Hematologic Malignancies

Martin Dreyling
Michael E. Williams *Editors*

Rare Lymphomas

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Editors
Martin Dreyling, MD
Medizinische Klinik und Poliklinik III
Klinikum Großhadern
Universität München
München
Germany

Michael E. Williams, MD, ScM
Division of Hematology/Oncology
and Cancer Center
University of Virginia Health System
Charlottesville, VA
USA

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To our families, patients and colleagues, with gratitude.

Preface

The non-Hodgkin lymphomas represent one of the most prevalent cancer types worldwide, comprised of a complex and fascinating array of malignancies that are in turn diverse in cell of origin, pathogenesis and clinical presentation. Rapid progress during the past decade in hematopathology and molecular diagnostics have defined more than 50 distinct entities in the current World Health Organization classification, leading to continuous refinement in treatment approach and, for many lymphomas, improving outcomes and survival.

Given these many subtypes, each of them "rare" to a greater or lesser extent, one may fairly ask why a "rare lymphoma" text devoted to selected topics is of value. The answers lie in the need for an up-to-date and comprehensive treatise for these highly treatable, if not always curable, cancers. The clinical importance of accurate diagnosis, a thorough understanding of the cellular and molecular biology of each subtype and optimized management has never been greater to offer patients the best outcome. There is a parallel need for basic and translational scientists to understand the spectrum of each lymphoma and the factors that underlie pathogenesis, prognostic stratification and therapeutic targets. Indeed, much of current-day cancer biology has been, and continues to be, informed by insights gained from lymphoma research. The purpose of this textbook thus is to be an up-to-date and comprehensive resource, and to do so at a time of dramatic progress in this rapidly evolving field.

Rare Lymphomas opens with chapters devoted to key concepts of pathology, cytogenetics and molecular genetics, as well as the critical signaling pathways that are increasingly important to targeted therapeutics. The following chapters are devoted to individual T-cell and B-cell lymphomas, organized to present key elements of diagnosis and staging, prognostic factors, pathologic and molecular features and current and emerging therapeutics. Each chapter is coauthored by well-recognized international experts, with bibliographies that are extensive but selectively organized to identify the most important advances.

The editors wish to express their appreciation to the authors for their generosity of time and expertise in preparing each chapter, and especially for their ongoing contributions to improve the outcomes for patients and families affected by lymphoma. We also acknowledge the editorial assistance of

Carmen Grimbs and the Springer-Verlag editorial staff including Annette Hinze, Karthikeyan Gurunathan and Jagannathan Prakash who diligently communicated with the many authors and assisted in final copy organization. Finally, we extend sincere appreciation to our readers for their devotion to improving the understanding, care and cure of non-Hodgkin lymphoma patients everywhere.

München, Germany — Martin Dreyling, MD
Charlottesville, VA, USA — Michael E. Williams, MD, ScM
January, 2014

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Part I

General

1 Principles of the Pathology and Biology of Malignant Lymphomas

German Ott, Eric D. Hsi, Jan Delabie, and Scott Rodig

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G. Ott, MD (✉)
Department of Clinical Pathology,
Robert-Bosch-Krankenhaus, Stuttgart, Germany
e-mail: german.ott@rbk.de

E.D. Hsi, MD
Department of Clinical Pathology,
Cleveland Clinic, L11, 9500 Euclid Ave,
Cleveland, OH 44195, USA
e-mail: hsie@ccf.org

J. Delabie, MD
Department of Pathology,
Oslo University Hospital, Oslo, Norway
e-mail: jandel@ous-hf.no

S. Rodig, MD
Department of Pathology,
Brigham and Women's Hospital,
Harvard Medical School, Boston, MA, USA
e-mail: srodig@partners.org

1.1 Classification of Malignant Lymphomas

The lymphoma classification currently in use, the fourth edition of the WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues (Swerdlow et al. 2008), is founded on the basic principles of the Revised European American Lymphoma (REAL) Classification of 1994 (Harris et al. 1994). These principles, however, had been recognized earlier. Divergent and conflicting proposals for lymphoma classifications had been formulated in the 1970s, resulting in the emergence of two classification systems widely used: the Kiel classification of non-Hodgkin's lymphomas (Stansfeld et al. 1988) and the working formulation for clinical usage (WF; Non-Hodgkin's Lymphoma Pathologic Classification Project 1982). Both classification systems relied upon entirely different principles. The Kiel classification was based on the exact morphological description and (later) immunological identification of the normal cellular counterparts of tumor cells and was updated several times. The working formulation was based on historical clinical survival data and, therefore, was not updated, although its usage was also adapted to modern findings. Of importance, there was a geographic split of categories in the diagnosis of lymphoid tumors, the Kiel classification being in use in Europe and the WF in the USA.

The taxonomic unit of the updated WHO classification, essentially formulated by the REAL classification, is the disease entity. Distinct entities

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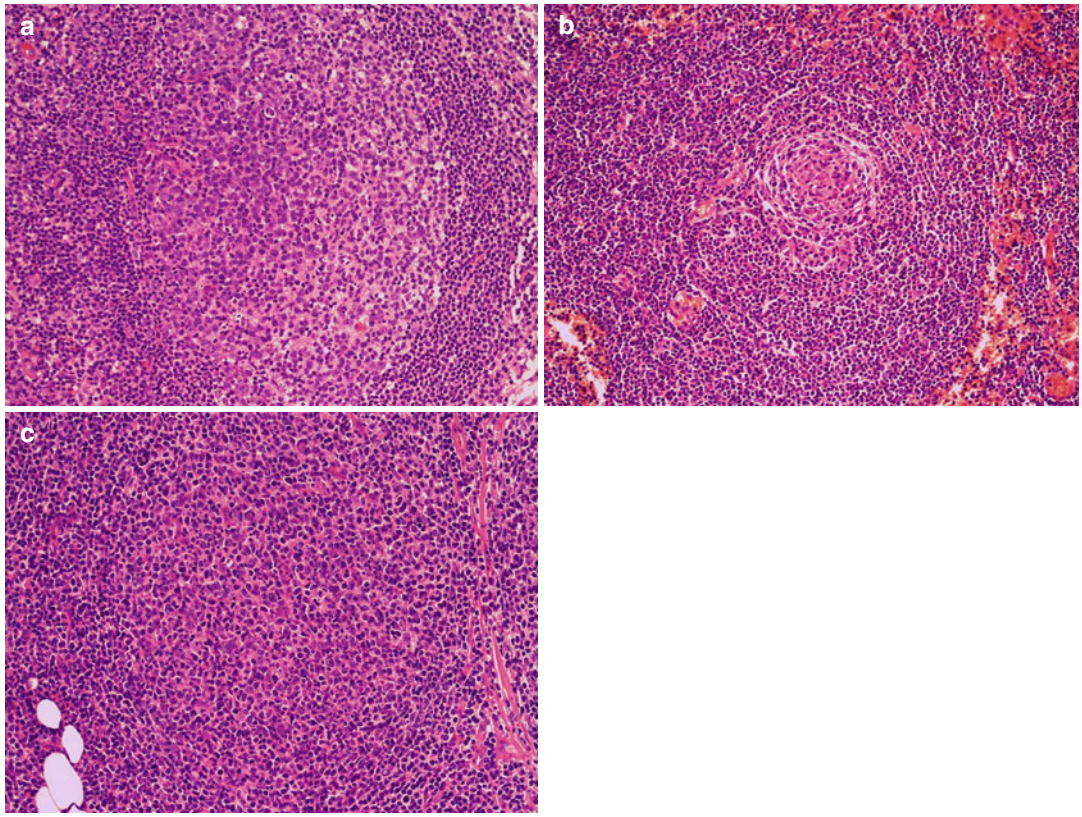

Fig. 1.1 Conventional morphology (H&E stain) of germinal centers (GCs) in different lymphoproliferations (×200). (**a**) Reactive germinal center. Note sharp circumscription of the GC, preserved perifollicular mantle zone, and polarization of the GC into a dark zone (*to the left*) and a light zone (*to the right*). (**b**) Germinal center in the hyaline-vascular variant of Castleman disease. Note small, regressively changed GC with onion-like proliferation of follicular dendritic cells. (**c**) GC in a typical case of follicular lymphoma. The GC is poorly demarcated to the surrounding cells, and there is no polarization

in malignant lymphomas, ideally, can be both – reproducibly – recognized by pathologists and hematologists and are of clinical relevance (Banks et al. 1992; Mason et al. 1994). For the definition of each disease entity, a combination of morphologic, immunophenotypic, genetic, and clinical features is used. Although morphology, naturally, is the most important and starting point of characterizing a given lymphoproliferation (Fig. 1.1), the relative value of each of these features may vary among different disease entities. It has been shown, e.g., that certain types of malignant lymphomas, like follicular lymphomas, can be readily recognized by experienced hematopathologists by morphology alone, while in others, such as mantle cell lymphoma, immunophenotypic and/or genetic studies are mandatory (The Non-Hodgkin's lymphoma classification project 1997). Variations in grade and aggressiveness, which may exist within a given disease entity and may be related to patients' survival and treatment response, must be distinguished from a different disease.

The basic rules for the definition of disease entities in the WHO classification are comparable to the general rules of tumor classification as used in many other organs and organ systems. A given lymphoma entity is defined in first line by the recognition of the predominant differentiated cell type using morphological and immunological features, a principle that follows the rules of the Kiel and REAL classifications (Stansfeld et al. 1988; Harris et al. 1994). In addition, the importance of the primary site of involvement is explicitly stated. In some entities, this feature is clinically relevant and important. The importance of site, e.g., is readily apparent in the definition

of extranodal marginal zone B-cell lymphoma of MALT type or of primary mediastinal large B-cell lymphoma (Isaacson and Du 2005; Rosenwald et al. 2003a; Savage et al. 2003). It must be stressed in general that knowledge of the clinical presentation of the disease is essential for making a correct diagnosis. The pertinent importance of disease presentation can be readily deduced from the single chapters following; it is of pivotal impact in some of the rarer lymphoma entities.

Second-line principles of classification are important for some entities. They may constitute specific etiological features, such as the association of certain infections to diseases, e.g., the Epstein-Barr virus to endemic Burkitt lymphoma or Hodgkin lymphoma. Similarly, Helicobacter pylori has been revealed as the causative agent in gastric marginal zone B-cell lymphomas of MALT type. Primary cytogenetic abnormalities are correlated to distinct biological features of lymphoma categories. Specific clinical features, as outlined above, are important in clinical and histopathological differential diagnoses and diagnostic procedures. Most lymphomas may be subdivided into mainly leukemic and generalized lymphomas, mainly nodal lymphomas, and/or mainly extranodal diseases. Furthermore, the International Prognostic Index (IPI), including its specification in follicular lymphoma and mantle cell lymphoma, is of highly relevant prognostic value if applied in defined lymphoma entities (Shipp et al. 1993).

It has to be kept in mind that minor or more evident exceptions from the proposed rules lead to a well-recognized heterogeneity in the presenting features of each type of lymphoid neoplasia. Therefore, within many entities, morphological or clinical subtypes have been recognized that may be of clinical importance. Thus, some specific immunophenotypic and genetic features have been described, for example, in t(14;18)/BCL2 expression negative follicular lymphomas (Katzenberger et al. 2009; Leich et al. 2009, 2011) providing clues for the recognition of a possible biological difference introduced by the presence or absence of basic transforming events. Morphological variants, on the other hand, reflect the diagnostic spectrum of a disease, which is important to be recognized in order to arrive at a correct differential diagnosis. They may or may not have clinical relevance, but often are of relevance with respect to clinical presentation or even prognosis.

For the sake of scientific accuracy and for more defined and specific treatment trials in the future, a clinical grouping of the entities recognized was not realized, and a complete list of diseases was promoted. It can be easily seen, however, that the list of lymphoid neoplasms, nevertheless, shows a certain grouping according to the main clinical presentation of diseases. The B-cell and T-cell lymphomas are divided primarily into those of the B- and the T-cell system. In both lineages, there is a primary distinction of lymphomas that arise from precursor cells (the lymphoblastic leukemias/lymphomas) and from peripheral effector cells (Jaffe et al. 2008).

Table 1.1 lists the recent 2008 WHO classification of B- and T-cell lymphomas and of Hodgkin lymphoma.

Table 1.1 Main categories of the 2008 WHO classification of lymphoid neoplasms

B-cell neoplasms
B-lymphoblastic leukemia/lymphoma (With/without recurrent genetic abnormalities)
Mature B-cell neoplasms
Chronic lymphocytic leukemia/small lymphocytic lymphoma
B-cell prolymphocytic leukemia
Lymphoplasmacytic lymphoma
Mantle cell lymphoma
Follicular lymphoma
Cutaneous follicle center lymphoma
Extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma)
Nodal marginal zone lymphoma
Splenic marginal zone lymphoma
Hairy cell leukemia
Diffuse large B-cell lymphoma (DLBCL) not otherwise specified
T-cell/histiocyte-rich large B-cell lymphoma
Primary mediastinal (thymic) large B-cell lymphoma
Intravascular large B-cell lymphoma
Primary effusion lymphoma
Lymphomatoid granulomatosis
ALK+ large B-cell lymphoma
Plasmablastic lymphoma

(continued)

Table 1.1 (continued)

Burkitt lymphoma
Heavy-chain diseases
Plasma cell myeloma
Solitary plasmacytoma of bone
Extraosseous plasmacytoma
T-cell neoplasms
T-lymphoblastic leukemia/lymphoma
Mature T-cell and NK-cell neoplasms
T-cell prolymphocytic leukemia
T-cell large granular lymphocyte leukemia
Aggressive NK-cell leukemia
Systemic EBV+ T-cell lymphoproliferative disease of childhood
Hydroa vacciniforme-like lymphoma
Extranodal NK/T-cell lymphoma, nasal type
Sezary syndrome
Mycosis fungoides
Angioimmunoblastic T-cell lymphoma
Peripheral T-cell lymphoma not otherwise specified
Adult T-cell leukemia/lymphoma
Anaplastic large-cell lymphoma ALK+
Anaplastic large-cell lymphoma ALK-
Primary cutaneous CD30-positive T-cell lymphoproliferative disorders
Primary cutaneous gamma-delta T-cell lymphoma
Subcutaneous panniculitis-like T-cell lymphoma
Enteropathy-associated T-cell lymphoma
Hepatosplenic T-cell lymphoma
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 Hodgkin lymphoma

1.2 Epidemiology and Distribution of Malignant Lymphomas

There are considerable differences in the frequencies of the different types of both Hodgkin as well as non-Hodgkin lymphomas (Jaffe et al. 2008; Fig. 1.2). For a given entity, significantly different relative frequencies may be seen in different geographic regions. However, regardless of geography or ethnic group, two distinct entities make up by far the most frequent types of malignant lymphomas worldwide, namely, diffuse large B-cell lymphoma and follicular lymphoma, roughly comprising 60–70 % of B-cell lymphomas. B-cell neoplasias, in general, constitute 90 % of all lymphomas and 4–5 % of all newly diagnosed cancers worldwide. Their incidence is increasing, especially in the developed countries. There is a variation in the relative frequencies of the individual lymphomas. Follicular lymphoma is a common lymphoid neoplasm in the Western world comprising 35 % of lymphomas, while it is rarer in Africa, South America, and Asia. Burkitt lymphoma, on the other hand, does not make up more than 1–2 % of lymphomas in the West, but is endemic in equatorial Africa and New Guinea (Leoncini et al. 2008). Mature T-cell and T-/NK- or NK-cell neoplasms, on the whole, are rare diseases worldwide. Their frequency is estimated at 10 %. The most frequent entities diagnosed are peripheral T-cell lymphoma, not otherwise specified, and angioimmunoblastic T-cell lymphoma with frequencies of 25 and 20 %, respectively.

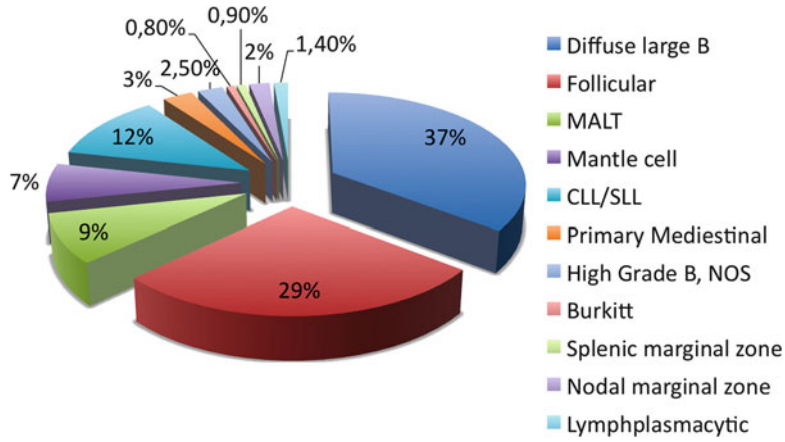

Fig. 1.2 Relative frequencies of B-cell lymphomas (Adapted from Jaffe et al. 2008)

There is a significant geographic variation in the occurrence of T-cell lymphomas. Generally, they do occur more frequently in Asia. In endemic HTLV-1 regions, i.e., Japan and the Caribbean, the frequency of adult T-cell lymphoma/leukemia is distinctly elevated (Oshima et al. 2008). EBV-associated NK-/T-cell neoplasias are far more common in Asia and in populations of Native American descent in South America and Mexico (who are genetically related to Asians) (Chan et al. 2008). Enteropathy-associated T-cell lymphoma is particularly frequent in individuals of Irish and Welsh descent characterized by the occurrence of certain HLA haplotype distributions conferring increased risk to enteropathy (Isaacson et al. 2008). Another factor strongly influencing the distribution of malignant lymphomas is age. Precursor cell B- and T-cell neoplasias are primarily diseases of children. A second peak occurs during old age. The great majority of precursor B-cell neoplasias clinically manifest as leukemias, while T-cell neoplasias comprise the large number of lymphoblastic lymphomas, especially in the mediastinum. Generally speaking, however, malignant lymphomas of B-cell types are diseases of older adults, with the peak of them manifesting in the sixth and seventh decades. Diffuse large B-cell lymphomas and Burkitt lymphoma are the only entities occurring to a significant extent in children. Mediastinal large B-cell lymphoma has a unique characteristic presentation in young women in the third life decade (Gaulard et al. 2008). Age in general seems to constitute an important feature also within entities, and age itself is a defining feature in some lymphomas. For example, pediatric variants of follicular lymphomas and of marginal zone lymphomas have been described that seem to behave differently from their adult counterparts. Pediatric follicular lymphomas presenting in the lymph nodes or in extranodal sites such as the testis seem to constitute indolent and especially localized disease variants that as a rule lack both t(14;18) chromosome translocations and also BCL2 protein expression (Finn et al. 1999; Lorsbach et al. 2002). Nodal marginal zone lymphomas occurring in children or young adults do present with a particular morphology. They arise in a background of follicular lymphoid hyperplasia and frequently exhibit a morphology similar to progressive transformation of germinal centers (Taddesse-Heath et al. 2003). Two rather characteristic EBV-associated lymphoproliferative disorders of T-cell type are described in the WHO classification occurring in children and, of note, in certain ethnic groups, namely, hydroa vacciniforme-like lymphoma and systemic EBV-positive T-cell lymphoproliferative disease of childhood both occurring in Asians and Native Americans from Central and South America and Mexico (Quintanilla-Martinez et al. 2008). EBV-associated diffuse large B-cell lymphoma of the elderly, on the other hand, is defined by the occurrence of EBV+ DLBCL in patients >50 years of age (Oyama et al. 2003, 2007). Of note, however, both pediatric as well as elderly lymphoma types also occur – sporadically – outside these characteristic age groups.

1.3 Early Lesions and Borderline Cases

As much as we would like to diagnose homogeneous diseases in every patient, daily clinical and pathological experience shows that there are borderline cases both within entities and between reactive and neoplastic lesions. In the 2008 WHO classification, there are two important provisional categories that had been coined owing to the fact that there are some cases within the borderlands of diffuse large B-cell lymphoma that bear resemblance – more or less – to other discrete entities. In *B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and Burkitt lymphoma* (Haralambieva et al. 2005; Kluin et al. 2008), the size of the tumor cells may be in between those of classical DLBCL and BL, or the immunophenotype of the cells may bear some particularities not easily fitting into the one or the other category, such as strong expression of BCL2 in a tumor morphologically equivalent to Burkitt lymphoma. Some of these cases with morphological overlap may show a distinct genotype with rearrangements of both *MYC* and *BCL2* or *BCL6*, or both. Another provisional

category is *B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and classical Hodgkin lymphoma*, frequently arising in the mediastinum. As is the case with the other category, overlap may be relying on either morphological or immunophenotypic features (Traverse-Glehen et al. 2005; Jaffe et al. 2008).

More recently, supposedly early events in lymphomagenesis have come to attention, because of the use of more refined detection methods, new insights into genetic alterations, or certain new morphological findings. One of these examples is monoclonal B-cell lymphocytosis that was also incorporated into the updated WHO classification (Marti et al. 2007; Rawstron et al. 2002, 2008) and that is defined as the detection of clonal expansions of small B cells in the peripheral blood $<5\times10^9$/L with or without a B-CLL phenotype and in the absence of cytopenias and tissue involvement. Although these populations have been found to carry certain chromosome aberrations characteristically found in B-CLL, they do but rarely progress to full-blown B-CLL. Likewise, the t(14;18) has been detected in the peripheral blood of up to 70 % of healthy persons (Limpens et al. 1991; Roulland et al. 2006), and there is currently no indication that these individuals may carry an increased risk of developing follicular lymphoma. In essentially reactive conditions, in about 2–3 % of cases in larger series (Henopp et al. 2011), there may be a partial or extended colonization of single follicles by BCL2-expressing B cells carrying the t(14;18) translocation, in an otherwise essentially reactive background of hyperplastic lymphadenitis (Fig. 1.3). This condition is discussed to represent the tissue equivalent of the phenomenon of t(14;18) recirculating cells. The morphology was initially described by Cong and associates in 23 patients, out of which 40 % had established FL in adjacent lymph nodes or elsewhere, but 60 % had not. Of those, only five patients subsequently developed overt FL (Cong et al. 2002). It is interesting to note that in these cases, no secondary aberrations (next to the BCL2 rearrangement) were observed, and this may be the reason why there – obviously – was only a subtle expansion of the t(14;18)-positive clone. This condition has initially been termed "in situ" follicular lymphoma or "intrafollicular neoplasia" (Harris et al. 2008), and its significance as a risk factor is currently debated. A similar phenomenon has been described for t(11;14)-positive cells exclusively colonizing mantle zones of reactive follicular – frequently hyperplastic – follicles, and this was termed "mantle cell lymphoma in situ" (Richard et al. 2006; Aqel et al. 2008; Fig. 1.4). Most interestingly, similar "in situ" manifestations of mantle cell lymphoma have been identified in

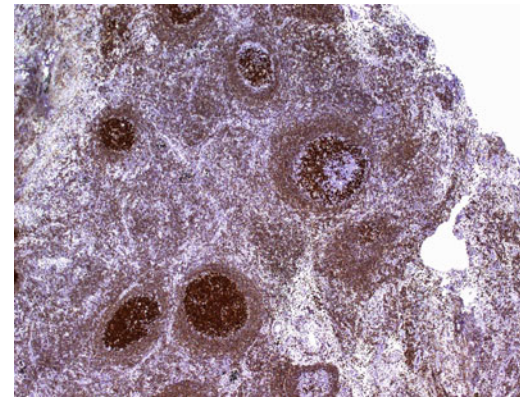

Fig. 1.3 Follicular lymphoma "in situ" (BCL2 stain, ×100). Darkly stained BCL2-positive neoplastic cells are colonizing reactive germinal centers. There are some remnants of reactive GSs (not stained), and the overall architecture of the lymph node parenchyma is preserved

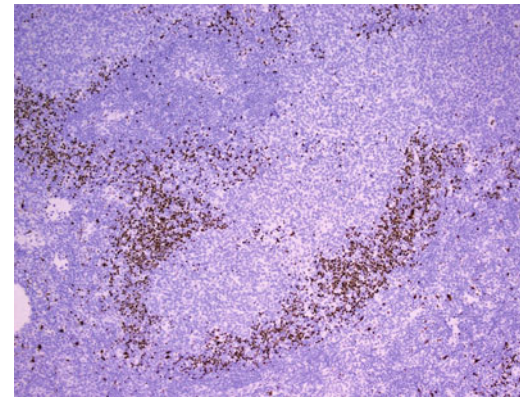

Fig. 1.4 Mantle cell lymphoma "in situ" (Cyclin D1 stain, ×100). Darkly stained neoplastic cells overexpressing cyclin D1 in their nuclei colonize slightly expanded mantle zones of irregularly shaped, hyperplastic germinal centers. Again, the overall architecture of the lymphatic parenchyma is preserved

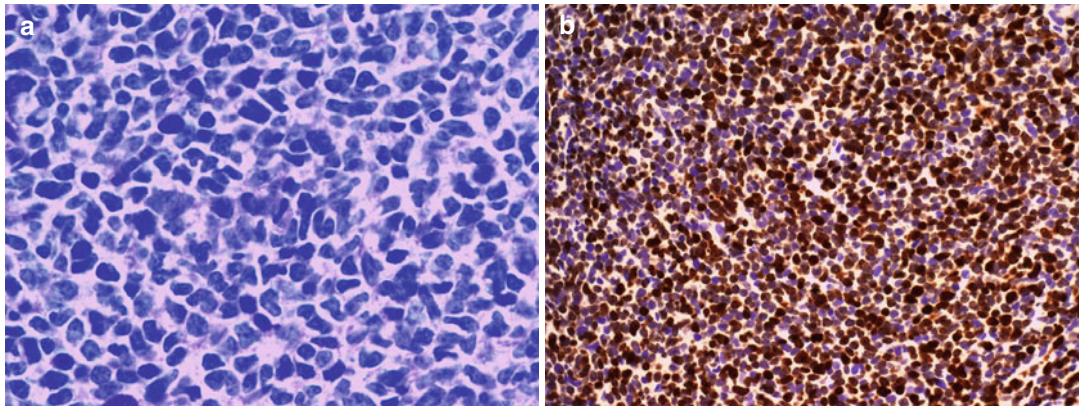

Fig. 1.5 Mantle cell lymphoma. Conventional morphology shows a diffuse proliferation of small- to medium-sized cells with scant cytoplasm and irregular nuclei corresponding to the classical variant (Giemsa ×1,000). On Ki67 staining, however, a high proliferation index of 80 % can be seen (×400). This latter feature places the tumor into the high-risk category in spite of the low-grade cytological appearance of the tumor cells

tissues from patients with established MCL that had been removed up to years prior to the diagnosis of overt mantle cell lymphoma.

1.4 Prognostic Factors

Having in mind the aforementioned principles of classification, it is clear that the most important prognostic factor is the correct definition of the disease entity, brought about by a combination of morphologic, immunohistochemical, genetic, and clinical features (International Non-Hodgkin's Lymphoma Classification Project 1997). Within these individual diseases, prognostic factors may influence clinical outcome. Prognostic factors and variations in grades *within* diseases are to be distinguished from *different* diseases. They may be histological, biological, or clinical in nature, such as stage or other features of the International Prognostic Index. Histological grading is one method to define types of prognostic factors. Usual approaches include the determination of cell size, nuclear features, mitotic rates, and growth pattern. In the last years, biological markers, such as genetic features, have turned out to be important prognostic factors and may even be more powerful than clinical or morphological features. Some of them may be recognized today by interphase cytogenetics, such as *TP53* and *ATM* deletions in B-CLL (Döhner et al. 2000) or *MYC* rearrangements in DLBCL, or by immunohistochemistry, such as the presence of MYC, BCL6, or BCL2 protein overexpression in DLBCL (Horn et al. 2013; Johnson et al. 2012). Determination of the proliferative index by gene expression profiling or using the Ki67 antibody has been shown to yield important prognostic information in mantle cell lymphoma (Rosenwald et al. 2003a, b; Katzenberger et al. 2006; Fig. 1.5).

1.5 Ancillary Methods for the Definition of Malignant Lymphomas

1.5.1 Immunophenotypic Studies

Immunohistochemistry plays a pivotal role in modern pathology and is indispensable in hematopathology. As has been convincingly shown, the diagnostic accuracy and reproducibility of diagnoses even among experienced hematopathologists is greatly enhanced by additional immunohistochemical studies of lymphoid tumors (The International Non-Hodgkin's lymphoma classification project 1997). Therefore, there is no doubt that the combination of sophisticated morphological studies in concert with the skilled application of immunohistochemical methods leads to a more reproducible – and

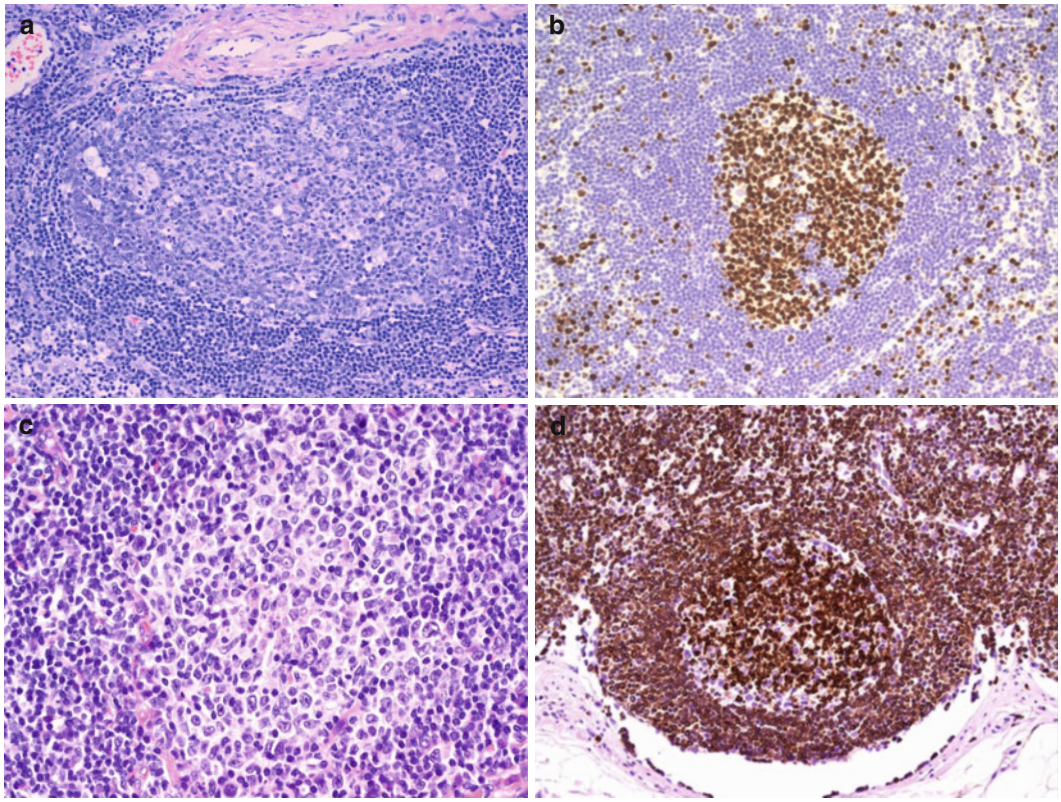

Fig. 1.6 Use of immunohistochemical staining in the delineation of reactive follicular hyperplasia (**a**, **b**) versus follicular lymphoma (**c**, **d**). The diagnosis can be suspected on the basis of conventional stainings (Giemsa ×200, **a**, **c**). Immunohistochemistry shows physiologically high proliferative activity of reactive GCs (Ki67 ×200, B). In follicular lymphoma, neoplastic GCs are, as a rule, BCL2 positive (D, ×200)

clinically more relevant – characterization of lymphoid proliferations. Because of the development, characterization, and commercial availability of antibodies enabling the detection of a large spectrum of lineage differentiation and other antigens, immunohistochemistry has gained particular importance with respect to the differentiation of the various subtypes of malignant lymphomas and in the differentiation of reactive versus neoplastic lymphoproliferative disorders (Fig. 1.6).

Immunophenotypic studies have become universally accepted as a valuable help in the recognition of lymphoma entities and have also largely contributed to our understanding of the histogenesis and pathogenesis of hematopoietic neoplasms in general. They may be used on a variety of materials including cell suspensions, flow cytometric analysis, as well as on frozen and paraffin-embedded tissue specimens. Immunohistochemical studies are not only useful with respect to the primary distinction of a neoplastic versus a benign lymphoid infiltrate but are also important in the revelation of a preserved or destroyed normal architectural pattern (Fig. 1.7). With the advent of epitope retrieval techniques applicable to paraffin sections of formalin-fixed specimens (Shi et al. 1991; Norton et al. 1994; Taylor et al. 1994), an even more intricate interplay between cytomorphology and antigen expression profile is possible, allowing for the exact identification of antigen-positive cells and hence, their unequivocal assignment to a given cell lineage or particular protein expression pattern (Fig. 1.8). With the use of antibodies to kappa and lambda immunoglobulin light chains and a determination of the light chain ratio, plasma cells or other immunoglobulin-producing lymphoid cells can be judged as polyclonal or monoclonal, the latter at least pointing to a monoclonal expansion of a B-cell population, if not to its malignant nature.

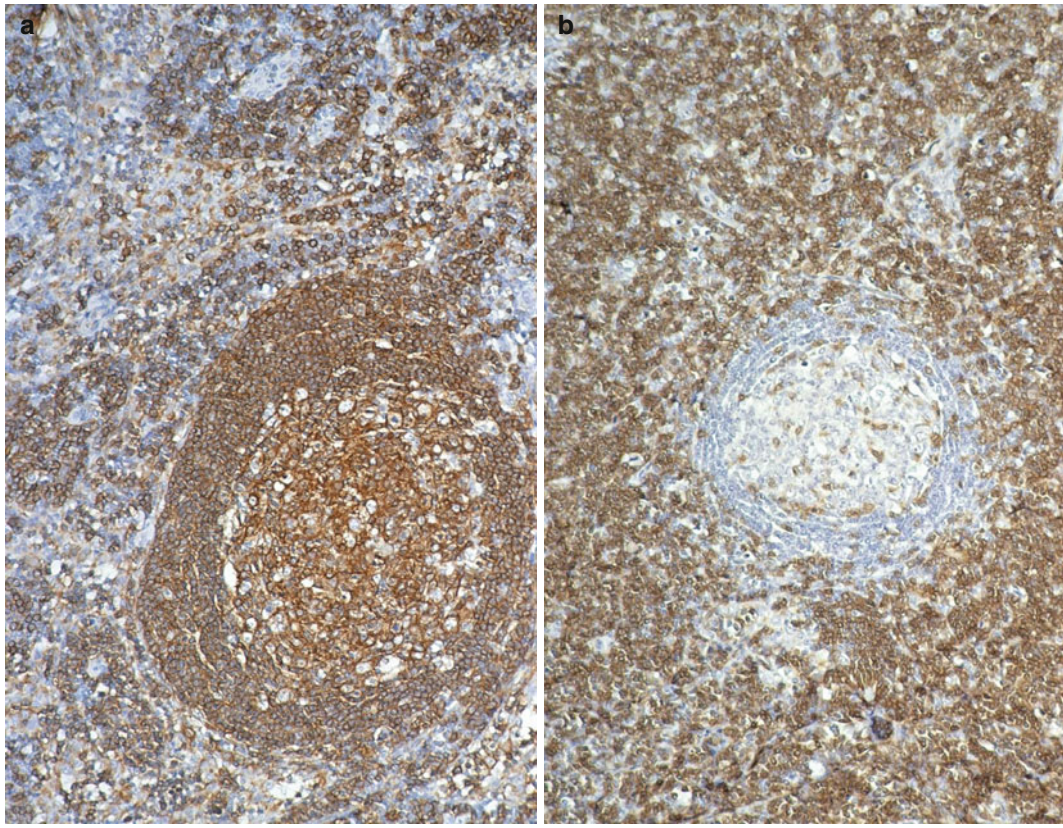

Fig. 1.7 Demonstration of immunohistochemical stains for B-cell and T-cell antigens in a reactive follicle (×200). The CD20 antibody (**a**) stains the B cells in the GC and the perifollicular mantle zone, while the T-cell area is stained with the T-cell marker CD5 (**b**) producing a nearly inverted pattern

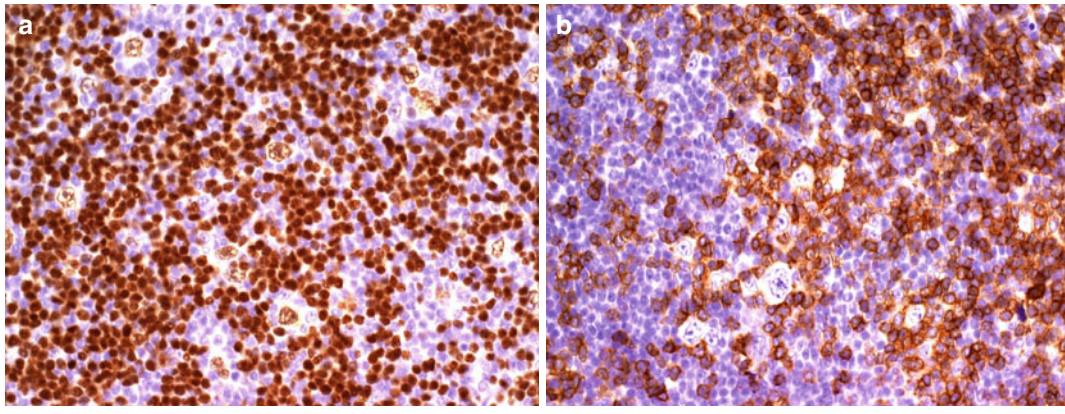

Fig. 1.8 A case of nodular lymphocyte-predominant Hodgkin lymphoma stained with the B-cell marker PAX5 (A ×400) and with the T-cell marker PD1 (B ×400). Note that in A, both the large tumor cells as well as small reactive cells in the background are positively stained revealing their B-cell nature. The T-cell marker PD1 highlights T cells surrounding the large tumor B cells and sets them apart from the small B-cell background in a rosetting pattern

By the application of other antibodies, certain biological features (e.g., the Ki67 antigen reflecting the proliferative index of a neoplasm; Gerdes et al. 1983) (Fig. 1.5) or prognostic features like the atypical expression of tumor suppressor genes (e.g., a mutated *TP53* gene) or the formation and particular–

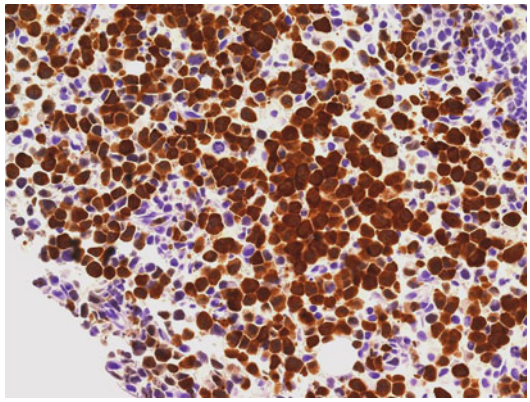

Fig. 1.9 Anaplastic large-cell T-cell lymphoma stained for the ALK antibody by immunohistochemistry (×400). In this case, both the tumor cell cytoplasm and the nuclei are positively stained suggesting ALK and NPM overexpression inferred by the t(2;5)(q23;q35) chromosome translocation, in which NPM shuttles ALK into the nucleus

nuclear or cytoplasmic – expression of a tumor-associated gene (e.g., ALK (Pulford et al. 1997; Mason et al. 1998) Fig. 1.9) may be determined.

Table 1.2 gives an overview of antibodies useful in the daily diagnostic practice of lymphoid neoplasms.

This table lists antibodies to CD antigens and other antibodies reliably working on paraffin sections provided antigen retrieval is performed. The specificity indicated represents only basic reactivity.

Table 1.2 Overview of antibodies used in the daily diagnostic work of lymphoma identification and classification

Antigen	Specificity
CD45	Leukocyte common antigen
CD2	T cells, NK cells
CD3	T cells
CD4	Helper T cells
CD5	T cells, B-cell subpopulation
CD7	T cells, myeloid cells/leukemias
CD8	Cytotoxic T cells
CD20	B cells
CD79a	B cells, plasma cells
CD138	Plasma cells
CD10	Germinal center B cells
BCL6	Germinal center B cells
PD1	Germinal center T cells
CD15	Neutrophils, Hodgkin, and Reed-Sternberg cells
CD21	B-cell subpopulation, follicular dendritic cells
CD23	B-cell subpopulation, follicular dendritic cells
CD30	Activated B and T cells, Hodgkin, and Reed-Sternberg cells
CD34	Precursor myeloid and lymphoid cells
CD43	T cells, B-cell subpopulation, myeloid and monocytic cells
CD56	T/NK cells, NK cells
CD57	T-cell subpopulation, NK cells
CD68	Monocytes, macrophages

Others	Specificity
TdT	Precursor B and T cells
TIA 1, perforin, granzyme B	Cytolytic and cytotoxic cells
Annexin A1	Hairy cell leukemia
BCL2	Proto-oncogene product
TP53	Proto-oncogene product
p27	Cell cycle kinase inhibitor
p21	Cell cycle kinase inhibitor
Cyclin D1	Cell cycle kinase

1.5.2 Genotypic Studies

The detection of a clonal population of B or T cells refers to the capability of these cells to either physically rearrange their immunoglobulin heavy (IGH)- and light (IGL)-chain genes or their T-cell receptor (TCR) genes. Therefore, in addition to the detection of a clonal cell population represented by a non-germline band (or bands), the technique may also be used for distinguishing T- or B-lineage neoplasms (Inghirami et al. 1993; van Krieken et al. 2007; Evans et al. 2007; Kneba et al. 1994).

Today, the most common technique used to demonstrate clonal cell populations is the polymerase chain reaction (PCR) technique. The PCR, in its basic principle, represents a technique in which small amounts of DNA can be amplified in vitro, provided that the DNA sequences flanking the regions looked for are known. Because of the requirement of only minimal amounts of DNA (or small degraded DNA particles), this technique can be used for the detection of clonal cell populations or DNA rearrangements also in paraffin-embedded formalin-fixed material (in which the DNA normally is largely degraded). Because of this inherent advantage, the PCR may also be used in the monitoring of minimal residual disease, especially if clonotypic primers are used. Moreover, by DNA sequence analysis of IG receptor genes, non-mutated (naive) prefollicular and mutated (memory) postfollicular B cells can

Table 1.3 Recurrent chromosome aberrations in malignant lymphomas

Diagnosis	Chromosome aberration	Genes involved
Precursor B-cell lymphoblastic lymphoma/ leukemia	t(1;19)(q23;p13) t(4,11)(q21;q23) del(6q) t(9;22)(q34;q11)	SKI (1q23) ETS1 (11q23–24) BCR-ABL
B-cell chronic lymphocytic leukemia/small lymphocytic lymphoma	del(11q) del(17p) del(13)(q14) Trisomy 12	ATM TP53
Mantle cell lymphoma	t(11;14)(q13;q32)	Cyclin D1
Follicular lymphoma	t(14;18)(q32;q21)	BCL2
Extranodal marginal zone B-cell lymphoma of MALT type	t(11;18)(q21;q21) t(14;18)(q32;q21)	API2/MALT1 IGH/MALT1
Splenic marginal zone B-cell lymphoma	t/del(7)(q22–32) del(10)(q22–24)	
Diffuse large B-cell lymphoma	t(3;14)(q27;q32) t(14;18)(q32:q21) t(8q24)	BCL6 BCL2 MYC/non-IG
Burkitt lymphoma	t(8;14)(q24;q32) t(2;8)(p12;q24) t(8;22)(q24;q11)	MYC/IG
Plasmacytoma	t(4;14)(p16;q32) t(6;14)(p25;q32)	FGFR3 IRF4/MUM1
Precursor T-cell lymphoblastic lymphoma/ leukemia	14q11 7p15 or 7q34–35	TCR genes TCR genes
T-cell prolymphocytic leukemia	inv(14)(q11q32)	TCL1
Angioimmunoblastic T-cell lymphoma	+3, +5, +X	
Anaplastic large-cell lymphoma	t(2;5)(p23;q35) Variants	NPM/ALK ALK
Hepatosplenic T-cell lymphoma	i(7)(q10)	

be distinguished in follicular cell populations, and some of their descendants, various "ongoing" somatic mutations, show a micro-polymorphism of the B-cell receptor repertoire. The detailed analysis of IG receptor genes, therefore, permits conclusions on the status of antigen-dependent selection and mutation as well as the V_H gene repertoire (Müller-Hermelink and Greiner 1998).

1.5.3 Chromosomal Rearrangements

Malignant lymphomas, especially the B-cell lymphomas, are quite well characterized – at least with respect to their primary genetic alterations – on the cytogenetic level. Apart from proving the neoplastic nature of a lymphoid cell proliferation, the description of characteristic cytogenetic aberrations in certain types of malignant lymphomas has greatly added to our understanding of their biology (Lee et al. 1987; Ott et al. 1997; Streubel et al. 2004) and has also influenced taxonomy, as is best exemplified by the close association of mantle cell lymphoma to the translocation t(11;14)(q13;q32), the recognition of which led to the worldwide acceptance of mantle cell lymphoma as an entity on its own (Banks et al. 1992; Weisenburger and Armitage 1996). Table 1.3 lists the most common and characteristic chromosome aberrations in malignant lymphoma.

Currently, malignant lymphomas are not classified according to their (primary) genetic aberrations alone, because in some entities, no

characteristic aberrations have been detected so far and because a recurring chromosomal translocation may be encountered in different lymphoma entities, e.g., the t(14;18)(q32;q21) in follicular lymphoma and diffuse large B-cell lymphoma and the t(11;14)(q13;q32) in mantle cell lymphoma and plasma cell myeloma. In addition, cytogenetically detectable alterations are recognized only in a fraction of tumors in a given lymphoma entity.

The use of fluorescent dye-conjugated DNA probes in in situ hybridization has overcome the limitations of conventional cytogenetics (Cremer et al. 1996). With the use of fluorescent or bright light in situ hybridization techniques, cytogenetic aberrations can today be reliably recognized also in FFPE tissues (Ventura et al. 2006). More recently, gene expression profiling of lymphoid neoplasms has led to some new and fascinating insights into the biology of malignant tumors including lymphomas (Golub et al. 1999; Alizadeh et al. 2000) and has since created highly interesting data on the specific activity of genes or gene signatures in different molecular types of lymphoid neoplasms not readily recognized by conventional morphology or immunohistochemistry (Rosenwald et al. 2002; Lenz et al. 2008a, b; Hummel et al. 2006; Dave et al. 2006). These data have been supported by the recent recognition of specific genetic aberrations exclusively occurring within these expression-defined (molecular) disease subtypes (Lenz et al. 2008a, b; Davies et al. 2010; Ngo et al. 2011).

Conclusion

It is due to the delicate interplay of all these new insights into the pathology, biology, and clinics of malignant lymphomas that has enabled us to reliably define entities of malignant lymphomas and, especially, rare entities. The particular morphological, immunological, genetic, and clinical features of these rarer lymphoma subtypes will be covered in the special chapters dealt with in this book.

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Cytogenetics

2

Gordana Raca, Jo-Anne van der Krogt,
Michelle M. Le Beau, and Iwona Wlodarska

Contents

G. Raca • M.M. Le Beau
Section of Hematology/Oncology and the Comprehensive Cancer Center,
University of Chicago, Chicago, IL, USA
e-mail: mlebeau@bsd.uchicago.edu

J.-A. van der Krogt • I. Wlodarska (✉)
Center for Human Genetics, KU Leuven,
Gasthuisberg, Herestraat 49, 602,
B-3000 Leuven, Belgium
e-mail: iwona.wlodarska@uzleuven.be

2.1 Cytogenetic and Molecular Cytogenetic Methods

2.1.1 Conventional Cytogenetic Analysis

Conventional cytogenetic analysis has the advantage that it provides a "whole genome view" and detects both balanced and unbalanced rearrangements. It has a long tradition of being an important tool in lymphoma research and diagnostics. The first recurrent cytogenetic abnormality was described in Burkitt lymphoma only a few years after the discovery of the Philadelphia chromosome (Jacobs et al. 1963) and was soon characterized as the t(8;14)(q24.2;q32) involving the *MYC* gene and immunoglobulin heavy-chain (*IGH*) locus (Zech et al. 1976). Many more recurrent chromosomal abnormalities were subsequently described in lymphomas and have been associated with various lymphoma subtypes,

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including the t(14;18) (q32;q21) in follicular lymphoma, t(3;14) (q27;q32) in diffuse large B-cell lymphoma, t(11;14) (q13;q32) in mantle cell lymphoma, and t(2;5) (p23;q35) and variants in anaplastic large cell lymphoma (Swerdlow et al. 2008; Heim and Mitelman 2009). Identification of consistent chromosomal alterations has greatly impacted the classification of non-Hodgkin lymphomas (NHLs), especially the B-cell lymphomas. Clonal and relatively complex chromosome patterns exist in the majority of NHLs at diagnosis (Dave et al. 2011; Kluin et al. 2011).

Despite its unquestionable clinical utility, karyotype analysis is not routinely performed on each case of newly diagnosed lymphoma at many institutions. This is largely due to logistical and technical problems, which complicate the routine use of conventional cytogenetic analysis, including the need to obtain fresh tumor tissue, the need for a relatively large sample, and the high level of skill required to culture the lymphoma cells, to prepare high-quality metaphase cells and to analyze the often complex karyotypes.

For optimal results, the lymphoid tissue must be transported in sterile media at room temperature to the cytogenetics laboratory as quickly as possible. The tissue is mechanically disaggregated, and depending on the specimen size, multiple unstimulated cultures are established (24- and 48-h) and incubated at 37 °C. Before the initiation of harvest, cell cultures are treated with Colcemid™ to block cell division. This is followed by a hypotonic treatment and fixation in 3:1 methanol/glacial acetic acid fixative. The microscope slides are made by dropping suspensions of fixed cells onto the glass surface. The slides are then stained by Giemsa, Leishman, or Wright stain, typically with a trypsin pretreatment (G-banding) (Roulston and LeBeau 1997). When available, at least 20 metaphase cells are analyzed. Karyotypes of Giemsa-banded metaphase chromosomes are described according to the most recent version of the International System of Cytogenetic Nomenclature (ISCN 2013) (Shaffer et al. 2013).

For most clinical laboratories, routine analysis requires 10–14 days. The traditional cytogenetic testing is labor-intensive, time-consuming, and expensive. Additionally, in indolent lymphomas and myelomas, the tumor cells may fail to grow in culture, and only cells with a normal karyotype may be seen.

A further limitation of conventional cytogenetic analysis is the low resolution of banding techniques, estimated at approximately 4–5 Mb. Small abnormalities are, therefore, undetected by karyotyping, and structural alterations (such as translocations) may appear similar at the light microscopic level, but differ at the molecular level. Taken together, these shortcomings of conventional cytogenetic analysis contributed to increased research and diagnostic use of molecular cytogenetics methods, such as fluorescence in situ hybridization (FISH) and conventional and array comparative genomic hybridization (CGH).

2.1.2 Fluorescence In Situ Hybridization

Interphase FISH has become a routine technique in clinical laboratories and is mainly used to detect well-characterized structural chromosomal abnormalities, such as specific recurrent translocations or rearrangements affecting etiologically important genes. FISH is based on using fluorescently labeled DNA probes to detect specific target sequences on metaphase chromosomes or in interphase nuclei. FISH is usually performed on samples prepared for standard cytogenetic analysis but can also be applied to a wide range of cellular preparations such as G-banded slides, air-dried bone marrow or blood smears, fresh tumor touch prints, frozen or paraffin-embedded tissue sections, or nuclear isolates from fresh or fixed tissues (Gozzetti and Le Beau 2000; Szeles 2002).

FISH is a rapid, reproducible, inexpensive, and relatively easily applied technique, allowing for short turnaround times of only 2 or 3 working days. Since nondividing (interphase) cells can be analyzed, the use of FISH does not depend on having vital, growing cells or specific culture systems, which is particularly advantageous for testing indolent tumors. Additionally, hundreds of nuclei can be scored quickly and easily, which

increases the sensitivity for detection of tumor cells present at low frequency in the sample. Finally, the relatively small size of FISH probes (80–200 kb) increases resolution for detecting submicroscopic abnormalities (Dave et al. 1999, 2002). The major limitation of FISH is that this method targets specific abnormalities, and the genome-wide perspective offered by conventional cytogenetic approaches is lost. This may be clinically important, as additional aberrations (or their absence) can be diagnostic or predictive for the course of disease.

A variety of FISH probes, targeting either a specific region or the entire chromosome, are available. Probes that are routinely used in the analysis of hematologic malignancies include chromosome-specific centromeric probes, gene- or locus-specific probes, whole chromosome painting probes, and telomeric probes. Commercially available probes are directly conjugated to fluorochromes, thereby simplifying the procedure.

Hybridization protocols are standardized, and well-optimized probe sets are commercially available for detection of the most common translocations in mature lymphomas, including those affecting *IGH* locus at 14q32, *MYC* locus at 8q24.2, *BCL2* locus at 18q21.3, *BCL6* locus at 3q27, and *MALT1* locus at 18q21.3. Commercial FISH assays are based on two-color probe sets which generate patterns that allow normal cells to be easily distinguished from the cells carrying the expected structural rearrangement. Two main probe design strategies are typically used in commercially available FISH assays. The so-called "dual-fusion" probe sets are used for detecting specific translocations, whereas the "break-apart" probe sets allow the detection of rearrangements of a specific gene, regardless of the translocation partner (Fig. 2.1a–d).

An algorithm for diagnostic FISH evaluation of lymphomas currently used by clinical laboratories consists of initial testing with a simple "break-apart" assay for the locus of interest. If this screening is positive, one or more dual-fusion assays for specific translocation(s) can be applied, if the identification of the specific partner gene is clinically relevant.

In addition to specific structural rearrangements, FISH methods can also be used to detect copy number gains, losses, and amplifications of specific loci. True amplifications, as seen in solid tumors, are relatively rare in lymphomas. Furthermore, the detection of low copy number gains and losses in tissue sections is much more problematic than the detection of breakpoints, for which probes have been developed that generate easily distinguishable signal patterns. Identification and enumeration of signals is particularly challenging on sections from formalin-fixed, paraffin-embedded (FFPE) tissues, where the accuracy is compromised by factors such as cutting artifacts and nuclear overlap related to the thickness and homogeneity of the tissue sections and the size of the nuclei, as well as the fixation.

2.1.2.1 FISH on FFPE Tissues

FISH on FFPE tissues is a powerful alternative for clinical evaluation of lymphomas for which cells from fresh or frozen tissues are not available. In addition, the use of fixed tissues provides the opportunity to analyze large numbers of archival cases of rare anomalies (Haralambieva et al. 2002; Ventura et al. 2006). FISH analysis of FFPE tissue can be performed on unstained histological tissue sections or on disaggregated, intact nuclei (Schofield and Fletcher 1992; Kuchinka et al. 1995). Unstained sections allow preservation of tissue morphology and precise histopathologic correlation of multiple foci of normal cells, premalignant lesions, and tumor cells within a single specimen, including study of intra-tumor heterogeneity. However, this approach results in many incomplete nuclei, which can lead to a loss of some chromosomal material (the so-called truncation artifact) and subsequently to underestimation of chromosome copy number. Interpretation of results, therefore, requires rigorous assay validation. Analysis of large numbers of control samples has to be performed initially, to establish laboratory cutoff values for each anticipated signal pattern. An alternative approach involving extraction of intact nuclei from 50 μm tissue sections is not frequently used in routine clinical testing. A comprehensive review of technical issues related to performing FISH analysis on

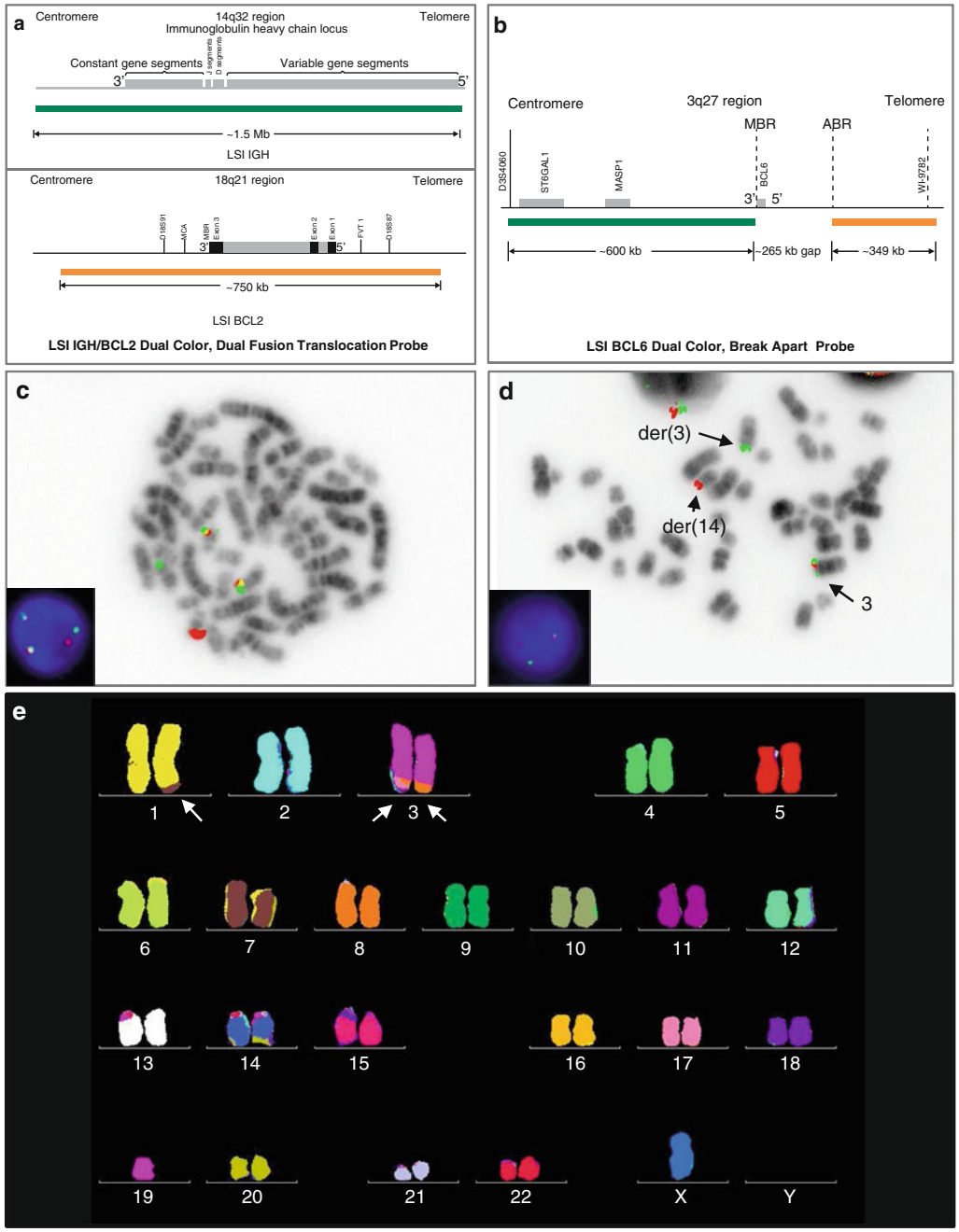

Fig. 2.1 Application of FISH in lymphoma diagnostics and research. (**a**) Scheme of the dual-color, break-apart probe for *BCL6* translocations (www.abbottmolecular.com); Scheme of the dual-color, dual-fusion translocation probe for the t(14;18)/*IGH-BCL2* (www.abbottmolecular.com) (**b**); (**c**–**d**) metaphase and interphase FISH with LSI *IGH/BCL2* and LSI *BCL6* performed on cases with t(3;14) and t(14;18) and t(3;14), respectively. Note the one fusion-one green-one red signal pattern in (**d**) the two fusions-one green-one red signal pattern in (**c**) and (**e**) Example of M-FISH. *Arrows* indicate abnormal chromosomes

FFPE tissues is beyond the scope of this text but can be found in other review articles and book chapters (Hopman et al. 1997; Weremowicz and Schofield 2007; Muller et al. 2009).

FISH results on tissue sections are typically available in 3–7 days. FISH on paraffin sections is labor-intensive and time-consuming; however, the opportunity to acquire valuable clinical information that otherwise would not be obtainable and to evaluate archival material far outweighs these considerations.

2.1.2.2 Multicolor FICTION

Multicolor fluorescence immunophenotyping and interphase cytogenetics technique (M-FICTION) has been developed as a tool for phenotypic and genotypic analyses of neoplastic cells (Martin-Subero et al. 2002). This approach enables the simultaneous detection of morphologic, immunophenotypic, and genetic features of single cells and is particularly useful in studies of tumors characterized by rare neoplastic cells, such as Hodgkin lymphoma. In practice, the multicolor FICTION combines a typical immunophenotypic detection of lineage- or tumor-specific antigens (e.g., CD20 in the case of mature B-cell lymphoma) with FISH using probe for the relevant loci (e.g., LSI BCL6) (for details, see Martin-Subero et al. 2002). FICTION can be applied on fresh (frozen) material, as well as on FFPE sections. Using DNA probes labeled with several fluorescence dyes and/or by combinatorial labeling schemes, multiple genetic aberrations may be simultaneously studied.

2.1.2.3 Spectral Karyotyping (SKY) and Multiplex Fluorescence In Situ Hybridization (M-FISH)

SKY and M-FISH are molecular cytogenetic techniques which allow simultaneous visualization of each chromosome in a different color, thus facilitating the identification of chromosomal aberrations (Speicher and Ward 1996; Veldman et al. 1997). Both methods use libraries of DNA probes produced from flow-sorted chromosomes that are specific for individual chromosomes or chromosomal regions. Chromosome-specific probes are labeled with five basic fluorescent dyes, which are used either alone or in combination with each other to create 24 color mixtures with distinct proportions of each dye. Thus, each chromosome is characterized by a unique spectral signature (Speicher et al. 1996; Tanke et al. 1999). Since the human eye is not capable of resolving small wavelength differences, a complex technical translation system has to be used for SKY/M-FISH analysis; the sophisticated equipment required is the major drawback of this technology.

Using SKY/M-FISH, complex structural abnormalities can be visualized readily and the chromosomal origin of abnormal structures, such as marker chromosomes, can be identified much more easily than by conventional cytogenetic analysis (Fig. 2.1e). However, because of the need for specific interpretative skills and more complex equipment than conventional cytogenetics, SKY and M-FISH are accessible to only a limited number of investigators, and it has remained a research tool.

