003;121(4):894–901.
31. Duvic M. Systemic monotherapy vs combination therapy for CTCL: rationale and future strategies. Review. Oncology (Williston Park). 2007;21(2 Suppl 1):33–40.
32. Duvic M, Foss FM. Mycosis fungoides: pathophysiology and emerging therapies. Semin Oncol. 2007;34(6 Suppl 5):S21–8.
33. Pimpinelli N, Olsen EA, Santucci M, Vonderheid E, Haeffner AC, Stevens S, et al. Defining early mycosis fungoides. J Am Acad Dermatol. 2005;53(6):1053–63.
34. Diamandidou E, Colome-Grimmer M, Fayad L, Duvic M, Kurzrock R. Transformation of mycosis fungoides/Sezary syndrome: clinical characteristics and prognosis. Blood. 1998;92(4):1150–9.
35. Diamandidou E, Colome M, Fayad L, Duvic M, Kurzrock R. Prognostic factor analysis in mycosis fungoides/Sezary syndrome. J Am Acad Dermatol. 1999;40(6 Pt 1):914–23.
36. Kim YHW, Pimpinelli N, Whittaker S, Olsen EA, Ranki A, Dummer R, et al. TNM classification system for primary cutaneous lymphomas other than mycosis fungoides and Sezary syndrome: a proposal of the International Society for Cutaneous Lymphomas (ISCL) and the Cutaneous Lymphoma Task Force of the European Organization of Research and Treatment of Cancer (EORTC). Blood. 2007;110(2):479–84.
37. Bunn Jr PA, Lamberg SI. Report of the Committee on Staging and Classification of Cutaneous T-Cell Lymphomas. Cancer Treat Rep. 1979;63:725–8.
38. Scarisbrick JJ, Whittaker S, Evans AV, Fraser-Andrews EA, Child FJ, Dean A. Prognostic significance of tumor burden in the blood of patients with erythrodermic primary cutaneous T-cell lymphoma. Blood. 2001;97:624–30.
39. Vidulich K, Bassett RL, Duvic M. Overall survival in erythrodermic cutaneous T-cell lymphoma: an analysis of prognostic factors in a cohort of patients with erythrodermic cutaneous T-cell lymphoma. Int J Dermatol. 2009;48(3):243–52.
40. Pujol RM, Gallardo F, Llistosella E, Blanco A, Bernadó S, Bordes R, et al. Invisible mycosis fungoides: a diagnostic challenge. J Am Acad Dermatol. 2002;47(2):S168–71.
41. Scheffer E, Meijer CJLM, van Vloten WA. Dermatopathic lymphadenopathy and lymph node involvement in mycosis fungoides. Cancer. 1980;45:137–48.
42. Sausville EA, Worsham GF, Matthews MJ, Makuch RW, Fischmann AB, Schechter GP, et al. Histologic assessment of lymph nodes in mycosis fungoides/Sézary syndrome (cutaneous T-cell lymphoma): clinical correlations and prognostic import of a new classification system. Hum Pathol. 1985;16:1098–109.
43. Clendenning WE, Rappaport HW. Report of the committee on pathology of cutaneous T cell lymphomas. Cancer Treat Rep. 1979;63:719–24.
44. Colby TV, Burke JS, Hoppe RT. Lymph node biopsy in mycosis fungoides. Cancer. 1981;47:351–9.
45. Kinney MC, Jones D. Cutaneous T-cell and NK-cell lymphomas: the WHO-EORTC classification and the increasing recognition of specialized tumor types. Am J Clin Pathol. 2007;127(5):670–86.
46. Berthelot C, Rivera A, Duvic M. Skin directed therapy for mycosis fungoides: a review. J Drugs Dermatol. 2008;7(7):655–66.

47. Horwitz SM, Duvic M, Porcu P, Kim YH. Review of the treatment of mycosis fungoides and Sézary syndrome: a stage-based approach. J Natl Compr Canc Netw. 2008;6(4):436–42.
48. Trautinger F, Knobler R, Willemze R, Peris K, Stadler R, Laroche L, et al. EORTC consensus recommendations for the treatment of mycosis fungoides/Sézary syndrome. Eur J Cancer. 2006;42(8):1014–30.
49. Kaye FJ, JrPA B, Steinberg SM, Stocker JL, Ihde DC, Fischmann AB, et al. A randomized trial comparing combination electron-beam radiation and chemotherapy with topical therapy in the initial treatment of mycosis fungoides. N Engl J Med. 1989;321(26):1784–90.
50. Zelenetz AD, Advani RH, Byrd JC, Czuczman MS, Damon LE, Duvic M, et al. National Comprehensive Cancer Network Non-Hodgkin's lymphomas. J Natl Compr Canc Netw. 2008;6(4):356–421.
51. Krathen RA, Ward S, Duvic M. Bexarotene is a new treatment option for lymphomatoid papulosis. Dermatology. 2003;206(2):142–7.
52. Apisarnthanarax N, Talpur R, Ward S, Ni X, Kim HW, Duvic M. Tazarotene 01 % gel for refractory mycosis fungoides lesions: an open-label pilot study. J Am Acad Dermatol. 2004;50(4):600–7.
53. Duvic M, Olsen EA, Omura GA, Maize JC, Vonderheid EC, Elmets CA, et al. A phase III, randomized, double-blind, placebo-controlled study of peldesine (BCX-34) cream as topical therapy for cutaneous T-cell lymphoma. J Am Acad Dermatol. 2001;44:940–7.
54. Farber EM, Zackheim HS, McClintock RP, et al. Treatment of mycosis fungoides with various strengths of fluocinolone acetonide cream. Arch Dermatol. 1968;97:165–72.
55. Farber EM, Cox AJ, Steinberg J, et al. Therapy of mycosis fungoides with topically applied fluocinolone acetonide under occlusive dressing. Cancer. 1966;19(2):237–45.
56. Cohen H, Baer R. Observations upon the use of topical triamcinolone acetonide preparations in various concentrations. Dermatologica. 1961;122:116–9.
57. Zackheim HS, Kashani-Sabet M, Amin S. Topical corticosteroids for mycosis fungoides Experience in 79 patients. Arch Dermatol. 1998;134(8):949–54.
58. Harrison A, Duvic M. Diagnosis and treatment of Sézary syndrome. Int J Dermatol. 2004;2(2).
59. Mrass P, Rendl M, Mildner M, et al. Retinoic acid increases the expression of p53 and proapoptotic caspases and sensitizes keratinocytes to apoptosis: a possible explanation for tumor preventive action of retinoids. Cancer Res. 2004;64(18):6542–8.
60. Hurst RE. Bexarotene ligand pharmaceuticals. Curr Opin Investig Drugs. 2000;1(4):514–23.
61. Duvic M, Martin AG, Kim Y, Olsen E, Wood G, Crowley CA, et al. Phase 2 and 3 clinical trial of oral bexarotene (Targretin capsules) for the treatment of refractory or persistent early-stage cutaneous T-cell lymphoma. Arch Dermatol. 2001;137(5):581–93.
62. Duvic M, Hymes K, Heald P, Martin AG, Myskowski P, Crowley C, et al. Bexarotene is effective and safe for the treatment of refractory advanced stage cutaneous T-cell lymphoma: multinational phase II-III trial results. J Clin Oncol. 2001;19(9):2456–71.
63. Heald P, Mehlmauer M, Martin AG, Crowley CA, Yocum RC, Reich SD, et al. Topical bexarotene therapy for patients with refractory or persistent early-stage cutaneous T-cell lymphoma: results of the phase III clinical trial. J Am Acad Dermatol. 2003;49(5):801–15.
64. Kuzel T, Breneman D, Duvic M, et al. Phase I-II trial of Targretin gel in the topical treatment of patients with cutaneous T-cell lymphoma. J Invest Dermatol. 2000;114:830.
65. Lebwohl M, Poulin Y. Tazarotene in combination with topical corticosteroids. J Am Acad Dermatol. 1998;39(4 Pt 2):S139–43.
66. Weinstein GD, Krueger GG, Lowe NJ, Duvic M, Friedman DJ, Jegasothy BV, et al. Tazarotene gel, a new retinoid, for topical therapy of psoriasis: vehicle-controlled study of safety, efficacy and duration of therapeutic effect. J Am Acad Dermatol. 1997;37:85–92.
67. Shalita AR, Chalker DK, Griffith RF, Herbert AA, Hickman JG, Maloney JM, et al. Tazarotene gel is safe and effective in the treatment of acne vulgaris: a multicenter, double-blind, vehicle controlled study. Cutis. 1999;63:349–54.
68. Sefton J, Kligman AM, Kopper SC, Lue JC, Gibson JR. Photodamage pilot study: a double-blind, vehicle controlled study to assess the efficacy and safety of tazarotene 01 % gel. J Am Acad Dermatol. 2000;43:656–63.
69. Duvic M, Ni X, Talpur R, Herne K, Schulz C, Sui D, et al. Tazarotene-induced gene 3 is suppressed in basal cell carcinomas and reversed in vivo by tazarotene application. J Invest Dermatol. 2003;121(4):902–9.
70. Bianchi L, Orlandi A, Campione E, Angeloni C, Costanzo A, Spagnoli LG, et al. Topical treatment of basal cell carcinoma with tazarotene: a clinicopathological study on a large series of cases. Br J Dermatol. 2004;151(1):148–56.
71. Duvic M, Helekar B, Schulz C, Cho M, DiSepio D, Hager C, et al. Expression of a retinoid-inducible tumor suppressor, Tazarotene-inducible gene-3, is decreased in psoriasis and skin cancer. Clin Cancer Res. 2000;6(8):3249–59.
72. Duvic M, Friedman-Kien AE, Looney DJ, Miles SA, Myskowski PL, Scadden DT, et al. Topical treatment of cutaneous lesions of acquired immunodeficiency syndrome-related Kaposi sarcoma using alitretinoin gel: results of phase 1 and 2 trials. Arch Dermatol. 2000;136:1461–9.
73. Bassiri-Tehrani S, Cohen DE. Treatment of cutaneous T-cell lymphoma with alitretinoin gel. Int J Dermatol. 2002;41:104–6.
74. Lain T, Talpur R, Duvic M. Long-term control of mycosis fungoides of the hands with topical bexarotene. Int J Dermatol. 2003;42(3):238–41.
75. Talpur R, Vu J, Bassett R, Stevens V, Duvic M. Phase I/II randomized bilateral half-head comparison of topical bexarotene 1 % gel for alopecia areata. J Am Acad Dermatol. 2009;61(4):592.

76. Zackheim HS. Cutaneous T, cell lymphoma: update of treatment. Dermatology. 1999;199:102–5.
77. Bunn Jr PA, Hoffman SJ, Norris D, Golitz LE, Aeling JL. Systemic therapy of cutaneous T-cell lymphomas (mycosis fungoides and the Sezary syndrome). Ann Intern Med. 1994;121(8):592–602.
78. Ramsay DL, Halperin PS, Zeleniuch-Jacquotte A. Topical mechlorethamine therapy for early stage mycosis fungoides. J Am Acad Dermatol. 1988;19(4):684–91.
79. Vonderheid EC, Tan ET, Kantor AF, et al. Long-term efficacy, curative potential, and carcinogenicity of topical mechlorethamine chemotherapy in cutaneous T cell lymphoma. J Am Acad Dermatol. 1989;20:416–28.
80. Hoppe RT, Abel EA, Deneau DG, et al. Mycosis fungoides: management with topical nitrogen mustard. J Clin Oncol. 1987;5:1796–803.
81. Hamminga L, Hermans J, Noordijk EM, et al. Cutaneous T-cell lymphoma: clinicopathological relationships, therapy and survival in ninety-two patients. Br J Dermatol. 1982;107:145–55.
82. Kim YH, Martinez G, Varghese A, et al. Topical nitrogen mustard in the management of mycosis fungoides: update of the Stanford experience. Arch Dermatol. 2003;2003(139):165–73.
83. Duvic M, Apisarnthanarax N, Cohen DS, Smith TL, Ha CS, Kurzrock R. Analysis of long-term outcomes of combined modality therapy for cutaneous T-cell lymphoma. J Am Acad Dermatol. 2003;49(1):35–49.
84. Esteve E, Bagot M, Joly P, et al. A prospective study of cutaneous intolerance to topical mechlorethamine therapy in patients with cutaneous T-cell lymphomas French Study Group of Cutaneous Lymphomas. Arch Dermatol. 1999;135(11):1349–53.
85. Zackheim HS. Treatment of mycosis fungoides with topical nitrosourea compounds. Arch Dermatol. 1972;106:177–82.
86. Zackheim HS, Epstein Jr EH, McNutt NS, et al. Topical carmustine (BCND) for mycosis fungoides and related disorders: a 10-year experience. J Am Acad Dermatol. 1983;9:363–74.
87. Zackheim HS, Epstein Jr EH, Crain WR. Topical carmustine (BCND) for cutaneous T cell lymphoma: a 15-year experience in 143 patients. J Am Acad Dermatol. 1990;22:802–10.
88. Grekin DA, Zackheim HS, Epstein Jr EH. Topical chemotherapy of mycosis fungoides. Cutis. 1979;24:543–6.
89. Zackheim HS, Feldmann RJ, Lindsay C, et al. Percutaneous absorption of 1,3-bis (2chloroethyl)-I-nitrosourea (BCND, carmustine) in mycosis fungoides. Br J Dermatol. 1977;97:65–7.
90. Sigmundsdottir H, Johnston A, Gudjonsson JE, Bjarnason B, Valdimarsson H. Methotrexate markedly reduces the expression of vascular E-selectin, cutaneous lymphocyte-associated antigen and the numbers of mononuclear leucocytes in psoriatic skin. Exp Dermatol. 2004;13(7):426–34.
91. Research USFaDACfDEa FDA Oncology Tools: product label details in conventional order for methotrexate http://wwwaccessdatafdagov/scripts/cder/onctools/labelscfm?GN=methotrexate. Accessed 28 Oct 2008.
92. Zackheim HS, Kashani-Sabet M, Hwang ST. Low-dose methotrexate to treat erythrodermic cutaneous T-cell lymphoma: results in twenty-nine patients. J Am Acad Dermatol. 1996;34(4):626–31.
93. Zackheim HS, Kashani-Sabet M, McMillan A. Low-dose methotrexate to treat mycosis fungoides: a retrospective study in 69 patients. J Am Acad Dermatol. 2003;49(5):873–8.
94. Tan AW, Giam YC. Lymphomatoid papulosis associated with recurrent cutaneous T-cell lymphoma. Ann Acad Med Singapore. 2004;33(1):110–2.
95. Bekkenk MW, Kluin PM, Jansen PM, Meijer CJ, Willemze R. Lymphomatoid papulosis with a natural killer-cell phenotype. Br J Dermatol. 2001;145(2):318–22.
96. Argyropoulos CL, Lamberg SI, Clendenning WE, Fischmann AB, Jegasothy B, Zackheim H, et al. Preliminary evaluation of 15 chemotherapeutic agents applied topically in the treatment of mycosis fungoides. Cancer Treat Rep. 1979;63:619–21.
97. Chatterjee DJ, Li WY, Koda RT. Effect of vehicles and penetration enhancers on the in vitro and in vivo percutaneous absorption of methotrexate and edatrexate through hairless mouse skin. Pharm Res. 1997;14(8):1058–65.
98. Demierre MF, Vachon L, Ho V, Sutton L, Cato A, Leyland-Jones B. Phase 1/2 pilot study of methotrexate-laurocapram topical gel for the treatment of patients with early-stage mycosis fungoides. Arch Dermatol. 2003;139:624–8.
99. Fam AG, Perez-Ordonez B, Imrie K. Primary cutaneous B cell lymphoma during methotrexate therapy for rheumatoid arthritis. J Rheumatol. 2000;27(6):1546–9.
100. Baird RD, van Zyl-Smit RN, Dilke T, Scott SE, Rassam SM. Spontaneous remission of low-grade B-cell non-Hodgkin's lymphoma following withdrawal of methotrexate in a patient with rheumatoid arthritis: case report and review of the literature. Br J Haematol. 2002;118(2):567–8.
101. Ruzicka T, Assmann T, Lebwohl M. Potential future dermatological indications for tacrolimus ointment. Eur J Dermatol. 2003;13:331–42.
102. Russell JJ. Topical tacrolimus: a new therapy for atopic dermatitis. Am Fam Physician. 2002;66:1899–902.
103. Poumaras CC, Liibbe J, Saurat JH. Staphylococcal colonization in atopic dermatitis treatment with topical tacrolimus (Fk506). J Invest Dermatol. 2001;116(3):480–1.
104. Rallis E, Economidi A, Verros C, Papadakis P. Successful treatment of patch type mycosis fungoides with tacrolimus ointment 01 %. J Drugs Dermatol. 2006;5(9):906–7.
105. Sauder DN. Immunomodulatory and pharmacologic properties of imiquimod. J Am Acad Dermatol. 2000;43:S6–11.
106. Kim YH, Girardi M, Duvic M, Kuzel T, Link BK, Pinter-Brown L, et al. Phase I trial of a toll-like receptor 9 (TLR9) agonist, PF-3512676 (CpG 7909) in patients with treatment-refractory CTCL. J Am Acad Dermatol. 2010;63(6):975–83.

107. Suchin KR, Junkins-Hopkins JM, Rook AH. Treatment of stage IA cutaneous T-Cell lymphoma with topical application of the immune response modifier imiquimod. Arch Dermatol. 2002;138:1137–9.
108. Deeths MJ, Chapman JT, Dellavalle RP, Zeng C, Aeling JL. Treatment of patch and plaque stage mycosis fungoides with imiquimod 5 % cream. J Am Acad Dermatol. 2005;52(2):275–80.
109. Herrmann JJ, Roenigk Jr HH, Honigsmann H. Ultraviolet radiation for treatment of cutaneous T-cell lymphoma. Hematol Oncol Clin North Am. 1995;9(5):1077–88.
110. Ramsay DL, Lish KM, Yalowitz CB, Soter NA. Ultraviolet B phototherapy for early-stage cutaneous T-cell lymphoma. Arch Dermatol. 1992;128(7):931–3.
111. Apisarnthanarax N, Talpur R, Duvic M. Treatment of cutaneous T cell lymphoma: current status and future directions. Am J Clin Dermatol. 2002;3:193–215.
112. Clark C, Dawe RS, Evans AT, Lowe G, Ferguson J. Narrowband TL-01 phototherapy for patch-stage mycosis fungoides. Arch Dermatol. 2000;136(6):748–52.
113. Mang R, Krutmann J. UVA-I Phototherapy. Photodermatol Photoimmunol Photomed. 2005; 21(2):103–8.
114. Gruner S, Hofmann T, Meffert H, Sönnichsen N. Studies on the effects of a high dose UVA-1 radiation therapy on surface markers and function of epidermal Langerhans cells. Arch Dermatol Res. 1993;285(5):283–6.
115. Duthie MS, Kimber I, Dearman RJ, Norval M. Differential effects of UVA1 and UVB radiation on Langerhans cell migration in mice. J Photochem Photobiol B. 2000;57(2–3):123–31.
116. Krutmann J, Schopf E. High-dose-UVA1 phototherapy: a novel and highly effective approach for the treatment of acute exacerbation of atopic dermatitis. Acta Derm Venereol Suppl (Stockh). 1992;176:120–2.
117. Plettenberg R, Stege R, Megahed M, Ruzicka T, Hosokawa Y, Tsuji T, et al. Ultraviolet A1(340–400 nm) phototherapy for cutaneous T-cell lymphoma. J Am Acad Dermatol. 1999;41(1):47–50.
118. Garcia-Zuazaga J, Cooper KD, Baron ED. Photodynamic therapy in dermatology: current concepts in the treatment of skin cancer. Expert Rev Anticancer Ther. 2005;5(5):791–800.
119. Coors EA, von don Driesch P. Topical photodynamic therapy for patients with therapy-resistant lesions of cutaneous T-cell lymphoma. J Am Acad Dermatol. 2004;50(3):363–7.
120. Ibbotson SH. Topical 5-aminolaevulinic acid photodynamic therapy for the treatment of skin conditions other than non-melanoma skin cancer. Br J Dermatol. 2002;146(2):178–88.
121. Orenstein A, Haik J, Tamir J, Winkler E, Trau H, Malik Z, et al. Photodynamic therapy of cutaneous lymphoma using 5-aminolevulinic acid topical application. Dermatol Surg. 2000;26(8):765–9.
122. Nisticò S, Costanzo A, Saraceno R, Chimenti S. Efficacy of monochromatic excimer laser radiation (308 nm) in the treatment of early stage mycosis fungoides. Br J Dermatol. 2004;151(4):877–9.
123. Passeron T, Zakaria W, Ostovari N, Perrin C, Larrouy JC, Lacour JP, et al. Efficacy of the 308-nm excimer laser in the treatment of mycosis fungoides. Arch Dermatol. 2004;140(10):1291–3.
124. Jones GW, Hoppe RT, Glatstein E. Electron beam treatment for cutaneous T-cell lymphoma. Hematol Oncol Clin North Am. 1995;9(5):1057–76.
125. Hoppe RT, Cox RS, Fuks Z, Price NM, Bagshaw MA, Farber EM. Electron beam therapy for mycosis fungoides: the Stanford University experience. Cancer Treat Rep. 1979;63:691–700.
126. Tadros AA, Tepperman BS, Hryniuk WM, Peters VG, Rosenthal D, Roberts JT, et al. Total skin electron irradiation for mycosis fungoides: failure analysis and prognostic factors. Int J Radiat Oncol Biol Phys. 1983;9(9):1279–87.
127. Nisce LZ, Safai B. Once weekly total-skin electron beam therapy for mycosis fungoides: 7 years' experience. Cancer. 1981;47(5):870–6.
128. Chinn D. Total skin electron beam therapy with or without adjuvant topical nitrogen mustard or nitrogen mustard alone as initial treatment of T2 and T3 mycosis fungoides. Int J Radiat Oncol Biol Phys. 1999;43(5):951–8.
129. Wilson LD, Jones GW, Kim D, Rosenthal D, Christensen IR, Edelson RL, et al. Experience with total skin electron beam therapy in combination with extracorporeal photopheresis in the management of patients with erythrodermic (T4) mycosis fungoides. J Am Acad Dermatol. 2000;43(1 Pt 1): 54–60.
130. Price NM. Radiation dermatitis following electron beam therapy: an evaluation of patients ten years after total skin irradiation for mycosis fungoides. Arch Dermatol. 1978;114(1):63–6.
131. Vowels BR, Lessin SR, Cassin M, Jaworsky C, Benoit B, Wolfe JT, et al. Th2 cytokine mRNA expression in skin in cutaneous T-cell lymphoma. J Invest Dermatol. 1994;103:669–73.
132. Duvic M, Sherman ML, Wood GS, Kuzel TM, Olsen E, Foss F, et al. A phase II open-label study of recombinant human interleukin-12 in patients with stage IA, IB, or IIA mycosis fungoides. J Am Acad Dermatol. 2006;55(5):807–13.
133. Rook AH, Kubin M, Cassin M, Vonderheid EC, Vowels BR, Wolfe JT, et al. IL-12 reverses cytokine and immune abnormalities in Sézary syndrome. J Immunol. 1995;154(3):1491–8.
134. Zhang C, Hazarika P, Ni X, Weidner DA, Duvic M. Induction of apoptosis by bexarotene in cutaneous T-cell lymphoma cells: relevance to mechanism of therapeutic action. Clin Cancer Res. 2002;8(5):1234–40.
135. Richardson SK, Lin JH, Vittorio CC, Kim EJ, Yoon JS, Junkins-Hopkins J, et al. High clinical response rate with multimodality immunomodulatory therapy for Sezary syndrome. Clin Lymphoma Myeloma. 2006;7(3):226–32.
136. Kuzel T, Roenigk HJ, Samuelson E, Herrmann JJ, Hurria A, Rademaker AW, et al. Effectiveness of

interferon alfa-2a combined with phototherapy for mycosis fungoides and the Sézary syndrome. J Clin Oncol. 1995;13:257–63.
137. Roenigk HJ, Kuzel T, Skoutelis A, Springer E, Yu G, Caro W, et al. Photochemotherapy alone or combined with interferon alpha-2a in the treatment of cutaneous T-cell lymphoma. J Invest Dermatol. 1990;95:198S–205.
138. Mostow E, Neckel S, Oberhelman L, Anderson TF, Cooper KD. Complete remissions in psoralen and UV-A (PUVA)-refractory mycosis fungoides-type cutaneous T-cell lymphoma with combined interferon alfa and PUVA. Arch Dermatol. 1993;129:747.
139. Olsen E, Bunn PA. Interferon in the treatment of cutaneous T-cell lymphoma. Hematol Oncol Clin North Am. 1995;9:1089–107.
140. Kuzel T, Roenigk HJ, Samuelson E, Rosen ST. Suppression of anti-interferon alpha-2a antibody formation in patients with mycosis fungoides by exposure to long-wave UV radiation in the a range and methoxsalen ingestion. J Natl Cancer Inst. 1992;84:119–21.
141. Braather LR. Interferon alfa 2a combined with etretinate: effective in the treatment of mycosis fungoides. Retinoids Today Tomorrow. 1987;9:17–9.
142. Knobler RM, Trautinger F, Radaszkiewicz T, Kokoschka EM, Micksche M. Treatment of cutaneous T cell lymphoma with a combination of low-dose interferon alfa-2b and retinoids. J Am Acad Dermatol. 1991;24(2 Pt 1):247–52.
143. Altomare GF, Capella GL, Pigatto PD, Finzi AF. Intramuscular low dose alpha-2B interferon and etretinate for treatment of mycosis fungoides. Int J Dermatol. 1993;32(2):138–41.
144. Straus DJ, Duvic M, Kuzel T, Horwitz S, Demierre MF, Myskowski P, et al. Results of a phase II trial of oral bexarotene (Targretin) combined with interferon alfa-2b (Intron-A) for patients with cutaneous T-cell lymphoma. Cancer. 2007;109(9):1799–803.
145. Avilés A, Guzman R, Garcia EL, Diaz-Maqueo JC. Biological modifiers (etretinate (changed from etetrinate) and alfa 2a) in the treatment of refractory cutaneous T-cell lymphoma. Cancer Biother Radiopharm. 1996;11(1):21–4.
146. Von Hoff DD, Dahlberg S, Hartstock RJ, Eyre HJ. Activity of fludarabine monophosphate in patients with advanced mycosis fungoides: a Southwest Oncology Group study. J Natl Cancer Inst. 1990; 82(16):1353–5.
147. Scheulen ME, Ohl S, Bamberg M, Goos M. Treatment of mycosis fungoides by recombinant leukocyte alpha-A interferon and vinblastine. J Cancer Res Clin Oncol. 1986;111:S40.
148. Foss FM, Ihde DC, Breneman DL, Phelps RM, Fischmann AB, Schechter GP, et al. Phase II study of pentostatin and intermittent high-dose recombinant interferon alfa-2a in advanced mycosis fungoides/Sézary syndrome. J Clin Oncol. 1992;10(12):1907–13.
149. Cummings FJ, Kim K, Neiman RS, Comis RL, Oken MM, Weitzman SA, et al. Phase II trial of pentostatin in refractory lymphomas and cutaneous T-cell disease. J Clin Oncol. 1991;9(4):565–71.
150. Greiner D, Olsen EA, Petroni G. Pentostatin (2′-deoxycoformycin) in the treatment of cutaneous T-cell lymphoma. J Am Acad Dermatol. 1997;36(6 Pt 1):950–5.
151. Tsimberidou AM, Giles F, Duvic M, Fayad L, Kurzrock R. Phase II study of pentostatin in advanced T-cell lymphoid malignancies: update of an MD Anderson Cancer Center series. Cancer. 2004;100(2):342–9.
152. Monfardini S, Sorio R, Cavalli F, Cerny TH, Van Glabbeke M, Kaye S, et al. Pentostatin (2′-deoxycoformycin, dCF) in patients with low-grade (B-T-cell) and intermediate- and high-grade (T-cell) malignant lymphomas: phase II study of the EORTC Early Clinical Trials Group. Oncology. 1996; 53(2):163–8.
153. Ho AD, Suciu S, Stryckmans P, De Cataldo F, Willemze R, Thaler J, et al. Pentostatin in T-cell malignancies—a phase II trial of the EORTC Leukemia Cooperative Group. Ann Oncol. 1999; 10(12):1493–8.
154. Dearden C, Matutes E, Catovsky D. Pentostatin treatment of cutaneous T-cell lymphoma. Oncology (Williston Park). 2000;14(6 Suppl 2):37–40.
155. Jumbou O, N'Guyen JM, Tessier MH, Legoux B, Dreno B. Long-term follow-up in 51 patients with mycosis fungoides and Sézary syndrome treated by interferon-alfa. Br J Dermatol. 1999;140(3):427–31.
156. Olsen EA, Rosen ST, Vollmer RT, Variakojis D, Roenigk Jr HH, Diab N, et al. Interferon alfa-2a in the treatment of cutaneous T-cell lymphoma. J Am Acad Dermatol. 1989;20:395–407.
157. Kaplan EH, Rosen ST, Norris DB, Roenigk Jr HH, Saks SR, Bunn Jr PA. Phase II study of recombinant human interferon gamma for treatment of cutaneous T-cell lymphoma. J Natl Cancer Inst. 1990;82(3):208–12.
158. Horikoshi T, Onodera H, Eguchi H, Hanada N, Fukuzawa K, Takahashi M, et al. A patient with plaque-stage mycosis fungoides has successfully been treated with long-term administration of IFN-gamma and has been in complete remission for more than 6 years. Br J Dermatol. 1996;134(1):130–3.
159. Rook AH, Suchin KR, Kao DMF. Photopheresis: clinical application and mechanism of action. J Investig Dermatol Symp Proc. 1999;4:85–90.
160. Gardner JM, Introcaso CE, Nasta SD, Kim EJ, Vittorio CC, Rook AH. A novel regimen of vorinostat with interferon gamma for refractory Sézary syndrome. J Am Acad Dermatol. 2009;61(1):112–6.
161. Raphael BA, Shin DB, Suchin KR, Morrissey KA, Vittorio CC, Kim EJ, et al. High clinical response rate of Sezary syndrome to immunomodulatory therapies: prognostic markers of response. Arch Dermatol. 2011;147(12):1410–5.
162. Zinzani PL, Mazza P, Gherlinzoni F. Beta interferon in the treatment of mycosis fungoides. Haematologica. 1988;73(6):547–8.
163. Koh LK, Greenspan FS, Yeo PP. Interferon-alpha induced thyroid dysfunction: three clinical presenta-

tions and a review of the literature. Thyroid. 1997;7(6):891–6.
164. Vowels BR, Cassin M, Vonderheid EC, Rook AH. Aberrant cytokine production by Sézary syndrome patients: cytokine secretion pattern resembles murine Th2 cells. J Invest Dermatol. 1992;99(1):90–4.
165. Gately MK, Wolitzky AG, Quinn PM, Chizzonite R. Regulation of human cytolytic lymphocyte responses by interleukin-12. Cell Immunol. 1992;143(2):127–42.
166. Rook AH, Wood GS, Yoo EK, Elenitsas R, Kao DM, Sherman ML, et al. Interleukin-12 therapy of cutaneous T-cell lymphoma induces lesion regression and cytotoxic T-cell responses. Blood. 1999;94:902–8.
167. Gollob JA, Mier JW, Veenstra K, McDermott DF, Clancy D, Clancy M, et al. Phase I trial of twice-weekly intravenous interleukin 12 in patients with metastatic renal cell cancer or malignant melanoma: ability to maintain IFN-gamma induction is associated with clinical response. Clin Cancer Res. 2000;6:1678–92.
168. Wang KS, Zorn E, Ritz J. Specific down-regulation of interleukin-12 signaling through induction of phospho-STAT4 protein degradation. Blood. 2001;97:3860–6.
169. Zaki MH, Wysocka M, Everetts SE, Wang KS, French LE, Ritz J, et al. Synergistic enhancement of cell-mediated immunity by interleukin-12 plus interleukin-2: basis for therapy of cutaneous T cell lymphoma. J Invest Dermatol. 2002;118(2):366–71.
170. Thomsen K, Molin L, Volden G, Lange Wantzin G, Hellbe L. 13-cis-retinoic acid effective in mycosis fungoides A report from the Scandinavian Mycosis Fungoides Group. Acta Derm Venereol. 1984;64(6):563–6.
171. Claudy AL, Rouchouse B, Boucheron S, Le Petit JC. Treatment of cutaneous lymphoma with etretinate. Br J Dermatol. 1983;109(1):49–56.
172. Molin L, Thomsen K, Volden G, Aronsson A, Hammar H, Hellbe L, et al. Oral retinoids in mycosis fungoides and Sézary syndrome: a comparison of isotretinoin and etretinate a study from the Scandinavian Mycosis Fungoides Group. Acta Derm Venereol. 1987;67(3):232–6.
173. Kessler JF, Jones SE, Levine N, Lynch PJ, Booth AR, Meyskens Jr FL. Isotretinoin and cutaneous helper T-cell lymphoma (mycosis fungoides). Arch Dermatol. 1987;123(2):201–4.
174. Suchin KR, Cucchiara AJ, Gottleib SL, Wolfe JT, DeNardo BJ, Macey WH, et al. Treatment of cutaneous T-cell lymphoma with combined immunomodulatory therapy: a 14-year experience at a single institution. Arch Dermatol. 2002;138:1054–60.
175. Lim HW, Harris HR. Etretinate as an effective adjunctive therapy for recalcitrant palmar/plantar hyperkeratosis in patients with erythrodermic cutaneous T cell lymphoma undergoing photopheresis. Dermatol Surg. 1995;21(7):597–9.
176. Duvic M, Lemak NA, Redman JR, Eifel PJ, Tucker SL, Cabanillas FF, et al. Combined modality therapy for cutaneous T-cell lymphoma. J Am Acad Dermatol. 1996;34(6):1022–9.
177. Boehm MF, Zhang L, Badea BA, White SK, Mais DE, Berger E, et al. Synthesis and structure-activity relationships of novel retinoid X receptor-selective retinoids. J Med Chem. 1994;37(18):2930–41.
178. Richardson SK, Newton SB, Bach TL, Budgin JB, Benoit BM, Lin JH, et al. Bexarotene blunts malignant T-cell chemotaxis in Sezary syndrome: reduction of chemokine receptor 4-positive lymphocytes and decreased chemotaxis to thymus and activation-regulated chemokine. Am J Hematol. 2007;82(9):792–7.
179. Talpur R, Ward S, Apisarnthanarax N, Breuer-Mcham J, Duvic M. Optimizing bexarotene therapy for cutaneous T-cell lymphoma. J Am Acad Dermatol. 2002;47(5):672–84.
180. Keun YK, Woodruff R, Sangueza O. Response of CD30+ large cell lymphoma of skin to bexarotene. Leuk Lymphoma. 2002;43(5):1153–4.
181. Sherman SI, Gopal J, Haugen BR, Chiu AC, Whaley K, Nowlakha P, et al. Central hypothyroidism associated with retinoid X receptor-selective ligands. N Engl J Med. 1999;340(14):1075–9.
182. Marks PA, Dokmanovic M. Histone deacetylase inhibitors: discovery and development as anticancer agents. Expert Opin Investig Drugs. 2005;14(12):1497–511.
183. Duvic M, Talpur R, Ni X, Zhang C, Hazarika P, Kelly C, et al. Phase II trial of oral vorinostat (suberoylanilide hydroxamic acid, SAHA) for refractory cutaneous T-cell lymphoma (CTCL). Blood. 2007;109(1):31–9.
184. Duvic M, Zhang C. Clinical and laboratory experience of vorinostat (suberoylanilide hydroxamic acid) in the treatment of cutaneous T-cell lymphoma. Br J Cancer. 2006;95 Suppl 1Suppl 1:S13–9.
185. Rangwala S, Duvic M, Zhang C. Trends in the treatment of cutaneous T-cell Lymphoma—critical evaluation and perspectives on vorinostat. Blood Lymp Cancer Targets Ther. 2012;2021(2):17–27.
186. De Ruijter AJ, van Gennip AH, Caron HN, Kemp S, van Kuilenburg AB. Histone deacetylases (HDACs): characterization of the classical HDAC family. Biochem J. 2003;370(Pt 3):737–49.
187. Gregoretti IV, Lee YM, Goodson HV. Molecular evolution of the histone deacetylase family: functional implications of phylogenetic analysis. J Mol Biol. 2004;338:17–31.
188. Sakajiri S, Kumagai T, Kawamata N, Saitoh T, Said JW, Koeffler HP. Histone deacetylase inhibitors profoundly decrease proliferation of human lymphoid cancer cell lines. Exp Hematol. 2005;33(1):53–61.
189. Kelly W, Richon VM, O'Connor O, Curley T, MacGregor-Curtelli B, Tong W, et al. Phase I clinical trial of histone deacetylase inhibitor: suberoylanilide hydroxamic acid administered intravenously. Clin Cancer Res. 2003;9(10 Pt 1):3578–88.
190. Butler L, Liapis MV, Bouralexis S, Welldon K, Hay S, le Thai M, et al. The histone deacetylase inhibitor, suberoylanilide hydroxamic acid, overcomes resistance of human breast cancer cells to Apo2L/TRAIL. Int J Cancer. 2006;119(4):944–54.