2.1.3 Comparative Genomic Hybridization (CGH), Array CGH, and SNP Arrays

Comparative genetic hybridization (CGH) and its successor, array CGH (aCGH), are genome-wide methods that allow detection of copy number abnormalities (deletions, duplications, chromosome loss or gain) with a very high resolution and do not require dividing tumor cells. As a result of its improved resolution (<50–100 kb) (Fig. 2.2), aCGH or "matrix CGH" has replaced conventional CGH. In aCGH, normal DNA and tumor DNA are differentially labeled with fluorescent dyes [frequently used dyes are cyanin 3 (Cy3) and cyanin 5 (Cy5)]. After blocking of repetitive DNA, these labeled DNAs are competitively hybridized to a high-density array of probes spotted on a glass slide and analyzed using a microarray scanner and specialized software. Equal hybridization of tumor and control DNA (comparable signal intensities between two colors) indicates the absence of deletions or duplications in the tumor sample. In contrast, increased or decreased signal intensities from tumor DNA relative to the control sample are

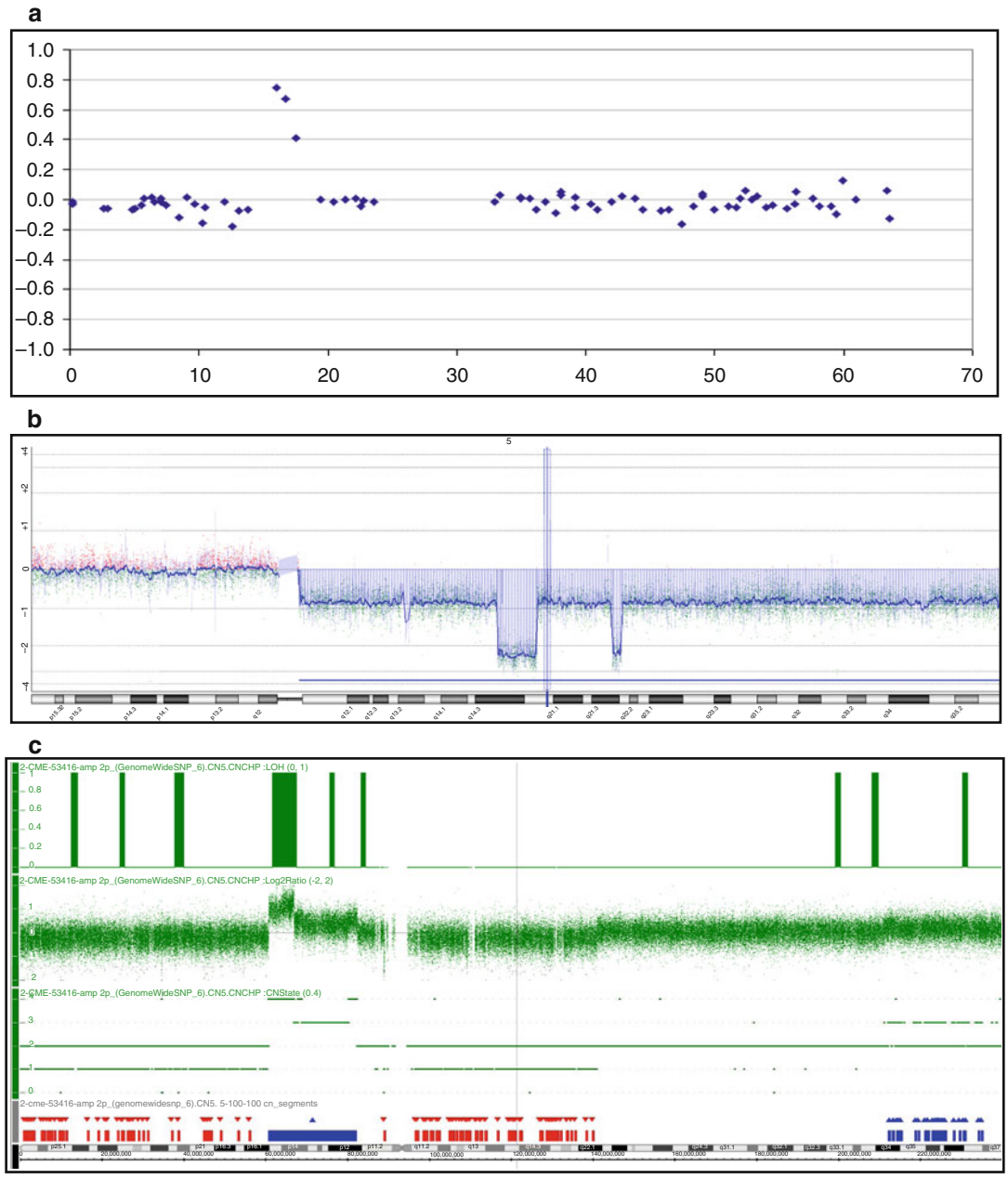

Fig. 2.2 Examples of array CGH analysis using platforms with an increased resolution. (**a**) 1 Mb BAC array (3,500 clones); (**b**) Agilent Oligo 400 k (400,000 oligos); (**c**) Affymetrix SNPs 6.0 (1.8 mln SNPs)

indicative of duplications or deletions of specific genomic regions in the tumor. DNA isolated from blocks of paraffin-embedded tissue can be used successfully for hybridization. Although the application of aCGH in mature B-cell and T-cell lymphomas has allowed the detection of distinct patterns of gains and losses characteristic of particular lymphoma subtypes, a major drawback of both conventional CGH and aCGH is that balanced chromosomal alterations such as translocations cannot be detected.

Single nucleotide polymorphism (SNP) array analysis is the newest, clinically applicable whole genome method. A unique advantage of SNP

analysis over other available methods is that not only changes in copy number but also regions of loss of heterozygosity (LOH) without loss or gain of DNA can be detected (i.e., copy-neutral LOH or uniparental disomy – UPD), indicating that both alleles are homozygous and derived from the same parental chromosome. The acquired LOH is very important to detect in tumors, since it represents a common mechanism of eliminating the wild-type alleles of tumor suppressor genes and oncogenes and duplicating mutated alleles. Regions of LOH, therefore, frequently harbor tumor suppressor genes with inactivating (loss-of-function) mutations or oncogenes with activating (gain-of-function) mutations. Alternatively, regions with UPD may contain biallelic hypermethylated and, thus, silenced (candidate tumor suppressor) genes. SNP analysis is a promising approach to genome-wide analysis in lymphoma and is now supported by efficient commercially available platforms.

2.2 Cytogenetic Aspects of Individual Lymphomas

Cytogenetic findings in individual subtypes of rare lymphomas are discussed below and summarized in Tables 2.1 and 2.2.

2.2.1 Mature T- and NK-Cell Neoplasms

The 2008 World Health Organization's (WHO's) classification of lymphoid malignancies recognized 18 subtypes of mature T- and NK-cell neoplasms (Swerdlow et al. 2008). These tumors are relatively uncommon and often clinically aggressive and their diagnosis remains a challenge. With rare exceptions, the molecular pathogenesis of T-cell and NK-cell lymphomas is largely unknown.

2.2.1.1 Adult T-Cell Leukemia

Adult T-cell leukemia/lymphoma (ATLL) is a mature T-cell neoplasm caused by the human T-lymphotropic virus 1 (HTLV-1, also called T-cell leukemia virus). This tumor is endemic in those parts of the world where HTLV-1 is prevalent in the population, including Japan, the Caribbean basin and parts of central Africa (Swerdlow et al. 2008). HTLV-1 is the first human retrovirus shown to cause malignant transformation, which is mediated by the viral protein Tax. One of its effects on cellular processes is to impair DNA repair mechanisms by repressing the expression of DNA polymerase-β, an enzyme involved in base excision repair, and by repressing nucleotide excision repair, involved in repairing UV irradiation and DNA replication-induced damage. Tax can also directly inactivate the TP53 protein (Kannian and Green 2010; Yasunaga and Matsuoka 2011). HTLV-1-induced destabilization of the genome may explain the complex karyotypes (3 or more cytogenetic abnormalities) that are typically observed in ATLL.

Chromosome abnormalities have been found by conventional cytogenetic analysis in almost all ATLL samples examined; the changes are typically complex and variable, with abnormalities affecting any chromosome pair. Nonetheless, several recurring abnormalities have been described. Translocations are identified in ~10 % of ATLL cases. The T-cell receptor α/Δ gene (*TCRA* and *TCRD*) locus at 14q11.2 is frequently rearranged, often as a t(14;14)(q11.2;q32), inv(14)(q11.2q32), or del(14)(q11.2q13) or as a rearrangement of 14q11.2 with one of several other chromosome arms, including Xq, 1p, 1q, 3p, 3q, 8q, 10p, 11p, 12q, and 18p (Kamada et al. 1992). The numerical abnormalities most frequently noted by conventional cytogenetic analysis comprised −X, −Y, −13, +X, +3, +5, and +7, whereas frequently observed segmental aneuploidies included deletions of 6q, 10p, 3q, 5q, 9q, 13q, 1p, or 7p (Schlegelberger et al. 1994a).

Genome profiles of ATLL have been extensively studied by conventional CGH. The most frequently observed imbalances were losses at 6q and 13q and gains at 7q and 3p. CGH analysis also showed abnormalities at numerous other chromosomal regions, including 1p, 1q, 3q, 5p, 5q, 9q, 10p, 10q, 11q, 12q, 18q, and Y. Comparison of genome profiles detected by CGH revealed differences between the acute and lymphoma types of ATLL, with the lymphoma

Table 2.1 Recurrent abnormalities in mature T- and NK-cell neoplasms

Lymphoma type	Recurrent abnormalities[a]	Primary (disease characteristic) abnormalities
Adult T-cell leukemia	Gains: X, 3, 5, 7; 1q, 3p (acute type), 2p, 4q, 7p, 7q (CGH) Losses: X, Y, 13, 6q, 10p, 3q, 5q, 9q, 13q, 1p, and 7p; 6q, 10p, 13q, 16q, 18p (CGH) Structural aberrations: t(14;14)(q11.2;q32), inv(14)(q11.2q32), del(14)(q11.2q13) Common breakpoints: 14q11.2 (*TCRA* and *TCRD*) Amplifications: 1p36, 6p25, 7p22 (*CARD11*), 7q, 14q32	Not described
Nasal NK-cell lymphoma	Gains: X; Xp, 2p, 10q (CGH); 2q (aCGH) Losses: 6q21-q25, 13q, 17p; 6q, 13q, 17p, 1p, 12q (CGH); 6q16–q27, 11q22–q23, 5p14, 5q34, 1p36, 2p16, 4q12, 4q31 (aCGH) Structural aberrations: i(1q), i(7q), i(17q)	Not described
Enteropathy-associated lymphoma	Gains: 1q22–q44, 5q, 9q31.3–qter (aCGH) Losses: 16q12.1 (aCGH) Amplifications: 8q24.2 (*MYC*)	Not described
Hepatosplenic T-cell lymphoma	Gains: 8 Losses: Y Structural aberrations: i(7)(q10)	i(7)(q10)
Cutaneous T-cell lymphomas:		
Mycosis fungoides and Sézary syndrome	Gains: 8, 18, 8q, 17q (CGH) Losses: 1p, 6q, 10q, 13q, 17p, 19 (CGH) Common breakpoints: 12q21 (*NAV3*)	Not described
Primary cutaneous anaplastic large cell lymphoma	Losses: 9p21 (*CDKN2A*) (CGH) Amplifications (CGH): 8p22 (*CTSB*), 3p25 (*RAF1*), 2p16 (*REL*), 19p13.2 (*JUNB*)	Not described
Peripheral T-cell lymphoma, not otherwise specified	Gains: 7q (*CDK6*), 8q (*MYC*), 17q, 22q (CGH and aCGH) Losses: 4q, 5q, 6q, 9q, 10q, 12q, 13q (CGH and aCGH) Structural aberrations: t(5;9)(q33;q22) in follicular variant	t(5;9)(q33;q22)/*ITK-SYK* in PTCL-F
Angioimmunoblastic T-cell lymphoma	Gains: X, 3, 5, 21 Losses: 6q, 13q	Not described
Anaplastic large cell lymphoma		
ALK-positive ALCL	Gains: 7, 17p, 17q (CGH) Losses: 4, 11q, 13q (CGH) Structural aberrations: t(2;5)(p23;q35) and variants – Table 2.3	t(2;5)(p23;q35)/*NPM1-ALK1*, variant 2p23/*ALK* aberrations
ALK-negative ALCL	Gains: 1q, 6p21, 6q, 7 Losses: 13q Structural aberrations: t(6;7)(p25.3;q32.3)	t(6;7)(p25.3;q32.3)

[a]Description of the listed abnormalities and references are provided in the text; unless otherwise specified, the abnormalities were detected by conventional cytogenetic analysis

type more frequently showing gains on 1q, 2p, 4q, 7p, and 7q and losses from 10p, 13q, 16q, and 18p and the acute type manifesting gains of 3p. Recurrent high-level amplifications were found at 1p36, 6p25, 7p22, 7q, and 14q32 in the lymphoma type, with *CARD11* identified as a candidate oncogene in 7p22 (Tsukasaki et al. 2001; Oshiro et al. 2006).

Table 2.2 Recurrent abnormalities in mature B-cell neoplasms

Lymphoma type	Recurrent abnormalities[a]	Primary (disease characteristic) abnormalities
B-cell prolymphocytic leukemia	Losses: 13q14, 11q23, 17p (FISH) Structural aberrations: chr 7	Not described
Marginal zone lymphoma		
MALT	Gains: 3, 6p, 7, 8q, 9q, 11q, 12, 18 Losses: 6q23 (aCGH and SNP array) Structural aberrations: t(11;18)(q21;q21.3), t(1;14)(p22;q32), t(3;14)(p13;q32), t(14;18)(q32;q21.3), t(X;14)(p12;q32)	t(11;18)/*API2-MALT1*, t(1;14)/*IGH-BCL10*, t(3;14)/*IGH-FOXP1*, t(14;18)/*IGH-MALT1*, t(X;14)/*IGH-GPR34*
Nodal MZL	Gains: 3, 18, 7q, 8q, 13q; 1q23–q25, 3q23–q24, 12q (CGH) Losses: 1p21–p22, 11q21–q22, 13q14, 15q25–q26 (CGH) Structural aberrations: t(X;14)/*IGH-GPR34*	t(X;14)/*IGH-GPR34* (rare)
Splenic MZL	Gains: 3, 18, 12 Losses: 7q, 6q, 13q Common breakpoints: 14q32 (*IGH*)	Not described
Mantle cell lymphoma	Gains and losses: see Tables 2.4 and 2.5 Structural aberrations: t(11;14)(q13;q32) or 11q13 variants	t(11;14)(q13;q32)/*IGH-CCND1*
CNS lymphoma	Gains: 12q, 18q21, 22q Losses: 9p21 (*CDKN2A*), 6q (CGH)	Not described
Primary mediastinal B-cell lymphoma	Gains: 2p, 9p, 12q, Xq (CGH); 7q22, 9q34, 11q23, 12q, 18q21 (aCGH) Losses: 6p21, 11q13.3 (aCGH) Amplification: 2p16 (*REL* and *BCL1A*), 9p24 (*JAK2*, *CD274/PDL1* and *PDCD1LG2/PDL2*) (aCGH)	Not described
Cutaneous B-cell lymphoma		
PCMZL	Structural aberrations: t(14;18)(q32;q21.3)/*IGH-MALT1*	
PCFCL	Structural aberrations: t(14;18)(q32;q21.3)/*IGH-BCL2*	
DLBL – leg type	Gains:18q, 1q, 7, 12q, Xp (CGH) Losses: 6q (CGH); 9p21.3 (aCGH) Amplification: 18q (*BCL2*, *MALT1*) (aCGH) Common breakpoints: 3q27 (*BCL6*), 8q24.2 (*MYC*), 14q32 (*IGH*) (FISH)	Not described
Waldenstrom macroglobulinemia	Gains:4 Losses: 6q	Not described
HIV-associated lymphoma		
Primary effusion lymphoma	Gains: 7, 12, 12q22–q23, 12q12–q23 (CGH) Common breakpoints: 1q21-q25	Not described
Adult Burkitt lymphoma	Gains: 1q, 7, 12 Losses: 6q, 17p, 13q32–q34 Structural aberrations: t(8;14)(q24.2;q32), t(2;8)(p12;q24.2), and t(8;22)(q24.2;q11.2)	t(8;14)(q24.2;q32)/*IGH-MYC*, t(2;8)(p12;q24.2)/*IGK-MYC*, t(8;22)(q24.2;q11.2)/*IGL-MYC*
Nodular lymphocyte-predominant Hodgkin lymphoma	Gains: 1q Losses: 4q28–q32, 7, 13q Common breakpoints: 14q32 (*IGH*), 3q27 (*BCL6*)	Not described

[a]Description of the listed abnormalities and references are provided in the text; unless otherwise specified, the abnormalities were detected by conventional cytogenetic analysis

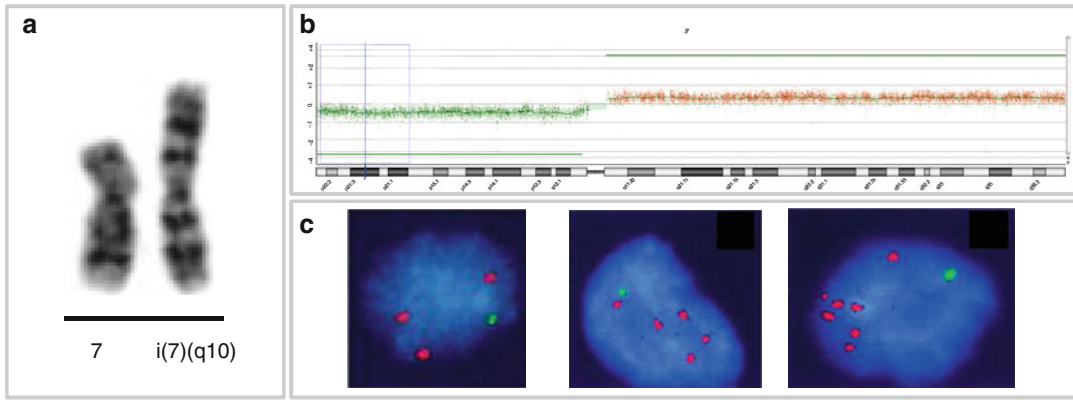

Fig. 2.3 Isochromosome 7q and HS**γδ**TCL. (**a**) Partial karyotype with i(7)(q10); (**b**) aCGH profile of chromosome 7 in case of HS**γδ**TCL showing loss of 7p and gain of 7q associated with i(7)(q10); (**c**) interphase FISH with probes for 7p (*green*) and 7q (*red*) in cases of HS**γδ**TCL with one (*left*), two (*middle*), and three (*right*) copies of the i(7)(q10) (Wlodarska et al. 2002, unpublished data)

Specific CGH profiles correlate with distinct clinical features. Aberrations of 1p, 1q, 1q10–21, 10p, 10p13, 12q, 14q, and 14q32 are associated with features such as hepatosplenomegaly, elevated LDH, and an unusual immunophenotype, which are all indicators of clinical severity in ATLL. Multiple changes; abnormalities of 1p, 1q22, 1q10–21, 2q, 3q, 3q10–12, 3q21, 14q, 14q32, and 17q; and partial losses from chromosome arms 2q, 9p, 14p, and 17q are correlated with shorter survival (Tsukasaki et al. 2001).

2.2.1.2 Nasal NK-Cell Lymphoma

Nasal NK-cell lymphoma is characterized by complex karyotypes, which do not appear to be disease specific. Wong et al. (1997) reported a common deletion at 6q21–q25 in three of seven cases studied by conventional cytogenetic analysis, suggesting that this may be a nonrandom chromosomal aberration in this disease. Other recurring abnormalities detected by metaphase analysis include +X, i(1q), i(7q), +8, del(13q), del(17p), i(17q), and rearrangements of 11q23 (Wong et al. 1999). Conventional CGH studies identified del(6q), del(13q), del(17p), del(1p), del(12q), and partial gain of Xp, 2p, or 10q as recurrent abnormalities (Siu et al. 1999). Genome-wide array-based CGH studies identified a number of recurrent imbalances in nasal-type extranodal NK-/T-cell lymphoma, including gain of 2q and loss of 6q16–q27, 11q22–q23, 5p14, 5q34, 1p36, 2p16, 4q12, and 4q31 (Nakashima et al. 2005).

2.2.1.3 Enteropathy-Associated Lymphoma

Enteropathy-associated lymphoma (ETL) is an intestinal tumor of intraepithelial T lymphocytes usually presenting as a tumor composed of large lymphoid cells that show varying degrees of transformation (Swerdlow et al. 2008). Genetic studies of ETL are limited, but most ETL cases (58–70 %) are characterized by frequent complex gains of the 9q31.3-qter chromosome region or by deletions of 16q12.1 (Deleeuw et al. 2007; Zettl et al. 2002). The affected 9q region harbors several candidate genes, including *ABL1* and *NOTCH1*, which are preferentially amplified in ETL (Cejkova et al. 2005). The 9q33–34 imbalances, however, are not specific for ETL, being found in almost 20 % of PTCL-NOS (Zettl et al. 2004). Other recurrent chromosomal aberrations include a partial trisomy of 1q22–q44 and gains of 5q reported in the classical form of ETL (Verkarre et al. 2003; Zettl et al. 2004) and amplifications of 8q24.2/*MYC* which are recurrently observed in the monomorphic variant (Zettl et al. 2002; Deleeuw et al. 2007). Furthermore, loss of heterozygosity at 9p21 is recurrent in classical ETL, and one or more genes in this region have been postulated to be involved in the pathogenesis of ETL (Obermann et al. 2004).

2.2.1.4 Hepatosplenic T-Cell Lymphoma

Hepatosplenic T-cell lymphoma (HSTCL) is a clinically aggressive subtype of PTCL, and the affected patients have a dismal outcome. This lymphoma accounts for 1.4 % of all mature T-cell neoplasms and represents <1 % of all non-Hodgkin lymphomas. The vast majority of HSTCLs express the γδ T-cell receptor (TCR) (HSγδTCL), but rare cases with the αβ TCR phenotype (HSαβTCL) have been also described (Lai et al. 2000; Suarez et al. 2000; Macon et al. 2001; Rashidi et al. 2012).

HSγδTCL is characterized by an isochromosome 7q [i(7)(q10)] (Fig. 2.3a), which has been identified by conventional cytogenetic and/or FISH analysis in almost all cases analyzed (Wang et al. 1995; Cooke et al. 1996; Jonveaux et al. 1996; Alonsozana et al. 1997). The i(7)(q10) can appear as the sole karyotypic abnormality, what suggests its primary and critical role in the development of HSγδTCL. The molecular consequences of i(7)(q10) are largely unknown, however, gene dosage effect resulting from the associated loss of 7p and gain of 7q (Fig. 2.3b) has been postulated. The tendency of HSγδTCL to select clones with increased copies of i(7)(q10) (Wlodarska et al. 2002) (Fig. 2.3c) or with the amplified 7q sequences (Shetty et al. 2006; Tamaska et al. 2006) suggests that this chromosome harbors genes potentially important for its pathogenesis. One of the candidate genes is cyclin-dependent kinase 6 (*CDK6*), known as a target of the t(2;7)(p12;q21) in splenic marginal zone lymphoma (Corcoran et al. 1999). Despite a strong association of the i(7)(q10) with HSγδTCL, this aberration is not specific for the entity, being observed at a lower frequency in a broad spectrum of hematological malignancies (Mertens et al. 1994). Secondary aberrations frequently associated with i(7)(q10) in HSγδTCL include trisomy 8 and loss of the Y chromosome (Alonsozana et al. 1997; Jonveaux et al. 1996; Wlodarska et al. 2002). The role of these abnormalities in the pathogenesis of HSγδTCL is unknown. Early cytogenetic studies reported rare HSγδTCL cases with abnormal karyotypes, but lacking i(7)(q10) (Ross et al. 1994; Salhany et al. 1997a, b; Weidmann et al. 2000); these findings, however, have not been validated by FISH.

Despite a different immunophenotype, HSαβTCL is also characterized by a recurrent i(7)(q10) (Suarez et al. 2000; Macon et al. 2001), suggesting a common pathogenesis of both lymphomas. Hence, it has been postulated that HSγδTCL and HSαβTCL represent phenotypic variants of the same disease entity.

2.2.1.5 Cutaneous T-Cell Lymphoma

T-cell lymphomas showing primary localization in the skin include mycosis fungoides and Sézary syndrome, primary cutaneous CD30+ lymphoproliferative disorders including primary cutaneous anaplastic large cell lymphoma and lymphomatoid papulosis, subcutaneous panniculitis-like T-cell lymphoma, and some rare lymphomas such as primary cutaneous aggressive epidermotropic CD8+ T-cell lymphoma (provisional), cutaneous γ/δ T-cell lymphoma (provisional), and primary cutaneous CD4+ small-/medium-sized pleomorphic T-cell lymphoma (provisional) (Swerdlow et al. 2008).

Complex karyotypes have been observed in mycosis fungoides (MF) and Sézary syndrome (SS), but no disease-specific abnormalities have been described (Prunieras 1974; Thangavelu et al. 1997). Karenko et al. reported recurrent rearrangements with a breakpoint at 12q21, targeting the *NAV3* (*POMF1L1*) gene (Karenko et al. 2007). Translocations involving TCR loci are notably absent in MF/SS (Salgado et al. 2011). CGH studies have revealed common deletions at 1p, 6q, 10q, 13q, and 17p and on chromosome 19 and gains of chromosomes 7 and 18 and at 8q and 17q (Mao et al. 2002).

Complex karyotypes are also characteristic for primary cutaneous anaplastic large cell lymphoma (C-ALCL). The t(2;5)(p23;q35), characteristic for ALK-positive ALCL, is detected in only rare cases. *NPM1-ALK* transcripts were detected by PCR in the absence of ALK protein expression in these tumors; thus, their pathogenetic significance is uncertain (Wood 1998). Deletions at 9p21, affecting the *CDKN2A/p16* locus, are present in some C-ALCLs. CGH studies also demonstrated oncogene amplifications

involving *CTSB* at 8p22, *RAF1* at 3p25, *REL* at 2p16, and *JUNB* at 19p13.2 (Mao et al. 2003; van Kester et al. 2010).

Cytogenetic studies of regressing lymphomatoid papulosis lesions demonstrated either a normal karyotype or abnormalities of chromosomes 7, 10, and 12. The t(2;5) has not been found (Mao et al. 2002).

2.2.1.6 Peripheral T-Cell Lymphoma, Not Otherwise Specified

Peripheral T-cell lymphoma, not otherwise specified (PTCL-NOS) is the largest and most common category of PTCL, accounting for approximately 30 % of all PTCLs in the Western world. This entity is very heterogeneous comprising cases that do not correspond to any of the PTCL subtypes recognized in the current WHO classification (Swerdlow et al. 2008). Cytogenetic analysis showed that karyotypes of PTCL-NOS are typically very complex. Both conventional and array CGH studies detected recurrent gains of 7q/*CDK6*, 8q/*MYC*, 17q, and 22q and recurrent losses of 4q, 5q, 6q, 9q, 10q, 12q, and 13q (Thorns et al. 2007; Nagel et al. 2008). Deletions of 5q, 10q, and 12q correlate with a better prognosis (Zettl et al. 2004). Notably, genomic profiles of PTCL-NOS differ from those observed in AITL and ALCL. The regions 6q16–q22, 9p21, and 11p11.2 are predominantly lost in PTCL-NOS when compared to AITL and gains of 7q22 and 8q24.1–q24.3 are more frequent in PTCL-NOS than in AITL- and ALK-negative ALCL. Common genetic events identified in these entities include a recurrent gain of 11q13 in both AITL and PTCL-NOS and loss of 6q21 in both ALK-negative ALCL and PTCL-NOS (Zettl et al. 2004; Thorns et al. 2007; Nelson et al. 2008).

The WHO classification recognized three variants of PTCL-NOS: lymphoepithelioid (Lennert's lymphoma), follicular, and T-zone variants (Swerdlow et al. 2008). Genetic data are mainly available for the follicular variant (PTCL-F), in which the growth pattern mimics follicular B-cell lymphoma or T-cell lymphomas with a perifollicular growth pattern (Rudiger et al. 2000; Ikonomou et al. 2006; Huang et al. 2009). This lymphoma is characterized by a recurrent t(5;9)(q33;q22), which involves two protein tyrosine kinase genes: *ITK*, the IL-2-inducible T-cell kinase gene located at 5q33, and *SYK*, the spleen tyrosine kinase gene mapped at 9q22 (Streubel et al. 2006b). The resulting *ITK-SYK* fusion protein revealed a constitutively active SYK tyrosine kinase, which has shown to be transforming both in vitro and in vivo (Rigby et al. 2009; Dierks et al. 2010; Pechloff et al. 2010). *SYK* plays an important key role in TCR signaling; overexpression and activation of SYK is a common feature of PTCL, and therefore, the gene represents a potential therapeutic target (Feldman et al. 2008; Wilcox et al. 2010). Although the t(5;9)/*ITK-SYK* has been detected in 17 % of PTCL-NOS, it seems to be restricted to PTCL-F (Streubel et al. 2006b; Feldman et al. 2008). Given the rarity of PTCL-F, the prognostic significance of the t(5;9) is unknown.

2.2.1.7 Angioimmunoblastic T-Cell Lymphoma

Angioimmunoblastic T-cell lymphoma (AITL) accounts for 1–2 % of all non-Hodgkin lymphomas and for 25–30 % of PTCL cases in Europe and North America (Rudiger et al. 2002). Clonal chromosomal aberrations have been detected in up to 90 % of the cases analyzed (Weiss et al. 1986; Tan et al. 2006; Attygalle et al. 2007). The recurrent aberrations include trisomies of chromosomes 3, 5, and 21; gain of X; and loss of 6q and 13q (Schlegelberger et al. 1994b; Thorns et al. 2007; Nelson et al. 2008; reviewed by Dogan et al. 2003). Chromosomal breakpoints affecting the TCR gene loci seem to be very scarce (Leich et al. 2007). Although the cellular derivation is uncertain, recent findings suggest that MAF-expressing follicular helper T cells (T_{FH}) represent the normal counterpart of AITL (Thielen et al. 2011).

2.2.1.8 Anaplastic Large Cell Lymphoma

ALK-Positive Anaplastic Large Cell Lymphoma

Anaplastic large cell lymphoma expressing the anaplastic lymphoma kinase (ALK-positive ALCL) is a well-defined subtype of PTCL accounting for approximately 3 % of all NHLs and for 60–80 % of all ALCLs. The tumor occurs

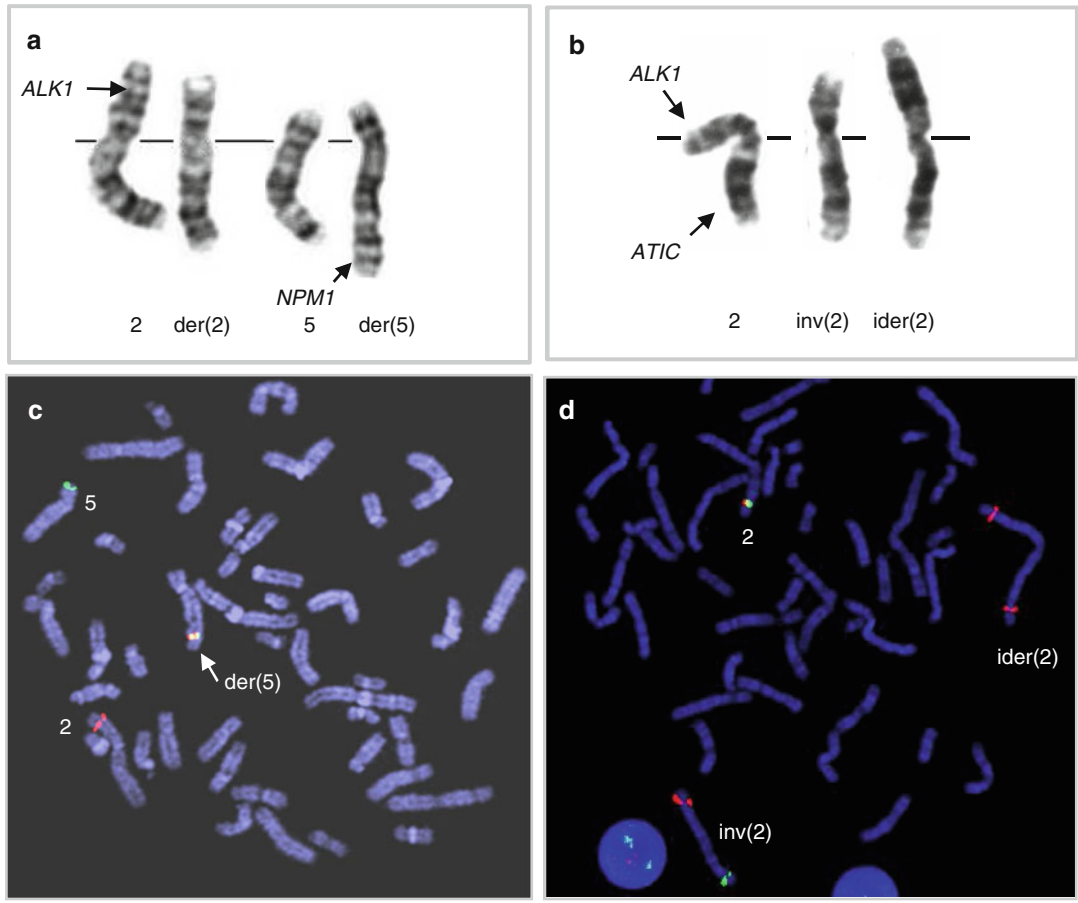

Fig. 2.4 The 2p23/*ALK* rearrangements in ALK-positive ALCL. (**a**) Classical t(2;5)(p23;q35)/*ALK-NPM1* rearrangement and (**b**) variant 2p23 aberration, inv(2)(p23q35), involving *ALK* and *ATIC*; this aberration is commonly accompanied by ider(2)(q10)inv(2)(p23q35); (**c**) FISH with probes covering the 3′ end of *ALK* (*red*) and the 5′ end of *NPM1* (*green*) in t(2;5)-positive lymphoma; note a fused signal on der(5); (**d**) FISH with LSI ALK break-apart probe in the inv(5)-positive tumor; note split *ALK* signals on inv(2) and two extra red signals (3′ *ALK*) on ider(2)(q10)inv(2)(p23q35) (Ma et al. 2000)

predominantly in children and young adults. The aberrant expression of ALK in these tumors is caused by chromosomal rearrangements of *ALK*/2p23. The gene was initially identified as a partner of nucleophosmin (*NPM1*) in ALCL-associated t(2;5)(p23;q35) (Morris et al. 1995) (Fig. 2.4a–c). The NPM1-ALK fusion protein contains the N-terminal portion of the NPM1 protein and the intracellular kinase domain of ALK. The oncogenic potential of NPM1-ALK has been proven by a number of in vitro and in vivo studies (Falini et al. 1999; Kuefer et al. 1997; Lange et al. 2003). *ALK* codes for a transmembrane receptor tyrosine kinase, which is a member of the insulin receptor superfamily. The biological function of ALK is largely unknown, but its normal expression is restricted to scattered cells in the central nervous system (Iwahara et al. 1997; Morris et al. 1997). The t(2;5) occurs in approximately 75 % of ALK-positive ALCL cases and the remaining cases harbor one of the variant *ALK*/2p23 rearrangements. The identified variant ALK fusions are listed in Table 2.3, and the inv(2) resulting in the *ATIC-ALK* rearrangement is illustrated in Fig. 2.4b–d. CGH analysis revealed that secondary genetic alterations are common in ALK-positive ALCL. Particularly frequent are gains of 7, 17p, and 17q and losses of 4, 11q, and 13q (Salaverria et al. 2008a; Swerdlow et al. 2008).

Table 2.3 Recurrent chromosomal rearrangements involving 2p23/*ALK* in ALK-positive ALCL

Chromosomal aberration	Fusion partner	Molecular weight of hybrid protein (kDa)	Cellular localization	References
t(2;5)(p23;q35)	NPM1	80	Nuclear, nucleolar, and diffuse cytoplasmic	Drexler et al. (2000), Morris et al. (1994)
t(1;2)(q25;p23)[a]	TPM3	104	Diffuse cytoplasmic and membranous	Elenitoba-Johnson et al. (2006), Lamant et al. (1999), Siebert et al. (1999), Stein et al. (2000)
inv(2)(p23;q35)	ATIC	96	Diffuse cytoplasmic	Colleoni et al. (2000), Ma et al. (2000), Matsubara et al. (2008), Trinei et al. (2000)
t(2;3)(p23,q21)[a]	TFG	85–97	Diffuse cytoplasmic	Hernandez et al. (1999, 2002)
t(2;17)(p23;q23)	CLTC	250	Granular cytoplasmic	Touriol et al. (2000)
t(2;19)(p23;p13.1)	TPM4	94–105	Cytoplasmic	Meech et al. (2001)
t(X;2)(q11;p23)[a]	MSN	125	Membranous	Tort et al. (2001, 2004)
t(2;17)(p23;q25)	ALO17	ND	Cytoplasmic	Cools et al. (2002)
t(2;22)(p23;q11)[a]	MYH9	220	Diffuse cytoplasmic	Lamant et al. (2003)

NPM1 Nucleophosmin, *ATIC* = *PurH* 5 aminoimidazole-4-carboxamide-1-beta-D-ribonucleotide transformylase/IMP cyclohydrolase, *TFG* Trk fusion gene, *TPM3/4* tropomyosin 3/4, *CLTC* clathrin heavy chain, *MSN* moesin, *MYH9* non-muscle myosin heavy-chain gene 9, *ND* not determined

[a]According to Ensembl (release 67, May 2012), TPM3, TFG, MSN, and MYH9 are located at 1q21.3, 3q12.2, Xq12, and 22q12.3, respectively

All 2p23 aberrations lead to overexpression of the ALK protein and constitutive tyrosine kinase activation of ALK. Of note, cellular localization of the ALK fusion in tumor cells is determined by the biological function of the partner gene (reviewed by Drexler et al. 2000 and Stein et al. 2000). Oncogenic potential of ALK, demonstrated in various lymphoid and non-lymphoid malignancies, is mediated by interaction in multiple signaling pathways, including the JAK3/STAT3 and PI3K/AKT pathways (reviewed by Amin and Lai 2007; Webb et al. 2009; de Leval and Gaulard 2011). ALK expression is an important prognostic factor for ALCL patients, since the 5-year survival rate of ALK-positive patients is significantly higher as compared to ALK-negative cases (Shiota et al. 1995; Falini et al. 1999; Gascoyne et al. 1999; Savage et al. 2008). Given that the ALK protein is not expressed by lymphoid cells, immunostaining with ALK-specific antibodies is routinely used for the diagnosis of ALK-positive tumors.

Interestingly, ALK fusions have been also identified in DLBCL, inflammatory myofibroblastic tumors, non-small cell lung cancer, and squamous cell carcinoma of the esophagus (Arber et al. 1996; Griffin et al. 1999; De Paepe et al. 2003; Jazii et al. 2006; Du et al. 2007; Rikova et al. 2007; Soda et al. 2007), highlighting the crucial role of ALK in tumorigenesis. Given that several small-molecule ALK inhibitors have been recently developed and tested preclinically, ALK-positive ALCL patients may also benefit from this novel targeted therapy.

ALK-Negative Anaplastic Large Cell Lymphoma

The genetic mechanisms underlying development of ALK-negative ALCL are poorly understood, but recent studies, including genome-wide molecular analyses, have identified novel genetic lesions in this entity and provided new insights into its pathogenesis. It has been shown that approximately 25 % of ALK-negative ALCLs harbor recurrent chromosomal translocations affecting 6q25.3 (Feldman et al. 2009, 2011; Pham-Ledard et al. 2010; Wada et al. 2011). Surprisingly, these aberrations appeared to target two different genes, *IRF4* and the telomerically located *DUSP22*. *IRF4* encodes a transcription factor which plays an important role in the regulation of normal lymphoid differentiation and tumorigenesis (Falini et al. 2000; Michaux et al. 2005). Thus far, partner

chromosomes involved by the 6p25.3/*IRF4* translocations have not been identified and molecular consequences of these rearrangements are also unclear, particularly that the expression of *IRF4* in these tumors is not dysregulated (Feldman et al. 2011). More than 50 % of cases with the 6p25.3 aberrations involving *DUSP22* affect 7q32.3. Interestingly, the recurrent t(6;7)(p25.3;q32.3) is associated with downregulation of *DUSP22* and overexpression of *MIR29* (Feldman et al. 2011). DUSP22 is a phosphatase that inhibits T-cell antigen receptor signaling in reactive T cells by inactivating the MAPK/ERK2 pathway (Alonso et al. 2002); thus, its downregulation as a result of the t(6;7) suggests that the gene acts as a tumor suppressor in ALK-negative ALCL. *MIR29* is known to target the *TCL1* oncogene (Ruiz-Ballesteros et al. 2007), which is not expressed in ALCLs. Further investigations are necessary to decipher the functional consequences of the 6p25.3 rearrangements in ALK-negative ALCL. Given that these aberrations occur only sporadically in other PTCL entities (Feldman et al. 2009, 2011; Pham-Ledard et al. 2010; Wada et al. 2011), their detection may be helpful in the diagnosis of ALK-negative ALCL.

Array CGH analysis of ALK-negative ALCL identified chromosomal imbalances in two-third of the cases analyzed; gains of 1q and 6p21 were more frequent in ALK-negative ALCL when compared to ALK-positive ALCL, but gains of chromosome 7 and 6q and loss of 13q were commonly seen in both subtypes (Salaverria et al. 2008a). In rare ALK-negative ALCL cases expressing PAX5, numerical changes of the *PAX5*/9p13 locus have been detected (Feldman et al. 2010).

2.2.2 Mature B-Cell Neoplasms

The 2008 WHO classification of lymphoid malignancies recognizes 30 entities of mature B-cell neoplasms (Swerdlow et al. 2008). Two of them, diffuse large B-cell lymphoma (DLBCL) and follicular lymphoma (FL) account for approximately 66 % of all B-cell lymphoma; the prevalence of the remaining 28 subtypes ranges from less than 1 to 12 %. The available (cyto)genetic data of these rare B-cell lymphomas are reviewed below.

2.2.2.1 B-Cell Prolymphocytic Leukemia

B-cell prolymphocytic leukemia (B-PLL) is a rare lymphoid neoplasm characterized by peripheral blood, bone marrow, and splenic involvement by a clonal proliferation of prolymphocytes. The cytogenetic pattern of B-PLL has been poorly characterized due to the rarity of the disease, the difficulty in obtaining mitoses of the rather mature leukemic cells, and the overlap with other entities that previously may have been considered to be B-PLL. The t(11;14)(q13;q32)/*CCND1/IGH* rearrangement has previously been reported in up to 20 % of B-cell PLL cases (Brito-Babapulle et al. 1987); however, these cases are now considered leukemic manifestations of MCL (Ruchlemer et al. 2004). More recent studies that focused on confirmed cases of B-PLL showed the presence of complex karyotypes, with frequent abnormalities of chromosome 7 (Schlette et al. 2001). FISH analysis has been used in B-PLL to test for abnormalities at specific genetic loci; this resulted in detection of 13q14 deletions involving the *RB1* gene in approximately 50 % of the cases. Additionally, deletions were frequently noted at band 11q23 and at 17p (*TP53* locus) (Lens et al. 2000). None of the aberrations reported thus far in B-PLL have been disease specific.

2.2.2.2 Marginal Zone Lymphoma

Marginal zone lymphoma (MZL) encompasses three distinct entities: extranodal MZL of the mucosa-associated lymphoid tissue (MALT lymphoma), nodal MZL (NMZL), and splenic MZL (SMZL) (Swerdlow et al. 2008). It is believed that these indolent malignancies, which develop in different anatomical sites, originate from postfollicular memory B cells. MZL share several morphologic, immunophenotypic, and genetic features, including trisomies 3 and 18; however, the pathogenesis of these lymphomas is poorly understood.

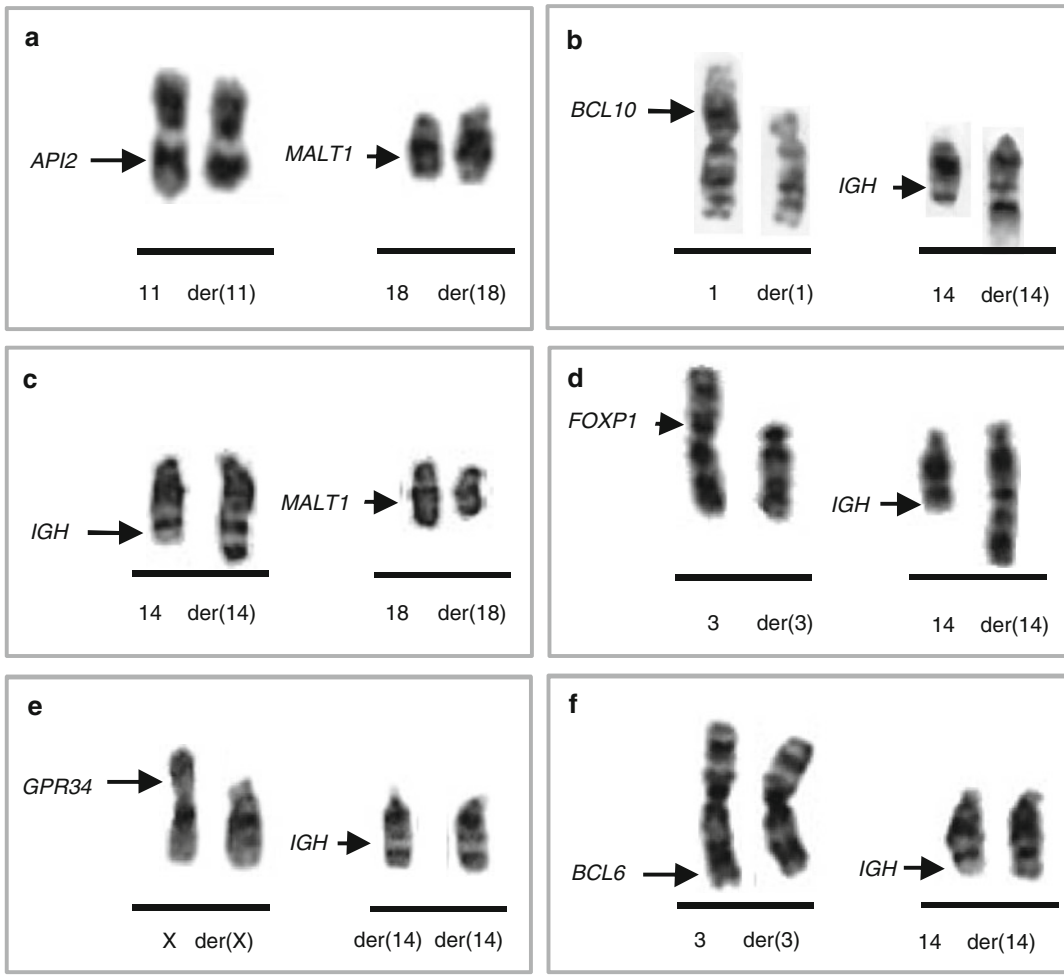

Fig. 2.5 MALT lymphoma-associated translocations and the involved genes. (**a**) t(11;18)(q21;q21.3)/*API2-MALT1*; (**b**) t(1;14)(p22;q32)/*IGH-BCL10*; (**c**) t(14;18)(q32;q21.3)/*IGH-MALT1*; (**d**) t(3;14)(p13;q32)/*IGH-FOXP1*; (**e**) t(X;14)(p11.2;q32)/*IGH-GPR34*; (**f**) t(3;14)(q27;q32)/*IGH-BCL6*

MALT Lymphoma

MALT lymphoma represents 50–70 % of MZLs and involves a variety of extranodal sites. Approximately 25 % of MALT lymphomas harbor balanced chromosomal translocations (Remstein et al. 2006), including the most frequent t(11;18)(q21;q21.3) and less common t(1;14)(p22;q32), t(3;14)(p13;q32), t(14;18)(q32;q21.3), and t(X;14)(p12;q32) (Fig. 2.5). These translocations are mutually exclusive and occur at variable frequencies in MALT lymphoma of different sites.

t(11;18)(q21;q21.3) typically occurs as the sole chromosomal aberration (Auer et al. 1997; Ott et al. 1997; Zhou et al. 2006). The translocation fuses the amino-terminus of the *API2* (*alias BIRC3*) gene (11q21) with three intact BIR domains to the carboxyl-terminus of the *MALT1* gene (18q21.3) containing an intact caspase-like domain, generating a functional API2-MALT1 fusion (Akagi et al. 1999; Morgan et al. 1999; Dierlamm et al. 2000a). API2 is an inhibitor of apoptosis, whereas MALT1 is involved in antigen receptor mediated NF-κB activation (Ruland et al. 2003). The t(11;18) is specific for extranodal MZL and occurs at variable frequencies in MALT lymphoma of different sites, being most frequent in tumors from lung (38–53 %), followed by those from stomach (24 %), conjunctiva (19 %), the intestine (12.5 %), and the ocular adnexa/orbit

(3–14 %) (Streubel et al. 2004; Ye et al. 2003a). The translocation is rare or absent in MALT lymphomas from the skin, thyroid, salivary gland, and liver. The t(11;18) has been associated with adverse clinical features. Gastric MALT lymphomas with the t(11;18) are negative for *H. pylori* (Ye et al. 2003b) and hence do not respond to *H. pylori* eradication (Liu et al. 2001). Despite controversial initial data, recent studies suggest that t(11;18)-positive gastric MALT lymphomas evolve to a more aggressive DLBCL, as the API2-MALT1 fusion was detected in both gastric MALT lymphomas and gastric DLBCLs at approximately equivalent frequencies (Toracchio et al. 2009).

t(1;14)(p22;32) occurs in a small minority of MALT lymphomas and has not been observed in other lymphoma subtypes. The translocation brings the entire *BCL10* gene under the regulatory control of *IGH* and, hence, dysregulates its expression (Willis et al. 1999; Zhang et al. 1999). *BCL10* encodes a protein containing a caspase recruitment domain (CARD) homologous to that found in several apoptotic molecules. Experimental data indicate that BCL10 is essential for both the development and function of B and T lymphocytes, specifically connecting antigen receptor signaling to the NF-κB pathway (Ruland et al. 2001; Xue et al. 2003). The t(1;14) is primarily seen in intestine (12.5 %) and pulmonary (6.7 %) MALT lymphoma (Streubel et al. 2004). Of note, gastric MALT lymphomas with t(1;14) and/or a strong BCL10 nuclear expression do not respond to *H. pylori* eradication (Ye et al. 2006). Interestingly, the *IGK* variant translocation, t(1;2)(p22;p12), has been identified in a case of pulmonary MALT lymphoma (Chuang et al. 2007).

t(14;18)(q32;q21.3) associated with MALT lymphoma is molecularly different from the follicular lymphoma-related t(14;18)/*IGH-BCL2*, as it targets the *MALT1* gene located 4.5 Mb proximal to *BCL2* (Sanchez-Izquierdo et al. 2003; Streubel et al. 2003). Thus, *MALT1* is rearranged by two different translocations in MALT lymphoma, either as a fusion partner of *API2* in the t(11;18)(q21;q21.3) or being upregulated by *IGH* due to the t(14;18)(q32;q21.3). The latter translocation has also been observed in rare cases of extranodal DLBCL (Cook et al. 2003; Sanchez-Izquierdo et al. 2003). Amplification of the 18q21.3/*MALT1* region, considered as an alternative mechanism of dysregulation of MALT1, was detected in cell lines derived from MZL as well as from Burkitt lymphoma and primary cutaneous DLBCL (Sanchez-Izquierdo et al. 2003). The t(14;18)/*IGH-MALT1* also occurs at variable frequencies in MALT lymphoma of different sites (Murga Penas et al. 2003; Streubel et al. 2003, 2004), primarily in lymphomas from the liver (17 %), ocular adnexa (7 %), and lung (6 %), but was not found in those from the stomach, salivary gland, thyroid, and skin (Ye et al. 2005).

t(3;14)(p13;q32) is present in approximately 4 % of MALT lymphoma (Goatly et al. 2008). The translocation is mediated by *IGH* and dysregulates expression of *FOXP1* (Streubel et al. 2005), which belongs to the Forkhead box (FOX) family of winged-helix transcription factors that play diverse biological functions. Experimental data indicate that FOXP1 is essential for development of B/T lymphocytes and monocytes (Hu et al. 2006; Shi et al. 2008; Feng et al. 2010). The t(3;14)/*IGH-FOXP1* was detected in MALT lymphomas arising in the thyroid, ocular adnexa, skin, and stomach and, also, in rare cases of extranodal DLBCL (Streubel et al. 2005; Wlodarska et al. 2005; Haralambieva et al. 2006; Fenton et al. 2006; Goatly et al. 2008). Rare translocations of *FOXP1* involving non-*IG* partner genes have also been reported (Wlodarska et al. 2005; Goatly et al. 2008). Notably, a significant fraction of MALT lymphomas and DLBCLs harbor a strong nuclear FOXP1 expression, which is independent from *FOXP1* rearrangements and gains (Wlodarska et al. 2005; Sagaert et al. 2006a; Barrans et al. 2007). Some studies indicate that aberrant expression of FOXP1 in these tumors predicts poor prognosis (Barrans et al. 2004; Banham et al. 2005; Wlodarska et al. 2005) and transformation to DLBCL in MALT lymphomas (Sagaert et al. 2006b; Han et al. 2009).

t(X;14)(p12;q32) is a recently identified *IGH*-mediated chromosomal translocation affecting *GPR34* (Baens et al. 2012), found in two cases of pulmonary MALT lymphoma and single cases of nodal MZL and gastric DLBCL.

GPR34 encodes a G-protein-coupled receptor, belonging to the largest family of cell surface molecules involved in signal transduction (Lappano and Maggiolini 2011). These proteins play important roles in many physiological and pathological processes, including tumorigenesis. Thus fra, the functional consequences of t(X;14) remain unknow.

In addition, the DLBCL-related t(3;14)(q27;q32)/*IGH-BCL6* was reported in sporadic cases of extranodal MZL (Dierlamm et al. 1997; Ye et al. 2008). Recently, novel *IGH* translocations targeting *CNN3* (1p21.3), *ODZ2* (5q34), or *JMJD2C* (9p24) were identified in single cases of MALT lymphoma (Vinatzer et al. 2008). Approximately 75 % of MALT lymphomas, however, do not harbor recurring translocations and their genetics is poorly understood. Trisomies or partial trisomies of several chromosomes including 3, 6p, 7, 8q, 9q, 11q, 12, and 18 are frequently observed in MALT lymphoma (Wotherspoon et al. 1995; Brynes et al. 1996; Dierlamm et al. 1996a; Streubel et al. 2004; Zhou et al. 2006; Kim et al. 2007; Rinaldi et al. 2011). Of note, trisomies 3, 12, and 18 are often concurrent and present in both translocation-negative and translocation-positive cases, with the exception of the (11;18). Recently, array CGH/SNP array studies identified loss of *TNFAIP3/A20* (6q23) in 21.8 % of MALT lymphomas (Kato et al. 2009), preferentially in translocation-negative MALT lymphoma of the ocular adnexa and salivary gland (Honma et al. 2008; Chanudet et al. 2009).

NMZL

Nodal MZL is a heterogeneous disorder accounting for less than 2 % of all lymphoid malignancies (Nathwani et al. 1999; Berger et al. 2000) and approximately 10 % of MZL (Braggio et al. 2012). Genetic data are scarce for NMZL, and thus far, no typical genetic defects have been identified in this entity. In addition, most genetic lesions observed in NMZL can also be found in extranodal MZL, especially in MALT lymphoma (Brynes et al. 1996; Dierlamm et al. 1996a; Traverse-Glehen et al. 2006; Arcaini et al. 2009; Rinaldi et al. 2011; Braggio et al. 2012). Trisomies 3 and 18 detected in 20–30 % of NMZL cases can occur either as a sole genetic aberration or together; hence, it is unclear whether they represent primary or secondary cytogenetic changes. The molecular consequences of these imbalances remain elusive; however, a dosage effect of tumor-associated genes located on chromosome 3 (e.g., *FOXP1*, *BCL6*) and 18 (*MALT1*, *BCL2*) has been hypothesized. Other recurrent cytogenetic alterations in NMZL include imbalances of chromosomes 1; gain of 7q, 8q, and 13q (Rinaldi et al. 2011); and structural rearrangements of chromosome 1 (Dierlamm et al. 1996b). Focal imbalances detected by CGH in 15–25 % of NMZL include loss of 1p21.2–p22.1, 11q21–q22/*ATM*, 13q14.3, and 15q25.3–q26.2 and gain of 1q23.3–q25.3, 3q23–q24, and 12q13.13–q21.31 (Braggio et al. 2012). Deletions of the known tumor suppressor genes, including *TP53*, *CDKN2A/p16*, *RB1*, and *TNFAIP3/A20*, are absent or sporadic in NMZL (Dierlamm et al. 2000b; Novak et al. 2009; Braggio et al. 2012). Chromosomal translocations occurring in MALT lymphoma are usually not detected in NMZL. Rare exceptions include the t(X;14)/*IGH-GPR34* (Baens et al. 2012) and t(3;14)/*IGH-BCL6* (Traverse-Glehen et al. 2006).

SMZL

Splenic MZL represents approximately 20 % of MZL (Braggio et al. 2012). Current knowledge on the genetic background of SMZL is limited. Early cytogenetic, FISH, and metaphase CGH studies performed on small series of SMZL showed that the disease is frequently associated with a complex karyotype, deletion of 7q, trisomy or partial trisomy 3, and alterations of chromosomes 1, 8, and 14 (Oscier et al. 1993; Dierlamm et al. 1996b, 1997; Sole et al. 2001; Callet-Bauchu et al. 2005). No single aberration was present in all cases. These findings were recently confirmed by a multicenter study of 330 cytogenetically documented SMZL cases published by the International Splenic Lymphoma Group (Salido et al. 2010). Clonal cytogenetic aberrations were found in 72 % of SMZL, of which 28 % harbored a single chromosomal aberration and 53 % had complex karyotypes. Deletion of 7q was the most common aberration

observed in 39 % of cases analyzed and also the most frequent single aberration (32 %); this anomaly was followed in frequency by gains of chromosome 3/3q (25 %), translocations involving 14q32 (12 %), deletion of 6q (11.7 %), trisomy 18 (10 %), deletion of 17p (8.7 %), trisomy 12 (8 %), and deletion of 13q (5 %). The breakpoints of the del(7q) were heterogeneous and the smallest overlapping region was defined as 7q32.1–q32.2. This aberration has been investigated by several groups (Mateo et al. 1999; Hernandez et al. 2001; Andersen et al. 2004; Vega et al. 2008; Watkins et al. 2010; Rinaldi et al. 2011), and currently the smallest commonly deleted region comprises approximately 3 Mb at 7q32.1–q32.2 (127.03–130.07 Mb) (Watkins et al. 2010). This region harbors 44 coding genes and a cluster of six microRNAs. Lack of evidence of homozygous deletions and/or microdeletions in this region, however, hampers identification of a putative tumor suppressor gene at 7q32. Given that del(7q) is preferentially associated with SMZL and rarely seen in other mature B-cell malignancies (Watkins et al. 2010; Rinaldi et al. 2011), this aberration is potentially valuable in SMZL diagnosis and differential diagnosis.

One-quarter of SMZL cases displayed gain of material from chromosome 3, particularly 3q (Gruszka-Westwood et al. 1999; Sole et al. 2001), due to various unbalanced translocations. Gain of chromosome 3 is frequently associated with trisomies 12 and 18, considered as secondary changes in SMZL (Brynes et al. 1996; Sole et al. 2001; Andersen et al. 2004).

Chromosomal translocations involving *IG* loci at 14q32, 2p12, and 22q11.2 were found in 12 % of SMZLs. These aberrations targeted at least 11 partner chromosomes/genes (1p34, 1p22, 1q21, 6p21/*CCND3*, 7q22/*CDK6*, 8q24.2/*MYC*, 9p13/*PAX5*, 9p11.3, 11q21, 12q23, 19q13.3/*BCL3*) and only sporadically occurred as a single anomaly. Some of these translocations were previously published in SMZL (Callet-Bauchu et al. 2005; Remstein et al. 2008). Of note, typical translocations associated with NHL, such as the t(11;14), t(14;18), and t(3;14), and MALT-lymphoma-associated translocations were not found in SMZL (Remstein et al. 2000; Salido et al. 2010). As in other B-NHL, a fraction of SMZL (18 %) showed loss of 17p/*TP53* (Gruszka-Westwood et al. 2001; Salido et al. 2010). This aberration and 14q abnormalities are associated with a shorter survival. The del(7q), gain of chromosome 3/3q, +18, and del(6q) have no impact on survival.