191. Lakshmikanthan V, Kaddour-Djebbar I, Lewis RW, Kumar MV. SAHA-sensitized prostate cancer cells to TNF alpha-related apoptosis-inducing ligand (TRAIL): mechanisms leading to synergistic apoptosis. Int J Cancer. 2006;119(1):221–8.
192. Glaser KB. HDAC inhibitors: clinical update and mechanism-based potential. Biochem Pharmacol. 2007;74(5):659–71.
193. Garcia-Manero G, Yang H, Bueso-Ramos C, Ferrajoli A, Cortes J, Wierda WG, et al. Phase 1 study of the histone deacetylase inhibitor vorinostat (suberoylanilide hydroxamic acid [SAHA]) in patients with advanced leukemias and myelodysplastic syndromes. Blood. 2008;111(3):1060–6.
194. Vrana JA, Decker RH, Johnson CR, Wang Z, Jarvis WD, Richon VM, et al. Induction of apoptosis in U937 human leukemia cells by suberoylanilide hydroxamic acid (SAHA) proceeds through pathways that are regulated by Bcl-2/Bcl-XL, c-Jun, and p21CIP1, but independent of p53. Oncogene. 1999;18:7016–25.
195. O'Connor OA. Clinical experience with the novel histone deacetylase inhibitor vorinostat (suberoylanilide hydroxamic acid) in patients with relapsed lymphoma. Br J Cancer. 2006;95:S7–12.
196. Piekarz RL, Robey RW, Zhan Z, Kayastha G, Sayah A, Abdeldaim AH, et al. T-cell lymphoma as a model for the use of histone deacetylase inhibitors in cancer therapy: impact of depsipeptide on molecular markers, therapeutic targets, and mechanisms of resistance. Blood. 2004;103(12):4636–43.
197. Marks PA, Jiang X. Histone deacetylase inhibitors in programmed cell death and cancer therapy. Cell Cycle. 2005;4:549–51.
198. Tiffon C, Adams J, van der Fits L, Wen S, Townsend P, Ganesan A, et al. The histone deacetylase inhibitors vorinostat and romidepsin downmodulate IL-10 expression in cutaneous T-cell lymphoma cells. Br J Pharmacol. 2011;162(7):1590–602.
199. Kelly WK, O'Connor OA, Krug LM, Chiao JH, Heaney M, Curley T, et al. Phase I study of an oral histone deacetylase inhibitor, suberoylanilide hydroxamic acid, in patients with advanced cancer. J Clin Oncol. 2005;23(17):3923–31.
200. Olsen E, Kim YH, Kuzel T, Pacheco TR, Foss FM, Parker S, et al. A Phase IIb multicenter trial of vorinostat (suberoylanilide hydroxamic acid, SAHA) in patients with persistent, progressive, or treatment refractory mycosis fungoides or Sézary syndrome subtypes of cutaneous T-cell lymphoma. J Clin Oncol. 2007;25(21):3109–15.
201. Mann BS, Johnson JR, He K, Sridhara R, Abraham S, Booth BP, et al. Vorinostat for treatment of cutaneous manifestations of advanced primary cutaneous T-cell lymphoma. Clin Cancer Res. 2007;13:2318–22.
202. Kim YH, Reddy S, Kim EJ, Rook AH, Lerner A, Demierre M, Duvic M, Whittaker S, Robak T, Becker JC, McCulloch W, Prentice AG. Romidepsin (depsipeptide) induces clinically significant responses in treatment-refractory CTCL: an international study. Blood. 2007;110(Abst 123).
203. Whittaker SJ, Demierre MF, Kim EJ, Rook AH, Lerner A, Duvic M, et al. Final results from a multicenter, international, pivotal study of romidepsin in refractory cutaneous T-cell lymphoma. J Clin Oncol. 2010;28(29):4485–91.
204. Piekarz RL, Frye R, Turner M, Wright JJ, Allen SL, Kirschbaum MH, et al. Phase II multi-institutional trial of the histone deacetylase inhibitor romidepsin as monotherapy for patients with cutaneous T-cell lymphoma. J Clin Oncol. 2009;27(32):5410–7.
205. Piekarz RL, Frye R, Prince HM, Kirschbaum MH, Zain J, Allen SL, et al. Phase 2 trial of romidepsin in patients with peripheral T-cell lymphoma. Blood. 2011;117(22):5827–34.
206. Marcellus DC, Vogelsang GB. Chronic graft-versus host disease. In: Ball DLJ, Law P, editors. Hematopoietic stem cell therapy. Oxford: Churchill Livingstone; 2000. p. 614–24.
207. Lazarus HM, Vogelsang GB, Rowe lM. Prevention and treatment of acute GVHD: the old and the new a report from the Eastern Cooperative Oncology group (BCOG). Bone Marrow Transplant. 1997;19:577–600.
208. Edelson RL, Berger CL, Gasparro FP, Jegasothy B, Heald P, Wintroub B, et al. Treatment of cutaneous T-cell lymphoma by extracorporeal photochemotherapy. N Eng J Med. 1987;316:297–303.
209. Pathak MA, Daniels F, Fitzpatrick TB. The presently known distributions of furocoumarins (psoralens) in plants. J Invest Dermatol. 1962;39:225–39.
210. Bladon J, Taylor PC. ECP induces apoptosis in the lymphocytes of cutaneous T-cell lymphoma and GVHD patients. Br J Haematol. 1999;107:707–11.
211. Ben-Nun A, Wekerle H, Cohen IR. Vaccination against autoimmune encephalomyelitis with T lymphocyte line cells reactive against myelin basic protein. Nature. 1981;292:60–1.
212. Yoo EK, Elonitsas R, Gasparro FP. Apoptosis induction by ultraviolet light A and photochemotherapy in coetaneous T-cell lymphoma: relevance to mechanism of therapeutic action. J Invest Dermatol. 1996;107:235–42.
213. Tambur AR, Ortegel JW, Morales A. Extracorporeal photopheresis induces lymphocyte but not monocyte apoptosis. Transplant Proc. 2000;32:747–8.
214. Albert ML, Sauter B, Bhardwaj N. Dendritic cells acquire antigen from apoptotic cells and induce class I-restricted CTLS. Nature. 1998;392:86–9.
215. Gatza E, Rogers CE, Clouthier SG, Lowler KP, Tawara I, Liu C, et al. Extracorporeal photopheresis reverses experimental graft-versus-host disease through regulatory T cells. Blood. 2008;112(4):1515–21.
216. Perseghin P. Extracorporeal photochemotherapy as a challenging treatment for cutaneous T-cell lymphoma, acute and chronic graft-versus-host disease, organ rejection and T-lymphocyte-mediated autoimmune diseases. Transfus Med Hemother. 2008;35:8–17.
217. Arulogun S, Prince HM, Gambell P, Lade S, Ryan G, Eaton E, et al. Extracorporeal photopheresis for the

treatment of Sézary syndrome using a novel treatment protocol. J Am Acad Dermatol. 2008;59:589–95.
218. Lim HW, Edelson RL. Photopheresis for the treatment of cutaneous T-cell lymphoma. Hematol Oncol Clin North Am. 1995;9(5):1117–26.
219. Bisaccia E, Gonzalez J, Palangio M, Schwartz J, Klainer AS. Extracorporeal photochemotherapy alone or with adjuvant therapy in the treatment of cutaneous T-cell lymphoma: a 9-year retrospective study at a single institution. J Am Acad Dermatol. 2000;43:263–71.
220. Oziemski MA, Rockman SP, Marks DI, Green MD, Varigos GA, Fox RM. Extracorporeal photochemotherapy in the treatment of cutaneous T-cell lymphoma Complexity of objective evaluation. Int J Dermatol. 1991;30:814–7.
221. Koh HK, Davis BE, Meola T, Lim HW. Extracorporeal photopheresis for the treatment of 34 patients with cutaneous T cell lymphoma (CTCL). J Invest Dermatol. 1994;102:567.
222. Heald PW, Perez MI, Christensen I, Dobbs N, McKiernan G, Edelson R. Photopheresis therapy of cutaneous T cell lymphoma: the Yale-New Haven Hospital experience. Yale J Biol Med. 1989;62:629–38.
223. Duvic M, Hester JP, Lemak NA. Photopheresis therapy for cutaneous T-cell lymphoma. J Am Acad Dermatol. 1996;35(4):573–9.
224. Zic J, Arzubiaga C, Salhany KE, Parker RA, Wilson D, Stricklin GP, et al. Extracorporeal photopheresis for the treatment of cutaneous T-cell lymphoma. J Am Acad Dermatol. 1992;27:729–36.
225. Armus R, Keyes B, Cahill C, Berger C, Crater D, Scarborough D, et al. Photopheresis for the treatment of cutaneous T-cell lymphoma. J Am Acad Dermatol. 1990;23:898–902.
226. Prinz B, Behrens W, Holzle E, Plewig G. Extracorporeal photopheresis for the treatment of cutaneous T-cell lymphoma—the Dusseldorf and Munich experience. Arch Dermatol Res. 1995;287:621–6.
227. Jiang SB, Dietz SB, Kim M, Lim HW. Extracorporeal photochemotherapy for cutaneous T-cell lymphoma: a 97-year experience. Photodermatol Photoimmunol Photomed. 1999;15:161–5.
228. Vonderheid EC, Zhang Q, Lessin SR, Polansky M, Abrams JT, Bigler RD, et al. Use of serum soluble interleukin-2 receptor levels to monitor the progression of cutaneous T-cell lymphoma. J Am Acad Dermatol. 1998;38:207–20.
229. Owsianowski M, Garbe C, Ramaker J, Orfanos CE, Gollnick H. Therapeutische Erfahrungen mit der Extracorporalen Photopherese. Hautarzt. 1996;47:114–23.
230. Dall'Amico R, Zacchello G, Heald P. Applicazione della fotoferesis nella terapia di malattie oncologiche ed autiommuni (The application of photopheresis in the therapy of cancerous and autoimmune disease). Recenti Prog Med. 1991;82:294–9.
231. Crovetti G, Carabelli A, Berti E, Guizzardi M, Fossati S, De Filippo C, et al. Photopheresis in cutaneous T-cell lymphoma: five-year experience. Int J Artif Organs. 2000;23:55–62.
232. Konstantinow A, Balda B-R. Treatment of cutaneous T-cell lymphoma with extracorporeal photochemotherapy. J Eur Acad Dermatol Venereol. 1997;9:111–7.
233. Russell-Jones R, Fraser-Andrews E, Spittle M, Whittaker S. Extracorporeal photopheresis in Sézary syndrome. Lancet. 1997;350(9081):886.
234. Stevens S, Bowen G, Duvic M, King LE, Knobler R, Lim HW, et al. Effectiveness of photopheresis in Sezary syndrome. Arch Dermatol. 1999;135:995–7.
235. Edelson RL. Sézary syndrome, cutaneous T-cell lymphoma, and extracorporeal photopheresis. Arch Dermatol. 1999;135:600–1.
236. Zic JA. The treatment of cutaneous T-cell lymphoma with photopheresis. Dermatol Ther. 2003;16:337–46.
237. Zic JA, Stricklin GP, Greer JP, Kinney MC, Shyr Y, Wilson DC, et al. Long-term follow-up of patients with cutaneous T-cell lymphoma treated with extracorporeal photochemotherapy. J Am Acad Dermatol. 1996;35:935–45.
238. Gollnick HP, Owsianowski M, Ramaker J, Chun SC, Orfanos CE. Extracorporeal photopheresis—a new approach for the treatment of cutaneous T cell lymphomas. Recent Results Cancer Res. 1995;139:409–15.
239. Heald P, Rook A, Perez M, Wintroub B, Knobler R, Jegasothy B, et al. Treatment of erythrodermic cutaneous T-cell lymphoma with extracorporeal photochemotherapy. J Am Acad Dermatol. 1992;27:427–33.
240. Gottlieb SL, Wolfe JT, Fox FE, DeNardo BJ, Macey WH, Bromley PG, et al. Treatment of cutaneous T-cell lymphoma with extracorporeal photopheresis monotherapy and in combination with recombinant interferon alfa: a 10-year experience at a single institution. J Am Acad Dermatol. 1996;35:946–57.
241. Fraser-Andrews ESP, Whittaker S, Russell-Jones R. Extracorporeal photopheresis in Sézary syndrome: no significant effect in the survival of 44 patients with a peripheral blood T-cell clone. Arch Dermatol. 1998;134:1001–5.
242. Vidulich K, Jones D, Duvic M. Cutaneous gamma/delta T-cell lymphoma treated with radiation and denileukin diftitox. Clin Lymphoma Myeloma. 2008;8(1):55–8.
243. McKenna KE, Whittaker S, Rhodes LE, Taylor P, Lloyd J, Ibbotson S, et al. Evidence-based practice of photopheresis 1987–2001: a report of a workshop of the British Photodermatology Group and the UK Skin Lymphoma Group. Br J Dermatol. 2006;154:7–20.
244. Demierre MF, Foss FM, Koh HK. Proceedings of the international consensus conference on cutaneous T-cell lymphoma (CTCL) treatment recommendations. Boston, Massachusetts, Oct 1 and 2, 1994. J Am Acad Dermatol. 1997;36:460–6.
245. Girardi M, Knobler R, Edelson R. Selective immmunotherapy through extracorporeal photochemotherapy: yesterday, today, and tomorrow. Hematol Oncol Clin North Am. 2003;17:1391–403.

246. Knobler R, Jantschitsch C. Extracorporeal photochemoimmunotherapy in cutaneous T-cell lymphoma. Transfus Apher Sci. 2003;28:81–9.
247. Stevens SR, Baron ED, Masten S, Cooper KD. Circulating CD4+CD7-lymphocyte burden and rapidity of response: predictors of outcome in the treatment of Sézary syndrome and erythrodermic mycosis fungoides with extracorporeal photopheresis. Arch Dermatol. 2002;138:1347–50.
248. Knobler E, Warmuth I, Cocco C, Miller B, Mackay J. Extracorporeal photochemotherapy—the Columbia presbyterian experience. Photodermatol Photoimmunol Photomed. 2002;18:232–7.
249. Miller JD, Kirkland EB, Domingo DS, Scull H, Jekutis B, Dallas M, et al. Review of extracorporeal photopheresis in early-stage (IA, IB, and IIA) cutaneous T-cell lymphoma. Photodermatol Photoimmunol Photomed. 2007;23(5):163–71.
250. Duvic M, Chiao N, Talpur R. Extracorporeal photopheresis for the treatment of cutaneous T-cell lymphoma. J Cutan Med Surg. 2003;7:3–7.
251. Di Renzo M, Rubegni P, De Aloe G, Paulesu L, Pasqui AL, Andreassi L, et al. Extracorporeal photochemotherapy restores Th1/Th2 imbalance in patients with early stage cutaneous T-cell lymphoma. Immunology. 1997;92:99–103.
252. Child FJ, Mitchell TJ, Whittaker SJ, Scarisbrick JJ, Seed PT, Russell-Jones RA. Randomized cross-over study to compare PUVA and extracorporeal photopheresis in the treatment of plaque stage (T2) mycosis fungoides. Clin Exp Dermatol. 2004;29:231–6.
253. Wollina U, Liebold K, Kaatz M, Looks A, Stuhlert A, Lange D. Survival of patients with cutaneous T-cell lymphoma after treatment with extracorporeal photochemotherapy. Oncol Rep. 2000;7:1197–201.
254. Rubegni P, De Aloe G, Fimiani M. Extracorporeal photochemotherapy in long-term treatment of early stage cutaneous T-cell lymphoma. Br J Dermatol. 2000;143:894–6.
255. Wollina U, Looks A, Meyer J, Knopf B, Koch HJ, Liebold K, et al. Treatment of cutaneous T cell lymphoma stage II with interferon-alpha-2a and extracorporeal photochemotherapy: a prospective controlled trial. Ann N Y Acad Sci. 2001;941:210–3.
256. Talpur R, Demierre MF, Geskin L, Baron E, Pugliese S, Eubank K, et al. Multicenter photopheresis intervention trial in early-stage mycosis fungoides. Clin Lymphoma Myeloma Leukemia. 2011;11(2):219–27.
257. Rook AH, Prystowsky MB, Cassin M, Boufal M, Lessin SR. Combined therapy for Sézary syndrome with extracorporeal photochemotherapy and low-dose interferon alfa therapy Clinical, molecular, and immunologic observations. Arch Dermatol. 1991;127(10):1535–40.
258. Zackheim HS. Evidence is lacking for a synergistic or additive effect of combination extracorporeal photopheresis with interferon alfa for cutaneous T-cell lymphoma. J Am Acad Dermatol. 2000;42(6):1087–8.
259. Wilson LD, Licata AL, Braverman IM, Edelson RL, Heald PW, Feldman AM, et al. Systemic chemotherapy and extracorporeal photochemotherapy for T3 and T4 cutaneous T-cell lymphoma patients who have achieved a complete response to total skin electron beam therapy. Int J Radiat Oncol Biol Phys. 1995;32(4):987–95.
260. Quaglino P, Fierro MT, Rossotto GL, Savoia P, Bemengo MG. Treatment of advanced mycosis fungoides/Sézary syndrome with fludarabine and potential adjunctive benefit to subsequent extracorporeal photochemotherapy. Br J Dermatol. 2004;150(2):327–36.
261. Duvic M, Talpur R. Optimizing denileukin diftitox (Ontak) therapy. Future Oncol. 2008;4(4):457–69.
262. Olsen EA, Duvic M, Frankel A, Kim Y, Martin A, Vonderheid E, et al. Pivotal phase III trial of two dose levels of denileukin diftitox for the treatment of cutaneous T-cell lymphoma. J Clin Oncol. 2001; 19:376–88.
263. Duvic M, Cather J, Maize J, Frankel AE. DAB389IL2 diphtheria fusion toxin produces responses in tumor stage cutaneous T-cell lymphoma. Am J Hematol. 1998;58:87–90.
264. Chin KM, Foss FM. Biologic correlates of response and survival in patients with cutaneous T-cell lymphoma treated with denileukin diftitox. Clin Lymphoma Myeloma. 2006;7(3):199–204.
265. Foss F, Demierre MF, DiVenuti G. A phase-1 trial of bexarotene and denileukin diftitox in patients with relapsed or refractory cutaneous T-cell lymphoma. Blood. 2005;106(2):454–7.
266. Prince HM, Duvic M, Martin A, Sterry W, Assaf C, Sun YS, et al. Phase III placebo-controlled trial of denileukin diftitox for patients with cutaneous T-cell lymphoma. J Clin Oncol. 2010;28(11):1870–7.
267. Foss FM. DAB(389)IL-2 (ONTAK): a novel fusion toxin therapy for lymphoma. Clin Lymphoma. 2000;1(2):110–6.
268. Foss FM, Bacha P, Osann KE, Demierre MF, Bell T, Kuzel T. Biological correlates of acute hypersensitivity events with DAB(389)IL-2 (denileukin diftitox, ONTAK) in cutaneous T-cell lymphoma: decreased frequency and severity with steroid premedication. Clin Lymphoma Myeloma. 2001;1(4):298–302.
269. Talpur R, Apisarnthanarax N, Ward S, Duvic M. Treatment of refractory peripheral T-cell lymphoma with denileukin diftitox (ONTAK). Leuk Lymphoma. 2002;43(1):121–6.
270. Lundin J, Hagberg H, Repp R, Cavallin-Ståhl E, Fredén S, Juliusson G, et al. Phase 2 study of alemtuzumab (anti-CD52 monoclonal antibody) in patients with advanced mycosis fungoides/Sézary syndrome. Blood. 2003;101:4267–72.
271. Querfeld C, Mehta N, Rosen ST, Guitart J, Rademaker A, Geram P, et al. Alemtuzumab for relapsed and refractory erythrodermic cutaneous T-cell lymphoma: a single institution experience from the Robert H Lurie Comprehensive Cancer Center. Leuk Lymphoma. 2009;50(12):1969–76.
272. Kim YH, Duvic M, Obitz E, Gniadecki R, Iversen L, Osterborg A, et al. Clinical efficacy of zanolimumab (HuMax-CD4): two Phase II studies in refractory cutaneous T-cell lymphoma. Blood. 2007;109(11): 4655–62.

273. Forero-Torres A, Bernstein SH, Gopal A, et al. SGN-30 (Anti-CD30 mAb) has a single-agent response rate of 21 % in patients with refractory or recurrent systemic anaplastic large cell lymphoma (ALCL). Blood. 2006;108:768a.
274. Duvic M, Reddy SA, Pinter-Brown L, Korman NJ, Zic J, Kennedy DA, et al. A phase II study of SGN-30 in cutaneous anaplastic large cell lymphoma and related lymphoproliferative disorders. Clin Cancer Res. 2009;15(19):6217–24.
275. Duvic M, Forero-Torres A, Foss F, Olsen EA, Kim Y, Bennett JC, et al. Oral Forodesine (BCX-1777) is clinically active in refractory cutaneous T-cell lymphoma: results of a Phase I/II study. Blood. 2006;108(11):698a.
276. Bantia S, Ananth SL, Parker CD, Horn LL, Upshaw R. Mechanism of inhibition of T-acute lymphoblastic leukemia cells by PNP inhibitor-BCX-1777. Int Immunopharmacol. 2003;3:879–87.
277. Kicska GA, Long L, Horig H, Fairchild C, Tyler PC, Furneaux RH, et al. A powerful transition-state analog inhibitor of purine nucleoside phosphorylase, selectively inhibits human T lymphocytes. Proc Nat Acad Sci USA. 2001;98:4593–8.
278. Nabhan C, Krett N, Gandhi V, Rosen S. Gemcitabine in hematologic malignancies. Curr Opin Oncol. 2001;13:514–21.
279. Zinzani PL, Magagnoli M, Bendandi M, Orcioni GF, Gherlinzoni F, Albertini P, et al. Therapy with gemcitabine in pretreated peripheral T-cell lymphoma patients. Ann Oncol. 1998;9(12):1351–3.
280. Sallah S, Wan JY, Nguyen NP. Treatment of refractory T-cell malignancies using gemcitabine. Br J Haematol. 2001;113(1):185–7.
281. Zinzani PL, Baliva G, Magagnoli M, Bendandi M, Modugno G, Gherlinzoni F, et al. Gemcitabine treatment in pretreated cutaneous T-cell lymphoma: experience in 44 patients. J Clin Oncol. 2000;18(13):2603–6.
282. Marchi E, Alinari L, Tani M, Stefoni V, Pimpinelli N, Berti E, et al. Gemcitabine as frontline treatment for cutaneous T-cell lymphoma: phase II study of 32 patients. Cancer. 2005;104(11):2437–41.
283. Duvic M, Talpur R, Wen S, Kurzrock R, David CL, Apisarnthanarax N. Phase II evaluation of gemcitabine monotherapy for cutaneous T-cell lymphoma. Clin Lymphoma Myeloma. 2006;7(1):51–8.
284. Grever MR, Bisaccia E, Scarborough DA, Metz EN, Neidhart JA. An investigation of 2′-deoxycoformycin in the treatment of cutaneous T-cell lymphoma. Blood. 1983;61(2):279–82.
285. Foss FM. Activity of pentostatin (Nipent) in cutaneous T-cell lymphoma: single-agent and combination studies. Semin Oncol. 2000;27(2 Suppl 5):58–63.
286. Kurzrock R, Pilat S, Duvic M. Pentostatin therapy of T-cell lymphomas with cutaneous manifestations. J Clin Oncol. 1999;17(10):3117–21.
287. Grem JL, King SA, Chun HG, Grever MR. Cardiac complications observed in elderly patients following 2′deoxycoformycin therapy. Am J Hematol. 1991;38:245–7.
288. Wollina U, Graefe T, Kaatz M. Pegylated doxorubicin for primary cutaneous T cell lymphoma: a report on ten patients with follow-up. Ann N Y Acad Sci. 2001;941:214–6.
289. Chanan-Khan AA, Lee K. Pegylated liposomal doxorubicin and immunomodulatory drug combinations in multiple myeloma: rationale and clinical experience. Clin Lymphoma Myeloma. 2007;7 Suppl 4Suppl 4:S163–9.
290. Ryan DH, Bickers JN, Vial RH, Hussein K, Bottomley R, Hewlett JS, et al. Doxorubicin and ifosfamide combination chemotherapy in previously treated acute leukemia in adults: a Southwest Oncology Group pilot study. Cancer Treat Rep. 1980;64(8–9):869–72.
291. Gabizon AA. Selective tumor localization and improved therapeutic index of anthracyclines encapsulated in long-circulating liposomes. Cancer Res. 1992;52(4):891–6.
292. Wollina U, Dummer R, Brockmeyer NH, Konrad H, Busch JO, Kaatz M, et al. Multicenter study of pegylated liposomal doxorubicin in patients with cutaneous T-cell lymphoma. Cancer. 2003;98(5):993–1001.
293. Winkelmann RK, Diaz Perez JL, Buechner SA. The treatment of Sézary syndrome. J Am Acad Dermatol. 1984;10:1000–4.
294. Tirelli U, Carbone A, Veronesi A, Galligioni E, Trovo MG, Tumolo S, et al. Combination chemotherapy with cyclophosphamide, vincristine, and prednisone (CVP) in TNM-classified stage IV mycosis fungoides. Cancer Treat Rep. 1982;66(1):167–9.
295. Grozea PN, Jones SE, McKelvey EM, Coltman Jr CA, Fisher R, Haskins CL. Combination chemotherapy for mycosis fungoides: a Southwest Oncology Group study. Cancer Treat Rep. 1979;63(4):647–53.
296. Hallahan DE, Griem ML, Griem SF, Medenica M, Soltan K, Lorincz AL, et al. Combined modality therapy for tumor stage mycosis fungoides: results of a 10-year follow-up. J Clin Oncol. 1988;6(7):1177–83.
297. Zakem MH, Davis BR, Adelstein DJ, Hines JD. Treatment of advanced stage mycosis fungoides with bleomycin, doxorubicin, and methotrexate with topical nitrogen mustard (BAM-M). Cancer. 1986;58:2611–6.
298. Sentis HJ, Willemze R, Van Vloten WA. Systemic polychemotherapy in patients with mycosis fungoides and lymph node involvement: a follow-up study of 17 patients. Acta Derm Venereol. 1985;65(2):179–83.
299. Molina A, Zain J, Arber DA, Angelopolou M, O'Donnell M, Murata-Collins J, et al. Durable clinical, cytogenetic, and molecular remissions after allogeneic hematopoietic cell transplantation for refractory Sezary syndrome and mycosis fungoides. J Clin Oncol. 2005;23(25):6163–71.
300. Duarte RF, Schmitz N, Servitje O, Sureda A. Hematopoietic stem cell transplantation for patients with primary cutaneous T-cell lymphoma. Bone Marrow Transplant. 2008;41(7):597–604.

Central Nervous System Lymphoma

20

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Abstract

Central nervous system (CNS) lymphomas comprise a diverse group of primary or secondary neoplasms of the brain and leptomeninges. Primary CNS lymphoma, most commonly a diffuse large B-cell lymphoma (DLBCL) confined to the CNS, is treated by chemotherapy or chemoradiation strategies that are different from the approaches used for DLBCL elsewhere in the body. Secondary CNS lymphoma occurs when a systemic lymphoma disseminates to the leptomeninges or the brain. The risk of secondary CNS lymphoma is dependent on the lymphoma subtype and the anatomic location. Although radiation and intrathecal or intravenous chemotherapy are commonly utilized in the treatment of secondary CNS lymphoma, prognosis remains poor. Neurotoxicity is a significant complication of CNS-directed therapy for primary and secondary CNS lymphomas and the risk is highest in older patients treated with whole brain radiation therapy.

Keywords

Brain • Leptomeninges • Lymphoma • Chemotherapy • Radiation

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Primary CNS Lymphoma

Primary central nervous system lymphoma (PCNSL) is a form of extranodal non-Hodgkin lymphoma (NHL) that is confined to the central nervous system (CNS). PCNSL can affect multiple parts of the neuraxis including the eyes, brain, leptomeninges, or spinal cord. An estimated 3,855 cases of PCNSL were diagnosed in the United States from 2004 to 2006, and the number of cases is expected to increase further with the aging of the United States population [1]. PCNSL accounts for approximately 3 % of all the primary CNS tumors diagnosed each year in the

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United States. Between 1970 and 2000, the incidence of PCNSL increased, largely due to the human immunodeficiency virus (HIV) pandemic. However, the incidence has stabilized or decreased over the last decade to about 0.47 cases per 100,000 persons [2, 3]. Congenital or acquired immunodeficiency is the only established risk factor for PCNSL and HIV-infected individuals are at greater risk of developing this tumor.

Pathobiology

The majority (90 %) of non-HIV associated PCNSL is the diffuse large B-cell (DLBCL) type with the remaining 10 % consisting of low-grade lymphomas, Burkitt lymphoma, or T-cell lymphomas [4]. Less is known about these rare variants of PCNSL. Primary CNS DLBCL has an angiocentric pattern of growth in the brain that is unique to this NHL subtype. Occasionally T-cell infiltrates are also present, making it difficult for the pathologist to discriminate between PCNSL and a reactive, inflammatory process.

PCNSL likely arises from late germinal center or post-germinal center lymphoid cells and localizes to the CNS because of a poorly understood neurotropism [5]. Gene expression studies have demonstrated 3 gene "signatures" associated with PCNSL: germinal center B cell, activated B cell, and type 3 large B-cell lymphoma [6]. While these 3 gene expression patterns parallel DLBCL, there are unique molecular features of PCNSL. For example, microRNA studies have demonstrated different expression patterns between PCNSL and systemic DLBCL [7]. Also, extracellular matrix-related genes are upregulated in PCNSL compared to systemic DLBCL [8]. Interaction between tumor cells and extracellular matrix proteins specific to the CNS may offer an explanation for the neurotropism of PCNSL.

Several genes associated with interleukin-4 (IL-4), a B-cell growth factor expressed by both tumor endothelium and tumor cells, are highly expressed in PCNSL including X-box binding protein 1 (XBP-1), a regulator of the unfolded protein response (UPR) signaling pathway. The expression of UPR-related genes is important for cell survival under stressful conditions such as hypoxia so activation of this pathway may promote tumor cell survival in the CNS. STAT6, a mediator of IL-4 signaling, is expressed by tumor cells and tumor endothelium in PCNSL. High expression levels of STAT6 are associated with reduced survival in PCNSL patients treated with chemotherapy [6].

Clinical Features

The median age of immunocompetent patients diagnosed with PCNSL is 60 [9]. In 248 immunocompetent patients, 43 % had neuropsychiatric signs, 33 % had symptoms of increased intracranial pressure, 14 % had seizures, and 4 % had ocular symptoms [10]. Seizures are less common than with other types of brain tumors probably because PCNSL involves predominantly subcortical white matter rather than epileptogenic gray matter. Unlike patients with systemic NHL, PCNSL patients rarely manifest B symptoms.

Diagnostic Evaluation

The International PCNSL Collaborative Group (IPCG) has established guidelines for the diagnostic evaluation of a patient with suspected PCNSL (Table 20.1) [11]. These guidelines establish the extent of disease and confirm that the disease is restricted to the CNS. Physical examination should include palpation of the lymphatic chain as well as testicular examination in males since testicular lymphoma has a predilection to disseminate to the brain parenchyma. Diagnostic studies include contrast-enhanced brain imaging; lumbar puncture, if not contraindicated (for cell count, protein, glucose, cytology, IgH gene rearrangement, and flow cytometry studies); ophthalmologic examination including slit lamp evaluation; computerized tomography (CT) scans of the chest,

Table 20.1 International Primacy CNS Lymphoma Collaborative Group (IPCG) guidelines for baseline evaluation for clinical trials

Pathology	Clinical	Laboratory	Imaging
Centralized review of pathology	Complete medical and neurological examination	HIV serology	Contrast-enhanced cranial MRI[c]
Immunophenotyping	Dilated eye examination including slit lamp evaluation	Serum LDH level	CT of chest, abdomen, and pelvis
	Record prognostic factors (age, performance status)	CSF cytology, flow cytometry, IgH PCR	Testicular ultrasound in elderly males
	Serial evaluation of cognitive function[a]	24-h urine collection for creatinine clearance[b]	
		Bone marrow biopsy with aspirate	

Adapted from Abrey et al. [11]
[a]Mini-mental status examination is used commonly although improved instruments are being developed
[b]For patients who will receive high-dose methotrexate
[c]Contrast-enhanced cranial CT should be obtained in patients who have a contraindication for MRI (e.g., pacemaker) or who cannot tolerate MRI (e.g., claustrophobia)

abdomen, and pelvis; and bone marrow biopsy. Blood tests for HIV, complete blood count, basic metabolic panel, and lactate dehydrogenase (LDH) level are also recommended. Testicular ultrasound should be considered in men. Body FDG-PET scans should also be considered in evaluating patients with PCNSL for subclinical systemic disease. In a retrospective study of 49 PCNSL patients evaluated with body FDG-PET studies, extraneural hypermetabolic lesions were identified in 15 % of subjects [12]. Subsequent biopsy was performed, and 11 % of the hypermetabolic lesions were found to be lymphoma, while 4 % were other types of cancer.

Occult systemic disease was observed in a subset of patients with CNS lymphoma when tumor, bone marrow, and blood specimens were concurrently assessed. Identical polymerase chain reaction (PCR) products of clonally rearranged immunoglobulin heavy-chain (IgH) genes were identified in the bone marrow aspirates, blood, and brain tumor biopsy specimens in 2 of 24 patients with "primary" CNS lymphoma. In one of these patients, follow-up IgH PCR 24 months after diagnosis yielded a persistent monoclonal blood product despite a complete radiographic response in the CNS [13]. Prospective, long-term follow-up studies will be necessary to further elucidate the frequency and importance of subclinical systemic disease in CNS lymphoma patients and whether the presence of these monoclonal cell populations increases the risk of relapse.

Neuroimaging

Contrast-enhanced cranial MRI is the imaging modality of choice in evaluating a patient with a suspected diagnosis of PCNSL. If MRI is not possible or contraindicated, a contrast-enhanced cranial CT scan is obtained. Typically, immunocompetent PCNSL patients present with a single, homogeneously enhancing brain mass on both contrast-enhanced cranial CT and MRI (Fig. 20.1) [14]. Since PCNSL is characterized by a high nuclear to cytoplasmic ratio and high cell density, there may be regions of restricted diffusion observed on diffusion-weighted MRI sequences, and apparent diffusion coefficient imaging may be useful as a biomarker of response to chemotherapy [15].

In immunocompetent PCNSL patients, lesions are solitary in 65 % of cases and are located in a cerebral hemisphere (38 %), thalamus/basal ganglia (16 %), corpus callosum (14 %), periventricular region (12 %), or cerebellum (9 %) [16]. Isolated spinal cord involvement is rare and observed in <1 % of cases, so spinal imaging is

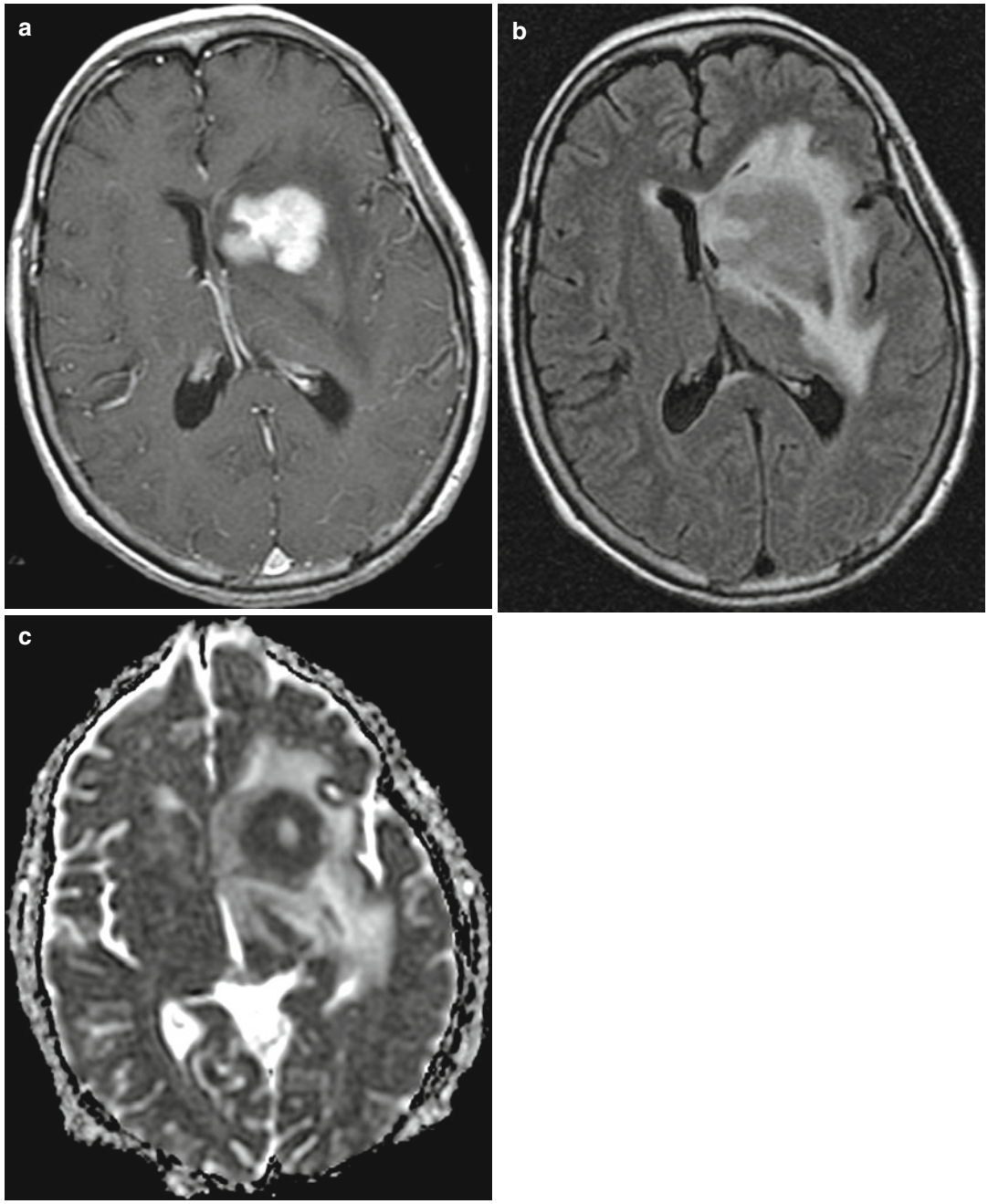

Fig. 20.1 Magnetic resonance imaging (MRI) scan from a patient with PCNSL. Note the homogeneous contrast enhancement (**a**) in a periventricular location with surrounding edema (FLAIR image) (**b**) and corresponding dark appearance on ADC imaging(**c**) suggestive of increased cell density

only necessary if warranted based on clinical suspicion or to screen for leptomeningeal involvement if lumbar puncture cannot be performed.

Prognostic Markers

Two prognostic scoring systems have been proposed for use in patients with PCNSL. In a

retrospective review of 105 PCNSL patients, the International Extranodal Lymphoma Study Group (IELSG) identified age >60, Eastern Cooperative Oncology Group (ECOG) performance status >1, elevated serum LDH level, elevated CSF protein concentration, and involvement of deep regions of the brain as independent predictors of poor prognosis [17]. In patients with 0–1 factors, 2–3 factors, and 4–5 factors, the 2-year survival proportions were 80, 48, and 15 %, respectively. Another group of investigators proposed a prognostic model that divides PCNSL patients into three groups based on age and performance status: those <50 years old, those >50 years old with a KPS >70, and those >50 with a KPS <70 [18]. Based on these divisions, significant differences in overall and failure-free survival were observed.

The search for biomarkers of prognosis for patients with PCNSL is an active area of investigation. BCL-6, a proto-oncogene expressed in 22–100 % of PCNSL patients, has been associated with prognosis in some studies but not others [19–21]. Progression free survival (20.5 vs. 10.1 months) [22] and overall survival (101 vs. 14.7 months) [19, 23] are longer in PCNSL patients with BCL-6 expression. These findings are consistent with the observation that BCL-6 expression is a favorable prognostic marker in patients with systemic NHL [21, 24, 25]. However, translocations of BCL6 may be associated with a worse prognosis [26]. In addition, expression of FOXP1, a transcription factor, is increased in some patients with PCNSL and may be associated with poor prognosis [27]. As noted, high expression levels of STAT6 are associated with reduced survival in PCNSL patients treated with methotrexate [5].

Treatment

Resection

Due to the infiltrative nature of the tumor, resection of PCNSL is not a recommended treatment except in the rare patient experiencing brain herniation due to mass effect. In addition, PCNSL may be multifocal involving the leptomeninges, the eyes, or the deep regions of the brain making complete resection impossible. Median survival following surgery alone is 1–4 months [28].

Corticosteroids

Corticosteroids cause tumor regression in up to 40 % of PCNSL patients likely through direct lymphocytolysis and reduced tumor-associated edema [29]. However, corticosteroids should be withheld, if possible, prior to a diagnostic biopsy as these drugs may disrupt cellular morphology making histopathological diagnosis difficult. Despite an initial positive response to corticosteroids, patients quickly relapse and require alternate treatment strategies. Nevertheless, initial radiographic response to corticosteroids in newly diagnosed PCNSL patients is a favorable prognostic marker with survival of 117 months in responders versus 5.5 months in non-responders in one study [30].

Radiation

Given the multifocal and infiltrative nature of PCNSL, whole brain radiation therapy (WBRT) was historically the treatment modality of choice. However, WBRT alone is inadequate therapy for PCNSL patients, particularly those with CSF dissemination of their tumor. Initial radiographic response to WBRT is observed in approximately 90 % of PCNSL patients but relapse usually occurs within a few months [31]. In patients receiving WBRT alone without chemotherapy, median survival varies from 12 to 18 months and 5-year survival ranges from 18 to 35 % [32, 33]. A radiation dose–response relationship exists for PCNSL as dose reduction from 45 to 30 Gy increased relapse risk in one nonrandomized study [34]. Despite initial control of disease, WBRT produces delayed neurotoxicity, especially in those older than 60.

Chemoradiation

Results of randomized trials in the PCNSL patient population are beginning to define regimens that will shape the management of this disease (Table 20.2) [35–40]. A randomized trial of WBRT versus WBRT and cyclophosphamide, doxorubicin, vincristine, and prednisone

Table 20.2 Selected treatment studies

Chemotherapy regimen		N	IT chemo	WBRT	CR	PR	OS (mo)	PFS (mo)
Chemotherapy with RT								
DeAngelis et al. [39]	MTX (2.5 g/m^2), procarbazine, vincristine, dexamethasone, cytarabine	102	MTX	45 Gy	58 %[a] (29/50)	36 %[a] (18/50)	36.9	24
Shah et al. [40]	MTX (3.5 g/m^2), rituximab, procarbazine, vincristine, cytarabine	30	None	23.4 Gy if CR, 45 Gy if not CR	77 % (23/30)	NA	2 year survival 67 %[b]	40[b]
Ferreri et al. [35]	MTX (3.5 g/m^2) ± cytarabine	MTX alone: 40 MTX + cyt: 39	None	Added based on response and age	MTX alone 18 % MTX + cyt 46 %	MTX alone 23 % MTX + cyt 23 %	3 year survival: MTX alone 32 % MTX + cyt 46 %	3 year survival: MTX alone 21 % MTX + cyt 38 %
Chemotherapy alone								
Pels et al. [38]	MTX (5 g/m^2), vincristine, ifosfamide, dexamethasone, cyclophosphamide, cyt, vindesine	65	Prednisolone, MTX, cytarabine	None	61 % (37/61)	10 % (6/65)	50	21
Gerstner et al. [36, 37][c]	MTX (8 g/m^2)	25	None	None	52 % (12/25)	NA	55.4	12.8

RT radiation therapy, *MTX* methotrexate, *i.v.* intravenous, *ACNU* nimustine, *cyt* cytarabine
[a]Prior to RT since post-RT results not available
[b]Estimated
[c]This study is an update of a previous study

(CHOP) was terminated early due to poor accrual although results demonstrated that a combination of WBRT and CHOP was not superior to WBRT alone [41]. Given that the agents in the CHOP regimen achieve poor CNS levels, this was not a surprising result and this treatment regimen was abandoned for patients with CNS lymphoma. A randomized trial of methotrexate monotherapy (3.5 g/m^2) versus methotrexate with cytarabine followed by WBRT in both arms demonstrated that more patients in the combination arm achieved a radiographic response (CR and PR) but grades 3 and 4 hematological toxicity were also higher in this arm [35]. Given the high risk of neurotoxicity of WBRT, there is a growing consensus to defer WBRT in the newly diagnosed PCNSL patient population. In the largest randomized trial conducted in the newly diagnosed PCNSL patient population, those subjects who achieved a CR to methotrexate-based chemotherapy were randomized to receive consolidation with WBRT versus observation [42]. The intent-to-treat analysis of this trial demonstrated improved PFS in the arm that included WBRT but no difference in OS. Although the results of this study have generated conflicting interpretations, the study provides support for the strategy of deferred WBRT in the newly diagnosed PCNSL patient population.

Nonrandomized, uncontrolled studies of lower doses of WBRT to reduce the risk of neurotoxicity have been conducted. However, as noted, reduction of WBRT dose from 45 to 30 Gy increased relapse risk in one such study [34]. In a study of methotrexate, vincristine, procarbazine, and rituximab (R-MVP) followed by a reduced dose of WBRT (23.4 Gy) for those patients who achieved a CR to chemotherapy, the risk of neurotoxicity appeared to be reduced although these results await confirmation with longer-term follow-up and a randomized trial. There is growing preclinical evidence that even single fractions of WBRT to mice are associated with irreversible injury to neural progenitor cell populations with negative behavioral consequences [43]. Thus, it would be surprising that any dose of WBRT sufficient for cytotoxicity would not result in some degree of neurotoxicity.

Chemotherapy

Given the risk of treatment-related neurotoxicity in regimens that include WBRT, a number of methotrexate-based regimens have been studied in nonrandomized, uncontrolled studies with no clear evidence of the superiority of any one regimen.

In a multicenter study of 25 patients treated with intravenous methotrexate (8 g/m^2) monotherapy, 52 % of patients achieved a CR, the median PFS was 12.8 months, the median OS was 55.4 months, and median disease-specific survival had not been reached at 72.3 months [36, 37]. In this study, 5 of the 25 patients treated with methotrexate alone achieved a CR and have not relapsed after a median follow-up of 6.8 years. Optimal consolidative therapy after a PCNSL patient achieves a CR remains unclear.

While methotrexate monotherapy may be effective for a small subset of patients, most patients will require combination chemotherapy to achieve a durable response. In patients >60 years of age, a regimen consisting of methotrexate, CCNU, procarbazine, methylprednisolone, intrathecal methotrexate, and intrathecal Ara-C was associated with a median OS of 14.3 months and a decreased risk of neurotoxicity relative to historical controls [44]. Another regimen including methotrexate, Ara-C, vincristine, ifosfamide, cyclophosphamide, and intrathecal methotrexate/Ara-C/prednisolone was associated with a 71 % ORR and a median OS of 50 months. Despite these promising results, however, 6 patients died from treatment-related complications and 12 patients had Ommaya reservoir infections [38]. The combination of methotrexate, temozolomide, and rituximab (MTR) induction followed by consolidation with etoposide and cytarabine (EA) has been utilized successfully in the multicenter setting as induction therapy in PCNSL [45, 46]. Each agent in the MTR regimen has been studied as monotherapy in PCNSL patients with activity of each agent demonstrated [36, 37, 47]. In this study, 63 % of patients treated with MTR induction achieved a complete radiographic response and the median 2-year progression-free survival (PFS2) after MTR+EA was 55 %. However, these preliminary results from nonrandomized, uncontrolled studies must be confirmed in prospective, randomized clinical trials.

High-Dose Chemotherapy with Stem Cell Rescue

Initial studies of high-dose chemotherapy (HDT) followed by autologous stem cell transplantation (ASCT) have involved limited numbers of patients and have yielded mixed results likely because of the use of heterogeneous therapies and outcome measures (Table 20.3) [48–53]. Studies of HDT/ASCT utilizing the BEAM (carmustine, etoposide, cytarabine, melphalan) conditioning regimen demonstrated disappointing results with a median event-free survival of 5.6 months [48]. However, results from studies of induction and conditioning regimens including thiotepa have been more encouraging. In a multicenter, uncontrolled study induction with methotrexate, Ara-C, and thiotepa was followed by a conditioning regimen consisting of carmustine (400 mg/m^2) and thiotepa (5 mg/kg × 2) and ASCT with WBRT administered at the end of all treatment as consolidation. With a median follow-up of 63 months, the 5-year OS was 69 % for all patients and 87 % for those completing HDT/ASCT [50]. However, neurotoxicity was observed in approximately one-quarter of the patients. In a follow-up trial, the protocol

Table 20.3 Studies of high-dose chemotherapy and ASCT in newly diagnosed PCNSL

Reference	# of pts.	Median age	Induction regimen	Conditioning regimen	WBI	CRR[%]	FU[mos]	Survival	TRM (%)
Colombat et al. [52].	25	51	MBVP +i.th. AraC	BEAM	yes	44	34	4-y EFS: 46 %	4
Abrey et al. [48]	28	53	MTX AraC	BEAM	no	18	27	mEFS: 9 month	0
Stewart et al. [53].	11	56	MTX	Thiotepa Busulfan Cy	yes	82	22	3-year OS: 61 %	18
Illerhaus et al. [50].	30	54	MTX (8 g) AraC/TT	Thiotepa (10 mg/kg) BCNU	yes	76	63	5-year OS: 69 %	3
Montemurro et al. [49].	23	55	MTX (8 g)	Bu/TT (10 mg/kg)	yes	69	15	2-year OS: 48 %	13
Illerhaus et al. [51].	13	54	MTX (8 g) AraC/TT	TT (20 mg/ kg)/ BCNU	no	54	25	3-year OS: 77 %	0

was modified with intensification of the chemotherapy dose and restriction of WBRT only to patients who did not achieve CR after induction therapy. Seven of 11 patients achieved CR following ASCT and 3/11 achieved PR with the latter group receiving WBRT consolidation. After a median follow-up of 25 months, 3-year OS was 77 %. None of the patients suffered from severe neurotoxicity during the follow-up period [51]. HDT/ASCT is likely to assume an increasingly important role in younger patients with PCNSL in the newly diagnosed and relapsed setting. ASCT may be effective in patients with poor prognostic features as well [54].

Intrathecal Chemotherapy

A beneficial role of intrathecal chemotherapy for patients with PCNSL has not been established. Historical comparisons have determined that there appears to be no improvement in OS when intrathecal methotrexate is added to regimens that already included high doses of intravenous methotrexate [55]. By administering methotrexate systemically, the risk of Ommaya reservoir placement, extra-CSF drug delivery, chemical meningitis, and infection are avoided. In a prospective study of 18 patients, a polychemotherapy regimen was administered without intrathecal chemotherapy [56]. Although the radiographic CR proportion was 53 %, the median time to progression for responders was only 10 months, shorter than that previously reported for this same regimen when intrathecal chemotherapy was included. Although the authors contend that this early relapse was possibly due to the omission of intrathecal chemotherapy, this conclusion is speculative and should be confirmed in a larger, randomized trial.

Salvage Therapy

Despite aggressive treatment, the majority of patients with PCNSL will progress or relapse and require salvage therapy. Optimal management of relapsed or refractory PCNSL has yet to be determined and has only been studied in small patient series or case reports using heterogeneous therapies. In general, prognosis for patients with relapsed or progressive PCNSL is poor with a median survival of approximately 4.5 months [57]. For patients who initially achieved a CR to a chemotherapy regimen that included methotrexate, re-treatment with methotrexate may be effective [58]. Temozolomide; topotecan; etoposide (VP-16), ifosfamide, and Ara-C (VIA); high-dose chemotherapy followed by ASCT; and procarbazine, lomustine (CCNU), and vincristine (PCV) have all been studied in patients with relapsed or refractory PCNSL with varying results [47, 59–61].

Rituximab has been assessed as a salvage therapy in PCNSL. In a study of patients with relapsed or refractory PCNSL, rituximab monotherapy was administered at a dose of 375 mg/m^2 for eight doses. Radiographic responses were observed in 5/12 (42 %) of patients and median PFS and OS were 2 and 21 months in all patients and 7.6 and 47 months in responders [62]. Rituximab was administered in combination with temozolomide in two studies of relapsed or progressive PCNSL, yielding median survival of 8 and 14 months [63, 64].

Radiation as a salvage therapy has also been explored. Following WBRT as a salvage strategy, 74–79 % of patients with relapsed or refractory PCNSL can achieve a radiographic response [65, 66]. Median survival after WBRT is 10.9–16 months, with those patients less than 60 years old faring better. These results with WBRT as salvage therapy are comparable to the results when WBRT alone is utilized in the newly diagnosed PCNSL setting.

Secondary CNS Lymphoma

Clinical Features

The risk of CNS relapse is dependent on the underlying type of lymphoma and the anatomic site(s) of involvement. Approximately 30–40 % of patients with Burkitt and lymphoblastic lymphomas develop CNS dissemination. In studies of "aggressive" lymphomas, excluding Burkitt and lymphoblastic subtypes, the incidence is approximately 5 %, but this risk is further dependent on the underlying clinical features of the specific lymphoma type [67]. In patients with DLBCL, factors that appear to increase the risk of CNS relapse include older age (>60), more advanced disease stage, increased serum levels of lactate dehydrogenase (LDH), involvement of >1 extranodal site, and the presence of B symptoms [67, 68]. In DLBCL patients who have four to five of these factors, the risk of CNS relapse may be as high as 25 % [67]. Involvement of specific anatomic sites may also confer higher risk of CNS relapse. Patients with testicular NHL have a risk of CNS relapse of approximately 15 % with remote relapses reported [69]. Other anatomic sites of NHL that may increase risk of CNS release include breast, bone, adrenal gland, lung, and skin [69]. Parameningeal location of NHL including the epidural space and sinonasal sinuses also appears to confer a higher risk of CNS relapse.

CNS relapse occurs in the leptomeninges, brain parenchyma, or both sites in approximately 55, 30, and 15 % of cases, respectively [70]. An exception to this distribution is testicular NHL in which 64 % of CNS relapses occur in the brain parenchyma [70]. The median time to CNS relapse is 5–12 months after lymphoma diagnosis and relapse is isolated to the CNS in approximately 50 % of cases [71]. CNS relapse is a poor prognostic marker with median survival of all cases ranging from 2 to 6 months [70].

The incidence of CNS relapse may be changing since the introduction of rituximab into the treatment regimens for DLBCL. In the RICOVER trial, the addition of rituximab to CHOP decreased the relative risk for CNS relapse to 0.58 (compared to CHOP alone) and prophylaxis with intrathecal methotrexate did not confer any benefit in these patients [72].

CNS Prophylaxis

There are no randomized trials that have defined a beneficial role of CNS prophylaxis in lymphoma. However, it is commonly accepted that certain subtypes of NHL with a high risk of CNS relapse should receive CNS prophylaxis including Burkitt lymphoma and lymphoblastic lymphoma. Testicular NHL patients also routinely receive prophylaxis. In one study incorporating intrathecal methotrexate as CNS prophylaxis, 3/50 patients relapsed in the CNS [73]. Beyond these subtypes it is not clear which patients should receive prophylaxis. DLBCL patients with multiple risk factors (age> 60, >1 extranodal site, elevated LDH levels, B symptoms) might benefit from CNS prophylaxis although this has not been definitively established. Lymphoma patients with parameningeal location of disease also could benefit although this remains to be proven.

The optimal therapeutic and route of delivery for CNS prophylaxis have not been defined. Prophylactic cranial irradiation is associated with a risk of neurotoxicity and does not treat the entire craniospinal axis and is not advisable. Although many protocols include intrathecal chemotherapy (methotrexate, Ara-C) as CNS prophylaxis, it is not clear that this is justified as approximately one-third of CNS relapses occur in the brain parenchyma and there is poor drug penetration of brain parenchyma with the intrathecal route of delivery. Incorporation of high-dose, intravenous methotrexate or Ara-C as CNS prophylaxis could be more effective although this has not been established [74].

Treatment

There have been few studies of treatment for lymphoma patients with brain relapse. In a retrospective series of 113 lymphoma (83 % DLBCL) patients who developed brain parenchymal relapse, the median survival was 1.6 years with 23 % of patients surviving ≥3 years [75]. Young age and use of methotrexate were associated with improved survival in a multivariate analysis. In patients with isolated brain or brain and leptomeningeal relapse, therapeutic approaches used for PCNSL can be employed. There is limited data that high-dose therapy and autologous stem cell transplantation could be beneficial in this setting [76].

Leptomeningeal relapse complicates 5–30 % of all lymphoma cases depending on the subtype [77]. Multifocal neurological symptoms and signs involving different parts of the neuraxis are common. Diagnosis is typically made after lumbar puncture and CSF analysis for cytology, flow cytometry, and IgH polymerase chain reaction. Prognosis is poor and median survival is typically 3–6 months [72]. Treatment includes chemotherapy or radiation. Craniospinal radiation would be required to treat the entire CSF axis but is rarely employed due to risk of toxicity and lack of demonstrated benefit. Focal radiation to areas of bulky disease or WBRT is sometimes used for palliation of symptoms. Agents typically administered by the intrathecal route include methotrexate, Ara-C, and liposomal Ara-C. Most agents are administered twice weekly although liposomal Ara-C is administered twice monthly. In a randomized study of 28 patients with lymphomatous meningitis, the cytological response proportion was 10/14 (71 %) in those patients receiving liposomal Ara-C and 2/13 (15 %) in those patients receiving Ara-C [78]. The risk of symptomatic chemical meningitis was higher in the patients receiving liposomal Ara-C and dexamethasone must be administered with this agent to mitigate this risk. In a phase I study of intrathecal rituximab in patients with lymphomatous meningitis, the maximal tolerated dose was 25 mg twice weekly and 6/10 patients experienced cytological responses [46]. Clinical trials of intrathecal rituximab are ongoing. High doses of intravenous methotrexate and Ara-C have also been used in patients with lymphomatous meningitis with anecdotal responses reported.

In patients who are to receive intrathecal chemotherapy, a ventricular reservoir is often recommended, as it is the most efficient, reliable, and safest method by which to deliver intrathecal chemotherapy. Repeated lumbar punctures are uncomfortable for patients and may result in inconsistent delivery of chemotherapy into the subarachnoid space. Distribution of chemotherapy along the CSF axis also appears to be better when administered through a ventricular reservoir versus lumbar puncture [79]. Radionuclide CSF flow studies should be obtained in patients with suspected CSF flow obstruction as the latter increases the risk of neurotoxicity with intrathecal chemotherapy.

Neurotoxicity

Delayed neurotoxicity is a common complication in CNS lymphoma patients treated with chemoradiation (WBRT + chemotherapy) or WBRT alone [80]. Treatment-related neurotoxicity most commonly occurs in patients older than 60 and may present as a subcortical dementia, gait ataxia,

and incontinence. MRI changes associated with neurotoxicity include periventricular white matter abnormalities, cortical atrophy, and ventricular enlargement. Less severely affected patients have problems with attention, executive function, memory (particularly verbal), and psychomotor speed. These clinical features may appear 6–12 months following WBRT, but it is important to note that radiographic changes do not always correlate with clinical symptoms, which may appear earlier. Pathological studies have demonstrated demyelination, neuronal loss, gliosis, and rarefaction of the white matter [81]. Large vessel atherosclerosis has been observed as well, implicating vascular injury and resultant tissue ischemia as one possible mechanism for neurotoxicity. Although the pathophysiology of treatment-related neurotoxicity is multifactorial, toxicity to neural progenitor cells is likely to play a pivotal role [43].

Studies examining neurotoxicity have several methodological limitations including lack of baseline evaluations, different definitions of cognitive impairment, and small patient sample sizes [82]. In one study of PCNSL patients, the 5-year cumulative incidence of neurotoxicity was 24 %, and the use of WBRT was the only significant predictor of development of neurotoxicity on multivariate analysis [83]. This is in contrast to chemotherapy alone in which less decline in cognitive function is observed despite evidence of white matter changes on MRI [82, 84, 85]. One treatment strategy has been to decrease the dose of WBRT to 23.4 Gy in patients who achieved a complete response to induction chemotherapy. In a small study of 12 PCNSL patients who had serial neuropsychological testing up to 24 months after R-MVP chemotherapy followed by low-dose WBRT, there was no significant decline in cognitive function compared to baseline [80]. However, the small sample size and high attrition rate may have limited the ability of the investigators to detect more subtle cognitive changes.

There is no effective treatment for neurotoxicity, and patients are often disabled or may die from the complications of neurotoxicity without evidence of relapsed lymphoma.

References

1. Central Brain Tumor Registry of the United States (CBTRUS) statistical report: primary brain and central nervous system tumors diagnosed in the United States in 2004–2007, Hinsdale, IL. http://www.cbtrus.org
2. Kadan-Lottick N, Sklusarek M, Gurney J. Decreasing incidence rates of primary central nervous system lymphoma. Cancer. 2002;95:193–202.
3. Panageas KS, Elkin EB, DeAngelis LM, Ben-Porat L, Abrey LE. Trends in survival from primary central nervous system lymphoma, 1975–1999: a population-based analysis. Cancer. 2005;104:2466–72.
4. Miller DC, Hochberg FH, Harris NL, Gruber ML, Louis DN, Cohen H. Pathology with clinical correlations of primary central nervous system non-Hodgkin's lymphoma. The Massachusetts General Hospital experience 1958–1989. Cancer. 1994;74:1383–97.
5. Camilleri-Broët S, Crinière E, Broët P, et al. A uniform activated B-cell–like immunophenotype might explain the poor prognosis of primary central nervous system lymphomas: analysis of 83 cases. Blood. 2006;107:190–6.
6. Rubenstein J, Fridlyand J, Shen A, et al. Gene expression and angiotropism in primary CNS lymphoma. Blood. 2006;107:3716–23.
7. Fischer L, Hummel M, Korfel A, Lenze D, Joehrens K, Thiel E. Differential micro-RNA expression in primary CNS and nodal diffuse large B-cell lymphomas. Neuro Oncol. 2011;13(10):1090–8.
8. Tun HW, Personett D, Baskerville KA, et al. Pathway analysis of primary central nervous system lymphoma. Blood. 2008;111:3200–10.
9. Newell M, Hoy J, Cooper S, et al. Human immunodeficiency virus-related primary central nervous system lymphoma: factors influencing survival in 111 patients. Cancer. 2004;100:2627–34.
10. Bataille B, Delwail V, Menet E, et al. Primary intracerebral malignant lymphoma: report of 248 cases. J Neurosurg. 2000;92:261–6.
11. Abrey L, Batchelor T, Ferreri A, et al. Report of an international workshop to standardize baseline evaluation and response criteria for primary CNS lymphoma. J Clin Oncol. 2005;23:5034–43.
12. Mohile NA, Deangelis LM, Abrey LE. The utility of body FDG PET in staging primary central nervous system lymphoma. Neuro Oncol. 2008;10(2):223–8.
13. Jahnke K, Hummel M, Korfel A, et al. Detection of subclinical systemic disease in primary CNS lymphoma by polymerase chain reaction of the rearranged immunoglobulin heavy-chain genes. J Clin Oncol. 2006;24:4754–7.
14. Fine HA, Mayer RJ. Primary central nervous system lymphoma. Ann Intern Med. 1993;119:1093–104.
15. Barajas Jr RF, Rubenstein JL, Chang JS, Hwang J, Cha S. Diffusion-weighted MR imaging derived apparent diffusion coefficient is predictive of clinical outcome in primary central nervous system lymphoma. AJNR Am J Neuroradiol. 2009;31(1):60–6.

16. Kuker W, Nagele T, Korfel A, et al. Primary central nervous system lymphomas (PCNSL): MRI features at presentation in 100 patients. J Neurooncol. 2005;72: 169–77.
17. Ferreri A, Blay J, Reni M, et al. Prognostic scoring system for primary CNS lymphomas: the International Extranodal Lymphoma Study Group experience. J Clin Oncol. 2003;21:266–72.
18. Abrey L, Ben-Porat L, Panageas KS, et al. Primary central nervous system lymphoma: the Memorial Sloan-Kettering Cancer Center prognostic model. J Clin Oncol. 2006;24(36):5711–5.
19. Braaten K, Betensky R, de Leval L, et al. BCL-6 expression predicts improved survival in patients with primary central nervous system lymphoma. Clin Cancer Res. 2003;9:1063–9.
20. Larocca LM, Capello D, Rinelli A, et al. The molecular and phenotypic profile of primary central nervous system lymphoma identifies distinct categories of the disease and is consistent with histogenetic derivation from germinal center-related B cells. Blood. 1998;92: 1011–9.
21. Takeshita M, Iwashita A, Kurihara K, et al. Histologic and immunohistologic findings and prognosis of 40 cases of gastric large B-cell lymphoma. Am J Surg Pathol. 2000;24:1641–69.
22. Levy O, Deangelis LM, Filippa DA, Panageas KS, Abrey LE. Bcl-6 predicts improved prognosis in primary central nervous system lymphoma. Cancer. 2007;112:151–6.
23. Lin C, Kuo K, Chuang S, et al. Comparison of the expression and prognostic significance of differentiation markers between diffuse large B-cell lymphoma of the central nervous system origin and peripheral nodal origin. Clin Cancer Res. 2006;12: 1152–6.
24. Rosenwald A, Wright G, Chan W. Lymphoma/leukemia molecular profiling project. The use of molecular profiling to predict survival after chemotherapy for diffuse large-B-cell lymphoma. N Engl J Med. 2002; 346:1937–47.
25. Lossos I, Jones C, Warnke R, et al. Expression of a single gene, BCL-6, strongly predicts survival in patients with diffuse large B-cell lymphoma. Blood. 2001;98:945–51.
26. Cady FM, O'Neill BP, Law ME, et al. Del(6)(q22) and BCL6 rearrangements in primary CNS lymphoma are indicators of an aggressive clinical course. J Clin Oncol. 2008;26:4814–9.
27. Courts C, Brunn A, Montesinos-Rongen M, et al. Preferential expression of truncated isoforms of FOXP1 in primary central nervous system lymphoma. J Neuropathol Exp Neurol. 2009;68:972–6.
28. Batchelor T, Loeffler J. Primary CNS lymphoma. J Clin Oncol. 2006;24:1281–8.
29. Ferreri A, Abrey L, Blay J, et al. Summary statement on primary central nervous system lymphomas from the eighth international conference on malignant lymphoma, Lugano, Switzerland, June 12 to 15, 2002. J Clin Oncol. 2003;21:2407–14.
30. Beela MS, Carson K, Grossman SA. Initial response to glucocorticoids: a potentially important prognostic factor in patients with primary CNS lymphoma. Cancer. 2006;106:383–7.
31. Nelson D, Martz K, Bonner H, et al. Non-Hodgkin's lymphoma of the brain: can high dose, large volume radiation therapy improve survival? Report on a prospective trial by the Radiation Therapy Oncology Group (RTOG): RTOG 8315. Int J Radiat Oncol Biol Phys. 1992;23:9–17.
32. Shibamoto Y, Ogino H, Hasegawa M, et al. Results of radiation monotherapy for primary central nervous system lymphoma in the 1990s. Int J Radiat Oncol Biol Phys. 2005;62:809–13.
33. Ishikawa H, Hasegawa M, Tamaki Y, et al. Comparable outcomes of radiation therapy without high-dose methotrexate for patients with primary central nervous system lymphoma. Jpn J Clin Oncol. 2003;33: 443–9.
34. Bessell E, Lopez-Guillermo A, Villa S, et al. Importance of radiotherapy in the outcome of patients with primary CNS lymphoma: an analysis of the CHOD/BVAM regimen followed by two different radiotherapy treatments. J Clin Oncol. 2002;20: 231–6.
35. Ferreri AJ, Reni M, Foppoli M, et al. High-dose cytarabine plus high-dose methotrexate versus high-dose methotrexate alone in patients with primary CNS lymphoma: a randomised phase 2 trial. Lancet. 2009; 374(9700):1512–20.
36. Gerstner ER, Carson K, Grossman S, Batchelor TT. Long-term outcome in PCNSL patients treated with high-dose methotrexate and deferred radiation. Neurology. 2008;70:401–2.
37. Batchelor T, Carson K, O'Neill A, et al. Treatment of primary CNS lymphoma with methotrexate and deferred radiation therapy: a report of the NABTT 96–07. J Clin Oncol. 2003;21:1044–9.
38. Pels H, Schmidt-Wolf IG, et al. Primary central nervous system lymphoma: results of a pilot and phase II study of systemic and intraventricular chemotherapy with deferred radiotherapy. J Clin Oncol. 2003;21: 4489–95.
39. DeAngelis L, Seiferheld W, Schold S, Fisher B, Schultz C. Combination chemotherapy and radiotherapy for primary central nervous system lymphoma: Radiation Therapy Oncology Group Study 93–10. J Clin Oncol. 2002;20:4643–8.
40. Shah G, Yahalom J, Correa D, et al. Combined immunochemotherapy with reduced whole-brain radiotherapy for newly diagnosed primary CNS lymphoma. J Clin Oncol. 2007;25:4730–5.
41. Mead GM, Bleehen NM, Gregor A, et al. A medical research council randomized trial in patients with primary cerebral non-Hodgkin lymphoma: cerebral radiotherapy with and without cyclophosphamide, doxorubicin, vincristine, and prednisone chemotherapy. Cancer. 2000;89:1359–70.
42. Thiel E, Korfel A, Martus P, et al. High-dose methotrexate with or without whole brain radiotherapy for

primary CNS lymphoma (G-PCNSL-SG-1): a phase 3, randomised, non-inferiority trial. Lancet Oncol. 2010;11:1036–47.
43. Monje M, Mizumatsu S, Fike J, Palmer T. Irradiation induces neural precursor cell dysfunction. Nat Med. 2002;8:955–62.
44. Hoang-Xuan K, Taillandier L, Chinot O, et al. Chemotherapy alone as initial treatment for primary CNS lymphoma in patients older than 60 years: a multicenter phase II study (26952) of the European Organization for Research and Treatment of Cancer Brain Tumor Group. J Clin Oncol. 2003;21:2726–31.
45. Issa S SA, Karch J, Kachoch C, Shuman M, Damon L, Rubenstein JL.Treatment of primary CNS lymphoma with induction high-dose methotrexate, temozolomide, rituximab followed by consolidation cytarabine/etoposide. J Clin Oncol. 2006 ASCO Annual Meeting Proceedings (Post-Meeting Edition), Vol 24, No 18S (June 20 Supplement); 2007.
46. Rubenstein JL, Fridlyand J, Abrey L, et al. Phase I study of intraventricular administration of rituximab in patients with recurrent CNS and intraocular lymphoma. J Clin Oncol. 2007;25:1350–6.
47. Reni M, Mason W, Zaja F, et al. Salvage chemotherapy with temozolomide in primary CNS lymphomas: preliminary results of a phase II trial. Eur J Cancer. 2004;40:1682–8.
48. Abrey L, Moskowitz C, Mason W, et al. Intensive methotrexate and cytarabine followed by high-dose chemotherapy with autologous stem-cell rescue in patients with newly diagnosed primary CNS lymphoma: an intent-to-treat analysis. J Clin Oncol. 2003;21:4151–6.
49. Montemurro M, Kiefer T, Schüler F, et al. Primary central nervous system lymphoma treated with high-dose methotrexate, high-dose busulfan/thiotepa, autologous stem-cell transplantation and response-adapted whole-brain radiotherapy: results of the multicenter Ostdeutsche Studiengruppe Hamato-Onkologie OSHO-53 phase II study. Ann Oncol. 2007;18:665–71.
50. Illerhaus G, Marks R, Ihorst G, et al. High-dose chemotherapy with autologous stem-cell transplantation and hyperfractionated radiotherapy as first-line treatment of primary CNS lymphoma. J Clin Oncol. 2006;24:3865–70.
51. Illerhaus G, Müller F, Feuerhake F, Schäfer AO, Ostertag C, Finke J. High-dose chemotherapy and autologous stem-cell transplantation without consolidating radiotherapy as first-line treatment for primary lymphoma of the central nervous system. Haematologica. 2008;93:147–8.
52. Colombat P, Lemevel A, Bertrand P, Delwail V, Rachieru P, Brion A, et al. High-dose chemotherapy with autologous stem cell transplantation as first-line therapy for primary CNS lymphoma in patients younger than 60 years: a multicenter phase II study of the GOELAMS group. Bone Marrow Transplant. 2006;38:417–20.
53. Stewart D, Forsyth P, Chaudhry A, et al. High dose thiotepa, busulfan, cyclophosphamide (TBC) and autologous hematopoietic stem cell transplantation (ASCT) without whole brain radiotherapy (WBRT) for primary central nervous system lymphoma (PCNSL). ASH Annual Meeting Abst. 2004;104:911.
54. Cheng T, Forsyth P, Chaudhry A, et al. High-dose thiotepa, busulfan, cyclophosphamide and ASCT without whole-brain radiotherapy for poor prognosis primary CNS lymphoma. Bone Marrow Transplant. 2003;31:679–85.
55. Khan R, Shi W, Thaler H, DeAngelis L, Abrey L. Is intrathecal methotrexate necessary in the treatment of primary CNS lymphoma? J Neurooncol. 2002;58:175–8.
56. Pels H, Juergens A, Glasmacher A, et al. Early relapses in primary CNS lymphoma after response to polychemotherapy without intraventricular treatment: results of a phase II study. J Neurooncol. 2009;91:299–305.
57. Jahnke K, Thiel E, Martus P, et al. Relapse of primary central nervous system lymphoma: clinical features, outcome and prognostic factors. J Neurooncol. 2006;80(2):159–65.
58. Plotkin S, Betensky R, Hochberg F, et al. Treatment of relapsed central nervous system lymphoma with high-dose methotrexate. Clin Cancer Res. 2004;10:5643–6.
59. Fischer L, Thiel E, Klasen H, et al. Prospective trial on topotecan salvage therapy in primary CNS lymphoma. Ann Oncol. 2006;17(7):1141–5.
60. Arellano-Rodrigo E, Lopez-Guillermo A, Bessell EM, Nomdedeu B, Montserrat E, Graus F. Salvage treatment with etoposide (VP-16), ifosfamide and cytarabine (Ara-C) for patients with recurrent primary central nervous system lymphoma. Eur J Haematol. 2003;70:219–24.
61. Herrlinger U, Brugger W, Bamberg M. PCV salvage chemotherapy for recurrent primary CNS lymphoma. Neurology. 2000;54:1707–8.
62. Batchelor TT, Grossman SA, Mikkelsen T, Ye X, Desideri S, Lesser GJ. Rituximab monotherapy for patients with recurrent primary CNS lymphoma. Neurology. 2011;76:929–30.
63. Wong ET, Tishler R, Barron L, Wu JK. Immunochemotherapy with rituximab and temozolomide for central nervous system lymphomas. Cancer. 2004;101:139–45.
64. Enting R, Demopoulos A, DeAngelis L, Abrey L. Salvage therapy for primary CNS lymphoma with a combination of rituximab and temozolomide. Neurology. 2004;63:901–3.
65. Hottinger AF, DeAngelis LM, Yahalom J, Abrey LE. Salvage whole brain radiotherapy for recurrent or refractory primary CNS lymphoma. Neurology. 2007;11:1178–82.
66. Nguyen P, Chakravarti A, Finkelstein D, Hochberg F, Batchelor T, Loeffler J. Results of whole-brain radiation as salvage of methotrexate failure for immunocompetent patients with primary CNS lymphoma. J Clin Oncol. 2005;23:1507–13.
67. Hollender A, Kvaloy S, Nome O, Skovlund E, Lote K, Holte H. Central nervous system involvement

following diagnosis of non-Hodgkin's lymphoma: a risk model. Ann Oncol. 2002;13:1099–107.
68. van Besien K, Ha CS, Murphy S, et al. Risk factors, treatment, and outcome of central nervous system recurrence in adults with intermediate-grade and immunoblastic lymphoma. Blood. 1998;91:1178–84.
69. Boehme V, Zeynalova S, Kloess M, et al. Incidence and risk factors of central nervous system recurrence in aggressive lymphoma—a survey of 1693 patients treated in protocols of the German High-Grade Non-Hodgkin's Lymphoma Study Group (DSHNHL). Ann Oncol. 2007;18:149–57.
70. Ferreri AJ, Assanelli A, Crocchiolo R, Ciceri F. Central nervous system dissemination in immunocompetent patients with aggressive lymphomas: incidence, risk factors and therapeutic options. Hematol Oncol. 2009;27:61–70.
71. Bollen EL, Brouwer RE, Hamers S, et al. Central nervous system relapse in non-Hodgkin lymphoma. A single-center study of 532 patients. Arch Neurol. 1997;54:854–9.
72. Boehme V, Schmitz N, Zeynalova S, Loeffler M, Pfreundschuh M. CNS events in elderly patients with aggressive lymphoma treated with modern chemotherapy (CHOP-14) with or without rituximab: an analysis of patients treated in the RICOVER-60 trial of the German High-Grade Non-Hodgkin Lymphoma Study Group (DSHNHL). Blood. 2009;113:3896–902.
73. Vitolo U, Chiappella A, Ferreri AJ, et al. First-line treatment for primary testicular diffuse large B-cell lymphoma with rituximab-CHOP, CNS prophylaxis, and contralateral testis irradiation: final results of an international phase II trial. J Clin Oncol. 2011;29:2766–72.
74. Abramson JS, Hellmann M, Barnes JA, et al. Intravenous methotrexate as central nervous system (CNS) prophylaxis is associated with a low risk of CNS recurrence in high-risk patients with diffuse large B-cell lymphoma. Cancer. 2010;116:4283–90.
75. Doolittle ND, Abrey LE, Shenkier TN, et al. Brain parenchyma involvement as isolated central nervous system relapse of systemic non-Hodgkin lymphoma: an International Primary CNS Lymphoma Collaborative Group report. Blood. 2008;111:1085–93.
76. Patil S, Spencer A, Schwarer A, et al. Disease status at autologous stem cell transplantation and the cell of origin phenotype are important predictors of outcome in patients with neurologic (central nervous system) relapse of diffuse large B-cell lymphoma undergoing autologous stem cell transplantation. Leuk Lymphoma. 2009;50:1964–8.
77. Gleissner B, Chamberlain M. Treatment of CNS dissemination in systemic lymphoma. J Neurooncol. 2007;84:107–17.
78. Glantz MJ, LaFollette S, Jaeckle KA, et al. Randomized trial of a slow-release versus a standard formulation of cytarabine for the intrathecal treatment of lymphomatous meningitis. J Clin Oncol. 1999;17:3110–6.
79. Shapiro WR, Young DF, Mehta BM. Methotrexate: distribution in cerebrospinal fluid after intravenous, ventricular and lumbar injections. N Engl J Med. 1975;293:161–6.
80. Correa DD, Rocco-Donovan M, DeAngelis LM, et al. Prospective cognitive follow-up in primary CNS lymphoma patients treated with chemotherapy and reduced-dose radiotherapy. J Neurooncol. 2009;91:315–21.
81. Lai RA, Lauren E, Rosenblum MK, DeAngelis LM. Treatment-induced leukoencephalopathy in primary CNS lymphoma: a clinical and autopsy study. Neurology. 2004;62:451–6.
82. Correa D, Maron L, Harder H, et al. Cognitive functions in primary central nervous system lymphoma: literature review and assessment guidelines. Ann Oncol. 2007;18:1145–51.
83. Omuro A, Ben-Porat L, Panageas K, et al. Delayed neurotoxicity in primary central nervous system lymphoma. Arch Neurol. 2005;62:1595–600.
84. Correa D, Anderson N, Glass A, Mason W, DeAngelis L, Abrey L. Cognitive functions in primary central nervous system lymphoma patients treated with chemotherapy and stem cell transplantation: preliminary findings. Clin Adv Hematol Oncol. 2003;1:490.
85. Fliessbach K, Helmstaedter C, Urbach H, et al. Neuropsychological outcome after chemotherapy for primary CNS lymphoma: a prospective study. Neurology. 2005;64(7):1184–8.

Monoclonal Antibodies for Lymphoma

21

Guillaume Cartron and Philippe Solal-Céligny

Abstract

Monoclonal antibodies are undoubtedly one of the therapeutic revolutions of the last 10 years in oncology. Because of the absolute specificity of the antibody for its target, they epitomize the concept of targeted therapy used in the late nineteenth century. Their recent success is due to advances in biotechnology in the 1980s that allowed the humanization of murine monoclonal antibodies. Today more than 200 monoclonal antibodies are in preclinical clinical development and more than a third of them in the field of oncology. Rituximab (MabThera®, Rituxan®) was the first recombinant anticancer monoclonal antibody marketed worldwide. The results obtained in non-Hodgkin lymphomas and its rapid clinical development explain much of the enthusiasm for this new drug class. Progress in understanding the mechanisms of action of this antibody and its ability to interact with the immune system should have consequences applicable to all monoclonal antibodies. Monoclonal antibodies can also be used as a tool for delivering radionuclide, toxin, or cytokine at cellular level. These successes explain why antibodies are now a great hope for patients and a model for physicians, scientists, and drug manufacturers.

Keywords

Anti-CD20 • Radioimmunotherapy • Antibody-drug conjugate

History

The history remembers that it is on Christmas day, 1891, that Emil von Behring infused for the first time a serum allowing to save a child from a diphtheria croup. Thus, long before the term "targeted therapy" was used, therapeutic antibodies (anti-diphtheria toxin antibodies) already illustrated this concept. During the nineteenth century, the success of this serotherapy strategy

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against tetanus and diphtheria largely confirmed the therapeutic potential of antibodies. The idea to use serotherapy in oncology could be attributed to Paul Gibier who submitted to the Academy of Sciences of Paris in 1893 the proposition "to infuse to an animal, the juice of human tumor and to use the blood or the serum of this animal to infuse in the human harboring this tumor." On March 12, 1895, Jules Héricourt and Charles Richet treated the first patient with a sarcoma by serotherapy. Despite numerous experiments, the modest clinical effects and the heaviness of the procedure did not allow an extended use.

Biotechnologies Era

During the first part of the last century, it appeared that the limitations of serotherapy were mainly related to both the polyclonality of the antibodies and the immunization induced by the animal origin of the serum. In this context, the discovery of hybridoma technology by Georges Köhler and Césare Milstein in 1975 [1] revolutionized antibodies use. It became then possible to produce murine monoclonal antibodies (mAbs) and therefore to characterize the target antigen of each of these antibodies. Thus, the production of monoclonal antibodies has allowed to the identification of many antigens, among them, leukocyte antigens gathered together into clusters of differentiation (CD). The identification that same CD could be expressed by both leukemic cells and their normal counterparts allowed fantastic progress in the comprehension of lymphopoiesis and classification of lymphoproliferative disorders. This raised also the therapeutic potential of these mAbs which are able to target a specific antigen expressed by tumor cells. In 1980, Stevenson team produced for the first time sheep mAbs against idiotype of chronic lymphocytic leukemia cells [2]. The first lymphoma patient was infused 2 years after and experienced a prolonged response to this antibody [3]. This clinical success illustrated perfectly nearly one century later the concept of personalized anticancer serotherapy suggested by Paul Gibier.

At the beginning of 1980s, it became clear that the non-humanization of mAbs explained immunization, side effects, poor cytolytic activity, and short efficacy duration leading to limiting the clinical use of murine mAbs. In 1984, Sherie Morrison [4] described for the first time the technology allowing the production of chimeric recombinant mAbs with heavy and light chains obtained by fusion of murine variable domains with human constant domains. This important discovery explains mainly the success of mAbs. More recently, biotechnology advances permitted to reduce the part of murine counterpart with the production of antibodies exclusively humanized.

Rituximab Era

In 1984, the second workshop on the human leukocyte cluster of differentiation defined 11 new CDs (CD16 to CD26), and among them, CD20 antigen was expressed by most of B cells and B-lymphoma cells. Among mAbs defining this cluster, the company *Oncogene* tested the clone 1F5 (murine IgG2a) in five lymphoma patients. The patients receiving the highest dose experienced a significant clinical response [5]. These results led *IDEC Pharmaceuticals* company to produce a chimeric anti-CD20 antibody constructed from the murine parental clone 2B8. This murine mAb was humanized with constant human κ (kappa) and γ1 (gamma1) (human IgG1 ant-CD20, C2B8) or κ (kappa) and γ4 (gamma4) (human IgG4 anti-CD20) domains [6]. The human IgG1 version (C2B8) only exhibited complement activation and effector cell activation and induced lymphopenia in macaque. It has been developed with the name of rituximab by *IDEC Pharmaceuticals* and *Genentech* companies. The first phase I clinical trial using rituximab began in 1993, and rituximab was the first recombinant anticancer mAb to have received approval to market in the USA (Rituxan®, 1997) and in Europe (MabThera®, 1998). This antibody was an undeniable revolution in the treatment of malignant non-Hodgkin lymphomas of B-cell origin, and this success has contributed to the development of this therapeutic class in the field of oncology. Beyond these considerations, rituximab is a model for studying mechanisms of

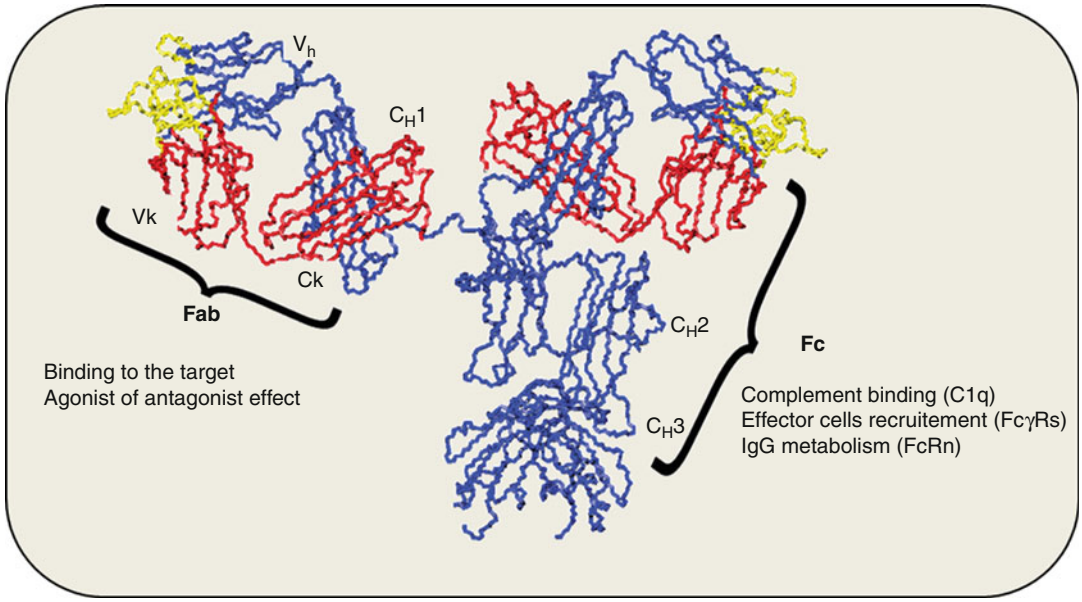


Fig. 21.1 Structure of an immunoglobulin G kappa. The Fc portion can activate complement cascade and recruitment of effector cells via receptors on the Fc portion of IgG1 (Fcγ(gamma)R). The Fab (fragment antigen binding) allows the binding of immunoglobulin to its target. The specificity of antigen recognition is carried by the complementarity determining region (CDR or, in *yellow*) carried by the Fv (fragment variable, including variable domain heavy chain VH and light Vκ[kappa]). (PDB and IMGT/3Dstructure-DB, http://imgt.cines.fr:1hzh) (Adapted from "Anticorps Monoclonaux: une révolution en marche," in "Histoire de la thérapie ciblée." Reproduced with the permission of John Libbey Eurotext Edition)

action of mAbs and this work should enable progress in the near future applicable to the majority of monoclonal antibodies for therapeutic use.

Naked Antibodies: The Rituximab Model

Rituximab is a "bifunctional" molecule bringing together functions related to antigen recognition (and therefore specific to the epitope) and other functions dependent on the Fc portion (fragment crystallizable) common to all IgG1 (Fig. 21.1). Properties due to the Fc portion can distinguish IgG1 and IgG3 classes (Tables 21.1a and 21.1b) that have the greatest ability to recruit the immune system (complement and effector cells) in human. In mice, however, it is mainly the IgG2a which has this property, highlighting the difficulties in interpreting experiments using humanized mAbs in mouse models. The Fc portion of IgG is also capable of binding to a receptor called FcRn (or Brambell receptor) expressed by endothelial, epithelial, and syncytiotrophoblastic cells. The interaction with the receptor ensures their transplacental or transepithelial passage and allows the IgG to escape lysosomal degradation, which explains their longer half-life compared to other isotypes of immunoglobulins.

Table 21.1a Ability to recruit effector cell with immunoglobulins based on their isotype

Human		Mouse	
IgG1	++	IgG1	+
IgG2	–	IgG2a	+++
IgG3	++	IgG2b	++
IgG4	–	IgG3	+
IgM	–	IgM	–

Table 21.1b Ability to recruit complement with immunoglobulins based on their isotype

Human	IgG1	IgG2	IgG3	IgG4
Classical pathway	+++	+	+++	–
Alternate pathway	–	+	–	–

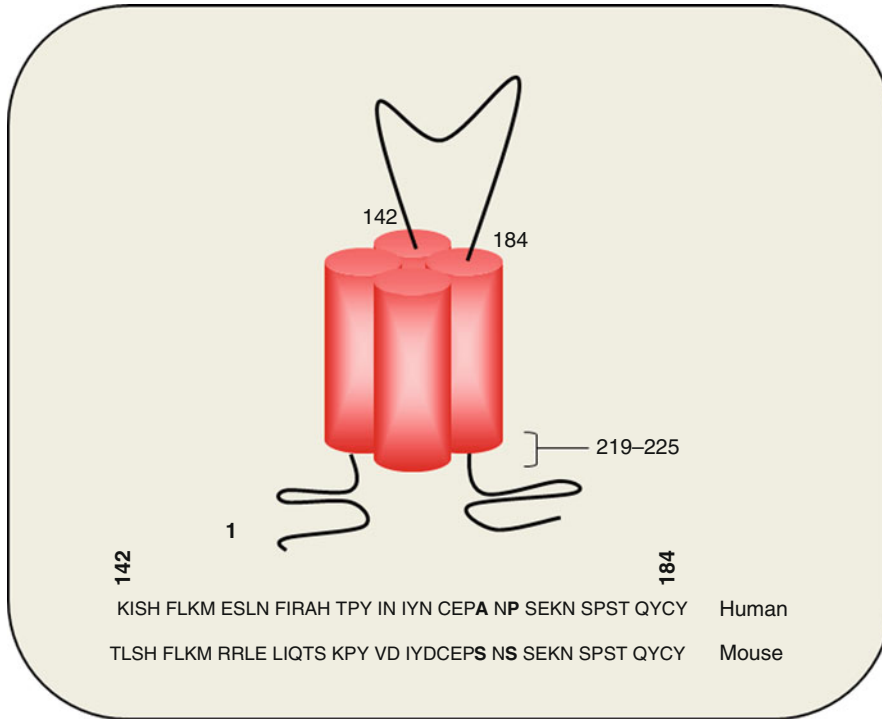


Fig. 21.2 Structure of human CD20. The CD20 protein is a no glycosylated protein with four transmembrane domains (tetraspan). The extracellular domain bears the epitopes recognized by different anti-CD20. The alanine and proline at position 170 and 172 residues are important in determining the epitope of rituximab. The sequence between residues 219 and 225 plays an important role during migration of CD20 in lipid rafts (Adapted from "Anticorps Monoclonaux: une révolution en marche," in "Histoire de la thérapie ciblée." Reproduced with the permission of *John Libbey Eurotext Edition*)

Mechanisms Involved in Target Recognition

The CD20 antigen is the target of rituximab and it is paradoxically the success of this therapeutic antibody that has attracted and led to important advances in the understanding of this protein and its functions. CD20 is a transmembrane protein (Fig. 21.2) which has characteristics that make it an ideal therapeutic target. CD20 is expressed by most B cells but is absent or weakly expressed on progenitor B or plasma cells, thus maintaining immunoglobulin levels and peripheral lymphoid reconstitution after treatment. After binding to the antibody, the CD20 is not internalized or stripped from the cell surface. The homology with the murine CD20 is 73 % and is mainly in the transmembrane regions. The extracellular domain of CD20 human which is the binding site of rituximab differs from that of murine CD20 by 16 of the 43 amino acids [7], explaining the absence of binding of rituximab to the murine CD20. The function of CD20 has long been misunderstood and the role of calcium channel has only been recently demonstrated [8]. However, knockout mice for the gene encoding CD20 do not display any phenotypic abnormality [9] which may reflect either the minor role of CD20 in B-cell physiology or a biological redundancy with other proteins. The use of anti-CD20 mAbs has been for a long time the only way to understand CD20 functions. Two types of properties related to two different epitopes have been originally identified: the first epitope which is the binding site of rituximab, but also other anti-CD20 (2H7, B1), leads to inhibitory signals inducing apoptosis and/or antiproliferative activity, while

the second is an activation of cell proliferation induced by antibody 1F5. In reality there are a variety of epitopes although some residues are critical for the antiproliferative activity [7]. The binding of the antibody to its target can induce the migration of the antigen within the lipid rafts located on the surface of the plasma membrane. This movement is dependent on a sequence of amino acids (219–225) that is not present in mice [10]. This property has allowed to the identification of mAbs inducing (antibody type 1: rituximab, 2H7, etc.) or not (type 2 antibodies: B1, LY1, etc.) this migration. The movement of CD20 in these structures allows the co-localization with proteins ensuring signal transduction. Recent work has demonstrated that anti-CD20 may mediate cell death in different ways (Fig. 21.3). A first type of anti-CD20 mAbs, such as rituximab, induces apoptotic cell death process which appears largely caspase dependent. Several activation pathways of apoptosis have been described including passing through the mitogen-activated protein kinase (MAP kinase), NF κ (kappa) B, protein kinase C (PKC), or ceramides or bcl-2. A second subgroup of anti-CD20 mAbs can kill cells in a caspase-independent manner. In this case, homotypic adhesion could mediate cell death through a lysosomal pathway [11].