2.2.2.3 Mantle Cell Lymphoma

Mantle cell lymphoma (MCL) is a well-defined neoplasm originating from naïve pre-germinal-center B cells, associated with an aggressive clinical presentation, poor response to therapy, and a short survival (Swerdlow et al. 2008). Cytogenetically, MCL is defined by the t(11;14)(q13;q32), a translocation that brings the *CCND1* gene (the breakpoint region was previously known as *BCL1*) under transcriptional control of the regulatory sequences of *IGH* (Fig. 2.6a–b) (Tsujimoto et al. 1984; Williams et al. 1991). *CCND1* encodes cyclin D1 which plays an important role in the regulation of the G1-S transition following mitotic growth factor signaling (Hunter and Pines 1994); its aberrant expression in MCL is routinely detected by IHC (Fig. 2.6c). Of note, the t(11;14)(q13;q32) is also noted in a subset of multiple myeloma and related disorders (Fonseca et al. 2003). Conventional cytogenetic analysis identifies the t(11;14) in 60–80 % of MCL (Li et al. 1999; Wlodarska et al. 1999; Au et al. 2002); however, FISH detects the *IGH-CCND1* rearrangement in almost all MCL cases examined (Li et al. 1999; Bentz et al. 2000, 2004; Frater et al. 2001). FISH analysis is particularly recommended in MCL cases with atypical karyotypes, because the t(11;14) may be cryptic or masked by complex secondary alterations (Gruszka-Westwood et al. 2002; Aventin et al. 2003; Gazzo et al. 2005) (Fig. 2.6d–e). Sporadic MCL cases display variant 11q13/*CCND1* translocations involving either *IGK*/2p12 or *IGL*/22q11.2 (Fig. 2.7) (Komatsu et al. 1994; Wlodarska et al. 2004a; Espinet et al. 2010; Rocha et al. 2011). Rare cases of MCL that are negative for the translocation and cyclin D1 have been identified. Interestingly, these lymphomas are similar to classical MCL with a typical morphology, transcriptome profile, and pattern of secondary

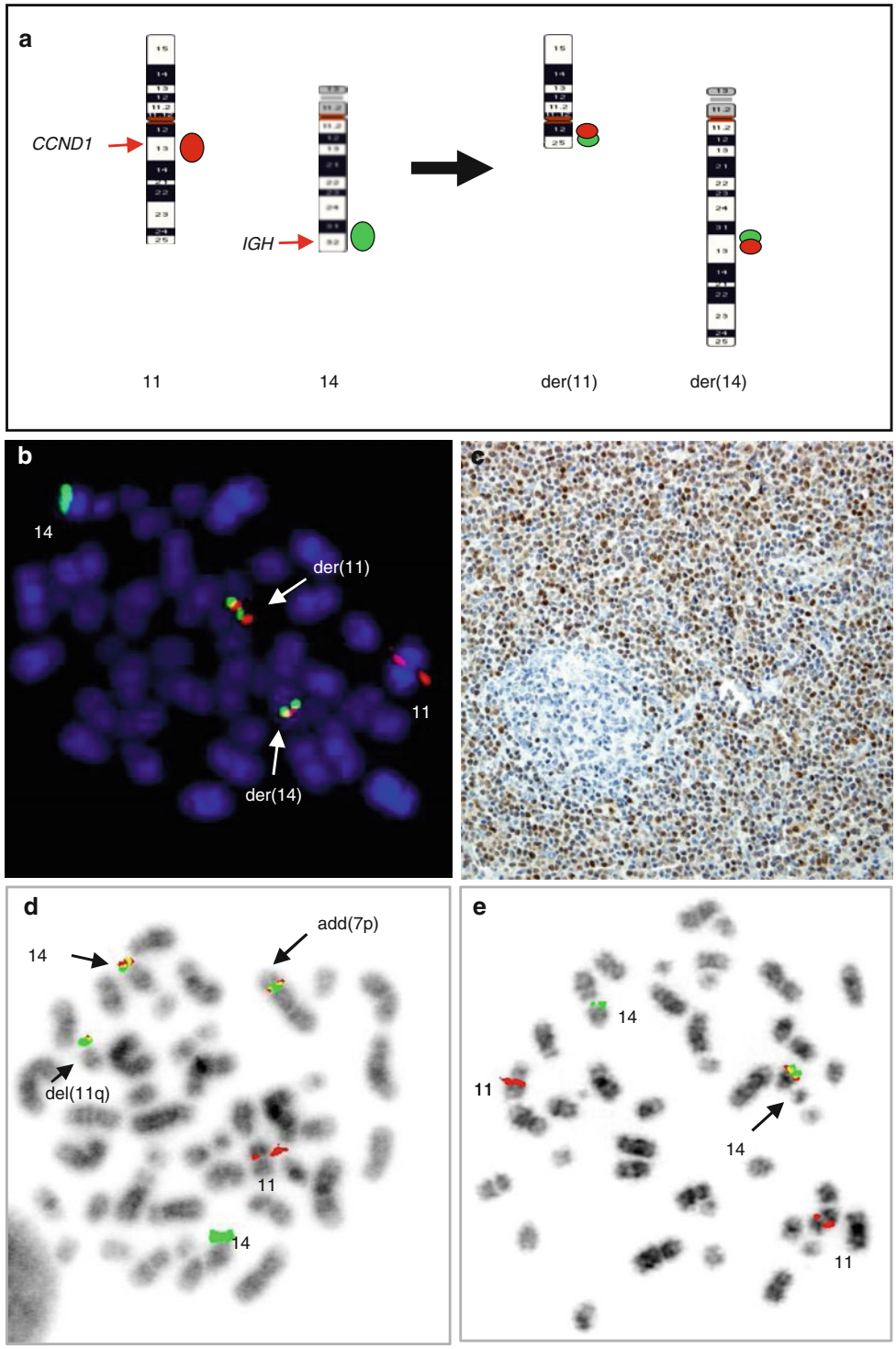

Fig. 2.6 Common and rare 11q13/*CCND1* rearrangements in MCL. (**a**) Scheme of a classical t(11;14)(q13;q32) and distribution of FISH signals from the dual-color, dual-fusion LSI IGH/CCND1 probe; (**b**) FISH image of a metaphase cell with LSI IGH/CCND1; (**c**) an aberrant expression of cyclin D1 in MCL shown by IHC; (**d**) cryptic *IGH-CCND1* fusion in case of t(11;14)-negative MCL detected with LSI IGH/CCND1; (**e**) cryptic insertion of *CCND1* at 14q32/*IGH* in a case of t(11;14)-negative MCL detected with LSI IGH/CCND1 (Wlodarska et al. 2004a, unpublished data)

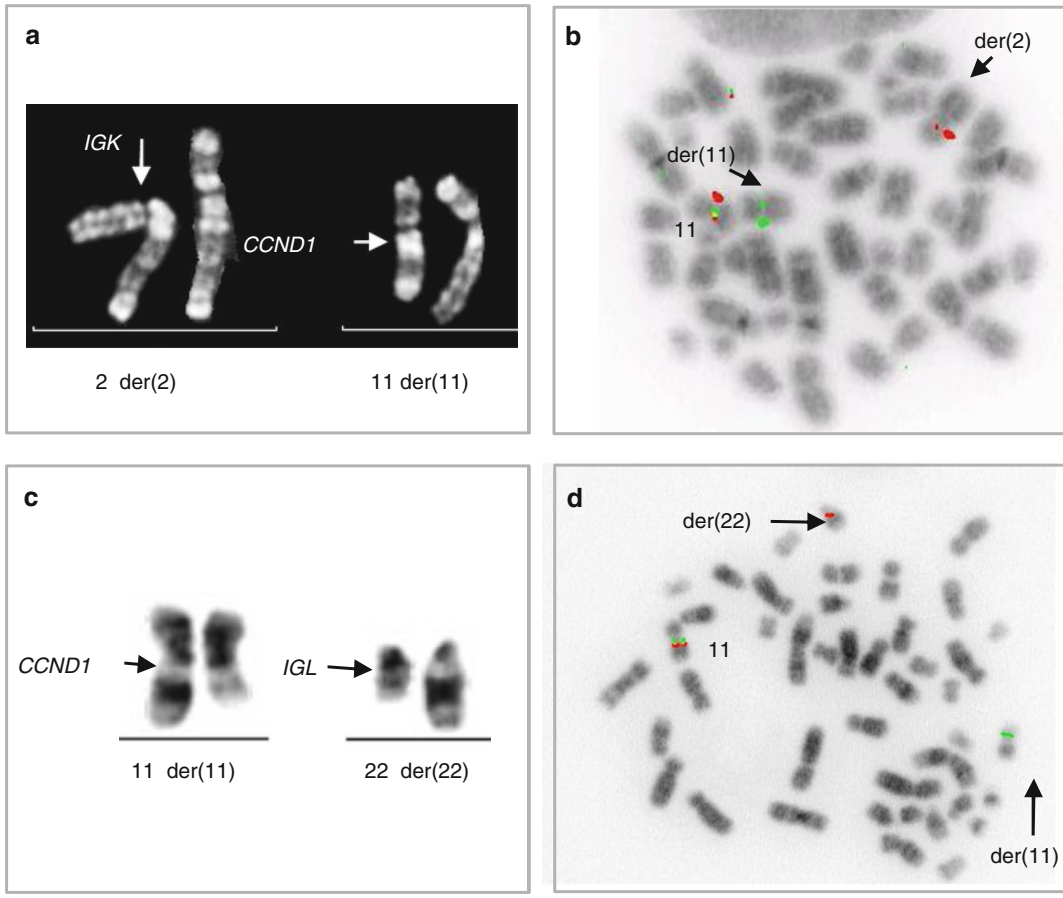

Fig. 2.7 Variant *CCND1* aberrations in MCL. (**a**) Partial R-banded karyotype of t(2;11)(p12;q13) and (**b**) the related FISH image showing a rearrangement of *CCND1* (*CCND1* break-apart probe); (**c**) partial karyotype of t(11;22)(q13;q11.2) and (**d**) the related FISH image showing a rearrangement of *CCND1*

genetic alterations, but they express high levels of either cyclin D2 or cyclin D3 (Fu et al. 2005; Salaverria et al. 2007; Hartmann et al. 2010) and do not express SOX11 (Mozos et al. 2009). As documented in several cases, an aberrant expression of cyclin D2 and D3 in t(11;14)-negative MCL is a consequence of *IG* translocations targeting *CCND2* and *CCND3*, respectively (Gesk et al. 2006; Herens et al. 2008; Wlodarska et al. 2008; Quintanilla-Martinez et al. 2009; Shiller et al. 2011) (Fig. 2.8).

Approximately one-third of MCLs have the t(11;14)(q13;q32) as a sole cytogenetic aberration or together with 1–2 additional chromosomal changes. Remarkably, these cases are usually associated with a leukemic (non-nodal) disease, an indolent clinical course, and a long survival (Fernandez et al. 2010; Royo et al. 2012). In contrast, classical aggressive MCLs display complex karyotypes characterized by a high number of nonrandom secondary chromosomal changes, initially shown by cytogenetic studies (Li et al. 1999; Wlodarska et al. 1999; Au et al. 2002) and further demonstrated by genome-wide screening approaches (reviewed by Royo et al. 2011). These aberrations are commonly associated with genomic gains and losses. Balanced translocations are infrequent in MCL. An exception is the t(8;14)(q24.2;q32)/*IGH-MYC* and variant *MYC* aberrations, which have been recurrently seen in MCLs with blastoid morphology (Tirier et al. 1996; Au et al. 2000; Vaishampayan et al. 2001; Hao et al. 2002; Michaux et al. 2004). As in the well-known *MYC/BCL2* "double-hit" lympho-

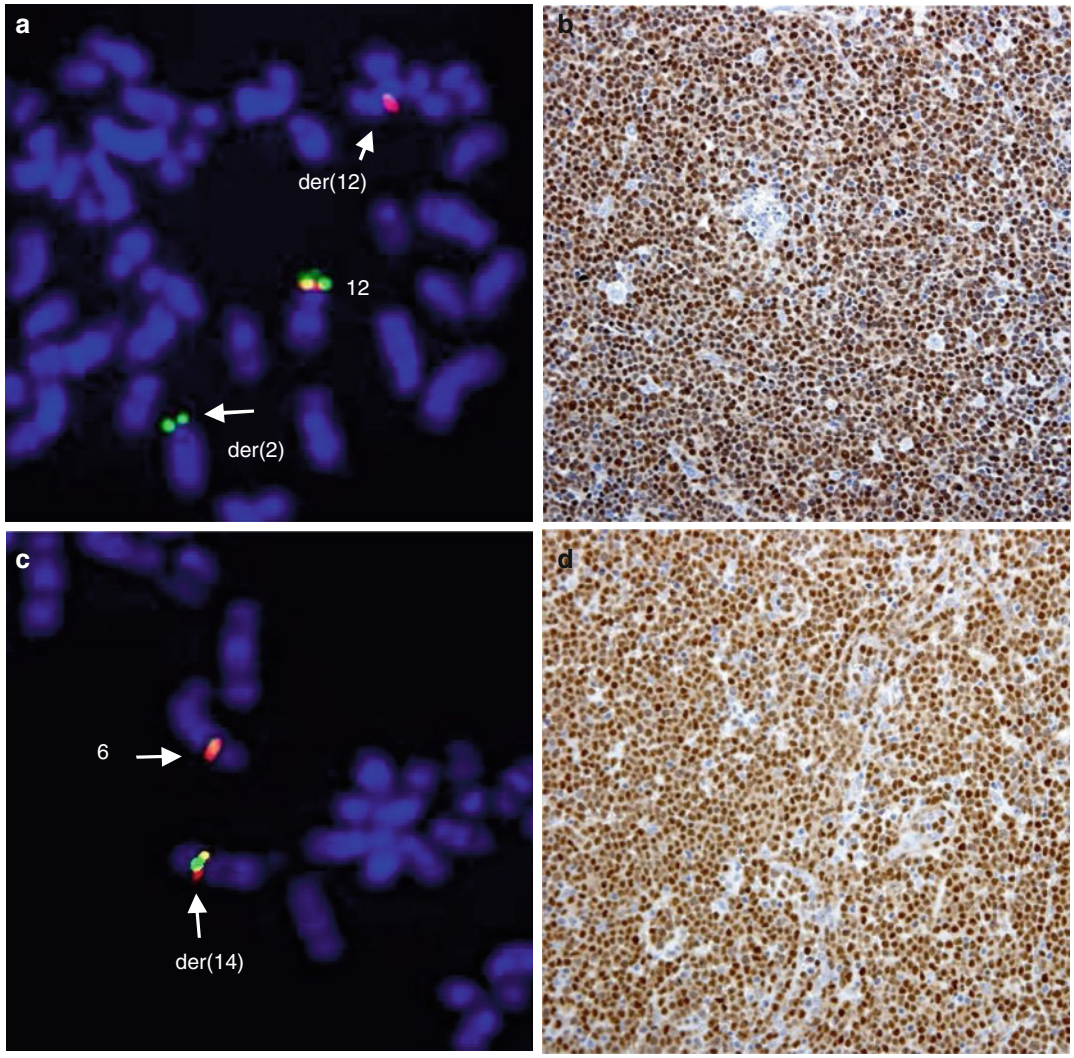

Fig. 2.8 Alternative translocations in t(11;14)-negative MCL. The t(2;12)(p12;p13)-associated rearrangement of *CCND2* (*CCND2* break-apart probe) (**a**) resulting in aberrant expression of cyclin D2 demonstrated by IHC (**b**); t(6;14)(p21;q32) affecting *CCND3* (*CCND3* break-apart probe) (**c**) and the underlying aberrant expression of cyclin D3 demonstrated by IHC (**d**) (Wlodarska et al. 2008)

mas, survival of patients with MCLs with *MYC*/8q24.2 alterations is extremely short.

The genomic profile of MCL was initially investigated using conventional CGH (Monni et al. 1998; Bea et al. 1999; Bentz et al. 2000; Martinez-Climent et al. 2001; Allen et al. 2002; Jarosova et al. 2004; Salaverria et al. 2007). These studies identified numerous recurrent genomic imbalances in MCL, of which the most frequent were losses of 1p, 6q, 8p, 9p, 10p, 11q, 13q, and 17p and gains of 3q, 7p, 8q, 12q, 15q, and 18q (Table 2.4, adapted from Royo et al. 2011). These findings were further confirmed using high-resolution CGH and SNP arrays that mapped the minimal regions of loss and gain and identified new submicroscopic deletions and duplications (Schaffner et al. 2000; Kohlhammer et al. 2004; Rubio-Moscardo et al. 2005; Schraders et al. 2005; Tagawa et al. 2005; Rinaldi et al. 2006; Flordal et al. 2007; Bea et al. 2009; Kawamata et al. 2009; Vater et al. 2009; Halldorsdottir et al. 2011). The results and candidate target genes are shown in Table 2.5. The genes postulated to be involved in the pathogen-

Table 2.4 Recurrent secondary genomic alterations in MCL detected by CGH and potential target genes[a]

	Monni et al. (1998)	Bea et al. (1999)		Bentz et al. (2000)	Martinez-Climent et al. (2001)	Allen et al. (2002)	Salaverria et al. (2007)	Jarosova et al. (2004)	
Number of cases	27	45		27	28	30	77	30	
% altered cases	100	89		85	95	100	90	80	
Mean number of alterations per case	4	6		4	6	7	4	6	
Chromosomal region	% cases	% cases	% cases	% cases	% cases	% cases	% cases	Minimal region	Potential target genes
Loss 1p	33	24	33	26	33	52	27	1p21–p22	
Loss 3p	4	7	–	5	–	5	–	3p13–p14	
Loss 6q	30	27	19	32	37	20	13	6q21–q22; 6q25–q26	
Loss 8p	7	7	30	79	23	13	33	8p21–p22	
Loss 9p	30	16	30	16	17	18	7	9p21	*CDKN2A*
Loss 9q	15	13	14	5	13	21	20	9q21–q22	
Loss 10p	–	18	11	10	17	3	17	10p14–p15	
Loss 11q	30	22	19	26	27	28	37	11q22–q23	*ATM*
Loss 13q	41	40	70	32	60	17	33	13q13–q14; 13q33–q34	
Loss 17p	19	16	4	26	20	13	30	17p13	*TP53*
Gain 3q	52	49	37	37	70	32	40	3q27–q28	
Gain 7p	15	27	7	5	23	8	7	7p22	

[a]Adapted from Royo et al. (2011)

esis of MCL include *TNFAIP3* (6q23), *CDKN2A* (9p21), *ATM* (11q22.3), *RB1* (13q14), and *TP53* (17p13) located in the recurrently deleted regions and *BMI1*(10p12), *CDK4/MDM2* (12q14), and *BCL2* (18q21.3), which are found to be gained and/or amplified in MCL. In addition, SNP array analysis identified CN-LOH events in up to 60 % of MCL (Bea et al. 2009; Kawamata et al. 2009; Vater et al. 2009; Fernandez et al. 2010; Hartmann et al. 2010; Halldorsdottir et al. 2011). CN-LOH frequently affects 6p, 9p, 11q, 17p, and 20q, which are recurrently deleted regions in MCL. Whether CN-LOH represents an alternative mechanism of biallelic inactivation of tumor suppressor genes remains to be determined.

It has been postulated that several genomic aberrations have a prognostic impact in MCL. For example, a complex karyotype, gain of 3q27–q29 and 12q, and mutations of *TP53* (but not loss of *TP53*/17p13) correlate with unfavorable outcome (Bea et al. 1999; Allen et al. 2002; Rubio-Moscardo et al. 2005; Salaverria et al. 2007; Katzenberger et al. 2008; Kawamata et al. 2009; Vater et al. 2009), and loss of 9p21/*CDKN2A* and 9q21–q22 predicts inferior prognosis (Bea et al. 1999; Rubio-Moscardo et al. 2005; Salaverria et al. 2007; Hartmann et al. 2010). Remarkably, biallelic inactivation of *CDKN2A* and *TP53* is frequently found in blastoid and progressed MCL, but has not been observed in leukemic/indolent MCL.

In summary, the genomic profile of MCL is unique and distinct from other lymphomas. The hallmark of this lymphoma is the t(11;14)(q13;q32)/*IGH-CCND1* rearrangement, which is an initial event in MCL lymphomagenesis. An aggressive behavior of this lymphoma, however, is related to a high level of genomic instability associated with the accumulation of numerous secondary chromosomal abnormalities mainly targeting genes involved in cell cycle regulation, DNA damage response, and cell survival pathways.

Table 2.5 Recurrent minimal regions and target genes detected in MCL by CGH and SNP arrays[a]

Loss/homozygous loss	SNPa (range %)	CGH (range %)	Candidate genes	Pathways
Loss 1p21.2	17–55	29–50		
Loss 1p22.2–p22.3	13–55	29–50		
Loss 1p32.3–p33[a]	4–14	–	*CDKN2C*, *FAF1*	Cell cycle/cell survival
Loss 1q32	5–18	–	*PROX1*	Proliferation
Loss 2q13[a]	3–4	17	*BCL2L11*	Cell survival
Loss 2q37.1	15–33	–	*SP100*, *SP140*	DNA damage
Loss 6q23.3	19–23	26–36	*TNFAIP3*	NF-κB inhibitor
Loss 6q25	19–28	23–36	*LATS1*	Hippo signaling pathway
Loss 8p21.3	25–31	17–34	*MCPH1*	DNA damage
Loss 9p21.2	19–24	10–36	*MOBKL2B*	Hippo signaling pathway
Loss 9p21.3[a]	10–36	10–36	*CDKN2A/B*, *MTAP*	Cell cycle
Loss 9q22.2–q22.31	17–29	18–31		
Loss 10p14–p13	18–27	18–28		
Loss 11q22.3	11–55	21–57	*ATM*	DNA damage response
Loss 13q12.3–q13.1	15–27	43–54		
Loss 13q14.2	27–38	25–55	*RB1*	Cell cycle
Loss 13q33.2–q33.3	35–36	28–54		
Loss 13q34	16–39	28–54	*CUL4A*, *ING1*	Cell cycle/DNA damage
Loss 17p13	21–32	22–45	*TP53*	Cell cycle, DNA damage
Loss 19p13.1	3–19	24		
Loss 19p13.3	10–19	24	*MOBKL2A*	Hippo signaling pathway
Loss 21q11.2	10–19	–		
Gain 3q26.1–q26.32	28–46	31–50		
Gain 7p22.1–p22.3	8–19	16–31		
Gain 8q24.21	6–32	17–19	*MYC*	Proliferation
Gain 10p12.2–12.31	6–7	12	*BMI1*	Cell cycle
Gain 11q13.3–q21	4–14	9–11	*CCND1*, *MAP6*	Cell cycle/microtubule dynamics
Gain 12q14	4–7	3	*CDK4*, *MDM2*, *CENTG1*	Cell cycle/apoptosis/DNA damage
Gain 13q31.3	6–11	5	*MIR17HG* (*miR-17-92*)	Cell cycle, apoptosis
Gain 15q23	10–23	9		
Gain 18q21.33	3–11	5–17	*BCL2*	Apoptosis

[a]Adapted Table 2.4 from Royo et al. (2011)

Abbreviations: *CGH* comparative genomic hybridization, *MCL* mantle cell lymphoma, *SNPa* single nucleotide polymorphism array. Potential target genes were only indicated if they were validated by other techniques and if there is previous evidence of oncogenic or tumor suppressor activity

2.2.2.4 Primary DLBCL of the CNS

Diffuse large B-cell lymphoma of the central nervous system frequently shows abnormalities of the *BCL6* locus, but rearrangements of the *BCL2* or *MYC* loci are rare. Homozygous and hemizygous deletions affecting the *CDKN2A/p16* locus at 9p21 are common (Cobbers et al. 1998). Conventional CGH studies showed recurrent deletions at 6q and gains at 12q, 18q21, and 22q (Weber et al. 2000). Small deletions, beyond the resolution of conventional CGH, have been detected by molecular methods at 6p21.3 (HLA locus) and are believed to result in loss of HLA classes I and II expression, which may allow the lymphoma cells to escape from immune surveillance (Riemersma et al. 2000; Booman et al. 2006).

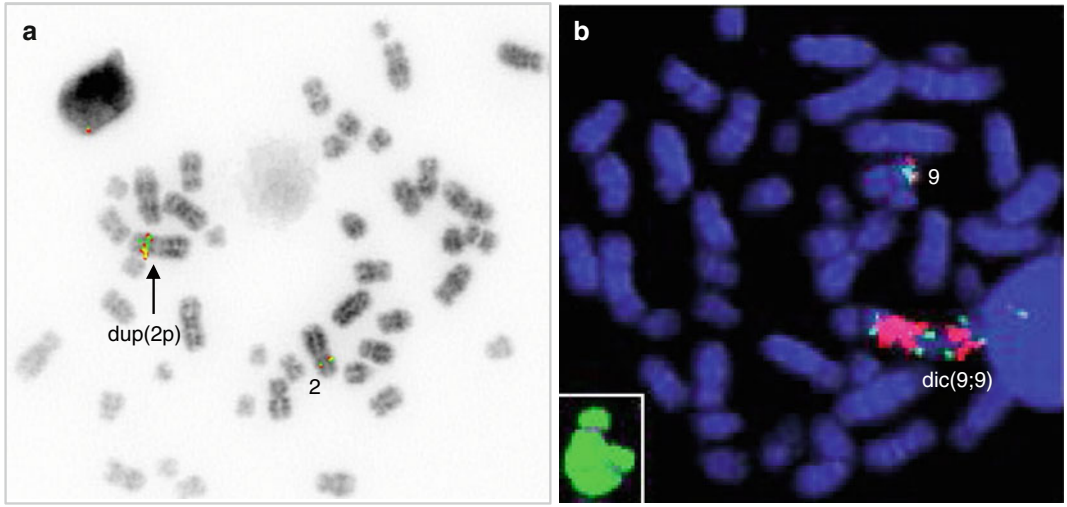

Fig. 2.9 Gains/amplifications of 2p and 9p are recurrent in PMBCL. (**a**) Metaphase FISH with a *REL* break-apart probe in a case of PMBCL with dup(2p); (**b**) metaphase FISH with the *JAK2*/9p24 (*red*), *CDKN2A*/9p21 (*green*), and WCP9 (*inset*) probes in case with dic(9;9)(p24;p24). Note two homogenously stained regions harboring amplified *JAK2* (*red*) and two extra copies of *CDKN2A* (Wlodarska unpublished data)

2.2.2.5 Primary Mediastinal (Thymic) Large B-Cell Lymphoma

Primary mediastinal large B-cell lymphoma (PMBCL) is a well-defined subtype of diffuse large B-cell lymphoma (DLBCL) that shares certain clinical, histological, and molecular features with classical Hodgkin lymphoma (cHL) (Rosenwald et al. 2003; Savage et al. 2003; Calvo et al. 2004; Swerdlow et al. 2008). Cytogenetic reports of original PMBCL tumors and PMBCL-derived cell lines are scanty (Nacheva et al. 1994; Bentz et al. 2001; Palanisamy et al. 2002; Stejskalova et al. 2006). The majority of information about the genomic alterations in this lymphoma has been obtained by CGH, interphase FISH, and molecular investigations. Initial studies using conventional CGH and FISH approaches showed that PMBCL is characterized by a frequent gain of 9p, 12q, and Xq (31–50 %) and a sporadic amplification of 2p/*REL* (Joos et al. 1996). Further studies using array CGH showed that the genomic profile of PMBCL is unique; the hallmark is the gain/amplification of chromosome bands 2p14–p16 and 9p24 detected in approximately 50 and 70 % of cases, respectively (Fig. 2.9). Additionally, PMBCL shows frequent gain of 7q22 (32 %), 9q34 (32 %), 11q23 (18 %), 12q (30 %), and 18q21 (24 %) and loss of 6p21.3/*MHC class II* (11 %) and 11q13.3/*FADD* (11 %) (Wessendorf et al. 2007). Other studies demonstrated a common gain of the X chromosome in up to 85 % of PMBCL cases (Bentz et al. 2001). The 2p16 amplicon covers two cancer-related genes, *REL* and *BCL11A* (Bea et al. 2005; Weniger et al. 2006, 2007; Wessendorf et al. 2007). Amplification of *REL* was associated with an activation of a canonical NF-κB pathway, suggesting a role for this pathway in the pathogenesis of PMBCL. This concept was further supported by the demonstration of frequent deletions/mutations of the gene encoding TNFAIP3 (6q23), a known inhibitor of *NF-kB*, in PMBCL (Kim et al. 2007; Schmitz et al. 2009) and recurrent gain of *BCL10* (1p22) and *MALT1* (18q21.3) in these tumors (Wessendorf et al. 2007). The 9p24 gain/amplification does not target a single gene and usually affects more than 5 Mb. Using high-resolution array CGH, a minimal amplified region has been delineated to an ~1.6 Mb region harboring three candidate genes, *JAK2*, *CD274/PDL1*, and *PDCD1LG2/PDL2* (Wessendorf et al. 2007), suggesting that these genes cooperate in the pathogenesis

of both lymphomas leading to increased cell proliferation and survival through activation of the JAK-STAT pathway (JAK2) and immune evasion (PD-1 ligands). The results of recent integrative studies suggest that *PDL1* and *PDL2* are key targets of the 9p24 amplification, commonly occurring not only in PMBCL but also in cHL (Green et al. 2010).

Specific balanced chromosomal translocations have not been identified in PMBCL; however, interphase FISH analysis performed on 12 cases with a documented gene expression profile of PMBCL revealed *BCL6* and *IGH-BCL2* rearrangements in 33 % (4/12) and 22 % (2/12) of analyzed cases, respectively (Iqbal et al. 2007). Of note, among 17 PMBCL cases with abnormal karyotypes published by Palanisamy et al. (2002), one had the t(3;14)(q27;q32) and two revealed the t(14;18)(q32;q21.3). Recent interphase FISH studies showed that *CIITA* (16p13.1) rearrangements identified in 15 % of cHL cases occur in 38 % of PMBCL cases (Steidl et al. 2011). CIITA which is the master regulator of MHC class II expression was found to be a promiscuous partner of various in-frame fusions in PMBCL, including two t(9;16)(p24;p13.1)-associated fusions with *CD274/PDL1* and *PDCD1LG2/PDL2*. The latter finding further underscores an important role of PD-1 ligands in pathogenesis of PMBCL. Notably, the presence of *CIITA* rearrangements in PMBCL significantly correlated with a shorter disease-specific survival.

Other recurrent genetic lesions in PMBCL include frequent mutations of *BCL6* (>70 %) (Iqbal et al. 2004), inactivating deletions/mutations in the suppressor of cytokine signaling 1 (*SOCS1*) observed at a high frequency (45 %) (Melzner et al. 2005), and mutations of *STAT6* (36 %) (Ritz et al. 2009), *MYC* (25 %), and *TP53* (13 %) (Scarpa et al. 1999).

In summary, PMBCL shows a unique pattern of genetic aberrations, which together with a distinctive gene expression profile support the recognition of this lymphoma as a distinct entity. Most of PMBCL-related genetic lesions, however, are not specific and are observed at a lower frequency in other malignancies. The similar pattern of genetic alterations in PMBCL and cHL underscores their close relationship (Joos et al. 2000; Lenz et al. 2008; Schmitz et al. 2009; Green et al. 2010; Steidl and Gascoyne 2011; Steidl et al. 2011).

Mediastinal Gray Zone Lymphoma

Mediastinal gray zone lymphoma (MGZL) shows overlapping morphologic and immunophenotypic features with PMBCL and the nodular sclerosis subtype of cHL (NSHL) (Traverse-Glehen et al. 2005). This variant is included in the WHO category of "B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma and classical Hodgkin lymphoma" (Swerdlow et al. 2008). Cytogenetic features of MGZL are largely unknown. In a recent interphase FISH study of 27 MGZL cases, Eberle et al. (2011) analyzed four loci (2p16.1/*REL*, 8q24.2/*MYC*, 9p24.1/*JAK2/PDL1/PDL2*, and 16p13.13/*CIITA*) known to be affected in PMBCL and NSHL. Gains/amplifications of 2p16.1, 8q24.2, and 9p24.1 were detected in 34.6, 27, and 50 % of analyzed cases, respectively, and their incidence was higher in cases with evident mediastinal involvement. *CIITA* breaks were observed in 27 % of cases, and these rearrangements were more common in cases without mediastinal involvement. This study demonstrated that MGZL carries the same genetic aberrations as PMBCL and NSHL and that their frequency is intermediate between that observed for the parental entities.

2.2.2.6 Cutaneous B-Cell Lymphoma

Multiple B-cell lymphomas show primary localization in the skin: primary cutaneous marginal zone lymphoma (PCMZL); primary cutaneous follicular center lymphoma (PCFCL); diffuse large B-cell lymphoma, leg type; diffuse large B-cell lymphoma, other; plasmacytoma; plasmablastic lymphoma; and B-lymphoblastic leukemia/lymphoma. Recurrent cytogenetic abnormalities have been reported for only a subset of these lymphomas.

PCMZL typically does not show the presence of translocations involving the *BCL2* and *IGH* genes, despite high BCL2 protein expression (Child et al. 2001). There are three recurrent translocations associated with extramedullary marginal zone lymphomas (EMZL): the t(11;18)(q21;q21.3) resulting in the *API2-MALT1* fusion, the t(14;18)(q32;q21.3) juxtaposing *MALT1* and the *IGH* locus, and the t(1;14)(p22.3;q32) deregulating the *BCL10* gene. The t(11;18)(q21;q21.3) has not been reported in PCMZL, but the t(14;18) occurs occasionally in this lymphoma (Schreuder et al. 2005).

Information about the cytogenetic pattern of PCFCL is sparse in comparison to the nodular follicular lymphoma. Whereas the t(14;18)(q32;q21.3) is the hallmark of nodular follicular lymphoma, there is conflicting data regarding its presence in PCFCL, and the detection rate for *BCL2* rearrangements by polymerase chain reaction (PCR) varies over a wide range (0–41 %) (Volkenandt et al. 1992; Cerroni et al. 1994; Geelen et al. 1998; Child et al. 2001; Goodlad et al. 2002, 2003,). Recent FISH studies suggest that the t(14;18)(q32;q21.3) occurs in 40 % of cases (Aguilera et al. 2001; Kim et al. 2005). It is unclear why *BCL2* rearrangements frequently escape detection by PCR in this entity, but this could be due to *BCL2* mutations, breakpoints outside the amplified DNA, or a high load of somatic mutations (Streubel et al. 2006a).

Diffuse large B-cell lymphoma, leg type is typically negative for the t(14;18)(q32;q21.3) but shows rearrangements of the *MYC*, *BCL6*, and *IGH* loci when investigated by interphase FISH (Hallermann et al. 2004a). Most frequent abnormalities detected by CGH in this lymphoma include gains of 18q, 1q, chromosome 7, 12q, and Xp and losses of 6q (Hallermann et al. 2004b). aCGH studies detected amplifications of the 18q region containing the *BCL2* and *MALT1* genes, which may explain high levels of *BCL2* expression in the absence of the t(14;18). In addition, small deletions were detected by aCGH at 9p21.3, affecting the *CDKN2A* and *CDKN2B* loci (Hallermann et al. 2004b; Dijkman et al. 2006).

2.2.2.7 Waldenstrom Macroglobulinemia

Lymphoplasmacytic lymphoma (LPL) is a disseminated B-cell lymphoproliferative disorder characterized by a spectrum of small B cells, plasmacytoid lymphocytes, and plasma cells. Waldenstrom macroglobulinemia (WM) is the clinical syndrome defined as LPL with an associated monoclonal IgM protein of any level (Swerdlow et al. 2008).

The t(9;14)(p13;q32), juxtaposing the *PAX5* gene at 9p13 and the *IGH* locus at 14q32, was initially reported to be specific for LPL and to be present in up to 50 % of cases (Iida et al. 1996). Recent studies did not confirm this association and suggested that the t(9;14) may also be found in other B-cell lymphomas (Cook et al. 2004; Baro et al. 2006). The discrepancy may relate to the more precise current definition of LPL and increased use of FISH techniques to confirm genetic abnormalities in lymphoma cells. FISH testing has demonstrated that *IGH* rearrangements in LPL are quite uncommon. The most frequent, although nonspecific, abnormality in LPL/WM appears to be a del(6q) in 40–60 % of the cases (Schop et al. 2002). Trisomy of chromosome 4 has also been reported in a subset of LPL cases. Cytogenetic abnormalities often associated with CLL, such as +12 or del(13q), are infrequent in LPL/WM.

2.2.2.8 HIV-Associated Lymphoma

After Kaposi's sarcoma, NHL is the second most common malignancy associated with HIV infection (Carbone 2002; Dal Maso and Franceschi 2003). The introduction of highly active antiretroviral therapies (HAART) resulted in a dramatic decrease in the incidence of opportunistic infections in HIV patients, but had lesser impact on the incidence of NHL (Carbone 2002). According to the WHO classification, HIV-associated lymphomas are divided into (1) lymphomas that are also diagnosed in non-immunocompromised patients, such as BL and DLBCL often involving the central nervous system (primary central nervous system lymphoma, PCNSL); (2) lymphomas predominantly seen

in the setting of HIV infection, such as primary effusion lymphomas (PEL), plasmablastic lymphoma of the oral cavity (PBL), and large B-cell lymphoma arising in human herpes virus 8 (HHV8)-associated multicentric Castleman disease; and (3) lymphoma also occurring in other immunodeficiency states, such as polymorphic lymphoid proliferations resembling post-transplant-associated lymphoproliferative disorder (PTLD) (Swerdlow et al. 2008).

The heterogeneity of HIV-associated lymphoma reflects multiple pathogenic mechanisms, including chronic antigenic B-cell stimulation by HIV itself, as well as other coinfecting viruses, such as Epstein-Barr virus (EBV) and HHV8 (Carbone 2002). EBV is detected in the neoplastic cells of approximately 60 % of HIV-related lymphomas (ranging from 30 to 50 % in BL to 70–80 % in systemic DLBCL and virtually 100 % of PCNSL). An additional factor contributing to the development of HIV-associated NHL is the production of B-cell stimulatory cytokines (such as interleukin (IL)-10 and IL-6), with their potential to support the growth and viability of neoplastic cells (Gaidano et al. 2000a). In lymphomas associated with HHV8 infection, induction of the macrophage inflammatory protein 1 (MIP-1) by the virus may contribute to lymphomagenesis through attraction of other growth factor-producing cells into the lymphoma environment (Nicholas et al. 1997). Genetic abnormalities known to be involved in lymphomagenesis in immunocompetent patients are also involved in the context of HIV infection (Lim and Levine 2005). *MYC* is activated in nearly all cases of HIV-associated BL (Subar et al. 1988). Secondary events such as *TP53* and *RAS* mutations have also been reported. Molecular alterations of the *BCL6* proto-oncogene are associated with a significant fraction of HIV-associated DLBCL: rearrangements are detected in 20 % (Gaidano et al. 1994) and mutations in 70 % of the cases (Gaidano et al. 1997).

Chromosomal alterations, however, are not well characterized in HIV-associated lymphoma, owing to limitations related to in vitro culture of infected lymphoid tumor cells and the complex nature of chromosomal changes. Only a few cytogenetic studies have been published, either on individual cases or on very small series. Gains of 1q (Polito et al. 1995) and chromosome 12 (Bernheim and Berger 1988) have been reported in BL, as well as deletions of 6q in DLBCL (Pastore et al. 1996). Complex karyotypes with frequent gains of chromosomes 7 and 12 and rearrangements at 1q21–q25 have been observed in PEL (Gaidano et al. 2000b).

HIV-associated lymphomas that also occur in non-immunocompromised individuals show similar CGH abnormalities in HIV patients as observed when the same lymphomas are not linked to the presence of an HIV infection (Vaghefi et al. 2006). Among lymphomas that are predominantly seen in HIV patients, only PEL has been systematically studied by CGH. Mullaney et al. analyzed eight cases of HIV-associated PEL and detected recurrent duplications on chromosome 12 (at 12q22–q23, 12q12–q23), together with copy number gains on the X chromosome (Mullaney et al. 2000).

2.2.2.9 Adult Burkitt Lymphoma

The defining feature of Burkitt lymphoma (BL) in the World Health Organization's (WHO's) classification of lymphoid malignancies is deregulation of the *MYC* gene, which in almost all cases occurs through translocation with one of three immunoglobulin loci (Swerdlow et al. 2008). Consequently, conventional cytogenetic analysis and FISH studies may be critical to confirm the diagnosis of BL, by validating the presence of the hallmark t(8;14)(q24.2;q32) or its variants, t(2;8)(p12;q24.2) and t(8;22)(q24.2;q11.2).

BL has an important place in the history of cancer cytogenetics, as it served as one of the earliest models for the exploration of chromosomal aberrations in cancer. BL was the first lymphoid neoplasm for which the underlying chromosomal rearrangement was characterized. The first cytogenetic study of BL was reported in 1963 by Jacobs and coworkers (Jacobs et al. 1963). Introduction of banding techniques allowed Manolov and Manolova to describe an additional band at the end of the long arm of chromosome 14 in fresh tumors and BL cell lines (Manolov and Manolova 1972). The nature

of the BL-specific abnormality was clarified by Zech et al. (1976), who proposed that the additional material on chromosome 14 originated from a translocation event between the long arm of chromosome 14 and the long arm of chromosome 8 (Zech et al. 1976). Variant translocations involving 2p and 22q were described in BL cell lines in 1979 (Berger et al. 1979; Miyoshi et al. 1979). The molecular targets of the recurring translocations in BL were soon discovered to be *MYC* (at 8q24.2) (Dalla-Favera et al. 1982; Taub et al. 1982) and the genes coding for the immunoglobulin heavy chain (*IGH* at 14q32) (Erikson et al. 1982), kappa light chain (*IGK* at 2p12) (Malcolm et al. 1982), and lambda light chain (*IGL* at 22q11.2) (de la Chapelle et al. 1983).

The t(8;14) is present in 75–85 % of all BL (Johansson et al. 1995). One of the variant translocations is found in the remaining 15–25 % of the cases, with the t(8;22) being twice as common as the t(2;8). These translocations are present in both the endemic, African tumor type, and in sporadic BL occurring in Europe, America, and Japan and in both EBV-infected and in EBV-negative BL. Although considered the hallmark of BL, translocations involving the *MYC* locus are not specific and are frequently seen in other types of lymphomas.

The molecular consequence of the t(8;14) and its variants is deregulation of the *MYC* oncogene at 8q24.2 through juxtaposition with the enhancer elements of the immunoglobulin loci (Willis and Dyer 2000). Activation of *MYC* occurs on the der(14) in the t(8;14) and on the der(8) in the t(2;8) and t(8;22). The breakpoint on the der(8) occurs centromeric of the *MYC* gene in the t(8;14) and telomeric of *MYC* in the t(2;8) and t(8;22). In endemic BL, the chromosome 8 breakpoints frequently fall far centromeric of the *MYC* locus, whereas in sporadic BL, these breakpoints typically occur between exons 1 and 2 or immediately 5′ of the *MYC* gene (Boxer and Dang 2001). The breakpoints of the t(8;14) are also different within the *IGH* locus: in endemic BL they regularly affect the J-segments of the *IGH* locus, whereas in sporadic BL, they typically involve the switch regions of the constant segments of *IGH*. This suggests that the t(8;14) arises during aberrant VDJ recombination in endemic BL and during aberrant class switch recombination in the sporadic form of the disease (Kuppers and Dalla-Favera 2001; Kuppers 2005). The molecular heterogeneity between different forms of BL can represent a considerable challenge when using molecular or molecular cytogenetic techniques to detect recurrent translocations in BL.

BL typically has a rather simple karyotype. The t(8;14) or a variant translocation is present as a sole abnormality in 40 % of BL cases. Secondary chromosomal changes, in particular when they result in very complex karyotypes, indicate disease progression in BL (Johansson et al. 1995; Boerma et al. 2009). The most frequent secondary aberration, present in >30 % of all BL cases, is a structural rearrangement of chromosome 1, typically affecting the long arm and leading to partial trisomy of 1q. Trisomy 7 and trisomy 12 are other common secondary changes. Abnormalities involving chromosomes 1, 7, and 12 tend to be mutually exclusive in BL progression (Boerma et al. 2009). Losses of chromosomal material in BL primarily involve 6q and 17p, as well as 13q32–q34. Secondary abnormalities in 13q32–q34 occur in ~15 % of the cases and most likely target the *miR-17-92* miRNA locus that appears to cooperate with *MYC* in transformation (He et al. 2005; O'Donnell et al. 2005).

Translocations involving typical lymphoma oncogenes other than *MYC*, such as *BCL2* (18q21.3), *BCL6* (3q27), and *CCND1* (11q13), do not occur in BL. The same is true for the so-called non-*IG-MYC* translocations that have been described in NHLs with some overlapping features with BL. Examples of these include the t(8;9)(q24.2;p13) juxtaposing *MYC* to the region containing *PAX5* and t(3;8)(q27;q24.2) juxtaposing *MYC* to *BCL6* (Bertrand et al. 2007; Sonoki et al. 2007). The precise mechanisms by which these translocations deregulate *MYC* are unclear.

Translocations involving *MYC* can be easily detected by FISH, using the *MYC* "break-apart" probes, a mixture of two fluorescently tagged DNA probes of two different colors that hybridize to the upstream and downstream segments flanking the gene (Fig. 2.10). Using these probes,

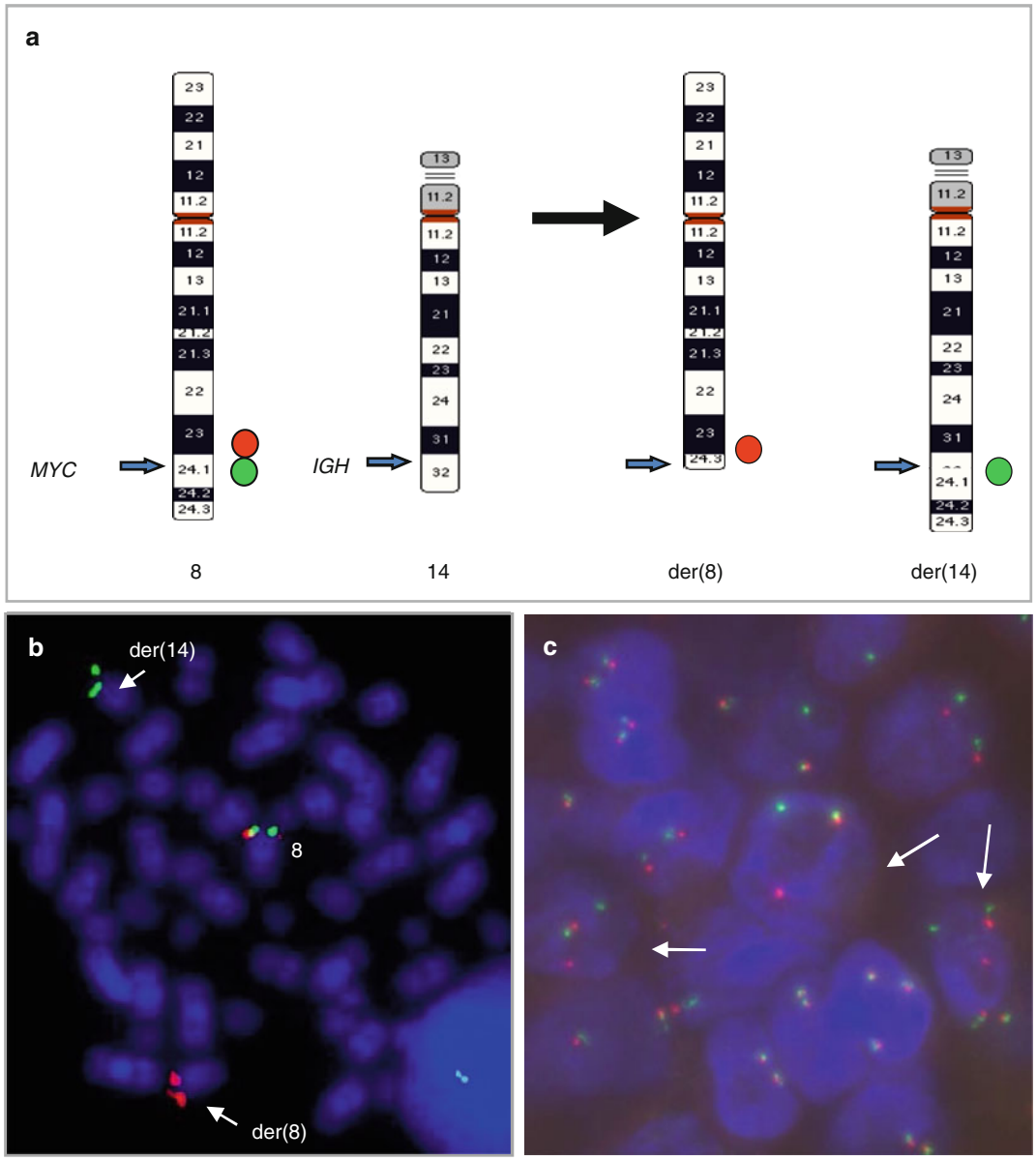

Fig. 2.10 Burkitt lymphoma-associated t(8;14)(q24.2;q32). (**a**) Scheme of t(8;14), targeted genes, and the distribution of FISH signals with the LSI MYC break-apart probe. (**b**–**c**) Examples of FISH with LSI *MYC* performed on metaphase chromosomes and a section of FFPE tissue, respectively. *Arrows* indicate interphase cells with an abnormal signal pattern

the detection of a translocation is independent of the partner genes. Therefore, translocation-specific probes and/or specific probes for *IGH* and *IGL* loci should also be used to confirm one of the immunoglobulin genes as the partner of *MYC*. If overlapping features with DLBCL are present, this can be further complemented with FISH assays for *BCL2* and *BCL6* translocations, which should show negative results in BL.

The question of whether *MYC*-negative typical BL exists is still controversial. Hummel et al. reported rare cases of mature aggressive B-cell lymphomas without detectable *MYC* aberrations, but showing the gene expression signature of BL

(Hummel et al. 2006). Similarly, Leucci et al. described a series of *MYC*-negative classic BLs with an alternative pathogenetic mechanism which involved microRNA deregulation (Leucci et al. 2008). It is important to remember that none of the techniques used to detect genetic changes in BL (cytogenetics, FISH, PCR, or Southern blot) can unambiguously rule out the presence of a *MYC* rearrangement. The current recommendation is that the diagnosis of BL in the absence of a demonstrable *MYC* abnormality should be reserved only for those cases where all other features support the diagnosis (Swerdlow et al. 2008).

The view that BL represents a homogeneous disease characterized by relatively few cytogenetic mutations is also supported by gene expression profiling and CGH/aCGH studies. Gene expression studies have shown that cases of typical BL share a recognizable expression signature (Dave et al. 2006; Hummel et al. 2006), whereas CGH and aCGH analyses performed on limited series of BLs failed to identify novel, cryptic genomic rearrangements (Hummel et al. 2006; Salaverria et al. 2008b).

The main diagnostic challenge in BL is to distinguish it from a small subset of DLBCLs with one or more overlapping features with BL. This distinction has important clinical implications for treatment and prognosis, as BL responds poorly to standard DLBCL therapy, but shows excellent response to high-intensity chemotherapy. According to the 2008 WHO classification, these "intermediate," "gray zone" lymphomas should be diagnosed as "B-cell lymphoma with features intermediate between DLBCL and BL" (Swerdlow et al. 2008). This is a disease subset that frequently shows the presence of the t(8;14) or its variants and might present with a BL-like morphology and immunophenotype (Boerma et al. 2009; Bellan et al. 2010). In contrast to BL, these intermediate lymphomas tend to occur at a significantly older age and typically have complex karyotypes. Many of them, in addition to a *MYC* translocation, harbor recurrent *IG* translocations with other oncogenes, such as *BCL2* and *BCL6* (the so-called double-hit lymphomas). Moreover, a wide range of *MYC* translocations not involving *IG* loci, such as t(3;8) and t(8;9), also occur. The detection of a double hit, the presence of a non-*IG*-*MYC* translocation, and the occurrence of a t(8;14) or variant as a part of a complex karyotype are all characteristics supporting the diagnosis of a "B-cell lymphoma with features intermediate between DLBCL and BL" (Boerma et al. 2009; Bellan et al. 2010).

From the practical, diagnostic perspective, it is advisable to submit material of any lymphoma suspicious for BL for cytogenetic analysis by metaphase karyotyping. Although lacking the molecular resolution of FISH methods, conventional cytogenetic analysis has the inherent advantage of providing an overview of chromosomal changes that are relevant for diagnosis; it also provides information about the partner of the *MYC* locus, the presence or absence of translocations involving *BCL2* and *BCL6*, and presence and number of other structural abnormalities. In addition, a simple *MYC* break-apart FISH test should be carried out in all cases. More elaborate FISH tests for *MYC*, *IG* loci, and also *BCL2* or *BCL6* and, eventually, *CCND1* should be carried out in suspected BL cases with unusual findings by morphology or immunohistochemistry.

2.2.2.10 Nodular Lymphocyte-Predominant Hodgkin Lymphoma

Nodular lymphocyte-predominant Hodgkin lymphoma (NLPHL) is a distinct Hodgkin lymphoma subtype accounting for 5 % of all HLs (Swerdlow et al. 2008). The diagnostic lymphocyte-predominant (LP) cells (previously known as L&H, lymphocytic and histiocytic cells, or popcorn cells) postulated to derive from late germinal-center (GC) B cells (Brune et al. 2008) are rare and usually comprise 0.1–10 % of the tumor mass, which is mainly composed of reactive small B and T cells. Genetic studies of NLPHL are hampered by a low incidence of LP cells and their low mitotic index. Thus far, clonal cytogenetic abnormalities have been reported in approximately 20 NLPHL cases and one NLPHL-derived cell line (DEV) (Falzetti et al. 1999; Franke et al. 2001; Stamatoullas et al. 2007; Wlodarska et al. 2003, 2004b; Atayar et al. 2006). These data indicate that karyotypes of NLPHL

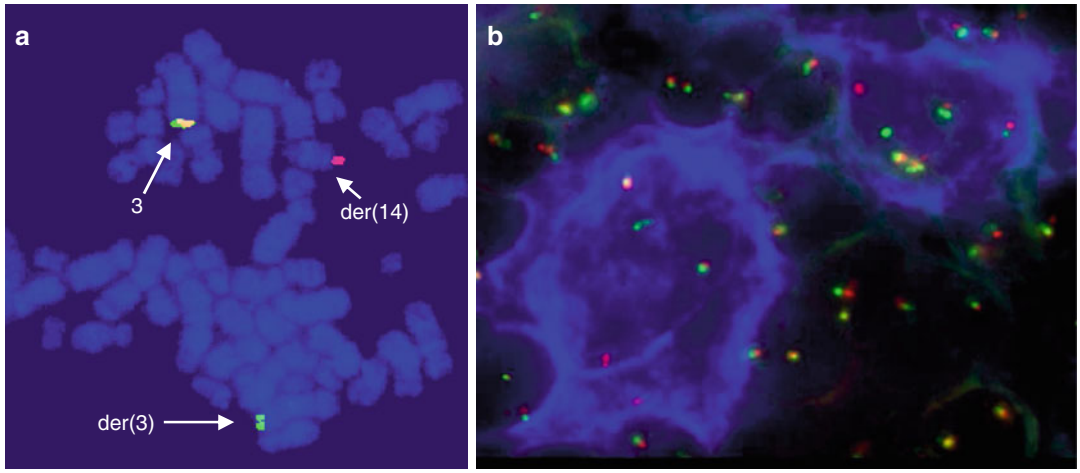

Fig. 2.11 Recurrent *BCL6* rearrangements in NLPHL. (**a**) FISH image of a metaphase cell with the t(3;14) using the LSI *BCL6* break-apart probe; (**b**) FICTION (CD20 and LSI *BCL6*) on a frozen lymph node section. Note the CD20-positive Hodgkin cells with split LSI *BCL6* signals (Wlodarska et al. 2003)

are usually in the diploid range (46–49 chromosomes) and characterized by complex numerical and structural chromosomal alterations. The most frequent changes include a gain of 1q, 3q27 rearrangements, loss of 4q28–q32 and chromosome 7, 7q22–33 rearrangements, deletion of 13q, and rearrangements of 14q32 (Stamatoullas et al. 2007). The 14q32/*IGH* translocations recurrently target the *BCL6* gene at 3q27 (Fig. 2.11). In addition, single cases involving *IGH*, inv(14)(q23q32), and t(9;14)(q22;q32) were reported. The observation of recurring translocations involving *BCL6* in NLPHL was confirmed by interphase FISH showing *BCL6* rearrangements in 48 % of cases (Wlodarska et al. 2003). The *BCL6* partners are promiscuous and comprise both *IG* and non-*IG* loci (4q27, 5q31, 6q22, 9p13) (Wlodarska et al. 2003, 2004b; Renne et al. 2005; Stamatoullas et al. 2007). These data support the hypothesis of the GC origin of NLPHL and indicate a significant role for *BCL6* in the pathogenesis of this lymphoma. The frequent occurrence of *BCL6* translocations in NLPLH contrasts with the absence (Wlodarska et al. 2003) or low incidence of *BCL6* rearrangements in classical HL (Martin-Subero et al. 2006), providing further evidence of the genetic diversity underlying the pathogenesis of both HL subtypes. Additional evidence was provided by recent molecular studies showing that mutations of NF-κB-related genes, including *TNFAIP3/A20*, recurrently detected in cHL, are uncommon in NLPHL (Schumacher et al. 2010). To date, only one study of genomic imbalances in NLPHL has been reported (Franke et al. 2001). The authors combined conventional CGH with DOP-PCR (degenerate oligonucleotide primed-polymerase chain reaction) amplification of DNA from 4 to 5 microdissected LP cells. All 19 cases analyzed with this approach showed a high number of genomic imbalances involving all chromosomes, except 19, 22, and Y. Gain of 2q, 4q, 5q, 6, and 11q seemed to be more frequent in NLPHL than in other B-cell lymphomas. These initial data, however, await validation by high-resolution CGH based on a large number of microdissected/sorted neoplastic cells.

Conclusions

The role of cytogenetic and FISH analyses remains a pivotal element for establishing the diagnosis, prognosis, and therapeutic decisions in the lymphomas, including the initiation of specific treatments and the follow-up of altered clinical behavior of the disease. The recurring abnormalities, while rarely specific for a disease entity, have provided not only insight into prognosis but also the molecular pathogenesis of these disorders. Coupling careful clinical observation with both classical

cytogenetic techniques and newer genomics technologies will refine our understanding of these diseases.

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Molecular Genetics of Rare Lymphomas

3

Sören-Sebastian Wenzel and Georg Lenz

Contents

3.1 Introduction

Non-Hodgkin's lymphoma represents a heterogeneous group of malignancies with diverse clinical manifestations, histologic characteristics, and biologic behavior. Recent improvements in pathologic diagnosis as well as the application of novel molecular biological techniques in clinical routine have led to the distinction of various subtypes of malignant lymphoma that occur at rather low frequencies. These lymphoma subtypes are here referred to as "rare lymphomas".

A correct pathologic diagnosis of these uncommon entities is a prerequisite for adequate treatment strategies especially as a significant number of these "rare lymphoma" subtypes are characterized by adverse survival. Therefore, a better understanding of the biology of these entities is critically warranted to substantially improve prognosis. This chapter summarizes current concepts of our understanding of the molecular pathogenesis of "rare lymphomas" subtypes. This is exemplified by focusing on selected subtypes, namely, primary mediastinal B-cell lymphoma, Burkitt lymphoma, and mantle cell lymphoma.

3.2 Primary Mediastinal B-Cell Lymphoma

Primary mediastinal B-cell lymphoma (PMBL), which originates from a B-cell subpopulation that resides in the thymus, is a rare subtype of diffuse

S.-S. Wenzel • G. Lenz, MD (✉)
Department of Hematology,
Oncology and Tumor Immunology,
Molecular Cancer Research Center,
Charité - Universitätsmedizin Berlin,
Augustenburger Platz 1, 13353 Berlin, Germany
e-mail: georg.lenz@charite.de

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large B-cell lymphoma (DLBCL), accounting for approximately 5 % of all aggressive lymphomas (van Besien et al. 2001). In contrast to other subtypes of DLBCL that commonly arise in elderly patients, PMBL usually occurs in females with a median age of only 30–35 years. It typically involves the mediastinum, whereas manifestation within other extranodal sites is rare (van Besien et al. 2001). Currently, the diagnosis of PMBL is based on pathological and clinical criteria only (Isaacson et al. 1987). However, gene expression studies showed that PMBL is characterized by a specific expression profile. These studies indicated that pathological and clinical parameters alone are not always sufficient to reliably differentiate PMBL from other subtypes of DLBCL with mediastinal involvement (Rosenwald et al. 2003a; Savage et al. 2003). In fact, roughly 25 % of all cases assigned as PMBL by conventional criteria alone were not confirmed by gene expression profiling suggesting that additional diagnostic techniques have to be implemented into clinical routine to reliably diagnose these patients (Rosenwald et al. 2003a).

3.2.1 Molecular Genetics

Various studies showed that PMBL is characterized by chromosomal gains or amplifications of the 9p24 locus in roughly 50 % of cases (Fig. 3.1) (Bea et al. 2005; Wessendorf et al. 2007; Lenz et al. 2008). One of the probable target genes of this genetic abnormality is the Janus kinase 2 (*JAK2*), as it is significantly upregulated in amplified cases compared to samples without this aberration (Lenz et al. 2008). JAK2 is involved in cytokine signaling through phosphorylation of STAT transcription factors, causing their translocation to the nucleus and the induction of STAT target genes (Fig. 3.1) (Ghoreschi et al. 2009). Interestingly, suppressor of cytokine signaling 1 (*SOCS1*) a known negative regulator of JAK signaling is recurrently deleted or mutated in PMBL (Melzner et al. 2005; Mestre et al. 2005; Weniger et al. 2006). Ectopic SOCS1 overexpression in the SOCS1-deficient PMBL cell line model MedB-1 induced growth arrest and significant reduction of phospho-JAK2 and its downstream interaction partner pSTAT5 implicating a tumor suppressor role in PMBL (Melzner et al. 2005). Interestingly, *SOCS1* mutations and *JAK2* amplifications are not mutually exclusive, suggesting that both molecular mechanisms can be utilized by the same lymphoma cell (Fig. 3.1) (Melzner et al. 2005).

A recent genome-wide small hairpin RNA (shRNA)-mediated interference screen by Rui and colleagues added another novel facet to JAK2 function in PMBL (Rui et al. 2010). In this study, an interaction between JAK2 and the histone demethylase JMJD2C was identified leading to epigenetic dysregulation (Fig. 3.1). Intriguingly, *JMJD2C* is located on chromosome 9p24 and recurrently amplified in PMBL cases that also harbor *JAK2* amplifications (Rui et al. 2010). Inhibition of JAK2 and JMJD2C cooperated in killing PMBL cell lines by decreasing tyrosine 41 phosphorylation and increasing lysine 9 trimethylation of histone H3, promoting heterochromatin formation. Interestingly, MYC appeared to be one of the main targets of JAK2-mediated histone phosphorylation, indicating a potentially important role in PMBL biology (Rui et al. 2010).

Another consequence of the 9p24 amplification is the overexpression of PD-L1 and PD-L2. Both proteins are ligands for the PD receptor on T-cell. Engagement of the PD receptor by its ligands inhibits signaling through the T-cell receptor, suggesting that amplification of these genes modulates the interaction between PMBL cells and surrounding T-cell (Rosenwald et al. 2003a).

Another characteristic feature of PMBL is the constitutive activation of the oncogenic nuclear factor-kappa B (NF-κB) pathway (Fig. 3.1). PMBLs seem to depend on NF-κB signaling, as inhibition of this cascade using a specific compound induced cell death (Lam et al. 2005). However, the genetic abnormalities leading to this constitutive activation have not yet been fully elucidated. A significant fraction of PMBL cases harbor gains or amplifications of the *c-rel* locus on chromosome 2p (Bea et al. 2005; Lenz et al. 2008). These amplifications were associated with nuclear localization of REL protein, indicating NF-κB activation in these cases (Weniger et al. 2007). Additionally, inactivating mutations and

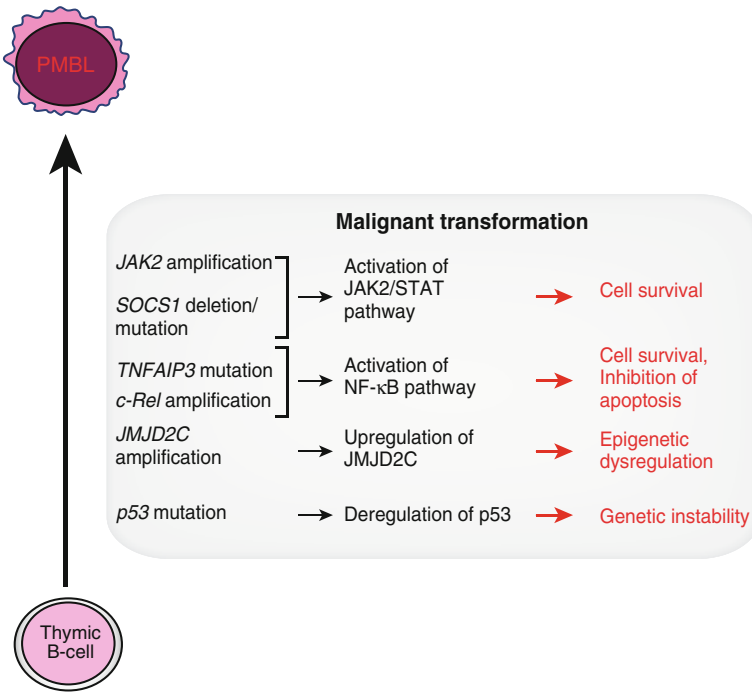

Fig. 3.1 PMBLs are derived from thymic B-cell and are characterized by deregulation of the JAK2-STAT pathway, constitutive activation of the oncogenic NF-**κ**B pathway, epigenetic dysregulation through *JMJD2C* amplifications, as well as *p53* mutations

deletions of the tumor suppressor *TNFAIP3* (A20) have been identified in roughly 30 % of PMBL samples (Schmitz et al. 2009). A20 is an ubiquitin-modifying enzyme that inhibits NF-κB signaling, and loss of function is associated with constitutive activity of NF-κB. Interestingly, these destructive *TNFAIP3* mutations have also been detected in several other lymphoma subtypes such as the activated B-cell-like (ABC) DLBCL subtype, marginal zone lymphoma, or Hodgkin's lymphoma (Compagno et al. 2009; Kato et al. 2009; Novak et al. 2009; Schmitz et al. 2009).

PMBL cases are additionally characterized by deregulation of various oncogenes and tumor suppressors that are frequently involved in lymphomagenesis. *MYC* rearrangements as well as *MYC* promoter sequence variations have been detected in a fraction of cases (Tsang et al. 1996; Scarpa et al. 1999). Aberrations affecting the known tumor suppressor genes *CDKN2A* (*INK4A-ARF*) and *p53* have been identified in a small number of PMBL samples and therefore might also contribute to the molecular pathogenesis of this entity (Fig. 3.1) (Tsang et al. 1996; Scarpa et al. 1999).

At last, clinically and biologically PMBL resembles nodular sclerosis Hodgkin's lymphoma. Both entities commonly present in younger women and display mediastinal tumors with prominent sclerosis. Gene expression profiling revealed that a significant number of overexpressed genes in PMBL are also highly expressed in Hodgkin's lymphoma models (Rosenwald et al. 2003a; Savage et al. 2003). In line with these data, both subtypes are addicted to the same oncogenic pathways, such as the NF-κB pathway as well as the JAK-STAT pathway. Interestingly, both lymphoma subtypes are furthermore characterized by chromosomal translocations involving the major histocompatibility complex (MHC) class II transactivator CIITA (MHC2TA). This abnormality was detected in 38 % of PMBL and 15 % of classical Hodgkin's lymphoma cases, respectively. An association of this genetic aberration with downregulation of surface HLA class II expression suggested an impact on antitumor immune responses (Steidl et al. 2011). However, despite these various similarities, PMBL and Hodgkin's lymphoma can reliably be differentiated by conventional pathological techniques or gene expression profiling.

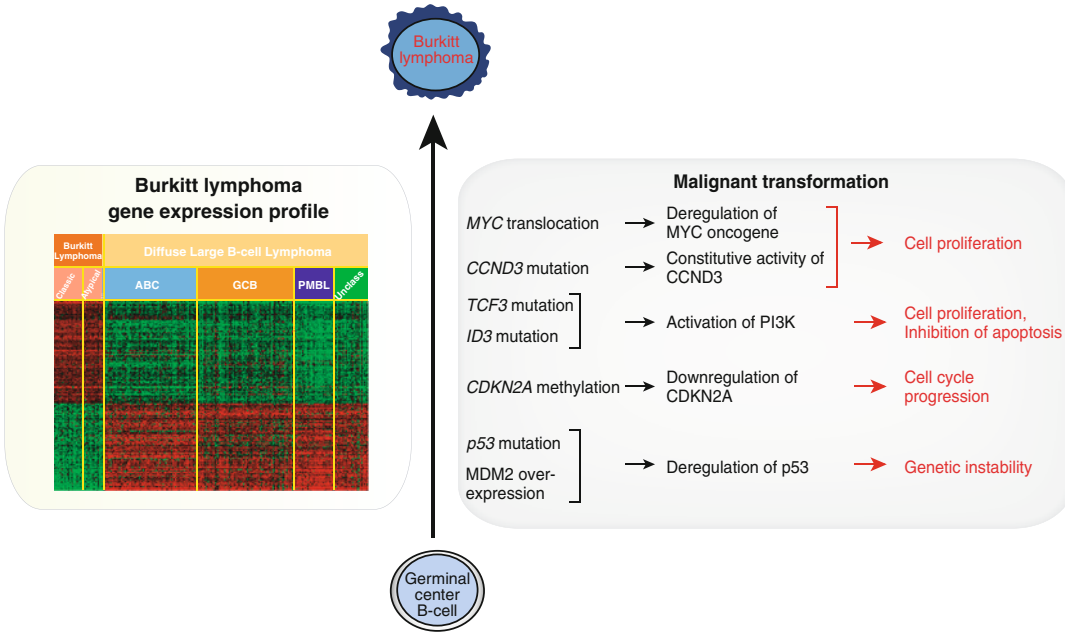

Fig. 3.2 Burkitt lymphoma is characterized by a specific gene expression signature that distinguishes it from other subtypes of aggressive lymphoma. Pathogenetically Burkitt lymphoma samples show deregulation in cell proliferation, inhibition of apoptosis, as well as genetic instability by diverse abnormalities

3.3 Burkitt Lymphoma

Burkitt lymphoma (BL) is a highly aggressive lymphoma subtype of germinal center origin characterized by a high proliferation rate and frequent extranodal manifestation. Three different variants of BL can be distinguished: sporadic, endemic, and immunodeficiency-associated BL. While the endemic subtype mainly occurs in equatorial Africa, no geographical predominance is observed for the sporadic and immunodeficiency-associated variants (Blum et al. 2004). Virtually all cases of endemic BL are associated with an Epstein-Barr virus (EBV) infection. In contrast, only 10–20 % of sporadic BL are positive for EBV, whereas roughly 40 % of the immunodeficiency-associated BL are associated with an EBV infection (Neri et al. 1991; Blum et al. 2004). Although the frequent association between BL and EBV suggests a role of EBV in the pathogenesis of BL, the exact molecular mechanisms leading to lymphomagenesis remain unknown.

Sporadic BL is a rare lymphoma subtype in adults accounting for only 1–2 % of all malignant lymphoma subtypes. In contrast, in patients infected with the human immunodeficiency virus (HIV), BL is significantly more frequent than in uninfected populations (Knowles 1996).

3.3.1 Molecular Genetics

Virtually all BL cases are characterized by chromosomal translocations affecting the *MYC* oncogene (Fig. 3.2) (Dalla-Favera et al. 1982, 1983; Taub et al. 1982). MYC is a transcription factor with known activating and repressing functions on its target genes and is involved in the regulation of various critical biologic processes such as cell cycle control, cell growth, protein synthesis, angiogenesis, and apoptosis (Meyer and Penn 2008). Approximately 80 % of BL cases harbor a t(8; 14) (q24; q32) translocation that juxtaposes *MYC* to the immunoglobulin heavy-chain (IgH) enhancer elements, whereas in the remaining cases, t(2;8)(p12;q24) to the Ig-kappa or t(8;22) (q24;q11) translocations to the Ig-lambda loci are detectable (Fig. 3.2) (Neri et al. 1988; Gerbitz

et al. 1999). The positions of the chromosomal breakpoints are dispersed over several hundred kilobases. Interestingly, the different BL subtypes harbor diverse *MYC* and *IgH* loci breakpoints. In endemic BL, *MYC* usually breaks outside the *MYC* region and within the joining region of *IgH*, indicating aberrant somatic hypermutation as the underlying molecular mechanism of this translocation. In contrast, the majority of sporadic and immunodeficiency-associated BL cases have their chromosomal breaks within the *MYC* locus and the *Ig* switch region suggesting a role of the activation-induced cytidine deaminase (AID, AICDA) recombinase in the development of these translocations (Neri et al. 1988). This hypothesis is supported by the ability of AID to induce *IgH-MYC* translocations in mouse B-cells (Ramiro et al. 2006).

In BL several additional oncogenic events have been detected (Fig. 3.2). The known tumor suppressor gene *CDKN2A* has been shown to be silenced by promoter methylation (Klangby et al. 1998). Additionally, p53 can be deregulated by different molecular mechanisms in BL. Somatic *p53* mutations occur in approximately 30 % of BL patient samples (Farrell et al. 1991; Bhatia et al. 1992). Alternatively, overexpression of MDM2 is detected in BL samples with wild-type *p53* leading to p53 degradation through ubiquitin-dependent proteolysis (Capoulade et al. 1998).

A recent high-throughput RNA sequencing and RNA interference screen in BL cell lines and 28 sporadic BL patient biopsies revealed novel insights into the molecular pathogenesis of BL (Schmitz et al. 2012). Using this combined approach several unappreciated oncogenic events were unraveled. In approximately 70 % of sporadic BL cases, mutations affecting the transcription factor TCF3 (E2A) or its negative regulator ID3 fostered TCF3 dependency (Fig. 3.2). TCF3 activated the pro-survival phosphatidylinositol-3 kinase (PI3K) pathway in BL, in part by augmenting tonic B-cell receptor signaling (Schmitz et al. 2012). These data suggest an oncogenic synergy between MYC and the PI3K pathway. Accordingly, a combination of constitutive MYC expression and PI3K activity in mouse germinal center B-cell induced BL-like tumors that strongly resembled human BL (Sander et al. 2012).

Another aspect of BL biology was revealed by the detection of recurrent *CCND3* mutations in roughly 40 % of sporadic BL cases (Fig. 3.2) (Schmitz et al. 2012). *CCND3* encodes cyclin D3 that regulates G1-S cell cycle transition in germinal center B-cell (Cato et al. 2011; Schmitz et al. 2012). CCND3 mutations produced highly stable cyclin D3 isoforms that drive cell cycle progression and knockdown of CCND3 was toxic to BL cell line models indicating a dependency on these mutant cyclin D3 forms (Schmitz et al. 2012).

Two large gene expression profiling studies using DNA microarrays revealed that BL is characterized by a specific gene expression signature that mirrors its oncogenic addictions (Fig. 3.2) (Dave et al. 2006; Hummel et al. 2006). Thus, BL can reliably be distinguished from other subtypes of aggressive lymphomas such as DLBCL which is of major clinical relevance, as BL can only be cured in a high proportion of cases if multi-agent chemotherapy regimens are applied (Dave et al. 2006; Hummel et al. 2006). In contrast, DLBCL is treated in the majority of cases with a combined approach of the anti-CD20 antibody rituximab and CHOP chemotherapy (cyclophosphamide, doxorubicin, vincristine, and prednisone; R-CHOP) (Dupire and Coiffier 2010). Therefore, an accurate diagnosis is mandatory for adequate treatment stratification of patients with aggressive lymphomas. Dave et al. showed that BLs are characterized by a gene expression signature consisting of MYC target genes that is expressed at significantly higher levels compared to DLBCL patient biopsies (Dave et al. 2006). Additionally, BL samples have high expression of a subset of germinal center B-cell genes such as *CD10* and *GCET2* consistent with its derivation from this stage of B-cell differentiation (Dave et al. 2006). In contrast, a NF-κB signature is expressed at lower levels compared to DLBCL implicating that this oncogenic pathway is not involved in the molecular pathogenesis of BL (Dave et al. 2006).

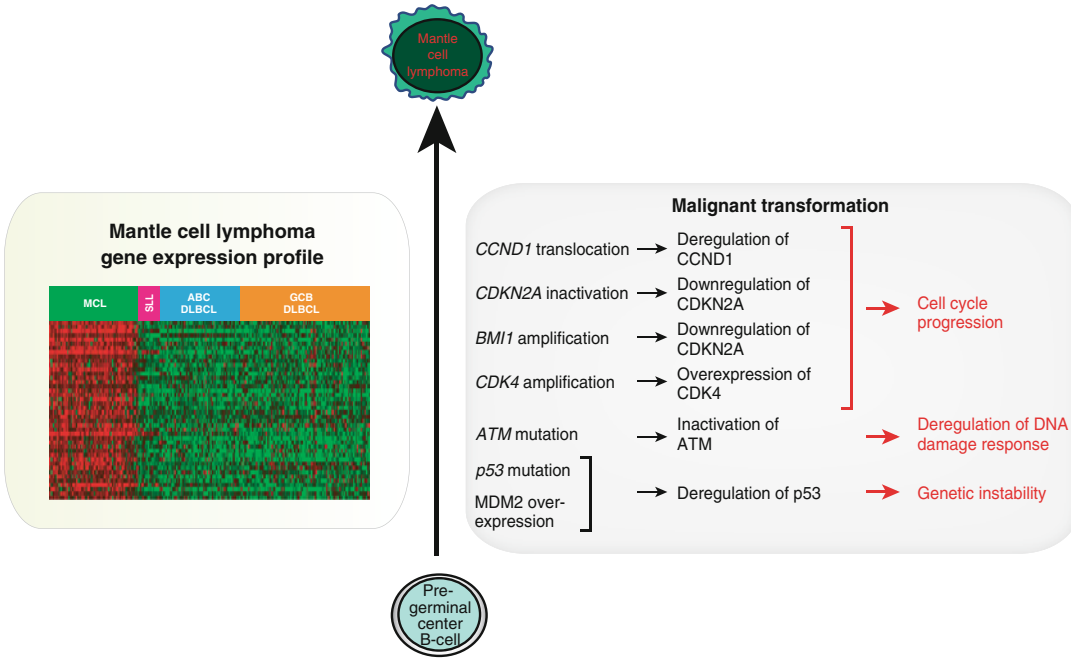

Fig. 3.3 Mantle cell lymphoma patient samples show a distinct gene expression profile. Deregulation of cell cycle control by different genetic alterations is the pathogenetic hallmark of mantle cell lymphoma. Additionally, mantle cell lymphoma is characterized by deregulation of DNA damage response and genetic instability

3.4 Mantle Cell Lymphoma

Mantle cell lymphoma (MCL) accounts for approximately 5–8 % of all malignant lymphoma cases and predominantly affects elderly males (Dreyling and Hiddemann 2009). It is characterized by an aggressive clinical course and poor prognosis with a median survival of only 3–5 years (Dreyling and Hiddemann 2009). MCL is derived in the vast majority of cases from a naive pre-germinal center B-cell, as the Ig variable regions are unmutated. Histologically, either a mantle zone, nodular, or diffuse growth pattern can be observed (Banks et al. 1992), whereas cytologically, two main variants can be distinguished, the classic subtype and the blastoid variant that is characterized by adverse survival (Campo et al. 1999).

3.4.1 Molecular Genetics

Deregulation of the cell cycle is the pathogenetic hallmark of MCL (Fig. 3.3). The vast majority of MCL cases are characterized by the chromosomal translocation t(11;14)(q13;q32), juxtaposing the *CCND1* gene to the *IgH* locus, leading to constitutive overexpression of the cell cycle regulator cyclin D1. Cyclin D1 drives cell cycle progression from G1 to the S phase by forming heterodimers with the cyclin-dependent kinases CDK4 and CDK6. Thus, the tumor suppressor retinoblastoma protein (RB) is inactivated, thereby losing its function as a molecular break of G1 to S phase progression. Additionally, cyclin D1-CDK4/6 heterodimers bind to p27kip1, an inhibitor of cyclin E-CDK2 complexes, leading to cyclin E-CDK2-driven entry into S phase (Nogai et al. 2011).

Other genetic abnormalities that deregulate cell cycle control are deletions affecting $p16^{INK4a}$ and $p14^{ARF}$ that have been detected in MCL cases (Fig. 3.3) (Pinyol et al. 1997). $p16^{INK4a}$ inhibits the interaction between CDK4/6 and cyclin D1 and thereby controls the phosphorylation of Rb (Sherr and McCormick 2002). A fraction of MCL cases are characterized by amplification and/or

overexpression of *BMI1* that acts as a transcriptional repressor of the $p16^{INK4A}$ locus and by amplifications of the *CDK4* locus (Fig. 3.3) (Bea et al. 2001; Hernandez et al. 2005). At last, the *RB* gene can be inactivated by deletions in some MCL cases (Pinyol et al. 2007).

Intriguingly, overexpression of cyclin D1 alone is not sufficient to induce lymphomagenesis. Thus, secondary genetic abnormalities are required for lymphoma development (Bodrug et al. 1994; Lovec et al. 1994). In line, MCLs are characterized by deregulation of DNA damage response. Mutations and deletions of the ataxia-telangiectasia-mutated (*ATM*) gene that plays an important role in the cellular response to DNA damage are highly prevalent in MCL (Schaffner et al. 2000). Additionally, *p53* mutations occur in roughly 15 % of MCL samples and are associated with poor prognosis (Greiner et al. 1996). Alternative molecular mechanisms to inactivate p53 are high MDM2 expression in a subset of MCL cases or loss of $p14^{ARF}$ that stabilizes p53 by inhibition of MDM2-mediated ubiquitination and degradation (Fig. 3.3) (Hernandez et al. 2005).

Additional molecular mechanisms contribute to MCL biology. High-throughput sequencing identified recurrent somatic *NOTCH1* mutations in more than 10 % of MCL patient samples (Kridel et al. 2012). However, its functional role in the molecular pathogenesis of MCL remains to be elucidated. Different groups have shown that the oncogenic PI3K-AKT signaling pathway is constitutively activated in MCL (Rudelius et al. 2006). However, its exact role in MCL development is unclear. Finally, a recent study by Hartmann and colleagues applied a combined approach of high-resolution gene expression and copy number profiling to more than 70 MCL patient biopsies (Hartmann et al. 2010). Inactivation of *CUL4A* and *ING1* that both may affect cell proliferation and DNA damage response pathways could be identified as well as inactivation of several members of the Hippo pathway. However, the functional significance of these findings remains unclear (Hartmann et al. 2010).

Gene expression profiling can be used to predict survival of MCL patients at diagnosis. Rosenwald et al. investigated the expression profiles of 101 MCL cases (Rosenwald et al. 2003b). A proliferation signature, consisting of 20 genes that are related to tumor cell proliferation, is a strong predictor of adverse survival. This signature includes genes involved in cell cycle control, DNA synthesis, and DNA repair as well as other cellular processes that are upregulated to enable cellular proliferation (Fig. 3.3). As expected the vast majority of MCL cases expressed high levels of cyclin D1. Interestingly, a small fraction of MCL samples seems to be cyclin D1 negative (Rosenwald et al. 2003b; Fu et al. 2005). These cases are histologically indistinguishable from cyclin D1-positive samples and have the same gene expression profile (Fu et al. 2005). They seem to express either cyclin D2 or cyclin D3 to substitute for the lacking cyclin D1 expression (Fu et al. 2005). Some of these cases are characterized by translocations involving cyclin D2 or cyclin D3 (Gesk et al. 2006; Wlodarska et al. 2008).

3.5 Perspectives

A better understanding of the molecular pathogenesis of "rare" malignant lymphoma subtypes is a prerequisite to improve future therapeutic strategies. A rapidly increasing number of novel targeted molecules are currently being evaluated in the laboratory as well as in early clinical trials. However, the use of these agents will only be successful, if the targeted pathway is utilized by the malignant cells. Therefore, we need to understand and determine to which signaling cascades the malignant cells are addicted to. Thus, we have to incorporate sophisticated scientific techniques such as gene expression profiling or high-throughput sequencing in the diagnostic routine to detect specific pathway dependencies in each patient. By this approach rational and individualized treatment strategies of patients affected by rare entities will become clinical reality.

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Signaling Pathways in Rare Lymphomas

4

Andrew Lipsky, Patricia Pérez-Galán, Claudio Agostinelli, Pier Paolo Piccaluga, Stefano A. Pileri, and Adrian Wiestner

Contents

A. Lipsky
Department of Internal Medicine,
Montefiore Medical Center,
Albert Einstein College of Medicine,
New York, NY, USA

A. Wiestner (✉)
Hematology Branch, National Heart, Lung,
and Blood Institute, National Institutes
of Health, Bethesda, MD, USA
e-mail: wiestnera@mail.nih.gov

P. Pérez-Galán
Department of Hemato-Oncology,
Institut d'Investigacions Biomédiques August
Pi I Sunyer (IDIBAPS), Barcelona, Spain

C. Agostinelli • P.P. Piccaluga • S.A. Pileri
Pathology and Haematopathology Unit,
Department of Specialty, Diagnostic
and Experimental Medicine, Bologna University
School of Medicine, Bologna, Italy

4.1 MCL, a Paradigm of Multiple Activated Pathways in a Mature B-Cell Lymphoma

Mantle cell lymphoma (MCL), a mature B-cell neoplasm defined as a distinct entity in the early 1990s, constitutes about 6 % of all non-Hodgkin's lymphomas (NHL) (O'Connor 2007; Dreyling and Hiddemann 2009; Ghielmini and Zucca 2009; Jares et al. 2007). MCL is typically disseminated at presentation, with a leukemic component in 20–30 % of patients. Classic and blastoid variants are recognized,

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the latter associated with inferior clinical outcome. MCL is one of the most difficult to treat B-cell lymphomas. While conventional chemotherapy induces high remission rates in previously untreated patients, relapse within a few years is common, contributing to a rather short median survival of 5–7 years (Herrmann et al. 2009; Martin et al. 2008). MCL is a complex disease, where several deregulated pathways contribute to its aggressiveness. Here we described those pathways that have been found to play a key role in MCL pathogenesis.

4.1.1 Cell Cycle Deregulation

The genetic hallmark of MCL is the translocation t(11;14)(q13;q32) which leads to constitutively high expression of cyclin D1. Additional mutations in the cyclin D1 transcript delete regulatory elements that normally shorten mRNA half-life. These mutations further increase cyclin D1 expression (Rosenwald et al. 2003). However, cyclin D1-negative cases having typical morphology and gene expression profile have been described and often show overexpression of cyclin D2 or D3 (Rosenwald et al. 2003). Cyclin D1 is labile protein of 30 kDa that forms a complex with the cyclin-dependent kinase CDK4 or CDK6 to promote cell cycle entry. The *CDK4* locus is frequently amplified (Bea et al. 1999), and decreased expression of miR-29, which targets CDK6, can lead to increased CDK6 expression and identifies patients with short survival (Zhao et al. 2010). Transcription, translation, assembly into holoenzyme complexes, subcellular localization, and degradation of cyclin D1 are tightly regulated. While overexpression of cyclin D1 is not transforming in nude mice, additional events that increase nuclear cyclin D1 levels and the activity of signaling pathways that regulate D1 translation and protein stability can enhance its oncogenic potential.

4.1.2 DNA Damage Pathway

MCL shows genetic alterations that affect DNA damage response pathways and are of particular interest because they may contribute to refractoriness to chemotherapy (Bea et al. 2009). 11q22–23 deletions affecting the *ATM* gene are recurrent in MCL. The ATM kinase is critically involved in the cellular response to DNA damage and may act as a tumor suppressor gene. Truncating or missense mutations involving the PI3K domain of *ATM* are found in a majority of MCL cases and are commonly accompanied by the loss of the other allele. The high frequency of *ATM* mutations in MCL is striking and has been linked to ATM expression in naïve B cells in the mantle zone. Another mechanism for the strong selective pressure on *ATM* mutant clones may be aberrant re-initiation of DNA replication during S-phase leading to double-strand DNA breaks and activation of the ATM pathway (Kim and Diehl 2009). Moreover, the tumor suppressor gene TP53, downstream of ATM, plays an important role in DNA damage responses. Mutations of *TP53* typically in conjunction with 17p13 deletions have been detected primarily in blastoid MCL cases. In addition, UPDs involving the chromosomal band 17p are associated with TP53 inactivation. An alternative mechanism to disrupt the p53 pathway involves overexpression of the negative regulators MDM2 and MDM4. MDM2 overexpression due to copy number gains correlates with inferior survival. Similarly, MDM4, which is also highly expressed in MCL, decreases expression of the CDK inhibitor p21, thereby promoting cell cycle progression (Liang et al. 2010).

4.1.3 B-Cell Receptor Pathway

Recent studies reported constitutive activation of the B-cell receptor (BCR) signal transduction components SYK and PKCβII (Boyd et al. 2009; Rinaldi et al. 2006). SYK was amplified in both Jeko-1 cells and some primary MCL samples, but constitutive activity of SYK was only demonstrated in the cell line (Rinaldi et al. 2006). Jeko-1 cells were more sensitive to a SYK inhibitor than MCL cell lines without constitutive SYK activation, indicating some dependence on the pathway. In a screen for phosphoproteins, PKCβII was found to be

phosphorylated in primary MCL samples in contrast to normal B cells (Boyd et al. 2009). However, inhibitors targeting these molecules have induced only minor clinical responses. On the contrary, targeting the downstream kinase BTK (Bruton's tyrosine kinase) has yielded objective responses in a few patients with MCL (Advani et al. 2010). These results may suggest a role for BCR signaling in MCL pathogenesis.

4.1.4 PI3K/AKT/mTOR Pathway

The PI3K/AKT pathway is involved in the transduction of a variety of extracellular signals and plays a prominent role in many cancers (Engelman 2009). In normal B cells, PI3K functions as a transducer of BCR signaling that regulates proliferation, differentiation, apoptosis, and survival. Gene expression profiling implicated the PI3K/AKT pathway in the pathogenesis of MCL (Rizzatti et al. 2005), and several key components of the PI3K/AKT/mTOR pathway are activated in MCL (Peponi et al. 2006), indicating a possible contribution of this pathway to MCL pathogenesis. Constitutive activation of AKT was found in most blastoid and many classic MCL tumors and was associated with the phosphorylation of downstream targets including MDM2, Bad, and p27 (Dal Col et al. 2008; Rudelius et al. 2006). Furthermore, AKT-mediated activation of mTOR and its downstream targets S6K and eukaryotic initiation factor 4E-binding protein-1 (4E-BP1) can increase translation of key proteins.

Several mechanisms may cause constitutive activation of AKT, including activation of upstream kinases such as SYK, and amplification of *PI3KCA*, the gene encoding the catalytic subunit p110α (Psyrri et al. 2009).In contrast to solid tumors, no activating somatic mutations of *PI3KCA* have been identified (Rudelius et al. 2006; Psyrri et al. 2009). Loss of PTEN, a phosphatase that turns the PI3K pathway off, is another recurrent feature in MCL and may be the result of mutations, deletions, or promoter methylation (Rudelius et al. 2006). PTEN can also be inactivated by phosphorylation at Ser380 and Thr382/383, which has been found in MCL cases with constitutively active AKT (Dal Col et al. 2008).

4.1.5 JAK/STAT Pathway

The JAK/STAT signaling pathway regulates growth, proliferation, differentiation, and survival in response to external stimuli especially cytokines (Sun et al. 2004). The JAK/ STAT pathway is aberrantly activated in several B-cell lymphomas, including in primary mediastinal B-cell lymphoma (discussed in the next section). In MCL, the active, phosphorylated form of STAT-3 was found in 47 % of nodal cases (Kawadler et al. 2008) and in 70 % of leukemic cases (Düwel et al. 2009). It has been hypothesized that STAT-3 activation may be through activation of the BCR and/or interleukins 6 and 10 (Düwel et al. 2009).

4.1.6 NOTCH Pathway

NOTCH receptors play a critical role in cell fate specification during development and participate in multiple biological processes. In the hematopoietic system, NOTCH1 activation plays a critical role at multiple stages in both T- and B-cell development (Pui et al. 1999). *The NOTCH1* receptor functions as a ligand-activated transcription factor that directly transduces extracellular signals in the cell surface into changes in gene expression in the nucleus. Activation of NOTCH receptors typically occurs via cell-cell contact and interaction of a NOTCH protein with a delta-like or jagged ligand expressed on the surface of a neighboring cell.

Whole transcriptome sequencing in MCL samples uncovered recurrent somatic mutations in NOTCH1 coding sequence in 12 % of clinical samples and 20 % of cell lines. These mutations generate a premature stop codon, resulting in a NOTCH1 protein lacking the C-terminal domain, which contains a PEST sequence (a sequence rich in proline, glutamic acid, serine, and threonine). Removal of this region results in the accumulation of an active protein isoform. *NOTCH1* mutations were associated with poor overall survival (Kridel et al. 2012). The pattern and frequency of *NOTCH1* mutations is very similar to what has recently been described in chronic lymphocytic leukemia (CLL) (Puente et al. 2011) but

different from T-cell acute lymphoblastic leukemia (T-ALL) (Paganin and Ferrando 2011), where *NOTCH1* mutations arise in more than 50 % of cases and target the heterodimerization and/or the PEST domains of NOTCH1.

4.2 Signaling Pathways in Other B-Cell Lymphomas

4.2.1 Primary Mediastinal B-Cell Lymphoma

Primary mediastinal B-cell lymphoma (PMBL) represents 2–4 % of NHL and since 2001 is considered a separate clinicopathologic entity, different from diffuse large B-cell lymphoma (DLBCL) and characterized by a distinct gene expression profile (Lenz et al. 2008). PBML often presents with a bulky tumor in the anterior mediastinum and progresses rapidly, affecting primarily women in the third or fourth decade. PBML shows similarities with classic Hodgkin lymphoma in terms of genetic alterations and gene expression profiling. In fact, both lymphomas show activation of Janus kinase-signal transducer and activator of transcription (JAK-STAT) and nuclear factor-kB (NF-kB) signaling pathways that increase proliferation and survival of tumor cells. However, PBML also has the ability to bypass the immune surveillance, adding another level of complexity and an advantage for the malignant clone.

4.2.1.1 JAK/STAT Pathway

A common genetic lesion in PBML present in more than half of cases is gain of chromosomal region 9p, that involves among other genes, JAK2 (Lenz et al. 2008; Wessendorf et al. 2007). Additional alterations further support the importance of this pathway in PBML pathogenesis. Gene expression studies have demonstrated overexpression of JAK2 in PMBL and have suggested constitutive activation of the IL-4 and IL-13 pathways as a result (Savage et al. 2003). Accordingly, STAT6, the transcription factor that is primarily regulated by IL-4 and IL-13, is constitutively activated in PMBL, and somatic mutations in the DNA-binding domain of STAT6 have been found in 36 % of PMBCL cases (Guiter et al. 2004; Ritz et al. 2009). Surprisingly, despite *JAK2* amplification and an increase in *JAK2* mRNA, only minimal changes in JAK2 protein have been documented in PMBL cell lines. However, a clear prolongation of the JAK2 protein half-life was observed, resulting in decreased protein turnover (Melzner et al. 2006). Moreover, a negative regulator of the JAK/STAT pathway, Src-homology 2 domain containing suppressor of cytokine signaling 1 (SOCS1), is frequently deleted in PMBL. SOCS1 targets JAK for proteasomal degradation. *SOCS1* is deleted in up to 45 % of cases making it the most common recurrently mutated tumor suppressor gene in PMBL. Biallelic deletions of *SOCS1* have also been detected in PMBL cell lines (Melzner et al. 2005).

4.2.1.2 NF-κB Pathway

Mutations and structural alterations of genes belonging *NF-κB* have been described in PBML leading to constitutive NF-κB activity. Frequent gains (~50–75 % of cases) of 2p14–16 affecting the *REL* proto-oncogene are present in PBML, and nuclear expression of this transcription factor has been described (Bentz et al. 2001; Joos et al. 1996; Weniger et al. 2007). In addition, gene expression profiling studies have demonstrated increased expression of a NF-κB gene signature expressed in PMBL, and a possible role for TNF-α signaling has been entertained. (Feuerhake et al. 2005) Another gene at this chromosomal location is the zinc finger transcriptional repressor *BCL11A* that encodes a protein critical to lymphoid development. BCL11A is thought to be the oncogene providing a selective advantage to cells harboring the 2p amplification. *BCL11A* is present at increased copy numbers in 75 % of cases, and high nuclear protein expression is seen in 88 % of cases. This suggests that *BCL11A-dependent* transcriptional repression may provide a survival advantage to the malignant B cell (Weniger et al. 2006). Other chromosomal imbalances affecting NF-κB-related proteins are amplification of *BCL10* (1p22) and *MALT1* (18q21). Recently, *TNFAIP3* (encoding A20) has been reported as a novel tumor suppressor gene in PMBL. The A20 protein acts

as a ubiquitin-modifying enzyme that inhibits NF-κB signaling downstream of TNF receptor engagement by interacting with RIP1, TRAF1, and TRAF2. Mutations in *TNFAIP3* leading to constitutive NF-κB activity were found in 36 % of PMBCL cases (Schmitz et al. 2009; Honma et al. 2009).

4.2.1.3 Dysregulation of Immune Surveillance Pathways

Histologically, PMLBL includes variable numbers of malignant cells within an inflammatory infiltrate, suggesting that these tumors escape immune surveillance and that this immune privilege may contribute to the cancer phenotype. To date, two groups of molecules have been identified as responsible of this privileged microenvironment: MHCII and PD ligands.

Early studies recognized that PBML shows reduced expression of major histocompatibility complex class II(MHC II) genes in a substantial number of cases (Moller et al. 1986). Subsequently, this decreased expression of MHCII was found to be associated with inferior survival (Roberts et al. 2006). Moreover, in a large series 65 % of PBML cases showed decreased MHCII expression which correlated with reduced numbers of cytotoxic T lymphocytes (Farinha et al. 2009). These data indicate a possible immune escape through downregulation of HLA class II molecules. Interestingly, loss of *MHCII* expression, at least in part, may be due to unbalanced genomic rearrangements of 16p13.13 affecting *CIITA*, a positive regulator of MHCII expression (Steidl et al. 2011). Of note, the SOCS1 gene, which is frequently deleted in PBML, resides close to *CIITA* on chromosome 16.

PDL1/CD274 and PDL2/CD273 belong to the CD28 superfamily of costimulatory receptors that regulate T-cell activation. PDL1 and PDL2 serve as negative regulators of CD8+ T-cell activation and proliferation. Gene expression profiling studies revealed PDL2/CD273 overexpression in PMBL. The genes encoding PDL1 and PDL2 are located at chromosome 9p, where gains have been found in more than half of PBML cases. Studies of copy number and mRNA expression confirmed simultaneous overexpression of JAK2 and the PD ligands in cases with 9p amplification and demonstrated that JAK2 further augments their expression through increased transcription. In fact, a direct correlation between 9p copy number changes, and mRNA expression of both PD ligands has been demonstrated (Green et al. 2010). In addition, both PDL1/CD274 and PDL2/CD273 have been found as recurrent gene fusion partners of *CIITA*. As a result of the translocation t(9;16)(p24.1;p13.13), both PDL1 and PDL2 are highly expressed under control of *CIITA* promoter III. Thus, overexpression of PD ligands could further contribute to immune evasion. Indeed, in vitro studies showed that U2940, a PMBL cell line expressing high wild-type levels of PD ligands, induced energy in cocultured Jurkat T cells.

Interestingly, detailed cytogenetic studies of the translocated *CIITA* locus at 16p13.13 found that a substantial number of cases harbor additional unbalanced rearrangements with loss of genetic material centromeric of the breakpoint. This finding is in keeping with previous reports describing deletions of the tumor suppressor gene *SOCS1* located in the vicinity of *CIITA* on chromosome 16 (Melzner et al. 2006). Thus, a single genetic event may trigger a complex genetic rearrangement that simultaneously contributes to immune evasion (MHCII downregulation as a consequence of CIITA disruption and inhibition of T-cell activation through upregulation of PD ligands) as well as activation of growth and survival pathways (JAK2 amplification and deletion of the negative regulator SOCS1).

4.2.2 Burkitt's Lymphoma

Burkitt's lymphoma (BL) is a mature aggressive B-cell NHL (Dalla-Favera et al. 1982). BL occurs in three distinct clinical varieties: an endemic variant, arising in children in equatorial Africa (coinciding with areas of malaria endemicity) that is often associated with Epstein-Barr virus (EBV); a sporadic variant, observed in adolescents and young adults in Europe and North America that is less frequently associated with

EBV; and an immunodeficiency-related variant, most commonly observed in HIV-infected individuals (Swerdlow et al. 2008; Molyneux et al. 2012). In high-risk areas endemic Burkitt's lymphoma has an incidence of 4–5 per 100,000 children, whereas in lower-risk areas the incidence of sporadic BL is about tenfold less (Cardy and Sharp 2001; Rainey et al. 2007). Each of the three variants is characterized by a chromosomal rearrangement resulting in the dysregulation and overexpression of the MCY proto-oncogene (Thorley-Lawson and Allday 2008). MCY encodes a basic helix-loop-helix (bHLH) transcription factor that is involved in regulating a diverse set of >1,500 downstream genes (Zeller et al. 2006). By forming a heterodimer with its bHLH partner protein Max, MCY exerts direct or indirect regulatory influence on 15 % of the genome including genes that play a role in cell cycle regulation, growth, protein biosynthesis, and apoptosis (Dang et al. 2006; Hecht and Aster 2000). In fact, MCY is thought to be a global transcriptional activator through its role in modulating chromatin structure (Klapproth and Wirth 2010). Recent work has demonstrated that in addition to regulating transcription, MCY can regulate microRNA networks that plays a role in the modulation of tumorigenesis (Thorley-Lawson and Allday 2008; Chang et al. 2007; Sander et al. 2009). The malignant B-cell clone expresses CD19, CD20, and low to intermediate levels of CD10. As one of the fastest growing human tumors, >95 % of tumor cells express Ki-67 (Hecht and Aster 2000). On tissue microscopy, BL is further characterized by the classical "starry sky" appearance which is produced by numerous apoptotic cells interspersed with pale macrophages. Though there remains some controversy, observations based in part on the patterns and rates of somatic hypermutations in the variable region of the immunoglobulin chain suggest that the likely precursor cell of origin is the germinal center B cell in the case of EBV-negative BL and the memory B cell in the setting of EBV-positive BL (Thorley-Lawson and Allday 2008; Hochberg et al. 2004).

Several translocations are commonly observed in BL which associate MYC to one of three immunoglobulin loci. The most prominent translocation, t(8:14)(q24;q32), has been observed in 85 % of all BL, including both EBV positive and negative forms of disease (Yustein and Dang 2007; Zech et al. 1976). It is associated with the juxtaposition of MYC and the enhancer element of the Ig heavy chain. Other translocations including t(2;8)(p12;q24) and t(8;22)(q24;q11) occur in 10–15 % of patients and involve the kappa and lambda light chains, respectively (Molyneux et al. 2012; Kornblau et al. 1991). While additional chromosomal abnormalities are detected in up 70 % of pediatric BL, the total number of such additional genetic events in any one case is relatively low (Salaverria et al. 2008). It has been known for some time that trisomies of chromosomes 7, 8, 12, and 18 are frequent, and more recent results from whole genome mapping have allowed for identification of recurrent aberrations at other loci.

4.2.2.1 MYC Expression, p53, and Apoptosis

Paradoxically, elevated expression of MCY has been demonstrated to induce apoptosis; (Hoffman and Liebermann 2008) this is accomplished via upregulation and expression of pro-apoptotic genes including TP53 and ARF1, as well as downregulation or disruption of anti-apoptotic genes such as BCL2 and BCL2L1 (Klapproth and Wirth 2010; Meyer and Penn 2008). The prevailing explanation of this observation is that apoptosis serves as a "built-in" failsafe, balancing any unrestrained growth resulting from aberrant MYC activation. Tumorigenesis is thought to result when this failsafe is circumvented. Indeed, *TP53* mutation is observed in the majority of BL cell lines and at least 30 % of BL tissue biopsies; thus, MCY activation and proliferation may select for *TP53* inactivation via point mutation (Lindström and Wiman 2002). Additional MCY-dependent mechanisms may also contribute to tumorigenesis. For example, MCY can increase expression of cyclin D and E, thereby dysregulating the phosphorylation of pRB, leading to promotion of the G1 to S cell cycle transition (Lindström and Wiman 2002; Santoni-Rugiu et al. 2000). Also, MCY may

directly repress the transcription of cell cycle inhibitors including p27 and p21 (Chandramohan et al. 2008; Gartel et al. 2001).

4.2.2.2 BL and the INK4a/ARF Network

The INK4a-ARF locus encodes two potent tumor suppressors, p16Ink4a and p14ARF, which contribute to the regulation of RB and p53 (Lowe and Sherr 2003). RB acts to prevent entry into the S phase of the cell cycle by suppressing E2F transcription factors responsible for activation of several genes involved in DNA replication (Sherr 2001). Phosphorylation of RB during the G1 phase is catalyzed by cyclin D and E resulting in the loss of RB's ability to suppress E2Fs. The Ink4 proteins, including the canonical protein P16INK4a, inhibit cyclin D-dependent kinases (CDK4 and CDK6), thereby maintaining RB-E2F repression (Lowe and Sherr 2003). Interestingly, MYC activation has been shown to activate cdc25A, a CDK-activating phosphatase that acts on CDK4 and CDK6, a process which might induce increased RB phosphorylation (Galaktionov et al. 1996). In addition, silencing of the p16/INK4a gene via methylation is commonly observed, occurring in 89 % of BL cell lines and 42 % of primary biopsies (Klangby et al. 1998). Interestingly, p73, a gene located on 1p36.2–3 that is functionally homologous to p53, has been shown to also undergo aberrant promoter methylation in 30 % of BL (Corn et al. 1999). Additionally, in a subset of BL cells that express wild-type p53, inactivation of p53 is mediated by MDM2 that targets of p53 for proteasomal degradation (Lindström and Wiman 2002; Capoulade et al. 1998). Therefore, in a subset of BL, inactivation of the ARF-MDM2-p53 pathway is thought to allow the escape from MCY-induced apoptosis early in BL development (Lindström et al. 2001). This conclusion was reinforced by the observation that the ARF-MDM2-p53 apoptotic pathway is disrupted in approximately 55 % of sporadic BL (Wilda et al. 2003).

4.2.2.3 Additional Genetic Abnormalities and Cofactors

Most recently somatic mutations in the transcription factor *TCF3* (E2A) or its negative regulator *ID3* have been described in 70 % of BL cases (Schmitz et al. 2012). TCF3 activates the pro-survival PI3K pathway in BL, in part by augmenting tonic B-cell receptor signaling. In addition, mutations of CCND3 were found in 38 % of sporadic BL cases, and were shown to result in the production of highly stable cyclin D3 isoforms that mediate cell cycle progression.

The BCL6 proto-oncogene, known to be implicated in chromosomal translocations in diffuse large-cell lymphomas, has also recently been shown to undergo mutations in the setting of BL (Capello et al. 2000). In one study, the frequency of aberration was detected to be 28.6 % in sporadic BL and 50 % in endemic BL (Capello et al. 1997). A mechanism for accrual of BCL-6 mutation similar to that of somatic hypermutation of IG genes is suggested; this accords well with both the observation that BCL-6 mutations arise during transit through the germinal center and the notion that BL arises from germinal or post-germinal center B cells (Capello et al. 2000).

Several cofactors are also thought to play an important role in BL. In the endemic variety, infection with *Plasmodium falciparum* is thought to contribute to BL pathogenesis via reactivation of EBV through the malaria parasite's cysteine-rich interdomain 1a (Donati et al. 2004). The induction of TLR-9 signaling via ligand activation is also implicated in pathogenesis, as in human B cells, it can induce cytidine deaminase, a key enzyme involved in chromosomal translocations. Despite the fact that several observations imply a link between EBV and tumorigenesis, the exact mechanism is controversial and remains to be elucidated. The prevailing assumption is that EBV plays a role in fixing genetic and epigenetic changes that either inhibit apoptosis directly or shield BL cells from the growth constrains established by the immune system and the tumor microenvironment (Gruhne et al. 2009).

4.2.3 MALT Lymphoma

Extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma) is an extranodal lymphoma that occurs

in a wide variety of sites including the stomach, lung, salivary gland, thyroid, and small bowel. Clinically, MALT lymphoma may be classified as low or high grade and presents in adults (with a median age of 61 years), the majority of whom exhibit localized disease (Isaacson 2005). Most cases originate in the gastric mucosa and are strongly associated with chronic *Helicobacter pylori* infection. Accounting for approximately 7 % of all NHLs and 50 % of gastric lymphomas, MALT lymphoma is the most common extranodal lymphoma and is characterized by the cytological features and immunophenotype of marginal zone B cells, including expression of CD19, CD20, and CD22 and negativity of CD5, CD10, and CD23 (Isaacson 2005; Zucca et al. 2000).

4.2.3.1 NF-κB Pathway

Karyotype studies of MALT lymphoma have revealed four recurrent and mutually exclusive chromosomal aberrations implicated in its pathogenesis: t(11;18)(q21;q21), t(1;14)(p22;q32), t(14;18)(q32;q21), and t(3;14)(p13;q32) (Du 2011). All four translocations promote activation of NF-κB activation. This effect is mediated via the uncoupling of the BCL-10/MALT1 signaling complex from physiologic upstream stimuli (Lucas et al. 2001).

Under physiologic conditions, activation of the canonical NF-κB pathway is initiated when antigen-receptor stimulation triggers the recruitment of the scaffolding adaptor CARMA1 (CARD11), phosphorylating its protein kinase C (PKC)-regulated domain (Rawlings et al. 2006). A conformational change in CARMA1 allows for recruitment of BCL10 and triggers a subsequent oligomerization cascade. Oligomerized BCL10 then induces oligomerization of MALT1 by binding to its immunoglobulin (Ig)-like domain, forming the CARMA1 complex. This CARMA1 complex directly interacts with TRAF6, conferring ubiquitin-ligase activity via the oligomerization of TRAF6 by oligomerized MALT1. The interaction of TRAF6 with the ubiquitin-conjugating enzyme E2 facilitates the K-63-linked polyubiquitylation of IκB kinase-γ (IKKγ) [alternatively designated nuclear factor-κB essential modulator (NEMO)]. An activated IκB kinase (IKK) complex results when ubiquitylated IKKγ, acting as the regulatory subunit of the complex, activates IKKα and IKKβ, the two catalytic subunits (Du 2011). The activated IKK complex phosphorylates IκB, resulting in its proteolytic degradation by the 26S proteasome. NF-κB dimers are thereby released and translocated to the nucleus where they upregulate genes involved in cellular activation, proliferation, and survival.

The translocation t(1;14)(p22;q32) found in 5 % of MALT lymphomas results in the overexpression of BCL10 by placing the whole BCL10 gene under the regulatory control of the IG gene enhancer (Du 2011; Willis et al. 1999; Zhang et al. 1999). As mentioned above, BCL10 is expressed primarily in the cytoplasm of normal lymphocytes where it functions as an adaptor protein, coupling the protein CARMA1 to MALT1. This effect is mediated upstream via CARD-CARD (caspase recruitment domain) interactions and downstream via BCL10 interaction with two N-terminal immunoglobulin-like domains on MALT1 (Lucas et al. 2001). In the setting of BCL10 overexpression and in the absence of upstream signaling, the N-terminal CARD facilitates the formation of BCL10 oligomers, leading to MALT1 oligomerization and constitutive NF-κB activation. Furthermore, recent work suggests that BCL10 may also play a role in activation of the noncanonical NF-κB pathway (Du 2011; Bhattacharyya et al. 2010).

The translocation (11;18)(q21;q21) causes fusion of the apoptosis inhibitor-2 gene (which inhibits the biological activity of caspases 3, 7, and 9) with the MALT1 protein (Isaacson and Du 2004). The action of this API2-MALT1 fusion protein is sufficient for NF-κB activation, a property that is not independently exhibited by either wild-type API2 or MALT1 (Lucas et al. 2001; Isaacson and Du 2004). The API2 gene contains three N-terminal baculovirus inhibitor of apoptosis repeats (BIR) that may mediate the oligomerization of the API2-MALT1 fusion protein, which when accompanied by TRAF2 binding renders the protein capable of NF-κB activation (Garrison et al. 2009).

The translocation t(14;18)(q32;q21) places the MALT1 gene under the control of the

enhancer region of the Ig-heavy-chain gene leading to dysregulation and overexpression of MALT1 (Sanchez-Izquierdo et al. 2003; Streubel et al. 2003). The paracaspase MALT1 has several known points of intersection with the NF-κB pathway. First, it plays a part in the aforementioned oligomerization cascade: after self-oligomerization, activated MALT1 binds to TRAF6 inducing its oligomerization and subsequent IKK complex activation (Sun et al. 2004). Second, MALT1 may activate the NF-κB pathway through heterodimerization and activation of caspase-8, a caspase required for lymphocyte proliferation (Kawadler et al. 2008). Additionally, through its protease activity and attenuation of the global NF-κB inhibitor A20, MALT1 also regulates NF-κB activation, although such protease activity is not essential for IκB phosphorylation (Du 2011; Düwel et al. 2009).

Recently, a translocation (*3*;*14*)(*p13*;*q32*) juxtaposing the immunoglobulin gene heavy-chain enhancer and the forkhead box protein P1(FOXP1) at 3p14.1 results in FOXP1 dysregulation (Streubel et al. 2005). Expressed in normal and neoplastic B cells, FOXP1 has been shown to be highly expressed in a subset of diffuse large B-cell lymphomas, and suspicion that it may regulate NF-κB activation has been bolstered by the observation that certain FOXP1 isoforms are capable of activating the NF-κB reporter in both B- and T-cell lines (Streubel et al. 2005; Barrans et al. 2004).

4.2.3.2 Translocation-Negative MALT Lymphoma and A20 Inactivation

While the analysis of chromosomal translocations has provided important insights into the pathogenesis of MALT lymphoma, it is important to note that the majority of MALT lymphomas – especially those of the ocular adnexa, salivary glands, and thyroid – do not harbor the aforementioned chromosome translocations (Du 2011). Work by Du and others has highlighted the role of A20 as a target of 6q23 deletion in translocation-negative MALT lymphomas (Du 2011; Chanudet et al. 2009). Through its dual ubiquitin-editing ability, the zinc finger protein A20 acts as a negative regulator of NF-κB activity attenuating the inflammatory and immune response (Rosebeck et al. 2011). Activation of immunoreceptors leads to rapid proteolytic cleavage and inactivation of A20 by MALT1 via its recruitment and formation of a complex with MALT1/BCL-10 (Rosebeck et al. 2011; Coornaert et al. 2008). Cleavage by MALT1 impairs the NF-κB inhibitory function of A20 (Malinverni et al. 2010). Additionally, A20 inhibits NF-κB signaling through disruption of the E2/E3 ubiquitin enzyme complex (Shembade et al. 2010). These observations have led some authors to suggest that restoring the molecular break via therapeutics targeting the proteolytic activity of MALT1 (rather than by inhibiting NF-κB activation directly) might represent a therapeutic alternative with lesser propensity for generalized immunodeficiency (Coornaert et al. 2008).

Du and others have stressed that neither the translocation product in translocation-positive MALT lymphoma nor A20 inactivation in translocation-negative MALT lymphoma is sufficient for malignant transformation. Rather, oncogenesis is thought to be dependent upon cooperation between genetic factors and immunologic drive. Evidence suggests that an important and complementary role is played by surface receptor stimulation in the translocation-positive setting and active immune response to antigen – including activation of NF-KB via TNF and other presently unidentified actors – in translocation-negative MALT lymphoma (Du 2011).

4.2.4 Waldenström's Macroglobulinemia/ Lymphoplasmacytic Lymphoma

Waldenström's macroglobulinemia (WM) is a rare B-cell malignancy characterized by infiltration of the bone marrow with lymphoplasmacytic cells, hence the name lymphoplasmacytic lymphoma and the presence of an IgM monoclonal gammopathy in the serum (Owen et al. 2003). Accounting for 1–2 % of hematological malignancies, WM has an incidence of approximately three per million, with 1,400 new cases

diagnosed each year in the United States (Fonseca and Hayman 2007). In contrast to the well-established clinical picture of the disease, less is known about its biological origins (Chng et al. 2006). The tumor clone is thought to evolve from a memory B cell that has undergone somatic hypermutation in the germinal center in the absence of antigenic selection and has failed to undergo clonotypic isotype class switching (Chng et al. 2006; Kriangkum and Taylor 2006; Sahota et al. 2002).

4.2.4.1 Cytokines and JAK/STAT Pathway Activation in the Tumor Microenvironment

The regulation of cell proliferation, dissemination, and trafficking through interactions between the malignant clone and the bone marrow microenvironment (BME) is a key area of research in WM (Ghobrial et al. 2011). Akin to multiple myeloma, a related plasma cell dyscrasia, the interaction with the BME results in cell proliferation and drug resistance through the activation of several proliferative signaling cascades including the PI3K, NF-κB, MAPK, and JAK/STAT pathways. BM stromal cells are crucial for the growth of WM cells, and expression of Jak1 and Stat3 protein is higher in the WM population than in controls (Hatjiharissi et al. 2007; Hodge and Ansell 2011). Aberrant activation of the JAK/STAT pathway is thought to depend on cytokine signaling with IL-6 likely playing a key role. IL-6 levels are increased in WM patients and decrease significantly during treatment (Hatzimichael et al. 2001). Elsawa and others have identified CCL5 as highly expressed in patients with WM (with CCL5 levels correlated to that of IL-6) and have established that CCL5 directly stimulates secretion of IL-6 in WM stromal cells through JAK/STAT signaling (Elsawa et al. 2011). Other additional cytokines, including IL-2, IL-7, IL-10, and IL-12, may also activate the JAK/STAT pathway and regulate IgM secretion (Hodge and Ansell 2011).

4.2.4.2 MYD88 and Oncogene Addiction

The TLR and IL-1 receptor adaptor protein MYD88 transduces signals to NF-κB transcription factors that induce expression of pro-survival and pro-proliferation cytokines (TNF, IL-6, IFNβ, IL-1β) and chemokines (CXCL1, CXCL2, CXCL10) (Ben-Neriah and Karin 2011). Via a complex interaction with its amino-terminal death domain, MYD88 acts to coordinate the IRAK-family kinases into a helical signaling complex (Lin et al. 2010). IRAK4 then phosphorylates IRAK1, enabling the recruitment of ubiquitin ligase TRAF6 and subsequent downstream signaling, including the NF-κB, p38 MAP kinase, and type I interferon pathways (Iwasaki and Medzhitov 2010; Staudt 2012). Recent work has implicated this innate immune signaling pathway in the pathogenesis of B-cell malignancies. In the case of activated B-cell-like subtype of diffuse large B-cell lymphoma (ABC-DLBCL), a somatic mutation in MYD88 giving rise to a mutant L265P variant (a single amino acid substitution located in the TIR domain) has been found to promote oncogenic activity via constitutive TLR signaling; this has been shown to occur via the assembly of the aforementioned complex leading to IRAK1 phosphorylation and downstream activation of the NF-κβ signaling pathway (Ngo et al. 2011). In WM, whole genome sequencing of bone marrow lymphoplasmacytic cells has documented expression of the same L265P somatic variant in a majority of cases (Treon et al. 2012). In this setting, greater phosphorylation of downstream proteins IRAK1, IκBα, NFκB-p65, and STAT3 was observed compared to wild type, and the disruption of MYD88 pathway signaling led the loss of constitutive activation of these proteins and induced apoptosis. (Treon et al. 2012; Yang et al. 2011) These findings, when combined with the observation from ABC-DLBCL that cell death after MYD88 knockdown may be rescued via mutant L265P but not via the wild type (Ngo et al. 2011), suggest that tumor maintenance depends upon the continued activity of MYD88, thus constituting a case of oncogene addiction (Weinstein 2002). Additionally, it is known that epigenetics also play a role in the regulation of cell proliferation within the bone marrow milieu, with recent studies highlighting the ability of miRNA-155 to modulate cellular growth (Sacco et al. 2011). WM cells are also

driven, in part by miRNA-206, to an unbalanced expression of histone deacetylases (HDACs) and histone acetyl transferases (HATs) (Roccaro et al. 2010). Furthermore, both miRNA-155 and miRNA-21 have been shown to be overexpressed in WM cells compared to healthy donor B cells (Cao et al. 2011). MYD88 is also implicated in such microRNA dysregulation, with knockdown of MYD88 resulting in decreased levels of both miRNAs, as well as reduction in miRNA-21 following cell treatment with an IRAK 1/4 kinase inhibitor (Cao et al. 2011).

4.3 Molecular Pathogenesis of Peripheral T-Cell Lymphomas

Conversely to B-cell lymphomas, the molecular pathogenesis of peripheral T-cell lymphomas (PTCLs) has been the object of a limited number of studies. This certainly reflects the much lower incidence of the latter that corresponds to about 12 % of all lymphoid tumors all over the world (Swerdlow et al. 2008). Accordingly, there is a limited availability of fresh or frozen material for the application of novel high-throughput technologies that can provide substantial contributions in this setting. Nevertheless, there is the cogent need to deepen this issue, since the vast majority of PTCLs run a very aggressive clinical course with dismal prognosis (Vose et al. 2008). Such behavior may be at least in part due to the lack of sensitivity to anthracyclines as shown retrospectively by the International T-Cell Lymphoma Project (Vose et al. 2008) and experimentally in primary cell cultures by Piccaluga et al. (2007a).

In the following, attention will be paid to four varieties of PTCL that per se represent about two thirds of T-cell neoplasms, i.e., the not otherwise specified (NOS), angioimmunoblastic (AITL), and systemic ALK+ and ALK- anaplastic large-cell (ALCL) types (Jaffe et al. 2008). Notably, in the fourth edition of the WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues, ALK-ALCL is quoted as a provisional entity – in contrast to the remaining ones that are regarded as distinct entities. Such attribution reflects the uncertainties as to the nosography of this tumor existing at the time of the classification writing, which have been largely overcome during the last 2 years (Agnelli et al. 2012; Piva et al. 2010). In spite of its "distinct" status, PTCL, NOS is by definition a Pandora's box as diffuse large B-cell lymphoma (DLBCL) was in the past in the field of B-cell tumors. In fact, it includes all T-cell neoplasms that cannot be classified in one of the other categories.

In particular, two issues will be separately discussed: the histogenesis and pathogenetic mechanisms of these four types of PTCL.

4.3.1 Histogenesis

Two independent gene expression profiling (GEP) studies, simultaneously published by de Leval et al. (2007) and Piccaluga et al. (2007b), have provided important contributions concerning the histogenesis of AITL. First of all, they showed that AITL has a gene signature related to follicular helper T lymphocytes (FHT), i.e., the T-cell subset which takes part in the life and function of germinal center B cells. Such derivation found its phenotypic surrogate in the immunohistochemical detection of a series of markers (i.e., CD10, BCL6, PD-1, ICOS, SAP, C-MAF, CXCL13, and CCR5) that are physiologically expressed by FHT. Importantly, at least three of these antigens should be simultaneously detected to postulate the FHT origin of a given tumor (Laurent et al. 2010). In fact, one of them can incidentally occur in normal and pathological T-cell populations of variable derivation because of cell plasticity. Worthy of note is the fact that the postulated histogenesis of AITL explains some morphologic characteristics of the tumor (Dogan et al. 2008). Among these are the hyperplasia of follicular dendritic cells (FDCs) and the intimately intermingled B-cell component. The latter is usually EBV infected and represents the substrate for the development of an independent DLBCL that is encountered in 10–20 % of instances. The interaction between neoplastic T lymphocytes and B cells can also explain some of

the clinical manifestations of AITL, such as polyclonal hypergammaglobulinemia, hemolytic anemia, and autoimmune phenomena in general.

Much more complex is the situation as to what PTCL, NOS is concerned. In 2007, Piccaluga et al. (2007a) first reported that by comparison with the gene signature of the main subsets of normal T-lymphocytes, the vast majority of PTCLs, NOS were closer to activated central memory T cells, only a small group revealing a cytotoxic profile. These findings were subsequently confirmed by Iqbal et al. (2010) who observed that cytotoxic tumors had an even worse prognosis than the others. Such assumption seems, however, to find an exception in the lymphoepithelioid variant of PTCL, NOS, also known as Lennert's lymphoma, which behaves much better than all the tumors belonging to this category, in spite of its cytotoxic profile (Hartmann et al. 2011). Furthermore, de Leval et al. (2007) described a few PTCLs, NOS carrying an FHT-related signature, thus suggesting that the FHT derivation might not be exclusive of AITL. The latter assumption has found support in a series of immunohistochemical observations pertaining PTCL, NOS, with special reference to the follicular variant (Pileri et al. 2008). The latter may architecturally mimic follicular B-cell lymphoma, nodular lymphocyte-predominant Hodgkin lymphoma, or marginal zone B-cell lymphoma. Conversely to these conditions, the growth is however sustained by clear cells similar to the ones seen in AITL. In the last edition of the WHO Classification (Pileri et al. 2008), such variant was maintained distinct from AITL for the following reasons: (a) the occurrence of a specific translocation [t(5;9)(q33;q22) fusing ITK to SYK] in about 25 % of cases (Streubel et al. 2006), (b) the limited extent of the disease at the time of presentation, (c) the lack of the symptomatic complex of AITL, which however has been recently questioned (Miyoshi et al. 2012), and (d) the absence of high endothelial venules (HEV) and FDC hyperplasia. In the meanwhile, the usage of markers raised against FHT-related antigens has shown that, besides the follicular variant, other PTCLs, NOS growing diffusely and consisting of clear cells but lacking the hallmark of AITL (i.e., HEV and FDC hyperplasia) are also related to FHT (Agostinelli et al. 2011). This prompts the question whether or not a new category of T-cell tumors should be envisaged ranging from AITL to cases belonging to the PTCL, NOS morphologic spectrum, all provided with the same derivation. Prospective clinical-pathological studies are warranted to definitely assess this point.

ALCLs, both ALK+ and ALK-, stem from activated cytotoxic T lymphocytes that have partially lost the expression of T-cell-associated antigens (Piccaluga et al. 2007a; Piva et al. 2010). They are morphologically and phenotypically undistinguishable with only one exception the lack of the small-cell variant in the setting of ALK- ALCL. Clinically, ALK- ALCL runs a more aggressive clinical course than the ALK+ one (Delsol et al. 2008; Mason et al. 2008). Retrospective studies have suggested that ALK- ALCL has anyhow a better prognosis than PTCL, NOS with CD30 expression (Savage et al. 2008), thus supporting the decision taken at the time of the WHO Classification writing to maintain it as a provisional entity. The assignment of a given tumor with the typical morpho-phenotypic features of ALCL (i.e., presence of hallmark cells, intrasinusoidal diffusion, cohesive growth pattern, strong CD30 expression, EMA positivity, and activated cytotoxic profile) to one or the other of the two categories is based on the occurrence of a translocation [more often t(2;5)(p23;q35)] leading part of the transcriptional domain of *ALK* under the control of a partner gene (usually *NPM*) (Delsol et al. 2008; Barreca et al. 2011; Inghirami and Pileri 2011). The newly formed hybrid gene encodes for a chimeric protein that can be easily detected with antibodies raised against the ALK protein. GEP studies have largely contributed to clarify the relationships between ALK+ and ALK- ALCLs and the borders between ALK- ALCL and PTCL, NOS, more than to assess their histogenesis. Thompson et al. (2005) were the first to apply this approach demonstrating the feasibility of this analysis in distinguishing ALK+ from ALK- ALCL, although both entities were found to share a com-

mon profile, suggesting putative common pathogenetic lesions. These findings were subsequently confirmed by Lamant et al. (2007) who demonstrated that a limited set of genes, including *BCL6*, *C/EBPb*, *SERPINA1*, and *PTPN12*, were preferentially expressed by ALK+ ALCL compared with ALK- ALCL. Moreover, common type ALCL and morphologic variants (small-cell, lympho-histiocytic and "mixed" variants) could also be distinguished (Lamant et al. 2007). Later on, Piva et al. (2010) demonstrated that ALCL could be differentiated from PTCL, NOS by a relatively small classifier and that ALK+ and ALK- cases could be stratified by the expression of selected genes. These included *perforin*, *IL2RA*, and *GAS1*. More recently, Agnelli et al. (2012) have undertaken a transcriptional profiling meta-analysis of 309 cases, including ALCL and other primary T-NHL samples. Pathway discovery and prediction analyses defined a minimum set of genes capable to recognize ALK- ALCL. Application of RT-qPCR in independent data sets from cryopreserved and formalin-fixed paraffin-embedded (FFPE) samples validated a three-gene model (*TNFRSF8*, *BATF3*, *TMOD1*) able to successfully separate ALK- ALCL from PTCL-NOS, with overall accuracy near 97 %.