Mechanisms Linked to the Fc Portion

The ability of the Fc portion of IgG1 to interact with effectors of cellular immunity or complement gives to the whole mAbs belonging to this class common cytolytic properties that explain much of their therapeutic activity.

Complement-Dependent Cell Lysis

Complement has a major role in the eradication of malignant cells. Activation of the classical complement pathway by immunoglobulins (IgG1, IgG3 and IgM) requires the prior binding of the antibody on its target and then setting the C1q protein on at least two Fc portions (Fig. 21.3). This binding will trigger a proteolytic cascade leading to the formation of large amounts of C3b which induces the formation of membrane attack complex (MAC) and the destruction of the cell (complement-dependant cell lysis, CDC). It also allows the chemotactic attraction of inflammatory cells (via C3a and C5a), while C3b opsonization of the target cell makes its interaction with complement receptors (CR3 and CR4) expressed by immune cells (natural killer cells, monocytes, neutrophils). The complement is therefore a system allowing both a direct lysis of the target cell and the establishment of a cytolytic response. Many in vitro studies have shown that rituximab induces a CDC on lymphoid cell lines or fresh lymphoma cells. Complement activation with rituximab was well demonstrated in a syngeneic mouse lymphoma model expressing human CD20 [12]. In this model, the therapeutic activity of rituximab was not found in mice deficient in C1q. In humans, infusion of rituximab increases levels of degradation products of complement (C3b/c, C4b/c) [13]. The role of the level of expression of CD20 or protein negatively regulating the complement (CD46, CD55, CD59) on this activity has long been discussed. Results have shown clearly that the CDC was correlated with the level of expression of CD20 by the target cell [14]. The ability of an anti-CD20 mAb to activate complement is also linked to the epitope recognized and the ability to relocate CD20 within lipid rafts [15]. Thus, rituximab or 2H7, which induces a migration, effectively leads CDC, while the B1 or LY1 antibodies do not induce CDC because of their inability to migrate CD20 within lipid rafts.

Cell Death Dependant on Receptors to the Fc Portion of Antibody

The Fc portion of rituximab is able to interact with receptors of the Fc portion of IgG or Fcγ(gamma) R (Fig. 21.4). By the recruitment of cells expressing these receptors (Table 21.2), immunoglobulins are involved in the development of immune effector mechanisms such as phagocytosis and antibody-dependent cell cytotoxicity (ADCC) (Fig. 21.3). The ability of rituximab to induce ADDC or to promote phagocytosis has been demonstrated in vitro in human lymphoma cell lines, and the involvement of Fcγ(gamma)Rs was fully described in a mouse model [16]. The involvement of these receptors, particularly the Fcγ(gamma)

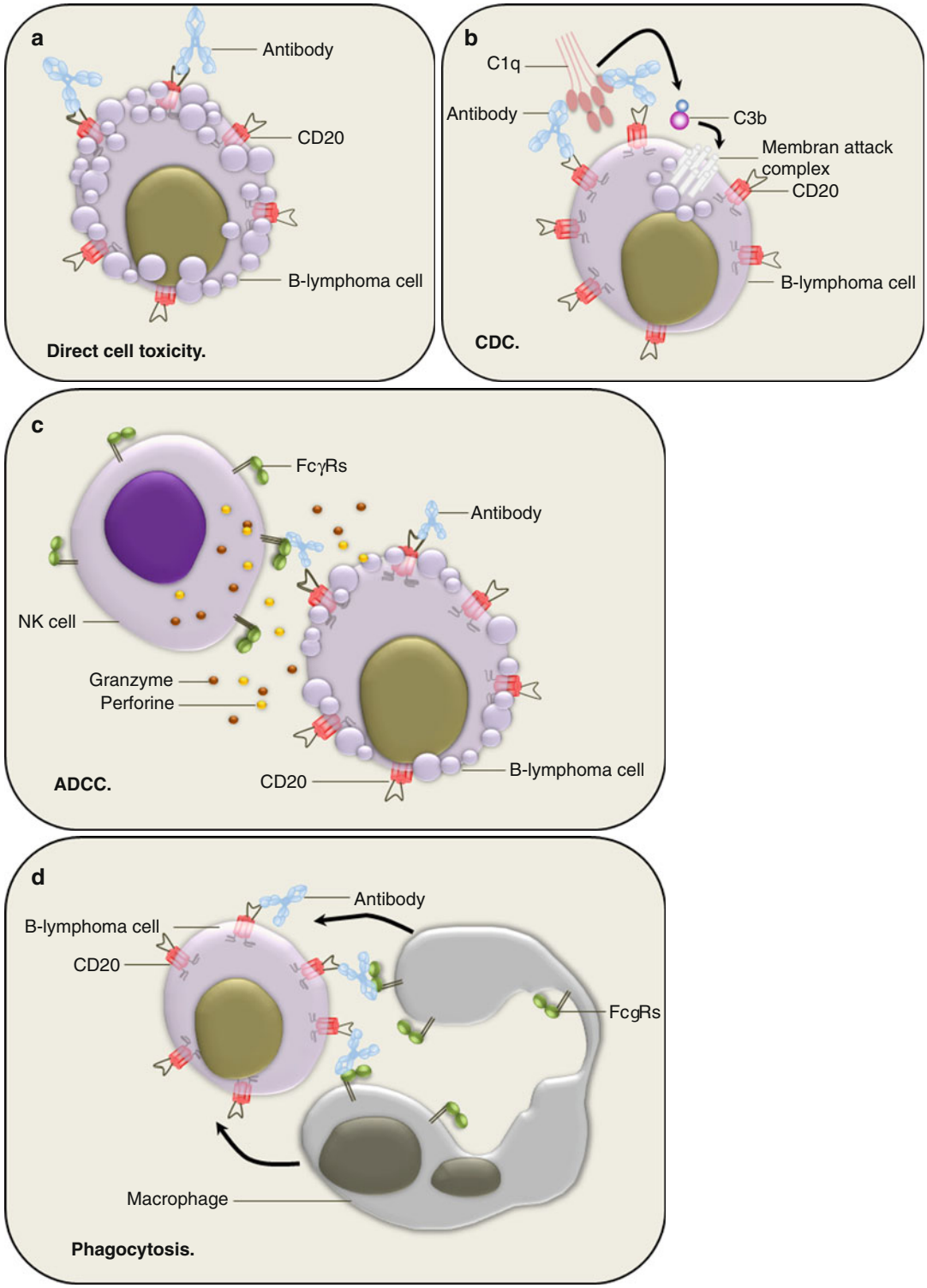
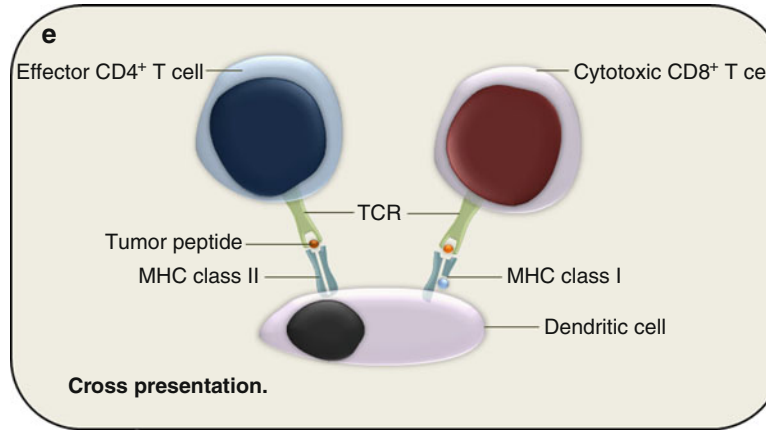

a
Antibody
CD20
B-lymphoma cell
Direct cell toxicity.
b
C1q
Antibody
C3b
Membran attack complex
CD20
B-lymphoma cell
CDC.
c
FcγRs
Antibody
NK cell
Granzyme
Perforine
CD20
B-lymphoma cell
ADCC.
d
Antibody
B-lymphoma cell
CD20
FcgRs
Macrophage
Phagocytosis.

Fig. 21.3 (continued)



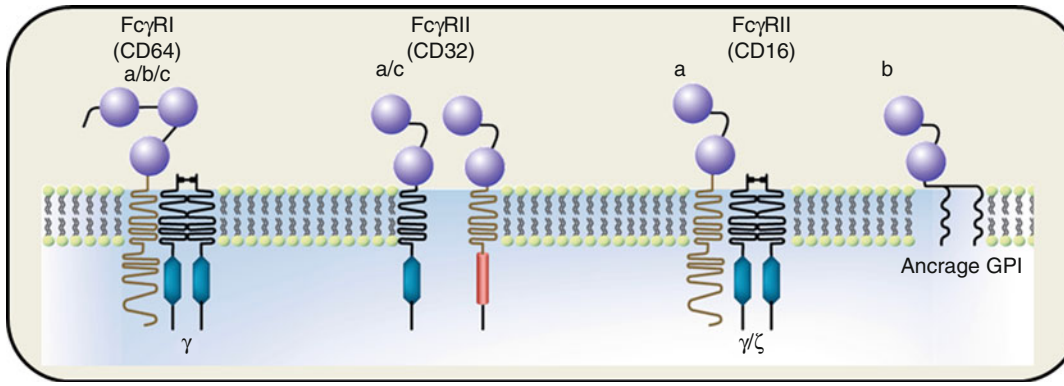


Fig. 21.4 Structure of the various receptors of the Fc portion of IgG (Fcγ(gamma)R). Fcγ(gamma)RIIb is the only inhibitory receptor because of the presence of an ITIM motif (immunoreceptor tyrosine-based inhibitory motif) in its intracytoplasmic portion (*red cylinder*). The presence of an ITAM motif (immunoreceptor tyrosine-based activatory motif) (*blue hexagon motifs*) in intracytoplasmic or within an accessory associated with channel gives the other Fcγ(gamma)R activating properties (Adapted from "Anticorps Monoclonaux: une révolution en marche," in "Histoire de la thérapie ciblée." Reproduced with the permission of *John Libbey Eurotext Edition*)

Fig. 21.3 (**a**) Direct cell toxicity. The binding of mAbs on CD20 antigen induces direct cell cytotoxicity which could be caspase dependant or not. (**b**) Complement-dependent cytotoxicity *(CDC)*. The binding of the antibody on its target will allow the activation of the classical complement pathway. This activation requires the binding of C1q to the Fc portion of the antibody. In addition to cell lysis, complement activation will allow migration to the site of effector cells and target cells opsonization by C3b. Effector cells can then be activated via their C3b receptors (CR3 and CR4). (**c**) Mechanism-dependent cellular cytotoxicity of the antibody (*ADCC*). The binding of the antibody to its target allows the recruitment of effector cells through their Fcγ(gamma)Rs, and cell activation induced by this binding will lead to cell lysis (by the degranulation of natural killer cells). (**d**) Phagocytosis. mAbs opsonized on tumor cell can bind Fc receptors on phagocyte cells initiating Fc-dependant phagocytosis. (**e**) Cross presentation. Peptides derived from phagocytosis of tumor cells by macrophages or dendritic cells can be loaded on to MHC molecules leading to activation of $CD4^+$ helper T cells and to prime cytotoxic $CD8^+$ T cells

Table 21.2 Cellular expression of different receptors of the portion of IgG1 (Fcγ(gamma)R)

	Fcγ(gamma) RI	Fcγ(gamma) RIIa	Fcγ(gamma) RIIb	Fcγ(gamma) RIIc	Fcγ(gamma) RIIIa	Fcγ(gamma) RIIIb
Monocyte/macrophage	+	+	+		+	
Natural killer cell				+	+	
Neutrophils	+/–	+				+
B lymphocyte			+			
Dendritic cell	+	+	+		+	
Mastocyte	+/–	+	+			
Platelets		+				

RIIIa has been established in human. Indeed, this receptor has a nucleotide polymorphism leading to substitution of the amino acid at position 158. And two receptor variants are possible, one with a valine at position 158 (Fcγ(gamma)RIIIa-158V), the other with a phenylalanine (Fcγ(gamma) RIIIa-158F). This substitution is accompanied by a change in the affinity of the Fcγ(gamma)RIIIa for the Fc portion of immunoglobulin IgG1 [17]. The influence of this amino acid is not surprising since it is the site of interaction between these two proteins. A study including patients with follicular lymphoma showed that patients homozygous for the high-affinity receptor (Fcγ(gamma) RIIIa-158V) had a better clinical and molecular response to rituximab [18]. Since this receptor is expressed by monocytes and natural killer cells, key players in the ADCC, this cell lysis mechanism is now considered as an important mode of action of rituximab. Above all, this work has highlighted the importance of the interaction between Fcγ(gamma)R and the Fc portion of the antibody.

Specific Anti-lymphoma Immunity

Several experimental results argue for the establishment of specific immunity in anti-lymphoma treatment with rituximab. Indeed, most of the antigen-presenting cells (dendritic cells, macrophages) express Fcγ(gamma)Rs whose role in the therapeutic activity of antibodies has been demonstrated. In addition, a number of clinical observations could account for this mechanism: delayed response in relation to treatment and increasing duration of response to retreatment. Cytolytic mechanisms caused by mAbs induce the cross presentation (Fig. 21.3) of lymphoma-specific antigens by antigen-presenting cells, leading to the establishment of a specific immune response. Some recent experimental data [19] seem to confirm this specific anti-lymphoma immunity. Such mechanism could lead to a change rituximab use and would open new avenues for optimizing its therapeutic activity.

Table 21.3 Characteristics of type I and type II anti-CD20 antibodies

Type I anti-CD20 mAbs	Type II anti-CD20 mAbs
Localize CD20 to lipid rafts	Do not localize CD20 to lipid rafts
High CDC	Low CDC
No homotypic adhesion	Homotypic adhesion
Low direct cell killing	High direct cell killing
Rituximab, ofatumumab, R-603	B1, obinutuzumab

Classification of Anti-CD20 Antibodies

According to mechanisms of action, anti-CD20 mAbs can be separated in two distinct subgroups called type I and II [20] (Table 21.3). Both type I and type II mAbs demonstrate efficient phagocytosis and ADCC. Type I anti-CD20 mAbs induce migration of the antibody/antigen complex into lipid rafts which cluster the antibody Fc regions, thus enabling improved C1q binding. In contrast, type II mAbs do not induce redistribution into lipid rafts leading to relatively ineffective CDC. Intriguingly, type II mAbs exhibit far more homotypic adhesion and direct killing of target cells. Reasons for this difference have long remained unknown, but new structural informations suggest that type I mAbs lead CD20 to adopt an "open" configuration likely linked to its role as a calcium channel, whereas type II mAbs leave

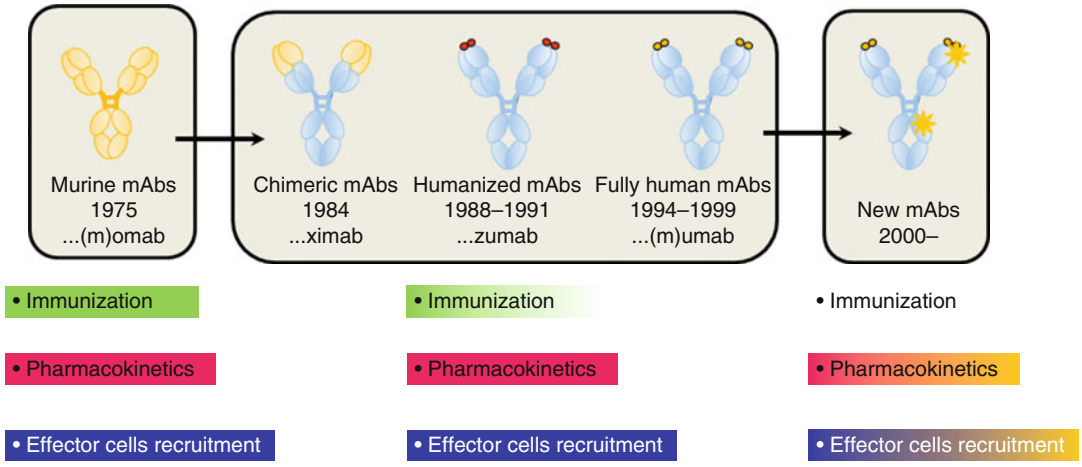


Fig. 21.5 Biotechnological advances applied to monoclonal antibodies. For 30 years technological advances have allowed the gradual humanization of murine antibodies (recognizable by the suffix-omab) produced by the hybridoma technique. The first step has allowed the production of chimeric antibodies such as rituximab (recognizable by the suffix-ximab) where only the Fc is of murine origin. It was then possible to obtain new generation humanized antibodies (identified by the suffix-zumab) where only the CDR (determining the idiotype) are of murine origin. Today, there are also fully human antibodies (suffix-mumab), obtained by techniques using transgenic mice or by screening phage libraries, but this pseudo-humanization of the idiotype does not remove its potential immunogenicity. In practice, it is essentially the humanizing of the Fc region (obtained from the chimeric antibodies) which has reduced the immunogenicity of recombinant antibodies and improved their pharmacokinetics and their ability to recruit effector cells. A third generation of modified antibodies appeared more recently in the aim of improving function, such as a longer half-life by mutation of amino acids involved in the interaction with FcRn. It is too early to know the immunogenic potential of these new drugs, clinical trials have just begun (Adapted from "Anticorps Monoclonaux: une révolution en marche," in "Histoire de la thérapie ciblée." Reproduced with the permission of *John Libbey Eurotext Edition*)

CD20 molecule in "closed" configuration without induction of calcium flux [11].

"New" Anti-CD20 Monoclonal Antibodies

Progresses in the field of mAbs are partly related to advances in biotechnology. Indeed, it was necessary to reduce the immunogenicity of these antibodies to improve their efficacy and safety (Fig. 21.5). In the field of hematology, the antibodies developed since the advent of rituximab are humanized antibodies in which only a very small portion of murine sequences remain. More recently, fully humanized monoclonal antibodies have been engineered. Rituximab appeared poorly immunogenic in the treatment of malignant lymphomas and further humanization should not significantly modify its efficacy in this indication. However, the frequency of antibodies against rituximab in autoimmune diseases currently justifies the development of fully humanized anti-CD20 antibodies.

The progress made in recent years in understanding the mechanisms of rituximab action should enable advances applicable to the whole therapeutic class (IgG1 antibodies). Thus, the influence of polymorphism of Fcγ(gamma)RIIIa has highlighted the critical role of its interaction with the Fc portion. It therefore seemed essential to improve the affinity of the Fc portion for Fcγ(gamma)RIIIa and to increase its ability to recruit effector cells. By 2002, the Genentech company produced monoclonal antibodies (anti-ErbB2) with mutations in the region of the Fc portion interacting with Fcγ(gamma)RIIIa. These antibodies have shown in vitro a higher affinity for both allotypic forms of Fcγ(gamma)RIIIa and an increase of their cytolytic capacity [21]. The clinical development of these "mutant" antibodies may, however, face problems of increased antigenicity

of the molecule and therefore immunization. In 1999, the Swiss group of Pablo Umana showed that the modification of oligosaccharides in the Fc portion could change the ADCC induced by a monoclonal antibody of IgG1 class [22]. Since then, it was established that the fucose content of the oligosaccharide influences the affinity of the Fc portion for Fcγ(gamma)RIIIa and ADCC. Thus, whatever the phenotype Fcγ(gamma)RIIIa-158V or Fcγ(gamma)RIIIa-158F of effector cells, a low-fucosylated anti-CD20 antibody induced a better ADCC [23]. It was then possible to produce antibodies mutated in the region of the Fc portion interacting with C1q and FcRn. Some of these mutants have improved affinity for FcRn and increased half-life in monkeys [24]. The clinical relevance of such antibodies is evident in terms of rate of administration. In contrast, mutations of the Fc portion of rituximab for a better affinity to C1q increased in vitro CDC but accompanied by a reduction in their ability to induce ADCC might limit its clinical development. Obinutuzumab (GA101, Roche Pharmaceuticals) is a humanized type II anti-CD20 antibody with low fucose content into the oligosaccharide of the Fc portion leading to an increased ADCC [25]. Similarly, this antibody exhibited a higher level of direct cell death activity than that of rituximab. This property appears to be related to a change of one amino acid (a valine instead of a leucin) located into the elbow-hinge region between the first constant domain and variable domain of the heavy chain. First clinical trials showed promising results in both non-Hodgkin's lymphoma and chronic lymphocytic leukemia and prospective phase III trials are ongoing.

The group of Martin Glennie selected two anti-CD20 mAbs exhibiting higher CDC than rituximab [26]. These type I antibodies showed similar ability to induce CD20 antigen migration into lipid raft but recognized a different CD20 epitope from that of rituximab leading to a lower capacity of dissociation from the target. Authors demonstrated that epitope recognized by these antibodies was located on the small extracellular loop of CD20 antigen, and they hypothesized that the low distance between target epitope and membrane cell could explain the high CDC. One of this mAb called ofatumumab (Arzerra®, GlaxoSmithKline) is currently in clinical development and indicated in refractory chronic lymphocytic leukemia.

Other Targets, Other Histories?

Monoclonal antibodies target different cell populations including tumor or immune cells and all the components of the microenvironment.

Targeting Tumor Cells

Rituximab success led companies to develop mAbs against new lymphoma cell targets (Table 21.4). Among them, the benefit of epratuzumab (anti-CD22), galiximab (anti-CD80), and

Table 21.4 Monoclonal antibodies targeting tumor cell antigen

Target	Antibody	Company
CD20	Rituximab (MabThera®, Rituxan®)	Roche, Genentech
	Ofatumumab (Arzerra®)	GlaxoSmithKline
	Obinutuzumab	Roche
	R-603	LFB
	AME-133v	Lilly
CD22	Epratuzumab	Immunomedics
CD23	Lumiliximab	Biogen Idec
CD37	TRU-016	Abbott
CD52	Alemtuzumab	Bayer
CD74	Milatuzumab	Immunomedics
CD80	Galiximab	Biogen Idec
CD194 (CCR4)	KW-0761	Kyowa Hakko Kirin

Table 21.5 Monoclonal antibodies targeting molecules expressed by immune cells or tumor microenvironment

Target	Name	Company
CD25	Daclizumab (Zenapax®)	Roche
CD32B	Anti-CD32B	MacroGenics
C40	Dacetuzumab	Seattle Genetics
	Lucatumumab	Novartis
	CP-870893	Pfizer
CD137	BMS-663513	Bristol-Myers-Squibb
PD1	CT-011	CureTech Ltd
	MK-3475	Merk
	BMS-936558, BMS-936569	Bristol-Myers-Squibb
OX40	Anti-OX40	Portland Providence Medical Center
CTLA4	Tremelimumab	Pfizer
	Ipilimumab	Bristol-Myers-Squibb

lumiliximab (anti-CD23) has been explored in prospective phase III studies. However, except anti-CD52 mAb (alemtuzumab, MabCampath®), approved in relapsed B-CLL, there are no new mAbs targeting other NHL antigens than CD20 approved for clinical use.

Targeting Immune Cells

More recently, numerous mAbs have been developed to target cells of the immune system with the goal of enhancing antitumor response (Table 21.5). Thus, targeting of immunoregulatory co-receptors such as PD1 or CD137 seems to be a promising strategy but has not been undergone extensive clinical testing. Cytotoxic T lymphocyte antigen 4 (CTLA4) is a negative regulator of T-cell activation that binds CD80 and CD86. CTLA4 blockade could therefore prevent and reverse antigen-specific $CD8^+$ T-cell tolerance, enhancing adaptive immunity and promoting tumor regression [27]. Preclinical data demonstrated that the blockade of the inhibitory receptor Fcγ(gamma) RIIb (CD32b) by antagonist mAb could enhance cross presentation of tumor antigens and promote adaptive immune responses [28].

Targeting Tumor Microenvironment

There are extensive data supporting the role of microenvironment in cancer development and progression. Thus, targeting cells or matrix proteins of tumor microenvironment (Table 21.5) could constitute an attractive strategy. The interest of targeting vascular endothelial growth factors (VEGF) has been well demonstrated in many solid tumors, and anti-VEGF mAbs (bevacizumab, Avastin®) have been recently explored in a large phase III study in aggressive NHLs. Among the other attractive targets in the tumor stroma, fibroblast activation protein (FAP), tenascin, or fibronectin extra-domain B have not been extensively studied in hematologic malignancies.

Immunoconjugates

Monoclonal antibodies could be considered as a tool targeting radionuclides or drugs to tumor cells. Potential advantages of such a strategy are to increase cytotoxic effects of the conjugates as well as to prevent cellular damages on normal cells. However, the binding of radionuclides or cytotoxic drug limits the immunologic effect of such a strategy.

Targeting Radionuclides

For 20 years, the linking of radionuclides to mAbs has been studied to increase their cytotoxic activity and to exploit their specific targeting. The FDA has approved the use of two radiolabeled anti-CD20 mAbs for the treatment of B-cell NHLs: ^{90}Y ibritumomab (Zevalin®) and ^{131}I-tositumomab (Bexxar®). Other radiolabeled mAbs using other B-cell antigens and/or other radionuclides have been developed and are currently under trials.

The mAbs used for radioimmunotherapy are usually of murine origin since there is no clear

advantage for using humanized mAbs other than the theoretical concern regarding the development of HAMA which has not been observed during the development of these radiolabeled antibodies. Pretreatment with either the unconjugated antibody or rituximab is required to coat the circulating antigens on B cells and to suppress low-affinity sites such as nonspecific Fc receptors. This predose results in prolongation of the plasma half-life of the radiolabeled antibody, thus allowing more time to remain in the circulation and to reach other sites than normal B-cell reservoirs (spleen).

Three types of radionuclides have been tested:

- β (beta)-emitters such as ^{131}I, ^{90}Y, ^{186}Re, and, more recently, ^{177}Lu which have long emission path lengths (around 300 μ(mu)m for ^{90}Y). They are able to bypass tumor antigen heterogeneity and to uniformly target a tumor whose radius does not exceed the emission range. Some of them, e.g., ^{131}I, are also γ(gamma) emitters and thus allow direct tumor imaging and dosimetry, while others, e.g., ^{90}Y, are pure β (beta)-emitters, lack imageable emission, and require dosimetry using ^{111}In (a gamma-emitting radiometal with chemistry similar to ^{90}Y) as a surrogate. In European countries, this imaging and dosimetry step is no more required for conventional dosage treatment with ibritumomab tiuxetan. Convenience, availability, low cost, and familiarity have favored the use of these radionuclide types [29].
- α (alpha)-emitters produce particles which have a much higher energy than β (beta)- emitters but on a short distance. Although their cytotoxic capacities are high, only the targeted cell and the immediate neighboring cells are killed, thus limiting their use in NHL radioimmunotherapy.
- Auger emitters such as ^{67}Ga or ^{125}I are extremely cytotoxic and can only be used to treat microscopic residual disease.

The link between the radionuclide and the mAbs has a crucial importance. ^{131}I allows a direct and easy iodination of the protein and has thus been most extensively used. However, deiodination occurs after internalization of the protein and compromises its efficacy. Anti-CD19 or anti-CD22 antibodies, which are internalized after binding to their epitope, are thus unsuitable for radioimmunotherapy using iodinated antibodies, while anti-CD20 antibodies are convenient. All other radionuclides require chemical chelation to the protein. Adequate chelating agents possess both a functional group which allows conjugation to the protein and a site which forms a stable complex with the metallic radionuclide.

Radioimmunoconjugates: Clinical Trials

Radioimmunotherapy has been used for the treatment of B-cell non-Hodgkin's lymphomas in several circumstances [30–35]:

Relapsed and Refractory Indolent NHLS

Numerous phase II studies of radioimmunotherapy of indolent (mostly follicular) NHLs have been reported. They show overall response rates of 60–80 % and complete response (CR) rates up to 40 %. The median time to progression (TTP) does not exceed 7–10 months.

Several features should be underlined:

- Radioimmunotherapy is active even in rituximab-refractory patients but with lower response rates and shorter TTP than in rituximab naïve or sensitive patients.
- In a small randomized trial [36] carried out on rituximab naïve patients, radioimmunotherapy using ibritumomab tiuxetan was superior to rituximab in terms of response and CR rates and TTP.
- Radioimmunotherapy is also active in patients with transformed indolent NHL, with lower response rates than in the absence of transformation.
- Treatment after more than two relapses, bulky disease, increased serum LDH levels, prior autologous stem cell transplantation (ASCT), and no response to last therapy have a negative influence on radioimmunotherapy efficacy.
- Although there is no randomized trial, ^{90}Y ibritumomab tiuxetan and ^{131}I-tositumomab seem to have similar efficacies.

Untreated Indolent NHLS

Only one study has been reported in 2005 with updated results in 2009 [37, 38]. In 76 previously

untreated patients with follicular lymphoma, a single treatment with ^{131}I-tositumomab yielded a 95 % response rate with 75 % CR rate. The median duration of response was 6 years and the median progression-free survival was 11 years in the 57 CR patients. For unknown reasons, these results have not been confirmed in other trials.

Consolidation After Initial Chemotherapy

Radioimmunotherapy has also been incorporated into the frontline treatment of patients with indolent NHL. Morschhauser et al. [39]. reported results of a randomized trial comparing consolidation with ^{90}Y ibritumomab tiuxetan versus observation in patients with follicular lymphoma who have reached complete or partial response following initial chemotherapy induction. Radioimmunotherapy significantly improved median PFS (36.5 vs. 13.3 months, $p<0.0001$). This effect of radioimmunotherapy consolidation was especially marked in patients in PR after induction chemotherapy and in patients who had detectable *BCL-2* rearrangement detectable before radioimmunotherapy and who became negative after radioimmunotherapy. A noted limitation of this trial was that the vast majority of patients did not receive rituximab-based chemotherapy prior to consolidation radioimmunotherapy. The results of an SWOG trial comparing R-CHOP followed by ^{131}I-tositumomab vs. observation are thus eagerly waited.

Radioimmunotherapy as a Component of Conditioning Regimen for ASCT

Radioimmunotherapy has been used either alone at high doses or in combination with high-dose chemotherapy.

Small phase II trials of radioimmunotherapy using either ^{131}I-tositumomab or ^{90}Y ibritumomab tiuxetan at high doses followed by ASCT have been reported establishing the feasibility of these procedures in some experimented groups. Interesting results in term of posttransplant progression-free survival have been reported. However, because of the complexity of the procedures, their toxicity requiring prolonged hospitalizations, and a significant transplant-related mortality, this conditioning regimen has not been largely used.

Several groups have tested the combination of a conventional weight-based radioimmunotherapy with BEAM chemotherapy and reported the feasibility of this approach. Trials comparing a conditioning regimen with chemotherapy alone vs. chemotherapy plus radioimmunotherapy are ongoing.

The toxicity of radioimmunotherapy at conventional dosage is mainly myeloid. The cytopenias are delayed (starting 6–8 weeks after treatment) and most often modest and rapidly reversible provided that contraindications to usual dose radioimmunotherapy (thrombocytopenia, bone marrow hypoplasia, significant bone marrow lymphoma infiltration) have been respected. In patients treated after first-line induction chemotherapy, the incidence of grade 4 neutropenia and thrombocytopenia were respectively 26.5 and 2 % [39]. There has been concern regarding myelodysplastic syndromes (MDS) occurring late after radioimmunotherapy. However, the risk seems modestly increased compared to patients treated with chemotherapy only.

Ways to Improve Radioimmunoconjugates

Several approaches are currently tested in order to improve the efficacy of radioimmunotherapy:

- Combining radioimmunotherapy with a novel agent that increases the sensitivity of tumor cells to ionizing radiation such as bortezomib or motexafin gadolinium.
- Combining radioimmunotherapy with an agent that upregulates CD20 expression such as CpG.
- Fractionating radioimmunotherapy in order to allow a higher cumulative whole-body dose (approximately +60 %) than with a single dose.
- Engineering of the antibody (single-chain Fv fragments, diabodies formed by dimerization of Fv fragments, minibodies formed by 2 Fv fragments fused to single constant domains, and other immunoproteins) in order to improve tumor penetration and to decrease circulation time and radiation exposure of normal organs.

- Increasing the tumor to normal organ ratios of absorbed radioactivity by multi-step pretargeting methods. In this method, the targeting mAb conjugated to a nonradioactive adapter molecule such as streptavidin is administered first. This large antibody molecule allows an optimal localization in tumor sites. Following this localization, a small molecular weight radiolabeled ligand (i.e., biotin) is administered, rapidly penetrates the tumor site, and binds tightly to the adapter [35]. This latter method has yet only been used in mice bearing lymphoma xenografts.

Antibody-Drug Conjugates (ADCS)

ADCs allow cytotoxic drugs, attached via chemical linkers to antibodies that recognize cancer cell antigens, to be delivered only to the cells of interest. ADCs have been developed for several decades, but, until recently, there use has been limited either by the systemic toxicity of the conjugated drug(s) that was used (ricin chain A or diphtheria toxin) or by the low clinical activity of drugs usually given by a systemic route (doxorubicin, antitubulin agents). It has only been in the past few years that the critical parameters for optimization have begun to be addressed [40, 41].

The antigens used for ADC in NHLs (e.g., CD19, CD22, CD79, and CD30) undergo rapid internalization once the ADC binds to the tumor cell through a process known as receptor-mediated endocytosis. With few exceptions, once internalized, the ADC is delivered to lysosomes where the drug takes advantage of the catabolic environment and binds to its pharmacological target.

Substantially more potent drugs than conventional cytotoxic agents that were too toxic to use in an untargeted manner have been more promising as ADCs. These include auristatins, maytansines, and calicheamicin. The latter is the active drug of gemtuzumab ozogamicin, the only clinically approved ADC in acute myeloid leukemias. While auristatins and maytansines exert their cytotoxic effects by binding to tubulin causing cycle arrest and apoptosis, calicheamicin is a DNA strand-cleaving agent. The linkage between the drug and the mAb incorporates two labile bonds, a hydrazone which is cleaved under acidic conditions within the lysosomes of target cells and a sterically hindered disulfide which undergoes intracellular reduction. Inotuzumab ozogamicin (CMC-544) is an anti-CD22 ADC which uses the same calicheamicin drug linkage. It is currently under phase III trials, given either alone or in combination with rituximab, in follicular and aggressive NHLs. In a phase I/II escalation dose trial, the overall response rate was 88 % in relapsed follicular NHL and 33 % in relapsed aggressive NHL [42]. However, CMC-544 has systemic toxicity (thrombocytopenia, bone marrow hypoplasia, hepatitis) most probably because of the release of cleaved calicheamicin.

This explains why stable linkers have been developed. These linkers are not cleaved from the antibodies. After internalization, the mAbs are degraded, thus releasing the drug still attached to the conjugating amino acids [39]. New ADCs combining either a humanized anti-CD22 or an anti-CD79b mAb with an auristatin derivative have been engineered and demonstrated promising efficacy in preclinical models [39, 43].

Brentuximab vedotin (SGN-35) is a chimeric IgG1 targeting CD30 conjugated to the antitubulin agent monomethyl auristatin E through a peptide linker that is cleaved after internalization into CD30-positive cells [44]. CD30 is expressed on the surface of Reed-Sternberg and Hodgkin cells, on anaplastic large-cell lymphomas (ALCLs), embryonal carcinomas, and select subtypes of B-cell- or T-cell-derived NHLs. Normal expression of CD30 is limited to a small population of activated B cells and T cells and a small portion of eosinophils.

Younes et al [45]. have recently reported the results of an extended phase I trial of brentuximab vedotin in 45 patients with CD30-positive lymphoma (Hodgkin's disease in 42/45). All these patients had relapsed or refractory disease and 73 % had previously received high-dose therapy with ASCT. The maximum tolerated dose (MTD) was of 1.8 mg/kg every 3 weeks. Responses were observed in 17/45 (38 %) of the patients, including 11 CR, and in 6/12 (4CR) of those treated at the MTD. Peripheral neuropathy

was the most noticeable adverse event. Several phase III trials of brentuximab vedotin are ongoing, especially a randomized trial comparing observation to brentuximab vedotin after ASCT for relapsed Hodgkin's disease.

Other targets of ADCs in lymphoproliferative disorders have been tested. For instance, the receptor kinase ROR1, which is selectively upregulated in CLL and in mantle cell lymphomas, is a promising target [46, 47].

Optimization of the parameters influencing ADC activity has led to the development of new agents with promising activities. Although several challenges lie ahead, it is apparent that continued research in this area will feed the clinical product pipeline and play an increasingly important role.

Other Structures

Advances in biotechnologies make now possible construction of customized mAbs-based molecules with optimized size and affinity or with appropriate additional functions. Immunocytokines, associating mAbs and cytokine (interferon, GM-CSF, etc.), could allow to improve effector cell recruitment and activity. Bispecific antibodies target simultaneously antigen on lymphoma cells (e.g., CD3) and molecules expressed by immune effector in order to increase cytotoxic effects. Experimental data using radiolabeled mAbs suggest tumor penetration does not exceed 0.01 % of the infused dose per gram of tumor. This lack of tumor distribution could make ineffective mAbs in certain types of malignancies. In order to increase this penetration rate, truncated monoclonal IgGs have been engineered. These constructs with lower molecular weight than IgG (150 KD) include Fab fragments (55 KD), single-chain Fv (sc-FV, 25 KD), diabody (50 KD), or minibody (80 KD). Although they have a low molecular weight favoring tumor penetration, most of these constructs have however lost the interaction site with FcRn increasing blood clearance. A wide range of strategies has been developed to improve the pharmacokinetic properties including the conjugation with polyethylene glycol (PEG or albumin). All these new format of mAbs are currently extensively evaluated in preclinical model or in early clinical studies.

Conclusion

Although the idea of treating cancer patients with antisera or antibodies has been a research topic for more than 100 years, their development and clinical usage has really begun 20 years ago. Since then, there has been an "explosion" as well as of the antibodies and of the clinical indications. As a proof, in 2011 approximately 140 and 120 novel mAbs are in phase I and phase II studies, respectively, as well as a dozen Fc fusion proteins. Among the neoplasias treated with mAbs, lymphoproliferative disorders have been not only the main indication, allowing to largely improve the outcomes of patients, but also constitute a model for testing new targets, for molecular engineering in order to increase specificity and activity, for designing clinical trials.

References

1. Hamblin TJ, Abdul-Ahad AK, Gordon J, Stevenson FK, Stevenson GT. Preliminary experience in treating lymphocytic leukaemia with antibody to immunoglobulin idiotypes on the cell surfaces. Br J Cancer. 1980;42(4):495–502.
2. Kohler G, Milstein C. Continuous cultures of fused cells secreting antibody of predefined specificity. Nature. 1975;256(5517):495–7.
3. Miller RA, Maloney DG, Warnke R, Levy R. Treatment of B-cell lymphoma with monoclonal anti-idiotype antibody. N Engl J Med. 1982;306(9):517–22.
4. Morrison SL, Johnson MJ, Herzenberg LA, Oi VT. Chimeric human antibody molecules: mouse antigen-binding domains with human constant region domains. Proc Natl Acad Sci U S A. 1984;81(21):6851–5.
5. Press OW, Appelbaum F, Ledbetter JA, et al. Monoclonal antibody 1F5 (anti-CD20) serotherapy of human B cell lymphomas. Blood. 1987;69(2):584–91.
6. Reff ME, Carner K, Chambers KS, et al. Depletion of B cells in vivo by a chimeric mouse human monoclonal antibody to CD20. Blood. 1994;83(2):435–45.
7. Polyak MJ, Deans JP. Alanine-170 and proline-172 are critical determinants for extracellular CD20 epitopes; heterogeneity in the fine specificity of CD20 monoclonal antibodies is defined by additional requirements imposed by both amino acid sequence and quaternary structure. Blood. 2002;99(9):3256–62.