One of the major limitations of the above-mentioned studies is the limited number of cases analyzed in each cohort, with the exception of the one of Agnelli et al. (2012) that however represented a meta-analysis based on in silico data. This is largely due to the need for frozen or fresh material when the Affymetrix technology is applied. Recently, new tools have been developed allowing GEP from FFPE samples (e.g., the Whole-Genome DASL Assay from Illumina). Such studies, which are ongoing at present, will further expand our histogenetic knowledge of PTCLs. For instance, preliminary data in this setting provide objective confirmation of the extreme heterogeneity of the PTCL, NOS category (Piccaluga et al. 2013). In fact, besides the expected distinctions into helper and cytotoxic as well as into central and effector memory subsets, the helper branch contains neoplasms related to Th1, Th2, THF, and Treg lymphocytes.

4.3.2 Pathogenetic Mechanisms

In their comprehensive GEP analysis, De Leval et al. (2007) found that by comparison with normal T-lymphocytes, AITL is characterized by the systematic overexpression of *VEGF* among others. This finding was originally thought to be related to the hyperplasia of HEV that – as mentioned above – is one of the morphologic hallmarks of the tumor. Almost at the same time, however, Piccaluga et al. (2007b) showed by immunohistochemistry on tissue microarrays (TMA) that neoplastic cells strongly express both VEGF and one of its receptors (KDR). Accordingly, as reported in the setting of some nonlymphoid tumors, *VEGF* overexpression (1) can actually correspond to an intrinsic attribute of lymphomatous elements more than of the microenvironment, (2) suggests an interaction between the two components, and (3) may be sustained by an autocrine phenomenon. The latter might represent a common trait among T-cell lymphomas affecting different pathways and different histotypes (see below). On this respect, similar features have been observed in biopsies from patients with mycosis fungoides (Pileri et al. 2012). Importantly, *VEGF* deregulation hints at possible sensitivity of the tumor to anti-angiogenetic drugs. This assumption has found some support in a series of reports in the literature showing that AITL patients refractory to several lines of conventional therapy had a quite favorable response to thalidomide or bevacizumab (Aguiar 2008; Bruns et al. 2005; Dogan et al. 2005; Gottardi et al. 2008; Strupp et al. 2002). More recently, Cairns et al. (2012) have reported that in a large set of PTCLs, *IDH2* mutations were identified in approximately 20 % of AITLs, but not in other tumors. These results were confirmed in an independent set of AITL patients, where the *IDH2* mutation rate was approximately 45 %. Interestingly, this is the second common genetic lesion identified in AITL after mutations of *TET2* coding sequence that, however, are not exclusive of the disease, occurring also in 38 % of PTCLs of the NOS type (see below) (Lemonnier et al. 2012).

Although several GEP studies have focused on PTCL, NOS, only those of Piccaluga et al. (2007a) and Iqbal et al. (2010) actually provided relevant contributions being based on the comparison between the main normal T-cell subsets and a sufficiently large number of well-characterized tumors. Such studies revealed the extensive deregulation of genes controlling functions typically damaged in malignant cells (e.g., matrix remodelling, cell adhesion, cytoskeleton organization, transcription regulation, translation, and cell kinetics). These observations might explain the dissemination pattern of PTCL/NOS, with frequent extranodal and bone marrow involvement and spread to peripheral blood, as well as their poor response to conventional chemotherapies. In line with this, there is the deregulation of (a) *FN1*, *LAMB1*, *COL1A2*, *COL3A1*, *COL4A1*, *COL4A2*, and *COL12A1*, genes promoting local invasion and metastasis in different types of human cancers; (b) *MOAP1*, *ING3*, *GADD45A*, and *GADD45B*, all involved in apoptosis; and (c) *CYR61* and *NNMT*, which may be responsible for chemoresistance. On the same line, Cuadros et al. (2007) observed that a gene signature related to proliferation has a negative impact on the prognosis of these neoplasms. Notably, Piccaluga et al. (2005, 2007a) first described the regular overexpression of *PDGFRA* in PTCLs of the NOS type. The relevance of this finding was supported by immunohistochemistry on TMA from about 200 neoplasms that showed consistent strong positivity for the protein, which in turn occurred in its activated (i.e., phosphorylated) form. The same approach displayed PDGFRA negativity of normal T lymphocytes with the exception of a few activated elements. Subsequent studies revealed that activation of the PDGFR cascade does occur also in other T-cell tumors, including T-prolymphocytic leukemia, extranodal, NK-/T-cell lymphoma of the nasal type, and ALK+ and ALK- ALCLs. The mechanism sustaining this phenomenon is still matter of investigation: preliminary data suggest an autocrine stimulation as previously reported in the setting of breast cancer. Importantly, *PDFGRA* or *B* overexpression might play a major biopathologic role by promoting both proliferation and rescue from apoptosis. In particular, to this hand it might vicariate the NF-kB pathway. The status of the latter has been the object of controversies in the literature. Martinez-Delgado et al. (2004, 2005) reported NF-kB activation in a proportion of PTCLs and correlated this with a more favorable clinical course. However, it was unclear whether or not this finding represented an intrinsic property of neoplastic cells or was influenced by the microenvironment. In fact, in the original report of Martinez-Delgado et al. (2004, 2005), the activation of the NF-kB pathway was more often detected in cases with a high amount of reactive T lymphocytes. In contrast to Martinez-Delgado et al., in tumors mostly consisting of neoplastic cells, Piccaluga et al. (2007a) found downregulation or malfunctioning of the NF-kB-related genes in 70–80 % of instances. Such observation was supported by the nuclear location (i.e., activation) of the components of both the canonical and alternative NF-kB pathways in only 20 % of PTCLs, NOS as shown by immunohistochemistry on TMA. Similar results were obtained by Hartmann et al. (2010) in a SNP array-based study carried out in an independent series of PTCLs of the NOS type. Last but not least, a recent GEP analysis performed by Rossi et al. (2012) in 40 PTCLs (28 NOS, 6 ALCLs, and 6 AITLs), 4 reactive lymph nodes, and 20 samples of normal T lymphocytes showed significantly lower *BCL10* expression in all tumors in comparison to normal samples, the lowest values being detected in ALCL. Immunohistochemistry on TMA revealed BCL10 positivity in only 10/52 PTCLs, NOS (19 %) with no significant correlation with either expression of Ki-67 and the T-cell markers or NF-κB activation, as well as with progression free survival and overall survival, although a favorable trend was recorded in BCL10+ cases. Besides the pathogenetic relevance, *PFGDFRA* overexpression may have therapeutic implications, suggesting possible sensitivity of neoplastic cells to tyrosine-kinase inhibitors. This is supported by the results obtained by Piccaluga et al. (2007a) and Huang et al. (2009) in primary cell cultures from PTCL, NOS and the MEC04 NKTCL-cell line, respectively. In both instances, in fact, the

usage of imatinib produced the dramatic deletion of neoplastic cells by blockage of proliferation and induction of apoptosis. Similar features were recorded by Laimer et al. (2012) in the setting of ALK+ ALCL by focusing on the PDGFRB pathway. Notably, the treatment of PTCL, NOS or ALK+ ALCL by imatinib or dasatinib in phase I clinical studies or mouse models produced the partial or complete regression of neoplastic masses. *PRDGFRA* does not represent the only deregulated gene which might be of potential therapeutic interest in PTCL, NOS. For instance, the downregulation of *GADD45 A* and *B* suggests possible sensitivity to histone deacetylase inhibitors (HDACi) (3), which has in fact been recorded in clinical trials (Foss et al. 2011). On the same line, Streubel et al. (2006) detected in a subset of PTCLs/NOS (see above) the t(5;9)(q33; q22) translocation leading to the fusion transcript *ITK-SYK* with constitutive kinase activity and possible pathogenetic significance. In this regard, overexpression and activation of SYK, a downstream molecule of T-cell receptor signaling, was recorded in PTCL/NOS, its inhibition being effective ex vivo. Finally, the above-mentioned *TET2* mutations have been detected in 38 % of PTCLs, NOS (Lemonnier et al. 2012). Interestingly, these cases more often express THF phenotype or have morphologic features reminiscent of AILD and are associated with advanced stage, thrombocytopenia, high IPI and shorter progression-free survival.

As mentioned above, in ALK+ ALCL the *ALK* gene located on chromosome 2 and coding for a tyrosine kinase undergoes a balance translocation in which the intracytoplasmic region of the gene is fused with different partners. The most common of these translocations is the t(2;5)(p23;q35) generating the NPM-ALK fusion protein with transforming properties. In about 20 % of cases, alternative translocations have been discovered involving various partners, such as *TPM3*, *TFG*, *ATIC*, *TSPYL2*, *MSN*, *KIAA1618*, and *MYH9* (Delsol et al. 2008; Barreca et al. 2011; Inghirami and Pileri 2011). Despite the well-defined primary *ALK* chromosomal translocations, the profile of secondary chromosomal aberrations in ALCL, ALK+, is not well known. The oncogenic potential of the ALK chimera has repeatedly been demonstrated using genetic, proteomic, and pharmacologic modalities. The deregulated expression of ALK in lymphoid cells leads to concomitant activation of multiple signaling pathways, which contribute synergistically to transformation, and they are required to control cell kinetics and survival, as well as to maintain the neoplastic phenotype and expression of key molecules, like CD30. These include phospholipase-Cγ, RAS/MEK/extracellular signal-related kinase (ERK), phosphatidylinositol 3 kinase (PI3K)/AKT, c-Src, and Jak/signal transducers and activators of transcription (STAT). Due to their pathogenetic role, ALK fusion proteins have become an ideal molecular target for small inhibitor molecules (Barreca et al. 2011; Inghirami and Pileri 2011). Moreover, ALK fusion proteins are antigenic and capable of eliciting relevant ALK-specific B- and T-cell responses in lymphoma patients. Thus, ALK proteins fulfil the major requirements for an ideal oncoantigen. Because the expression of ALK native protein in adult tissues is largely restricted, it is therefore anticipated that the use of ALK inhibitors or, alternatively, ALK-based vaccination protocols should not be linked to major toxic effects (Barreca et al. 2011; Inghirami and Pileri 2011). This hypothesis is supported by in vivo models taking advantage of novel anti-ALK small molecules and ALK-based vaccination protocols. As described above, ALK oncogenic properties rely on the constitutive activation of multiple signaling pathways; thus, it is reasonable that the use of small molecules targeting key effectors within these pathways might be applied in therapeutic modalities for ALK+ ALCL. Toward this end, because ALK+ ALCL is exclusively dependent on STAT3, the inhibition of this transcription factor could provide a novel therapeutic avenue.

Similar to ALK+ ALCL, the profile of genetic alterations of ALK- ALCL is still largely unclear. Feldman et al. (2011) have described a recurrent translocation in a subset of ALK- ALCLs involving the IRF4 gene. More recently, the same group has discovered, using a bioinformatic algorithm for translocation discovery, a novel balance t(6;7)

(p25.3;q32.3) translocation, disrupting the *DUSP22* phosphatase gene on 6p25.3 and adjoining the *FRA7H* fragile site on 7q32.3 in systemic ALK- ALCL. Some studies are ongoing at present that might lead to the discovery of novel genetic aberrations in the setting of ALK- ALCL, which might explain the overlap of morphologic and phenotypic features with ALK+ ALCL.

Recently, microRNAs (miRNAs) deregulated expression has been referred to PTCL pathogenesis as well. In particular, Merkel et al. (2010) identified a distinct profile of miRNA characteristic of ALCLs; moreover, based on miRNA profiles, they could distinguish ALK+ from ALK- forms, pointing toward novel potential mechanisms of tumorigenesis induced by aberrant ALK function. Interestingly, using both a transgenic mouse model and human ALCL cells (including primary ALCL tumor tissues and human ALCL-derived cell lines), a set of deregulated miRNAs was identified that might be implicated in the development and progression of ALCL. Importantly, ALK+ and ALK- ALCL could be distinguished by a distinct profile of "oncomirs." In particular, five members of the miR-17-92 cluster were expressed more highly in ALK+ ALCL, whereas miR-155 was expressed more than tenfold higher in ALK- ALCL. Furthermore, miR-101 was downregulated in all ALCL model systems, but its forced expression attenuated cell proliferation only in ALK+ and not in ALK- cell lines, perhaps suggesting different modes of ALK-dependent regulation of its target proteins. Noteworthy, inhibition of mTOR, which is targeted by miR-101, led to reduced tumor growth in engrafted ALCL mouse models (Merkel et al. 2010).

Finally, most recently, our group provided evidences that miRNA deregulation can contribute to PTCL/NOS pathogenesis (Piccaluga et al. 2011). In particular, a series of miRNA differentially expressed in tumoral and normal tissues was identified; noteworthy, target genes of such miRNAs were deregulated as well in PTCLs, indicating the potential tumorigenicity of these miRNAs in this setting. In addition, using a microarray-based technology in a training set of cases, we identified miRNA signatures able to discriminate the commonest nodal PTCL subtypes, including PTCL/NOS, AITL, and ALCLs. Noteworthy, we could validate these findings by qRT-PCR in an independent test set of cases, the molecular classifier showing high sensitivity and specificity. Therefore, our data supported the use of miRNA profiling in the diagnostic of nodal PTCLs, as also proposed for CTCLs (Piccaluga et al. 2011).

4.3.3 Adult T-Cell Leukemia/Lymphoma (ATLL)

Adult T-cell leukemia/lymphoma (ATLL) is an uncommon mature peripheral T-cell neoplasm of post-thymic pleomorphic lymphocytes associated with infection by the retrovirus human T-cell lymphotropic virus type 1 (HTLV-1). First recognized as a distinct clinical entity by Takatsuki in 1977, ATLL manifests with characteristic physical findings including skin lesions, lymphadenopathy, hepatosplenomegaly, and hypercalcemia and presents in a leukemic form in two thirds of patients (with a median age in the mid-1960s) and a lymphomatous form in the remaining third (Matutes 2007; Takatsuki 2005). The causative etiological agent, the deltaretrovirus HTLV-1, was originally identified after it was isolated from cells derived from patients with cutaneous T-cell lymphoma (Poiesz et al. 1980). HTLV-1 exhibits a distinct geographical distribution and is endemic to southwestern Japan, Africa, the Caribbean basin, and South America (Proietti et al. 2005). The prevalence of HTLV-1 infection in blood donors varies greatly, from up to 0.37 % in some areas of Japan to less than .0039 % in France. However, only 2–5 % of infected individuals will progress to ATLL, often 20–40 years after infection implying that viral infection cooperates with cellular genetic changes that accumulate over time.

Clinically, the disease may present as any of several variants; aggressive acute and lymphomatous forms and more indolent chronic and smoldering forms have been described. Each variant is thought to be associated with specific genomic alterations (Oshiro et al. 2006). Histologically, the mature helper T cells display highly indented or lobulated flowerlike nuclei and exhibit a CD2+ CD4+ CD25+ phenotype with approximately

58–68 % of cells expressing the Foxp3 protein, a marker of regulatory T cells. The clinical course is typically highly aggressive and generally fatal. While newer therapies can lead to remissions, these are generally transient, and only very few patients achieve long complete remissions with bone marrow transplantation.

The initiation of malignant transformation of ATLL is considered to be dependent upon TAX, an oncogenic viral protein which functions as a transactivator of HTLV-1 gene expression and is itself capable of immortalizing human primary T cells and inducing leukemia in transgenic mice (Hasegawa et al. 2006; Yasunaga and Matsuoka 2011). TAX is known to have several functions in addition to activating the viral genome. Through a wide range of molecular interactions within the host cell, TAX produces chromosomal instability, amplifies centrosomes, abrogates DNA repair, mediates cell growth via activation of cyclin-dependent kinases, and silences both tumor suppressor proteins (e.g., p53) and spindle assembly checkpoints (Matsuoka and Jeang 2007). TAX also induces several important cellular signal transduction pathways via its effect on the transcription factors NF-κB, CREB, SRF, and AP-1 (Azran et al. 2004; Grassmann et al. 2005). Interestingly, immune stimulation of HTLV-1-infected cells may play an important role in enhancing TAX expression (Swaims et al. 2010). TAX changes the expression of hundreds of cellular genes, playing a role as both activator and repressor of transcription (Ng et al. 2001).

As previously discussed, the NF-κB family of transcription factors is known to regulate a wide range of cellular processes necessary for proliferation and survival. NF-κB has been shown to undergo constitutive activation in HTLV-1-infected cells, regardless of whether transformation has occurred (Swaims et al. 2010; Watanabe et al. 2005). TAX acts via interactions in both the cytoplasm and the nucleus to produce canonical NF-κB activation, which appears to be required for CD4+ cell transformation (Qu and Xiao 2011). Specifically, TAX binds NEMO via two homologous leucine zipper domains, facilitating the docking of TAX to the catalytic subunits IKKα and IKKβ and resulting in IKK complex activation. The complex also induces (via IKK1) the phosphorylation of the C terminus of RelA/p65, an event which is required for full NF-κB activity. Additionally, TAX co-localizes to the nucleus, accompanied by transcription and splicing complexes as well as the NFκB subunits p50 and RelA, forming distinct subnuclear foci termed "TAX nuclear bodies," interchromatin granules that form functional transcriptional hot spots (Qu and Xiao 2011).

In noncanonical NF-κB activation, precursor proteins p100 and p105 function as IκB-like inhibitors of NFκB activation; their proteolytic processing, forming the products p50 and p52, results in NFκB activation via both the liberation and generation of specific NFκB complexes (Xiao et al. 2006). Under physiologic conditions, the processing of p100 is dependent on the NF-κB-inducing kinase (NIK), and not on IKKβ or IKKγ (Senftleben et al. 2001). Conversely, TAX has been shown to activate the noncanonical NFκB pathway in a manner that is dependent on both IKKα and IKKγ but not on NIK (Harhaj et al. 2000). In such noncanonical activation, TAX acts to physically recruit IKKα to p100 by directly binding the two short helices of the precursor protein, triggering its phosphorylation-dependent ubiquitylation and processing. The exact relationship between the canonical and noncanonical pathways in ATL tumorigenesis remains unknown; however, recent work has demonstrated that the tumor suppressor gene WWOX may form a link between the two pathways, acting as a negative target of the noncanonical pathway and a potent suppressor of the canonical pathway (Fu et al. 2011).

The cyclic AMP response element-binding protein (CREB) is a transcription factor that plays an important role both in regulating a significant percentage of human genes and in HTLV-1 viral transcription (Geiger et al. 2008). TAX's viral promoter contains three conserved CREs which are recognized by CREB (Kim et al. 2007). Furthermore, TAX is able to bind to the transcriptional activator CBP, forming a bridge between CREB and CBP, bypassing the requirement for direct binding between the two coactivators which is normally a necessary antecedent for CREB phosphorylation (Taylor 2007). There exists some controversy regarding the exact role of CREB phosphorylation in TAX transactivation,

and it should be noted that the role of CREB in HTLV-1 infected cells has only been well studied at the level of the HTLV-1 promoter (Kim et al. 2010). Notably, recent observations suggest that the cyclin D1 gene may be upregulated by TAX through both the NF-κB and CREB pathways, the latter process involving cooperation between pCREB TORC2 and p300. Furthermore, TAX is able to transactivate the TAL1 gene promoter 1b through both NF-κB and CREB-binding sites, resulting in the upregulation of the HTLV-1 promoter by removing the negative effect of the E protein bHLH transcription factor E47 (Terme et al. 2008). Interestingly, although most of the existing data stresses the role of NF-κB signaling, PI3K/Akt activation, observed in HTLV-1-transformed cells, may play a role in apoptotic resistance. Recent work has demonstrated that in the absence of NF-κB signaling, TAX may activate activator protein-1 through the aforementioned PI3k/mTor pathway, producing growth independently of IL-2 (Grassmann et al. 2005; Ikezoe et al. 2007; Yoshita et al. 2012). Investigation is continuing into TAX's anti-apoptotic abilities through its affects on CREB phosphorylation and interaction with the PI3K/AKT and possibly Raf/MEK/ERK pathways (Saggioro 2011). TAX has also been shown to mediate activation of serum response factor (SRF)-dependent gene expression, leading to formation of dimeric transcription factors such as AP1, Erg-1, and Erg-2. This is accomplished through a mechanism similar to that of CREB activation via the interaction with various coactivators including ternary complex factors, CPB/p300 and P/CAF (Boxus et al. 2008).

Although a great deal of work has focused on the role of TAX in ATLL pathogenesis, its transcripts cannot be detected in about 60 % of ATL samples (Matsuoka and Jeang 2007). Observations have suggested that TAX may not always be necessary for leukemogenesis and that other factors may be involved. For example, the HTLV-1 basic leucine zipper factor (HBZ), a novel antisense protein and RNA, has recently been shown to be oncogenic in vivo (Satou et al. 2011). HBZ, which is ubiquitously expressed in all ATL cells, has also been demonstrated to promote T-lymphocyte proliferation and is the only HTLV-1 gene that is able to evade the generation of nonsense mutations by APOBEC3G, a human cytidine deaminase that acts as a defense against retroviruses (Arnold et al. 2008; Fan et al. 2010). For these and other reasons, HBZ is thought to be essential for the maintenance of ATL transformation (Matsuoka and Jeang 2007).

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Part II

Disease-Specific: T-NHL

Adult T-cell Leukemia-Lymphoma

5

Kunihiro Tsukasaki and Kensei Tobinai

Contents

5.1 Introduction

Adult T-cell leukemia-lymphoma (ATL) is a rare T-cell malignancy associated with human T-lymphotropic virus type 1 (HTLV-1). ATL was first described in 1977 by Uchiyama and Takatsuki as a distinct progressive T-cell leukemia of peculiar morphology, so-called flower cells, with a suspected viral etiology because of the clustering of the disease in the southwest region of Japan (Uchiyama et al. 1977). Subsequently, a novel RNA retrovirus, human T-cell leukemia/lymphotropic virus type I (HTLV-1), was first isolated by Poiesz and associates (1980) in the United States from cultured cells from one patient with an aggressive variant of mycosis fungoides and from one with Sézary syndrome. Although both patients, who were African Americans, were diagnosed clinically as having cutaneous T-cell lymphoma (CTCL) at the time of reporting, their clinical features were later found to closely resemble those of Japanese patients with ATL (Poiesz et al. 1980; Hinuma et al. 1981; Miyoshi et al. 1981; Yoshida et al. 1982). In the mid-1980s and 1990s, several inflammatory diseases were reported to be associated with HTLV-1 including tropical spastic paraparesis (TSP)/HTLV-1-associated myelopathy (HAM), HTLV-associated uveitis, and infective dermatitis (Gessain et al. 1985; Osame et al. 1986; LaGrenade et al. 1990; Mochizuki et al. 1992). At the same time, endemic areas for the virus and diseases have been found such as the Caribbean islands, tropical Africa, South

K. Tsukasaki, MD, PhD (✉) • K. Tobinai, MD, PhD
Department of Hematology, National Cancer Center Hospital East, Kashiwa City, Japan
e-mail: tsukasak@net.nagasaki-u.ac.jp; ktobinai@ncc.go.jp

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America, Middle East, and northern Oceania. Only a few percent of HTLV-1 carriers, who were transmitted through breast-feeding, develop the disease suggesting multistep carcinogenesis (Takatsuki 1994; IARC 1996). The diversity in clinical features and prognosis of patients with this disease has led to its subtype classification into four categories, acute, lymphoma, chronic, and smoldering types, defined by organ involvement and LDH and calcium values (Shimoyama 1991; Ohshima et al. 2008). ATL is very refractory to chemotherapy but sensitive to allogeneic hematopoietic stem cell transplantation (allo-HSCT) and possibly to interferon (IFN)/zidovudine (AZT) therapy. Recent phase 2 trial revealed that anti-CC chemokine receptor (CCR4) antibody was effective for relapsed ATL. Furthermore, other promising new agents for T cell including ATL are under development. This chapter will review this rare disease focusing on molecular epidemiology and biology and treatment of the disease.

5.2 Epidemiology and Biology of HTLV-1-Associated ATL

5.2.1 Molecular Epidemiology of HTLV-1 and ATL

HTLV-1 is spread throughout the world with small clusters of hyperendemicity located within endemic areas (Takatsuki 1994; IARC 1996). Population-based study in Japan revealed that the HTLV-1 antibody prevalence in the adult population varies from 0.2 to 15 %. Endemic areas (>2 % prevalence) in the world include southwestern coast of Japan, Caribbean islands (African), tropical Africa (African), South America (Mongoloid), and northern Oceania (Melanesian). Many patients who have been diagnosed with ATL in Western countries are immigrants from the West Indies and tropical Africa. It has been estimated that worldwide between 15 and 20 million individuals are infected with HTLV-1 (Takatsuki 1994; IARC 1996). The annual rate of ATL development among HTLV-1 carriers older than 40 years is estimated at 1.5 per 1,000 in males and 0.5 per 1,000 in females, and the cumulative risk of ATL development among the HTLV-1 carriers is estimated to be 2.5 to 5 % over the course of a 70-year life span (Kondo et al. 1989).

The root of global-wide spread of HTLV-1 remains undetermined but an African origin has been suggested by the occurrence of HTLV-1 clusters in Africa, the coincidence of a highly related simian T-cell lymphotropic virus (STLV)-1 in African primates and some findings in the phylogenetic analysis of HTLV-1/STLV-1 and HTLV-2/STLV-2 (Takatsuki 1994; IARC 1996). However, the clusters of HTLV-1 in southwestern Japan, aboriginal peoples of Papua New Guinea, Australia and the Solomon Island, and northern Iran could not be easily explained by such an African origin. Similarly, the origin of HTLV-2, which is endemic in aboriginals in southern America and drug abusers in developed countries in northern America and Europe, remains determined (Takatsuki 1994; IARC 1996). Based on sequence and/or RFLP analysis of HTLV-1 from distinct epidemiologic locales, three major clades have emerged. Furthermore, the results revealed that there is remarkably little difference in viral sequences in isolates from many locales, suggesting little evolutionary pressure to change in contrast to the lente-retroviruses such as HIV-1. In contrast to HIV-1 which mutates rapidly to escape from immune surveillance during viral expansion with its expression, HTLV-1 appears to be a very ancient virus in man with less viral expression and clonal expansion of infected T cells (IARC 1996).

Three modes of HTLV-1 transmission have been demonstrated for HTLV-1 consisting of mother-to-child, sexual, and blood transmissions (Takatsuki 1994; IARC 1996). Mother-to-child transmission represents a major mode of transmission of HTLV-1 in endemic areas, mainly due to breast-feeding beyond 6 months (Hino et al. 1985). Maternal factors associated with transmission include high HTLV-1 antibody titers, presence of anti-Tax antibodies, and in vitro maternal HTLV-1 antigen expression in short-term culture. The overall infection rate for HTLV-1 in children with seropositive mothers has been estimated to be 10 to

30 % (IARC 1996). HTLV-1-infection has also been reported in about 3 % of children not breast-fed which suggests the possibility of an intrauterine or transvaginal infection. However, the intrauterine route was unlikely considering the discordance of HTLV-1 DNA in cord blood and the subsequent seroconversion of the babies (Katamine et al. 1994). Several types of intervention have been conducted in HTLV-1-endemic areas in Japan, where seropositive pregnant women are advised not to breast-feed. Recently, a nationwide intervention has been initiated in Japan.

HTLV-1 is sexually transmissible and this is more effective from men to women than the reverse. The risk for transmission, over 10 years, from seropositive husbands to wives has been calculated at 60 %, whereas that for transmission from wives to husbands was only 0.4 % (IARC 1996).

Infection by blood transfusion appears to be the most efficient mode of HTLV-1 transmission, with a 15–60 % risk of infection among recipients of a contaminated cellular blood product. Also, sharing of needles by intravenous drug abusers is infectious. Fresh frozen plasma, which is acellular, is not infectious (IARC 1996; Okochi et al. 1984; Brown et al. 1991).

Most of that of ATL is after mother-to-child infection but rarely the other two. In contrast, HAM/TSP developing after blood transfusion is not rare; incubation time is frequently only several months and rapidly progressive. Rare cases of definite ATL development after blood transfusion and probable HAM/TSP development after sexual transmission have been recognized (IARC 1996).

Recently, the prevalence of HTLV-1 in Japan as determined by screening of blood donors was surveyed (Satake et al. 2012). The seroprevalence of HTLV-1 among 1,196,321 Japanese first-time blood donors from 2006 to 2007 was investigated. A total of 3,787 such donors were confirmed to be positive for the anti-HTLV-1 antibody. By applying a fitness curve to the age ranges outside the blood donor age range, the present number of HTLV-1 carriers covering ages from 0 to 99 years was estimated to be at least 1.08 million in Japan; this value was 10 % lower than that reported in 1988 (Hashimoto et al. 1991). The adjusted overall prevalence rates were estimated to be 0.66 % and 1.02 % in men and women, respectively. The peak in carrier numbers was found among individuals in their 70s, which is a shift from the previous peak observed in the 1988 database among individuals in their 50s. As compared to the survey in the 1980s, carriers were distributed not only in the endemic southwestern region of Japan, but throughout the country, particularly in the greater Tokyo metropolitan area (Satake et al. 2012).

Factors reportedly associated with the development of ATL include HTLV-1 infection early in life, increase in age, male sex, family history of ATL, past history of infective dermatitis, smoking of tobacco, serum titers of antibody against HTLV-1, HTLV-1 proviral load, and several HLA subtypes (Kondo et al. 1989; Kawano et al. 1984 Jul 1; Tokudome et al. 1993; Tsukasaki et al. 1994; Hisada et al. 1998; Usuku et al. 1988). However, definitive risk factors for the development of ATL among asymptomatic HTLV-1 carriers have not been elucidated. Recently, Iwanaga and colleagues evaluated 1,218 asymptomatic HTLV-1 carriers (426 males and 792 females) who were enrolled during 2002–2008 for a prospective study on the development of ATL (Iwanaga et al. 2010) The proviral load at enrollment was significantly higher in males than females (median, 2.10 vs. 1.39 copies/100 peripheral blood mononuclear cells (PBMC)), in those aged 40 or more years, and in those with a family history of ATL. During the follow-up period, 14 participants developed acute ATL. Their baseline proviral loads were high (range, 4.17–28.58 copies/100 PBMC). Multivariate Cox regression analyses indicated that not only a higher proviral load but also advanced age, a family history of ATL, and the first opportunity for HTLV-1 testing during treatment for other diseases were independent risk factors for the progression of ATL from a carrier status.

5.2.2 Molecular Biology of HTLV-1-Associated ATL

ATL is a single disease entity etiologically associated with HTLV-1. However, only a few percent

of HTLV-1 carriers develop ATL after several decades, suggesting multistep leukemogenesis in contrast to retroviral carcinogenesis in which carcinoma development requires only several weeks in most of the infected animals (IARC 1996).

HTLV-1 does not carry viral oncogene, expression of the virus including Tax appears just after in vitro culture, integration sites of the provirus into host genome is random, and chromosomal/genetic abnormality is complex (Takatsuki 1994; IARC 1996). Therefore, ATL is a single HTLV-1 disease entity with diverse molecular features. Instability of HTLV-1 infected cells in HTLV-1 carriers and ATL patients consists in molecular, cytogenetic, and clonal levels resulting accumulation of genetic alterations and ATL development (Tsukasaki 2002).

The HTLV-I gene encodes three structural proteins, Gag, Pol and Env, and complex regulatory proteins such as Tax, which not only activate viral replication but also induces the expression of several cellular genes important in proliferation and anti-apoptosis of ATL cells including NF-kB (Takatsuki 1994; IARC 1996). The expression of these cellular proteins may enhance the multistep carcinogenesis of ATL. Recently, a new viral factor, HTLV-1 basic Zip factor (HBZ), encoded from the minus strand mRNA was discovered and is thought to be implicated in viral replication and T-cell proliferation (Satou et al. 2006). Several isoforms of HBZ transcripts were reported to be steadily expressed in HTLV1-infected cells and primary ATL cells in contrast to Tax. The functions of these transcripts and putative protein in the context of cellular transformation are now under investigation. More recently, polycomb-mediated epigenetic silencing of miR-31 is implicated in the aberrant and constitutive activation of NF-kB signaling in ATL cells (Yamagishi et al. 2012). HBZ and miR-31 are apparently good targets for the prevention as well as treatment of ATL.

Prototypical ATL cells have a mature helper T-cell phenotype (CD3+, CD4+, CD8-). Recent studies have suggested that the cells of some ATL may be the equivalent of Th2/Treg cells because of the high frequency of expression of CD25/CCR4 and about half of FoxP3 (Ishida et al. 2003; Kohno et al. 2005). By Southern blotting for both HTLV-1 integration and TCR rearrangement, about 10–20 % of ATL cases showed clonal change during the transformation from indolent to aggressive disease (Tsukasaki et al. 1997). Oligoclonal expansion of HTLV-1-infected premalignant cells was detected in asymptomatic HTLV-1 carriers by HTLV-1-integrated site-specific PCR (Wattel et al. 1995; Gillet et al. 2011). A high rate of chromosomal abnormalities has been detected in HTLV-1-infected T-cell clones derived from HTLV-1 carriers (Fujimoto et al. 1999). Abnormalities in tumor suppressor genes are frequent and rare in acute- and chronic-type ATL, respectively, and associated in poor prognosis in both (Tawara et al. 2006). Chromosomal abnormalities detected by cytogenetics or comparative genomic hybridization are often more frequent and more complex in acute ATL than in chronic ATL, with aneuploidy and several hot spots such as 14q and 3p (Tsukasaki 2002; Itoyama et al. 2001). DNA microarray analyses of the transcriptomes of ATL cells at the chronic and acute stages to elucidate the mechanism of stage progression in this disease revealed that several hundred genes were modulated in expression including those for MET, a receptor tyrosine kinase for hepatocyte growth factor and cell adhesion molecule, TSLC1 (Choi et al. 2007; Sasaki et al. 2005).

5.3 Clinical Features of ATL

Reflecting the molecular diversity of ATL cells, the clinical features and prognosis of patients with this disease is diverse. Subtype classification into four categories, acute, lymphoma, chronic, and smoldering types, defined by organ involvement and LDH and calcium values has been proposed in 1990s and has been applied for the decision of treatment strategy (Shimoyama 1991; Major prognostic factors of patients with adult T-cell leukemia- lymphoma: a cooperative study. Lymphoma Study Group 1984) (Table 5.1).

Organ involvements of ATL resemble the mixture of those of peripheral T-cell lymphoma (PTCL), cutaneous T-cell lymphoma (CTCL),

Table 5.1 Diagnostic criteria for clinical subtypes of adult T-cell leukemia-lymphoma

	Smoldering	Chronic	Lymphoma	Acute
Anti-HTLV-1 antibody	+	+	+	+
Lymphocyte ($\times 10^3/\mu L$)	<4	≥4	<4	[a]
Abnormal T lymphocytes	≥5 %[b]	+[c]	≤1 %	+[c]
Flower cells with T-cell marker	[d]	[d]	No	+
LDH	≤1.5 N	≤2 N	[a]	[a]
Corrected Ca^{2+} (mEq/L)	<5.5	<5.5	[a]	[a]
Histology-proven lymphadenopathy	No	[a]	+	[a]
Tumor lesion				
Skin and/or lung	[a]	[a]	[a]	[a]
Lymph node	No	[a]	Yes	[a]
Liver	No	[a]	[a]	[a]
Spleen	No	[a]	[a]	[a]
Central nervous system	No	[a]	[a]	[a]
Bone	No	No	[a]	[a]
Ascites	No	No	[a]	[a]
Pleural effusion	No	No	[a]	[a]
Gastrointestinal tract	No	No	[a]	[a]

From Shimoyama (1991)

HTLV-1 human T-lymphotropic virus type I, *LDH* lactate dehydrogenase, *N* normal upper limit

[a]No essential qualification except terms required for other subtype(s)

[b]Histologically proven skin and/or pulmonary lesion(s) is required if there are fewer than 5 % abnormal T lymphocytes in peripheral blood

[c]If the proportion of abnormal T lymphocytes is less than 5 % in peripheral blood, a histologically proven tumor lesion is required

[d]Typical "flower cells" may be seen occasionally

and chronic lymphocytic leukemia (CLL), showing nodal and extranodal involvement including cutaneous and leukemic manifestation (Shimoyama 1991; Ohshima et al. 2008). ATL patients show a variety of clinical manifestations because of various complications of organ involvement by ATL cells, opportunistic infections and/or hypercalcemia (Shimoyama 1991; Ohshima et al. 2008). These three often contribute to the extremely high mortality of the disease. Lymph node, liver, spleen, and skin lesions are frequently observed. Although less frequently, digestive tract, lungs, central nervous system, bone, and/or other organs may be involved (Fig. 5.1). Large nodules, plaques, ulcers, and erythroderma are common skin lesions (Shimoyama 1991; Ohshima et al. 2008; Bittencourt et al. 2007; Amano et al. 2008; Sawada et al. 2011). Immune suppression is common. Approximately 26 % of 854 patients with ATL had active infections at diagnosis in a prior nationwide study in Japan (Shimoyama 1991). The incidence was highest in the chronic and smoldering types (36 %) and lower in the acute (27 %) and lymphoma types (11 %). The infections were bacterial in 43 %, fungal in 31 %, protozoal in 18 %, and viral in 8 % of patients. The immunodeficiency at presentation in ATL patients can be exacerbated by cytotoxic chemotherapy. Individuals with indolent ATL might have no manifestation of the disease and are identified only by health check-ups and laboratory examinations as in case of CLL.

ATL cells are usually detected quite easily in the blood of affected individuals except for the smoldering type with mainly skin manifestations and lymphoma type (Shimoyama 1991). These so-called flower cells have highly indented or lobulated nuclei with condensed chromatin, small or absent nucleoli, and an agranular and basophilic cytoplasm (Bennett et al. 1989; Tsukasaki et al. 1999). The histological analysis of aberrant cutaneous lesions or lymph nodes is essential for the diagnosis of the smoldering type

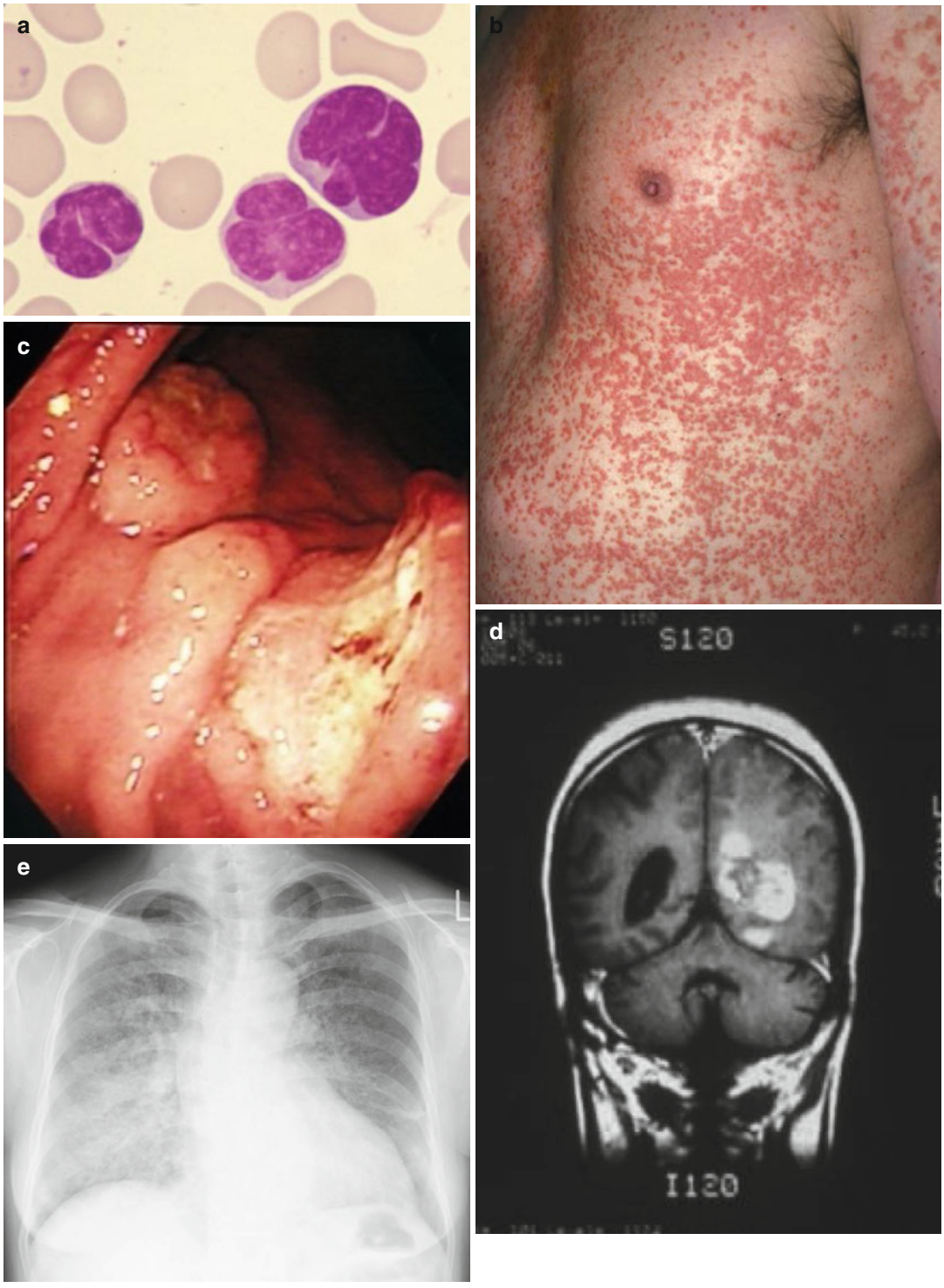

Fig. 5.1 (**a**) Leukemic cells (the so-called flower cells) showing characteristic polymorphic nuclei in a peripheral blood smear from a patient with acute-type ATL. (**b**) Skin involvement of ATL. (**c**) Gastric involvement of ATL. (**d**) Central nervous system involvement of ATL. (**e**) Pneumocystis jirovecii pneumonia in a patient with chronic ATL

with mainly skin manifestations and lymphoma type of ATL, respectively. Because ATL cells in the skin and lymph node can vary in size from small to large and in form from pleomorphic to anaplastic and Hodgkin-like cell with no specific histological pattern of involvement, differentiating between mycosis fungoides/Sezary syndrome, other PTCLs, and Hodgkin lymphoma versus ATL can at times be difficult without examinations for HTLV-1 serotype/genotype (Ohshima et al. 2008; Ohshima 2007).

Hypercalcemia is the most distinctive laboratory abnormality in ATL as compared to other lymphoid malignancies and is observed in 31 % of patients (50 % in acute type, 17 % in lymphoma type, and 0 % in the other two types by definition) at onset (Shimoyama 1991). Individuals with hypercalcemia do not usually have osteolytic bone lesions. Parathyroid hormone-related protein or receptor activator of nuclear factor kappa B ligand (RANKL) produced by ATL cells is considered the main factor causing hypercalcemia (Watanabe et al. 1990; Nosaka et al. 2002).

Similar to serum LDH, β2-microglobulin, and serum thymidine kinase levels reflecting disease bulk/activity, the level of the soluble form of interleukin (IL)-2 receptor alpha-chain is elevated in the order acute-/lymphoma-type ATL, smoldering-/chronic-type ATL, and HTLV-1 carriers as compared with normal individuals, perhaps with better accuracy than the other markers (Tsuda et al. 1992; Sadamori et al. 1991; Kamihira et al. 1994). These serum markers are useful for detecting the acute transformation of indolent ATL as well as the early relapse of ATL after achieving responses by therapy.

5.4 Diagnosis of ATL

The diagnosis of typical ATL is not difficult and is based on clinical features, ATL cell morphology, mature helper T-cell phenotype, and anti-HTLV-1 antibody in most cases (Shimoyama 1991). Those rare cases which might be difficult to diagnose can be shown to have the monoclonal integration of HTLV-1 proviral DNA in the malignant cells as determined by Southern blotting. However, the monoclonal integration of HTLV-1 is also detected in some HAM/TSP patients and HTLV-1 carriers in PBMNCs (Furukawa et al. 1992; Ikeda et al. 1993). After the diagnosis of ATL, subtype classification of the disease is necessary for the selection of appropriate treatment (Shimoyama 1991; Tsukasaki et al. 2009).

5.5 Treatment and Prevention of HTLV-1-Associated ATL

Treatment of ATL is based on subtype classification of ATL. Treatment strategy is diverse ranging from watchful waiting to intensive chemotherapy followed by allo-HSCT.

Major prognostic indicators for ATL, which have been elucidated in 854 patients with ATL in Japanese nationwide survey by multivariate analysis, were advanced performance status, high LDH level, age of 40 years or more, more than three involved lesions, and hypercalcemia (Major prognostic factors of patients with adult T-cell leukemia- lymphoma: a cooperative study. Lymphoma Study Group 1984). Additional factors reportedly associated with a poor prognosis include cutaneous involvement, thrombocytopenia, eosinophilia, bone marrow involvement, a high interleukin-5 serum-level, C-C chemokine receptor 4 (CCR4) expression, lung resistance-related protein (LRP), p53 mutation, and p16 deletion by multivariate analysis (Ishida et al. 2003; Tawara et al. 2006; Sawada et al. 2011; Yamada et al. 1997; Utsunomiya et al. 2007; Takasaki et al. 2007; Ohno et al. 2001; Inagaki et al. 2006). Specific for the chronic type of ATL, high LDH, high blood urea nitrogen (BUN), and low albumin levels were identified as factors for a poor prognosis by multivariate analysis (Takatsuki 1994). Primary cutaneous tumoral type although generally included among smoldering ATL had a poor prognosis in one univariate analysis [Bit].

Since 1978, chemotherapy trials have been consecutively conducted for patients newly diagnosed with aggressive non-Hodgkin lymphoma including ATL by JCOG's Lymphoma Study Group (LSG) and the following results were obtained for this disease (Shimoyama et al. 1988a, b; Tobinai

et al. 1992, 1994; Tsukasaki et al. 2003, 2007, 2012; Yamada et al. 2001):

1. As compared to other aggressive NHLs including DLBCLs and PTCLs other than ATL, response and survival rates were far worse in ATL in clinical trials evaluating 1st- and 2nd-generation chemotherapy regimens in 1980s (JCOG7801, 8101, 8701).
2. The disappointing results with conventional chemotherapies in the 1980s and the proposal for a subtype classification of ATL led to a search for standard combination chemotherapy focusing exclusively on aggressive ATL consecutively by JCOG-LSG since 1990s.
3. The first phase II study of combination chemotherapy with pentostatin (2′-deoxycoformycin, an inhibitor of adenosine deaminase) was conducted exclusively against aggressive ATL; however, the results were disappointing with a median survival time (MST) of 7 months similar to previous studies by JCOG-LSG (Tsukasaki et al. 2003).
4. The next phase II trial (JCOG9303) consisting of vincristine, cyclophosphamide, doxorubicin, and prednisone (VCAP); doxorubicin, ranimustine, and prednisone (AMP); and vindesine, etoposide, carboplatin, and prednisone (VECP) intensified with the prophylactic use of G-CSF revealed a promising response rate and MST superior to those obtained by our previous trials despite considerable hematological toxicity (Yamada et al. 2001).
5. Based on the promising results of JCOG9303, we conducted a phase III trial comparing modified (m)-LSG15 (VCAP-AMP-VECP) with CHOP-14 both supported with G-CSF and intrathecal prophylaxis. The longer survival at 3 years and higher %CR with VCAP-AMP-VECP compared with CHOP-14 in this first phase III trial against PTCL including aggressive ATL suggest that the former is a more effective regimen at the expense of greater toxicity, providing the basis for future investigations in the treatment of aggressive ATL (Tsukasaki et al. 2007). However, the MST of 13 months still compares unfavorably to other hematologic malignancies.

A treatment strategy based on the clinical subtypes, prognostic factors, and response to initial therapy is suggested in an international consensus report (Tsukasaki et al. 2009) (Table 5.2). Patients with acute, lymphoma, or

Table 5.2 Strategy for the treatment of adult T-cell leukemia-lymphoma

Smoldering- or favorable chronic-type ATL
Consider inclusion in prospective clinical trials
Symptomatic patients (skin lesions, opportunistic infections, etc): Consider AZT/IFN or Watch and Wait
Asymptomatic patients: Consider Watch and Wait
Unfavorable chronic- or acute-type ATL
If outside clinical trials, check prognostic factors (including clinical and molecular factors if possible):
Good prognostic factors: consider chemotherapy (VCAP-AMP-VECP evaluated by a phase III trial against biweekly-CHOP) or AZT/IFN (evaluated by a meta-analysis on retrospective studies)
Poor prognostic factors: consider chemotherapy followed by conventional or reduced intensity allo-HSCT (evaluated by retrospective and prospective Japanese analyses, respectively)
Poor response to initial therapy: Consider conventional or reduced intensity allo-HSCT
Lymphoma-type ATL
If outside clinical trials, consider chemotherapy (VCAP-AMP-VECP)
Check prognostic factors (including clinical and molecular factors if possible) and response to chemotherapy:
Good prognostic factors and good response to initial therapy: Consider chemotherapy followed by observation
Poor prognostic factors or poor response to initial therapy: Consider chemotherapy followed by conventional or reduced intensity allo-HSCT
Options for clinical trials (relapse or progressive disease)
Test the effect of promising targeted therapies such as arsenic trioxide and IFN-, bortezomib, a purine nucleotide phosphorylase inhibitor, histone deacetylase inhibitors, monoclonal antibodies, antiangiogenic therapy, and survivin, -catenin, syk, and lyn inhibitors, etc.
Consider conventional or reduced-intensity allogeneic HSCT when possible

unfavorable chronic subtypes (aggressive ATL) generally have a very poor prognosis due to multidrug resistance of ATL cells, a large tumor burden with multiorgan failure, hypercalcemia, and/or opportunistic infections. In case aggressive ATL, intensive chemotherapy such as VCAP-AMP-VECP (mLSG15) is usually recommended based on the results of a phase3 trial (JCOG 9801) (Tsukasaki et al. 2007). In case of favorable chronic or smoldering ATL (indolent ATL), watchful waiting until disease progression has been recommended although the long-term prognosis was inferior to those of, for instance, chronic lymphoid leukemia (Takasaki et al. 2010). Retrospective analysis suggested that the combination of interferon alpha and zidovudine (IFN/AZT) was apparently promising for the treatment of ATL, especially for types with leukemic manifestation (Gill et al. 1995; Hermine et al. 1995; Bazarbachi et al. 2010). Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is very promising for the treatment of aggressive ATL possibly reflecting graft vs. ATL effect (Hishizawa et al. 2010; Kanda et al. 2012; Ishida et al. 2012; Okamura et al. 2005; Tanosaki et al. 2008).

Recently, defucosylated humanized anti-CCR4 monoclonal antibody (mogamulizumab) was approved for the treatment of relapsed/refractory ATL based on the results of phase1 and phase2 studies in Japan (Yamamoto et al. 2010; Ishida et al. 2010). Subsequent randomized phase2 study of full dose of mLSG15 +/− mogamulizumab for untreated patients with aggressive ATL is ongoing. This is anticipated because the former was more effective for ATL cells in lymph node than those in peripheral blood, and the latter was vice versa. Other new agent trials for ATL ongoing or in preparation in Japan include IL2 fused with diphtheria toxin targeting CD25; histone deacetylase inhibitors; a novel purine nucleoside phosphorylase inhibitor, which induces apoptosis mainly in T cells; an NF-kB targeting proteasome inhibitor; and an immunomodulatory agent, lenalidomide.

ATL still has a worse prognosis than the other T-cell malignancies in general. There is no plateau with an initial steep slope and subsequent gentle slope in the survival curve for aggressive and indolent ATL treated with chemotherapy and watchful waiting, respectively, although the prognosis is much better in the latter, suggesting the need of refinement of subtype classification. Recently, a retrospective review of 807 patients in Japan made a prognostic index for acute- and lymphoma-type ATL based on the 5 prognostic factors, stage, PS, age, serum albumin, and sIL2R (Katsuya et al. 2012). In the validation sample, the index was reproducible with MSTs of 3.6, 7.3, and 16.2 months for patients at high, intermediate, and low risk, respectively. Japan Clinical Oncology Group (JCOG)-Lymphoma Study Group (LSG) conducted a meta-analysis of three consecutive trials exclusively for aggressive ATL (see below) (Fukushima et al. 2011). OS analysis of a total 276 patients with acute, lymphoma, or unfavorable chronic ATL enrolled identified two significant prognostic factors, PS and hypercalcemia. In the validation sample, a proposed prognostic index using the two factors into two strata revealed MSTs of 6.3, and 17.8 months for patients at high and low risk, respectively. In both studies, however, the 5-year OS rate was less than 15 % even in the low-risk group, which could not identify the subgroup of patients with sufficient prognosis. ATL with abnormalities in tumor suppressor gene such as p53 was reportedly resistant to IFN/AZT therapy as well as chemotherapy. Allo-HSCT might overcome the resistance.

Two steps should be considered for the prevention of HTLV-1-associated ATL. The first is the prevention of HTLV-1 infections. This has been achieved in some endemic areas by screening for HTLV-1 among blood donors and asking mothers who are carriers to refrain from breast-feeding. The second step is the prevention of ATL among HTLV-1 carriers. This has not been achieved partly because only about 5 % of HTLV-1 carriers develop the disease in their lifetime although several risk factors have been identified. Also, no agent has been found to be effective in preventing the development of ATL among HTLV-1 carriers. Further investigation on the pathogenesis of ATL is crucial for the development of prevention and treatment of this refractory leukemia-lymphoma.

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Anaplastic Large Cell Lymphoma

6

Anas Younes, Pier Luigi Zinzani, Scott Rodig, and Jan Delabie

Contents

Pathology: Scott Rodig and Jan Delabie

A. Younes (✉)
MD Anderson Cancer Center,
The University of Texas, Houston, TX, USA
e-mail: ayounes@mdanderson.org

P.L. Zinzani
Institute of Hematology "L. e A. Seràgnoli",
University of Bologna, Bologna, Italy
e-mail: pierluigi.zinzani@unibo.it

S. Rodig, MD
Department of Pathology,
Brigham and Women's Hospital,
Harvard Medical School, Boston, MA, USA
e-mail: srodig@partners.org

J. Delabie, MD
Department of Pathology,
Oslo University Hospital, Oslo, Norway
e-mail: jandel@ous-hf.no

6.1 Introduction

Anaplastic large cell lymphoma (ALCL) is a peripheral T-cell-derived malignancy, representing around 2–3 % of all lymphoid neoplasms, according to the World Health Organization (WHO) estimates (Delsol et al. 2008; Mason et al. 2008). Originally described by Stein et al. (1985), it has undergone a series of revisions, which have led to a more refined and restrictive definition of the process (Delsol et al. 2008; Mason et al. 2008; Stansfeld et al. 1988; Harris et al. 1994). In particular, two different entities are recognized as systemic forms, the ALK+ and ALK− ALCL (Delsol et al. 2008; Mason et al. 2008; Savage et al. 2008), on genetic and clinical features, the first one being characterized by the deregulated expression of chimeric proteins expressing the intracytoplasmic domain of the anaplastic lymphoma kinase (ALK) gene. Noteworthy, in the last edition of the WHO classification, ALK− ALCL was regarded only as a provisional entity (Delsol et al. 2008; Mason et al. 2008). However, emerging evidences suggest the existence of two real tumors (Piva et al. 2010). Whereas ALK-positive ALCL typically presents in children and young adults (mean age 22 years) with a male predominance (Falini et al. 1999) ALK-negative ALCL presents in older individuals (mean age 43 years) and does not show a sex

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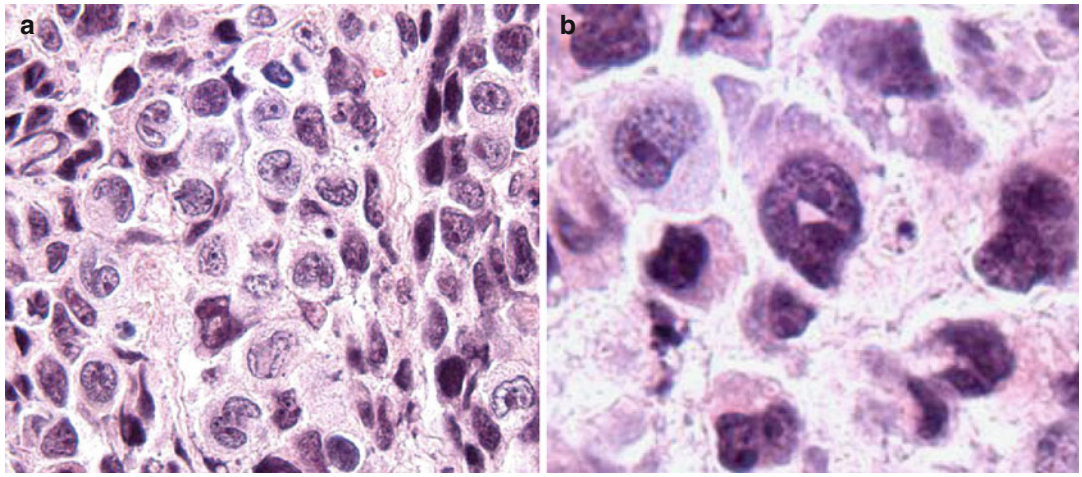

Fig. 6.1 (**a** and **b**) ALCL, ALK- with large tumor cells containing pleomorphic nuclei

predilection (Falini et al. 1999; Gascoyne et al. 1999). Both subtypes can present in lymph nodes and in extranodal sites including bone, soft tissue, and skin. On the other hand, differently from what initially reported by Stein et al. (1985), the cutaneous variant was recognized as a different disease (Ralfkiaer et al. 2008).

Primary systemic ALCL has a peak incidence in childhood, accounting for approximately 40 % of NHL cases diagnosed in pediatric patients (Gascoyne et al. 1999), whereas it accounts for <5 % of NHL in adults (Delsol et al. 2008; Mason et al. 2008; Ralfkiaer et al. 2008), and it is seen mostly in males. Patients present with stage III to IV disease, often with multiple extranodal sites of involvement (Delsol et al. 2008; Mason et al. 2008; Rizvi et al. 2006; Savage 2007).

6.2 Morphology

The tumor cells of ALCL are distinguished by their large size with large, pleomorphic nuclei showing extensive lobation (Chott et al. 1990; Benharroch et al. 1998). The tumor nuclei characteristically demonstrate "horseshoe" or "embryoid" shapes, and when multinucleated, the tumor nuclei may be arranged in a "wreath-like" pattern. Nucleoli may be distinct (Fig. 6.1). Intermixed with these large, distinctive cells, there are frequently small- to intermediate-sized, neoplastic cells with a single large nucleus. The tumor may completely replace normal lymph node tissue or may be restricted within the lymph node to the T-cell zone or even the sinuses in a pattern resembling metastatic carcinoma. Small cell variants and variants closely resembling Hodgkin Reed-Sternberg cells exist (Stein et al. 1985; Kinney et al. 1993).

6.3 Immunophenotype

The tumor cells of ALCL, ALK-positive and ALK-negative, universally express CD30 and a majority express CD43. Most ALK+ ALCL additionally express epithelial membrane antigen (EMA). Although ALCLs often express one or more general T-cell and cytotoxic T-cell antigens (i.e., CD2, CD5, CD4, TIA1, granzyme B), most have lost at least a proportion of the normal complement of antigens expressed by normal, mature T-cells (Benharroch et al. 1998; Foss et al. 1996). Most notably, CD3 is absent in up to 70 % of tumors (Kinney et al. 1993). Occasional cases have lost all detectable T-cell markers and express only CD30. Cases with a "null-cell" phenotype demonstrate genetic evidence of the T-cell lineage. Cases of ALK+ ALCL are distinguished by the expression of the kinase domain of the

ALK oncoprotein which is detected with specific antibodies either in the cytoplasm only or both in the nucleus and cytoplasm (Pileri et al. 1997).

6.4 Genetics

Molecular studies demonstrate a clonal rearrangement of one or more genes encoding the T-cell receptor (TCR) in the tumor cells of ALCL regardless of the overall immunophenotype (Foss et al. 1996). ALK+ ALCL is additionally characterized by balanced translocations involving the 3′ portion of the ALK gene, which encodes the kinase domain of ALK, with the 5′ portion of a variety of partner genes, most commonly the NPM gene which encodes nucleophosmin (NPM) (Morris et al. 1995). The NPM-ALK fusion, as a result of the t(2;5)(p23;q35) (Gascoyne et al. 1999; Benharroch et al. 1998; Kadin and Morris 1998; Sandlund et al. 1994) balanced translocation, encodes a fusion protein with kinase activity and which promotes lymphoid transformation (Chiarle et al. 2003). ALK in these cases is usually expressed both in the cytoplasm and nucleus. In addition to NPM, there are at least eight additional known fusion partners with ALK− all of which preserve the ALK kinase domain and the resulting fusion proteins are usually expressed only in the cytoplasm. In contrast to ALK+ ALCL, ALK− ALCL is not distinguished by a unique chromosomal or known molecular abnormality.

6.5 Differential Diagnosis

The spectrum of morphologic appearances and immunophenotypes in ALCL frequently raise the differential diagnoses of classical Hodgkin lymphoma (cHL) and peripheral T-cell lymphoma, not otherwise specified (PTCL, NOS). With the advent of better diagnostic markers of Reed-Sternberg cells that illuminate their B-lineage origin (such as PAX5), distinguishing ALCL from cHL has become more straightforward. In contrast, distinguishing ALK-negative ALCL from PTCL, NOS with CD30 expression has remained challenging despite recent attempts to clarify the distinction. The histologic appearance of the tumors cells and degree of expression of CD30 on the tumor cells remains a primary means of distinguishing these tumor types (Went et al. 2006). ALCL, as described here, is a systemic disease that may involve or present in the skin. However, care must be taken to distinguish cutaneous involvement by ALK-negative ALCL from primary cutaneous CD30+ T-cell lymphoproliferative disorders including primary cutaneous ALCL, lymphomatoid papulosis, and transformed mycosis fungoides.

6.6 Clinical Presentation

ALCL is an aggressive lymphoma which frequently presents in advanced clinical stage (III–IV) with systemic symptoms and extranodal involvement, as other PTCLs do (Falini et al. 1999; Stein et al. 2000). Bone marrow involvement is detected in up to 30 % of cases, being a relevant prognostic feature (Fraga et al. 1995; Mussolin et al. 2005; Kalinova et al. 2008).

Importantly, ALCLs display quite different clinical features depending on the expression of the ALK protein (Savage et al. 2008; Falini et al. 1999; Stein et al. 2000). In particular, ALK+ tumors most frequently occur among patients in the first or second decade of life, while ALK− ones are usually recorded among people aged 50–70 (Savage et al. 2008; Falini et al. 1999; Stein et al. 2000). Moreover, advanced-stage disease and B symptoms are slightly more common in ALK+ ALCL (Savage et al. 2008; Stein et al. 2000). B symptoms were observed in both groups, and patients with ALCL, ALK+ had significantly better performance status, and fewer had above normal LDH levels. Patients in both groups showed a nodal presentation, and >40 % of children with disseminated disease had inguinal lymph node involvement. Mediastinal involvement was less common than in Hodgkin lymphoma. Skin, bone, and soft tissues were commonly affected extranodal sites. Pelvic muscle involvement is not infrequent and can be

mistaken for a soft tissue sarcoma. Central nervous system involvement seems rare in adults and especially in children with ALK+ ALCL. Bone marrow involvement is considered to be an uncommon event and can be difficult to detect on routine histologic examinations alone (Falini et al. 1999). ALCL, ALK+ is rare in patients after transplant (Costes-Martineau et al. 2002) and those infected with human immunodeficiency virus (Tirelli et al. 1995; Gabarre et al. 2001). In this setting, most ALCL cases appear to be related to the anaplastic variant of diffuse large B-cell lymphoma (Tirelli et al. 1995). Secondary ALCL may arise in the progression of other lymphomas, most commonly during the course of mycosis fungoides, PTCLs, Hodgkin lymphoma, or lymphomatoid papulosis (Stein et al. 2000), and is usually characterized by a poor prognosis (Salhany et al. 1988).

6.7 Prognostic and Predictive Factors

Comparison of ALK+ vs. ALK− ALCL in adult patients identified risk groups described in the International Prognostic Index (IPI) with a significant difference in favor of patients with ALK+, although those with an IPI score of three were in the poor risk regardless of ALK status (Savage et al. 2008). The T-cell prognostic index for T-cell (PIT) (Gallamini et al. 2004) was predictive of overall survival and failure-free survival in both groups. These clinical findings further support the inclusion of ALCL, ALK+ as a distinct entity in the new WHO classification. Interestingly, comparison of ALK+ and ALK− ALCL in patients >40 years of age revealed no difference in survival (Savage et al. 2008), suggesting that age is a predominant factor driving outcome difference. Suzuki et al. (2000) found CD56 expression to be a prognostic factor independent of IPI and ALK expression in multivariate analysis. In fact, in both ALK+ and ALK− subgroups, CD56+ cases showed a poorer prognosis than did CD56- cases.

Several series of childhood and adult ALCL have been reported but were often difficult to compare because of problems in defining entities, the heterogeneity of the treatments, or the lack of a common staging system (Brugieres et al. 1998; Dearden et al. 2011). St. Jude's Hospital's classification has been used only for children, whereas the Ann Arbor staging system and the IPI scoring are used for adults. Although ALK absence or presence in ALCL is useful, in association with the IPI score, for discriminating a patient's prognosis and to evaluate the impact of treatment in adult patients, it is not applicable for children because 90 % of ALCLs at this age are ALK+ (Brugieres et al. 1998).

6.8 Treatment

6.8.1 Frontline Treatment

Multi-agent chemotherapy is the standard frontline treatment for patients with systemic ALCL, plus the addition of involved-field radiation therapy for patients who present with stage I–II locoregional disease (Dearden et al. 2011; Kwong et al. 2009; National Comprehensive Cancer Network (NCCN) 2012). However, the optimal chemotherapy regimen remains a bit unclear, mostly because of the lack of robust clinical trial data focusing on patients with ALCL and the need to extrapolate from trials of patients with PTCL.

A meta-analysis of studies examining anthracycline-based therapy in patients with PTCL showed good outcomes with this approach, similar to that seen in aggressive B-cell lymphomas (Abouyabis et al. 2011), and CHOP-based regimens have been shown to yield good responses and prolonged clinical benefit in patients with favorable prognosis ALC (Savage et al. 2008). However, data from the International Peripheral T-Cell Lymphoma Project show that outcomes in patients with ALCL are similar regardless of whether an anthracycline-based therapy is used as frontline chemotherapy (Vose et al. 2008). One possible approach to selecting an optimal treatment is to look more specifically at subgroup populations. The International Peripheral T-Cell Lymphoma Project found that, for patients with ALK+ disease, a CHOP-based regimen was

shown to yield a 5-year overall survival rate of 75 % for patients with an IPI 0-1, but the rate fell to 25 % for patients with IPI ≥ 2. In patients with ALK− disease, higher-risk IPI also predicted a poorer outcome from CHOP-based chemotherapy: ALK− patients with IPI 0-1 showed a 5-year survival rate of 50 % vs. 18 % for patients with IPI ≥ 2 (Savage et al. 2008). Similar findings were seen in an examination of 363 patients with T-cell lymphoma enrolled on 7 prospective trials of different CHOP-based regimens from the German High-Grade Non-Hodgkin's Lymphoma Study Group. Within this cohort, 78 patients (24 %) had ALK+ ALCL and 113 (35 %) had ALK− ALCL (Schmitz et al. 2010). Across all trials, the 3-year event-free and overall survival rates were 76 and 90 %, respectively, for patients with ALK+ ALCL, vs. 46 and 62 %, respectively, for patients with ALK− ALCL. In younger, favorable-risk patients, event-free survival rates were improved for patients treated with CHOP plus etoposide (CHOEP), while CHOP alone yielded better results for older patients with less favorable risk (Schmitz et al. 2010).

Of note, attempts at intensification by shortening the treatment interval in older patients or by escalating the treatment doses in younger patients did not improve outcomes (Schmitz et al. 2010). By contrast, the use of the intense NHL-Berlin-Frankfurt-Münster (BFM)-90 regimen, which had previously showed good results in a pediatric ALCL population (Seidemann et al. 2001), also showed good results in an adult ALCL population with a median age of 26 (range, 17–65). Based on these data, it would be reasonable to conclude that CHOP-21 is an appropriate frontline treatment strategy for older patients and those with less favorable ALCL (e.g., ALK− disease, higher-risk IPI), and CHOEP-14, CHOEP-21, or even the dose-intense BFM-90 protocol is an appropriate frontline treatment strategy for younger patients and those with more favorable ALCL (e.g., ALK+, lower-risk IPI).

In an attempt to improve outcomes beyond CHOP and dose intensification, researchers have explored alternative treatment strategies in select populations. For example, in 653 older patients with poorer-risk aggressive lymphoma, 22 of whom had ALCL, the French GELA compared standard CHOP with the ACVBP regimen (Tilly et al. 2003). Five-year event-free and overall survival rates were higher with ACVBP than with CHOP, even after adjusting for IPI and after excluding disease subtypes thought to have better outcomes. Rates of hematologic toxicity, infection, and the need for growth factor support were higher with ACVBP, again underscoring the importance of careful patient selection in determining the optional treatment approach (Tilly et al. 2003).

6.8.2 Consolidation with Autologous Stem Cell Transplant

Response rates in patients with ALK+ ALCL are typically high, and consolidation with autologous stem cell transplant (ASCT) is not recommended (Dearden et al. 2011; Kwong et al. 2009). By contrast, ASCT consolidation in patients with ALK− ALCL may be beneficial in some subpopulations. Although patients with ALCL, even those with ALK− disease, tend to fare better from transplantation than do patients with other types of PTCL (Jagasia et al. 2004), most trials enrol only a handful of patients with ALCL and fewer still differentiate between good prognosis ALK+ and poor-prognosis ALK− disease, making it difficult to truly assess the potential benefits of ASCT in these patients. Nevertheless, results of a few studies that specifically isolated patients with ALK− ALCL suggest that appropriately selected patients may benefit from this more aggressive treatment approach.

Extrapolating from a larger clinical trial, researchers with the Nordic Lymphoma Group evaluated 31 patients with ALK− ALCL who were treated with CHOP-14 (age 60–65) or CHOEP-14 (under age 60) followed by high-dose chemotherapy and ASCT. The response rate in transplanted patients was 96 % vs. 74 % for the group as a whole, and the 3-year overall survival rate was 73 % (Relander et al. 2012). Data from a German multicenter, prospective trial also showed good results with ASCT consolidation. In this

trial, 83 patients with PTCL, 13 of whom had ALK− ALCL, were treated with CHOP followed by total body irradiation, high-dose chemotherapy, and ASCT. The estimated 3-year overall survival rate was 71 % for patients who underwent transplantation vs. 48 % for those who did not, and subgroup analysis showed no significant differences in overall survival between ALK− ALCL and other types of PTCL (Reimer et al. 2009).

As with other types of lymphoma, not all patients with ALK− ALCL achieve complete or partial response after frontline chemotherapy, yet this remains a key prognostic factor in determining outcome from ASCT (Hosing and Champlin 2011). In the trial from the Nordic group, only 77 % underwent ASCT, while in the German study, only 66 % did; in most cases, patients were excluded from treatment because they showed a poor response to frontline chemotherapy (Relander et al. 2012; Reimer et al. 2009). Of note, single-institution retrospective data from MD Anderson Cancer Center show that the presence of refractory disease, as indicated by an inability to achieve first complete response to induction therapy, independently predicts for a worse outcome from ASCT regardless of ALK status (Beitinjaneh et al. 2011). Thus, available data suggest that, for patients who show good response to frontline chemotherapy and who remain good candidates for transplantation, consolidation with ASCT can further improve outcomes (Dearden et al. 2011; Kwong et al. 2009). Nevertheless, until better regimens that are more effective at inducing high response rates are available, the potential benefits of ASCT consolidation will not be fully realized.

6.8.3 Second-Line Therapy

The relatively small population of patients with progressive systemic ALCL precludes large-scale clinical trials from definitively determining whether second-line chemotherapies, either multi-agent or single-agent, might be beneficial. Extrapolations are typically made from trials in patients with PTCL that may or may not relate to patients with ALCL, or even from trials in patients with NHL, in which patients with ALCL may represent only a tiny fraction of enrolees. Indeed, current clinical practice guidelines from the United States and Europe both describe the use of platinum-based multi-agent regimens, even though the trials evaluating those regimens were often primarily done in patients with aggressive B-cell NHL (Dearden et al. 2011; Kwong et al. 2009). Thus, although these and other chemotherapy regimens can be considered in patients with systemic ALK− ALCL, the outcomes remain unknown and their potential benefit remains difficult to assess.

The benefit of salvage SCT is also unclear. A review of records on patients with T-cell lymphoma by the Center for International Blood and Marrow Transplant Research suggest that a greater number of prior chemotherapy regimens and chemoresistance independently predict for worse outcomes from both autologous and allogeneic SCT, indicating that these treatment modalities should be considered earlier in the course of disease (Schmitz et al. 2010). Nevertheless, studies in patients with ALCL seem to suggest that certain patient populations can benefit from autologous and allogeneic SCT in the salvage setting. Trials with salvage ASCT in patients with ALK− ALCL have shown little benefit for its use in this population. In a series of 16 patients with ALK− ALCL, only 1 patient showed long-term disease-free survival, while 14 showed relapsed after a median of only 12 weeks despite achieving complete or partial response after salvage chemotherapy (Zamkoff et al. 2004). By contrast, patients with ALK+ disease seem to fare well from salvage ASCT: in one series, patients with ALK+ ALCL showed very high event-free survival rates (Jagasia et al. 2004). Thus, in patients with ALK+ disease, salvage ASCT can prolong remission but the treatment modality seems to offer little benefit to patients with ALK− ALCL.

Salvage allogeneic SCT might be an option for patients with systemic ALK− ALCL. In particular, patients with ALK− ALCL who underwent allogeneic SCT with reduced-intensity conditioning showed good response. In a series of 17 patients with PTCL, after at least 10

months' follow-up, 1 of 4 patients with ALK– ALCL had stable disease, 1 achieved partial response after donor lymphocyte infusion, and 2 achieved complete response (Corradini et al. 2004). Of note, the partial responder and one of the complete responders had relapsed after a prior autologous SCT, underscoring that patients with chemosensitive disease can continue to benefit from further treatment modalities.

Several recent reports have described the occurrence of ALK-negative ALCL in patients with breast implants (Popplewell et al. 2011; Lazzeri et al. 2011; Kim et al. 2011). A recent review of the literature identified 29 cases, of which 25 had information on ALK staining and all were negative. Interestingly, 72 % were stage IE, involving the breast only and in particular the capsule (a fibrous layer of tissue that forms around the implant after surgery). Unfortunately, treatment information was detailed for only 17 cases, but it appears that this entity has an indolent course, with 75 % remaining disease-free with a median duration of follow-up of 5.5 years. These data suggest that ALK-negative ALCL that develops in the context of a breast implant may have a clinical course that resembles primary cutaneous ALCL, but further studies are needed (Kim et al. 2011).

6.8.4 Targeted Therapies

Although our knowledge of ALCL molecular biology has significantly increased over the past two decades, the development of molecular targeted agents for ALCL has been slow. This is mainly due to the high cure rate in a relatively rare disease. Despite these challenges, a new antibody-drug conjugate (ADC) was approved last year by the US Food and Drug Administration (FDA) for the treatment of patients with relapsed ALCL (Younes 2011a, b). A second drug, crizotinib, which targets ALK kinase was approved for a rare type of lung cancer but is also expected to be approved in the near future for patients with ALCL (Shaw et al. 2011). To date, the leading therapeutic targets are CD30 and ALK.

Shortly after the discovery of CD30 receptor in 1982, several investigators developed a variety of monoclonal antibodies against CD30 for diagnostic and therapeutic purposes. CD30 is highly restricted receptor and therefore considered an ideal target for monoclonal antibody therapy. Unfortunately, first-generation naked monoclonal antibodies targeting CD30 failed to produce meaningful clinical responses, most likely due to their poor antigen binding properties, ineffective activation of effector cells, and neutralization by soluble CD30 (Younes 2011a; Ansell et al. 2003; Forero-Torres et al. 2009). Because CD30 can be internalized, it was recently explored for the development of ADCs. Brentuximab vedotin (SGN-35) is a novel ADC that was developed by conjugating the anti-CD30 antibody cAC10 to a synthetic antitubulin agent monomethyl auristatin E (MMAE) using a cleavable dipeptide linker (Younes et al. 2011; Katz et al. 2011). Brentuximab vedotin was initially evaluated in a phase I study that predominantly included patients with relapsed classical HL but also included two patients with relapsed ALCL (Younes et al. 2010). Both patients with ALCL achieved complete remissions. In a follow-up phase II study, 58 patients with relapsed ALCL, of whom, 72 % had ALK-negative disease (Pro et al. 2012). Patients were treated with 1.8 mg/kg of brentuximab vedotin by short intravenous infusion every 3 weeks. Sixty-two percent of the patients had primary refractory disease, and 50 % were refractory to their last qualifying therapy. The overall response rate was 86 %, and the CR rate was 53 %. These results lead to the approval of brentuximab vedotin by the US FDA for the treatment of patients with relapsed ALCL. Ongoing studies are evaluating the contribution of brentuximab vedotin to frontline therapy.

ALCL is frequently associated with the presence of several chromosomal translocations involving the ALK kinase (Falini et al. 1999). The most common translocation is the t(2;5)(p23;q35), which fuses the ALK and the nucleophosmin (NPM) genes, resulting in an NPM-ALK fusion protein. Other less common ALK partners also exist (Stein et al. 2000). ALK-positive ALCL is more common in children and young adults and usually have better prognosis than the ALK-negative cases. The fusion of the N-terminal of

NPM to the kinase domain of ALK results in its constitutive activation. Consequently, a constitutively active ALK kinase activates several downstream pro-survival proteins, including STAT3, PI3K/AKT/mTOR, cJun, and c-Myc. Preclinical experiments using a variety of small molecule inhibitors of ALK kinase activity demonstrated antiproliferative activity in ALK-driven tumors, including ALCL. The most clinically advanced inhibitor is crizotinib (PF-02341066), which was recently approved by the US FDA for the treatment of lung carcinoma bearing the ELM4-ALK translocation (Trial watch: success for crizotinib in ALK-driven cancer 2010; Ou 2011). Although there is no major clinical experience with crizotinib in the treatment of patients with ALCL, preclinical and a recent limited clinical experience suggested that this drug should also be active in this disease. In a recent report, two patients with relapsed ALCL were treated with crizotinib at 250 mg twice daily, and both achieved complete remissions (Gambacorti-Passerini et al. 2011). These results are currently being confirmed in a prospective phase II clinical trial.

Conclusions

In conclusion, based on the most recent findings, the WHO classification currently considers two ALCL types, ALK+ and ALK–. In fact, though the latter is still quoted as a provisional entity, increasing evidence, both biological and clinical, suggests the real existence of two distinct, though similar, tumors. ALK represents an ideal therapeutic target for innovative strategies, including small inhibiting molecules and even vaccination. On the other hand, ALK– cases may benefit, in the future, from monoclonal antibodies (i.e., antiCD30), tyrosine-kinase inhibitors, or other signal transduction inhibitors.

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Extranodal NK/T-Cell Lymphoma, Nasal Type

7

Won Seog Kim, Seok Jin Kim, and Young Hyeh Ko

Contents

W.S. Kim (✉)
Divison of Hematology-Oncology,
Department of Medicine, Samsung Medical Center,
Seoul, South Korea
e-mail: wskimsmc@skku.edu

S.J. Kim
Divison of Hematology-Oncology,
Samsung Medical Center, Seoul, South Korea
e-mail: kstwoh@skku.edu

Y.H. Ko
Department of Pathology,
Samsung Medical Center, Seoul, South Korea
e-mail: yhko310@skkuy.edu

Extranodal natural killer (NK)/T-cell lymphoma (ENKL) is a unique disease entity that is strongly associated with Epstein–Barr virus (EBV) infection. This disease is more common in Asia and Central and South America but is rare in North America and Europe. Pathologically, ENKL involves polymorphous infiltration of variable-size lymphocytes with accompanying inflammation and necrosis. The malignant cells express cytoplasmic CD3 and CD56. EBV is detected by EBV-encoded small RNA (EBER) in situ hybridization.

Unlike other aggressive lymphomas, around 80 % of cases are localized to the nasal and upper airway region. Skin, intestine, and testis are the common sites of involvement outside the nasal area. The prognosis is generally poor, with 30–40 % long-term survival. The Korean Prognostic Index (KPI) is more powerful than the International Prognostic Index (IPI) for predicting the prognosis. The response to anthracycline-based chemotherapies, such as CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone) or CHOP-like regimens is disappointing: 30–40 % long-term survival, even for stage I or II cases. Concurrent chemoradiation strategies have a higher efficacy for localized ENKL treatment: 70–80 % long-term survival. For advanced disease, L-asparaginase-based polychemotherapy regimens are producing promising results. The roles of high-dose chemotherapy and autologous hematopoietic stem cell transplantation have not been determined.

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7.1 Background and Epidemiology

ENKL is a distinctive lymphoid malignancy of mature T or NK cells and comprises 5–8 % of non-Hodgkin lymphoma cases (Vose et al. 2008; Au et al. 2009; Lee et al. 2005a, 2006). Before the revised European–American lymphoma (REAL) classification, this lymphoma was not listed in many classifications including the Rappaport, Lukes–Collins, Kiel, and Working formulation. ENKL has different names such as angiocentric immunoproliferative lesion (grades 2 and 3), polymorphic reticulosis, and lymphomatoid granulomatosis. In the REAL classification, ENKL is termed "angiocentric lymphoma" following the characteristic pathological features of angiocentric and angioinvasive infiltration (Harris et al. 1994). In the current WHO classification, the term "extranodal NK/T-cell lymphoma (ENKL), nasal type" has been adopted (Campo et al. 2011). Most understanding of the clinical behavior and biology of ENKL has been obtained recently.

ENKL is invariably associated with EBV infection (Harris et al. 1994; Campo et al. 2011; Ng et al. 2011a). It has a skewed pattern of geographic distribution, i.e., it is more common in Asia and Central and South America but is less common in southern Asia (Vose et al. 2008; Au et al. 2009; Aviles et al. 2000; Naresh et al. 2000). In far eastern Asian countries (including Korea, Hong Kong, China, and Japan), ENKL accounts for 5–9 % of all cases of lymphoma and up to 30 % of all mature T-cell lymphoid malignancies (Ko et al. 1998; Kim et al. 2002; Suzuki et al. 2005). However, in Europe and North America, ENKL accounts for 4–5 % of mature T-cell lymphomas and only 1 % of all lymphomas (Vose et al. 2008).

7.2 Pathogenesis

The geographic variation in the incidence suggests genetic susceptibility, possibly based on certain HLA types. A study of natives of New Guinea has shown that HLA A11 may provide the basis for the higher frequency of EBV-positive tumors, including ENKL (de Campos-Lima et al. 1993). By contrast, the low frequency of the HLA-A*0201 allele in patients with ENKL suggests an HLA-A*0201-restricted T-cell response in suppressing the development of overt lymphoma (Kanno et al. 2000).

The most common cytogenetic aberration is deletion of 6q. Recent studies show loss of tumor suppressor genes such as HACE1 (HECT domain and ankyrin receptor containing E3 ubiquitin protein kinase 1), PRDM1 (PD domain zinc finger domain 1), and FOXO3 (forkhead transcription factor of O class 3) (Karube et al. 2011; Huang et al. 2010). Other reported chromosomal changes are gains on chromosomes 2q, 13q, 10q, 21q, 3q, 5q, and 17q and losses on 1p, 17p, 12q, 13q, 11q22.3–q23.3, 5p14.1–p14.3, 5q34–q35.3, 1p36.23–p36.33, 2p16.1–p16.3, 4q12, and 4q31.3–q32 (Ko et al. 2001; Nakashima et al. 2005).

A gene expression study suggested that PDGFR overexpression is also a major mechanism responsible for lymphomagenesis (Huang et al. 2010). Another study reported alterations in the expression of PLK1 (polo-like kinase 1), CDK1 (cyclin-dependent kinase 1), Aurora-A, c-Myc, p53, NF-κB, and survivin (Ng et al. 2011a). Dysregulation of microRNA (miR) has also been suggested as an important mechanism responsible for lymphomagenesis. The downregulated microRNAs are miR-101, miR-26a, miR26b, miR-28-5, and miR-363 (Ng et al. 2011b).

Genetic alterations in the tumor suppressor genes and several oncogenes have been reported with different frequencies. Mutations of p53 are present in more than 60 % of cases in Indonesia and Japan, in 45 % of cases in China, in 30 % of cases in Korea, and in 24 % of cases in Mexico. The rate of mutations in c-kit is also significantly higher in China (70 %) than in Japan, Korea, northeast China, and Indonesia (<20 %). Almost half of cases harbor a Fas gene mutation (Hoshida et al. 2003; Kurniawan et al. 2006; Hongyo et al. 2005; Shen et al. 2002; Takakuwa et al. 2002).

7.3 Pathology

All ENKLs share similar histological changes irrespective of the disease location. The tumor causes mucosal ulceration with diffuse necrosis because of angiocentric infiltration or angioinvasion.

Many apoptotic bodies appear scattered in the necrotic tumor tissue. The cytological composition varies widely between cases. A common feature is mixed small and medium atypical lymphocytes with serpentine and hyperchromatic nuclei. Mitotic figures are frequent. Inflammatory cells are admixed with tumor cells. In some cases, tumor cells appear to be large and anaplastic with prominent nucleoli. The neoplastic cells often comprise only small lymphocytes lacking atypia and necrosis, resulting in a misdiagnosis as chronic inflammation. Therefore, EBER in situ hybridization is needed to avoid overlooking the tumor (Fig. 7.1).

In the skin, the tumor presents as large ulcerating lesions or multiple erythematous skin rashes. Tumor cells infiltrate throughout the skin, including subcutaneous adipose tissue. Diffuse necrosis of the skin with ulceration of the epidermis is common (Kost et al. 2009; Chia et al. 2009). Pyogenic granuloma-like lesions have also been reported (Fernandez-Torres et al. 2009).

The immunophenotype of typical ENKL is positive for CD2, CD56, and cytoplasmic CD3ε and negative for surface CD3 (Fig. 7.1). Typical ENKL is also positive for cytotoxic granules including granzyme B, perforin, and T-cell–restricted intracellular antigen. Occasionally,

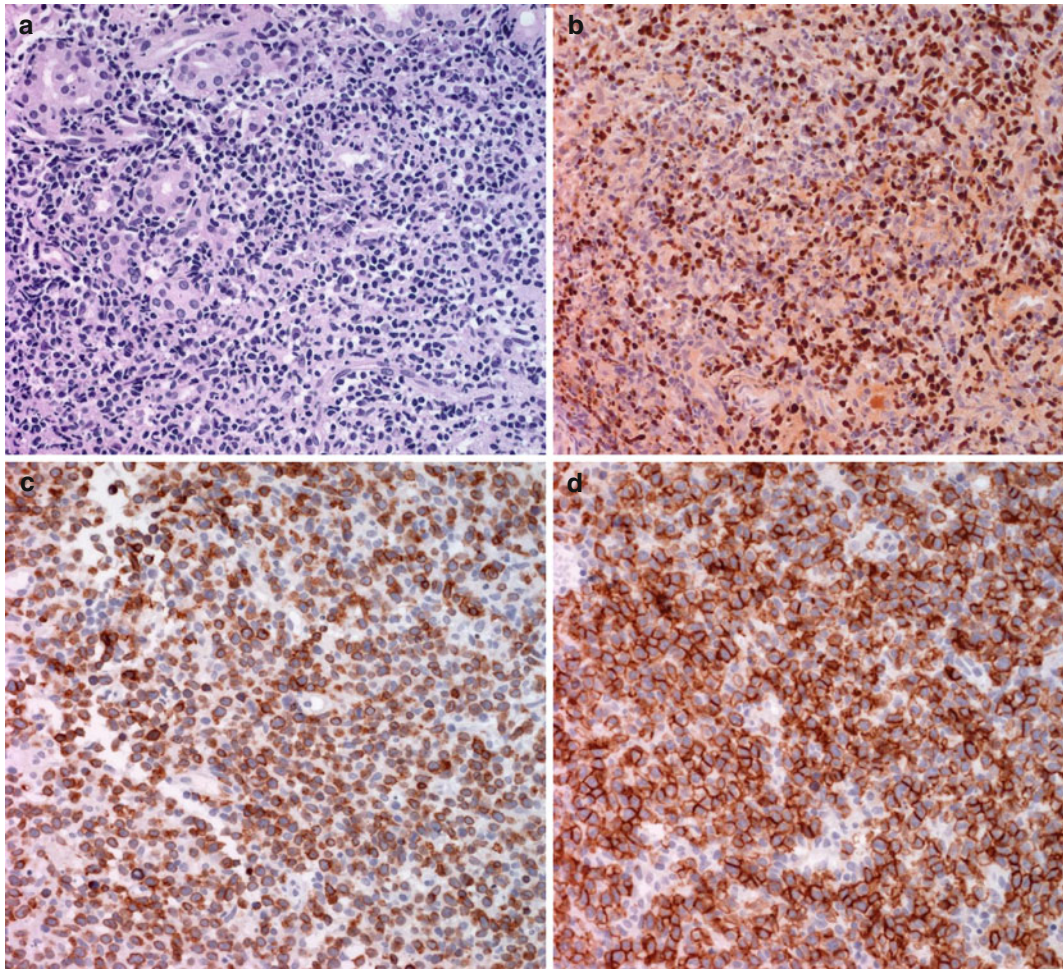

Fig. 7.1 (**a**) Extranodal NK/T-cell lymphoma of the nasal cavity. Small- to medium-sized tumor cells are intermingled with plasma cells. (**b**) EBER in situ hybridization revealed positive signal in the nuclei of tumor cells. (**c**) Tumor cells express cytoplasmic CD3. (**d**) Tumor cells express CD56

ENKL is also positive for CD30 and CD7. Some cases are CD56 negative. Cases that are CD56 negative and cytoplasmic CD3ε positive are also classified as ENKL if they are positive for both cytotoxic granules and EBER. However, cases that are CD56 negative and cytoplasmic CD3ε positive but are negative for EBV and cytotoxic molecules should be classified as peripheral T-cell lymphoma, unspecified (Au et al. 2009).

7.4 Clinical Presentation

The most typical clinical presentation of ENKL is ulceration and destruction because of the characteristic angioinvasion and necrosis. The median onset age is the mid-40s to 50 years. This disease is quite uncommon in children and adolescents. There is a male predominance, especially nasal cases (Au et al. 2009; Lee et al. 2005a, b, 2006; Suzuki et al. 2010). Because around 80 % of cases occur in the upper airway tract, common symptoms are nasal obstruction, ulceration in the nasal cavity and palate, hemorrhage, and discharge. Severe destruction of the hard palate can cause midline perforation, which is why it was previously called "lethal midline granuloma" (Fig. 7.2a, b). Extranasal disease occurs in the skin, gastrointestinal tract, testis, and other organs (Au et al. 2009; Lee et al. 2005a, b; Suzuki et al. 2010). In cutaneous ENKL, the most typical lesion is nonhealing ulceration (Fig. 7.2c). Gut perforation, bleeding, and acute abdomen are common symptoms in cases of gastrointestinal tract involvement. The lesions are characterized endoscopically by erosion, ulceration, or ulceroinfiltrative lesions without mass formation (Fig. 7.2d) (Kim et al. 2007). Interestingly, there are clinical differences between nasal and extranasal ENKL even though they share the same histological features. Patients with extranasal ENKL have more adverse clinical features including advanced stage, elevated lactate dehydrogenase (LDH) level, presence of B symptoms, and poor performance status (Au et al. 2009; Lee et al. 2005a; Suzuki et al. 2010).

Hepatic dysfunction caused by cytokines and/or hepatosplenic involvement can occur even in clinically stage I or II disease. Hemophagocytosis presenting as fulminant multiorgan failure with elevated LDH level, fever, and pancytopenia can occur; this is sometimes misdiagnosed as sepsis due to secondary infection from the ulcerative lesions.

7.5 Staging and Prognostic Factors

No specific staging system for ENKL has been proposed. Ann Arbor staging is usually applied even though it is not satisfactory. For initial assessment, a complete history and physical examination are necessary (Kwong et al. 2009). Recent studies show the prognostic importance of local tumor invasiveness and regional lymph node involvement (Lee et al. 2006; Suzuki et al. 2010; Kim et al. 2005). The use of appropriate computed tomography or magnetic resonance imaging may provide good information about the extent of the disease. The role of positron emission tomography (PET) remains controversial. ENKL lesions are generally fluorodeoxyglucose avid. Therefore, it seems to be helpful to detect other systemic lesions before treatment. The role of posttreatment PET requires further studies (Kako et al. 2007; Fujiwara et al. 2011; Khong et al. 2008). To detect bone marrow involvement accurately, EBER in situ hybridization should be performed routinely in all patients with ENKL (Kwong et al. 2009; Lee et al. 2007). CNS involvement has been a concern for a long time because this lymphoma usually involves the nasal and paranasal sinuses. A recent report showed CNS involvement in <6 % of cases. Among patients at low risk (groups I and II by the KPI), CNS involvement was noted in <2 % of cases. Therefore, routine CNS evaluation is not recommended, although the role of CNS evaluation has not been determined for patients in KPI groups III and IV (Kim et al. 2010).

EBV DNA can be released from the apoptotic tumor cells. Serial monitoring of circulating EBV DNA can be a valuable marker of tumor burden and disease control. The test is recommended for

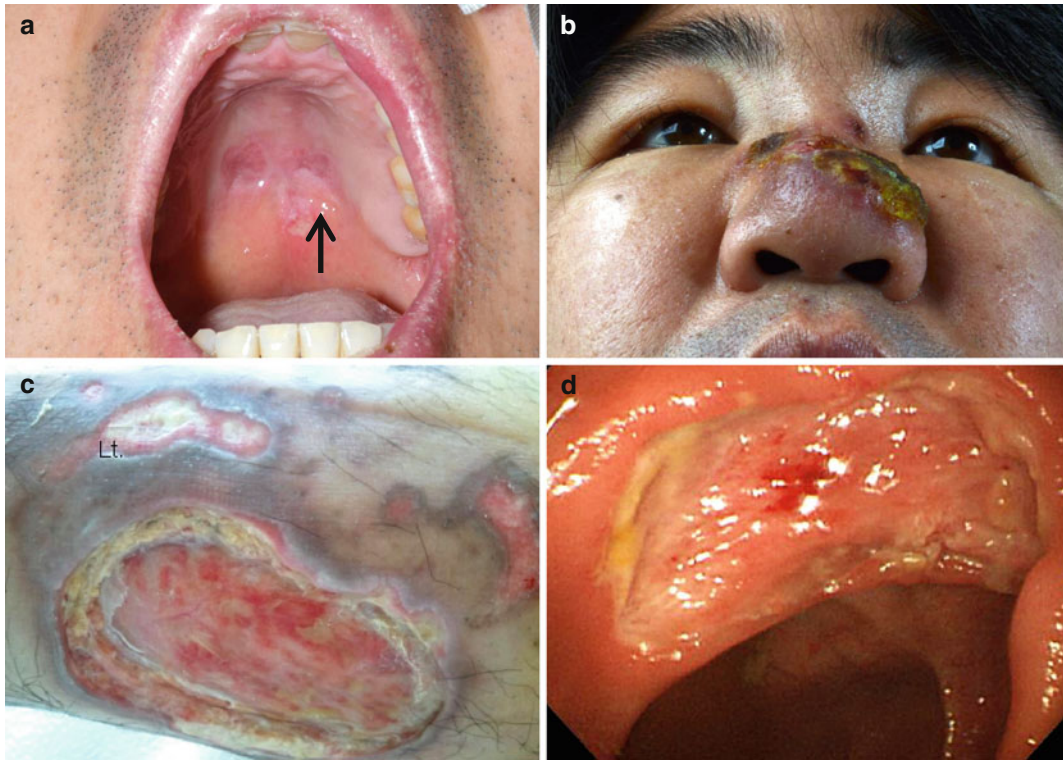

Fig. 7.2 (**a**, **b**) Nasal ENKL presented as palatal ulcer and perinasal skin infiltration. (**c**) Skin involvement as multiple nonhealing ulceration. (**d**) Intestine involvement as large ulceration. This lesion can easily bleed

staging, if quantitative Polymerase chain reaction (PCR) is available (Au et al. 2004; Kim et al. 2009a; Suzuki et al. 2011).

The IPI has good power for predicting the prognosis. However, most patients are classified into low- and low-intermediate-risk groups by IPI even though they have a poor prognosis. Besides the IPI, the following parameters including local tumor invasiveness, extra-upper aerodigestive tract origin, high proliferation index of the tumor tissue, and quantification of circulating EBV DNA seem to be prognostically relevant for individual patients (Kim et al. 2002, 2005, 2009a; Au et al. 2004; Suzuki et al. 2011). Therefore, other prognostic models have been proposed (Lee et al. 2006; Suzuki et al. 2010). Currently, the KPI, which is based on B symptoms, stage, LDH level, and regional lymph node involvement, has the strongest predictive power (Fig. 7.3) (Au et al. 2009; Lee et al. 2006).

7.6 Treatment

Anthracycline-based chemotherapies such as CHOP or CHOP-like regimens are not efficient because of high expression of P-glycoprotein. Instead, drugs that are not affected by P-glycoprotein are recommended as a part of a polychemotherapy regimen. L-asparaginase-containing chemotherapies have produced promising outcomes recently. In cases of localized disease, the addition of radiation is important.

7.6.1 Localized Disease

The upfront use of CHOP or CHOP-like regimens produces only a 35–44 % complete response (CR) rate (Kim et al. 2001a, 2003, 2006a; Wang et al. 2007). Frequent early disease progression during chemotherapy has been a problem. This lack of efficacy of

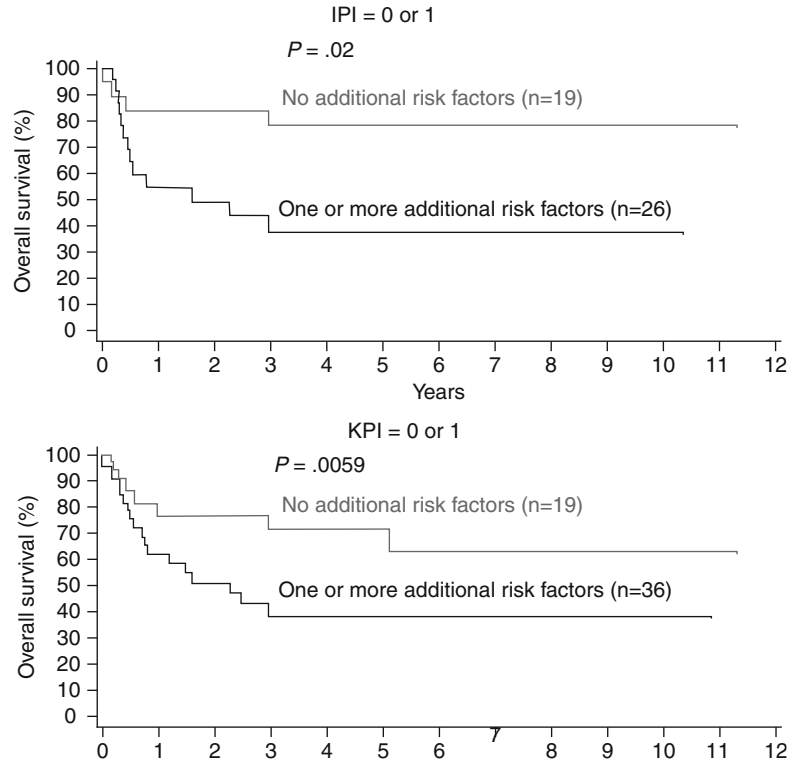

Fig. 7.3 Prognostic models (**a**) IPI form DLBCL: age/stage/extranodal/LDH/PS. (**b**) KPI: B symptom/stage/LDH/lymph node

anthracycline-based chemotherapy is associated with the high expression of P-glycoprotein (Yamaguchi et al. 1995).

The outcome is better for upfront use of radiotherapy than anthracycline-based chemotherapy (Kim et al. 2000; Chim et al. 2004; You et al. 2004). The largest series with radiotherapy alone produced a much higher CR rate (69 %) compared with previously reported CR rates (< 60%) of frontline chemotherapy (Kim et al. 2001b), and the frequency of early disease progression during radiotherapy is also lower than that of chemotherapy (22.2 % vs. 50.8 %) (Cheung et al. 2002). Therefore, radiotherapy is recommended as an initial treatment for localized ENKL (Suzuki et al. 2008; Kim and Kim 2010). However, there is no consensus on the radiation dose. Some studies reported that at least 52 or 54 Gy was required to obtain in-field control in patients with localized ENKL (Sakata et al. 2006; Huang et al. 2008), whereas a study of concurrent chemoradiotherapy showed that a median 40 Gy was enough to control the disease (Kim et al. 2009b). Considering the significant radiation-related toxicity, the adequate radiation dose should be defined more accurately.

Recent phase II trials demonstrated very impressive outcomes with concurrent chemoradiation (Table 7.1) (Kim et al. 2009b; Yamaguchi et al. 2009). In both trials, the response CR rates were >80 %, resulting in ~80 % long-term survival rates. Therefore, concurrent chemoradiation followed by nonanthracycline-based chemotherapy is now recommended as the treatment for localized ENKL.

7.6.2 Advanced and Relapse/Refractory Disease

As mentioned above, anthracycline-based chemotherapy such as CHOP only yielded a response CR rate of <20 % (Lee et al. 2005b; Suzuki et al. 2010). Instead, L-asparaginase has been suggested for the treatment of ENKL because NK/T-lymphoma cells are unable to

Table 7.1 Prospective trials with concurrent chemoradiotherapy for stage I/IIE ENKL

Authors (year)	No.	Treatment	Stage IE/IIE	KPI group[1]	CR	OR	Local/systemic relapse	Main toxicity	OS
Yamaguchi M et al. (2009) [56]	27	Concurrent radiotherapy with chemotherapy (RT-DeVIC) RT: 50 Gy for stage IE, 50.4 Gy for stage IIE CT: 3 courses of DeVIC[2]	18/9	17/10	77%	81%	4%/33%	mucositis	2-year 78%
Kim SJ et al. (2009) [55]	30	Concurrent chemoradiotherapy plus chemotherapy CCRT: RT (Median 40 Gy) with weekly cisplatin 30mg/m^2 CT: 3 courses of VIPD[3]	15/15	21/9	80%	83%	7%/7%	leucopenia	3-year 86%

RT: radiotherapy; CT: chemotherapy; CCRT: concurrent chemoradiotherapy; KPI group: NK lymphoma international prognostic index
This table is excerpted from Kim and Kim (2010)
RT radiotherapy, *CT* chemotherapy, *CCRT* concurrent chemoradiotherapy, *KPI group* NK lymphoma international prognostic index group, *CR* complete response, *OR* overall response, *OS* overall survival
[a]KPI group 1 (no risk factor)/group 2 (1 risk factor) versus group 3 (2 risk factors)/group 4 (3 or 4 risk factors)
[b]DeVIC: Dexamethasone 40 mg D 1–3, etoposide 67 mg/m^2 D 1–3, ifosfamide 1 g/m^2 D 1–3, carboplatin 200 mg/m^2 D 1 every 3 weeks
[c]VIPD: Etoposide 100 mg/m^2 D 1–3, ifosfamide 1.2 g/m^2 D 1–3, cisplatin 33 mg/m^2 D 1–3, dexamethasone 40 mg D 1–4 every 3 weeks

synthesize L-asparagine (Ando et al. 2005). Therefore, agents that are not affected by P-glycoprotein, such as methotrexate and ifosfamide, are recommended as part of polychemotherapy. A recent phase II study of patients with stage IV or relapsed/refractory ENKL demonstrated the efficacy and feasibility of SMILE (steroid, methotrexate, ifosfamide, L-asparaginase, and etoposide) chemotherapy (Yamaguchi et al. 2011). The overall response and CR rates after two cycles of SMILE were 79 and 45 %, respectively. Thus, SMILE chemotherapy can be recommended for both advanced disease and relapsed/refractory disease. L-Asparaginase-based regimens showed outstanding response rates of >80 % in patients with refractory and relapsed ENKL (Yong et al. 2008; Jaccard et al. 2008). Based on the results of these studies, current nonanthracycline-based intensive chemotherapy regimens including L-asparaginase can be recommended as frontline treatment.

7.6.3 Hematopoietic Stem Cell Transplantation (SCT)

High-dose chemotherapy followed by autologous SCT may be considered as a consolidation treatment for patients with a high risk of relapse. Initial experience suggested that patients with a poor prognosis should be considered for autologous SCT at the time of the first CR (Au et al. 2003; Kim et al. 2006b). A recent multinational, matched-control study of 47 patients who underwent autologous SCT suggested that autologous SCT could confer a survival benefit in patients who attained a CR with high-risk scores of KPI at diagnosis (Lee et al. 2008).

Allogeneic SCT may be another appealing treatment option for patients with advanced disease or relapsed/refractory disease. The largest series of 28 patients showed a 2-year overall survival rate of 40 % (Shustov et al. 2010). However, treatment-related mortality may present a hurdle to the application of allogeneic SCT for ENKL. Considering the limited data and heterogeneity of information, a firm recommendation cannot be made for allogeneic SCT in ENKL (Kwong 2010).

7.6.4 Prophylaxis Against Secondary CNS Involvement

Because ENKL frequently affects the nasal cavity and the paranasal area near the CNS, ENKL may have a risk of CNS involvement. Previous studies report a variable incidence of CNS involvement in 0–6 % of ENKL cases (Cheung et al. 1998; Cuadra-Garcia et al. 1999; Kim et al. 2004). Thus, it is unclear whether CNS prophylaxis should be included in the treatment of ENKL. A recent large retrospective analysis reported that 5.76 % (12/208) of cases had CNS involvement and that CNS involvement was associated with the KPI score (Kim et al. 2010). Therefore, routine CNS

evaluation and prophylaxis is not necessary in patients at low risk of KPI. For high-risk patients with KPI, the role of CNS prophylaxis has not been determined yet.

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Cutaneous T-Cell Lymphoma

8

Jasmine Zain, Michael Weichenthal,
Scott Rodig, and Jan Delabie

Contents

Pathology: Scott Rodig and Jan Delabie

J. Zain (✉)
Columbia University Medical Center,
New York, NY, USA
e-mail: jmz45@mail.cumc.columbia.edu

M. Weichenthal
Department of Dermatology,
University of Kiel, Kiel, Germany
e-mail: mweichenthal@dermatology.uni-kiel.de

S. Rodig, MD
Department of Pathology,
Brigham and Women's Hospital,
Harvard Medical School, Boston, MA, USA
e-mail: srodig@partners.org

J. Delabie, MD
Department of Pathology,
Oslo University Hospital, Oslo, Norway
e-mail: jandel@ous-hf.no

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8.1 Introduction

By conceptual definition primary cutaneous T-cell lymphomas (CTCL) constitute a heterogeneous group of non-Hodgkin's lymphomas originating from skin-homing T lymphocytes. Clinically, they appear in considerable variability as cutaneous lesions, but mostly without signs of further nodal or systemic involvement and in many instances they keep confined to the skin for an indefinite time. Historically, mycosis fungoides (MF) and Sézary syndrome (SS) have been the primarily recognized forms of CTCL, and together they account for the majority of cases in this category. The clinical and pathological definition of additional subtypes of CTCL apart from MF and SS has led to an evolving classification of CTCL over the years, finally settled as the consensus classification of WHO and EORTC in 2005 (Willemze et al. 2005) which was then fully incorporated into the 2008 revised WHO lymphoma classification.

The CTCL category now consists of more than a dozen distinct CTCL entities leading to enhanced diagnostic and therapeutic consistency and providing a solid foundation for clinical practice and research (Table 8.1). The knowledge on the etiology of the various CTCL forms is still sparse, but recent findings have enlightened that many of the CTCL subtypes may have a physiological counterpart in the human skin-associated lymphoid tissue.

Improvements in classification and the development of new drugs during the recent years have also led to more differentiated disease and stage-specific treatment options that allow for effective disease control in many cases.

8.2 Mycosis Fungoides

First described in 1818 by Alibert (Alibert 1818), Mycosis fungoides (MF) represents the prototype of CTCL. It is characterized by a variety of more or less specific skin findings that can occur sequentially in a stage-dependent manner but also present synchronously in more advanced stages. It is in most cases a chronic indolent lymphoma and many patients will not progress to a nodal or systemic involvement of the disease.

Table 8.1 Cutaneous T-cell lymphoma (CTCL) subtypes according to WHO 2008 classification (Swerdlow et al. 2008). Their relative frequencies among the CTCLs are mainly derived from the original publication by Willemze et al. (2005)

Indolent subtypes	Frequency (%)	Aggressive subtypes	Frequency (%)
Mycosis fungoides variants and subtypes	61	Sézary syndrome	4
Folliculotropic mycosis fungoides	6	Adult T-cell leukemia/lymphoma	<1
Pagetoid reticulosis	<1	Extranodal NK/T-cell lymphoma, nasal type	<1
Granulomatous slack skin	<1	Primary cutaneous peripheral T-cell lymphoma, unspecified	3
Primary cutaneous CD30+ lymphoproliferative disorders	26	Primary cutaneous aggressive epidermotropic CD8+ T-cell lymphoma (provisional)	<1
Primary cutaneous anaplastic large-cell lymphoma (C-ALCL)	10	Cutaneous γ/δ T-cell lymphoma (provisional)	<1
Lymphomatoid papulosis (LyP)	16		
Primary cutaneous CD4+ small-/medium-sized TCL	3		
Subcutaneous panniculitis-like T-cell lymphoma (provisional)	1		

The diagnosis can be difficult and particularly in the very early stages, repeated biopsies and clinical follow-up may be necessary in order to differentiate it from benign eczematous disorders or certain types of parapsoriasis. Once established, the disease can be managed with a variety of skin-directed and systemic treatment options.

8.2.1 Epidemiology

MF occurs worldwide and it is the most common form of CTCL accounting for approximately 60–75 % of CTCL cases. The incidence of CTCL is estimated to be around 0.3–0.6 per 100,000 persons per year in different countries (Criscione and Weinstock 2007; Saunes et al. 2009). Increases in incidence of CTCL up to 1.0/100,000/year have been reported (Bradford et al. 2009), but the significance of these findings is uncertain. Regarding MF, males are more often affected than females with a ratio of around 1.3–1.6, and the incidence of MF is higher among blacks as compared to white or hispanic patients.

8.2.2 Clinical Presentation

The clinical presentation of MF in many cases follows the classical presentation of Bazin (Bazin 1870) with (1) erythematous patches, (2) plaques, and (3) tumors (Fig. 8.1). While in many cases there is an evolvement from (1) to (2) to (3) over time, all three clinical presentation can be present in parallel in advanced cases.

In the early stage of MF, many patients report a long-standing history of nonspecific erythema without further clinical symptoms. During this phase the histological diagnosis may be hard to establish, and the term "premycotic" stage has been used in this clinical situation. As a complicating fact, the so-called parapsoriasis disorders show some relationship to MF. The term was introduced in the beginning of the last century to

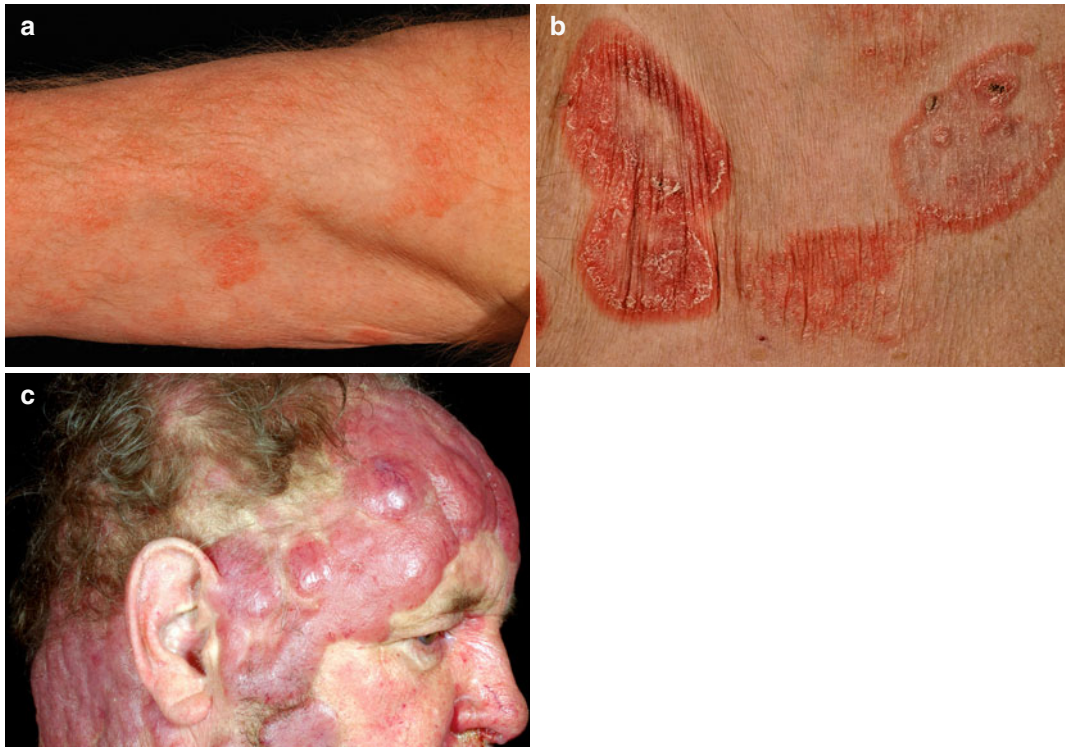

Fig. 8.1 Mycosis fungoides—clinical appearance. (**a**) Patches. (**b**) Plaques. (**c**) Tumor stage

describe a set of skin diseases that bear some similarities to psoriasis, lichenoid dermatosis, and MF while often being chronic, symptomless, and benign. While the large plaque type of "parapsoriasis en plaque" is now regarded as an early form of MF, in many cases the controversy over the so-called small plaque parapsoriasis is ongoing. While some authors tend to call this disease "chronic superficial dermatitis," others consider it as an early and/or abortive form of MF. While the majority will persist as a chronic benign condition, there are several cases of evolution into MF.

The diagnosis of early MF often requires an experienced clinicopathological review of the findings. Frequently, repeated biopsies will be necessary to establish a firm diagnosis.

Traditionally MF stages have been described according to their clinical appearance, including early, eczema-like "patches"; infiltrated plaques; and eventually "fungoid" (mushroom-like) tumors (Fig. 8.1). Erythroderma has been observed in patients with CTCL as part of the clinical spectrum with Sézary syndrome (SS) representing a particular type of erythrodermic CTCL accompanied by gross lymph node involvement and leukemic spread.

8.2.3 Morphology

Patch-stage MF is characterized by a superficial, dermal band-like distribution of small- to intermediate-sized lymphocytes with irregular to cerebriform nuclei, condensed chromatin, and scant cytoplasm (Fig. 8.2) (Smoller et al. 1995). Macrophages may be intermixed with the lymphocytes but granulocytes are rarely seen. Individual lymphoid cells often extend into the lower epidermis (epidermotropism). The epidermal keratinocytes show only minimal change in response to the infiltrating tumor cells. Plaque-stage MF is characterized by more extensive involvement of the epidermis by the neoplastic cells, often collected within small microabscesses (Pautrier microabscesses) (Smoller et al. 1995; Nickoloff 1988). Tumor-stage disease is characterized by a pronounced dermal proliferation of atypical lymphocytes (Diamandidou et al. 1998). Morphologic variants include folliculotropic MF, which targets hair follicles and generally spares the epidermis, and pagetoid reticulosis (Woringer-Kolopp disease) which shows a marked epidermotropism and a generally good prognosis (Haghighi et al. 2000). When >25 % of the lymphoid infiltrate consists of large-sized cells, CTCL is considered as having undergone large-cell transformation (Diamandidou et al. 1998). Lymph node involvement or extensive peripheral blood involvement is associated with higher stages of disease and are poor prognostic indicators (Willemze et al. 2005).

8.2.4 Differential Diagnosis

The sparse lymphoid infiltrate that characterizes skin lesions in early patch-stage MF can be

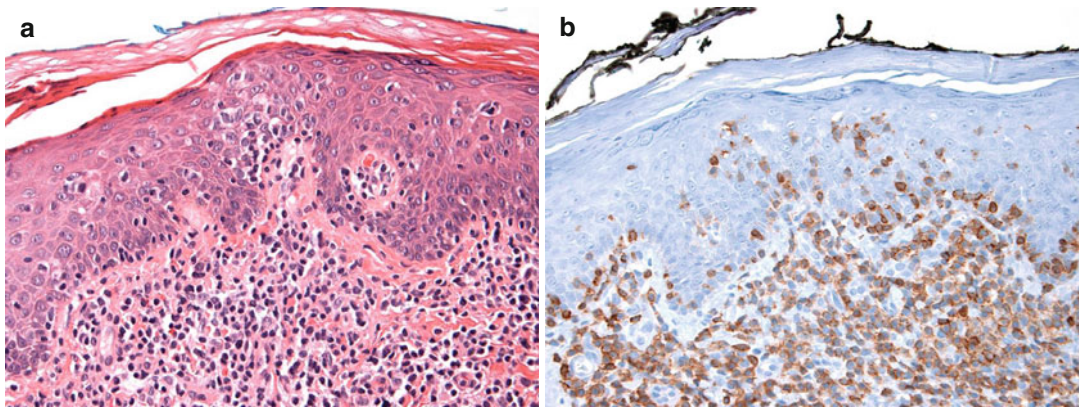

Fig. 8.2 Mycosis fungoides—histology. (**a**) Stained with hematoxylin and eosin stains and showing a superficial dermal and intraepidermal infiltrate of small lymphocytes and (**b**) stained with anti-CD3 antibody and showing a predominance of T cells

difficult to distinguish from a normal, reactive immune response. However, a mixed cellular infiltrate consisting of plasma cells, granulocytes, and B lymphocytes in addition to T lymphocytes is more characteristic of an immune response than MF. Difficult cases can be further evaluated by molecular testing for T-cell receptor (TCR) clonality. Advanced-stage or transformed MF that is characterized by a profound infiltrate of highly atypical lymphoid cells can be difficult to distinguish from cutaneous anaplastic large-cell lymphoma (C-ALCL); lymphomatoid papulosis (LyP); cutaneous involvement by peripheral T-cell lymphoma, not otherwise specified (PTCL, NOS); or cutaneous involvement by ALK-negative, anaplastic large-cell lymphoma (ALK-negative ALCL). Careful assessment of the patient's medical history, tumor staging, and previous pathology is necessary to distinguish among these diagnostic possibilities.

8.2.5 Genetics

The neoplastic lymphocytes of MF, SS, C-ALCL, and up to 60 % of LyP demonstrate a clonal rearrangement of their T-cell receptor genes by PCR analysis (Ponti et al. 2005). Primary gamma-delta T-cell lymphoma (PGD-TCL) shows clonal rearrangement of the TCRδ locus (Przybylski et al. 2000).

Karyotypes of tumor cells from advanced SS often reveal complex abnormalities. Losses of TP53, p16ink4a, and PTEN genes have been associated with disease progression. However genetic lesions specific to MF and SS have yet to be described (Mao et al. 2003). In contrast, between 20 and 30 % of C-ALCL demonstrate rearrangement of the IRF4 locus by fluorescent in situ hybridization (FISH) using specific probes. This genetic lesion is only rarely seen in LyP and transformed MF, and not seen in systemic ALCL (Wada et al. 2011).

8.2.6 Staging

In 1979, the WHO/UICC and AJCC published a staging system based on what was called the Mycosis Fungoides Cooperative Group (MFCG) classification (Mycosis fungoides cooperative study 1975), which was modified by the EORTC and the International Society for Cutaneous Lymphoma (ISCL) in 2007 (Olsen et al. 2007) and eventually adapted by the TNM staging manual in 2010 (Table 8.2A) (Edge et al. 2010).

The skin (T) status includes patches and plaques grouped together and graded by the extent of body surface area (BSA) involvement (T1 < 10 %; T2 ≥ 10 % BSA). Tumor stage (T3) and erythroderma (T4) will lead to an upstaging of the disease. Remarkably, only a minority of patients will undergo a formal transition to T3 and consequently T4. Rather, the development of tumors (T3) is associated with further nodal or visceral organ involvement in a proportion of cases, while most T4 cases develop directly from T1/T2 and are associated with the risk of blood (B) involvement, sometimes called "secondary" Sézary syndrome (Quaglino et al. 2012).

For nodal involvement a revised grading system has to be applied (Table 8.2B) either using the NCI-LN (Sausville et al. 1985; Vonderheid et al. 1994b) or the so-called Dutch system (Scheffer et al. 1980). They both take into account the observation that enlarged lymph nodes in MF might represent an initially benign infiltration of what has been called the dermatopathic lymphadenopathy which can also be observed in inflammatory skin disease. Nevertheless, the presence of dermatopathic lymphadenopathy is a significant predictor for the development of further nodal progression (Quaglino et al. 2012) and therefore leads to upstaging.

The combined, cutaneous (T), nodal (N), visceral (M), and blood (B) involvement will lead to assessment of different clinical stages highly predictive of prognosis (Table 8.2C) (Agar et al. 2010).

8.2.7 MF Subtypes

The clinical variants of the classical clinical presentation of MF show a broad range of different clinical pictures. One of the very early descriptions of MF variants is that of disease occurring primarily as tumors thereby sparing the patch/plaque stages, formerly termed MF "d'emblée"

Table 8.2 Staging of mycosis fungoides and sezary syndrome as adapted by 2010 TNM maual

A: **ISCL/EORTC TNM classification**				
Skin				
T1	Limited patches, papules, and/or plaques covering <10 % of the skin surface.			
	May further stratify into T1a (patch only) vs. T1b (plaque ± patch)			
T2	Patches, papules, or plaques covering ≥10 % of the skin surface. May further stratify into T2a (patch only) vs. T2b (plaque ± patch)			
T3	≥1 Tumor (≥1 cm diameter)			
T4	Confluence of erythema covering ≥80 % of body surface area			
Nodes				
N0	No clinically abnormal peripheral lymph nodes; biopsy not required			
N1	Clinically abnormal peripheral lymph nodes; pathology Dutch grade 1 or NCI LN0–2. May further stratify into N1a (clone negative) vs. N1b (clone positive)			
N2	Clinically abnormal peripheral lymph nodes; pathology Dutch grade 2 or NCI LN3.			
	May further stratify into N2a (clone negative) vs. N2b (clone positive)			
N3	Clinically abnormal peripheral lymph nodes; pathology Dutch grades 3–4 or NCI LN4; clone positive or negative			
Nx	Clinically abnormal peripheral lymph nodes; no histologic confirmation			
Visceral involvement				
M0	No visceral organ involvement			
M1	Visceral involvement (pathology confirmation and organ involved should be specified)			
Blood involvement				
B0	Absence of significant blood involvement: ≤5 % of peripheral blood lymphocytes are atypical (Sézary) cells.			
	May further stratify into B0a (clone negative) vs. B0b (clone positive)			
B1	Low blood-tumor burden: >5 % of peripheral blood lymphocytes are atypical (Sézary) cells but does not meet the criteria of B2			
	May further stratify into B1a (clone negative) vs. B1b (clone positive)			
B2	High blood-tumor burden: ≥1,000/μL Sézary cells with positive clone			
B: **Revised nodal staging for lymph node involvement in MF/SS**				
	Dutch system		*NCI Grading*	
N1	Grade 1: dermatopathic lymphadenopathy (DL)		LN0: no atypical lymphocytes	
			LN1: occasional and isolated atypical lymphocytes (not arranged in clusters)	
			LN2: many atypical lymphocytes or in 3-6 cell clusters	
N2	Grade 2: DL w/ early involvement by MF (presence of cerebriform nuclei > 7.5 μm)		LN3: aggregates of atypical lymphocytes; nodal architecture preserved	
N3	Grade 3: partial effacement of LN architecture; many atypical cerebriform mononuclear cells (CMCs)		LN4: partial/complete effacement of nodal architecture by atypical lymphocytes or frankly neoplastic cells	
	Grade 4: complete effacement			
C: **Clinical staging schema**				
	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
IIIA	4	0-2	0	0
IIIB	4	0-2	0	1
IVA1	1-4	0-2	0	2
IVA2	1-4	3	0	0-2
IVB	1-4	0-3	1	0-2

Revised classification rules for mycosis fungoides (*MF*) and Sézary syndrome (*SS*) (Olsen et al. 2007); (Scheffer et al. 1980) or NCI criteria (Colby et al. 1981)

(Vidal and Brocq 1885). Meanwhile these forms are mainly considered as representing either pleomorphic CTCL, anaplastic CD30-positive CTCL, or as CTCL, unspecified, and the term "d'emblée" is abandoned by most authors (Keehn et al. 2007; Olsen et al. 2007). During the past decades a wide range of additional clinical and/or histopathological subtypes have been described ranging from only subtle clinicopathological variations of classical MF to distinct entities that deserve particular diagnostic and therapeutic attention.