8. Li H, Ayert LM, Lytton J, Deans JP. Store-operated cation entry mediated by CD20 in membrane rafts. J Biol Chem. 2003;278(43):42427–34.
9. O'Keefe TL, Williams GT, Davies SL, Neuberger MS. Mice carrying a CD20 gene disruption. Immunogenetics. 1998;48(2):125–32.
10. Polyak MJ, Tailor SH, Deans JP. Identification of a cytoplasmic region of CD20 required for its redistribution to a detergent-insoluble membrane compartment. J Immunol. 1998;161(7):3242–8.
11. Cragg MS, Morgan SM, Chan HTC, et al. Complement-mediated lysis by anti-CD20 mAb correlates with segregation into lipid rafts. Blood. 2003; 101(3):1045–52.
12. Beers SA, Chan CHT, French RR, Cragg MS, Glennie MJ. CD20 as a target for therapeutic type I and II monoclonal antibodies. Semin Hematol. 2010;47:107–14.
13. Di Gaetano N, Cittera E, Nota R, et al. Complement activation determines the therapeutic activity of rituximab in vivo. J Immunol. 2003;171(3):1581–7.
14. van der Kolk LE, Grillo-Lopez AJ, Baars JW, Hack CE, van Oers MH. Complement activation plays a key role in the side-effects of rituximab treatment. Br J Haematol. 2001;115(4):807–11.
15. van Meerten T, van Rijn RS, Hol S, Hagenbeek A, Ebeling SB. Complement-induced cell death by rituximab depends on CD20 expression level and acts complementary to antibody-dependent cellular cytotoxicity. Clin Cancer Res. 2006;12(13):4027–35.
16. Clynes RA, Towers TL, Presta LG, Ravetch JV. Inhibitory Fc receptors modulate in vivo cytotoxicity against tumor targets. Nat Med. 2000;6(4):443–6.
17. Dall'Ozzo S, Tartas S, Paintaud G, et al. Rituximab-dependent cytotoxicity by natural killer cells: influence of FCGR3A polymorphism on the concentration-effect relationship. Cancer Res. 2004;64(13):4664–9.
18. Cartron G, Dacheux L, Salles G, et al. Therapeutic activity of humanized anti-CD20 monoclonal antibody and polymorphism in IgG Fc receptor FcγRIIIa gene. Blood. 2002;98:754–8.
19. Abès R, Gélizé E, Fridman WH, Teillaud JL. Long-lasting anti tumor protection by anti-CD20 antibody through cellular immune response. Blood. 2010;116:926–34.
20. Cragg MS, Glennie MJ. Antibody specificity controls in vivo effector mechanisms of anti-CD20 reagents. Blood. 2004;103:2738–43.
21. Shields RL, Namenuk AK, Hong K, et al. High resolution mapping of the binding site on human IgG1 for Fc gamma RI, Fc gamma RII, Fc gamma RIII, and FcRn and design of IgG1 variants with improved binding to the Fc gamma R. J Biol Chem. 2001;276(9):6591–604.
22. Umana P, Jean-Mairet J, Moudry R, Amstutz H, Bailey JE. Engineered glycoforms of an antineuroblastoma IgG1 with optimized antibody-dependent cellular cytotoxic activity. Nat Biotechnol. 1999;17(2):176–80.
23. Shields RL, Lai J, Keck R, et al. Lack of fucose on human IgG1 N-linked oligosaccharide improves binding to human Fcgamma RIII and antibody-dependent cellular toxicity. J Biol Chem. 2002;277(30):26733–40.
24. Hinton PR, Xiong JM, Johlfs MG, Tang MT, Keller S, Tsurushita N. An engineered human IgG1 antibody with longer serum half-life. J Immunol. 2006;176(1):346–56.
25. Mössner E, Brünker P, Moser S, Püntener U, Schmidt C, Herter S, et al. Increasing the efficacy of CD20 antibody therapy through the engineering of a new type II anti-CD20 antibody with enhanced direct and immune effector cell-mediated B-cell cytotoxicity. Blood. 2010;115:4395–402.
26. Teeling JL, French RR, Cragg MS, et al. Characterization of new human CD20 monoclonal antibodies with potent cytolytic activity against non-Hodgkin lymphomas. Blood. 2004;104(6):1793–800.
27. Shrikant P, Khoruts MF, Allison JP. CTLA-4 blockade reverse CD8+ T cell tolerance to tumor by a CD4+ T cell- and IL-2 dependant mechanism. Immunity. 1999;11: 483–93.
28. Dhodapkar KM, Kaufman JL, Ehlers M, et al. Selective blockade of inhibitory Fcg receptor enables human dendritic cell maturation with IL-2p70 production and immunity to antibody-coated tumor cells. Proc Natl Acad Sci U S A. 2005;102:2910–5.
29. Milenic DE, Brady ED, Brechbiel MW. Antibody-targeted radiation cancer therapy. Nat Rev Drug Discov. 2004;3:488–98.
30. Buchegger F, Press OW, Delaloye AB, Ketterer N. Radiolabeled and native antibodies and the prospect of cure of follicular lymphoma. Oncologist. 2008;13:657–67.
31. Zinzani PL, Tani M, Pulsoni A, Gobbi M, Perotti A, De Luca S, et al. Fludarabine and mitoxantrone followed by yttrium-90 ibritumomab tiuxetan in previously untreated patients with follicular non-Hodgkin lymphoma trial: a phase II non-randomised trial (FLUMIZ). Lancet Oncol. 2008;9:352–8.
32. Emmanouilides C. Review of Y-ibritumomab tiuxetan as first-line consolidation radio-immunotherapy for B-cell follicular non-Hodgkin's lymphoma. Cancer Manag Res. 2009;21:131–6.
33. Goldsmith SJ. Radioimmunotherapy of lymphoma: Bexxar and Zevalin. Semin Nucl Med. 2010;40:122–35.
34. Ahmed S, Winter JN, Gordon LI, Evens AM. Radioimmunotherapy for the treatment of non-Hodgkin lymphoma: current status and future applications. Leuk Lymphoma. 2010;51:1163–77.
35. Palanca-Wessels MC, Press OW. Improving the efficacy of radioimmunotherapy for non-Hodgkin lymphomas. Cancer. 2010;116(4S):1126–33.
36. Witzig TE, Gordon LI, Cabanillas F, Czuczman MS, Emmanouilides C, Joyce R, et al. Randomized controlled trial of yttrium-90-labeled ibritumomab tiuxetan radioimmunotherapy versus rituximab immunotherapy for patients with relapsed or refractory low-grade, follicular, or transformed B-cell non-Hodgkin's lymphoma. J Clin Oncol. 2002;20:2453–63.
37. Kaminski MS, Tuck M, Estes J, et al. 131I-tositumomab therapy as initial treatment for follicular lymphoma. N Engl J Med. 2005;352:441–9.
38. Kaminski MS, Tuck M, Estes J, et al. Tositumomab and iodine 131I-tositumomab for previously untreated,

advanced-stage, follicular lymphoma: median 10-years follow-up results. Blood. 2009;114:3759.
39. Morschhauser F, Radford J, Van Hoof A, Vitolo U, Soubeyran P, Tilly H, et al. Phase III trial of consolidation therapy with yttrium-90-ibritumomab tiuxetan compared with no additional therapy after first remission in advanced follicular lymphoma. J Clin Oncol. 2008;26:5156–64.
40. Teicher BA. Antibody-drug conjugate targets. Curr Cancer Drug Targets. 2009;9:982–1004.
41. Alley SC, Okeley NM, Senter PD. Antibody-drug conjugates: targeted drug delivery for cancer. Curr Opin Chem Biol. 2010;14:529–37.
42. DiJoseph JF, Dougher MM, Kalyandrug LB, Armellino DC, Boghaert ER, Hamann PR, et al. Antitumor efficacy of a combination of CMC-544 (inotuzumab ozogamicin), a CD22-targeted cytotoxic immunoconjugate of calicheamicin, and rituximab against non-Hodgkin's B-cell lymphoma. Clin Cancer Res. 2006;12:242–9.
43. Polson AG, Williams M, Gray AM, Fuji RN, Poon KA, McBride J, et al. Anti-CD22-MCC-DM1: an antibody-drug conjugate with a stable linker for the treatment of non-Hodgkin's lymphoma. Leukemia. 2010;24:1566–73.
44. Dornan D, Bennett F, Chen Y, Dennis M, Eaton D, Elkins K, et al. Therapeutic potential of an anti-CD79b antibody-drug conjugate, anti-CD79b-vc-MMAE, for the treatment of non-Hodgkin lymphoma. Blood. 2009; 114:2721–9.
45. Francisco JA, Cerveny CG, Meyer DL, Mixan BJ, Klussman K, Chace DF, et al. cAC10-vcMMAE, an anti-CD30-monomethyl auristatin E conjugate with potent and selective antitumor activity. Blood. 2003;102: 1458–65.
46. Younes A, Bartlett NL, Leonard JP, Kennedy DA, Lynch CM, Sievers EL, et al. Brentuximab vedotin (SGN-35) for relapsed CD30-positive lymphomas. N Engl J Med. 2010;363:1812–21.
47. Yang J, Baskar S, Kwong KY, Kennedy MG, Wiestner A, Rader C. Therapeutic potential and challenges of targeting receptor tyrosine kinase ROR1 with monoclonal antibodies in B-cell malignancies. PLoS One. 2011;6:e21018. Epub 2011 Jun 15.

Novel Agents Targeting Oncogenic Pathways in Lymphoma

22

Anas Younes

Abstract

With increasing number of new drugs and molecular targets, drug development continues to suffer from a high failure rate. The two major obstacles are unexpected toxicity and lack of antitumor efficacy in unselected patients. To increase response rates of new agents, it will be important to preselect patients based on predictive biomarkers. Furthermore, rather than developing drugs that target specific mutant or overexpressed oncogenic proteins, it is more efficient to group several proteins in "oncogenic pathways" that can be targeted with a variety of small molecules. This brief chapter will cover the most promising agents targeting oncogenic pathways under development for the treatment of lymphoma.

Keywords

PI3K • AKT • mTOR • BTK • Syk • JAK • STAT • Hodgkin • Signal transduction • Apoptosis

Introduction

In 2011, it is estimated that 75,190 people in the United States will be diagnosed with lymphoma, and approximately 21,000 are expected to die of their disease [1]. Worldwide, the incidence of non-Hodgkin lymphoma (NHL) is estimated to be 355,000 [2]. Current frontline treatment regimens primarily include established chemotherapy drugs with or without the monoclonal antibody rituximab and, in some cases, may also include radiation therapy [3, 4]. The standard backbone chemotherapy regimen consists of cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP), which has been in use for almost four decades. Attempts to empirically add more chemotherapy drugs or increase the doses of the CHOP regimen and—in some cases—incorporate more intensive regimens with stem cell support failed to improve treatment outcome of most lymphomas. These failures underline the importance of developing novel agents for the treatment of lymphoma and incorporating these novel treatment strategies in a comprehensive development strategies based on the disease molecular and genetic characteristics.

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During the past decade, genomic technology has dramatically improved which now allows a robust, comprehensive, and efficient profiling of the cancer genome. These advances led to a series of breakthroughs in our understanding of carcinogenesis and identified several new molecular targets. Importantly, some of these discoveries were successfully translated into novel therapies for a variety of cancers, including leukemia, lung cancer, and melanoma. Similarly, scientific advances also identified a variety of molecular and genetic defects that are associated with potentially druggable targets in lymphoid malignancies. However, the process of drug development for lymphoma continues to face serious challenges. Many drugs evaluated in phase I studies are discontinued because excessive toxicity or lack of significant efficacy. Furthermore, although the number of phase II studies enrolling lymphoma patients continues to increase, many trials lack focus, do not significantly advance the field, and compete for a relatively small pool of eligible patients. It remains a challenge to advance drugs with promising clinical activity from early, small phase I/II studies to large-scale pivotal trials that enroll patients in a timely manner.

Over the past three decades, the pathologic classification of lymphoma has significantly improved. The early Rappaport classification included a handful of subtypes that did not reflect the cell of origin and, not surprisingly, resulted in diagnostic inaccuracies. Today, the World Health Organization (WHO) currently classifies lymphoma into 30 major distinctive types. While this classification improved the accuracy and consistency of the histological diagnosis of lymphoma, it had little impact on advancing therapy and improving the cure rate [4]. Furthermore, basing treatment decisions of different cancers on histopathological features results in grouping tumors with different underlying molecular characteristics into one category. This treatment strategy is inefficient for drug development and exposes a large number of patients to potentially toxic drugs without providing any benefits. Importantly, even though the number of lymphoma histological subtypes has increased, recent developments in cancer genetics and gene expression profiling (GEP) demonstrated that these histological subtypes are not homogeneous. For example, diffuse large B-cell lymphoma (DLBCL) comprises at least three distinctive subtypes: germinal center B-cell type (GCB), activated B-cell type (ABC), and primary mediastinal B-cell lymphoma (PMCL) [5]. It is therefore not surprising to find that patients with different molecular subtypes of DLBCL have different treatment outcomes when they are treated with the same regimens.

Targeting Oncogenic Pathways in Lymphoma

Genetic alterations of human cancer frequently result in deregulation of signal transduction pathways that contribute to the oncogenic process. This observation generated new strategies for pathway-based cancer therapy [4]. This concept represents a potential paradigm shift in cancer therapy, as it advocates basing treatment decisions on the presence of specific deregulated oncogenic signaling pathway irrespective of the histological tissue subtype. However, for this strategy to be successful, it will be imperative to identify clinical biomarkers that measure pathway activation that can be used to match pathway-targeted drugs with patients whose tumors are associated with an oncogenic pathway. In this chapter, we will review the current data on promising new agents that target well-defined activated oncogenic pathways in lymphoma.

The PI3K/Akt/mTOR Pathway

The phosphatidylinositol 3-kinase (PI3K)/Akt/mammalian target of rapamycin (mTOR) signaling pathway is one of the most aberrantly activated oncogenic signaling pathway in cancer, including lymphoma, and therefore, it is intensively explored as a target for cancer drug development [6, 7]. Oncogenic activation of the PI3K pathway is associated with gain-of-function mutations in the PI3K p110α[alpha] or p85α[alpha] isoforms, loss-of-function of the *PTEN*, *and less frequently*, *activation mutations*

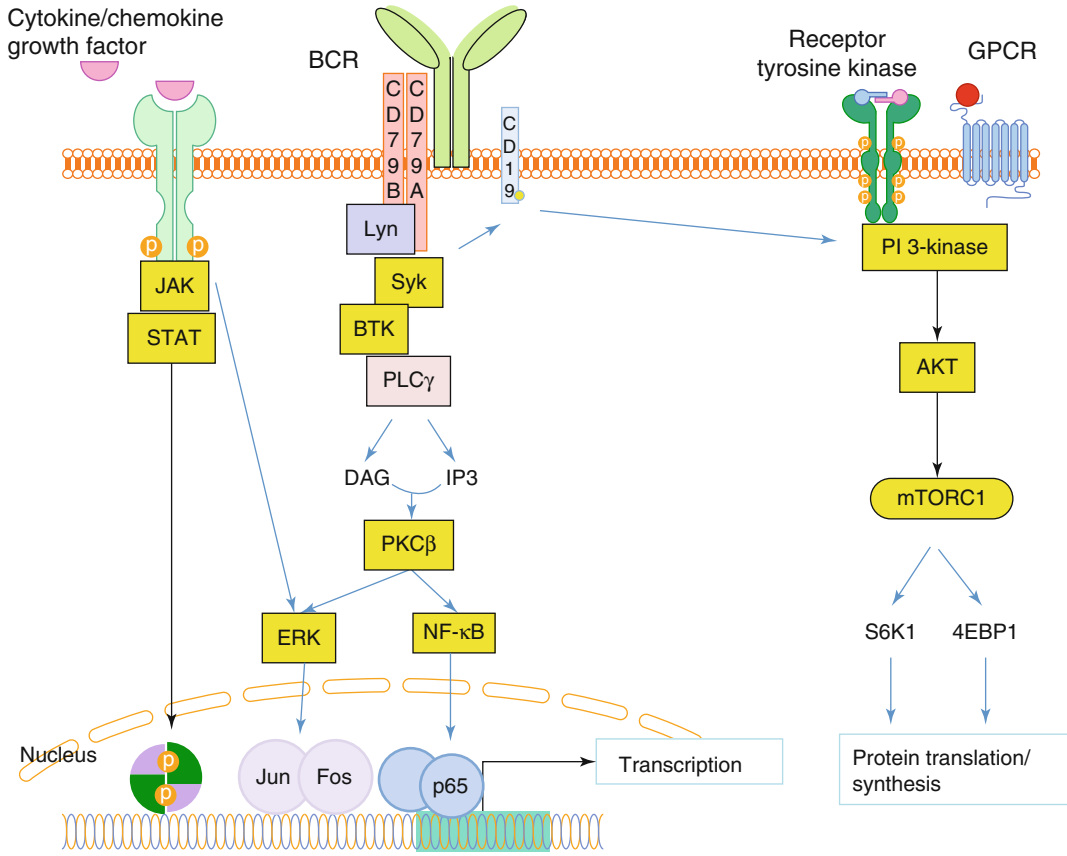
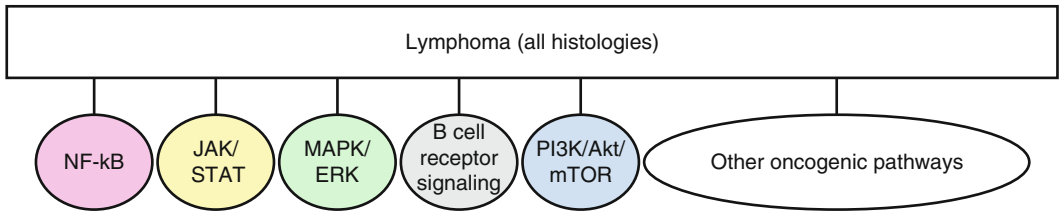


Fig. 22.1 Activation of signaling pathways may be initiated by receptor activation or by receptor-independent mechanisms that may involve genetic mutations of key pathway components. Some of these receptors may activate more than one signaling pathways. Cross talk and simultaneous activation of more than one signaling pathway may require rationally designed combination strategies. *Abbreviations*: *JAK* Janus kinase, *mTOR* mammalian target of rapamycin, *PI3K* phosphatidylinositol 3-kinase, *STAT* signal transducers and activators of transcription

in AKT (Fig. 22.1) [8–10]. In lymphoid malignancies, PI3K pathway activation is rarely associated with these mutations, but rather linked to constitutive B-cell receptor (BCR) activation and/or to exposure to survival factors present in the microenvironment through activation of receptor tyrosine kinases and/or G protein-coupled receptors (GPCR). For example, chemokines, CD30, CD40, BAFF, and RANK have all been reported to activate PI3K [11–16].

Several pharmacologic inhibitors of mTOR have been recently evaluated in preclinical and clinical studies. The first-generation small molecules are allosteric inhibitors (rapalogues), two of which have been approved by the FDA and/or the EMEA for the treatment of renal cell carcinoma (temsirolimus and everolimus), mantle cell lymphoma (temsirolimus), and pancreatic neuroendocrine tumors (everolimus). The rapalogues bind to FK506-binding protein 12 (FKBP12), preferentially inhibiting mTORC1, with no effect on mTORC2 [17, 18]. Recently, more potent small molecules that inhibit the kinase domain of mTORC1 and mTORC2 have been developed and have demonstrated in vitro activity even in rapamycin-resistant cancer cell lines [18]. The anticancer property of mTOR inhibitors is somewhat complex, as it involves several mechanisms, including induction of autophagy, anti-angiogenesis, immunoregulation, and inhibition of protein translation of critical cell survival proteins [19–21]. Because mTOR inhibitors



Pathway	Drug	Target	% response rate in different histologies					
			DLBCL %	FL %	MCL %	SLL/CLL %	T-Cell %	HL %
PI3K/AKT/ mTOR	Everolimus	mTOR	30	50	32	18	63	53
	Temsirolimus	mTOR	36	56	38	10	–	–
	CALI-101/ GS-1101	PI3K	0	55	67	30	–	–
B cell receptor (BCR)	Fostamatinib	Syk	22	10	11	55	0	–
	Ibrutinib	Btk	17	23	69	67	–	–

Fig. 22.2 Summary results of single-agent activity of agents that target the PI3K pathway and B-cell receptor pathway in lymphoma. Clinical responses are observed across different histologic subtypes, suggesting that the growth and survival of a fraction of these tumor types may depend on the presence of these activated oncogenic pathways

primarily induce cell cycle arrest and autophagy rather than cell death, in vitro, it is believed that their in vivo activity is augmented by modulation of the microenvironment, immunity, and angiogenesis [21–23].

Temsirolimus (CCI-779) and everolimus (RAD-001) have demonstrated broad clinical activity in a wide range of lymphoma subtypes in phase II studies (Fig. 22.2) [24]. In a phase II study reported by the Mayo Clinic group, temsirolimus produced an overall response rate of 38 % (13 of 34 patients) in patients with relapsed mantle cell lymphoma, and almost all responses were partial [25]. The most common adverse events were thrombocytopenia, anemia, neutropenia, hyperglycemia, hyperlipidemia, mucositis, and fatigue. However, lower responses were observed in a follow-up multicenter phase III randomized trial that compared temsirolimus with the investigators' choice of commercially available chemotherapy drugs (22 % vs. 2 %) [26]. Despite the low response rate, patients treated with temsirolimus had a longer progression-free survival. These modest results led to the approval of temsirolimus by the European EMEA for the treatment of patients with relapsed mantle cell lymphoma. In a separate phase II study that was led by the group from the University of Chicago, temsirolimus produced an overall response rate of 56 % in patients with relapsed follicular lymphoma, 36 % in diffuse large B-cell lymphoma, and 10 % in small lymphocytic lymphoma (Fig. 22.2) [24]. Everolimus also demonstrated clinical activity in both non-Hodgkin and Hodgkin lymphomas, with an overall response rates ranging between 18 % in small lymphocytic lymphoma and 63 % in patients with relapsed T-cell lymphoma (Fig. 22.2). These results have not been independently confirmed.

Several inhibitors of AKT and PI3K have demonstrated more potent in vitro anticancer activity compared with mTOR inhibitors. However, the clinical development of such agents was delayed because of the excessive toxicity and the nonspecificity of the earlier compounds [8]. An improved understanding of the PI3K signaling pathway has led to the identification of PI3K isoforms that can be targeted for cancer treatment with reasonable safety [6, 27]. Three different PI3K classes have been identified, but only class I has been linked with oncogenesis [28]. GS-1101

(formerly CAL–101) is a potent oral selective inhibitor of the PI3K isoform p110δ[delta]. In human lymphoma cell lines, p110δ[delta] expression was observed in >90 % of cases and was frequently associated with constitutive phosphorylation of Akt. CAL–101 decreased levels of phosphorylated Akt and other downstream effectors, such as S6 kinase and GSK–3β[beta], resulting in inhibition of growth and induction of apoptosis in a variety of lymphoma cell lines [29]. In a phase I study in patients with lymphoid malignancies, GS-1101 was administered at increasing doses (50–350 mg) orally twice daily in 28-day cycles. Although no hematologic DLTs were observed, serious hepatic toxic effects and infections were reported. Remarkably, 10 (56 %) of 18 patients achieved partial response (5 with indolent lymphoma and 5 with MCL) [30]. These data, together with results achieved using mTOR inhibitors, confirm that targeting the PI3K/Akt/mTOR pathway is a promising strategy for the treatment of lymphoma.

B-Cell Receptor (BCR) Signaling Pathway

The functional BCR complex consists of the BCR itself and the CD79a/CD79b heterodimer. The cytoplasmic domains of both CD79a and CD79b have an ITAM (immunoreceptor tyrosine-based activation motif). BCR signaling is initiated by the activation of Src family tyrosine kinases that phosphorylate ITAMs, leading to the recruitment and activation of protein tyrosine kinases (PTKs) such as Lyn, Syk, and Btk and finally the transduction of signal cascades (Fig. 22.1). Src homology 2 (SH2) domain—containing leukocyte adaptor protein of 65 kD (SLP-65, also known as BLNK)—is an adaptor protein that links Syk to the activation of phospholipase C-γ[gamma] (PLC-γ[gamma]). Moreover, Syk phosphorylates several key proteins, including CD19, B-cell adaptor for phosphoinositide 3-kinase (BCAP), and the guanine nucleotide exchange factor Vav, which contribute to the activation of phosphatidylinositol 3-kinases (PI3K) (Fig. 22.1). PI3Ks are a family of enzymes that phosphorylate the 3-position of the phosphatidylinositol ring. Class I PI3Ks use the substrate phosphatidylinositol-4,5-bisphosphate (PIP2) to generate phosphatidylinositol-3,4,5-trisphosphate (PIP3). In turn, PIP3 serves as a docking module for the downstream proteins kinases Akt and Btk (Bruton's tyrosine kinase) in B cells. This leads to the activation of a cascade of signaling molecules that regulate cell survival, growth, and immunity. For example, Akt activates the serine and threonine kinase mammalian target of rapamycin (mTOR), whereas Btk contributes to the activation of PLC-γ[gamma].

An augmented BCR signaling has been observed in a variety of B-cell lymphomas, which may promote their survival, suggesting that interrupting BCR signaling cascades by small molecules may have a potential therapeutic value in B-cell malignancies [31–33]. Both Syk and Btk inhibitors have been recently developed. In a phase II study, fostamatinib demonstrated clinical activity in a variety of B-cell malignancies; the highest ORR, 55 %, was observed in patients with relapsed SLL or CLL (Fig. 22.2). A large phase II study of fostamatinib is currently enrolling patients to further confirm the agent's activity in patients with CLL. Similarly, a phase I study of the Btk small-molecule inhibitor ibrutinib (PCI32765) demonstrated clinical activity in a variety of B-cell lymphoid malignancies.

JAK-STAT Pathway

The Janus kinases (JAKs) are a family of four intracellular non-receptor tyrosine kinases (JAK1, JAK2, JAK3, and TYK2) that primarily transduce signals from cell surface receptors that are activated by cytokines and growth factors. JAK3 expression is restricted to hematopoietic cells, whereas the remaining three JAK family members are ubiquitously expressed. After a cytokine is engaged with its receptor, members of the JAK family are phosphorylated, leading to the recruitment and phosphorylation of signal transducers and activators of transcription (STAT) proteins on tyrosine residues. Subsequently, phosphorylated STATs dimerize and translocate to the nucleus,

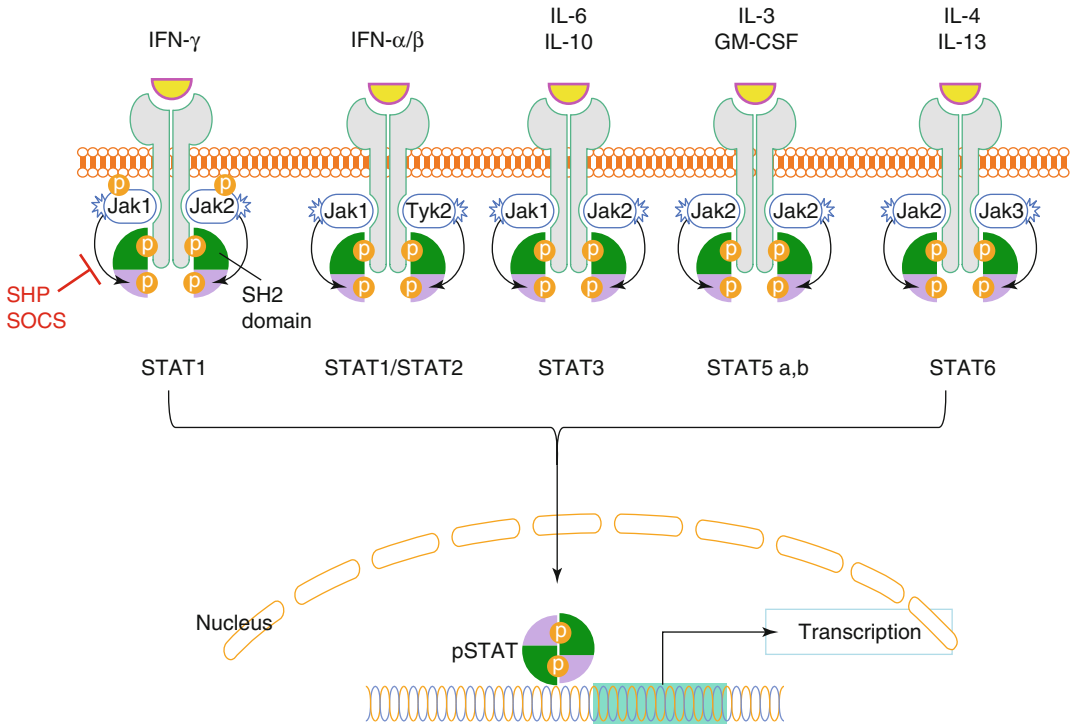


Fig. 22.3 B-cell receptor signaling pathway and its interaction with the PI3K and JAK/STAT pathways. Clinical responses have been observed with agents targeting Syk and Btk

triggering the transcription of target genes that are involved in cell proliferation, survival, angiogenesis, and immunity (Fig. 22.3). In humans, the STAT family of transcription factors consists of seven members; STATs 2, 4, and 6 are activated specifically by a small subset of cytokines (IFN-α[alpha], IL-6, IL-12, IL-13, respectively) [34]. In contrast, STATs 1, 3, 5a, and 5b can be activated not only by a large array of cytokines but also by growth factors and some G protein-coupled receptor agonists.

Aberrant activation of the JAK/STAT pathway has been linked to the oncogenic process in a variety of cancers, including Hodgkin lymphoma (HL) and non-Hodgkin lymphoma (NHL), making it an appealing target for pathway-directed therapy. In rare cases, aberrant activation of the JAK/STAT pathway in a variety of lymphomas has been linked to genomic gains of JAK2, inactivating mutations of suppressors of cytokine signaling (SOCS) proteins, or epigenetic silencing of SOCS1 and SHP1 proteins. However, in most cases, no genetic abnormalities can be detected.

JAK/STAT pathway may also play a role in the mechanism of immune escape in HL. STAT6 activation in HRS cells leads to the secretion of the immunosuppressive thymus- and activation-regulated chemokine (TARC/CCL17) with consequent attraction and homing of Th2 cells in areas surrounding HRS cells and consequent impairment of immune response. Another mechanism of tumor immune evasion is the interaction between the programmed cell death 1 (PD-1) receptor in tumor infiltrating T cells with its PD-ligands 1 and 2 [PD-L1 (CD274, B7-H1) and PD-L2 (CD273, B7-DC)], expressed on the cell surface of a variety of tumor types, including Hodgkin lymphoma, primary mediastinal B-cell lymphoma, and anaplastic large T-cell Lymphoma. The engagement of PD-1 receptor by PD-L1 and PD-L2 leads to inhibition of T-cell function and promotes apoptosis of cytotoxic T cells and the induction of immunosuppressive T regulatory (Treg) cells, leading to a decrease in tumor killing. Recently, the JAK-STAT pathway has been shown to be involved in the regulation of PD-L1

and PD-L2 expression in HL and anaplastic large cell lymphoma (ALCL) cells.

On the basis that activated STAT3 and STAT5 signaling promotes the growth and survival of a variety of lymphomas, the novel oral JAK2 small-molecule inhibitor SB1518 was evaluated in patients with relapsed Hodgkin lymphoma and non-Hodgkin lymphoma in a phase I study. Thirty-four patients received doses of 100–600 mg/day. Treatment was well tolerated, with mostly grade 1/2 toxicities. Gastrointestinal toxicities were the most common treatment-related events. Cytopenias were infrequent and modest. Pharmacologically active concentrations were achieved at all doses. SB1518 inhibited JAK2 signaling at 4 h postdose at all levels. Increases in FLT3-L, reflecting FLT-3 inhibition, were seen in most patients. There were three partial remissions and 15 stable diseases, with most responses lasting >2 months. Seven of 13 patients who had a stable disease demonstrated reductions in their tumor measurements ranging between 4 and 46 %. These encouraging results support a phase 2 trial of SB1518 in selected lymphomas.

Conclusions and Future Directions

As more targeted agents are developed for cancer therapy, most agents continue to produce modest response rates in unselected patients. Pretreatment biopsies to examine biomarker status and linking biomarkers of oncogenic pathway activation to clinical responses will be an important step before designing clinical trials that preselect patients based on biomarker status. While the identification of driver genetic abnormalities that lead to a druggable target is ideal for selecting patients for targeted therapy, such single-driver genetic abnormalities rarely exist in lymphomas. Regardless, many lymphomas seem to be addicted to one or more activated oncogenic pathways. An emerging strategy is to target different components of activated oncogenic pathways, such as mTOR and AKT, even though their genes are normal. Because most tumors utilize more than one oncogenic pathway to promote their survival, single-agent targeted therapy will rarely produce high response rates or durable responses, indicating that rapid combination strategies to target several oncogenic pathways are likely to be more successful. Furthermore, pharmacologic inhibition of a signaling pathway is frequently associated with upregulation of an alternative survival pathway by a negative feedback loop. Rationally designed combinations will be needed to inhibit these negative feedback loops Eventually, one or more targeted agents should be rapidly incorporated with standard frontline regimens, in a phase I/II studies, and new combinations that prove to be safe and effective should be randomized against standard regimens in biomarker-selected patients. Ultimately, this personalized treatment approach will improve the cure rate for selected patients with lymphoma.

References

1. Siegel R, Ward E, Brawley O, Jemal A. Cancer statistics, 2011: the impact of eliminating socioeconomic and racial disparities on premature cancer deaths. CA Cancer J Clin. 2011;61(4):212–36. doi:10.3322/caac.20121. Epub 2011 Jun 17. PMID:21685461.
2. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA Cancer J Clin. 2011; 61(2):69–90. doi:10.3322/caac.20107. Epub 2011 Feb 4. Erratum in: CA Cancer J Clin. 2011;61(2):134. PMID:21296855.
3. Zelenetz AD, Abramson JS, Advani RH, Andreadis CB, Bartlett N, Bellam N, et al. Non-Hodgkin's lymphomas. J Natl Compr Canc Netw. 2011;9(5):484–560. No abstract available. PMID:21550968.
4. Younes A. Beyond chemotherapy: new agents for targeted treatment of lymphoma. Nat Rev Clin Oncol. 2011;8(2):85–96. doi:10.1038/nrclinonc.2010.189. Epub 2010 Dec 14. Review. Erratum in: Nat Rev Clin Oncol. 2011;8(3):124. PMID:21151205.
5. Alizadeh AA, Eisen MB, Davis RE, et al. Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. Nature. 2000;403:503–11.
6. Ihle NT, Powis G. Take your PIK: phosphatidylinositol 3-kinase inhibitors race through the clinic and toward cancer therapy. Mol Cancer Ther. 2009;8:1–9.
7. Franke TF. PI3K/Akt: getting it right matters. Oncogene. 2008;27:6473–88.
8. Engelman JA. Targeting PI3K signalling in cancer: opportunities, challenges and limitations. Nat Rev Cancer. 2009;9:550–62.
9. Jaiswal BS, Janakiraman V, Kljavin NM, et al. Somatic mutations in p85alpha promote tumorigenesis through class IA PI3K activation. Cancer Cell. 2009;16: 463–74.

10. Martin-Berenjeno I, Vanhaesebroeck B. PI3K regulatory subunits lose control in cancer. Cancer Cell. 2009;16: 449–50.
11. Georgakis GV, Yazbeck VY, Li Y, Younes A. Preclinical rationale for therapeutic targeting of mTOR by CC-I779 and rapamycin in Hodgkin lymphoma. ASCO Meet Abstr. 2006;24:10070.
12. Jucker M, Sudel K, Horn S, et al. Expression of a mutated form of the p85alpha regulatory subunit of phosphatidylinositol 3-kinase in a Hodgkin's lymphoma-derived cell line (CO). Leukemia. 2002;16:894–901.
13. Morrison JA, Gulley ML, Pathmanathan R, Raab-Traub N. Differential signaling pathways are activated in the Epstein-Barr virus-associated malignancies nasopharyngeal carcinoma and Hodgkin lymphoma. Cancer Res. 2004;64:5251–60.
14. Nagel S, Scherr M, Quentmeier H, et al. HLXB9 activates IL6 in Hodgkin lymphoma cell lines and is regulated by PI3K signalling involving E2F3. Leukemia. 2005;19:841–6.
15. Renne C, Willenbrock K, Martin-Subero JI, et al. High expression of several tyrosine kinases and activation of the PI3K/AKT pathway in mediastinal large B cell lymphoma reveals further similarities to Hodgkin lymphoma. Leukemia. 2007;21:780–7.
16. Dutton A, Reynolds GM, Dawson CW, Young LS, Murray PG. Constitutive activation of phosphatidylinositide 3 kinase contributes to the survival of Hodgkin's lymphoma cells through a mechanism involving Akt kinase and mTOR. J Pathol. 2005;205:498–506.
17. Gough NR. Focus Issue: demystifying mTOR signaling. Sci Signal. 2009;2:eg5.
18. Dancey J. mTOR signaling and drug development in cancer. Nat Rev Clin Oncol. 2010;7(4):209–19. doi:10.1038/nrclinonc.2010.21. Epub 2010 Mar 16. Review. PMID:20234352.
19. Thomson AW, Turnquist HR, Raimondi G. Immunoregulatory functions of mTOR inhibition. Nat Rev Immunol. 2009;9:324–37.
20. Ma XM, Blenis J. Molecular mechanisms of mTOR-mediated translational control. Nat Rev Mol Cell Biol. 2009;10:307–18.
21. Younes A. Therapeutic activity of mTOR inhibitors in mantle cell lymphoma: clues but no clear answers. Autophagy. 2008;4(5):707–9. Epub 2008 May 6. Review. PMID:18469512.
22. Zheng Y, Collins SL, Lutz MA, et al. A role for mammalian target of rapamycin in regulating T cell activation versus anergy. J Immunol. 2007;178:2163–70.
23. Del Bufalo D, Ciuffreda L, Trisciuoglio D, et al. Antiangiogenic potential of the Mammalian target of rapamycin inhibitor temsirolimus. Cancer Res. 2006;66: 5549–54.
24. Smith SM, Pro B, Cisneros A, et al. Activity of single agent temsirolimus (CCI-779) in non-mantle cell non-Hodgkin lymphoma subtypes. ASCO Meet Abstr. 2008;26:8514.
25. Witzig TE, Geyer SM, Ghobrial I, et al. Phase II trial of single-agent temsirolimus (CCI-779) for relapsed mantle cell lymphoma. J Clin Oncol. 2005;23:5347–56.
26. Hess G, Herbrecht R, Romaguera J, Verhoef G, Crump M, Gisselbrecht C, et al. Phase III study to evaluate temsirolimus compared with investigator's choice therapy for the treatment of relapsed or refractory mantle cell lymphoma. J Clin Oncol. 2009;27(23): 3822–9. doi:10.1200/JCO.2008.20.7977. Epub 2009 Jul 6. PMID:1958153.
27. Courtney KD, Corcoran RB, Engelman JA. The PI3K pathway as drug target in human cancer. J Clin Oncol. 2010;28:1075–83.
28. Zhao L, Vogt PK. Class I PI3K in oncogenic cellular transformation. Oncogene. 2008;27:5486–96.
29. Lannutti BJ, Meadows SA, Kashishian A, et al. CAL-101, an oral p110{delta} selective phosphatidylinositol-3-kinase (PI3K) inhibitor for the treatment of B cell malignancies inhibits PI3K signaling, cellular viability and protective signals of the microenvironment. Blood. 2009;114:286.
30. Flinn IW, Byrd JC, Furman RR, et al. Evidence of clinical activity in a phase 1 study of CAL-101, an oral P110{Delta} isoform-selective inhibitor of phosphatidylinositol 3-kinase, in patients with relapsed or refractory B-cell malignancies. Blood. 2009;114: 922.
31. Chen L, Monti S, Juszczynski P, Daley J, Chen W, Witzig TE, et al. SYK-dependent tonic B-cell receptor signaling is a rational treatment target in diffuse large B-cell lymphoma. Blood. 2008;111(4):2230–7. Epub 2007 Nov 15. PMID:18006696.
32. Friedberg JW, Sharman J, Sweetenham J, et al. Inhibition of Syk with fostamatinib disodium has significant clinical activity in non Hodgkin's lymphoma and chronic lymphocytic leukemia. Blood. 2010;115(13):2578–85. doi:10.1182/blood-2009-08-236471.
33. Pollyea DA, Smith S, Fowler N, et al. A phase I dose escalation study of the Btk inhibitor PCI-32765 in relapsed and refractory B cell non-Hodgkin lymphoma and use of a novel fluorescent probe pharmacodynamic assay. Blood. 2009;114:3713.
34. Murray PJ. The JAK-STAT signaling pathway: input and output integration. J Immunol. 2007;178: 2623–9.

Stem Cell Transplantation for Non-Hodgkin's Lymphomas

23

Chitra Hosing and Richard E. Champlin

Abstract

Both autologous and allogeneic transplants have been extensively studied in the management of non-Hodgkin's lymphomas and may offer a chance of a long-term cure for some patients. Autologous stem cell transplantation is considered standard therapy for patients with diffuse large B cell lymphoma in chemotherapy-sensitive relapse. It is also widely applied for patients with T cell histologies both as consolidation of first remission and in the salvage setting. It is also often used in patients with other histologic subtypes like follicular lymphoma, primary central nervous system lymphoma, and mantle cell lymphoma. Allogeneic stem cell transplantation also confers an immune-mediated graft-versus-lymphoma effect which can produce long-term remissions in selected patients in settings where autologous transplants may not be effective. However, when compared to autologous transplants, allogeneic transplants are associated with higher complication rates and higher transplant-related mortality rates. This is primarily due to the toxicity of the high-dose regimen, acute and chronic graft-versus-host disease, and the associated risk of infections. Therefore, allogeneic transplants have historically been restricted to patients who are young and have a good performance status. There is recent interest in non-myeloablative or reduced-intensity conditioning regimen allogeneic transplants as a means of exploiting the graft-versus-lymphoma effect with less toxicity and lower regimen-related mortality. Several trials in lymphoma have been published evaluating the role of high-dose therapy and comparing the outcomes of allogeneic or autologous hematopoietic stem cell transplantation. In this chapter, we review the use of autologous and allogeneic transplants for the common subtypes of non-Hodgkin's lymphoma.

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Introduction

The overall cure rate in adult patients with non-Hodgkin's lymphoma (NHL) is around 30 % depending on the histology. Patients with follicular lymphoma (FL) are rarely cured with conventional treatments, whereas in patients with intermediate histology or diffuse large B cell lymphomas (DLBCL), the cure rate is approximately 50 % depending upon prognostic factors. T cell NHLs are a heterogeneous group of lymphomas accounting for approximately 10 % of aggressive lymphomas. Patients with T cell NHLs are more likely to present with aggressive clinical features, and standard treatments are less effective for T cell lymphomas than for B cell lymphomas, with the possible exception of anaplastic lymphoma kinase (ALK)-positive anaplastic large cell lymphoma (ALCL) [1, 2].

Because NHLs are highly sensitive to myelosuppressive chemotherapy and radiation and exhibit a steep dose–response curve, administration of high-dose therapy (HDT) followed by hematopoietic transplantation is an attractive strategy to improve cytoreduction. Both autologous and allogeneic transplantation have been widely studied and may offer a chance of a long-term cure for selected categories of patients. Autologous stem cell transplantation (ASCT) may be considered standard therapy for patients with DLBCL in chemotherapy-sensitive relapse. It is also widely applied for patients with PTCL-NOS as consolidation of first remission although the evidence is limited. Some patients with other histologic subtypes like FL, primary central nervous system lymphoma (PCNSL), and mantle cell lymphoma (MCL) may also benefit from this strategy in first remission, and studies are ongoing to identify the optimal role of this approach. Allogeneic stem cell transplantation (allo-SCT) also confers an immune-mediated graft-versus-lymphoma (GVL) effect [3] which can produce long-term remissions in selected patients in settings where autologous transplants are generally ineffective. Several registry studies have shown that allo-SCT is associated with lower relapse rates in patients with lymphoma when compared to ASCT using purged bone marrow [4–6]. Other observations which support the presence of a GVL effect are the induction of complete remissions by modulation of immunosuppressive therapy [7, 8] and by infusion of donor lymphocytes [9] in patients relapsing after allo-SCT. Aggressive lymphomas are less affected by GVL effects when compared to those with indolent histology [10, 11]. However, when compared to ASCT, high-dose myeloablative therapy and allogeneic transplants are associated with higher complication rates and higher transplant-related mortality (TRM) rates of approximately 30–40 % at 5 years. This is primarily due to the toxicity of the high-dose regimen, acute and chronic graft-versus-host disease (GVHD), and the associated risk of infections [11]. This risk further increases with age; therefore, allogeneic transplants have historically been restricted to patients who are young and have a good performance status. There is recent interest in non-myeloablative (NMA) or reduced-intensity conditioning regimen (RIC) as a means to therapeutically exploit GVL, with less toxicity and regimen-related mortality. This approach markedly reduces the risk of treatment-related mortality and allows use of allogeneic transplants in older patients (up to approximately age 75) and those with comorbidities who could not tolerate myeloablative regimens [12]. In addition, the risk of acute severe GVHD may also be lower in these patients because development of GVHD is in part related to the toxicity of the conditioning regimen and subsequent cytokine production [13, 14]. The GVL effect may be augmented by the infusion of additional donor lymphocytes in patients who achieve successful engraftment.

Several trials in NHL have been published evaluating the role of HDT and comparing the outcomes of allogeneic or autologous hematopoietic stem cell transplantation. However, comparison is difficult because of the small number of patients enrolled, different selection criteria

(often reserving allogeneic transplants for higher risk patients or patients who fail autografts), different transplant regimens, and variable follow-up. The patient populations studied are often highly selected group, and thus the results may not be applicable to all patients [15]. We separately review the use of autologous and allogeneic transplants for each category of lymphoma.

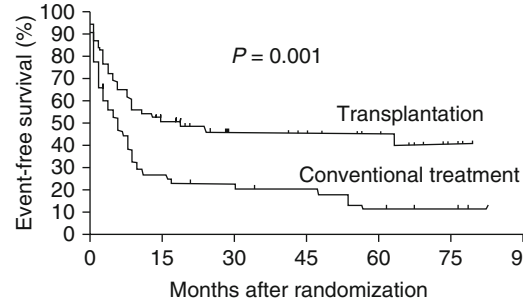

Fig. 23.1 Kaplan–Meier curves for event-free survival of patients in the transplantation and conventional treatment groups (Published with permission from Philip et al. [17])

Diffuse Large B Cell Lymphoma

DLBCL is the most common form of adult NHL, accounting for 25–30 % of cases. This category is highly heterogenous based on cytogenetic, molecular, and gene expression profiling analyses. DLBCL can be divided into two major molecular subgroups: germinal center B cell-like (GCB) and activated B cell-like (ABC). Other histologic and clinical subgroups include entities such as primary mediastinal large B cell lymphoma, or DLBCL with features intermediate between DLBCL and Burkitt's lymphoma. In most studies, these subgroups are lumped together, and this may affect the study outcome. Although DLBCL can be cured by current chemotherapy regimens, the prognosis of this disease also varies considerably based upon prognostic features, and for high-risk patients, long-term survival is less than 50 % [16]. Thus, at least half of the patients in high-risk groups will fail initial therapy. In patients with recurrent or refractory disease, the prognosis with chemotherapy alone is generally poor. These patients are generally recommended to undergo HDT followed by ASCT.

Autologous Transplantation for Relapsed DLBCL

ASCT has been the standard of care for relapsed, chemosensitive DLBCL since the results of the PARMA trial were published in 1995. This study demonstrated a superior event-free survival (EFS) and OS for chemosensitive patients undergoing HDT and ASCT compared with those randomized to receive conventional chemotherapy. In this study, a total of 215 patients with relapsed NHL were enrolled. All patients received two courses of conventional chemotherapy with DHAP (dexamethasone, cytarabine, cisplatin). The 109 patients who had a response to chemotherapy were randomly assigned to receive four additional courses of DHAP chemotherapy plus radiotherapy (54 patients) or radiotherapy plus intensive chemotherapy with BEAC (carmustine, etoposide, cytarabine, cyclophosphamide) and autologous bone marrow transplantation (55 patients). The two groups did not differ in terms of prognostic factors. With a median follow-up time of 63 months, the response rate was 84 % after bone marrow transplantation and 44 % after chemotherapy without transplantation. At 5 years, the rate of EFS was 46 % in the transplantation group and 12 % in the group receiving chemotherapy without transplantation ($P = 0.001$), and the rate of OS was 53 and 32 %, respectively ($P = 0.038$) (Fig. 23.1) [17]. Initial remission duration of fewer than 12 months was an adverse prognostic indicator [18]. In an update of the PARMA study, when patients were further classified according to the age-adjusted international prognostic index (aaIPI) it was predictive of outcome. The aaIPI at relapse correlated highly with OS in patients treated on the DHAP arm (5-year OS: 48, 21, 33, and 0 % for IPI 0, 1, 2, and 3, respectively; $P = 0.006$), but not on the BEAC arm (5-year OS: 51, 47, 50, and 50 % for IPI 0, 1, 2, and 3, respectively; $P = 0.90$). OS was significantly superior in the BEAC arm as compared with the DHAP arm in patients with an IPI >0 ($P < 0.05$), but not in patients with an IPI of 0 [19].

Prince et al. sought to retrospectively identify major prognostic factors predicting outcome in patients with relapsed, chemotherapy-sensitive intermediate-grade NHL who underwent HDT and ASCT. They evaluated a number of variables (age, histology, stage at diagnosis, immunophenotype, extranodal disease, at diagnosis, prior BM involvement, bulky disease at diagnosis, duration of prior complete remission [CR], number of cycles of conventional-dose salvage chemotherapy, tumor burden at relapse, relapse in a previous radiation field, and remission status immediately prior to ASCT) in a multivariate model. Remission status at ASCT was the only significant variable that predicted for improved OS and PFS ($P=0.0001$). Patients who received transplants in CR had a significantly better 4-year OS and progression-free survival (PFS) than those who received transplants in partial remission (PR) [OS 72 % vs. 26 %; PFS 61 % vs. 25 %] [20]. In general, patients with refractory disease and multiple relapses or those with marrow involvement tend to have a worse outcome, and less than 20 % achieve durable remissions [21–28]. Other variables which have been found to have prognostic significance include an elevated LDH [24, 27], extensive previous therapy [24, 29], bulky disease [30], poor performance status [31], and high-grade histology [29, 32].

Recently, the CORAL (Collaborative Trial in Relapsed Aggressive Lymphoma) study was performed as a multicenter collaborative effort involving 396 patients with refractory or relapsed DLBCL. Patients were randomly assigned to either rituximab, ifosfamide, etoposide, and carboplatin (R-ICE) or rituximab, dexamethasone, high-dose cytarabine, and cisplatin (R-DHAP). Responding patients received HDT and ASCT. There was no significant difference between R-ICE and R-DHAP for 3-year EFS or OS. Three-year EFS was affected by prior rituximab treatment versus no rituximab (21 % vs. 47 %, respectively), relapse less than versus more than 12 months after diagnosis (20 % vs. 45 %, respectively), and IPI of 2–3 versus 0–1 (18 % vs. 40 %, respectively). In the Cox model, these parameters were significant ($P<0.001$). In patients who experienced relapse more than 12 months after diagnosis, prior rituximab treatment did not affect EFS. Patients with early relapses after rituximab-containing first-line therapy had a poor prognosis, with no difference between the effects of R-ICE and R-DHAP. OS according to time of relapse onset (more or less than 1 year after initial diagnosis) in 241 DLBCL patients previously treated with rituximab was highly significant ($P=0.0005$). After HDT and ASCT, there was no significant difference between R-ICE and R-DHAP with regard to 3-year EFS (26 % vs. 35 %; $P=0.6$) or OS (47 % vs. 51 %; $P=0.5$) [33].

Rituximab has also been used along with the HDT and as maintenance after ASCT [34, 35]. Khouri et al. studied the feasibility of high-dose rituximab in combination with high-dose BEAM chemotherapy and ASCT in patients with recurrent B cell aggressive NHL. Sixty-seven consecutive patients were treated. Rituximab was administered during stem cell mobilization and then again on days 1 and 8 after ASCT. The results of this treatment were retrospectively compared with those of a historical control group receiving the same preparative regimen but without rituximab. With a median follow-up time for the study group of 20 months, the OS rate at 2 years was 80 % for the study group and 53 % for the control group ($P=0.002$). The DFS rate was 67 % for the study group and 43 % for the control group ($P=0.004$) [35].

Vose et al. treated 23 patients with chemotherapy-refractory or multiply relapsed B cell NHL in a phase I trial combining iodine-131 tositumomab with high-dose BEAM followed by ASCT. Patients with all histologies were eligible for this phase I trial. Short-term and long-term toxicities were similar to historical control patients treated with BEAM alone. With a median follow-up of 38 months, the OS rate was 55 %, and the EFS rate was 39 % [36]. In a matched-cohort analysis of autologous transplant patients with DLBCL, Krishnan et al. studied 92 patients who were treated with either radioimmunotherapy or TBI-based conditioning regimens. The radioimmunotherapy regimen consisted of (90)Y-ibritumomab tiuxetan plus BEAM (Z-BEAM). The TBI-based regimen combined fractionated TBI with etoposide and cyclophosphamide. Patients in the TBI group had higher rates of cardiac toxicity and

Table 23.1 Prospective randomized studies of up-front high-dose therapy and autologous stem cell transplantation versus conventional chemotherapy for aggressive diffuse large B cell non-Hodgkin's lymphomas

Study	*N*	Chemotherapy regimen	Comments
Gianni et al. [40]	50	MACOP-B	EFS, FFR, CR rates superior in HDT arm
	48	HDT + ASCT	
Santini et al. [42]	61	VACOP-B	No difference in intent-to-treat analysis
	63	VACOP-B + ASCT	
Haioun et al. [38]	111	ACBVP × 4 + consolidation	DFS, OS superior in HDT arm
	125	ACVBP × 4, MTX, +ASCT	
Kluin-Melemans et al. [43]	96	CHmP/BV × 8	No difference
	98	CHmP/BV × 6 + ASCT	
Milipied et al. [44]	99	CHOP × 8	EFS superior in HDT arm
	98	CEEP × 2 + ASCT	
Gisselbrecht et al. [39]	181	ACVBP	EFS, OS superior in standard chemotherapy arm
	189	ECVBP × 3 + ASCT	
Kaiser et al. [41]	154	CHOEP × 5	No difference
	158	CHOEP × 3 + ASCT	
Martelli et al. [45]	75	MACOP-B × 12	No difference
	75	MACOP-B × 8 + ASCT	

Abbreviations: *MACOP-B* methotrexate, doxorubicin, cyclophosphamide, vincristine, prednisone, and bleomycin, *HDT* high-dose therapy, *ASCT* autologous stem cell transplantation, *FFR* freedom from relapse, *CR* complete remission, *VACOP-B* etoposide, doxorubicin, cyclophosphamide, vincristine, prednisone, and bleomycin, *EFS* event-free survival, *OS* overall survival, *CHmP/BV* combination of cyclophosphamide, doxorubicin, teniposide, and prednisone, with bleomycin and vincristine added at mid-cycle, *CHOP* cyclophosphamide, vincristine, doxorubicin, prednisone, *CHOEP* CHOP + etoposide, *ACVBP* doxorubicin, cyclophosphamide, vindesine, bleomycin, and prednisone, *ECVBP* cyclophosphamide, epirubicin, vindesine, bleomycin, and prednisone

mucositis, while Z-BEAM patients had a higher incidence of pulmonary toxicity. Overall survival at 4 years was 81 % for the Z-BEAM and 53 % for the TBI group ($P = 0.01$). The 4-year cumulative incidence of relapse/progression was 40 and 42 % for Z-BEAM and TBI, respectively ($P = 0.63$). Non-relapse mortality was lower in the Z-BEAM group: 0 % compared to 16 % for TBI at 4 years ($P < 0.01$). This non-randomized retrospective study demonstrated that radioimmunotherapy-based conditioning was similar to TBI-based regimen as far as relapse was concerned but with lower toxicity, resulting in improved OS especially in patients who had received ≥2 prior regimens [37].

Autologous Stem Cell Transplantation for DLBCL in First Complete Remission

Because of the good results obtained with HDT and ASCT in patients with relapsed chemosensitive DLBCL, a number of investigators have evaluated its role in the up-front treatment of high-risk lymphoma. Results of some of these studies are summarized in Table 23.1 [38–45]. The LNH-87 trial was a randomized study that compared consolidative sequential chemotherapy with induction therapy followed by HDT and ASCT in patients with aggressive NHL in first CR. There was no difference in outcomes between conventional chemotherapy and HDT arms. However, in the final analysis of the study with a median follow-up of 8 years and focusing on high-intermediate and high-risk patients identified by the aaIPI scores of 2–3 (451 of 956 patients), 61 % of patients achieved CR after induction treatment. After reaching CR to induction therapy, 236 of these higher risk patients were assessable for the consolidation phase, with 125 patients in the HDT arm and 111 in the sequential chemotherapy arm. In the 236 randomized patients, HDT was superior to sequential chemotherapy, with 8-year DFS rates of 55 and 39 %, respectively ($P = 0.02$; relative risk [RR], 1.56). The 8-year OS rate was significantly superior in the

HDT arm (64 %) compared with the sequential chemotherapy arm (49 %) ($P=0.04$; RR, 1.51). On the basis of the final analysis of this prospectively treated series of patients, but retrospectively analyzed on the basis of the aaIPI, the authors concluded that HDT and ASCT benefit patients with high-risk disease who achieve CR after induction treatment. A subset analysis showed that patients who were high or high-intermediate risk demonstrated both OS and DFS advantage with HDT and ASCT [38].

Randomized trial LNH93-3 was conducted on patients who had poor-prognosis aggressive lymphoma and were younger than 60 years with two to three factors of the aaIPI to evaluate the benefit of early HDT with ASCT. Patients were randomized between doxorubicin, cyclophosphamide, vindesine, bleomycin, and prednisone (ACVBP) chemotherapy followed by sequential consolidation and an experimental shortened treatment consisting of three cycles with escalated doses of cyclophosphamide, epirubicin, vindesine, bleomycin, and prednisone and collection of peripheral blood stem cells. On day 60, HDT was administered with BEAM followed by ASCT. Three hundred and seventy patients with aggressive lymphoma were analyzed, ACVBP (181 patients) and HDT (189 patients). With a median follow-up of 60 months, 5-year OS and EFS for ACVBP and HDT were 60 and 46 % ($P=0.007$) and 52 and 39 ($P=0.01$), respectively. Survival was independently affected by age greater than 40 years ($P=0.0003$), T cell phenotype ($P=0.009$), bone marrow involvement ($P=0.003$), and HDT treatment group ($P=0.04$). In this study, early HDT with ASCT in high-risk patients was inferior to the ACVBP chemotherapy regimen [39].

Gianni et al. compared a regimen of six chemotherapeutic agents administered sequentially at high doses, followed by HDT and ASCT. Ninety-eight eligible patients were randomly assigned to receive either MACOP-B (methotrexate, doxorubicin, cyclophosphamide, vincristine, prednisone, and bleomycin) (50 patients) or high-dose sequential therapy (48 patients). The study design allowed for crossover to the other treatment group. After a median follow-up of 55 months, the patients given high-dose sequential therapy, as compared with those treated with MACOP-B, had significantly higher rates of CR (96 % vs. 70 %, $P=0.001$), freedom from disease progression (84 % vs. 49 %, $P<0.001$), freedom from relapse (88 % vs. 70 %, $P=0.055$), and EFS (76 % vs. 49 %, $P=0.004$). The difference in OS at 7 years favored the group assigned to high-dose sequential therapy (81 % vs. 55 %, $P=0.09$). In this study, high-dose sequential therapy was superior to standard-dose MACOP-B in patients with DLBCL [40].

Another trial of the German High-Grade Non-Hodgkin's Lymphoma Study Group compared the use of HDT as part of primary treatment with cyclophosphamide, doxorubicin, vincristine, and prednisone plus etoposide (CHOEP) followed by involved field radiotherapy in a phase III randomized, multicenter study. Three hundred twelve patients with "aggressive" NHL aged≤60 years with elevated serum LDH levels were included. Patients with at least a minor response after two cycles of CHOEP received three further cycles of CHOEP followed by involved field radiotherapy (arm A) or one further cycle of CHOEP followed by ASCT and involved field radiotherapy (arm B). Among 158 patients randomized to arm B, 65 % received HDT. With a median observation time of 45.5 months, OS after 3 years was 63 % for arm A and 62 % for arm B ($P=0.68$). The EFS was 49 % for arm A versus 59 % for arm B ($P=0.22$). Relapse in arm B was associated with a significantly worse OS than relapse in arm A. Results of this randomized trial comparing CHOP-like chemotherapy with early HDT did not support the use of HDT following shortened standard chemotherapy [41].

In order to assess the efficacy of HDT and ASCT compared to conventional chemotherapy as first-line therapy in patients with aggressive NHL, Greb et al. performed a systematic meta-analysis of published studies. They identified 15 randomized controlled trials including 2,728 patients. HDT and ASCT improved CR when compared to conventional chemotherapy (RR 1.11, CI 1.04–1.18). Overall, there was no evidence for improved OS (HR 1.05, 95 % CI 0.92–1.19) or EFS (HR 0.92, 95 % CI 0.80–1.05) with ASCT when compared with conventional chemotherapy. However, subgroup analysis indicated OS differences ($P=0.032$)

between good- (HR 1.46, 95 % CI 1.02–2.09) and poor-risk (HR 0.95, 95 % CI 0.81–1.11) patients. Conflicting results were reported for poor-risk patients, where some studies reported improved and others reduced OS and EFS after HDT and ASCT. Therefore, one can conclude from this analysis that there is no evidence that HDT and ASCT improve OS and EFS in good-risk NHL patients. The evidence for poor-risk patients remains inconclusive, and further high-quality randomized studies are needed [46].

The Groupe d'Etude des Lymphomes de l'Adulte (GELA) group treated 330 patients with poor-risk DLBCL with HDT and ASCT. After ASCT, 269 responders were re-randomized to receive either maintenance rituximab or observation alone. At a median of 4 years' follow-up from the second randomization, there was a trend ($P=0.1$) toward increased EFS for patients who received rituximab compared with observation. The type of induction therapy did not significantly affect OS at a median 51 months' follow-up [47]. The Gruppo Italiano Terapie Innovative nei Linfomi (GITIL) studied the combination of rituximab and HDT in 112 patients with previously untreated DLBCL and aaIPI score of 2–3. They reported an impressive CR rate of 80 %. The 4-year OS rate and EFS rates were projected to be 76 and 73 %, respectively. In their study, the life expectancy of younger patients with aaIPI 2–3 DLBCL was improved with the early administration of rituximab-supplemented intensive chemotherapy compared with the poor outcome following conventional chemotherapy [48]. The LNH2003-3 was a phase 2 trial including patients with DLBCL with 2–3 factors according to IPI. Patients received 4 cycles of intensive biweekly chemotherapy with rituximab, doxorubicin, cyclophosphamide, vindesine, bleomycin, and prednisolone (R-ACVBP) followed by ASCT in responding patients. A case–control study was performed by matching (1:1) 181 patients treated with R-ACVBP with ACVBP patients not given rituximab but submitted to ASCT from a previous LNH1998-3 trial. With a median follow-up of 45 months, there was no difference in outcomes between patients with 2 or 3 IPI factors. The 4-year PFS was significantly higher in R-ACVBP than ACVBP patients (74 % vs. 58 % $P=0.0005$). The gain in 4-year OS was also significant (76 % vs. 68 %, $P=0.0494$) [49]. Vitolo et al. compared the results of rituximab and HDT to those for a historical cohort treated with the same dose dense and HDT but without rituximab. The 4-year failure-free survival rates for the rituximab and historical groups were 73 % versus 44 %, respectively ($P=0.001$); the 4-year OS rates were 80 and 54 %, respectively ($P=0.002$). A Cox's multivariable model was applied to adjust the effect of treatment for unbalanced or important prognostic factors: failure and death risks were significantly reduced in the rituximab group compared to the historical group [50]. In another study, maintenance rituximab administered weekly or monthly to patients with relapsed DLBCL in CR post-ASCT led to statistically significant superior PFS and OS [51].

Autologous Transplantation in Patients Never Achieving a Complete Remission

Vose et al. evaluated 184 patients with diffuse aggressive NHL who never achieved a CR with conventional chemotherapy and subsequently underwent HDT and ASCT. Seventy-nine percent of patients achieved a CR or a CR with residual imaging abnormalities of unknown significance after ASCT. The probabilities of PFS and OS at 5 years after transplantation were 31 and 37 %, respectively. For patients who achieved a CR after transplantation, the 3-year probability of survival was 68 % compared to 11 % for patients with a partial or no response ($p<0.0001$). In multivariate analysis, chemotherapy resistance, poor Karnofsky performance status, age ≥55 years at transplantation, receiving three or more prior chemotherapy regimens, and not receiving pre- or post-transplant involved field radiation therapy were adverse prognostic factors for OS. Thus, HDT and ASCT should be considered for patients with diffuse aggressive NHL who never achieve a CR but who have chemotherapy-sensitive disease [52].

Kewalramani et al. similarly retrospectively analyzed outcomes for 85 primary refractory NHL

patients who underwent HDT and ASCT. Forty patients had a PR after induction therapy, and 45 patients had primary induction failure. In an intent-to-treat analysis, the 3-year OS and EFS were 25 and 22 %. The PR group had a statistically significantly higher OS compared to the induction failure group ($P=0.015$). There was no significant difference in EFS between the groups ($P=0.081$). In the subset of patients who underwent ASCT, there was no difference in the OS or EFS between the PR and induction failure groups [53].

Burkitt's and Burkitt-Like NHL

Most of the published transplant series of aggressive lymphomas include only a small percentage of patients with Burkitt's, Burkitt-like, or lymphoblastic NHL. Although these diseases are highly curable in children, the long-term prognosis in adults is generally poor. In 1996, Sweetenham et al. published the results of adult patients with Burkitt's and Burkitt-like NHL undergoing HDT and ASCT. This was a retrospective analysis of 117 adult patients who were reported to the lymphoma registry of the European Group for Blood and Marrow Transplantation (EBMT). Seventy of these patients received HDT and ASCT in first CR. The actuarial OS rate for the entire group was 53 % at 3 years. The major factor predicting for outcome after transplantation was disease status. The 3-year actuarial OS rate was 72 % for patients transplanted in first CR, compared with 37 % for patients in chemosensitive relapse, and 7 % for chemoresistant patients. For patients transplanted in first CR, disease bulk at the time of ASCT was the only factor predictive of PFS and OS. The results of HDT and ASCT for patients with relapsed disease, particularly chemosensitive relapse, were superior to those reported for conventional-dose salvage regimens. However, the favorable results for patients transplanted in first CR noted in this analysis require comparison with newer dose-intensive regimens [54].

Lymphoblastic lymphoma is a rare, clinically aggressive lymphoma that frequently involves the BM and/or central nervous system. Because lymphoblastic lymphoma is similar to acute lymphoblastic leukemia, many prefer allo-SCT to ASCT. Single-center studies have shown a long-term DFS rates of 31–77 % with HDT and ASCT [55, 56]. A retrospective analysis of 214 patients with lymphoblastic lymphoma who underwent HDT and ASCT was reported by the lymphoma registry of the EBMT. This included 105 patients who underwent ASCT in first CR. The actuarial OS rate at 6 years for the entire group was 42 %. Disease status at transplant was the major determinant of outcome: 6-year actuarial OS was 63 % for patients transplanted in first CR, compared with 15 % for those with resistant disease at the time of transplantation. Transplantation in second CR resulted in a 31 % rate of actuarial OS at 6 years. Results for patients transplanted in second CR were superior to those reported for conventional-dose salvage regimens [57]. In another study, 119 adult patients with lymphoblastic lymphoma were enrolled on to a prospective randomized. Patients received standard remission induction therapy, and responding patients were randomized either to continue with a conventional consolidation/maintenance protocol or to receive HDT and ASCT. A total of 111 were assessable for response to induction therapy. The overall response rate was 82 % (56 % complete response, 26 % partial response). Of the 98 patients eligible for randomization, 65 were randomized, 31 to ASCT and 34 to conventional chemotherapy. With a median follow-up of 37 months, the actuarial 3-year relapse-free survival rate is 24 % for the chemotherapy arm and 55 % for the ASCT arm (hazards ratio = 0.55 in favor of the ASCT arm; $P=0.065$). The corresponding figures for OS were 45 and 56 %, respectively (hazards ratio = 0.87 in favor of the ASCT arm; $P=0.71$). In this randomized, prospective study, HDT and ASCT in adults with lymphoblastic lymphoma in first remission produced a trend for improved relapse-free survival but did not improve OS compared with conventional-dose therapy [58].

Another area of controversy in the field of ASCT is whether contamination of stem cell grafts with tumor cells increases the risk of relapse. Sharp et al. demonstrated a 5-year relapse-free survival rate of 64 % for patients receiving a

tumor-negative peripheral blood stem cell transplant versus 17 % for those with a contaminated graft ($p<0.01$) [59]. On the other hand, data from the EBMT showed no difference in OS or DFS in patients receiving purged versus unpurged grafts. However, when the indolent lymphomas were analyzed separately, there was an improvement in OS with graft purging [6, 60].

Allogeneic Transplantation for DLBCL

The role of allo-SCT in intermediate/aggressive lymphomas is uncertain. Myeloablative allo-SCT has been examined in a number of phase I and II studies in patients with intermediate- or high-grade NHL. Most were in young patients with advanced disease. Results are difficult to interpret because of the lack of randomized controlled trials and the impact of eligibility criteria and patient selection on outcome.

A case-controlled study of patients who reported to the EBMT Group was performed to investigate the relative roles and efficacy of allo-SCT and ASCT in NHL. Of 1,060 patients who were reported to the lymphoma registry, 938 patients had an ASCT and 122 patients had an allo-SCT. Majority of the patients had aggressive histologies. One hundred and one allo-SCT patients were matched with 101 ASCT patients. The case matching was performed after the selection of the main prognostic factors for PFS by a multivariate analysis. The PFS was similar in both types of transplants (49 % vs. 46 %). The overall relapse and progression rate for the allo-SCT patients was 23 % compared with 38 % in the ASCT patients. This difference was not significant statistically. In the lymphoblastic lymphoma subgroup, allo-SCT was associated with a lower relapse rate than ASCT (24 % vs. 48 %; $P=0.035$). The PFS, however, was not significantly different because patients with lymphoblastic lymphoma who underwent allo-SCT had a higher TRM (24 % vs. 10 %; $P=0.06$). A significantly lower relapse/progression rate was also observed in patients with chronic GVHD compared with those patients without [61].

Peniket et al. analyzed 1,185 allogeneic transplants for lymphoma reported to the EBMT registry between 1982 and 1998 and compared the results with those of 14,687 autologous procedures performed over the same period. Patients receiving allo-SCT were subdivided according to histology: low-grade NHL ($N=231$), intermediate-grade NHL ($N=147$), high-grade NHL ($N=255$), lymphoblastic NHL ($N=314$), Burkitt's lymphoma ($N=71$), and Hodgkin's disease ($N=167$). These patients received allogeneic transplants as their first transplant procedure. Actuarial OS at 4 years from transplantation was as follows: intermediate-grade NHL 38 %, high-grade NHL 41 %, lymphoblastic lymphoma 42 % years, and Burkitt's lymphoma 37 %. Multivariate analysis showed that disease status at transplantation significantly affected outcome. A matched analysis was performed: for all categories of lymphoma, OS was better for ASCT than for allo-SCT. Relapse rate was lower in the allo-SCT group for low-, intermediate-, and high-grade and lymphoblastic NHL. It was equivalent for Burkitt's lymphoma [4].

Bierman et al. compared the results of syngeneic, allogeneic, and autologous hematopoietic stem cell transplantation for NHL. The databases of the International Bone Marrow Transplant Registry (IBMTR) and the EBMT were used to identify 89 NHL patients who received syngeneic transplants; these patients were compared with NHL patients who had received allogeneic (T cell depleted and T cell replete) and autologous (purged and unpurged) transplants. No significant differences in relapse rates were observed when results of allo-SCT were compared with syngeneic transplantation for any histology. T cell depletion of allografts was not associated with a higher relapse risk but was associated with improved OS for patients with low-grade and intermediate-grade histology. Patients who received unpurged autografts for low-grade NHL had a fivefold ($P=0.008$) greater risk of relapse than recipients of syngeneic transplants, and recipients of unpurged autografts had a twofold ($P=0.0009$) greater relapse risk than patients who received purged autografts. Among low-grade NHL patients, the use of purging was associated with significantly better DFS ($P=0.003$) and OS ($P=0.04$) when compared with patients who received unpurged autografts. Contrary to

other studies, this study failed to find evidence of a GVL effect but did provide indirect evidence to support the hypothesis that tumor contamination may contribute to lymphoma relapse and that purging may be beneficial for patients undergoing ASCT for low-grade NHL [6].

Reduced-Intensity Conditioning Regimens

The EBMT database was scanned for a first allo-SCT in relapsed DLBCL after a previous ASCT. A total of 101 patients (57 males; median age, 46 years) were included. Median follow-up for survivors was 36 months. Myeloablative conditioning regimen was used in 37 patients, and RIC was used in 64 patients. Three-year non-relapse mortality (NRM) was 28 %, response rate (RR) was 30 %, PFS was 42 %, and OS was 54 %. The NRM was significantly increased in patients ≥45 years ($P=0.01$) and in those with an early relapse (<12 months) after ASCT ($P=0.01$). RR was significantly higher in refractory patients ($P=0.03$). A time interval to relapse after ASCT of <12 months was associated with lower PFS ($P=0.03$). The use of RIC regimens was followed by a trend to a lower NRM ($P=0.1$) and a trend to a higher RR ($P=0.1$), with no differences in PFS and OS. No differences were seen between HLA-identical sibling and matched unrelated donor transplants [62].

The French Society of Marrow Transplantation and Cellular Therapy registry reported promising results of RIC and allo-SCT in 68 patients (median age: 48 years). Patients had received a median of 2 regimens of therapy prior to allo-SCT, and 79 % had undergone prior ASCT. Prior to transplantation, 47 % were in CR. In eighty-two percent of patients the donor was an HLA-matched sibling. With a median follow-up of 49 months, estimated 2-year OS, PFS, and the cumulative incidence of relapse were 49, 44, and 41 %, respectively. The 1-year cumulative incidence of NRM was 23 %. According to multivariate analysis, patients in CR before transplantation had a significantly longer PFS and a lower incidence of relapse than patients transplanted during partial remission or stable or progressive disease [63].

Thus, HDT with ASCT may be effective and potentially curative in patients with DLBCL with partial responses to induction chemotherapy, chemotherapy-sensitive first relapse, and high-risk patients in first remission. The impact of the preparative regimen, graft purging, and maintenance therapy post-transplant on outcomes is uncertain. Younger patients with a good performance status, patients with refractory disease or with multiple prior relapses, stem cell compromise due to prior therapy, bone marrow involvement with tumor or bone marrow fibrosis, and relapse after an autologous transplant should be considered for allogeneic transplantation. Allogeneic transplantation appears to be superior to autologous transplant in terms of producing a lower relapse rate, but the non-relapse mortality continues to be higher in allogeneic transplants. Results of allogeneic transplantation using NMA or RIC regimens appear to be promising; however, further studies are needed as higher relapse rates have been described in some studies. Thus, the toxicity of allogeneic procedures must be further reduced before this can translate into an improvement in survival. Use of alternative sources of stem cells remains investigational although preliminary results are promising [64, 65]. Use of novel preparative regimens with incorporation of monoclonal antibodies or radioimmunoconjugates may improve outcomes in both allogeneic and autologous transplants. Role of maintenance therapy also needs to be defined.

Primary Central Nervous System Lymphoma

PCNSL are rare but aggressive lymphomas and represent approximately 4 % of all intracranial neoplasms and 4–6 % of extranodal lymphomas. In general, the prognosis of untreated PCNSL patients is poor with a median survival of 1.5–3.3 months [66]. Durable remissions are possible with the newer treatment regimens; however, the outcome of PCNSL remains unsatisfactory, particularly when compared with that of patients with extra-central nervous system lymphomas of a similar stage and histotype. Therefore, consolidation therapies like whole brain irradiation (WBI)

or HDT followed by ASCT have been studied in these patients. Because of the high incidence of neurotoxicity associated with WBI, especially in patients over the age of 60, there is interest in pursuing non-radiation-based consolidative therapies [67, 68]. There is encouraging data from prospective, non-randomized studies utilizing HDT/ASCT as consolidation therapy in the newly diagnosed PCNSL patient population [69].

HDT/ASCT was initially studied in the PCNSL patient population in the setting of relapsed or refractory disease [70]. Based on promising median PFS and OS results of 41 and 58 months, respectively, this strategy was employed in the newly diagnosed PCNSL population, initially in combination with WBI. Although initial results in this setting were disappointing using the BEAM conditioning regimen [71], subsequent studies employed regimens with potentially better CNS penetration including busulfan, thiotepa, and carmustine [72]. Recent studies with the latter agents have eliminated WBI, and the median PFS and OS achieved in these studies are promising. In a pilot study, the 3-year progression-free survival was 77 % without the use of WBI [73]. Most studies have typically included patients younger than 60–65 years of age given the potential for increased risk of toxicity in older patient populations treated with HDT/ASCT. Recently, a number of randomized trials in the newly diagnosed PCNSL patient population have been completed or initiated; data from these randomized trials will define the standard of care for PCNSL in the future.

HIV Lymphoma

The introduction of highly active antiretroviral therapy (HAART) has significantly improved the survival of HIV-infected patients, allowing the use of more aggressive chemotherapy to treat lymphomas in this setting. Although the feasibility of harvesting stem cells and using ASCT for NHL was demonstrated before HAART, it was after the introduction of these drugs that the efficacy of high-dose chemotherapy and transplantation was demonstrated [74, 75]. Three-year PFS rates of 45–75 % have been reported for ASCT recipients (although in the Italian experience, only 27 of 50 patients for whom transplantation was planned actually received it). The Blood and Marrow Transplant Clinical Trials Network is conducting a US study of ASCT for HIV-infected lymphoma patients to expand on the observations from these smaller series. Experience with allo-SCT is very limited.

Mantle Cell Lymphoma

Mantle cell lymphoma (MCL) has an aggressive clinical course with a median survival <3 years and is incurable with conventional chemotherapy. HDT and ASCT may be effective in chemosensitive patients in first CR, but patients with resistant or recurrent disease have a high rate of treatment failure [76, 77].

Autologous Transplantation in First Complete Remission

A number of retrospective, prospective phase II, and registry studies evaluating the role of HDT and ASCT in MCL have been published [78–82]. In a large retrospective study of the ABMTR and EBMT registry, transplanted patients had a median survival of 59 months, which was longer than historical (1990s) series of patients treated conventionally who had a median survival of only 36 months. One hundred and ninety-five patients were included in the analyses with a median follow-up of 3.9 years. The 2-year and 5-year OS were 76 and 50 %, and PFS was 55 and 33 %, in the transplant and control arms respectively. Disease status at transplant was the most significant factor affecting survival: patients with chemosensitive disease but not in CR1 were 2.99 times ($P<0.001$) more likely to die than patients transplanted in CR1 [83]. The European MCL Network's randomized trial comparing consolidation with myeloablative radiochemotherapy followed by ASCT to interferon-alpha (α[alpha]) maintenance in CR1 showed a median PFS benefit with ASCT (median of 39 months vs. 17 months for patients in the interferon-α arm; $P=0.0108$).

The 3-year OS was similar in the two arms, and longer follow-up of these patients may be necessary to show an impact on the OS [84]. In this study, the absence of minimal residual disease (MRD) after ASCT strongly predicted for longer failure-free interval [85].

Geisler et al. reported the results of the second Nordic MCL trial which enrolled 160 consecutive, untreated patients younger than 66 years in a phase 2 protocol with dose-intensified induction immunochemotherapy with rituximab. Responding patients received HDT and ASCT. The 6-year OS, EFS, and PFS rates were 70, 56, and 66 %, respectively. There were no relapses beyond 5 years. The NRM was acceptable at 5 %. Multivariate analysis showed Ki-67 to be the sole independent predictor of EFS. The majority of stem cell products and patients assessed with PCR after transplantation were negative. Compared with their historical control, (patients who were enrolled in the Nordic MCL-1 trial), the EFS, OS, PFS, the duration of molecular remission, and the proportion of PCR-negative stem cell products were significantly increased ($P<0.001$). The lack of relapse after 5 years may suggest a possible cure [86].

Khouri et al. analyzed the long-term results of ASCT in patients with diffuse MCL in first CR. Thirty-three patients with advanced MCL were treated with hyper-CVAD regimen (hyperfractionated intense-dose cyclophosphamide, vincristine, continuous intravenous infusion of doxorubicin, and dexamethasone, alternating with high doses of cytarabine and methotrexate) followed by ASCT (cyclophosphamide and TBI). At a median follow-up of 49 months, the OS and DFS rates at 5 years were estimated to be 77 and 43 %, respectively, for those transplanted in CR1. A beta2-microglobulin level ≤3 mg/L at the time of diagnosis or transplantation was found to be strongly predictive of longer survival ($P=0.0001$). Tam et al. further updated these results of a risk-adapted strategy at the MD Anderson Cancer Center. Of a total of 121 patients enrolled in sequential transplant protocols over a 17-year study period, 86 underwent ASCT. At a median follow-up of 6 years, the actuarial PFS and OS were 39 and 61 %, respectively, with median PFS and OS durations of 42 and 93 months for patients transplanted in CR1. The addition of rituximab resulted in an improvement of PFS for those getting ASCT in CR1 [87]. Gianni et al. treated 28 previously untreated advanced-stage MCL patients younger than 61 years of age with three cycles of standard-dose chemotherapy followed by a high-dose rituximab-supplemented chemotherapy. All 27 patients who were assessable for response achieved a CR, of which 24 remained in continuous complete remission (CCR) after a median follow-up of 35 months. The OS and EFS rates at 54 months were 89 and 79 %, respectively. These results compared favorably with the 42 % OS rate and the 18 % EFS rate observed in 35 age-matched historical controls treated with standard-dose chemotherapy at the participating centers [81].

A joint analysis of two parallel single-center studies of sequential high-dose therapy for induction of minimal disease followed by a TBI-containing myeloablative regimen and ASCT enrolled 46 patients with advanced-stage MCL. Thirty-four patients were accrued to the protocol immediately after diagnosis ("up-front ASCT" group). The remaining 12 patients were put on the protocol later during the course of their disease ("delayed ASCT" group). All patients were in remission after mobilization chemotherapy and proceeded to ASCT. With a follow-up time of 24 months post-transplant, the EFS and OS probabilities at 2 years were 77 and 100 % for the up-front ASCT group compared to 30 % ($P=0.0007$) and 54 % ($P=0.0016$) for the delayed ASCT group. Timing of ASCT and spleen size was identified as an independent predictor of survival on multivariate analysis [88]. An important retrospective analysis of 118 MCL patients who underwent ASCT at 3 different referral centers in Germany was reported by Dietrich et al. Cox regression analysis of the incidence of relapse identified not receiving rituximab before ASCT and undergoing salvage ASCT as predictive factors for relapse [89]. Budde et al. found that MIPI scores were independently associated with survival after ASCT in 118 patients studied (HR 3.5; $P<0.001$) and in the 85 patients who underwent ASCT as initial consolidation (HR, 7.2; $P<0.001$). OS rates were 93 60, and 32 % at 2.5 years from ASCT for all patients with low-, intermediate-, and

high-risk mantle cell lymphoma international prognostic index (MIPI), respectively. After adjustment for the MIPI, an intensive induction regimen was not associated with improved survival after transplantation in all patients (HR, 0.5; $P=0.10$), the initial consolidation group (HR, 1.1; $P=0.86$), or in patients ≤60 years old (HR, 0.6; $P=0.50$) [90].

Relapsed Mantle Cell Lymphoma: Autologous Transplantation

The results of HDT and ASCT in relapsed/refractory MCL have been disappointing. In a study from the MD Anderson group, of a total of 121 patients enrolled in sequential transplant protocols over a 17-year study period, 86 underwent ASCT. The actuarial 6-year PFS and OS rates were 10 and 35 %, respectively, for patients transplanted beyond CR1 ($P=0.01$ and 0.02 compared with ASCT in CR1). The median PFS and OS durations were 27 and 52 months, respectively. These results were inferior for both PFS and OS compared with CR1 patients and were maintained in a multivariate analysis that accounted for differences in baseline factors. Surprisingly, in this study, PFS durations were similar regardless of disease status at transplantation (medians, 31, 27, and 23 months for CR, PR, and refractory relapse, respectively; $P=$NS for all comparisons). The presence of B symptoms, elevated β[beta]2 m, use of TBI, and hematopoietic stem cell transplantation-comorbidity index (HSCT-CI) score of 3 or greater were associated with inferior OS on the univariate analysis. However, none of these factors was independently prognostic on the multivariate analysis [87].

In order to improve the results of ASCT, Gopal et al. tested the safety and efficacy of using a CD20-specific monoclonal antibody conjugated with $^{(131)}$I to deliver high-dose radiation selectively to all lymphoma sites. Patients with relapsed or refractory MCL received $^{(131)}$I-labeled CD20-specific monoclonal antibody (Tositumomab®) followed 10 days later by high-dose etoposide (30–60 mg/kg), cyclophosphamide (60–100 mg/kg), and autologous stem cells. Among the patients with measurable disease at the time of transplant, the respective CR and overall response rates were 91 and 100 %. The OS at 3 years from transplantation was estimated at 93 %, and PFS was estimated at 61 % in this heavily pretreated group of patients [91].

Relapsed Mantle Cell Lymphoma: Allogeneic Transplantation

Case reports of patients with chemotherapy-refractory MCL having prolonged remission after allo-SCT and patients with disease detectable by PCR following allo-SCT converting to negative status several months later suggest the presence a GVL effect [92, 93]. Some of the studies evaluating the role of allogeneic transplantation in MCL are summarized in Table 23.2 [93–100].

Table 23.2 Allogeneic transplantation for mantle cell lymphoma

Study	*N*	Preparatory regimen	TRM/NRM (%)	DFS/EFS/PFS (%)	OS (%)
Khouri et al. [93]	16	cy/TBI		FFP 55 % at 3 years	55 % at 3 years
Rifkind et al. [98]	6	bu/cy	TRM 0	No relapses	Median survival post-transplant of 4.3+ years
Ganti et al. [94]	17	cy/TBI		EFS 44 % at 5 years	49 % at 5 years
Robinson et al. [96]	22	Multiple	TRM at 1 year 46 %	PFS 31 % at 1 year	38 % at 1 year
Morris et al. [99]	10	FM-alemtuzumab		PFS 50 % at 3 years	60 % at 3 years
Maris et al. [95]	33	Flu-2 Gy TBI	NRM 2 years 24 %	DFS 60 % at 2 years	65 % at 2 years
Sorror et al. [97]	53	Flu-2 Gy TBI	5-year NRM 27 %	PFS 52 % at 5 years	58 % at 5 years
Tam and Khouri [100]	35	FCR/PFA	TRM at 1 year 9 %	PFS 46 % at 6 years	53 % at 6 years

Abbreviations: *Cy/TBI* cyclophosphamide/total body irradiation, *bu/cy* busulfan/cyclophosphamide, *PFS* progression-free survival, *EFS* event-free survival, *DFS* disease-free survival, *OS* overall survival, *TRM* transplant-related mortality, *NRM* non-relapse mortality, *FM* fludarabine/melphalan, *TBI* total body irradiation, *PFA* cisplatin, fludarabine, cytarabine

Myeloablative Regimens

In a pilot study, 16 patients from MD Anderson Cancer Center with diffuse MCL received allo-SCT. Eleven patients were previously treated, including one who failed prior ASCT, and five patients were newly diagnosed. Conditioning regimen was cyclophosphamide and TBI or BEAM. Two additional patients received a NMA regimen. One patient who received NMA regimen relapsed post-transplant but later achieved CR after developing GVHD. Residual lymphoma was assessed in seven patients by polymerase chain reaction assay (PCR) for bcl-1 or immunoglobulin gene rearrangement. All had detectable disease at the time of transplant. When tested within 4-month post-transplant, four of these patients attained molecular remission, and one converted to a negative PCR status 7 months later. The OS and failure from progression (FFP) at 3 years were both 55 %. For patients with chemosensitive disease, FFP and OS at 1 year were both 90 % compared with 44 % ($P=0.04$) for those who were refractory to conventional chemotherapy at the time of transplantation [93]. The University of Nebraska group reported outcomes in patients with chemotherapy-sensitive MCL undergoing autologous ($N=80$) or allogeneic ($N=17$) stem cell transplants. Five-year estimated EFS (44 % vs. 39 %) and OS (49 % vs. 47 %) were similar in both groups. The 5-year relapse rate was lower at 21 % in the allo-SCT group, compared with 56 % in the ASCT group. This was balanced, however, by higher day 100 mortality rate in patients receiving allo-SCT (19 %) [94].