In the following sections we will describe the variants that have a significant impact on diagnosis, prognosis, or treatment.

8.2.7.1 Folliculotropic MF

Patients with folliculotropic MF (fMF) exhibit a particular pattern of neoplastic infiltration of the skin. Hair follicles are predominantly affected and the invasion of the hair follicle epithelium leads to characteristic clinical findings including acne-like cysts and comedones as well as a certain pattern of hair loss which is histologically often accompanied by mucin deposits, so-called mucinosis follicularis. While follicular mucinosis has sometimes been described as being a specific finding in fMF, there are clear examples of nonmalignant follicular mucinosis in the literature, in particular in children (Zvulunov et al. 2012). Conversely, fMF may occur without signs of mucinosis (van Doorn et al. 2002).

There is a suggestion that fMF has a worse prognosis as compared to classical MF which may be due to the inaccessibility of the deeper hair follicle epithelium to skin-directed treatments (van Doorn et al. 2000, 2002).

8.2.7.2 Pagetoid Reticulosis/ Unilesional MF

Historically, the term pagetoid reticulosis was referred to as a specific infiltration pattern of this subtype of T-cell lymphoma. However, lately it has been distinguished into a localized (Woringer-Kolopp) and a disseminated (Ketron-Goodman) variant. Nowadays, the latter term is obsolete and this subtype is generally included in the classical MF, as the histological features are not as distinctive, and some cases that were reported would now be classified as CD8-positive cytotoxic CTCL. Likewise, there is no sharp distinction between pagetoid reticulosis and cases that have been published as unilesional MF, since they apparently lacked the histopathological features of pagetoid reticulosis.

Typically the unilesional variants including classical pagetoid reticulosis present as a single plaque with predilection for the lower leg as a site. This is a chronic disease with no propensity to spread systemically and has an excellent prognosis (Steffen 2005).

8.2.7.3 Granulomatous MF and Granulomatous Slack Skin

Granulomatous forms of MF have repeatedly been reported, and in 1978 a granulomatous variant with particular features was named "granulomatous slack skin" (GSS) (Ackerman 1978), sometimes also called "cutaneous elastolytic lymphoma." Clinically the disease shows features of cutis laxa with abundant skin folds overlying the infiltrative process. It is often observed in the big flexures of the axillary or inguinal regions and the course is usually mild. However, a remarkable association with preceding, synchronous, or subsequent Hodgkin's or other lymphoproliferative disease including classical MF has been reported (Clarijs et al. 2003).

It could be shown in a larger series of cases that despite some clinical differences, granulomatous MF and GSS show an overlapping histological spectrum. Usually there is a diffuse dermal infiltrate of small or small- to medium-sized lymphocytes with cerebriform nuclei. Epidermotropism is often absent. Sarcoid-like granuloma formation and scattered multinucleated giant cells are prominent along with loss of elastic fibers and phagocytosis of elastic fibers by histiocytic giant cells. No feature was found to discriminate between granulomatous MF and GSS based on histological findings alone (Kempf et al. 2008).

8.3 Sézary Syndrome

Sézary and Bouvrain described this CTCL variant in 1938 based on the classical triad of (1) erythroderma, (2) generalized lymphadenopathy,

and (3) leukemic spread of a particular type of neoplastic cells (Sézary and Bouvrain 1938). The definition of Sézary syndrome (SS) has been changing over the years, and newer technologies and biomarkers have been introduced to differentiate SS from erythrodermic MF.

8.3.1 Epidemiology

Sézary syndrome accounts for approximately 5 % of CTCL cases and affects mainly the elderly population with a mean age at presentation of 66 years. Like in MF, there is an approximately 1.6:1 preponderance for males and unlike MF caucasians are more likely to be affected than individuals of colored skin (Kubica et al. 2012).

8.3.2 Clinical Presentation

The clinical presentation includes erythroderma with a total body surface involvement of 80 % or more (Fig. 8.3). Frequently palmoplantar hyperkeratosis, hair loss, and extensive nail changes can be found. Pruritus, virtually present in all patients, is often much more pronounced than in other forms of CTCL. Some patients present with concurrent cutaneous tumors or plaques or have a history of an MF diagnosis, though it has been a matter of debate whether these patients have "true" SS.

The distinction between erythrodermic MF and Sézary syndrome has been notoriously difficult, and the discussion whether SS represents a particular variant of MF was a long-standing matter of debate. Recently it has been shown that both MF and SS seem to develop from different precursor cells (Campbell et al. 2010). In MF the phenotype of the malignant clone is compatible with an effector memory T cell baring additional skin-homing receptors which may explain the long-standing confinement of MF to the skin and eventually the skin-draining lymph nodes. In contrast, a central memory T cell-like phenotype can be found in Sézary syndrome, suggesting that both MF and SS are different entities. As a consequence, erythrodermic MF is also assigned a different disease stage than a fully developed Sézary syndrome.

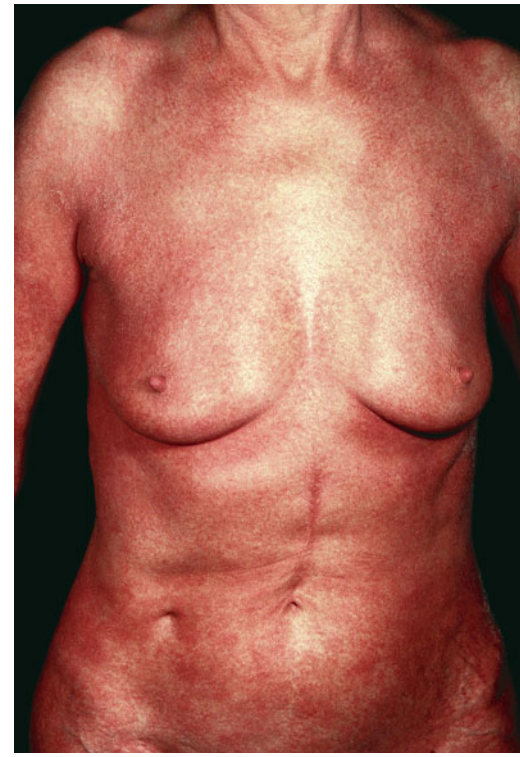

Fig. 8.3 Sézary syndrome—clinical appearance

8.3.3 Morphology

The neoplastic cells, like those in MF, consist of small- to intermediate-sized lymphoid cells with convoluted or "cerebriform" nuclei, condensed chromatin, inconspicuous nucleoli, and scant cytoplasm. Despite widespread skin involvement, epidermotropism may or may not be prominent. Involved lymph nodes show gross replacement of the normal architecture by tumor cells, but bone marrow may demonstrate only a sparse, interstitial infiltrate (Scheffer et al. 1986).

8.3.4 Prognosis

The prognosis of SS is much worse than for MF with a 5-year survival rate being reported between 10 and 50 %. Much of the heterogeneity regard-

ing the incidence and prognosis is thought to be related to the diagnostic criteria differentiating SS from erythrodermic forms of MF. The recent revision to the staging of MF and SS has given consensus based diagnostic criteria especially for the grading of blood involvement. Following this definition, significant blood involvement requires at least 1,000/μl tumor cells, either by blood smear counts of "Sézary cells" or by quantifying a characteristic aberrant population via FACS analysis.

Using strict diagnostic criteria for the diagnosis of SS, the prognosis remains poor despite new treatment options with a median survival of 4 years after diagnosis (Kubica et al. 2012).

8.4 Treatment of Mycosis Fungoides and Sézary Syndrome

Early aggressive therapy is not warranted in the management of MF as it has not been shown to impact survival (Kaye et al. 1989). Hence, early-stage disease can often easily be managed for years by topical therapies that can improve symptoms and skin appearance. These treatments are most appropriate for early-stage T1 and T2 disease states and can also be combined with systemic treatments for late-stage disease.

8.4.1 Skin-Directed Treatment

8.4.1.1 Topical Steroids

Topical or intralesional steroids are a mainstay of initial therapy for many patients with early patch-/plaque-stage disease and can provide good control for several years (Zackheim et al. 1998). Topical application to the sites of disease ensures minimal systemic absorption and side effects. Highly potent group I (US system) or class IV (non-US) steroids are the best option and it is recommended that they be applied vigorously to the lesions twice a day. Occasionally it is beneficial to use occlusive therapy especially at night in addition to the topical therapy. The treatment should be continued for at least 2–3 months to assess maximal response. At least one prospective study has looked at the response rates of topical steroids in MF patients and has reported complete response rates of 60–65 % in T1 disease and a partial response of 30 % and a CR rate of 25 % and PR of 57 % in T2 disease (Zackheim et al. 1998). Intralesional steroids can be used in the treatment of thicker lesion or tumor deposits.

8.4.1.2 Topical Cytotoxic Agents

This includes topical mechlorethamine (nitrogen mustard) and topical carmustine. The former is used more often usually in an aqueous or ointment preparation that can be cumbersome to prepare and apply. Care must be taken to avoid contact with family members and other house hold contacts. Long-term remissions lasting 4–14 years have been documented with aggressive topical therapy including a maintenance schedule for stage 1A and 1B (Vonderheid et al. 1989) but carries the risk of skin irritation and secondary malignancies. Topical nitrogen mustard has been used in sequentially or in combination therapy with TSEB (Price et al. 1977), systemic chemotherapy, and following other treatments as maintenance.

8.4.1.3 Topical Bexarotene

This is a useful therapy for patients with a limited number of patch-/plaque-type lesions (Breneman et al. 2002). The recommended dose is a 1 % gel applied twice a day to the affected areas. There are no systemic side effects and any adverse events are mild and limited to the site of application. These include skin irritation and generally increase with gel exposure. Overall response rates are around 63 % with a CR of 21 % with a median time to respond of 20.1 weeks (range 4.0–86).

8.4.1.4 Other Topical Agents

These include agents like imiquimod, an immune response modifier that is a potent inducer of interferon alpha at the site of administration. A response rate of 50 % has been demonstrated with the topical use of this agent in early-stage MF (Deeths et al. 2005).

8.4.1.5 Phototherapy

PUVA and UVB are the two most common forms of phototherapy used in the treatment of MF

usually for widespread disease that has failed to respond or is too extensive for topical therapy. As the main mechanism of action, induction of apoptosis of exposed cells is assumed in both modalities (Weichenthal and Schwarz 2005).

In PUVA, ingested psoralen is activated by exposure to UV light at a wavelength range of 320–400 nm resulting in its binding covalently to DNA forming bifunctional adducts to pyrimidine bases. This results in lymphocyte toxicity and a decrease in the number of helper T cells. Response rates are over 95 % with CR rates of 58–83 % (Berthelot et al. 2008). A taper and maintenance schedule is recommended after the initial therapy and responses can last for a median duration of 43 months. Side effects include nausea, photosensitivity accelerated photodamage to skin, and an increased risk of melanomas and squamous cell malignancies of the skin (Lindelöf et al. 1999).

Narrowband UVB (NBUVB) at 311 nm does not require the use of a sensitizing agent and suppresses Langerhans cells and cytokine production and has largely replaced the use of broadband UVB (290–320 nm). NBUVB is more readily available and avoids the side effects of psoralen, i.e., nausea and sun photosensitivity. It is less effective in thicker lesions as compared to PUVA. Phototherapy can be combined with other therapies, including interferon and retinoids, and has a role as maintenance therapy after the use of other modalities like chemotherapy (Rupoli et al. 1999; Stadler et al. 1998; Quiros et al. 1997).

8.4.1.6 Radiation Therapy

CTCL are radiosensitive tumors, and for most patch-/plaque-stage disease, the target volume of treatment, i.e., epidermis and dermis, can be only a few mm in depth, meaning that most lesions can be treated with low-penetrance beams like 50–145 kvp or 4–9 MeV electron beams. Deeper lesions like tumors and ulcers require higher energy beams. The dose of radiation is determined by the goals of treatment. Effective palliation of lesions can be achieved by 15–20 Gy though there is a dose response effect and higher doses are required to completely clear the lesions. Durable remissions after RT alone are rare except in cases of T1 lesions in a bathing trunk distribution that can be "cured" with long-term remission with RT doses of up to 30cGY. The 5-year relapse-free survival after RT is 40–60 % for T1 disease but less than 10 % for T4 disease. XRT is an excellent option for palliation and pain control of large tumors and ulcerated lesions. Radiation can be given concurrently with many other agents including retinoids, antibodies, and several chemotherapy agents though the dose of these agents may need to be modified.

8.4.1.7 Total Skin Electron Beam (TSEB) Therapy

This is an effective palliative strategy for patients with extensive skin and blood involvement. Best results are seen at doses of 2,500–3,000 Gy given on a fractionated regimen of 32–36 Gy with appropriate shielding over a time period of 9 weeks given at centers that are experienced in the technique. Side effects include skin erythema, hair loss, and nail dystrophy. Some patients will also have decreased sweating and changes in body temperature control that may be long lasting. Combination of TSEB with chemotherapy has been studied with the best results seen in patients receiving chemotherapy followed by TSEB.

8.4.2 Systemic Treatment

Once skin-directed therapies fail or if the disease is advanced (IIB and beyond), it becomes necessary to start systemic therapies. The principles of treatment are to minimize immunosuppression, reduce the risk of infections, and palliate symptoms. The disease remains largely incurable unless the patient undergoes an allogeneic stem cell transplant; hence it is prudent to select therapies that can be given for prolonged periods of time and have minimal side effects. Many of the treatments can be used in a recurrent setting and combination therapies are encouraged in progressive disease. Consensus-based guidelines are

used to determine treatment options in a given situation.

8.4.2.1 Biologic Agents

Interferon

Interferons are a class of TH1 cytokines and function as immune modifiers. Recombinant interferons are a good therapeutic option for patients with mycosis fungoides for all stages of disease including SS. Alpha interferon is the most common formulation available for clinical use and can be given subcutaneously or intramuscularly and has also been used for intralesional injections. The usual dose is three million to ten million units given subcutaneously in various schedules that range from three times a week to daily dosing. Data from studies involving more than 12 patients have reported partial response rates of 17–53 % and complete remission rates of 4–27 %. These studies included varying stages of disease (Olsen 2003), and the responses were higher if this was the first line of systemic therapy (Bunn and Norris 1990). It has limited and reversible dose-dependent side effects that include fever, chills, influenza-like symptoms, myalgias, and arthralgias. More chronic effects include fatigue, anorexia, weight loss, sleep disturbance, and hepatitis. Alpha Interferon can be used alone or can be combined with other treatment modalities to improve response rates and outcomes. These include extracorporeal photopheresis (Olsen et al. 1989), low-dose chemotherapy (Foss et al. 1992, 1994), retinoids (Stadler et al. 1998; Knobler et al. 1991), and phototherapy (Rupoli et al. 1999). While alpha interferon is the most commonly used formulation, there is data using interferon gamma as well in the treatment of MF resulting in prolonged responses (Kaplan et al. 1990).

Interleukins

Interleukins as immune response modifiers that can be used in the treatment of MF. Interleukin-2 and interleukin-12 have been used in clinical trials with good responses, but the excessive toxicity and limited availability make them impractical for general use (Duvic et al. 2006c).

8.4.2.2 Thalidomide-Derived Immunomodulatory Drugs (IMiDs)

Lenalidomide is currently being used in various hematological malignancies and solid tumors. The mechanism of action is unknown but appears to be immune-mediated with stimulation of T- and NK cell function, induction of Th1 cytokine production, and cytotoxic activity. A phase II trial in relapsed CTCL showed a RR of 32 % with partial remissions and stabilization of disease for a median of 5 months. A decrease was noted in the number of CD4+ T cells and CD4+ CD25+ T-regulatory cells and seemed to correlate with response (Querfeld et al. 2011). The main side effects are myelosuppression and an increased incidence of thrombotic events.

8.4.2.3 Proteasome Inhibitors

Proteasome inhibitors are a new group of anticancer agents that block the proteasome degradation system resulting in effects on cell survival pathways and apoptosis. The following 2 proteasome inhibitors are in clinical use for MF/SS.

Bortezomib

Bortezomib is a dipeptide boronic acid that binds the catalytic site of the 26S proteasome with high affinity and specificity (Bonvini et al. 2007). This agent is used in the treatment of MF/SS but the data supporting its use is limited to one study of ten patients. Bortezomib was administered at a dose of 1.3 mg/m^2 IV on days 1,4,8, and 11 every 21 days for 6 cycles, and an ORR of 70 % was noted with 1 CR lasting over 12 months (Zinzani et al. 2007). The main side effects associated with its use are myelosuppression, particularly thrombocytopenia, and sensory neuropathy that was seen in 50 % of the patients treated on the MF study. Other effects include diarrhea, asthenia, and headaches. A subcutaneous route of administration is being explored in other diseases and has shown to be just as efficacious but compared to IV infusions is associated with a lower incidence of neuropathy (38 % vs. 53 % $P = 0.044$) (Moreau et al. 2011). Similar trials are warranted in MF.

Carfilzomib

Carfilzomib irreversibly binds to and inhibits the chymotrypsin-like activity of the 20S proteasome, an enzyme that degrades unwanted cellular proteins. It is very well tolerated and can be administered for prolonged time periods without significant effects of neuropathy or myelosuppression. Current trials are under way to establish its activity in this group of lymphomas in combination with other agents (ClinicalTrials.gov Identifier: NCT01276717, (Dasmahapatra et al. 2011).

8.4.2.4 Retinoids

Retinoids are derivatives of vitamin A that bind to retinoid receptors in the nucleus and trigger downstream events of transcription, cell differentiation, and apoptosis (Mukherjee et al. 1997). Retinoid receptors come in two major flavors, i.e., retinoic acid receptor (RAR) and the retinoid X receptor (RXR) with isotypes (*a,b,gamma)* that vary in the degree of expression in different tissues. Skin tissues express both RAR and RXR receptors and various retinoids are in use for various skin disorders including MF and SS.

Bexarotene

This is a synthetic retinoid that selectively binds to the RXR receptors and is formulated both as an ointment for topical use as well as an oral formulation. Both forms are approved for the treatment of MF/SS both in the USA and Europe for both early-stage disease and advanced disease including SS (Talpur et al. 2002; Breneman et al. 2002; Duvic et al. 2001b; Gniadecki et al. 2007). Response rates and side effect profiles are dose dependent. In phase II/III studies response rates of 54 % were observed at a dose of 300 mg/m^2 per day and up to 67 % at higher doses (Duvic et al. 2001b) in early-stage MF (stage I–IIA). For advanced-stage disease (IIIB–IVB) the response rate was 48–55 % (Duvic et al. 2001b). The median time to respond was noted to be 8.1 weeks (range 4–16) and 25.7 weeks (2–28), respectively. Main side effects are reversible hyperlipidemia and hypercholesterolemia occurring within 2–4 weeks of initiating therapy that often require therapy with lipid-lowering agents, a decrease in thyroid-stimulating hormone (TSH) resulting in reduced levels of T4, hepatitis, anemia, leucopenia, headache, and dry skin. Bexarotene is contraindicated in pregnancy due to its effect on fetal development (Duvic et al. 2001a, b).

Other Retinoids

These are non-RXR selective and include oral etretinate, arotinoid, acitretin, and isotretinoin (13-*cis*-retinoic acid). There are no comparative trials but overall response rates based on studies range from 5 to 65 % either as single agents or in combination with PUVA, interferon, or cytotoxic chemotherapy (Burg and Dummer 2000; Zachariae et al. 1982; Stadler et al. 1998; Thomsen et al. 1989; Knobler et al. 1991).

8.4.2.5 Antibodies

Alemtuzumab (Anti-CD52)

Alemtuzumab is a humanized monoclonal antibody targeting CD52 on the surface of lymphocytes that has activity against many T-cell lymphoproliferative disorders (Piccaluga et al. 2007; Rowan et al. 1998). Alemtuzumab is thought to mediate its effects through antibody-dependent cellular toxicity and activation of complement-dependent and complement-independent cytolysis (Rowan et al. 1998; Dyer et al. 1989). Initial trials in heavily pretreated patients with MF and SS have reported response rates of 55 % with 31 % CRs (Lundin et al. 2003) and a duration of response of less than 12 months. Another small trial of eight patients reported a response rate of only 31 % with a median duration or response lasting 4 months (Kennedy et al. 2003). Main toxicity is hematological and an increase incidence in infections including CMV and EBV reactivation. Alternative dosing and routing schedules have been attempted including subcutaneous administration to reduce the associated toxicity (Bernengo et al. 2007; Zinzani et al. 2005; Querfeld et al. 2009). Alemtuzumab has shown particularly high-response rates of 86–87 % with a CR seen in 37 and 21 % cases, in small studies focused on patients with erythroderma and SS (Lundin et al. 2003; Bernengo et al. 2007; Querfeld et al. 2009), indicating that

this may be an effective therapy in otherwise difficult to treat patients with SS.

Zanolimumab (Anti-CD4)

Zanolimumab (HuMax-CD4) is a humanized monoclonal antibody directed against CD4 expressed universally on helper T cells and blocks the interaction of CD4 receptor and the major histocompatibility complex class II on cells thus preventing the activation of the T cell. It results in cell death via antibody-dependent cellular toxicity (ADCC) but does not induce complement-dependent cytotoxicity (CDC). It results in depletion for CD4-expressing T cells and has been studied in the setting of MF and SS. Two simultaneous phase 2 multicenter trials were conducted in patients with CTCL, one for early-stage disease and the other for advanced disease. Responses were seen in patients with MF and SS, with a median response rate of 56 % and a median duration of response at 81 weeks with more responses noted at the higher dose level. The agent was well tolerated with mild eczema and low-grade infections in spite of effective lowering of the CD4 count in patients (Kim et al. 2007). Further evaluation of this promising agent is warranted.

Anti-CCR4

CCR4 is a chemokine receptor expressed on CD4+ helper T cells and regulatory cells (Tregs) and in varying proportions in T-cell malignancies (Imai and Umezu 1999; Iellem et al. 2001; Ito et al. 2009). KW-0761 is a humanized monoclonal antibody directed against CCR4 that has a defucosylated Fc region that enhances the ADCC due to increased binding affinity to the Fc gamma receptor on cells. A phase 1 study of the antibody given once a week for 4 weeks indicated promising responses in CTCL (Yamamoto et al. 2010).

8.4.2.6 Conjugated Antibodies

Denileukin Diftitox

Denileukin diftitox (Ontak) is a novel fusion protein consisting of the membrane translocation sequence for the diphtheria toxin and the receptor-binding sequence of the human interleukin-2 that has affinity for the human IL-2 receptor (Williams et al. 1990; Taniguchi and Minami 1993). Initial phase I/II trial confirmed the antitumor activity of denileukin diftitox in patients with CTCL (Saleh et al. 1998). A phase III trial comparing two dose levels of denileukin diftitox at 9 and 18 μg/kg given daily for 5 days every 21 days was conducted in patients with CD25-expressing CTCL and SS. This trial led to the accelerated approval of the agent by the FDA in the USA for the treatment of relapsed and refractory CTCL with more than 25 % expression of CD25. A response rate of 30 % (20 % PRs and 10 % CRs) was reported in the trial. The median duration of response was 6.9 months (2.7–46.1 months) with no difference between the two dose levels. The main side effects were flu-like symptoms, infusional sensitivity reactions, vascular leak syndrome, hypoalbuminemia, and transaminitis with a statistical hint that the side effects may be worse in the higher-dose arm (Olsen et al. 2001). A second placebo-controlled phase III trial was conducted to evaluate the efficacy of the two dose levels of denileukin diftitox, i.e., 9 and 18 μg/kg, compared with placebo in CD25-expressing CTCL and SS patients who had received up to three prior systemic therapies (Prince et al. 2010). One hundred and forty-four patients were enrolled. The ORR was 44 % (34 % PRs, 10 % CRs) with the response rate being higher in the 18μg/kg group, i.e., 49 % vs. 37.8 % in the 9μg/kg group vs. 12 % in the placebo group. Progression-free survival was 124 days better in both dose groups as compared to placebo. There was no difference in the side effect profile at the two doses. This led to the full approval of the agent in 2010 for the treatment of CTCL if there is expression of CD25. The recommended dose of the agent is either 9 or 18μg/kg and is left to the discretion of the treating physician. There is a black box warning in the label for fatal vascular leak syndromes and loss of visual acuity and color vision which may not be reversible. Combination therapies have been evaluated, the most notable being the combination of denileukin diftitox with bexarotene (Foss et al. 2005). The combination was well tolerated with an overall response rate of 67 %. The study also

demonstrated that even low doses of bexarotene at 150 mg/day were capable of inducing upregulation of CD25 expression which may have led to the higher response rate.

Brentuximab Vedotin

Brentuximab vedotin is an antibody conjugate consisting of a chimeric monoclonal antibody that targets CD30 (a member of the transmembrane tumor necrosis factor family of proteins) linked to the antimitotic agent monomethyl auristatin E (MMAE). The binding of the agent to CD30 results in internalization of the compound which is then released intracellularly—mitosis is interrupted and the cell undergoes apoptosis. The agent is approved at a dose of 1.8 mg/kg given once every 3 weeks for CD30+ anaplastic large-cell lymphoma including the cutaneous variant of the disease. The response rate using this agent in the relapsed setting is 87 % in ALCL. Side effects are tolerable with the most common being sensory and motor neuropathy. Variable CD30 expression is seen in MF and up to 41 % of the time in the transformed MF (Arulogun et al. 2008). Hence, it is likely that there will be efficacy of this agent in CD30-expressing MF and SS. Early trials are encouraging and a phase III randomized trial is being conducted in CD30-expressing MF patients who need systemic therapy that will compare brentuximab vedotin with standard-dose methotrexate or bexarotene (physician's choice) in the comparator arm.

8.4.2.7 HDAC Inhibitors

Targeting histone acetylation processes has shown to be an important therapeutic intervention for the treatment of T-cell lymphomas and CTCL in particular (Bhalla 2005; Zain et al. 2010). While the exact mechanism of action is still unknown, most of these agents have shown remarkable antitumor activity in these diseases as well as clinical benefits like the effect on pruritus. While many HDAC inhibitors are in clinical trials, two are already approved by the FDA in the USA for the treatment of CTCL in the relapsed setting. A brief description of these follows below.

Vorinostat

Belongs to the class of hydroxamic acids and has both oral and IV formulations that inhibits both class I and II histone deacetylases. The recommended dosing schedule for CTCL is 400 mg orally once a day with dose adjustments recommended for toxicities. In the pivotal phase 2 trial that led to the approval of this agent, the overall response rate was 24 % with a 58 % reduction in pruritus (Olsen et al. 2001; Duvic et al. 2007). Responses were seen across all stages of diseases for stage IIB or higher. The most common toxicities are gastrointestinal, constitutional symptoms, dysgeusia, and hematological especially reversible thrombocytopenia (Mann et al. 2007). Long-term therapy with vorinostat in patients with stable disease or partial responses is feasible with manageable toxicity (Duvic et al. 2009). Combination studies using vorinostat have been conducted with promising results including a phase 1 trial of the combination of bexarotene and vorinostat (Dummer et al. 2008). Case reports of patients receiving vorinostat in addition to their ongoing therapies to improve responses have included combinations with IFN-α, phototherapy, and photopheresis (Geskin 2010).

Romidepsin

Romidepsin (FR901228, FK228, depsipeptide) is a potent HDAC inhibitor belonging to the class of cyclic peptides that mainly inhibits HDAC1 and HDAC2 class I enzymes has an intravenous formulation and is approved in the USA for the treatment of relapsed and refractory CTCL after failing at least one prior systemic therapy. The prescribing dose is 14 mg/m^2 given over a 4-h infusion once a week for 3 weeks followed by a 1-week rest. Two independent phase II trials (Piekarz et al. 2009; Coiffier et al. 2012) have been conducted, and the pooled data from both these trials has shown an ORR of 34 % with a median duration of response of 15 months (Demierre 2009). One of the striking features of romidepsin is the long duration of response that extended beyond 3 years, observed in some patients even after discontinuation of the drug. Main side effects were nausea, asthenia, anorexia,

vomiting, and fatigue. The drug needs to be administered with caution in patients with significant preexisting cardiac abnormalities and concomitant medications that prolong QT interval or inhibit CYP3A4. A topical formulation of romidepsin is currently in clinical trials in limited stage CTCL.

Other HDAC Inhibitors

At least two other HDAC inhibitors are currently undergoing investigation for efficacy in the treatment of CTCL/MF with indications of activity. These include the Novartis compound LBH589 (panobinostat) and belinostat (Duvic et al. 2008; Ellis et al. 2008; Pohlman et al. 2009).

8.4.2.8 Single-Agent Chemotherapy

Several chemotherapeutic agents have shown activity in CTCL/MF. Initial response rates with either single-agent or combination chemotherapy remain high but the responses are short lived. Given the immunosuppressive state and propensity to infections due to a compromised skin barrier, the best strategy remains to avoid multiagent chemotherapy for as long as possible and to treat with lowest possible doses of single agents to allow more frequent administration of drug and avoid systemic infectious complications. Some of the agents with the best known activity in CTCL are as follows.

Antifolates

Methotrexate

MTX has shown significant clinical activity in many types of non-Hodgkin's lymphoma including T-cell lymphoma and has immunosuppressive properties (Olsen 1991). Low-dose methotrexate given weekly has long been used for the treatment of MF and SS. The dose is less than 100 mg a week and is usually administered orally though it can be given intramuscularly or intravenously as well.

In spite of frequent use at doses that range between 2.5 and 25 mg a week, there are few studies that have looked at the response rates. Zackheim et al. published the first report at doses of 2.5–10 mg a week and reported responses at 58 % in erythrodermic MF and 33 % in plaque-stage disease (Zackheim et al. 1996, 2003). High-dose MTX with leucovorin rescue at doses between 60 and 240 mg/m^2 has shown responses up to 80 % in patients with more advanced-stage MF (McDonald and Bertino 1978). Case reports have confirmed activity of single-agent MTX in SS patients (Zackheim and Epstein 1989).

Combinations of MTX have also shown promising results though all objective data consists of small studies and case reports. Most patients treated have advanced (at least stage IIB) disease. The few published reports of these combinations have been either with biologic agents like IFN-alpha (Aviles et al. 2007) or other chemotherapy agents like etoposide (Hirayama et al. 2000) or fluorouracil (Schappell et al. 1995). No specific recommendations can be made with these small studies with very heterogeneous groups of patients. In transformed disease, there is a trend to use systematic and combination therapy including MTX-containing regimens used for aggressive lymphomas. A topical formulation is currently being investigated and has shown to be safe in an early phase 1/2 trial (Demierre et al. 2003). Major side effects are nausea, mucositis, bone marrow suppression, alopecia, hepatitis, and cirrhosis with cumulative dosing. The side effects are dose dependent and folate supplementation can help alleviate the severity of mucositis. High-dose methotrexate is combined with folinic acid (leucovorin) rescue to minimize mucositis (Olsen 1991).

Pralatrexate

Pralatrexate is a rationally designed antifolate with a higher affinity for the reduced folate carrier (RFC) that carries the molecule into the cell and a higher affinity for the enzyme folylpolyglutamate synthase (FPGS) as compared to MTX. It has shown activity in T-cell lymphomas and has been studied in CTCL in a multicenter phase I/II trial that enrolled over 54 patients in multiple centers. At 15 mg/m^2 weekly given during 3 out of 4 weeks, the ORR was reported at 45 % with a median duration of response that could not be assessed due to censoring in the study design. Responses were seen in both SS and MF patients. Most common side effects were mucositis and

leucopenia at this dose level (Horwitz et al. 2012).

Nucleoside Analogues

Nucleoside analogues are antimetabolites that are phosphorylated and incorporated into the growing DNA strand of a dividing cell in the S phase. Some of these agents are also active in MF and SS as described below.

Gemcitabine

This agent is widely used in the treatment of NHL and CTCL. However, published data to support the use of this agent in CTCL and SS is limited to a few small studies. The agent is given weekly for 3 weeks with a 1 week break at the end of each cycle. Dosing ranges from 1,000 to 1,200 mg/m^2 given intravenously. Some of the larger studies have included up to 30 patients with advanced-stage disease and have reported responses of up to 70 % with a few CRs as well (Duvic et al. 2006d; Zinzani et al. 2000; Marchi et al. 2005; Sallah et al. 2001). Main side effects are myelosuppression, fever, nausea, vomiting, interstitial pneumonitis, alopecia, and radiation sensitivity. Cases of hyperpigmentation in SS patients have been seen. Rare incidences of hemolytic uremic syndrome have been reported.

Fludarabine

Use of single-agent fludarabine has been studied in CTCL and SS. Trials are small but response rates vary from 19 to 30 % with CR of 9 %. Response may be higher in the SS group with a RR of 35 % and CRs of 18 % (Quaglino et al. 2004). Combination chemotherapy with fludarabine has also been evaluated with interferon alpha. 35 patients were treated and 11 % (4/35) reached a CR including 11 patients with SS maintained for 18 months in 3 patients (Foss et al. 1994). Infectious complications remain the major side effect.

Cladarabine

Small case series of up to eight patients reported responses in patients with MF including 1 CR (O'Brien et al. 1994; Trautinger et al. 1999). Differing dosing schedules have been employed. The largest series consists of 24 patients with MF/SS treated at 0.1 mg/kg/day by continuous infusion over 5–7 days repeated every 28 days. The RR was 24 % with 3 out of 24 patients reaching a CR (Kong et al. 1997). Based on an analysis of several small series, a RR of 50 % has been reported in SS (Saven et al. 1992; Zaucha et al. 1997; Bouwhuis et al. 2002). Main side effects are immunosuppression and prolonged leucopenia. Occasional constitutional symptoms of fever and nausea can occur.

Pentostatin (Deoxycoformycin)

One of the most widely studied agents in CTCL as activity was established in the first phase I study of this agent (Grever et al. 1983). Several trials have been published that support the use of single-agent pentostatin in the treatment of MF and SS including ECOG and EORTC but there is no consensus on the dose or schedule. These trials are also marked by patient heterogenicity and lack of uniform response criteria. Reported response rates are 31–66 % with higher responses seen in SS (71 %) with reports of CR lasting up to 76 months (Tsimberidou et al. 2004; Cummings et al. 1991; Greiner et al. 1997; Ho et al. 1999; Kurzrock et al. 1999). A combination of pentostatin and interferon produced a response rate of 41 % in a group of 41 patients with 2 CRs in SS patients and 15 PRs (Foss et al. 1992). The most common side effects are myelosuppression, nausea, fever, and elevation of liver enzymes that is transient. Prolonged suppression of CD4 counts can occur putting the patient at risk of potentially life-threatening infections. Neurologic symptoms, pulmonary toxicity, or unexpected nephrotoxicity has been reported.

Forodesine

Two phase I/II studies of forodesine have established its activity in patients with MF/SS using an IV and an oral formulation. Small studies have reported a ORR of up to (Duvic 2007; Duvic et al. 2004, 2006a). Side effects are fatigue, edema, nausea, pruritus, dyspnea, and headaches. Lymphopenia and low CD4 counts have been noticed in patients but opportunistic infections are not common (Duvic et al. 2006b).

Alkylating Agents

Most of the alkylating agents that are used in MF/SS are part of the combination chemotherapy regimens, but single agents used at lower doses have been attempted to enable prolonged use with fewer side effects.

Single-agent use for MF/Ss has been established for the following agents:

Nitrogen Mustard (Mechlorethamine)

The first alkylating agent was used as early as 1950 to treat 21 cases of MF at 0.1 mg/kg/day for 10 days resulting in responses to initial cycles of therapy (Karnofsky 1950). Topical formulation is used extensively in early-stage disease. A 27-patient study was conducted with MF/SS patients using nitrogen mustard at varying doses for a total of 0.4 mg/kg per session along with topical nitrogen mustard. An ORR of 54 % was demonstrated with one response lasting for more than 1 year. IV nitrogen mustard can cause phlebitis, myelosuppression, rashes, and GIT disturbance (Van Scott et al. 1975).

Chlorambucil

The earliest documented use of chlorambucil in MF was in the 1960s when it was used in four patients to treat erythroderma at doses of 4.5–56 mg/kg for a 4-week cycle with clinical responses lasting 4–24 months. Several case reports of single-agent chlorambucil in MF have shown variable results at varying doses. Best results in MF/SS have been shown with concomitant use of steroids particularly in SS (Hamminga et al. 1979; Winkelmann et al. 1984) and in combination with leukapheresis (McEvoy et al. 1989) resulting in an ORR of 100 % and a DOR of 1–3 years with improved survival of 6.5–8 years as compared to historical control survival of 3 years. Pulse chlorambucil given as 10–12 mg/day with a steroid on 3 successive days every 2 weeks also produced 54 % CRs and 46 % PRS (100 % RR) in a series of 13 SS patients (Coors and von den Driesch 2000). Medians response duration was 16.5 months.

Cyclophosphamide

This was first used to treat MF/SS as early as 1960s (Abele and Dobson 1960). The initial four patients were treated at 200 mg/m^2 for 14–220 days, and 3 out of 4 had a response to the initial therapy that required weekly maintenance dosing of 400–700 mg. There are several case reports in the literature regarding responses in MF/SS patients obtained with lower doses of Cytoxan used as a single agent given weekly (Suter 1964; Auerbach 1970; Maguire 1968), but the main use of Cytoxan remains as part of combination chemotherapy. The main side effects are alopecia, nausea, vomiting, and myelosuppression at higher doses. Like all alkylators, it carries the potential for germ cell damage in younger patients.

Temozolomide

Temozolomide is an oral alkylating agent that functions as a prodrug and undergoes rapid nonenzymatic conversion to active 5-(3-metyltriazen-1-yl) imidazole-4-carboxamide (Newlands et al. 1992, 1997). In a phase 1 study of this agent in advanced cancers, one patient with MF had a CR of 7 months duration (Newlands et al. 1992). This led to a prospective phase II study of nine patients at a dose of 150 mg/m^2/day × 5 days for the first 28 days cycle and then 200 mg/m^2 × 5 days for cycles 2 and 3. The ORR was 33 % including 1 CR and 2 PRs with a duration of response of 6–9 months (Tani et al. 2005). Myelosuppression is the main side effect with counts nadiring at day 22 of the treatment cycle (Newlands et al. 1997).

Topoisomerase Inhibitors

Pegylated Liposomal Doxorubicin

Doxorubicin is an anthracycline and the pegylated form is encapsulated in liposomes allowing selective accumulation in tumor vasculature and decreased clearance by mononuclear phagocytic system allowing improved availability of the agent in the cells (Bao et al. 2004). There is data to support single-agent activity of adriamycin in MF/SS (Levi et al. 1977) and is an important component of many combination regimens that are used in the treatment of advanced MF/SS. Pegylated liposomal doxorubicin is used extensively in the treatment of MF/SS with response rates that vary between 30 and 80 % and CR rates of 20–60 % given every 3–4 weeks (Wollina et al. 2003; Quereux et al. 2008; Di Lorenzo et al.

2005). Cardiac toxicity can occur with a total calculative dose of doxorubicin exceeding 550 mg/m^2 or lower at 400 mg/m^2 if used concomitantly with cyclophosphamide. Other side effects include flushing, shortness of breath, facial swelling, headache, chills, back pain, tightness in the chest or throat, and hypotension. A unique toxicity of doxil is inflammation of the skin of palms and soles. Myelosuppression is another serious side effect that can be potentiated with the use of other cytotoxic agents.

Etoposide (VP-16)

This agent was first used in CTCL in 1975 when a patient with tumor-stage MF was reported to have a CR after receiving VP-16 at a dose of 60 mg/m^2 IV × 5 days, repeated with maintenance injections (Jacobs et al. 1975). This was followed by several case reports of single cases showing responses to differing doses of the agent at different schedules including a maintenance schedule (Jacobs et al. 1975; Onozuka et al. 2004; Nasuhara et al. 1995). Small studies using an induction and maintenance approach or combination with cyclophosphamide or methotrexate have reported promising results (Molin et al. 1979; Miyoshi and Noda 2006). This agent is well tolerated but can cause myelosuppression, nausea, vomiting, mucositis, alopecia, and rarely hypersensitivity reactions. VP-16 use has been associated with the occurrence of secondary leukemias (Pui et al. 1991).

8.4.2.9 Combination Chemotherapy

The use of multiagent chemotherapy is usually restricted to advanced-stage MF/SS or cases of transformed disease with high tumor burden. Almost all regimens used for aggressive lymphomas have been used for advanced disease but their use is limited by a short duration of response. The optimal use of these regimens remains as a bridge to a sustained consolidation therapeutic regimen like TSEB or stem cell transplantation.

When the need for multiagent chemotherapy is indicated, most patients are referred to an oncologist who tend to treat these patients with their favorite regimen for aggressive lymphomas, generally some combination of cyclophosphamide, anthracycline, vinca alkaloids, methotrexate, and steroids. A review of the literature indicates that there have been some dedicated studies to look at the use of multiagent chemotherapy in Mf/SS patients. The regimens incorporate varying agents and the responses vary from 95 to 100 % (Grozea et al. 1979; Case 1984; Fierro et al. 1997) but DOR remains less than a year. The data is not sufficient to support the use of a particular regimen or agent. Other regimens have incorporated skin-directed therapies in addition to multiagent chemotherapy in an attempt to improve the outcome. These have included electron beam therapy in combination with multiagent chemotherapy (Bunn et al. 1979; Kaye et al. 1989; Zakem et al. 1986) and topical nitrogen mustard (Zakem et al. 1986) (increased response duration of 105 months).

8.4.2.10 Extracorporeal Photopheresis (ECP)

ECP is considered an immunomodulatory procedure that was approved by the FDA in 1988 for the treatment of CTCL (Edelson et al. 1987). It requires the process of leukapheresis followed by the injection of liquid 8-methoxypsoralen into the bag of collected WBCs. The bag is then exposed to a UVA source following which all products are returned to the patient. The process takes about 1.5–4 h and results in apoptosis of a portion (10 %) of the malignant T cells and the conversion of blood monocytes to dendritic cells with the expression of CD83$^+$ and CD36$^+$ that can phagocytose apoptotic T cells (Vowels et al. 1992). The apoptotic cells after infusion are phagocytosed by the antigen-presenting cells (APCS) that may activate cytotoxic T cells to produce an immune response against the tumor cells (Yoo et al. 1996; Martino et al. 2012). Together these processes are thought to induce an immune response against the tumor cells in the skin and blood. ECP has been shown to normalize the CD4/CD8 ratio and mature the CD4 cells towards a Th1 phenotype as opposed to the TH2 (immunosuppressive) type that is a hallmark of CTCL (Di Renzo et al. 1997; Knobler and Jantschitsch 2003).

The optimal frequency of the procedure is 2 treatments on consecutive days every 4 weeks. There is still some controversy regarding the need for blood involvement for this procedure to be effective. A hgb of less than 8 g/dl, platelets of less than 20 k, an intolerance to 8-MOP, and aphakia are contraindications to its use. Most common side effects are photophobia, pyrexia, nausea, and hypotension (Martino et al. 2012; Zic 2012). It is not myelosuppressive and can be combined with other modalities of treatment including interferon, bexarotene, radiation, and chemotherapy. The treatment is more effective in patients who are not heavily immunosuppressed with previously administered extensive chemotherapy.

The initial trials with ECP showed a response rate of 60 % in 37 patients who were treated for 4–6 months, and the antitumor effect correlated with the appearance of CD8+ cytotoxic T lymphocytes in the peripheral blood and tumor infiltrates of the skin. Responses were noted after a median of 22 weeks and the median survival of the group was 60 months vs. 33 months in the historical control. Other trials have confirmed these results, and for stage IB,IIA,III, and IVA, erythrodermic MF and SS, the responses are favorable ranging between 36 and 83 % in patients who have been treated for at least 3 months though randomized data to support this therapy is lacking (Zic 2012; Duvic et al. 2003; Russell-Jones 2000). The use of accelerated protocols where some patients received treatments every 2 weeks and underwent 9 vs. 6 cycles of collection had response rate of up to 50 % and a CR of up to 18 % (Duvic et al. 2003) with erythrodermic and SS patients having the highest likelihood of response (Stevens et al. 2002; Knobler et al. 2002; Jiang et al. 1999). Responses are better when ECP is combined with other agents particularly interferon alpha confirmed with a combined analysis of over 400 patients from mostly retrospective studies showing an ORR of 55.7 % (244 out of 438) and 17.6 % CRs (77out of 438) using adjunct therapies with ECP including interferon, bexarotene, granulocyte monocyte colony-stimulating factor, and ECP (Ferenczi et al. 2003; Vonderheid et al. 1994a; Booken et al. 2010; Zic et al. 1996; Richardson et al. 2003, 2006; Wilson et al. 1995). In a study of 18 patients, the use of ECP was explored with bexarotene or interferon and showed a response rate of 61 % in heavily pretreated patients. Median survival was 51 months, progression-free survival was 28 months, and response duration was 29 ± 23.9 months (Siakantaris et al. 2012).

Due to lack of randomized trials, it is difficult to establish a survival benefit from ECP, but long-term follow-up has shown survival of up to 60 months in stage III and IV CTCL patients, which is double that of historical controls. A high Sézary count and erythrodermic skin stage have been identified as the most useful predictors of response to this modality. Clinically, a meaningful reduction >50 % in skin lesions by 6 months seems to correlate with the best long-term response.

8.4.2.11 Stem Cell Transplant

Autologous Stem Cell Transplants

For chemosensitive relapsed aggressive lymphomas, high-dose therapy followed by autologous stem cell rescue remains a standard of care. However, for CTCL the results for this approach have paralleled those of other low-grade lymphomas resulting in frequent relapses. Even though the data is limited, there are a few small series that have shown high-response rates but early relapses within a year (mean of 5.8 months). These series of 9–10 patients have indicated that high-dose therapy and TBI-based regimens can be delivered to these patients without an increased incidence of transplant-related complications. T-cell depletion or CD34 selection did not make a difference in terms of relapse (Bigler et al. 1991; Olavarria et al. 2001; Russell-Jones et al. 2001; Ingen-Housz-Oro et al. 2004; Sterling et al. 1995).

Allogeneic Stem Cell Transplant

Allogeneic stem cell transplants have been performed in select patients with advanced disease to harness the graft versus lymphoma effect with success. Due to the general immunosuppressive state and a broken skin barrier, infectious complications remain a major challenge in the management of these patients. However, the data in the literature is supportive of this approach and the complication rate remains acceptable (Duarte

et al. 2008; Oyama et al. 2003; Duvic et al. 2010; Molina et al. 1999, 2005; Guitart et al. 2002; Wu et al. 2009; Paralkar et al. 2011). A review of these cases in detail indicates that an allogeneic transplant can achieve a CR even in the most refractory cases and reduced intensity conditioning (RIC) appears to be just as effective and less toxic than fully ablative regimen. The largest series is from Spain with a retrospective analysis of the outcome of 60 patients that included 36 patients with MF and 24 with SS. OS at 1 year in this series was 66 % and at 3 years it was 54 %. RIC resulted in decreased non-relapse mortality (NRM) without increasing the rate of relapse leading to a higher OS whereas T-cell depletion (TCD) resulted in an increased risk of relapse. Donor lymphocyte infusion (DLI) was used to successfully treat relapsed disease (Duarte et al. 2008). Other smaller series have confirmed this data. There may be increased skin toxicity and flaring associated with TBI-containing regimens. Disease recurrence in the skin is not uncommon after an allogeneic transplant but seems to respond to adjusting the immunosuppression, DLI infusion, or mild skin directed therapies. There appears to be no blood or systemic recurrences. The numbers are too small to say if the recurrences are more common after RIC as opposed to fully ablative conditioning, but it seems like the trend among physicians is to use RIC regimens.

8.4.3 Supportive Therapy

Pruritus is an important and debilitating symptom of MF particularly in the extensive stages of the disease and SS where patients describe it as burning, tightness, and sharp pain as well as a pins and needles sensation similar to neuropathic pain. In clinical trials pruritus is measured by visual analogue scale (VAS), quality of life questionnaire (QLQ-C30), and similar other objective scales. The pathophysiology of pruritus in MF and SS remains unclear but appears to have several contributing factors including cytokine imbalance, skin irritation by tumor cell infiltration, impaired epidermal function resulting in excessive water loss and dryness, and superinfection particularly with gram-positive bacteria (Talpur et al. 2008). Gabapentin, mirtazapine, and aprepitant have shown promising results.

8.5 CD30-Positive Lymphoproliferative Disorders of the Skin

These are the second largest subgroup of CTCL comprising around 30 % of cases. It covers a spectrum of diseases including primary cutaneous anaplastic large T-cell lymphoma, different types of LyP, and a proportion of borderline cases where a clear distinction cannot be made accordingly.

8.5.1 Anaplastic Large T-Cell Lymphoma

C-ALCL shows a prominent male predominance of around 2–3:1 in the commonly middle-aged to elderly patients. Clinically they show solitary or groups of nodules or tumors that show a tendency to ulcerate and—as in general for the CD30+ lymphoproliferative disorders—for spontaneous regression (Fig. 8.4). In around 10 % of cases, lymph node involvement can be found which is apparently not related to a significantly worse prognosis.

Primary staging should include the search for systemic ALCL in all cases regardless of ALK status, as cutaneous involvement is not uncommon in ALK-positive systemic ALCL, but may also occur in ALK-negative cases (Yang et al. 2011).

8.5.1.1 Morphology

C-ALCL is typically characterized by sheets of large cells with highly atypical, pleomorphic nuclei, prominent nucleoli, and moderate to abundant cytoplasm. Variant forms of C-ALCL demonstrate tumor cells with large, but less frankly pleomorphic, nuclei. The presenting site of C-ALCL can show ulceration along with a

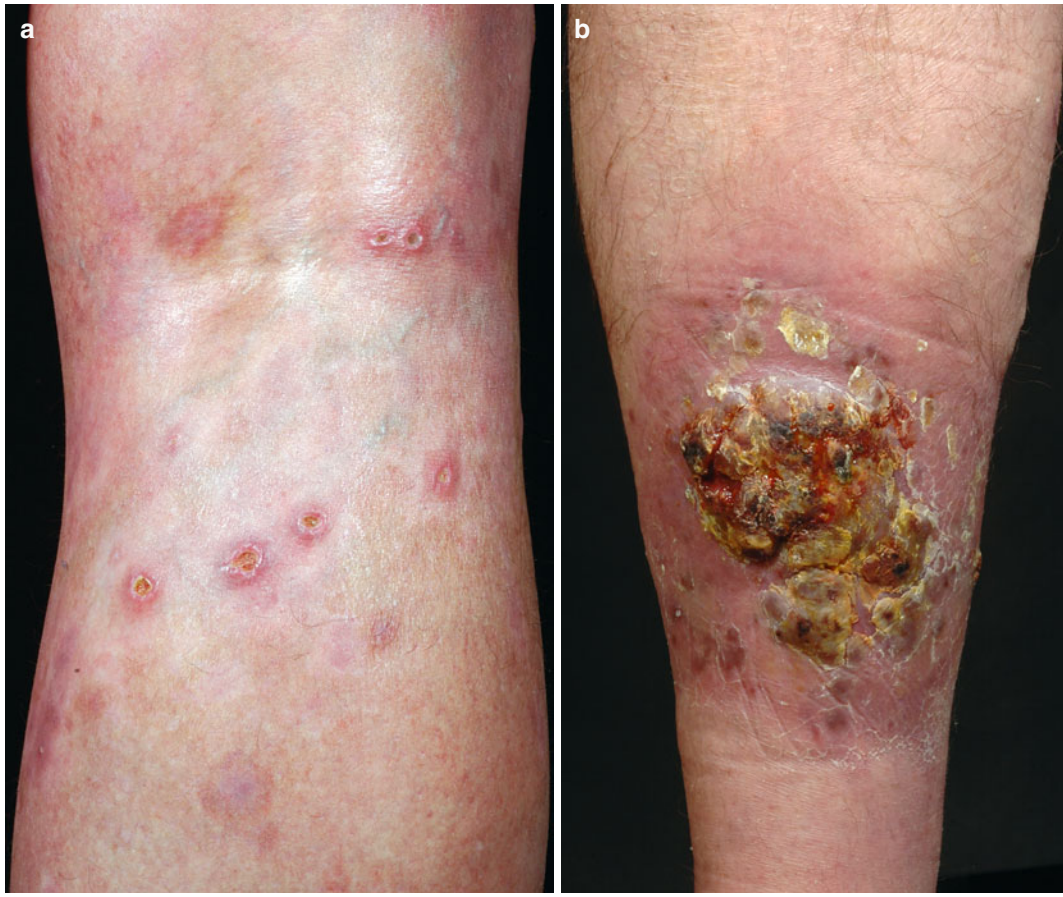

Fig. 8.4 CD30-positive lymphoproliferative disorders of the skin. (**a**) Anaplastic large-cell lymphoma. (**b**) Lymphoid papulosis

mixed inflammatory reaction consisting of histiocytes, neutrophils, and eosinophils. A substantial infiltrate of small, CD4+ T cells should raise the suspicion of transformed MF rather than the diagnosis of C-ALCL (Willemze et al. 2005).

C-ALCL may be distinguished from its morphological and phenotypic mimics with the detection of an IRF4 rearrangement in a subset of cases, and systemic ALCL may be distinguished by expression of EMA and ALK in some cases. However, for cases with limited clinical information, it is possible that the pathologist will only be able to classify a lesion as a "cutaneous CD30+ T-cell lymphoproliferative disorder" and provide a differential diagnosis. An agressive clinical course and histologic feature of the tumor are suspicious for PGD-TCL. This can be confirmed by immuno histochemical stains (IHC) for TCRd.

8.5.1.2 Treatment and Prognosis

In contrast to its nodal ALCL counterpart, the overall prognosis of primary cutaneous ALCL is very favorable with a 10-year survival rate of around 90 %. Although most cases of C-ALCL are shown to be ALK-negative, this does not impact prognosis.

Treatment of solitary lesion is most often surgical excision and/or radiotherapy (RT). Response to either is excellent and relapses occur in a similar range of about 40–50 % (Kempf et al. 2011).

Systemic treatment is warranted in disseminated and more advanced cases. Reported

systemic treatment options include a whole range of agents like anthracycline-based polychemotherapy, monochemotherapy with gemcitabine, etoposide or methotrexate, interferons, retinoids, steroids, and thalidomide (Kempf et al. 2011). While response rates might be somewhat higher with polychemotherapy, relapse rates tend to be similar as with the other treatment modalities in a range of 60–80 %. Therefore, multiagent chemotherapy is warranted mostly for cases with extracutaneous spread.

8.5.2 Lymphomatoid Papulosis

Being described as a clinically benign condition with a malignant histological phenotype, LyP has only recently been included formally into a malignant class of diseases which is now known as the CD30-positive lymphoproliferative disorders of the skin. The mean age of onset in LyP is in the middle age, but LyP can also occur in children and young adults (Boccara et al. 2012).

Clinically it is characterized by the occurrence of brownish-reddish papules, occasionally also larger nodules, on any part of the skin (Fig. 8.4). There is a constant tendency for ulceration and subsequently the lesions heal spontaneously within a period of 3–8 weeks.

8.5.2.1 Morphology

LyP has been subdivided into three major subtypes. Type A is characterized by small groups of large cells with pleomorphic nuclei and prominent nucleoli that are accompanied by a prominent, mixed inflammatory reaction (Fig. 8.5). In contrast, type B is characterized by a predominance of small lymphoid cells that cytologically resemble the tumor cells of MF. As in MF, the lesional cells exhibit epidermotropism. Type C is characterized by large groups or sheets of large atypical cells with little of the mixed inflammatory reaction observed for type A. Given the waxing and waning nature of LyP, the overall histological appearance of individual lesions can vary widely, from a sparse infiltrate of highly atypical cells with little accompanying reaction to a marked infiltrate of lesional cells, histiocytes, granulocytes, and scar formation (Willemze et al. 2005).

8.5.2.2 Treatment and Prognosis

Usually, LyP is a self-limited disease, generally confined to the skin, and of little to no impact on the patient's life expectancy. Therefore, a wait-and-see strategy is appropriate in many patients, given the fact that most of the treatment strategies have only a limited long-term effect and side effects need to be observed. Topical steroids may be helpful in mitigating the course in individual cases.

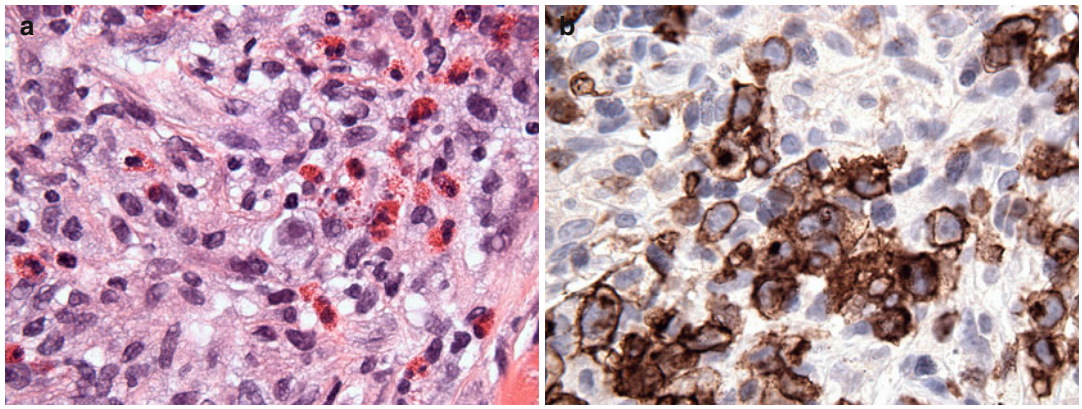

Fig. 8.5 Histopathology LyP type A. (**a**) Stained with hematoxylin and eosin stains and showing large lymphoid cells intermixed with histiocytes and eosinophils and (**b**) stained with anti-CD30 antibody and showing robust expression of the antigen in the large lymphoid cells

In cases with more extensive disease, phototherapy with PUVA and low-dose MTX are the best documented treatment options. However, a variety of other topical options (narrowband UVB, imiquimod, tacrolimus, nitrogen mustard, carmustine) and systemic treatments (retinoids, interferon, antibiotics, extracorporeal photopheresis, and multiagent chemotherapy) as well as radiotherapy have been reported (Kempf et al. 2011).

8.6 Rare Subtypes of CTCL

8.6.1 Subcutaneous Panniculitis-Like T-Cell Lymphoma (SPTCL)

The term subcutaneous panniculitis-like CTCL was created after several reports of CTCL involving the subcutaneous fat tissue simulating panniculitis clinically and in some instances also histopathologically. The cases that were described were often associated with a hemophagocytic syndrome (HPS) and frequently had a rapid unfavorable course with early fatality. In recent years, several cases of SPCTL have been reported that are not associated with HPS and have a rather favorable prognosis. In a multicenter analysis of 83 cases, the suggestion could be confirmed that the different clinical behavior could be linked to the respective phenotypes of SPCTL. SPTCL variants that showed HPS and a dismal course were found to be most often γδ type CTCL, while cases of αβ lineage showed a much better prognosis and could often be controlled by appropriate treatment (Willemze et al. 2008).

For that reason, the 2008 revision of the WHO classification excluded the γδ type of subcutaneous TCL from the category of SPCTL and included it into the provisional category of cutaneous γδ T-cell lymphoma (Jaffe et al. 2008).

Histologically, a lobular rather than septal infiltration of the panniculus by atypical lymphocytes showing a rim of pale-staining cytoplasm can be found. The cells may vary in size from case to case with irregular and hyperchromatic nuclei (Kumar et al. 1998). The epidermis and dermis are mostly uninvolved which is an important contrast to the γδ CTCL which often show involvement of all layers of the skin (Massone et al. 2006).

The preferable treatment strategy probably differs from MF and other CTCL types. Prednisone and methotrexate have frequently been reported to achieve complete and durable remissions for SPCTL, suggesting immunosuppressive rather than cytostatic regimens may be appropriate as first choice for this entity.

8.6.2 Primary Cutaneous Gamma-Delta T-Cell Lymphoma

Primary cutaneous γδ T-cell lymphoma (PCGD-TCL) represents less than 1 % of CTCL.

PCGD-TCL shows a variety of skin findings ranging from papules and plaques with or without necrosis and ulceration to extensive involvement of the subcutaneous tissue. Latter cases were formerly classified as subcutaneous panniculitis-like TCL (SPTCL) but are now designated as PCGD-TCL depending on their TCR lineage.

The neoplastic cells of PGD-TCL are intermediate to large in size with condensed chromatin. The cells often present as a dense infiltrate involving the epidermis, superficial dermis, deep dermis, and/or the subcutaneous adipose tissue. Necrosis and apoptosis can be present. The tumor cells are characteristically positive for TCRδ, and also positive for CD2, CD3, granzyme B, TIA1 and/or perforin. These cells are negative for TCRβ (βF1) and generally negative for CD4 and CD8 (Toro et al. 2000). PGD-TCL shows clonal rearrangement of the TCRγ locus (Przybylski et al. 2000).

The prognosis of PGD-TCL is poor with a 5-year survival rate of only 11 % reported in a gamma/delta SPTCL series (Willemze et al. 2008). With very few exceptions the clinical course is extremely aggressive requiring consideration for intensive therapies early in the course of the disease including stem cell transplantation.

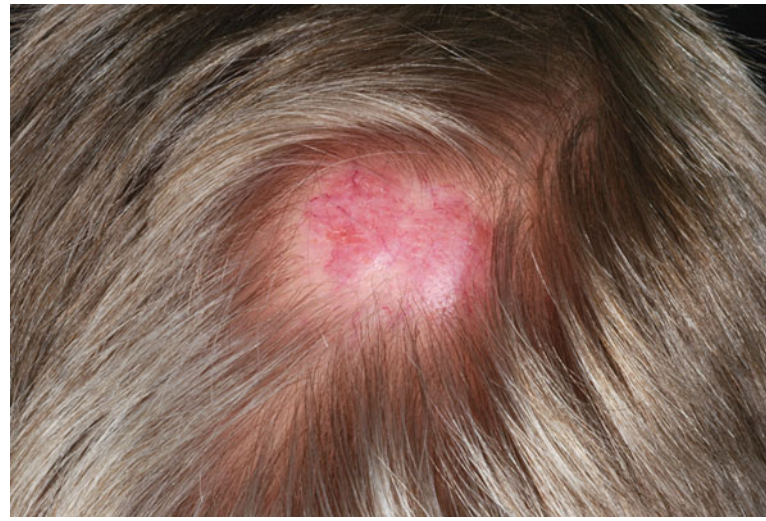

Fig. 8.6 CD8-positive aggressive epidermotropic cytotoxic CTCL

8.6.3 CD8-Positive Aggressive Epidermotropic Cytotoxic CTCL

Primary cutaneous CD8-positive aggressive epidermotropic cytotoxic TCL is a distinct CTCL subset that expresses a cytotoxic phenotype and is characterized by an aggressive ulcerating phenotype (Berti et al. 1999). The disease is rare, accounting for less than 1 % of CTCL cases and affects adults with no known gender predisposition.