Non-myeloablative Regimens

Preliminary data suggests that allo-SCT after NMA conditioning is a promising salvage strategy for patients with relapsed/refractory MCL. The high response and low relapse rates with this approach suggest that MCL is susceptible to GVL response. Updated results on 35 patients (median age 58), all with relapsed or refractory MCL, who underwent NMA transplant were published in 2009 by Tam et al. All patients had advanced-stage disease, and 83 % had chemosensitive disease at the time of transplant. With a long median follow-up of 56 months (range, 19–110 months), the median PFS duration was 60 months, and the median OS had not yet been reached. Major determinants of disease control were use of PBSC versus BM as source of stem cells and achievement of 95 % donor chimerism. Among 24 patients meeting both criteria, no lymphoma relapses had occurred at a median follow-up of 60 months. The 6-year actuarial PFS rate was 46 %, and the 6-year actuarial OS rate was 53 %. Importantly, plateaus in the survival curves were observed for both PFS and OS, with no relapses or deaths occurring in patients followed beyond 63 months. These outcomes were significantly superior to a cohort of patients who underwent ASCT in salvage setting where relapses and deaths occurred in a continuous fashion. Compared with ASCT in CR1, NMA allo-SCT had an initially lower OS; however, this reversed at 8 years because of the lack of late deaths among allo-SCT recipients [87].

Maris et al. carried out HLA-matched hematopoietic allo-SCTs in 33 patients with relapsed and refractory MCL after NMA conditioning regimen of fludarabine and 2 Gy TBI. The overall response rate in the 20 patients with measurable disease at the time of transplant was 85 %. The median follow-up was 24.6 months. Relapse and non-relapse mortalities were 9 and 24 %, respectively, at 2 years. The Kaplan–Meier probabilities of OS and DFS rates at 2 years were 65 and 60 %, respectively [95].

Most studies HDT and ASCT in first CR of MCL report a PFS advantage for ASCT. However, up-front ASCT has not been tested in randomized trials against intensive chemotherapy regimens (like rituximab/hyper-CVAD). ASCT beyond CR1 are suboptimal and may be beneficial in only a highly selected group of patients. The role of allo-SCT is difficult to define because of the paucity of data, with only a few small phase II trials reported. Patients whose disease progresses after ASCT generally have a very poor prognosis and, if they are suitable candidates, will likely benefit from an allo-SCT. It is unclear if a subgroup of patients, i.e., those who are young and have high MIPI scores are candidates for allo-SCT up-front, especially if they are relatively young and fit and have a sibling donor. Trials are

Table 23.3 Up-front high-dose therapy and autologous stem cell transplantation for peripheral T cell non-Hodgkin's lymphomas: prospective studies

Study	*N*	% going to transplant	Overall response rates	Median follow-up (months)	Overall survival	PTCL subtypes included
Corradini [101]	62	74	72	76 months	21 % non-ALK-pos ALCL	Including ALK-pos ALCL
Rodriguez [104]	26	73	81	35 months	73 % at 3 years	PTCL-NOS
D'Amore [103]	166	70	83	45 months	57 % at 3 years	Excluding ALK-pos ALCL
Mercadal [105]	41	41	59	3.2 years	39 % at 4 years	PTCL-NOS
Reimer [102]	83	66	71	33 months	48 % at 3 years	PTCL-NOS

Abbreviations: *PTCL-NOS* peripheral T cell lymphoma-not otherwise specified, *ALK* anaplastic lymphoma kinase

also needed to optimize conditioning regimens and investigate post-transplant maintenance therapy. Radioimmunotherapy is currently being investigated as part of the conditioning regimens for both autologous and allogeneic transplantation and may improve outcomes.

T Cell Lymphomas

The most common subtypes are PTCL-NOS, angioimmunoblastic lymphoma (AIL), and ALCL which may be ALK positive or negative. Standard chemotherapy treatments are less effective for T cell lymphomas than for B cell lymphomas, with the possible exception of ALK-positive ALCL [1, 2]. A number of retrospective and a few prospective studies of up-front HDT and ASCT to improve outcomes in T cell NHL have been reported. Most studies are small and have included all subtypes of T cell lymphomas including the ALK-positive lymphomas that generally have a better outcome than other T cell lymphomas. Some studies have included patients with relapsed disease and those in transplanted in first complete remission.

Autologous Transplantation in First Remission

There have been no randomized PTCL-restricted clinical trials evaluating ASCT in first CR to standard chemotherapy alone. However, there have been a number of prospective phase 2, retrospective, and randomized cooperative group studies of ASCT which have included all types of high-risk lymphomas (including PTCL). Some of these are summarized in Table 23.3 [101–105]. Corradini et al. reported the combined results of two prospective phase II studies investigating the efficacy of high-dose sequential chemotherapy, followed by ASCT in 62 patients with advanced-stage PTCL (including ALCL). Seventy-four percent of patients were able to complete the program. At a median follow-up time of 76 months, the estimated 12-year overall OS, DFS, and EFS rates were 34 55 and 30 %, respectively. The results of ALK-positive ALCL were significantly better than other PTCL patients. Multivariate analysis showed that patients who had a CR before ASCT had a statistically significant benefit in terms of OS and EFS [101]. A German study by Reimer et al. enrolled 83 patients with PTCL who received standard chemotherapy followed by HDT and ASCT if they were able to achieve a CR or PR. Only 66 % of patients were able to receive the HDT and ASCT. The main reason for not receiving ASCT was progressive disease. In an intent-to-treat analysis, the overall response rate after ASCT was 66 %. With a median follow-up time of 33 months, the estimated 3-year OS and DFS rates for patients in CR and 3-year PFS rate were 48, 53 and 36 %, respectively. The 3-year OS for patients who underwent HDT and ASCT was 71 %, whereas it was only 11 % for those who did not receive a transplant [102].

D'Amore from the Nordic Lymphoma Group performed one of the largest prospective multicenter phase II study to study the role of intensified treatment schedules using up-front ASCT as first-line therapy in newly diagnosed PTCL. A total of

166 patients with various T cell histologies (excluding ALK-positive ALCL) were enrolled. Seventy percent of patients were able to proceed to transplant. With a median follow-up time of 45 months, the 5-year OS and PFS rates were 50 and 43 %, respectively. Most common cause of death was lymphoma relapse [103]. A number of retrospective studies have also shown a benefit for ASCT in first CR or PR in patients with T cell NHLs. A retrospective study from the Grupo Espanol de Linfomas/Trasplante Autologo de Medula Osea (GEL-TAMO) group showed a survival benefit for patients receiving HDT and ASCT in first CR. They analyzed 74 patients with high-risk PTCL (including ALCL, ALK status unknown) who were transplanted in first CR. After a median follow-up time of 67 months from diagnosis, the 5-year OS and PFS rates were 68 and 63 %, respectively. Multivariate analysis showed that the only factor associated with a shorter OS and PFS was the presence of more than two risk factors from the prognostic index for peripheral T cell lymphoma-unspecified (PIT) risk system. Patients with ALCL had a better outcome than non-ALCL patients, with a 5-year OS of 84 % versus 61 %, respectively ($P=0.058$). Similarly, the PFS was significantly higher in the ALCL group than in the non-ALCL group (80 % vs. 55 %, $P=0.036$) [106].

Two randomized GELA trials (LNH-87 and LNH-93) evaluated the benefit of up-front ASCT in aggressive NHLs including a subgroup of patients with PTCL. In LNH-93, a shortened chemotherapy course followed by HDT and ASCT was compared with chemotherapy alone, and there was no overall benefit from ASCT including in those patients with a T cell immunophenotype [39]. A matched control analysis was also performed on patients with T cell lymphoma from this trial as well as from LNH-87 trial (consolidative sequential chemotherapy vs. ASCT) confining the analysis to those who achieved a confirmed or unconfirmed CR and who were able to receive either HDT/ASCT (case group) or sequential chemotherapy (control group). In this analysis, among the 29 patients with non-anaplastic PTCL, there was no difference in DFS or OS between the two groups [107].

Angioimmunoblastic Lymphoma

In a retrospective analysis from the EBMT, 146 patients with AIL who received ASCT were reported. After, a median follow-up time of 31 months, the actuarial OS was 59 % at 48 months. The cumulative incidence of relapse was estimated to be 51 % at 48 months. Disease status at transplantation was the major factor affecting outcome. Patients who underwent ASCT in first CR had significantly superior PFS and OS rates. The estimated PFS rates for patients who received their transplants in CR were 56 % at 48 months and 23 % for patients with chemotherapy-refractory disease [108].

Enteropathy-Associated T Cell Lymphoma

Enteropathy-associated T cell lymphoma (ETCL) tends to have a very poor outcome [109, 110]. In the Nordic Lymphoma Group study, there were 21 patients with ETCL. Their 3-year OS and PFS rates were 52 and 47 %, respectively [103]. Sieniawski et al. tested a novel regimen of ifosfamide, vincristine, and etoposide/methotrexate plus ASCT in 26 patients with ETCL. The 5-year PFS and OS rates of 52 and 60 %, respectively, were significantly improved compared with the historical group treated with conventional anthracycline-based chemotherapy [111].

ALK-Negative Anaplastic Large Cell Lymphoma

Few studies have evaluated the role of ASCT in ALK-negative patients. In the Nordic Lymphoma Group study, there were 31 patients with ALK-negative ALCL. The 5-year OS and PFS rates were 73 and 64 %, respectively [103].

Results of prospective studies demonstrate that not all patients achieve a CR or PR to front-line chemotherapy and are able to move forward with HDT and ASCT. The percentage of patients who can proceed to ASCT varies from 40 to 73 % [103–105]. Those who are able to proceed to ASCT have outcomes similar to those seen after

front-line treatments for B cell lymphomas. After ASCT, the CR rates vary between 59 and 81 %, and OS rates vary from 73 % at 3 years to 39 % at 4 years. Prognostic factors for better outcomes have included low PIT score at transplant, LDH levels, and able to achieve a CR prior to transplant.

Relapsed or Refractory Disease

A number of retrospective, single-institution, and registry studies have been published evaluating the role of HDT and ASCT in relapsed/refractory T cell NHL. In general, the reported results in most studies are comparable to those seen with ASCT for relapsed B cell NHL [112–121]. Others have reported inferior outcomes. In a study by Smith et al., the relapse-free survival was only 18 %, and OS was 34 % at 5 years following ASCT for PTCL [122]. Most studies have found that the best outcome is seen in patients who are in a CR at the time of transplant [120, 123, 124].

A registry analysis of 123 patients with PTCL from the GEL-TAMO database who underwent ASCT as salvage therapy was reported by Rodriguez et al. The median age at ASCT was 44 years, and majority of patients had chemosensitive disease. After HDT and ASCT, 73 % of patients achieved a CR. At a median follow-up time of 61 months, the 5-year OS and PFS rates were 45 and 34 %, respectively. The presence of more than one factor of the adjusted IPI score and a high beta (β[beta]) 2-microglobulin at transplantation were identified as adverse prognostic factors for both OS and PFS [125]. Vose et al. reported equivalent long-term OS and DFS in both relapsed T and B cell NHLs. In their study, patients with T cell immunophenotype had a slightly better CR rate than those with B cell immunophenotype (59 % vs. 42 %, P=NS). The actuarial 2-year OS was 35 % in the T cell group compared with 30 % in the B cell group (P=NS). The 2-year DFS was 28 % for the T cell and 17 % for the B cell patients [126]. Rodriguez et al. transplanted 78 patients in first or subsequent PR, second or more CR, or refractory disease. In this salvage setting, actuarial OS and DFS at 5 years were 45 and 49 %, respectively. Interestingly, there were no statistically significant differences between the 41 patients who were transplanted in PR and the 28 patients who were transplanted in second or subsequent CR as consolidation therapy. In the six patients who had refractory disease at the time of transplant, the 5-year OS was 0 % indicating that HDT and ASCT may not benefit this group of patients. Levels of LDH, adjusted IPI score, and disease status pre-transplant correlated with outcome [123]. EBMT registry analysis of 64 adult and pediatric patients with relapsed T cell and null cell ALCL was published by Fanin et al. At the time of transplant, 47 % of patients were in CR. The actuarial OS rate at 10 years was 70 %. Multivariate analysis showed that good performance status at transplant, younger age, absence of B symptoms, and absence of extranodal disease indicated a better prognosis [127]. Zamkoff et al. [116] identified 16 patients with ALK-negative ALCL who had HDT and ASCT at the time of first relapse. The median PFS in this group was only 3 months. Thus, HDT and ASCT may not benefit patients with relapsed ALK-negative ALCL [116].

Cutaneous T Cell Lymphomas

Primary cutaneous T cell lymphomas (CTCL) are characterized by infiltration of the skin by malignant T cells. The most common subtypes of these rare cutaneous lymphomas are mycosis fungoides (MF) and Sezary syndrome (SS). Patients with advanced disease have a shortened survival. ASCT for CTCL has generally yielded disappointing results with most patients' disease progressing within 1 year from transplant [128, 129]. In the relapsed and refractory setting, most series of ASCT for T cell NHLs show comparable response rates and survival durations to those seen in patients with B cell lymphomas if transplanted with chemosensitive disease. Patients who have refractory disease and CTCL subtype do not benefit from this approach.

Allogeneic Transplantation

Allogeneic transplantation has also been studied in patients with relapsed and/or refractory T cell

lymphomas. Most studies of allo-SCT are also of a retrospective nature, have a small number of patients, and include a variety of conditioning regimens. Patients who undergo allogeneic transplantation are generally younger than those undergoing autologous transplantation. Feyler et al. studied 18 patients with relapsed and/or refractory T cell lymphomas who underwent an allogeneic transplant. After a median follow-up time of 57 months, the 3-year OS and PFS rates were 39 and 33 %, respectively. The 3-year relapse rate was 28 %, and the NRM rate was significant at 39 % [124].

Angioimmunoblastic Lymphoma

A retrospective registry analysis from the EBMT reported on the results of allo-SCT in 45 patients with AIL. The cumulative incidence of NRM in this study was 25 % at 12 months. Patients with poor performance status had a significantly higher NRM. Relapse rate was estimated as 20 % at 3 years and was lower in patients who developed chronic GVHD. The PFS and OS rates were 53 and 64 % at 3 years, respectively, and were significantly better in chemotherapy-sensitive patients. The authors therefore concluded that there is a clinically relevant GVL effect in AIL patients [130].

Cutaneous T Cell Lymphoma

In recent years, several authors have reported favorable results of allo-SCT in patients with CTCL [131, 132]. A recent meta-analysis compared the outcome of allogeneic versus autologous stem cell transplantation in patients with MF/SS using 39 cases from reported literature. The OS and EFS rates were better in patients who received allogeneic transplant as compared to those who received an autologous transplant. In allogeneic group, the most common cause of death was GVHD, while majority of the deaths in the autologous group were because of progressive disease. Thus, allo-SCT may offer a better survival and disease-free outcome versus ASCT in MF/SS, likely because of a GVL effect [133].

NK/T Cell Lymphoma

HDT and ASCT have been studied in patients with relapsed/refractory in natural killer cell lymphoma and leukemia (NK/T cell). Results of pooled ASCT studies show that remission status prior to transplant is most significant factor predicting survival. Lee et al. matched 47 patients according to NK/T cell lymphoma international prognostic index (NKIPI) risk groups and disease status at transplantation with 107 patients from a historical control group. After a median follow-up of 116.5 months, the median survival time was not determined for the ASCT group, but it was 43.5 months for the control group ($P=0.127$). In patients who were in CR at the time of ASCT or at surveillance after remission, disease-specific survival rates were significantly higher in the ASCT group compared with the control group (disease-specific 5-year survival rate, 87 % for ASCT vs. 68 % for non-ASCT; $P=0.027$). In contrast, in subgroup analysis on non-CR patients at the time of ASCT, disease-specific survival rates were not significantly prolonged in the ASCT group compared with the control group (1-year survival rate, 67 % for ASCT vs. 29 % for non-ASCT; $P=0.141$). The impact of ASCT on the survival of all patients was significantly retained at the multivariate level with a reduced risk of death in these patients ($P=0.006$) [134]. For patients with relapsed disease achieving CR2, data on survival without further therapy are limited. In these patients, HDT and ASCT also have a poor outcome. Based on available data, ASCT is not recommended for patients with NK/T cell lymphoma in CR1, particularly those with early-stage disease. Patients who are at high risk for relapse based on the NKIPI may benefit from upfront HDT and ASCT in CR1. For patients with advanced/refractory disease, the outcome of ASCT is poor. Limited data is available for allo-SCT [135]. Role of NMA conditioning regimen is questionable as clear-cut graft-versus-NK cell lymphoma effect has not been established. NMA conditioning regimens should be reserved for patients who are not suitable candidates for myeloablative regimens. Whenever possible, these patients should be treated on clinical trials.

Table 23.4 Autologous hematopoietic stem cell transplantation for follicular lymphomas in first complete remission (randomized)

Study	No. of patients	Preparatory regimen	Follow-up (years)	PFS (%)	OS (%)
Lenz et al. [143]	240	ASCT	4.2	65 at 5 years	84 at 5 years
		Chemo		33 at 5 years ($P<0.0001$)	
Gyan et al. [144]	172	ASCT	9	64 at 9 years	76 at 9 years
		Chemo		39 at 9 years ($P=0.004$)	80 at 9 years ($P=$NS)
Sebban et al. [145]	401	ASCT	7.5	38 at 7 years	71 at 7 years
		23		28 at 7 years ($P=$NS)	76 at 7 years ($P=$NS)

Abbreviations: *ASCT* autologous stem cell transplantation, *PFS* progression-free survival, *OS* overall survival, *NS* not significant

PTCL responds favorably to chemotherapy, but relapse rates are high. HDT and ASCT in first CR or PR should be considered for patients who present with advanced disease and high PIT scores. However, there are no randomized PTCL-restricted studies comparing HDT and ASCT to conventional chemotherapy. A European intergroup phase III trial for primary T cell NHL will compare standard CHOP with alemtuzumab–CHOP therapy followed by up-front ASCT for patients younger than 60 years of age and may answer this question. Most studies suggest that results of ASCT used as a salvage therapy for PTCL improve outcomes compared to conventional chemotherapy and therefore should be offered to eligible patients with chemosensitive disease. For patients with relapsed and/or refractory disease and for those who relapse after ASCT, allo-SCT should be considered if they have an available donor and a good performance status. NMA and RIC conditioning regimens may provide GVL effect with acceptable NRM. Alternative donor transplants could be considered as part of a clinical trial. Studies of allo-SCT as part of frontline therapy in patients with T cell lymphoma who present with aggressive disease are under way.

Follicular Lymphoma

FL has been traditionally considered to be incurable with conventional treatment. Disease course is characterized by long median survival but a continuous pattern of relapse [136]. Eventually, the lymphoma becomes resistant to chemotherapy or undergoes transformation to the more aggressive large cell histology [137, 138]. HDT and ASCT as well as allogeneic transplantation have been extensively studied in this group of patients.

Autologous Transplantation

HDT with ASCT in patients with relapsed or recurrent disease has produced DFS rates of 42–60 % and OS rates of 50–86 % at 2–8 years of follow-up [139–141]. For patients transplanted in first remission, the DFS rates vary from 63 to 69 % and OS of 85 % at 3–10 years follow-up [142] (Table 23.4) [143–145].

Relapsed/Refractory FL

There are a number of phase II studies evaluating the role of ASCT in patients with relapsed/refractory FL. Rohatiner et al. published mature data from a retrospective analysis of myeloablative therapy supported by autologous BM transplantation in patients with FL as consolidation of second or subsequent remission. A total of 121 patients received cyclophosphamide and TBI supported by ex vivo purged autologous BM transplantation. With a relatively long median follow-up of 13.5 years, there was an apparent plateau on the remission duration curve of 48 % at 12 years. The 10-year OS and PFS were 54 and 48 %, respectively. Outcomes in patients treated

in second remission were significantly better than the survival of patients treated beyond second remission. Both remission duration and OS were also significantly longer for patients treated in second remission compared with an age-matched, remission-matched group of patients treated at St Bartholomew's Hospital before the introduction of this treatment. However, the development of secondary myelodysplasia (s-MDS) and secondary acute leukemia (s-AL) resulted in 15 patient deaths [139]. Another study from Dana–Farber Cancer Institute of 153 patients with relapsed FL who received monoclonal antibody-purged autologous BM transplantation showed a DFS and OS at 8 years of 42 and 66 %, respectively. In their study, patients whose BM was negative by PCR for bcl-2/IgH gene rearrangement after purging experienced longer freedom from recurrence than those whose BM remained PCR positive. Continued PCR negativity in follow-up BM samples was also strongly predictive of CCR. The 12-year survival from diagnosis for these 153 patients was an impressive 69 % [140].

Bierman et al. presented a retrospective analysis of 100 patients who underwent ASCT for relapsed FL. The median follow-up duration of surviving patients was 2.6 years (range, 1.0–11.7). The OS at 4 years was 65 %, and the FFS was estimated to be 44 %. They did not observe a definite plateau in the FFS curve. The only factor that was significantly associated with OS and FFS was the number of chemotherapy regimens received prior to transplantation. They did not observe any differences in outcomes between patients with who received peripheral blood stem cell transplants and unpurged autologous bone marrow transplants [141]. Rohatiner et al. found that the number of treatment episodes prior to transplantation (≤3 vs. >3) was statistically significant for OS ($P=0.01$) but not for remission duration ($P=0.9$) [146]. Number of prior chemotherapy regimens (<3 vs. ≥3) was also found to be statistically significant for OS by Cao et al. [147].

The CUP trial analyzed the value of purging in patients with relapsed chemosensitive FL undergoing ASCT. After three cycles of chemotherapy, responsive patients were randomized to either three more cycles of the same chemotherapy (C), HDT followed by autologous unpurged (U), or purged (P) ASCT. Purging was performed using a cocktail of monoclonals. A total of 140 patients were registered, of whom 89 fulfilled the criteria for randomization (C, 24; U, 33; and P, 32). With a median follow-up time of 26 months from randomization, 66 % in C arm progressed or relapsed, in contrast to 39 % of U and 37 % of the P patients ($P = 0.002$). OS was not reported due to short follow-up, but there was a suggestion of improved OS as well. Patients in U and P arms had higher PFS/relapse-free survival rate. Unfortunately, the trial was terminated early due to slow accrual; therefore, the question whether of ex vivo purging improved outcomes could not be answered [148].

With the availability of rituximab in vivo purging strategies have replaced in vitro purging [149–151]. Le Gouill et al. studied the impact of using HDT and ASCT and/or rituximab administration at first progression. With a median follow-up of 31 months, 3-year EFS and OS rates after progression were 50 % and 72 %, respectively. The 3-year EFS rate of rituximab-re-treated patients was 52 % versus 40 % for those not receiving rituximab second line ($P=0.075$). The 3-year OS was significantly and greatly different for patients receiving HDT/ASCT or not: 92 % versus 63 % ($P=0.0003$), respectively. In multivariate analysis, both HDT/ASCT and period of progression/relapse affected EFS and OS. This study supports incorporating HDT/ASCT in the therapeutic approach at first relapse for FL patients regardless of prior rituximab exposure [152].

To evaluate the long-term results of ASCT in FL with specific emphasis on the prognostic significance of PCR-detectable Bcl-2/IgH rearrangements, Apostolidis et al. treated 99 FL patients with ASCT as consolidation of second or subsequent remission. In vitro purging of the BM graft was accomplished by treatment with anti-B cell antibodies and complement. After a median follow-up of 5.5 years, 65 patients remained alive and 49 patients remained failure free. Overall, 12 % of patients developed s-MDS or s-AL. Kaplan–Meier estimates of freedom from recurrence (FFR) and survival rates at 5 years were 63 and 69 %, respectively. On multivariate analysis, absence of the Bcl-2/IgH rearrangement at the time of diagnosis

($P=0.04$) and three or fewer treatment episodes before ASCT ($P=0.001$) were significant prognostic factors for improved survival. For patients bearing Bcl-2/IgH rearrangements, absence of a PCR-detectable Bcl-2/IgH rearrangement during follow-up was associated with a significantly lower risk of recurrence ($P<0.001$) and death ($P=0.02$), whereas the PCR status of the reinfused stem cell graft did not correlate with outcome. There was an improvement in FFR after HDT but no survival advantage compared with conventional treatment. However, this study confirmed that the elimination of cells bearing the Bcl-2/IgH rearrangement improves outcomes [153].

A GELA study analyzed two cohorts of patients treated in two successive randomized studies with the same induction chemotherapy to evaluate the role of rituximab and ASCT after first disease progression or relapse. Of the 364 patients included in these two studies, 254 progressed or relapsed and constituted the population of the analysis. Among them, 98 were treated with ASCT including 33 of them after rituximab-containing salvage regimen and 69 with rituximab alone or combined with chemotherapy but without ASCT. ASCT was associated with a statistically significant benefit in terms of EFS from relapse and survival after relapse. Use of rituximab was associated with a greater benefit than ASCT for these two end points. When both treatments were combined, patients treated with rituximab-containing salvage regimen followed by ASCT had 5-year survival after relapse of more than 90 %. Thus, in FL patients treated with first-line chemotherapy, the combination of a salvage regimen containing rituximab with or without ASCT led to a dramatic improvement of long-term outcome [154].

Some of the factors that have found to be statistically significant for DFS and/or OS after ASCT for relapsed/refractory FL are age of the patient at the time of transplantation [141], presence of MRD before and after transplantation [140, 153], and chemotherapy-resistant versus sensitive disease at the time of transplantation [141]. Rohatiner et al. [146] found that the number of treatment episodes prior to transplantation (≤3 vs. >3) was statistically significant for OS ($P=0.01$) but not for remission duration ($P=0.9$). In a retrospective analysis, patients who underwent autologous BM transplantation in second remission were compared to patients who were treated with conventional chemotherapy. Those patients who underwent transplantation had a better DFS when compared to those receiving conventional chemotherapy, but there was no difference in OS [139].

First Complete Remission

Good results have also been reported with ASCT in patients with FL in first remission [142–145, 155]. However, because of the long natural history of the disease, concern regarding the development of secondary malignancies, and lack of survival benefit, ASCT high-risk patients with FL in first CR remain investigational (Table 23.4). The German Low-Grade Lymphoma Study Group (GLSG) initiated a randomized trial to compare the effect of potentially curative HDT followed by ASCT with interferon-alpha (IFN-α[alpha]) maintenance therapy in first remission. Three hundred and seven patients, younger than 60 years of age with FL, were enrolled. After two cycles of induction chemotherapy, patients were randomly assigned to either the ASCT or the IFN-α[alpha] group. The respective therapy was started when patients achieved CR or PR after induction chemotherapy. Two hundred and forty patients with FL were evaluable for the comparison of ASCT and IFN-α[alpha]. In patients who underwent ASCT, the 5-year PFS rate was 65 %, and in the IFN-α[alpha] arm, it was 33 % ($P<0.0001$). However, longer follow-up would be needed to determine the effect of ASCT on OS [143].

GOELAMS multicenter study randomized 172 patients with untreated FL for either conventional chemotherapy or purged ASCT. The 9-year OS was similar in the ASCT and conventional chemotherapy groups (76 and 80 %, respectively). The 9-year PFS was higher in the ASCT than the chemotherapy group (64 % vs. 39 %; $P=0.004$). A PFS plateau was observed in the ASCT group after 7 years. On multivariate analysis, OS and PFS were independently affected by the performance status score, the number of nodal

areas involved, and the treatment group. Secondary malignancies were more frequent in the transplant group. The occurrence of a PFS plateau may suggest that a subgroup of patients with FL might be cured by ASCT. As in other studies, of concern was the increased rate of secondary malignancies in the transplant arm [144]. In a randomized multicenter study conducted by Ladetto et al., six courses of CHOP chemotherapy followed by rituximab (CHOP-R) were compared with rituximab-supplemented high-dose sequential chemotherapy with ASCT (R-HDS) to assess the value of intensified chemotherapy as a first-line treatment for high-risk FL. The CR rates were 62 % with CHOP-R and 85 % with R-HDS ($P<0.001$). At a median follow-up of 51 months, the 4-year EFS was 28 and 61 %, respectively ($P<0.001$), with no difference in OS. Molecular remission was achieved in 44 % of CHOP-R and 80 % of R-HDS patients ($P<0.001$) and was the strongest independent outcome predictor. Patients relapsing after CHOP-R underwent salvage R-ASCT in 71 % of cases. Salvage R-ASCT had an 85 % CR rate and a 68 % 3-year EFS (median follow-up, 30 months). Achieving a molecular remission appears critical for effective disease control, regardless of treatment used. In this study, R-ASCT resulted in superior disease control and molecular outcome than CHOP-R, but no OS improvement. CHOP-R failures have a good outcome after salvage R-ASCT, suggesting that relapsed/refractory FL could be the most appropriate setting for transplantation [156].

With the exception of the study led by Ladetto et al., all studies mentioned were conducted in the pre-rituximab era. Therefore, the impact of rituximab cannot be fully evaluated in patients undergoing ASCT. Two recent studies have addressed the role of ASCT in patients with FL who have received front-line rituximab therapy [157, 158]. Hiddemann et al. conducted a retrospective analysis of two GLSG studies, which showed that R-CHOP followed by IFN maintenance achieved a 5-year PFS of 67 % and was comparable to CHOP followed by ASCT [158]. R-CHOP followed by ASCT, however, revealed a 5-year PFS of 79 %, with only one relapse after 24 months. This study suggested that ASCT may have a role in the era of R-CHOP front-line therapy, particularly for intermediate- or high-risk patients with advanced-stage FL [157].

Allogeneic Transplantation

Several studies have suggested that allogeneic transplantation for FL may improve the DFS when compared with autologous transplantation because of the presence of the GVL effect. The probability of relapse after allogeneic transplantation has ranged from 10 to 15 % [159]. In the past, the low relapse rate observed after allogeneic transplantation has not translated into an improvement in OS because of the high TRM associated with the use of high-dose chemotherapy [159, 160]. For example, in a study of 113 patients published by the IBMTR in 1998, the probability of DFS 3 years after myeloablative allo-SCT was 49 %, but the recurrence rate was only 16 %. However, the NRM was 40 %. Factors which were associated with improved survival were age <40 years, improved performance status at the time of transplantation, chemosensitive disease, and use of TBI-based conditioning regimens [159, 161]. Although data is limited on the use of unrelated versus related donors, a recent analysis from the National Marrow Donor Program (NMDP) included 52 patients with FL who had received myeloablative allo-SCT between 1991 and 2004. The 1-year TRM was 42 % and the 2-year PFS was 42 % [162]. Most of these published studies comparing allogeneic and autologous transplantation are retrospective or registry based. A prospective study by Bone Marrow Transplant-Clinical Trials Network was closed early due to slow accrual [163]. Van Besien et al. reported on 904 patients who had undergone transplants for FL. A total of 176 patients had received allogeneic transplants, 131 patients had received purged autologous transplants, and 597 patients had received unpurged autologous transplants. Five-year TRM rates were 30, 14, and 8 %, and 5-year recurrence rates were 21, 43, and 58 % after allotransplantation, purged autotransplantation, and unpurged autotransplantation, respectively. In multivariate analyses, allotransplantation had higher TRM and lower disease recurrence. The 5-year probabilities

of survival were similar in the three groups (51, 62, and 55 % after allogeneic, purged autotransplantation, and unpurged autotransplantation, respectively). Advanced age, prolonged interval from diagnosis to transplantation, high lactate dehydrogenase, refractory disease, bone marrow involvement, low performance scores, and transplantation between 1990 and 1993 were associated with adverse outcomes. TBI use was associated with higher TRM but lower recurrence. No association existed between acute or chronic GVHD disease and recurrence after allo-SCT [5].

Hosing et al. retrospectively compared the outcomes of myeloablative allo-SCT versus ASCT in patients with refractory or recurrent indolent NHL. Of 112 patients, 68 patients had undergone ASCT and 44 had undergone allo-SCT. In the allo-SCT group, the median follow-up time was 53 months (range, 21–113), and the OS and DFS rates were 49 and 45 %, respectively. After a median follow-up time of 71 months (range, 22–109) in the ASCT group, the OS and DFS rates were 34 and 17 %, respectively. The probability of disease progression was significantly higher in the autologous HSCT group than it was in the allogeneic HSCT group (74 % vs. 19 %, $P=0.003$) [164]. Ingram et al. analyzed 126 patients with relapsed advanced-stage FL who received BEAM–alemtuzumab allo-SCT ($N=44$) or BEAM-ASCT ($N=82$). The allogeneic group had a younger median age but had received a higher median number of therapies pre-transplant. The allogeneic group had a higher NRM than did the autologous group at 1 year (20 % vs. 2 %, $P=0.001$). Older age and heavily pretreated patients were associated with a high NRM and poor survival in the allogeneic group. There was a significantly lower relapse rate (20 % vs. 43 %, $P=0.01$) at 3 years in the allo-SCT group with no relapses after 2 years compared with a continued pattern of relapse in the autologous group. No difference in OS or DFS was identified at 3 years, whereas a plateau in OS and DFS with crossing of the survival curves in favor of the allogeneic group was observed [165].

Using the EBMT registry, Peniket et al. analyzed 1,185 allogeneic transplants for lymphoma reported to the registry between 1982 and 1998 and compared the results with those of 14,687 autologous procedures performed over the same period [4]. Two hundred and thirty-one patients had low-grade histology, and actuarial 4-year OS after transplantation was 51 % for this subgroup. These outcomes were relatively poor because of the high procedure-related mortality. Multivariate analysis showed that status at transplantation significantly affected outcome. A matched analysis was performed: for all categories of lymphoma, OS was better for autologous than for allogeneic transplantation, and the relapse rate was lower in the allogeneic group [4].

Preliminary results using NMA preparative regimens for allo-SCT have been very encouraging, with less than 15 % TRM [96, 166–169]. One of the first reports was published by Khouri et al., who treated 20 patients with indolent histology NHL with NMA allo-SCT. The day 100 mortality was 10 %, and after a median follow-up duration of 21 months, the actuarial probability of being alive and in remission was 84 % [170]. In a follow-up report on 47 patients with FL who had received a NMA conditioning regimen followed by allo-SCT, all patients achieved a CR after transplant. With a median follow-up time of 60 months (range, 19–94), the estimated OS and PFS rates of that study were 85 and 83 %, respectively. The incidence of grade II–IV acute GVHD was only 11 % (Fig. 23.2) [171]. Rezvani et al. studied 62 patients with indolent ($N=46$) or transformed NHL ($N=16$) who had received allo-SCT from related or unrelated donors after a NMA conditioning of 2 Gy of TBI +/− fludarabine. The median follow-up time after transplant was 36.6 months. At 3 years, the estimated OS and PFS rates were 52 and 43 % for patients with indolent disease. Among survivors, the median Karnofsky performance status at last follow-up was 85 %. It is encouraging to note that long-term survivors reported good overall functional status [166].

In a retrospective analysis of 208 transplants reported to the CIBMTR between 1997 and 2002, Hari et al. compared traditional myeloablative conditioning regimens with RIC for FL. Patients who had received RIC were older and had had a longer time interval from diagnosis to transplant. Median follow-up of survivors was 50 months after myeloablative conditioning versus 35 months after

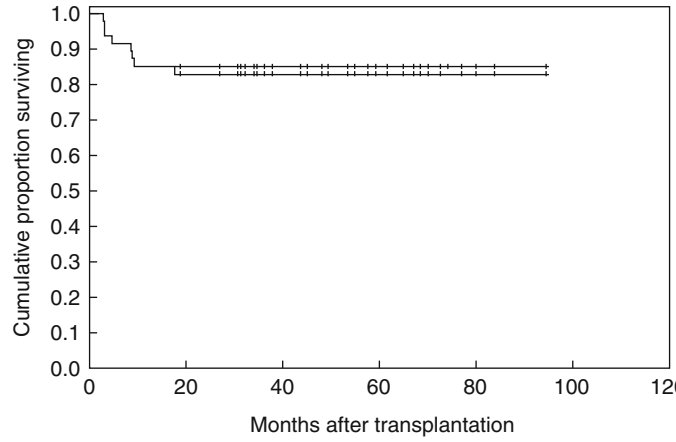

Fig. 23.2 Overall survival (*solid line*) and progression-free survival were 85 and 83 %, respectively, with a median follow-up of 60 months (range, 19–94 months) (This research was originally published in Khouri et al. [171]; © the American Society of Hematology)

RIC ($P<0.001$). Surprisingly, the OS, PFS, and TRM did not differ between the two groups. Lower performance score and resistance to chemotherapy were associated with higher TRM and lower OS and PFS rates. On multivariate analysis, an increased risk of lymphoma progression after RIC was observed ($RR=2.97$, $P=0.04$) [168]. Sorror et al. stratified outcomes by hematopoietic stem cell transplantation-comorbidity index (HSCT-CI). Patients in the NMA group were older, had more previous treatment and more comorbidities, more frequently had unrelated donors, and more often had malignancy in remission than did patients in the myeloablative group. After transplant, patients without comorbidities both in the NMA and myeloablative cohorts had comparable NRM, OS, and PFS. Patients with comorbidities experienced lower NRM and better OS after NMA conditioning. NMA allo-SCT recipients with comorbidities had favorable adjusted PFS compared with the patients in the myeloablative group [97].

Transformed FL

A prospective phase II study of patients who had relapsed transformed FL was performed before rituximab was included in standard treatment. Patients in CR or PR after salvage chemotherapy were eligible for HDT and ASCT. Forty-seven patients from five Norwegian centers were included, of whom 63 % received ASCT. Median follow-up for the surviving patients was 75 months; median PFS and OS were 26 and 47 months, respectively. Median OS for all patients was 43 months, compared to only 10 months for patients not eligible for ASCT. Patients receiving CD34(+) enriched/B cell-depleted grafts had inferior PFS and a trend for inferior OS compared to patients receiving non-purged grafts. The study found that after ASCT for transformed FL, majority of patients achieved CR and a significant number had prolonged OS. The use of in vitro purged grafts did not result in a survival benefit compared to that of non-purged grafts [172]. Rezvani et al. examined the outcome of NMA allo-SCT in patients with transformed FL. Sixty-two patients with indolent ($N=46$) or transformed NHL ($N=16$) were treated with allo-SCT from matched donors after conditioning with 2 Gy of TBI +/− fludarabine. Twenty patients had undergone prior ASCT. Median age was 54 years, and patients had received a median of six lines of treatment before transplant. Median follow-up time after transplant was 36.6 months. At 3 years, the estimated OS and PFS rates were 18 and 21 %, respectively, for patients with transformed disease. Thus, NMA allo-SCT can produce durable DFS in patients with transformed FL although the results are inferior to those without transformation [166].

Secondary Malignancies

One of the long-term side effect of HDT and ASCT has been the development of secondary

malignancy. A recent analysis of 1,347 patients with lymphoma treated with a high-dose sequential (HDS) program studied this issue. A total of 1,024 patients with B cell lymphoma, 234 patients with Hodgkin's lymphoma, and 89 patients with T cell lymphoma were included. The cumulative incidence at 5 and 10 years of s-MDS/AL was 3.09 and 4.52 %, respectively, and that of solid tumors was 2.54 and 6.79 %, respectively. Factors associated with the development of s-MDS/AL were male sex and use of the second harvest PBPC for the graft; factors found to be associated with solid tumor were advanced age, post-HDS radiotherapy, and rituximab addition to HDS. However, despite the increased risk of solid tumors, rituximab addition to HDS was still associated with survival advantages [173].

Selected patients with FL may benefit from ASCT, but it is still uncertain which subset of patients may receive the most benefit. Patients with relapsed, chemosensitive FL should be considered for ASCT as several phase II studies have shown survival advantage and prolonged remissions [139, 174]. It is reasonable to expect that the addition of monoclonal antibodies during stem cell collection for in vivo purging will reduce tumor contamination of the graft and reduce relapse rates. The role of ASCT for FL in rituximab era has been questioned by some, but preliminary data suggests that ASCT is still beneficial in patients with FL who relapse following rituximab-containing regimens [158, 175]. Similarly incorporation of monoclonal antibodies or radioimmunoconjugates during the ASCT may improve the long-term outcomes. Concern remains however regarding the increased risk of s-MDS/AL observed by some investigators. The patients who appear to benefit most from this strategy are patients who have chemosensitive disease, have received less than three chemotherapy regimens, and do not have high-risk FLIPI scores [176]. Maintenance therapy after transplantation may also be considered in a subset of patients although data is lacking in FL patients. Despite four large randomized studies comparing up-front ASCT with standard chemotherapy in FL, no survival advantage was noted for the transplant arm [143, 144]. Only one up-front randomized study included rituximab in the treatment regimen, and that study also failed to show a survival advantage for ASCT despite longer EFS [156]. Allo-SCT can cure patients with FL and should be offered to patients who are young, have a matched donor, and are beyond first CR. Use of NMA or RIC regimens appears very promising, but more studies and longer follow-up are needed before definite treatment recommendations can be made. There is concern regarding higher relapse rates after NMA or RIC regimens. Use of monoclonal antibodies and radioimmunoconjugates in the allo-SCT regimens may improve outcomes [171, 177]. All patients should be enrolled in clinical trials whenever possible.

References

1. Schmitz N, Trumper L, Ziepert M. Treatment and prognosis of mature T-cell and NK-cell lymphoma: an analysis of patients with T-cell lymphoma treated in studies of the German High-Grade Non-Hodgkin Lymphoma Study Group. Blood. 2010;116:3418–25.
2. Gisselbrecht C, Gaulard P, Lepage E, et al. Prognostic significance of T-cell phenotype in aggressive non-Hodgkin's lymphomas. Groupe d'Etudes des Lymphomes de l'Adulte (GELA). Blood. 1998;92:76–82.
3. Gale RP, Champlin RE. How does bone-marrow transplantation cure leukaemia? Lancet. 1984;2:28–30.
4. Peniket AJ, Ruiz de Elvira MC, Taghipour G. An EBMT registry matched study of allogeneic stem cell transplants for lymphoma: allogeneic transplantation is associated with a lower relapse rate but a higher procedure-related mortality rate than autologous transplantation. Bone Marrow Transplant. 2003;31:667–78.
5. van Besien K, Loberiza Jr FR, Bajorunaite R, et al. Comparison of autologous and allogeneic hematopoietic stem cell transplantation for follicular lymphoma. Blood. 2003;102:3521–9.
6. Bierman PJ, Sweetenham JW, Loberiza Jr FR, et al. Syngeneic hematopoietic stem-cell transplantation for non-Hodgkin's lymphoma: a comparison with allogeneic and autologous transplantation—The Lymphoma Working Committee of the International Bone Marrow Transplant Registry and the European Group for Blood and Marrow Transplantation. J Clin Oncol. 2003;21:3744–53.
7. Rondon G, Giralt S, Huh Y, et al. Graft-versus-leukemia effect after allogeneic bone marrow transplantation for chronic lymphocytic leukemia. Bone Marrow Transplant. 1996;18:669–72.
8. van Besien KW, de Lima M, Giralt SA, et al. Management of lymphoma recurrence after allogeneic transplantation: the relevance of graft-versus-lymphoma effect. Bone Marrow Transplant. 1997;19:977–82.

9. Kolb HJ, Schattenberg A, Goldman JM, et al. Graft-versus-leukemia effect of donor lymphocyte transfusions in marrow grafted patients. Blood. 1995;86:2041–50.
10. Armand P, Kim HT, Ho VT, et al. Allogeneic transplantation with reduced-intensity conditioning for Hodgkin and non-Hodgkin lymphoma: importance of histology for outcome. Biol Blood Marrow Transplant. 2008;14:418–25.
11. van Besien KW, Mehra RC, Giralt SA, et al. Allogeneic bone marrow transplantation for poor-prognosis lymphoma: response, toxicity and survival depend on disease histology. Am J Med. 1996;100:299–307.
12. Khouri IF, Keating M, Korbling M, et al. Transplant-lite: induction of graft-versus-malignancy using fludarabine-based nonablative chemotherapy and allogeneic blood progenitor-cell transplantation as treatment for lymphoid malignancies. J Clin Oncol. 1998;16:2817–24.
13. Antin JH, Ferrara JL. Cytokine dysregulation and acute graft-versus-host disease. Blood. 1992;80:2964–8.
14. Hill GR, Crawford JM, Cooke KR, et al. Total body irradiation and acute graft-versus-host disease: the role of gastrointestinal damage and inflammatory cytokines. Blood. 1997;90:3204–13.
15. Surbone A, Armitage JO, Gale RP. Autotransplantation in lymphoma: better therapy or healthier patients? Ann Intern Med. 1991;114:1059–60.
16. Sehn LH, Berry B, Chhanabhai M, et al. The revised International Prognostic Index (R-IPI) is a better predictor of outcome than the standard IPI for patients with diffuse large B-cell lymphoma treated with R-CHOP. Blood. 2007;109:1857–61.
17. Philip T, Guglielmi C, Hagenbeek A, et al. Autologous bone marrow transplantation as compared with salvage chemotherapy in relapses of chemotherapy-sensitive non-Hodgkin's lymphoma. N Engl J Med. 1995;333:1540–5.
18. Guglielmi C, Gomez F, Philip T, et al. Time to relapse has prognostic value in patients with aggressive lymphoma enrolled onto the Parma trial. J Clin Oncol. 1998; 16:3264–9.
19. Blay J, Gomez F, Sebban C, et al. The International Prognostic Index correlates to survival in patients with aggressive lymphoma in relapse: analysis of the PARMA trial. Parma Group. Blood. 1998;92:3562–8.
20. Prince HM, Imrie K, Crump M, et al. The role of intensive therapy and autologous blood and marrow transplantation for chemotherapy-sensitive relapsed and primary refractory non-Hodgkin's lymphoma: identification of major prognostic groups. Br J Haematol. 1996; 92:880–9.
21. Colombat P, Gorin NC, Lemonnier MP, et al. The role of autologous bone marrow transplantation in 46 adult patients with non-Hodgkin's lymphomas. J Clin Oncol. 1990;8:630–7.
22. Weisdorf DJ, Haake R, Miller WJ, et al. Autologous bone marrow transplantation for progressive non-Hodgkin's lymphoma: clinical impact of immunophenotype and in vitro purging. Bone Marrow Transplant. 1991;8:135–42.
23. Lazarus HM, Crilley P, Ciobanu N, et al. High-dose carmustine, etoposide, and cisplatin and autologous bone marrow transplantation for relapsed and refractory lymphoma. J Clin Oncol. 1992;10:1682–9.
24. Vose JM, Anderson JR, Kessinger A, et al. High-dose chemotherapy and autologous hematopoietic stem-cell transplantation for aggressive non-Hodgkin's lymphoma. J Clin Oncol. 1993;11:1846–51.
25. Wheeler C, Strawderman M, Ayash L, et al. Prognostic factors for treatment outcome in autotransplantation of intermediate-grade and high-grade non-Hodgkin's lymphoma with cyclophosphamide, carmustine, and etoposide. J Clin Oncol. 1993;11:1085–91.
26. Mills W, Chopra R, McMillan A, et al. BEAM chemotherapy and autologous bone marrow transplantation for patients with relapsed or refractory non-Hodgkin's lymphoma. J Clin Oncol. 1995;13:588–95.
27. van Besien K, Tabocoff J, Rodriguez M, et al. High-dose chemotherapy with BEAC regimen and autologous bone marrow transplantation for intermediate grade and immunoblastic lymphoma: durable complete remissions, but a high rate of regimen-related toxicity. Bone Marrow Transplant. 1995;15: 549–55.
28. Ladenstein R, Pearce R, Hartmann O, et al. High-dose chemotherapy with autologous bone marrow rescue in children with poor-risk Burkitt's lymphoma: a report from the European Lymphoma Bone Marrow Transplantation Registry. Blood. 1997;90:2921–30.
29. Petersen FB, Appelbaum FR, Hill R, et al. Autologous marrow transplantation for malignant lymphoma: a report of 101 cases from Seattle. J Clin Oncol. 1990;8: 638–47.
30. Rapoport AP, Lifton R, Constine LS, et al. Autotransplantation for relapsed or refractory non-Hodgkin's lymphoma (NHL): long-term follow-up and analysis of prognostic factors. Bone Marrow Transplant. 1997;19:883–90.
31. Phillips GL, Fay JW, Herzig RH, et al. The treatment of progressive non-Hodgkin's lymphoma with intensive chemoradiotherapy and autologous marrow transplantation. Blood. 1990;75:831–8.
32. Weaver CH, Petersen FB, Appelbaum FR, et al. High-dose fractionated total-body irradiation, etoposide, and cyclophosphamide followed by autologous stem-cell support in patients with malignant lymphoma. J Clin Oncol. 1994;12:2559–66.
33. Gisselbrecht C, Glass B, Mounier N, et al. Salvage regimens with autologous transplantation for relapsed large B-cell lymphoma in the rituximab era. J Clin Oncol. 2010;28:4184–90.
34. Horwitz SM, Negrin RS, Blume KG, et al. Rituximab as adjuvant to high-dose therapy and autologous hematopoietic cell transplantation for aggressive non-Hodgkin lymphoma. Blood. 2004;103:777–83.
35. Khouri IF, Saliba RM, Hosing C, et al. Concurrent administration of high-dose rituximab before and after autologous stem-cell transplantation for relapsed aggressive B-cell non-Hodgkin's lymphomas. J Clin Oncol. 2005;23:2240–7.

36. Vose JM, Bierman PJ, Enke C, et al. Phase I trial of iodine-131 tositumomab with high-dose chemotherapy and autologous stem-cell transplantation for relapsed non-Hodgkin's lymphoma. J Clin Oncol. 2005;23: 461–7.
37. Krishnan A, Palmer JM, Tsai NC. Matched-cohort analysis of autologous hematopoietic cell transplantation with radioimmunotherapy- versus total body irradiation-based conditioning for poor-risk diffuse large cell lymphoma. Biol Blood Marrow Transplant. 2011;18(3):441–50.
38. Haioun C, Lepage E, Gisselbrecht C, et al. Survival benefit of high-dose therapy in poor-risk aggressive non-Hodgkin's lymphoma: final analysis of the prospective LNH87–2 protocol—a groupe d'Etude des lymphomes de l'Adulte study. J Clin Oncol. 2000; 18:3025–30.
39. Gisselbrecht C, Lepage E, Molina T, et al. Shortened first-line high-dose chemotherapy for patients with poor-prognosis aggressive lymphoma. J Clin Oncol. 2002;20:2472–9.
40. Gianni AM, Bregni M, Siena S, et al. High-dose chemotherapy and autologous bone marrow transplantation compared with MACOP-B in aggressive B-cell lymphoma. N Engl J Med. 1997;336:1290–7.
41. Kaiser U, Uebelacker I, Abel U, et al. Randomized study to evaluate the use of high-dose therapy as part of primary treatment for "aggressive" lymphoma. J Clin Oncol. 2002;20:4413–9.
42. Santini G, Salvagno L, Leoni P, et al. VACOP-B versus VACOP-B plus autologous bone marrow transplantation for advanced diffuse non-Hodgkin's lymphoma: results of a prospective randomized trial by the non-Hodgkin's Lymphoma Cooperative Study Group. J Clin Oncol. 1998;16:2796–802.
43. Kluin-Nelemans HC, Zagonel V, Anastasopoulou A, et al. Standard chemotherapy with or without high-dose chemotherapy for aggressive non-Hodgkin's lymphoma: randomized phase III EORTC study. J Natl Cancer Inst. 2001;93:22–30.
44. Milpied N, Deconinck E, Gaillard F, et al. Initial treatment of aggressive lymphoma with high-dose chemotherapy and autologous stem-cell support. N Engl J Med. 2004;350:1287–95.
45. Martelli M, Gherlinzoni F, De Renzo A, et al. Early autologous stem-cell transplantation versus conventional chemotherapy as front-line therapy in high-risk, aggressive non-Hodgkin's lymphoma: an Italian multicenter randomized trial. J Clin Oncol. 2003;21: 1255–62.
46. Greb A, Bohlius J, Trelle S, et al. High-dose chemotherapy with autologous stem cell support in first-line treatment of aggressive non-Hodgkin lymphoma—results of a comprehensive meta-analysis. Cancer Treat Rev. 2007;33:338–46.
47. Haioun C, Mounier N, Emile JF, et al. Rituximab versus observation after high-dose consolidative first-line chemotherapy with autologous stem-cell transplantation in patients with poor-risk diffuse large B-cell lymphoma. Ann Oncol. 2009;20:1985–92.
48. Tarella C, Zanni M, Di Nicola M, et al. Prolonged survival in poor-risk diffuse large B-cell lymphoma following front-line treatment with rituximab-supplemented, early-intensified chemotherapy with multiple autologous hematopoietic stem cell support: a multicenter study by GITIL (Gruppo Italiano Terapie Innovative nei Linfomi). Leukemia. 2007;21:1802–11.
49. Fitoussi O, Belhadj K, Mounier N, et al. Survival impact of rituximab combined with ACVBP and upfront consolidative autotransplantation in high risk diffuse large B-cell lymphoma for GELA. Haematologica. 2011;96:1136–43.
50. Vitolo U, Chiappella A, Angelucci E, et al. Dose-dense and high-dose chemotherapy plus rituximab with autologous stem cell transplantation for primary treatment of diffuse large B-cell lymphoma with a poor prognosis: a phase II multicenter study. Haematologica. 2009;94:1250–8.
51. Tsirigotis P, Dray L, Resnick IB. Post-autologous stem cell transplantation administration of rituximab improves the outcome of patients with aggressive B cell non-Hodgkin's lymphoma. Ann Hematol. 2010;89: 263–72.
52. Vose JM, Zhang MJ, Rowlings PA, et al. Autologous transplantation for diffuse aggressive non-Hodgkin's lymphoma in patients never achieving remission: a report from the Autologous Blood and Marrow Transplant Registry. J Clin Oncol. 2001;19:406–13.
53. Kewalramani T, Zelenetz AD, Hedrick EE, et al. High-dose chemoradiotherapy and autologous stem cell transplantation for patients with primary refractory aggressive non-Hodgkin lymphoma: an intention-to-treat analysis. Blood. 2000;96:2399–404.
54. Sweetenham JW, Pearce R, Taghipour G, et al. Adult Burkitt's and Burkitt-like non-Hodgkin's lymphoma—outcome for patients treated with high-dose therapy and autologous stem-cell transplantation in first remission or at relapse: results from the European Group for Blood and Marrow Transplantation. J Clin Oncol. 1996;14:2465–72.
55. Jost LM, Jacky E, Dommann-Scherrer C, et al. Short-term weekly chemotherapy followed by high-dose therapy with autologous bone marrow transplantation for lymphoblastic and Burkitt's lymphomas in adult patients. Ann Oncol. 1995;6:445–51.
56. Santini G, Congiu AM, Coser P, et al. Autologous bone marrow transplantation for adult advanced stage lymphoblastic lymphoma in first CR. A study of the NHLCSG. Leukemia. 1991;5(Suppl 1):42–5.
57. Sweetenham JW, Liberti G, Pearce R, et al. High-dose therapy and autologous bone marrow transplantation for adult patients with lymphoblastic lymphoma: results of the European Group for Bone Marrow Transplantation. J Clin Oncol. 1994;12:1358–65.
58. Sweetenham JW, Santini G, Qian W, et al. High-dose therapy and autologous stem-cell transplantation versus conventional-dose consolidation/maintenance therapy as postremission therapy for adult patients with lymphoblastic lymphoma: results of a randomized trial of the European Group for Blood and

Marrow Transplantation and the United Kingdom Lymphoma Group. J Clin Oncol. 2001;19:2927–36.
59. Sharp JG, Kessinger A, Mann S, et al. Outcome of high-dose therapy and autologous transplantation in non-Hodgkin's lymphoma based on the presence of tumor in the marrow or infused hematopoietic harvest. J Clin Oncol. 1996;14:214–9.
60. Williams CD, Goldstone AH, Pearce RM, et al. Purging of bone marrow in autologous bone marrow transplantation for non-Hodgkin's lymphoma: a case-matched comparison with unpurged cases by the European Blood and Marrow Transplant Lymphoma Registry. J Clin Oncol. 1996;14:2454–64.
61. Chopra R, Goldstone AH, Pearce R, et al. Autologous versus allogeneic bone marrow transplantation for non-Hodgkin's lymphoma: a case-controlled analysis of the European Bone Marrow Transplant Group Registry data. J Clin Oncol. 1992;10:1690–5.
62. van Kampen RJ, Canals C, Schouten HC. Allogeneic stem-cell transplantation as salvage therapy for patients with diffuse large B-cell non-Hodgkin's lymphoma relapsing after an autologous stem-cell transplantation: an analysis of the European Group for Blood and Marrow Transplantation Registry. J Clin Oncol. 2011;29:1342–8.
63. Sirvent A, Dhedin N, Michallet M. Low nonrelapse mortality and prolonged long-term survival after reduced-intensity allogeneic stem cell transplantation for relapsed or refractory diffuse large B cell lymphoma: report of the Societe Francaise de Greffe de Moelle et de Therapie Cellulaire. Biol Blood Marrow Transplant. 2010;16:78–85.
64. Rodrigues CA, Sanz G, Brunstein CG, et al. Analysis of risk factors for outcomes after unrelated cord blood transplantation in adults with lymphoid malignancies: a study by the Eurocord-Netcord and lymphoma working party of the European group for blood and marrow transplantation. J Clin Oncol. 2009;27:256–63.
65. Wang HX, Yan HM, Liu J, et al. Haploidentical hematopoietic stem-cell transplantation for non-Hodgkin lymphoma with bone marrow involvement. Leuk Lymphoma. 2009;50:1488–93.
66. Reni M, Ferreri AJ, Garancini MP, et al. Therapeutic management of primary central nervous system lymphoma in immunocompetent patients: results of a critical review of the literature. Ann Oncol. 1997;8:227–34.
67. Correa DD, Maron L, Harder H, et al. Cognitive functions in primary central nervous system lymphoma: literature review and assessment guidelines. Ann Oncol. 2007;18:1145–51.
68. Thiel E, Korfel A, Martus P. High-dose methotrexate with or without whole brain radiotherapy for primary CNS lymphoma (G-PCNSL-SG-1): a phase 3, randomised, non-inferiority trial. Lancet Oncol. 2010;11:1036–47.
69. Abrey LE, Batchelor TT, Ferreri AJ, Gospodarowicz M, Pulczynski EJ, Zucca E, et al. Report of an international workshop to standardize baseline evaluation and response criteria for primary CNS lymphoma. J Clin Oncol. 2005;23(22):5034–43. Epub 2005 Jun 13.
70. Soussain C, Hoang-Xuan K, Taillandier L, et al. Intensive chemotherapy followed by hematopoietic stem-cell rescue for refractory and recurrent primary CNS and intraocular lymphoma: Societe Francaise de Greffe de Moelle Osseuse-Therapie Cellulaire. J Clin Oncol. 2008;26:2512–8.
71. Abrey LE, Moskowitz CH, Mason WP, et al. Intensive methotrexate and cytarabine followed by high-dose chemotherapy with autologous stem-cell rescue in patients with newly diagnosed primary CNS lymphoma: an intent-to-treat analysis. J Clin Oncol. 2003;21:4151–6.
72. Illerhaus G, Marks R, Ihorst G, et al. High-dose chemotherapy with autologous stem-cell transplantation and hyperfractionated radiotherapy as first-line treatment of primary CNS lymphoma. J Clin Oncol. 2006;24:3865–70.
73. Illerhaus G, Muller F, Feuerhake F, et al. High-dose chemotherapy and autologous stem-cell transplantation without consolidating radiotherapy as first-line treatment for primary lymphoma of the central nervous system. Haematologica. 2008;93:147–8.
74. Krishnan A, Palmer JM, Zaia JA. HIV status does not affect the outcome of autologous stem cell transplantation (ASCT) for non-Hodgkin lymphoma (NHL). Biol Blood Marrow Transplant. 2010;16:1302–8.
75. Re A, Michieli M, Casari S, et al. High-dose therapy and autologous peripheral blood stem cell transplantation as salvage treatment for AIDS-related lymphoma: long-term results of the Italian Cooperative Group on AIDS and Tumors (GICAT) study with analysis of prognostic factors. Blood. 2009;114:1306–13.
76. Freedman AS, Neuberg D, Gribben JG, et al. High-dose chemoradiotherapy and anti-B-cell monoclonal antibody-purged autologous bone marrow transplantation in mantle-cell lymphoma: no evidence for long-term remission. J Clin Oncol. 1998;16:13–8.
77. Khouri IF, Romaguera J, Kantarjian H, et al. Hyper-CVAD and high-dose methotrexate/cytarabine followed by stem-cell transplantation: an active regimen for aggressive mantle-cell lymphoma. J Clin Oncol. 1998;16:3803–9.
78. Milpied N, Gaillard F, Moreau P, et al. High-dose therapy with stem cell transplantation for mantle cell lymphoma: results and prognostic factors, a single center experience. Bone Marrow Transplant. 1998;22:645–50.
79. Vigouroux S, Gaillard F, Moreau P, et al. High-dose therapy with autologous stem cell transplantation in first response in mantle cell lymphoma. Haematologica. 2005;90:1580–2.
80. Murali S, Winton E, Waller EK, et al. Long-term progression-free survival after early autologous transplantation for mantle-cell lymphoma. Bone Marrow Transplant. 2008;42:529–34.
81. Gianni AM, Magni M, Martelli M, et al. Long-term remission in mantle cell lymphoma following high-dose sequential chemotherapy and in vivo rituximab-purged stem cell autografting (R-HDS regimen). Blood. 2003;102:749–55.

82. Vose JM, Bierman PJ, Weisenburger DD, et al. Autologous hematopoietic stem cell transplantation for mantle cell lymphoma. Biol Blood Marrow Transplant. 2000;6:640–5.
83. Vandenberghe E, Ruiz de Elvira C, Loberiza FR. Outcome of autologous transplantation for mantle cell lymphoma: a study by the European Blood and Bone Marrow Transplant and Autologous Blood and Marrow Transplant Registries. Br J Haematol. 2003;120:793–800.
84. Dreyling M, Lenz G, Hoster E, et al. Early consolidation by myeloablative radiochemotherapy followed by autologous stem cell transplantation in first remission significantly prolongs progression-free survival in mantle-cell lymphoma: results of a prospective randomized trial of the European MCL Network. Blood. 2005;105:2677–84.
85. Pott C, Schrader C, Gesk S, et al. Quantitative assessment of molecular remission after high-dose therapy with autologous stem cell transplantation predicts long-term remission in mantle cell lymphoma. Blood. 2006;107:2271–8.
86. Geisler CH, Kolstad A, Laurell A, et al. Long-term progression-free survival of mantle cell lymphoma after intensive front-line immunochemotherapy with in vivo-purged stem cell rescue: a nonrandomized phase 2 multicenter study by the Nordic Lymphoma Group. Blood. 2008;112:2687–93.
87. Tam CS, Bassett R, Ledesma C. Mature results of the M. D. Anderson Cancer Center risk-adapted transplantation strategy in mantle cell lymphoma. Blood. 2009;113:4144–52.
88. Dreger P, Martin S, Kuse R, et al. The impact of autologous stem cell transplantation on the prognosis of mantle cell lymphoma: a joint analysis of two prospective studies with 46 patients. Hematol J. 2000;1:87–94.
89. Dietrich S, Tielesch B, Rieger M. Patterns and outcome of relapse after autologous stem cell transplantation for mantle cell lymphoma. Cancer. 2011;117(9): 1901–10.
90. Budde LE, Guthrie KA, Till BG. Mantle cell lymphoma international prognostic index but not pretransplantation induction regimen predicts survival for patients with mantle-cell lymphoma receiving high-dose therapy and autologous stem-cell transplantation. J Clin Oncol. 2011;29(22):3023–9.
91. Gopal AK, Rajendran JG, Petersdorf SH, et al. High-dose chemo-radioimmunotherapy with autologous stem cell support for relapsed mantle cell lymphoma. Blood. 2002;99:3158–62.
92. Adkins D, Brown R, Goodnough LT, et al. Treatment of resistant mantle cell lymphoma with allogeneic bone marrow transplantation. Bone Marrow Transplant. 1998;21:97–9.
93. Khouri IF, Lee MS, Romaguera J, et al. Allogeneic hematopoietic transplantation for mantle-cell lymphoma: molecular remissions and evidence of graft-versus-malignancy. Ann Oncol. 1999;10:1293–9.
94. Ganti AK, Bierman PJ, Lynch JC, et al. Hematopoietic stem cell transplantation in mantle cell lymphoma. Ann Oncol. 2005;16:618–24.
95. Maris MB, Sandmaier BM, Storer BE, et al. Allogeneic hematopoietic cell transplantation after fludarabine and 2 Gy total body irradiation for relapsed and refractory mantle cell lymphoma. Blood. 2004;104:3535–42.
96. Robinson SP, Goldstone AH, Mackinnon S, et al. Chemoresistant or aggressive lymphoma predicts for a poor outcome following reduced-intensity allogeneic progenitor cell transplantation: an analysis from the Lymphoma Working Party of the European Group for Blood and Bone Marrow Transplantation. Blood. 2002;100:4310–6.
97. Sorror ML, Storer BE, Maloney DG, et al. Outcomes after allogeneic hematopoietic cell transplantation with nonmyeloablative or myeloablative conditioning regimens for treatment of lymphoma and chronic lymphocytic leukemia. Blood. 2008;111:446–52.
98. Rifkind J, Mollee P, Messner HA, et al. Allogeneic stem cell transplantation for mantle cell lymphoma—does it deserve a better look? Leuk Lymphoma. 2005;46:217–23.
99. Morris E, Thomson K, Craddock C, et al. Outcomes after alemtuzumab-containing reduced-intensity allogeneic transplantation regimen for relapsed and refractory non-Hodgkin lymphoma. Blood. 2004;104:3865–71.
100. Tam CS, Khouri IF. Autologous and allogeneic stem cell transplantation: rising therapeutic promise for mantle cell lymphoma. Leuk Lymphoma. 2009;50:1239–48.
101. Corradini P, Tarella C, Zallio F, et al. Long-term follow-up of patients with peripheral T-cell lymphomas treated up-front with high-dose chemotherapy followed by autologous stem cell transplantation. Leukemia. 2006;20:1533–8.
102. Reimer P, Rudiger T, Geissinger E, et al. Autologous stem-cell transplantation as first-line therapy in peripheral T-cell lymphomas: results of a prospective multicenter study. J Clin Oncol. 2009;27:106–13.
103. D'Amore F, Relander T, Lauritzen G, et al. Dose-dense induction followed by autologous stem cell transplant (ASCT) leads to sustained remissions in a large fraction of patients with previously untreated peripheral t-cell lymphomas (PTCLs)—overall and subtype-specific results of a phase II study from the Nordic Lymphoma Group. [abstract #53]. Presented at the annual meeting of the European Hematology Association, Berlin, 2009. EHA, 2009.
104. Rodriguez J, Conde E, Gutierrez A, et al. Frontline autologous stem cell transplantation in high-risk peripheral T-cell lymphoma: a prospective study from The Gel-Tamo Study Group. Eur J Haematol. 2007;79:32–8.
105. Mercadal S, Briones J, Xicoy B, et al. Intensive chemotherapy (high-dose CHOP/ESHAP regimen) followed by autologous stem-cell transplantation in previously untreated patients with peripheral T-cell lymphoma. Ann Oncol. 2008;19:958–63.
106. Rodriguez J, Conde E, Gutierrez A, et al. The results of consolidation with autologous stem-cell transplantation in patients with peripheral T-cell lymphoma (PTCL) in first complete remission: the

Spanish Lymphoma and Autologous Transplantation Group experience. Ann Oncol. 2007;18:652–7.
107. Mounier N, Gisselbrecht C, Briere J, et al. Prognostic factors in patients with aggressive non-Hodgkin's lymphoma treated by front-line autotransplantation after complete remission: a cohort study by the Groupe d'Etude des Lymphomes de l'Adulte. J Clin Oncol. 2004;22:2826–34.
108. Kyriakou C, Canals C, Goldstone A, et al. High-dose therapy and autologous stem-cell transplantation in angioimmunoblastic lymphoma: complete remission at transplantation is the major determinant of Outcome-Lymphoma Working Party of the European Group for Blood and Marrow Transplantation. J Clin Oncol. 2008;26:218–24.
109. Al-Toma A, Verbeek WH, Visser OJ, et al. Disappointing outcome of autologous stem cell transplantation for enteropathy-associated T-cell lymphoma. Dig Liver Dis. 2007;39:634–41.
110. Bishton MJ, Haynes AP. Combination chemotherapy followed by autologous stem cell transplant for enteropathy-associated T cell lymphoma. Br J Haematol. 2007;136:111–3.
111. Sieniawski M, Angamuthu N, Boyd K. Evaluation of enteropathy-associated T-cell lymphoma comparing standard therapies with a novel regimen including autologous stem cell transplantation. Blood. 2010;115:3664–70.
112. Rodriguez J, Munsell M, Yazji S, et al. Impact of high-dose chemotherapy on peripheral T-cell lymphomas. J Clin Oncol. 2001;19:3766–70.
113. Blystad AK, Enblad G, Kvaloy S, et al. High-dose therapy with autologous stem cell transplantation in patients with peripheral T cell lymphomas. Bone Marrow Transplant. 2001;27:711–6.
114. Rodriguez J, Conde E, Gutierrez A, et al. Prolonged survival of patients with angioimmunoblastic T-cell lymphoma after high-dose chemotherapy and autologous stem cell transplantation: the GELTAMO experience. Eur J Haematol. 2007;78:290–6.
115. Schetelig J, Fetscher S, Reichle A, et al. Long-term disease-free survival in patients with angioimmunoblastic T-cell lymphoma after high-dose chemotherapy and autologous stem cell transplantation. Haematologica. 2003;88:1272–8.
116. Zamkoff KW, Matulis MD, Mehta AC, et al. High-dose therapy and autologous stem cell transplant does not result in long-term disease-free survival in patients with recurrent chemotherapy-sensitive ALK-negative anaplastic large-cell lymphoma. Bone Marrow Transplant. 2004;33:635–8.
117. Jagasia M, Morgan D, Goodman S, et al. Histology impacts the outcome of peripheral T-cell lymphomas after high dose chemotherapy and stem cell transplant. Leuk Lymphoma. 2004;45:2261–7.
118. Jantunen E, Wiklund T, Juvonen E, et al. Autologous stem cell transplantation in adult patients with peripheral T-cell lymphoma: a nation-wide survey. Bone Marrow Transplant. 2004;33:405–10.
119. Kewalramani T, Zelenetz AD, Teruya-Feldstein J, et al. Autologous transplantation for relapsed or primary refractory peripheral T-cell lymphoma. Br J Haematol. 2006;134:202–7.
120. Kim MK, Kim S, Lee SS, et al. High-dose chemotherapy and autologous stem cell transplantation for peripheral T-cell lymphoma: complete response at transplant predicts survival. Ann Hematol. 2007;86:435–42.
121. Rodriguez J, Caballero MD, Gutierrez A. High dose chemotherapy and autologous stem cell transplantation in patients with peripheral T-cell lymphoma not achieving complete response after induction chemotherapy. The GEL-TAMO experience. Haematologica. 2003;88:1372–7.
122. Smith SD, Bolwell BJ, Rybicki LA, et al. Autologous hematopoietic stem cell transplantation in peripheral T-cell lymphoma using a uniform high-dose regimen. Bone Marrow Transplant. 2007;40:239–43.
123. Rodriguez J, Caballero MD, Gutierrez A, et al. High-dose chemotherapy and autologous stem cell transplantation in peripheral T-cell lymphoma: the GEL-TAMO experience. Ann Oncol. 2003;14:1768–75.
124. Feyler S, Prince HM, Pearce R, et al. The role of high-dose therapy and stem cell rescue in the management of T-cell malignant lymphomas: a BSBMT and ABMTRR study. Bone Marrow Transplant. 2007;40:443–50.
125. Rodriguez J, Conde E, Gutierrez A, et al. The adjusted International Prognostic Index and beta-2-microglobulin predict the outcome after autologous stem cell transplantation in relapsing/refractory peripheral T-cell lymphoma. Haematologica. 2007; 92:1067–74.
126. Vose JM, Peterson C, Bierman PJ, et al. Comparison of high-dose therapy and autologous bone marrow transplantation for T-cell and B-cell non-Hodgkin's lymphomas. Blood. 1990;76:424–31.
127. Fanin R, Ruiz de Elvira MC, Sperotto A. Autologous stem cell transplantation for T and null cell CD30-positive anaplastic large cell lymphoma: analysis of 64 adult and paediatric cases reported to the European Group for Blood and Marrow Transplantation (EBMT). Bone Marrow Transplant. 1999;23:437–42.
128. Oyama Y, Guitart J, Kuzel TM. High-dose therapy and bone marrow transplantation in cutaneous T-cell lymphoma. Hematol Oncol Clin North Am. 2003;17:1475–83. xi.
129. Russell-Jones R, Child F, Olavarria E, et al. Autologous peripheral blood stem cell transplantation in tumor-stage mycosis fungoides: predictors of disease-free survival. Ann N Y Acad Sci. 2001;941:147–54.
130. Kyriakou C, Canals C, Finke J, et al. Allogeneic stem cell transplantation is able to induce long-term remissions in angioimmunoblastic T-cell lymphoma: a retrospective study from the lymphoma working party of the European group for blood and marrow transplantation. J Clin Oncol. 2009;27:3951–8.
131. Lechowicz MJ, Agovi M, Carreras J, et al. Allogeneic hematopoietic cell transplantation (AHCT) for primary cutaneous T cell lymphoma (CTCL): a Center for International Blood and Marrow Transplant Research (CIBMTR) review. Blood. 2010;116:364.

132. Duvic M, Donato M, Dabaja B, et al. Total skin electron beam and non-myeloablative allogeneic hematopoietic stem-cell transplantation in advanced mycosis fungoides and Sezary syndrome. J Clin Oncol. 2010;28:2365–72.
133. Wu PA, Kim YH, Lavori PW, et al. A meta-analysis of patients receiving allogeneic or autologous hematopoietic stem cell transplant in mycosis fungoides and Sezary syndrome. Biol Blood Marrow Transplant. 2009;15:982–90.
134. Lee J, Au WY, Park MJ, et al. Autologous hematopoietic stem cell transplantation in extranodal natural killer/T cell lymphoma: a multinational, multicenter, matched controlled study. Biol Blood Marrow Transplant. 2008;14:1356–64.
135. Shustov AR, Gooley TA, Sandmaier BM. Allogeneic haematopoietic cell transplantation after nonmyeloablative conditioning in patients with T-cell and natural killer-cell lymphomas. Br J Haematol. 2010; 150:170–8.
136. Horning SJ. Follicular lymphoma: have we made any progress? Ann Oncol. 2000;11(Suppl 1):23–7.
137. Fisher RI, LeBlanc M, Press OW, et al. New treatment options have changed the survival of patients with follicular lymphoma. J Clin Oncol. 2005;23: 8447–52.
138. Swenson WT, Wooldridge JE, Lynch CF, et al. Improved survival of follicular lymphoma patients in the United States. J Clin Oncol. 2005;23:5019–26.
139. Rohatiner AZ, Nadler L, Davies AJ, et al. Myeloablative therapy with autologous bone marrow transplantation for follicular lymphoma at the time of second or subsequent remission: long-term follow-up. J Clin Oncol. 2007;25:2554–9.
140. Freedman AS, Neuberg D, Mauch P, et al. Long-term follow-up of autologous bone marrow transplantation in patients with relapsed follicular lymphoma. Blood. 1999;94:3325–33.
141. Bierman PJ, Vose JM, Anderson JR, et al. High-dose therapy with autologous hematopoietic rescue for follicular low-grade non-Hodgkin's lymphoma. J Clin Oncol. 1997;15:445–50.
142. Freedman AS, Gribben JG, Neuberg D, et al. High-dose therapy and autologous bone marrow transplantation in patients with follicular lymphoma during first remission. Blood. 1996;88:2780–6.
143. Lenz G, Dreyling M, Schiegnitz E, et al. Myeloablative radiochemotherapy followed by autologous stem cell transplantation in first remission prolongs progression-free survival in follicular lymphoma: results of a prospective, randomized trial of the German Low-Grade Lymphoma Study Group. Blood. 2004;104:2667–74.
144. Gyan E, Foussard C, Bertrand P, et al. High-dose therapy followed by autologous purged stem cell transplantation and doxorubicin-based chemotherapy in patients with advanced follicular lymphoma: a randomized multicenter study by the GOELAMS with final results after a median follow-up of 9 years. Blood. 2009;113:995–1001.
145. Sebban C, Mounier N, Brousse N, et al. Standard chemotherapy with interferon compared with CHOP followed by high-dose therapy with autologous stem cell transplantation in untreated patients with advanced follicular lymphoma: the GELF-94 randomized study from the Groupe d'Etude des Lymphomes de l'Adulte (GELA). Blood. 2006;108:2540–4.
146. Rohatiner AZ, Freedman A, Nadler L, et al. Myeloablative therapy with autologous bone marrow transplantation as consolidation therapy for follicular lymphoma. Ann Oncol. 1994;5(Suppl 2):143–6.
147. Cao TM, Horning S, Negrin RS, et al. High-dose therapy and autologous hematopoietic-cell transplantation for follicular lymphoma beyond first remission: the Stanford University experience. Biol Blood Marrow Transplant. 2001;7:294–301.
148. Schouten HC, Kvaloy S, Sydes M, et al. The CUP trial: a randomized study analyzing the efficacy of high dose therapy and purging in low-grade non-Hodgkin's lymphoma (NHL). Ann Oncol. 2000;11(Suppl 1): 91–4.
149. Tarella C, Zanni M, Magni M, et al. Rituximab improves the efficacy of high-dose chemotherapy with autograft for high-risk follicular and diffuse large B-cell lymphoma: a multicenter Gruppo Italiano Terapie Innovative nei linfomi survey. J Clin Oncol. 2008;26:3166–75.
150. Arcaini L, Montanari F, Alessandrino EP, et al. Immunochemotherapy with in vivo purging and autotransplant induces long clinical and molecular remission in advanced relapsed and refractory follicular lymphoma. Ann Oncol. 2008;19:1331–5.
151. Arcaini L, Orlandi E, Alessandrino EP, et al. A model of in vivo purging with Rituximab and high-dose AraC in follicular and mantle cell lymphoma. Bone Marrow Transplant. 2004;34:175–9.
152. Le Gouill S, De Guibert S, Planche L. Impact of the use of autologous stem cell transplantation at first relapse both in naive and previously rituximab exposed follicular lymphoma patients treated in the GELA/GOELAMS FL2000 study. Haematologica. 2011;96(8):1128–35.
153. Apostolidis J, Gupta RK, Grenzelias D, et al. High-dose therapy with autologous bone marrow support as consolidation of remission in follicular lymphoma: long-term clinical and molecular follow-up. J Clin Oncol. 2000;18:527–36.
154. Sebban C, Brice P, Delarue R, et al. Impact of rituximab and/or high-dose therapy with autotransplant at time of relapse in patients with follicular lymphoma: a GELA study. J Clin Oncol. 2008;26:3614–20.
155. Horning SJ, Negrin RS, Hoppe RT, et al. High-dose therapy and autologous bone marrow transplantation for follicular lymphoma in first complete or partial remission: results of a phase II clinical trial. Blood. 2001;97:404–9.
156. Ladetto M, De Marco F, Benedetti F, et al. Prospective, multicenter randomized GITMO/IIL trial comparing intensive (R-HDS) versus conventional (CHOP-R) chemoimmunotherapy in high-risk

follicular lymphoma at diagnosis: the superior disease control of R-HDS does not translate into an overall survival advantage. Blood. 2008;111:4004–13.
157. Buske C, Hoster E, Dreyling M, et al. Rituximab in combination with CHOP in patients with follicular lymphoma: analysis of treatment outcome of 552 patients treated in a randomized trial of the German Low Grade Lymphoma Study Group (GLSG) after a follow up of 58 months. Blood. 2008;112:901.
158. Hiddemann W, Buske C, Kneba M, et al. Autologous stem cell transplantation after myeloablative therapy in first remission may be beneficial in patients with advanced stage follicular lymphoma after front-line therapy with R-CHOP. An analysis of two consecutive studies of the German Low Grade Lymphoma Study Group (GLSG). Blood. 2008;112:286–7.
159. van Besien K, Sobocinski KA, Rowlings PA, et al. Allogeneic bone marrow transplantation for low-grade lymphoma. Blood. 1998;92:1832–6.
160. Stein RS, Greer JP, Goodman S, et al. High-dose therapy with autologous or allogeneic transplantation as salvage therapy for small cleaved cell lymphoma of follicular center cell origin. Bone Marrow Transplant. 1999;23:227–33.
161. Kuruvilla J, Pond G, Tsang R, et al. Favorable overall survival with fully myeloablative allogeneic stem cell transplantation for follicular lymphoma. Biol Blood Marrow Transplant. 2008;14:775–82.
162. van Besien K, Carreras J, Bierman PJ, et al. Unrelated donor hematopoietic cell transplantation for non-hodgkin lymphoma: long-term outcomes. Biol Blood Marrow Transplant. 2009;15:554–63.
163. Laport G, Bredeson C, Tomblyn MR, Kahl BS, Goodman SA, Ewell M, et al. Autologous versus reduced-intensity allogeneic hematopoietic cell transplantation for patients with follicular non-hodgkins lymphoma (FL) beyond first complete response or first partial response. Clin Oncol. 2008;26:382a.
164. Hosing C, Saliba RM, McLaughlin P, et al. Long-term results favor allogeneic over autologous hematopoietic stem cell transplantation in patients with refractory or recurrent indolent non-Hodgkin's lymphoma. Ann Oncol. 2003;14:737–44.
165. Ingram W, Devereux S, Das-Gupta EP, et al. Outcome of BEAM-autologous and BEAM-alemtuzumab allogeneic transplantation in relapsed advanced stage follicular lymphoma. Br J Haematol. 2008;141:235–43.
166. Rezvani AR, Storer B, Maris M, et al. Nonmyeloablative allogeneic hematopoietic cell transplantation in relapsed, refractory, and transformed indolent non-Hodgkin's lymphoma. J Clin Oncol. 2008;26:211–7.
167. Rodriguez R, Nademanee A, Ruel N, et al. Comparison of reduced-intensity and conventional myeloablative regimens for allogeneic transplantation in non-Hodgkin's lymphoma. Biol Blood Marrow Transplant. 2006;12:1326–34.
168. Hari P, Carreras J, Zhang MJ, et al. Allogeneic transplants in follicular lymphoma: higher risk of disease progression after reduced-intensity compared to myeloablative conditioning. Biol Blood Marrow Transplant. 2008;14:236–45.
169. Kusumi E, Kami M, Kanda Y, et al. Reduced-intensity hematopoietic stem-cell transplantation for malignant lymphoma: a retrospective survey of 112 adult patients in Japan. Bone Marrow Transplant. 2005;36:205–13.
170. Khouri IF, Saliba RM, Giralt SA, et al. Nonablative allogeneic hematopoietic transplantation as adoptive immunotherapy for indolent lymphoma: low incidence of toxicity, acute graft-versus-host disease, and treatment-related mortality. Blood. 2001;98:3595–9.
171. Khouri IF, McLaughlin P, Saliba RM, et al. Eight-year experience with allogeneic stem cell transplantation for relapsed follicular lymphoma after nonmyeloablative conditioning with fludarabine, cyclophosphamide, and rituximab. Blood. 2008;111:5530–6.
172. Eide MB, Lauritzsen GF, Kvalheim G. High dose chemotherapy with autologous stem cell support for patients with histologically transformed B-cell non-Hodgkin lymphomas. A Norwegian multi centre phase II study. Br J Haematol. 2011;152:600–10.
173. Tarella C, Passera R, Magni M. Risk factors for the development of secondary malignancy after high-dose chemotherapy and autograft, with or without rituximab: a 20-year retrospective follow-up study in patients with lymphoma. J Clin Oncol. 2011;29:814–24.
174. Montoto S, Canals C, Rohatiner AZ, et al. Long-term follow-up of high-dose treatment with autologous haematopoietic progenitor cell support in 693 patients with follicular lymphoma: an EBMT registry study. Leukemia. 2007;21:2324–31.
175. Kang TY, Rybicki LA, Bolwell BJ, et al. Effect of prior rituximab on high-dose therapy and autologous stem cell transplantation in follicular lymphoma. Bone Marrow Transplant. 2007;40:973–8.
176. Vose JM, Bierman PJ, Loberiza FR, et al. Long-term outcomes of autologous stem cell transplantation for follicular non-Hodgkin lymphoma: effect of histological grade and Follicular International Prognostic Index. Biol Blood Marrow Transplant. 2008;14:36–42.
177. Bethge WA, Wilbur DS, Sandmaier BM. Radioimmunotherapy as non-myeloablative conditioning for allogeneic marrow transplantation. Leuk Lymphoma. 2006;47:1205–14.

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