As originally reported, patients develop rapidly progressive generalized ulcerating and necrotizing patches, plaques, and tumors (Fig. 8.6), lacking a chronic phase of indolent precursor lesions which is typical in MF (Agnarsson et al. 1990; Gormley et al. 2010). Mucosal involvement is not unusual and often a rapid progression with visceral involvement occurs leading to a fatal course in many cases.

The histopathology shows a nodular or diffuse infiltrate of CD8+ CD4− lymphocytes. Epidermotropism is often prominent and a constant feature of reported cases and the skin appendages are frequently involved. One or more cytotoxic markers are positive in nearly all of the cases (Berti et al. 1999; Nofal et al. 2012).

Of notice, not all CD8-positive epidermotropic CTCL belong to this entity, even with the expression of cytotoxic markers. In particular, indolent cases of CD8+ MF, pagetoid reticulosis, small-/medium-sized pleomorphic CTCL, and CD30+ lymphoproliferative disorders have been described (Cho et al. 2012; Xu et al. 2011; Geraud et al. 2011; Plaza et al. 2010; Martin et al. 2010; Beltraminelli et al. 2010; Khamaysi et al. 2006; Ameen et al. 2000). Therefore, the clinicopathological correlation is of importance when making the diagnosis.

The prognosis of CD8+ aggressive epidermotropic cytotoxic TCL is poor with a reported 5-year survival rate of only 18 % (Willemze et al. 2005). As with the γδ CTCL, treatment options should consider early aggressive therapy including stem cell transplantation protocols.

8.6.4 CD4-Positive Small- to Medium-Sized CTCL

Primary cutaneous CD4-positive small-/medium-sized pleomorphic T-cell lymphoma (PCSM-TCL) has only recently been included in the WHO classification as a provisional entity. It may account for up to 3 % of CTCL following one series (Willemze et al. 1997), but with refined diagnostic criteria its frequency is possibly less.

Morphologically it is characterized by a predominance of small- to medium-sized CD4-positive pleomorphic T cells (large cells not exceeding 30 %). There is no epidermotropism and the infiltrate often extends into the subcutaneous

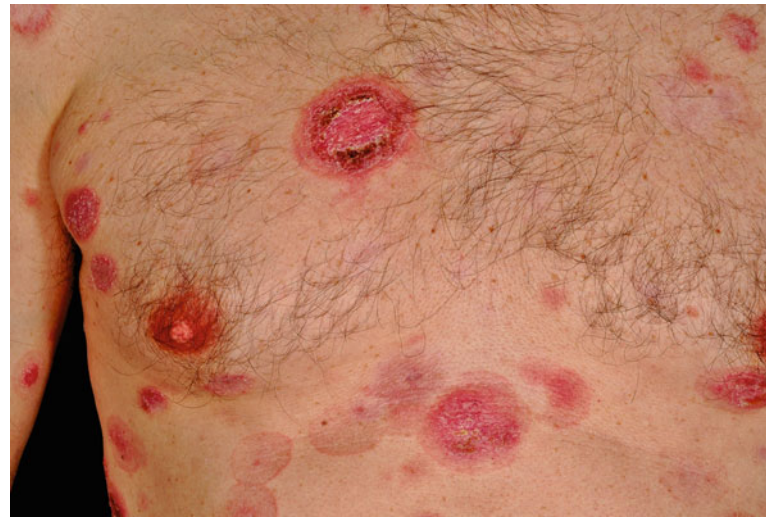

Fig. 8.7 CD4-positive small- to medium-sized CTCL

tissue. A prominent reactive infiltrate can frequently be found consisting of plasma cells, small lymphocytes, and histiocytes (Grogg et al. 2008).

Recent findings of positivity for the programmed death receptor 1 (PD-1) in PCSM-TCL suggest that it might be a neoplasia originating from follicular interdigitating cells.

The clinical picture differs from MF by lacking typical patches and plaques (Beltraminelli et al. 2009). Instead, a majority of cases present with a solitary skin lesion, often located at the head and neck area (Fig. 8.7).

Several reports demonstrate that the typical cases with solitary lesions have an excellent prognosis, often achieved by excision and/or radiotherapy alone (Grogg et al. 2008).

8.6.5 Cutaneous NK/T-Cell Lymphoma

Extranodal NK/T-cell lymphoma is a clinically aggressive entity, with a characteristic "nasal-type" involvement of the upper aerodigestive tract, formerly known as "lethal midline granuloma." While being rare in western countries, extranodal NK/T-cell lymphomas are much more frequent in Asia. Extranasal occurrence shows varying presentations, often including the skin with ulcerative nodules and tumors or a cellulitis-like picture, and in rare occasions the skin might be the sole initial site of involvement (Choi et al. 2009).

Histologically cutaneous NK/TCL is similar to other types of extranodal NK/T-cell lymphoma characterized by an Epstein-Barr virus (EBER)-positive atypical lymphoid cytotoxic infiltrate, extensive vascular destruction, and prominent tissue necrosis. Cells may vary in size and shape ranging from uniformly small lymphocytes to a mixture of small- and medium-sized or even very large cells. An admixture with inflammatory cells can often be seen and in a portion of cases there is an angiocentric growth pattern (Hasserjian and Harris 2007).

In comparison with the "nasal" subtype, the extranasal manifestation in the skin shows a tendency towards a slightly better prognosis, but many cases are still fatal despite aggressive treatment, with radiotherapy or multimodal treatment (Choi et al. 2009).

8.6.6 Cutaneous Adult T-Cell Leukemia/Lymphoma (cATLL)

Adult T-cell leukemia/lymphoma is a T-cell neoplasia due to infection with human T-lymphotropic virus 1 (HTLV-1) that occurs in about 2.5 % of HTLV-1 infected patients (Shimizu et al. 2007). It can be distinguished into (i) acute, (ii) lymphoma, (iii) chronic, and (iv) smoldering subtypes, of which (i) and (ii) show an aggressive course.

Cutaneous manifestations are relatively common and include a variety of clinical findings that often resemble those in classical MF like patches, plaques, tumor nodule, and erythroderma. A "cutaneous type" of ATLL has been proposed involving the skin only, together with a limited proportion of malignant cells in the peripheral blood (Miyata et al. 2010).

Histopathology of skin lesions shows an infiltrate of medium- to large-sized pleomorphic cells in different skin layers. It commonly shows a marked epidermotropism including the formation of Pautrier-like microabscesses. The immune phenotype is most often CD3+, CD4+, CD25+, and CD45RO+. Markers for CD8 and CD7 are usually lacking.

It has been demonstrated that the extent and type of skin lesions are of prognostic value, and skin-directed treatments analogous to the various MF stages may be helpful (Sawada et al. 2011).

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Part III

Disease-Specific: B-NHL

Adult Burkitt Lymphoma and Leukemia

9

Nicola Gökbuget, Paul Barr, Jonathan W. Friedberg, Eric D. Hsi, and German Ott

Contents

Pathology: Eric D. Hsi and German Ott

N. Gökbuget (✉)
Department of Medicine II,
Goethe University Hospital, Theodor Stern Kai 7,
60590 Frankfurt, Germany
e-mail: goekbuget@em.uni-frankfurt.de

P. Barr • J.W. Friedberg
James P. Wilmot Cancer Center, University of Rochester Medical Center, 601 Elmwood Avenue, Box 704,
Rochester, NY 14642, USA
e-mail: paul_barr@urmc.rochester.edu;
jonathan_friedberg@urmc.rochester.edu

E.D. Hsi, MD
Department of Clinical Pathology,
Cleveland Clinic, L11, 9500 Euclid Ave,
Cleveland, OH 44195, USA
e-mail: hsie@ccf.org

G. Ott, MD
Department of Clinical Pathology,
Robert-Bosch-Krankenhaus, Stuttgart, Germany
e-mail: german.ott@rbk.de

9.1 Definition

Burkitt lymphoma (BL) is an aggressive B-cell lymphoma frequently presenting at extranodal sites or, rarely, as leukemia. BL has – characteristically – a very high proliferation rate (>95 % measured by Ki-67 immunohistochemistry) and harbors chromosomal translocations involving the *MYC* gene (Leoncini 2008). For its accurate definition, a combination of diagnostic techniques is essential. The distinction between BL and diffuse large B-cell lymphoma (DLBCL) is of great clinical importance, because it entails therapeutic consequences. While DLBCL patients are usually treated with R-CHOP, this therapy is not appropriate for BL patients, who may be cured by intensified chemotherapy regimens including intrathecal prophylaxis.

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BL occurs in three clinical variants: the endemic, the sporadic, and the immunodeficiency-associated form. The endemic variant affects predominantly extranodal sites in children aged 4–7 years in the malaria belt of equatorial Africa and in Papua, New Guinea, and is associated with the Epstein-Barr virus (EBV) (Burkitt 1970). The sporadic form occurs throughout the world, not uncommonly in the gastrointestinal tract, and accounts for approximately 1–2 % of lymphomas in the Western hemisphere in adults but for 30–50 % of childhood lymphomas. Finally, BL constitutes a large group of lymphomas arising in the setting of immunodeficiency comprising roughly 30 % of cases (see Chap. 9.10).

9.2 Pathology

BL displays distinct morphological and immunophenotypical features, and many classical BL can be diagnosed with high inter- and intra-observer agreement. In its classical form, BL is composed of cohesive sheets of medium-sized blasts with deeply basophilic cytoplasm containing lipid vacuoles. The nuclei are round, with finely dispersed chromatin and usually several paracentric nucleoli. Normally, there are a lot of mitotic figures within the tumor but few accompanying reactive small lymphocytes. Characteristically, a starry sky pattern is present, induced by large numbers of tingible body macrophages interspersed between the tumor cells (Fig. 9.1a–c). Some cases, previously classified as "atypical" BL, have slightly larger nuclei with greater nuclear pleomorphism. Usually, the architecture of BL is diffuse, but rare cases of tumors with a follicular pattern occur (Warnke 1994). A subset of cases may show a prominent granulomatous reaction (Hollingsworth et al. 1993). It has to be stated here, however, that application of these criteria does not always allow the precise discrimination between BL (with/without atypical features) and DLBCL, since their morphological features may overlap.

9.3 Immunophenotype

Burkitt lymphoma cells express the B-cell associated antigens CD19, CD20, CD22, and PAX5 and, characteristically, are also positive for germinal center associated antigens such as CD10 and BCL6. In contrast, they are usually negative for BCL2 (some cases do express weakly BCL2) and virtually always for TdT. IRF4/MUM1 is usually not expressed, and the proliferation fraction as measured by Ki67 staining is always near 100 % (Lai et al. 1998; Dogan et al. 2000; Capello et al. 2000) (Fig. 9.2a–c). More recently, a new MYC protein monoclonal antibody has been validated allowing for the in situ demonstration of MYC protein overexpression (Green et al. 2012; Ruzinova et al. 2010) (Fig. 9.2d). These immunophenotypic features, however, are not unique to BL as DLBCL, especially of the GCB-like type, may have the same antigen expression pattern including overexpression of MYC.

9.4 Molecular Genetics

The cytogenetic hallmark of BL are translocations involving chromosomal band 8q24 encountered in >95 % of cases. The molecular consequence of these translocations, usually involving the sites of the immunoglobulin heavy chain (*IGH*) or of the IG light chain (*IGL*) genes, and, infrequently, also other non-immunoglobulin translocation partners is the deregulation and overexpression of the *MYC* oncogene (Willis and Dyer 2000). Translocations of *MYC*, however, are not unique to BL but are also seen in 5–10 % of *bona fide* DLBCL. More recently, other mechanisms of MYC activation have come to attention (reviewed in Slack and Gascoyne 2011).

Studies using global gene expression profiling have shed light on the special relation of BL and DLBCL (Dave et al. 2006; Hummel et al. 2006). Dave and colleagues (Dave et al. 2006) initially searched for a *MYC* target gene signature, defined by RNA interference experiments. Following these experiments, they were able to create a robust BL classifier, which was able to clearly identify cases of BL. Moreover, the analysis of

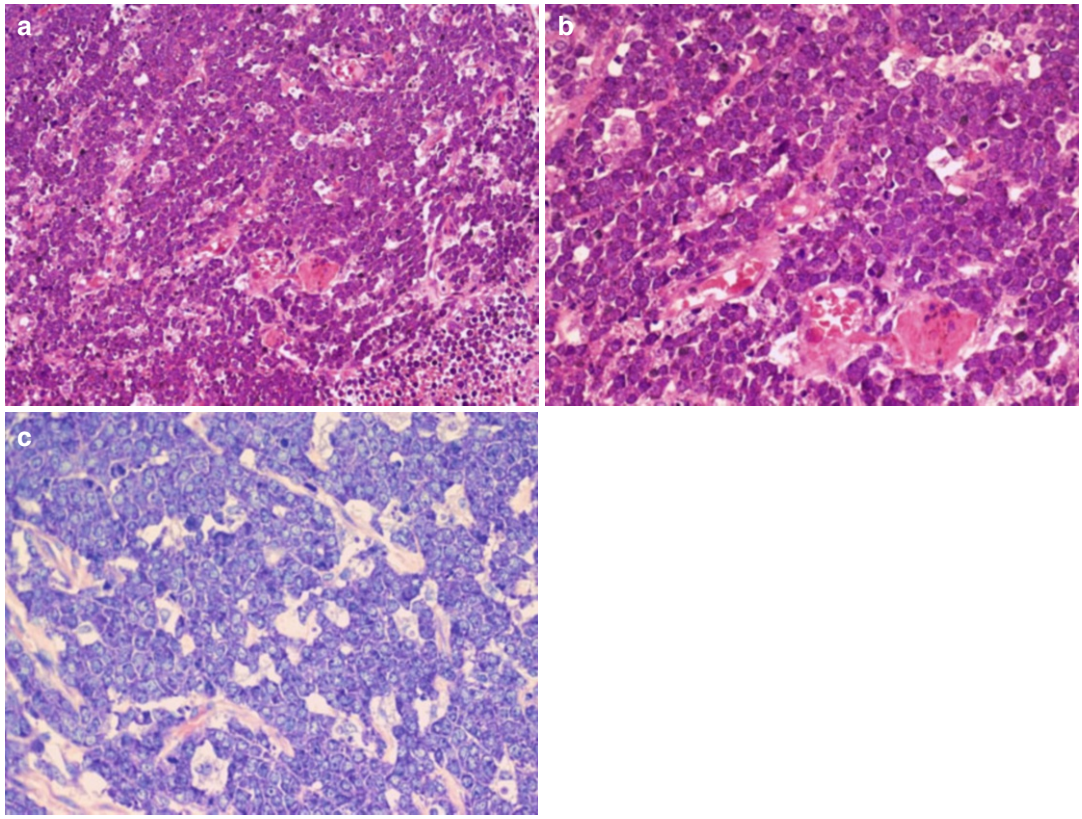

Fig. 9.1 *Burkitt lymphoma*. (**a**) Burkitt lymphoma shows an infiltration of uniform tumor cells with cohesive growth pattern. A starry sky pattern is present, imparted by the admixture of macrophages with ingestion of apoptotic tumor cells. (**b**) The tumor cells are medium-sized with deeply basophilic cytoplasm nicely appreciated in a Giemsa stain (**c**). The nuclei are round with finely clumped chromatin and several paracentric nucleoli

the genes included in the classifier provided further insights in the biological differences between BL and DLBCL. Hierarchical clustering identified four prominent clusters of differentially expressed genes. Not unexpectedly, since BL usually harbor *MYC* rearrangements, a gene expression signature including *MYC* and its target genes was found to be more highly expressed in BL than in DLBCL. In addition, BL is characterized by high expression of a distinct subgroup of germinal center B-cell genes. Two gene expression signatures were expressed at lower levels in BL compared to the DLBCL subgroups, one including MHC class I genes and the other containing NF-kB target genes. Importantly, DLBCL that harbor a *MYC* translocation could be distinguished from BL using the gene expression-based classifier. Hummel and coworkers (Hummel et al. 2006) carried out gene expression profiling and simultaneous array-based comparative genomic hybridization (array CGH) and fluorescence in situ hybridization (FISH) in BL and DLBCL. A "BL similarity index" including gene expression levels of 58 genes was calculated for all cases. Using this approach, cases were classified as molecular BL (mBL; 20 %), non-molecular BL (non-mBL; 58 %), and intermediate cases (20 %) according to their expression level of the mBL signature genes. In accordance with the results of Dave and associates (Dave et al. 2006), the mBL signature included several genes involved in the NF-kB signaling pathway, and these genes were found

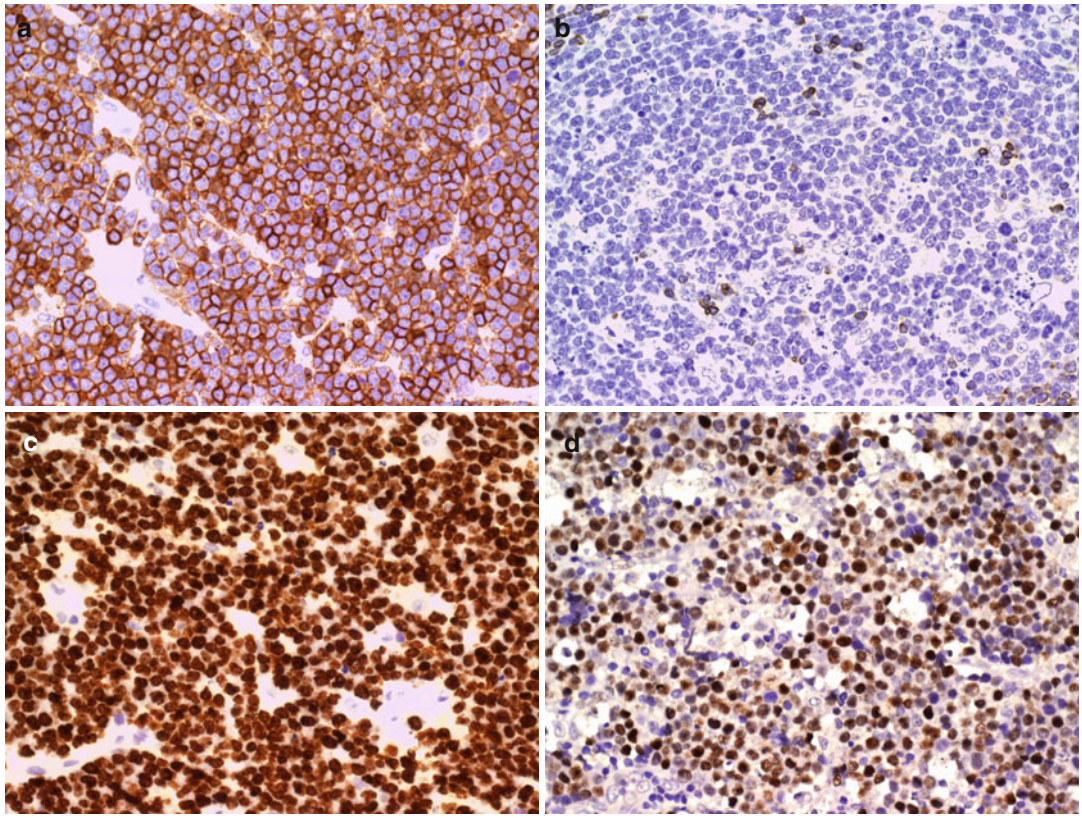

Fig. 9.2 *Burkitt lymphoma* (**a**–**d**) On immunohistochemistry, classically, Burkitt lymphoma is CD10 positive (**a**) and BCL2 negative (**b**) and has a high Ki67 index with almost all cells in cycle (**c**) (x400). A MYC protein overexpression characterizes the large majority of MYC rearranged lymphomas (**d**)

to be expressed at lower levels in BL compared to DLBCL. On the genetic level, the mBL cases were characterized by rearrangements of the *MYC* gene but relatively few additional chromosomal imbalances (low chromosomal complexity). In contrast, the cases that were assigned to the intermediate and non-mBL group based on their gene expression profile displayed a high chromosomal complexity, i.e., a high number of chromosomal alterations. Moreover, the presence of a *MYC* rearrangement in these subgroups was associated with a poor clinical outcome if treated with R-CHOP.

These studies provide a more accurate definition of BL on the molecular level and, moreover, allow insights into the underlying biological mechanisms of this neoplasm. However, at the same time these studies expand the spectrum of molecular BL including a subset of cases that would be diagnosed as DLBCL based on current WHO criteria but, nonetheless, show a gene expression profile typical of BL, the "molecular" BL. It is unclear whether intensified treatment approaches may be beneficial for these patients.

9.5 Differential Diagnosis

The differential diagnosis of Burkitt lymphoma, naturally, targets the borderlands between BL and DLBCL. BL is usually composed of medium-sized cells with immunohistochemical evidence of CD10 and BCL6 expression, lack of BCL2 positivity, and *MYC* rearrangement. Therefore, in the context of a highly proliferative lymphoid tumor with blastic cells of medium size, lack of CD10 and/or BCL6 expression and strong reactivity for BCL2 argue against BL (Harris and Horning 2006). On the other hand, daily practice tells us that there is some architectural and cytological variation in tumors, as evidenced, e.g., by pediatric *MYC*

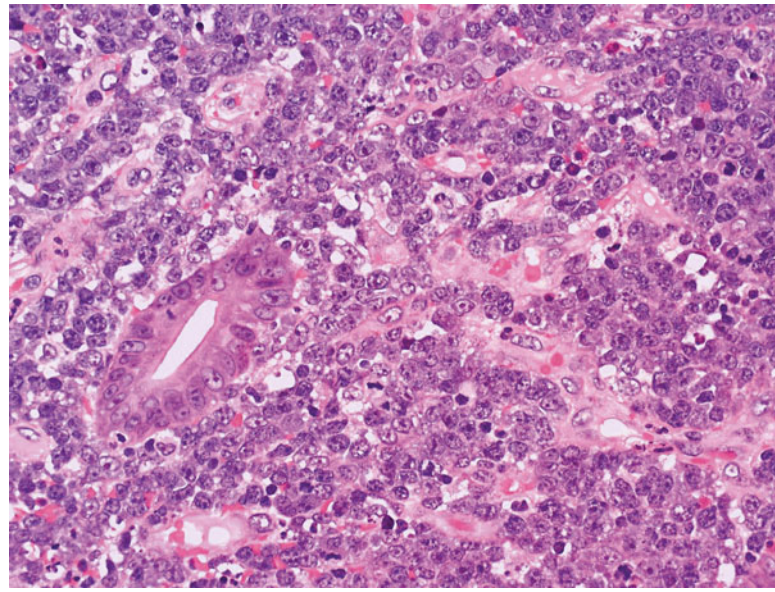

Fig. 9.3 *Pediatric MYC rearranged lymphoma*. This CD10+, BCL2-, BCL6+, and Ki67high MYC rearranged lymphoma arose in a 6-year-old boy. It might be classified as BL-DLBCL intermediate lymphoma or as *MYC* rearranged DLBCL

rearranged lymphomas not easily subclassified as BL or DLBCL (Fig. 9.3). In the WHO 2008 classification, a provisional category of "B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and BL" has been coined, paying tribute to the fact that a clear-cut differentiation between BL and DLBCL is not possible in all cases (Kluin 2008). It is considered a heterogeneous category comprising both de novo lymphomas as well as cases possibly representing transformed follicular lymphoma. Specifically, this category comprises cases presenting with morphological and/or genetic features of both and in between BL and DLBCL. Cytologically, the tumor cells are small to medium sized or in between those of typical BL and DLBCL, with greater variation in nuclear size than observed in BL (Fig. 9.4). A high proliferative index and a starry sky pattern can usually be appreciated, and an immunophenotype consistent with BL (CD10+, BCL6+, BCL2-/weak, IRF4/MUM1-) may be observed. Other tumors do cytologically resemble BL but have a discordant immunophenotype (BCL2 moderate/strong). 8q24 translocations and/or *MYC* rearrangements have been reported in 30–50 % of these cases; however, in contrast to BL, non-IG-MYC translocations do frequently occur (Bertrand et al. 2007; Johnson et al. 2009). Also, intermediate cases tend to have secondary chromosomal alterations in addition to *MYC* rearrangements. A fraction of intermediate

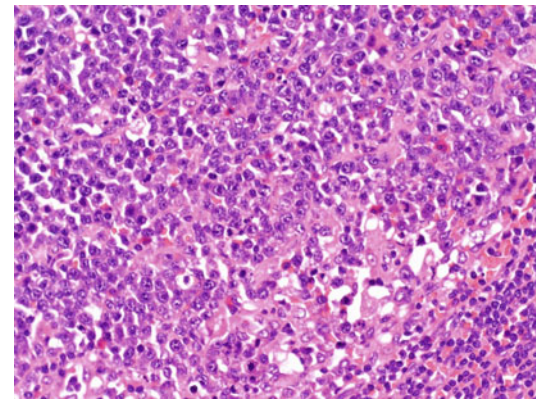

Fig. 9.4 *BL-DLBCL intermediate lymphoma*. In this lymphoma, the tumor cells are larger, and the cells are more pleomorphic. Nevertheless, the tumor cells were CD10+, BCL2-, BCL6+, and Ki67high, and *MYC* was rearranged (x400)

lymphomas with features between BL and DLBCL will present with "double-hit" features harboring both *MYC* rearrangements and translocations involving either *BCL2* or *BCL6* or, rarely, both. Some of these tumors, obviously, arise from a preexisting follicular lymphoma (Fig. 9.5a–d). Gene expression profiling of double-hit cases has shown an intermediate profile between BL and DLBCL.

It is important to note that aggressive lymphomas with typical DLBCL morphology and *MYC* rearrangement (Fig. 9.6) do *not* fall within this category, neither do morphological DLBCL

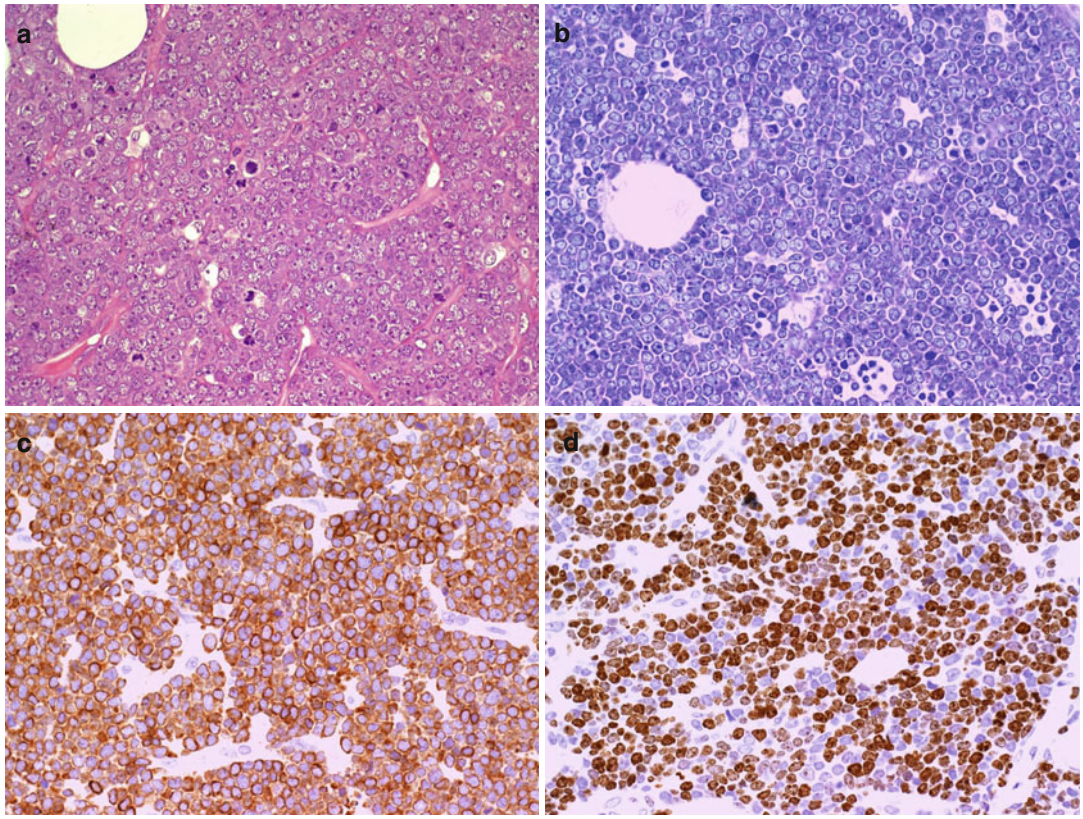

Fig. 9.5 *High-grade "double-hit" lymphoma arising from preexistent follicular lymphoma*. This high-grade lymphoma arose in a background of FL, and FISH disclosed both rearrangements of *BCL2* and *MYC*. (**a** and **b**) There is a diffuse infiltration of medium-sized to large blasts (**a**) with basophilic cytoplasm and round nuclei with open chromatin (x400). A starry sky pattern can be appreciated in the Giemsa stain (**b**). In contrast to Burkitt lymphoma, BCL2 is moderately strong to strongly expressed in the tumor cells (**c**), and Ki67 is lower than in classical BL (**d**) (x400). Morphologically, this case was classified as DLBCL

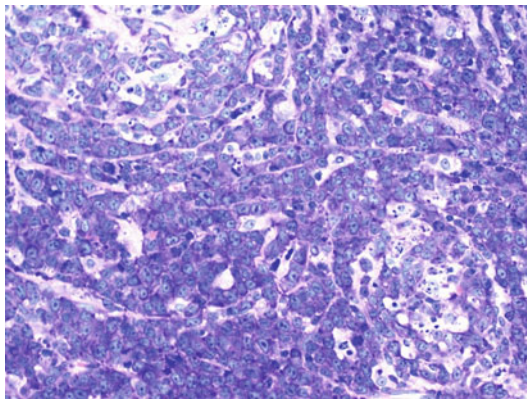

Fig. 9.6 *MYC rearranged testicular DLBCL*. This tumor arose in a 35-year-old male patient in the testis. It was found to be CD10+, BCL2-, BCL6+, and Ki67high, and MYC was rearranged (Giemsa x400)

cases with a BL gene expression profile – the so-called molecular BL (mBL). Table 9.1 summarizes morphological, immunological, and genetic features that may assist in differentiating between BL, DLBCL, and intermediate cases.

9.6 Epidemiology and Pathogenesis

BL accounts for roughly 30–50 % of pediatric lymphomas but only 1–2 % of adult non-Hodgkin lymphomas in Western countries (Morton et al. 2006; Sant et al. 2010). It is however 100-fold more common in tropical Africa (Ogwang et al. 2008; Parkin et al. 1985) leading to the distinction

Table 9.1 Differential diagnosis of BL, intermediate DLBCL/BL, and DLBCL (Adapted from (Leoncini 2008))

Feature	BL	Intermediate BL-DLBCL	DLBCL
Cytomorphology	Small- to medium-sized cells with paracentric nucleoli	Small- to medium-sized cells	Predominantly large cells
Proliferation (Ki67)	>90 %	Variable, often <90 %	Usually <90 %
BCL2 expression	Negative or weak	Negative/weak or strong	Negative/weak or strong
Genetics			
MYCR	95 %	50 %	5–10 %
MYC-IGH	Yes	Yes	Yes
MYC-non-IGH	No	Yes	Rare
BCL2R only	No	Rare	20 %
BCL6R only	No	Rare	30 %
Double hit	No	50 %	No/rare
MYC simple	Yes	Rare	Rare
MYC complex	Rare	Yes	Rare

between sporadic and endemic BL. The study of children afflicted with the African variant provided several clues to the etiology of BL. Parasitic induction of enzymes capable of promoting the genetic abnormalities, viral-induced evasion of normal immunosurveillance, and the discovery of a characteristic chromosomal translocation form much of our understanding of the pathogenesis of BL. A *third* variant, immunodeficiency-related BL, was recognized with the observation of an increased incidence in HIV-infected patients. Much less is known about the disease pathogenesis in this setting. While HIV is a well-described risk for BL in Western countries, the association has not been demonstrated in equatorial Africa.

In equatorial Africa, BL is estimated to *occur* in 5–10 per 1 million as compared to 1–3 per 1 million in North America and Europe (Gascoyne et al. 2010). Characteristic jaw tumors in children were first described by pathologists in equatorial Africa during the first part of the 20th century. Noting this recurring presentation, Denis Burkitt provided the first detailed clinical description of BL in 1958 (Burkitt 1958). Subsequently traveling throughout Africa, Burkitt mapped the incidence of this unique lymphoma, demonstrating an equatorial concentration, a distribution similar to viral diseases vectored by mosquitoes. The geographical coincidence with malaria was suggested, as the lymphoma seemed to not only mirror the disease distribution but also the intensity of infection (Dalldorf et al. 1964; Morrow 1985; Rainey et al. 2007). For example, within this "lymphoma belt," BL was less common in more arid climates. Additionally, its incidence fell with malarial eradication programs using chloroquine prophylaxis (Geser et al. 1989a, b).

While the association is incompletely understood, the ability of malaria to induce B-cell hyperplasia *increasing* the chance of genetic change supported its pathogenic role. Investigations have demonstrated the infection to further play a direct role in generating chromosomal translocations. Parasitic induction of B-cell activation-induced cytidine deaminase (AID) via interactions with Toll-like receptors leads to double-strand DNA breaks in the immunoglobulin heavy chain constant regions, normally occurring in somatic hypermutation and class switch recombination in B-cell development (Edry et al. 2008). Sustained AID activity is sufficient to generate MYC-IGH translocations in primary B cells further implicating this enzyme (Ramiro et al. 2006).

Endemic BL was additionally shown to contain Epstein-Barr virus (EBV) genomes in nearly all cases after the presence of the herpes virus was first demonstrated in 1964 (Schulte-Holthausen and zur Hausen 1972; Epstein et al. 1964a, b). The causal relationship between EBV and BL was demonstrated in a large cohort of children in east Africa. Prospectively, collected serum samples demonstrated an increase in EBV antibody titers just prior to the development of BL (de-The G et al. 1978). Despite the ability of EBV to

"immortalize" B cells in vitro, it appears that its pathogenic role may be to help the BL cell evade immunosurveillance mechanisms in vivo. Long and colleagues demonstrated the EBV containing cell to avoid the cytotoxic T-cell response normally responsible for its elimination (Long et al. 2011). However, only 10 % of sporadic BL and 40 % of immunodeficiency-associated BL cases appear to associated with EBV (Brady et al. 2007). The ubiquitous nature of EBV as well as the absence of EBV in the majority of non-endemic cases indicates that it is not essential for BL development.

The characteristic translocation involving *MYC* on chromosome 8 and *IGH* on chromosome 14 was first reported in 1976 (Zech et al. 1976). The frequent involvement with *IGH* further implicates AID in the genesis of this characteristic translocation (Robbiani et al. 2008). While different partners with the *MYC* gene have been described, recent genetic profiling investigations have demonstrated a relative lack of other genetic abnormalities as well as a striking similarity of sporadic, endemic, and HIV-related BL, all being very genetically distinct from DLBCL (Hummel et al. 2006; Lenze et al. 2011). The predominance of *MYC* expression, the prevention of apoptosis in *MYC* overexpressing cells, and the lack of other abnormalities in BL suggest its primary oncogenic role in driving neoplastic cell proliferation as opposed to its secondary acquisition in DLBCL.

The experience in equatorial Africa led to BL being primarily characterized as a childhood disease where it is the most common childhood cancer. Despite still accounting for a significant portion of pediatric malignancies in Western countries, the sporadic variant may occur more often in an adult population. Kelly and colleagues, using the Survival Epidemiology and End Results database, determined that 59 % of BL cases occurred in patients older than 40 in the United States, with 30 % being 60 years or older (Kelly et al. 2009). Having been previously underrepresented in the clinical literature, there appears to be a substantial increase in the inclusion of older patients in more recent reports.

The association between the acquired immunodeficiency syndrome and lymphoma was recognized early in the 1980s (Ziegler et al. 1984). While not accounting for the majority of presentations, Burkitt lymphoma was observed as well in these patients, now recognized to occur at an increased incidence in HIV-affected patients (Ziegler et al. 1982; Engels et al. 2008). It is well recognized that the immunosuppression caused by the HIV infection allows for the development of malignancy, in some cases caused by other viruses. More profound immunosuppression, as measured by absolute numbers of CD4+ lymphocytes, has been linked with a higher risk of DLBCL. BL, on the other hand, appears less likely to occur in those with the lowest levels of CD4+ cells (Guech-Ongey et al. 2010). HIV infection is known to lead to B-cell hyperplasia and predisposes patients to EBV reactivation (Bonnet et al. 2006). However, the observation that the disease occurs in those with higher numbers CD4+ cells suggests a role for this subset of T lymphocytes in the pathogenesis of BL, perhaps suppressing normal immune responses to *MYC* overexpressing cells.

9.7 Clinical Presentation

Endemic BL often presents as a rapidly progressive jaw or orbital tumor, occurring more frequently in young children. Metastases to distant extranodal sites occur in the absence of treatment. This presentation is relatively rare with sporadic BL. Abdominal presentations dominate, typically including nodal and extranodal sites. A variety of symptoms can be observed ranging from nausea, vomiting, and a change in bowel habits to gastrointestinal bleeding and intestinal perforation. When involved lymph nodes are palpable, involved sites can progress rapidly over hours to days, potentially leading to bowel obstruction or airway compromise. Extranodal disease is not uncommon, with numerous reports describing organ invasion, pleural effusions, and ascites, as well as pharyngeal and sinus involvement. Bone marrow involvement is documented in roughly 20 % of cases of sporadic Burkitt lymphoma and may be much less common in the endemic variant. Central nervous system (CNS) involvement has been reported in up to one-third of patients, is typically associated with other sites of extranodal involvement, and appears more frequently in endemic BL.

In making the diagnosis of BL, the rapid nature of disease presentation and progression is the most salient feature obtained in the patient's history. Rapidly growing tumors in conjunction with laboratory evidence of tumor lysis and an elevated lactate dehydrogenase (LDH) can be observed. Patients may have systemic complaints or B symptoms defined as fever of more than 38 °C, weight loss of more than 10 % of body weight, or the presence of drenching night sweats. Enlarged lymphadenopathy, abdominal masses, or organomegaly may be palpable on physical exam. In conjunction, a rapidly declining performance status may be evident. Routine staging studies including computed tomography of the neck, chest, abdomen, and pelvis and bone marrow aspiration/biopsy are indicated. The Ann Arbor staging system continues to be used in adult patients while the St Jude/Murphy system continues to be used in children and young adults (Blum et al. 2004). In addition, a serum LDH may be used in treatment planning. Routine laboratory testing includes an evaluation of renal and hepatic function as well as testing for HIV. A study of cardiac ejection fraction is necessary for patients who will receive anthracyclines as part of their treatment. A lumbar puncture is indicated to exclude involvement of the cerebral spinal fluid, present in roughly 1/3 of patients. Despite the presence or absence of neurologic symptoms, this is frequently performed in conjunction with intrathecal chemotherapy as systemic treatment is initiated.

Other non-Hodgkin lymphoma histologies need to be differentiated from Burkitt lymphoma. The most common NHL, DLBCL, may have a MYC rearrangement in 5–10 % of cases. Given the relative incidence of DLBCL, the MYC-positive subset is more commonly encountered in developed countries than Burkitt lymphoma. Differentiating these entities via expert hematopathologic review and appropriate molecular testing is critical given the major differences in treatment. Other lymphoma histologies including lymphoblastic lymphoma and blastic mantle cell lymphoma call present in a similar fashion to sporadic Burkitt lymphoma in adults. In younger patients, Wilms' tumor, neuroblastoma, and soft tissue sarcomas should be considered in the differential.

9.8 Prognostic Factors

A number of risk factors have been identified to predict for outcome in BL. Based on the need for short-duration dose-intense regimens to treat patients in general, age remains one of the strongest prognostic factors. Additionally, early studies in children demonstrated CNS involvement predict for a markedly poorer outcome (Patte et al. 1986). While outcomes have improved with the inclusion of CNS directed therapy, this risk remains prognostic in adults. Additional risk factors include LDH, WHO performance status, Ann Arbor stage, tumor mass size, and number of extranodal sites similar to the international prognostic index developed for DLBCL (Mead et al. 2002, 2008; Rizzieri et al. 2004). Patients not having any risk factors appeared to have superior outcomes allowing abbreviation of dose-intensive regimens. Patients not having any risk factors appeared to have superior outcomes allowing abbreviation of dose-intensive regimens. In adult patients, those with Burkitt leukemia appeared to have an inferior prognosis compared to those with Burkitt lymphoma (Hoelzer et al. 2007). Additional prognostic factors identified predominantely, in pediatric patients include surgical resection of the disease, leukemic presentation, and early response to treatment (Woessmann et al. 2005; Patte et al. 2007).

Posttreatment factors including minimal residual disease (MRD) have yet to be evaluated in adults. Bone marrow evaluation for the t(8;14) translocation using long-distance PCR demonstrated a 31 % positive rate in 84 pediatric patients, with only 18 % being positive by standard morphologic analysis. An inferior 3-year progression free survival was demonstrated in the patients have minimal disseminated disease, the only factor predicting a higher risk of treatment failure by multivariate analysis in this study (Mussolin et al. 2011).

The above risk factors likely apply in patients with immunodeficiency-related BL as well. A recent analysis demonstrated performance status to retain its prognostic ability. Additionally, a CD4 cell count of $<200 \times 106$/L was also predictive of a poor outcome (Galicier et al. 2007).

9.9 Therapy

9.9.1 Overall Results

Approximately 30 years ago Burkitt lymphoma and Burkitt leukemia were largely incurable diseases. It was important to acknowledge disease biology for definition of optimal treatment and these considerations were pioneered by Denis Burkitt (1967). BL has a high-growth fraction of malignant cells with a doubling time of approximately 1 day. Therefore, although the malignant cells are highly sensitive to different chemotherapeutic drugs, remaining lymphoma cells rapidly reenter the cell cycle and proliferate between chemotherapy cycles. In addition, ineffective cell-kill increases the risk of drug resistance. In the meantime, some general principles for successful treatment of BL have been defined:

- Fractionated cyclophosphamide or ifosfamide
- High-dose methotrexate as 24-h infusion
- Combination with other alternating non-cross-resistant drugs
- Short cycles with minimal treatment delays
- Intensive prophylaxis of CNS relapse
- Intensive supportive care

Successful regimens were first developed for pediatric BL. With these regimens, cure rates of above 80–90 % were achieved even in advanced disease (Patte et al. 1991; Reiter et al. 1992, 1999; Cairo et al. 2007, 2012). Pediatric regimens usually included methotrexate at very high doses from 3 up to 8 g/m^2 as 24-h infusion.

In adult BL and B-AL, no long-term survival was achieved with ALL-type regimens (Hoelzer et al. 1996). Several cooperative study groups have adopted pediatric-based regimens for adults such as the German Multicenter Study Group for Adult ALL (GMALL) protocols, the French LMB studies, and the CODOX-M/IVAC regimens. The hyper-CVAD regimen was primarily developed for adult ALL. The results of different regimens are often difficult to compare. Case numbers are small, and age distribution variable, diagnostic criteria, staging systems, and risk stratifications are different. Remission rates for regimens without rituximab range between 68 and 92 % and survival rates between 38 and 74 % (Table 9.2). Rituximab-based regimens yielded remission rates between 79 and 100 % and survival rates between 77 and 100 % (Table 9.3).

Clinical trial results often refer to somewhat selected patients. From the Swedish lymphoma registry data on 156 adults with BL diagnosed between 2000 and 2010 were reported. Interestingly the median age of 56 years was higher than in all published studies. The authors identified age, performance status, and LDH as significant prognostic factors. Patients older than 60 years had 35 % survival only. Regimens with different intensity were used, and data were available in 69 patients only. Pediatric-based (BFM) regimens and hyper-CVAD regimen yielded OS rates around 80 % which was significantly superior to CHOP-based regimens with 62 % survival (Wasterlid et al. 2011).

9.9.2 Short Intensive Regimens Without Rituximab

CODOX-M/IVAC is a regimen based on alternating cycles with high-dose methotrexate (HDMTX), cyclophosphamide, vincristine, and doxorubicin (CODOX-M) and cycles with ifosfamide, etoposide, and high-dose cytarabine (HDAC) (Magrath et al. 1996). The regimen is risk stratified. Low-risk patients (normal LDH, WHO performance status 0–1, Ann Arbor stages I–II, and no more than one extranodal manifestations) received three cycles CODOX-M. All other patients received four alternating CODOX-M/IVAC cycles. For the original protocol, applied in patients with a rather young median age of 25 years, complete remissions (CR) were reported in 24 of 26 patients with 22 patients being alive more than 12 months after diagnosis (Adde et al. 1998).

The protocol was then studied in an international consortium (Mead et al. 2002). In patients with a median age of 35 years, the overall survival rate (OS) was 73 % (Mead et al. 2002). In a subsequent, modified version, the HDMTX dose was 3 g/m^2 for patients younger than 65 years and 1 g/m^2 for those older than 65 years. The study also included patients with other aggressive B-cell lymphoma and a MKI67 fraction approaching 100 %. OS in 53 patients with BL was 67 %. Although toxicity appeared to be reduced by dose modifications, survival was still significantly poorer in

Table 9.2 Results with short intensive chemotherapy in Burkitt leukemia/lymphoma

Author	Year	Disease	Age (median)	*N*	Regimen	CR (%)	OS (%)
Soussain et al.	1995	BL	26 (17–65)	65	LMB 81,84,86,89	89	74
Divine et al.	2005	BL	33 (18–76)	51	LMB 81,84, 86, 89	83	70
Choi et al.	2009	BL, B-AL	47 (18–70)	38	LMB	74	68
Hoelzer et al.	1996	B-AL	33 (15–38)	24	B-NHL83	63	49
	1996	B-AL	36 (18–65)	35	B-NHL86	74	51
Hoelzer et al.	2002	BL, BLL	36 (15–63)	118	B-NHL90	83	70
		B-AL	15–65	89	B-NHL90	75	38
Rizzieri et al.	2004	SCNL,L3	50 (17–78)	92	Modified B-NHL90	68	50
		Cohort 1	*44*	*52*		*79*	*54*
		Cohort 2	*50*	*40*		*68*	*50*
Adde et al.	1998	BL	25 (18–59)	26	CODOX-M/IVAC	92	n.r.
Mead et al.	2002	BL	35 (15–60)	52	CODOX-M +/– IVAC	77	73
Mead et al.	2008	BL	37 (17–76)	53	CODOX-M +/– IVAC	n.r.	67
Lacasce et al.	2004	BL	47 (18–65)	14	CODOX-M +/– IVAC	86	71
Thomas et al.	1999	BL, B-AL	58 (17–79)	26	Hyper-CVAD	81	49
Thomas et al.	2006	BL, B-AL	48	48	Hyper-CVAD	85	53

CR rate of complete remissions, *OS* overall survival, *B-AL* mature B-ALL or Burkitt leukemia, *BL* Burkitt lymphoma, *BLL* Burkitt-like lymphoma, *SCNL* small non-cleaved cell lymphoma

Table 9.3 Results with short intensive chemotherapy combined with rituximab in Burkitt leukemia/lymphoma

Author	Year	Disease	Age (median)	*N*	Regimen	CR	OS
Thomas et al.	2006	BL, L3	46	31	Hyper-CVAD + R	86 %	89 %
							<60y: 90 %
							>60y: 89 %
Hoelzer et al.	2007	B-AL	46 (15–78)	70	B-NHL2002+R	79 %	<55y:79 %
							>55y:39 %
		BL, BLL	36 (15–78)	115	B-NHL2002+R	90 %	<55y:91 %
							>55y:84 %
Oriol et al.	2008	BL, BLL, B-AL	36 (55–55)	17	B-NHL2002+R	88 %	82 %
Rizzieri et al.	2010	BL, B-AL	19–79	105	Modified B-NHL90+R	82 %	79 %
							<60y:87 % EFS
							>60y:67 % EFS
Dunleavy et al.	2011	BL	35 (16–88)	29	DA-EPOCH-R	100 %	100 %
Corazzelli et al.	2012	BL, BLL	52 (25–77)	30	CODOX-M/IVAC+D+R	93 %	78 % PFS
Barnes et al.	2011	BL	46 (17–76)	40	CODOX-M/IVAC+R[a]	90 %	77 %

CR rate of complete remissions, *OS* overall survival, *B-AL* mature B-ALL or Burkitt leukemia, *BL* Burkitt lymphoma, *BLL* Burkitt-like lymphoma, *+R* rituximab added, *D* liposomal cytarabine, *R* rituximab

[a]Included patients with unclassifiable B-Cell lymphoma with features intermediate between diffuse large B-cell lymphoma and BL

patients older than 65 years. OS was also significantly different for high- and low-risk patients (52 % versus 88 %) (Mead et al. 2008). Compared to the previous studies, the overall results appeared to be comparable despite dose reductions.

In France the pediatric *LMB protocols* were adopted for adult BL and B-AL. A retrospective review of adult patients treated according to the LMB 81–89 protocols revealed an OS of 74 % in a rather young patient population with a median age of 26 years (Soussain et al. 1995). A subsequent prospective study confirmed the results. The protocol was risk stratified into group A (resected stage I or abdominal stage II disease), group C (CNS involve-

ment and/or BM involvement <30 %), or group B (all remaining patients). The protocol has several different elements: COP (cyclophosphamide, vincristine, and prednisone) as a pre-phase, COPAD (vincristine, doxorubicin, cyclophosphamide, and prednisone), COPADM (COPAD with HDMTX), CYM (cytarabine and HDMTX), CYVE (HDAC and VP16), and M cycles (vincristine, prednisone, cyclophosphamide, doxorubicin with or without HDMTX, VP16, or cytarabine). Group A was treated with 3 cycles COPAD; group B with COP, 2 cycles COPADM and 2 cycles CYM, and one cycle M; and group C similar to group B but with CYVE instead of CYM, three more M cycles, and additional cranial irradiation in case of CNS involvement. The HDMTX dose in this protocol was 3 g/m^2. Group A consisted of 6 patients only and OS was 83 %. OS was similar for groups B and C with 70 and 67 %, respectively. OS for the whole group of 72 patients was 70 %. Age above 33 years, elevated LDH, and the lack of response to COP were identified as poor risk factors (Divine et al. 2005).

The *hyper-CVAD* regimen was developed at the MD Anderson Cancer Center. The regimen consists of eight alternating cycles with cycle 1 (cyclophosphamide, doxorubicin, vincristine, and dexamethasone) and cycle 2 (HDMTX, HDAC). The HDMTX dose in this protocol was 1 g/m^2 and the HDAC dose 3 g/m^2. In a smaller trial with a rather high median age and 26 treated patients, the OS was 49 %. OS was significantly poorer in patients older than 60 years with 17 % survival compared to 77 % in younger patients (Thomas et al. 1999). Considerable neurologic toxicity has been reported for this regimen as well (Koh and Lim 1999).

The *GMALL* adopted a pediatric protocol developed by the Berlin-Frankfurt-Münster (BFM) group. After a pre-phase (cyclophosphamide and prednisone), the regimen consists of six alternating cycles A (ifosfamide, teniposide, vincristine, cytarabine, dexamethasone, and HDMTX) and B (cyclophosphamide, doxorubicin, vincristine, dexamethasone, and HDMTX). Initially, the regimen was administered in B-AL only. In the first study (B-NHL 83), the HDMTX dose was 500 mg/m^2 only, and in 24 patients, OS of 49 % was observed. In the second study (B-NHL 86), besides other modifications, the HDMTX dose was increased to 1.5 g/m^2 and in 35 patients survival rate was 51 % (Hoelzer et al. 1996).

Based on these results, the regimen was also administered in adult patients with BL (B-NHL 90). In this study, the HDMTX dose was further increased to 3 g/m^2. Furthermore, prophylactic CNS irradiation after two cycles, which was part of the original protocol, was omitted. Despite the increased HDMTX dose, the survival in B-AL was not improved (38 %) compared to the previous protocol. Survival in 118 patients with BL was significantly better (70 %). The regimen was also well tolerated in older patients with BL. The major reason for poorer outcome of B-AL was the increased toxicity and early mortality associated with the elevated dose of HDMTX, particularly in older patients. On the other hand, no increase in terms of CNS relapse rate was observed, despite omission of CNS irradiation (Hoelzer et al. 2002).

With a modified version of the GMALL protocol, similar results with OS of 50 % in 92 patients with BL or B-AL were achieved by the Cancer and Leukemia Group B (CALGB). In this version of the protocol, CNS irradiation was included between the cycles 3 and 4 and considerable neurologic toxicity was described. Later on, CNS irradiation was omitted and this modification contributed to reduced neurotoxicity (Rizzieri et al. 2004).

Results are summarized in Table 9.2. Details of many of the regimens including the variability of details such as ifosfamide, cyclophosphamide, HDMTX, and HDAC dose and schedule and application intrathecal therapies are shown in a review by Blum and colleagues (Blum et al. 2004).

9.9.3 Short Intensive Regimens with Rituximab

Malignant cells in BL and B-AL usually express CD20 on their surface. Based on the significant impact of immunotherapy with rituximab in combination with chemotherapy in DLBCL, rituximab was added to the successful short intensive therapies for BL and B-AL by several groups, although no randomized study had been performed. This decision was made in studies for adult BL but not in pediatric studies, probably because options for intensification of chemotherapy were considered to be very limited in adults

and alternative approaches were needed. An overview on results is given in Table 9.3.

The *GMALL* study group added rituximab at a dose of 375 mg/m^2 before each of the six chemotherapy cycles. The regimen was followed by two consolidation cycles with rituximab at 3-week intervals. Furthermore, in patients younger than 55 years, two C cycles were added. These cycles contained besides other drugs HDAC at 2 g/m^2, which had not been part of the GMALL B-NHL protocols before. Thus, the regimen was now defined as ABCABC in patients younger than 55 years and ABABAB with dose-reduced chemotherapy in patients older than 55 years. The dose for HDMTX was 1.5 g/m^2 in younger and 0.5 g/m^2 in older patients. The CR rate in 115 patients with BL was 90 %. Three-year OS was 91 % in younger patients (15–55 years) and 84 % in older patients (>55 years). Treatment-related mortality was 3 %. Among 70 B-AL patients, the CR rate was 83 %. The OS was significantly different for patients younger and older than 55 years, 79 and 39 %, respectively. Mortality was increased compared to BL patients (11 %), particularly in patients older than 55 years. CNS relapses occurred in 3 of 22 older CR patients with B-AL, which affected the outcome in this group and could be related to exclusion of HDAC from the protocol for older patients (Hoelzer et al. 2007). Therefore, intensified supportive care and CNS-directed therapy appear to be required in older B-AL patients treated with reduced doses of HDMTX. If results were compared to the previous B-NHL90 study without rituximab, OS was improved from 54 to 80 %; OS was 56 % versus 85 % in younger than 55 years and 39 % versus 65 % in older patients (Hoelzer et al. 2007).

Similar results with this regimen were observed by a Spanish group in HIV-negative BL and B-AL patients. The CR rate was 88 % and OS was 82 % (Oriol et al. 2008). A Croatian group reported in 12 adult patients with BL/B-AL a CR rate of 83 % and OS of 83 % (Dujmovic et al. 2012).

Results of a modified version of the protocol were reported by the CALGB. In contrast to the GMALL protocol, rituximab was administered during cycle 2, day 8 with 50 mg/m^2, and days 10 and 12 at 375 mg/m^2. During cycles 3 to 7, rituximab was given at 375 mg/m^2 on day 8. HDMTX dose was 1.5 g/m^2. No HDAC-based cycle was added. The CR rate was 82 % and OS 79 %. The authors reported that significant differences were associated with the IPI score with 92 % OS for low-risk compared to 55 % for high-risk patients. Patients older than 60 years had a lower CR rate of 75 % versus 85 % in the younger ones and a lower rate of continuous complete remissions of 54 % versus 77 % (Rizzieri et al. 2010).

Rituximab was also added to the *hyper-CVAD* regimen. The early dose intensity was increased with two doses of rituximab in each of the first four cycles. The median age in this trial was 46 years, with 29 % of the patients older than 60 years. The OS of 89 % was improved compared to the regimen without rituximab, which yielded 53 % survival. The improved survival was particularly due to a reduced relapse rate with the rituximab-based regimen. Compared to the previous studies, the outcome of older patients was significantly improved (89 % versus 19 %), which may in addition have been due to improved supportive care. In this study, no difference in terms of survival was observed between younger and older patients (Thomas et al. 2006).

Several groups reported results of *CODOX-M/IVAC* combined with rituximab with different conclusions. In a retrospective analysis results of the regimen combined with rituximab were compared to a historic control. 40 patients were treated with a median number of 4 doses rituximab. The cohort included HIV-positive patients. The CR rate was 90 % versus 85 % and the OS 77 % versus 66 % in the historic control. This improvement was not statistically significant. However, significantly fewer relapses were observed in the rituximab group. The only significant prognostic factor for OS in the total cohort was age above or below 60 years (Barnes et al. 2011).

An Italian group used in addition liposomal cytarabine for intrathecal CNS prophylaxis. HDMTX was reduced to 3 and 1 g/m^2 in patients older than 60 years. HDAC was given at 2 or 1 g/m^2 in patients younger and older than 60 years, respectively. The cohort included 15 patients with BL and 15 patients with “unclassifiable” B-NHL with features between BL and DLBCL. Results were compared to a historic control group treated with the original CODOX-M/IVAC regimen with higher MTX doses. CR rate was 93 % in the rituximab-based regimen compared to 70 % in the historic

control. Progression free survival (PFS) was 78 % versus 55 %. No difference in outcome was observed for BL compared to unclassifiable lymphoma. Patients older than 60 years still achieved a CR rate of 83 and 49 % PFS (Corazzelli et al. 2012).

A Japanese group used the CODOX-M/IVAC regimen with reduced dose of HDMTX (3 g/m^2) in patients with BL, B-AL, and lymphoma with intermediate features between BL and DLBCL. Rituximab was added only in part of the patients and a relevant effect could not be demonstrated (Maruyama et al. 2010).

Several study groups reported retrospective studies with cohorts including treatments with or without rituximab. A pediatric-based regimen was used in pediatric and adult patients. Rituximab had been added in half of the adult patients. No difference was observed comparing children and adults with 96 and 94 % CR rates, respectively. The event-free survival was however poorer in adults compared to children with 72 % compared to 92 %, respectively. More toxic death (9 %) was observed in adults older than 40 years compared to the younger adults (4 %) and children (0 %). No difference was observed comparing adults with or without rituximab (Todeschini et al. 2012).

The *EPOCH* regimen represents a different approach to treat BL without methotrexate but based on etoposide, vincristine, doxorubicin, cyclophosphamide, and prednisone combined with rituximab. In 29 patients with a median age of 35 years, including HIV-positive patients, the CR rate and OS were reported to be 100 %. Results of a confirmatory larger multicenter study have to be awaited (Dunleavy et al. 2011).

The only study testing rituximab as a single agent in BL and B-AL was conducted by an international pediatric study group in mature B-cell NHL and B-AL. Before onset of chemotherapy, rituximab single dose was administered in conjunction with rasburicase, steroids, and intrathecal triple therapy in patients with CNS involvement. The response rate, defined as more than 25 % decrease of at least one lesion or bone marrow or peripheral blasts, was overall 41 %. The response rate was higher in BM involvement (67 %) compared to solid lesions (33 %). Although the response rate was lower than expected in the trial, the study still confirms that rituximab has even single drug efficacy in these rapidly proliferating diseases (Meinhardt et al. 2010).

It is still a matter of debate whether outcome in older patients with BL and B-AL can be treated with short intensive regimens combined with rituximab. According to the SEER registry, median age at diagnosis of Burkitt is 45 years. 59 % of the patients are older than 40 years and 30 % are aged above 60 years (Kelly et al. 2009). A meta-analysis of trials mostly conducted in the pre-rituximab era showed that patients older than 40 years had an inferior survival in 10 of 14 publications. However, if only studies published after 2000 were considered, the majority of trials reported survival rates above 60 %. Short intensive regimens with moderate-intensity chemotherapy yield favorable survival rates even in BL patients above 60 years (Hoelzer et al. 2007; Rizzieri et al. 2010; Thomas et al. 2006). Taking the poor results of CHOP-based regimens in BL into account, the application of dose-reduced short intensive regimens should be considered in older adults as well.

What is the role of rituximab in the treatment of BL and B-AL and which regimen is optimal? Firstly, neither rituximab-based studies nor historic comparators are comparable to each other due to different age median age, proportion of older patients, inclusion of HIV-positive patients, proportion of B-AL patients, and other factors. Most of the studies are small, oligocentric, or monocentric – with the exception of GMALL B-NHL backbone regimen (Hoelzer et al. 2007; Rizzieri et al. 2010). For the historical comparison not only the addition of rituximab may be important but also improved supportive care and optimized chemotherapy regimens. Despite worries, no study demonstrated an increase of toxicity by the addition of rituximab. Most importantly, several studies showed excellent results with chemo immunotherapy and a significant improvement compared to historic controls despite reduction of chemotherapy dose intensity (Hoelzer et al. 2007; Corazzelli et al. 2012). In younger patients, it is of interest to note that outcome with rituximab-based regimens designed for adults leads to similar or even better results than more intensive regimens without rituximab designed for younger patients. The BFM group has reported for a survival rate of 82 % for 101 adolescent patients (15–18 years)

with BL or B-AL compared to 88 % in children younger than 15 years (Burkhardt et al. 2011). In conclusion, rituximab-based regimens will become the standard treatment for adult BL and B-AL, whereas no preferred chemotherapy backbone can be defined.

9.9.4 CNS Prophylaxis

CNS involvement at diagnosis is observed frequently in BL and B-AL, and historic data show a high rate of CNS involvement at relapse if no CNS prophylaxis was administered. Contemporary regimens include a combination of several CNS active elements, such as intrathecal therapy, either methotrexate or triple combination, systemic HDMTX, and/or HDAC. It is important to note that CNS activity of HDMTX depends on dose. In the GMALL studies, higher CNS relapse rates were observed in older B-AL patients treated at a lower HDMTX dose of 0.5 g/m^2 only (Hoelzer et al. 2007). Some historic trials used CNS irradiation after two (Hoelzer et al. 1996) or four (Rizzieri et al. 2004) cycles. Intermittent irradiation leads to delays of systemic treatment and should therefore be avoided. Furthermore, the CALGB reported increased neurologic toxicity with CNS irradiation after four cycles, which was mitigated after omission of irradiation. Combination of short intensive regimens with intrathecal liposomal cytarabine is controversial. Severe toxicities were reported for the combination with hyper-CVAD at dense intervals and with high doses of systemic cytarabine (Jabbour et al. 2007). A combination of liposomal cytarabine with the CODOX-M/IVAC regimen was however reported to be feasible (Corazzelli et al. 2012). It remains open to discussion whether liposomal cytarabine can offer an advantage in terms of CNS prophylaxis compared to conventional intrathecal prophylaxis or whether it can contribute to improved feasibility of systemic therapy since systemic effects exerted by conventional intrathecal drugs are avoided. Whereas prophylactic CNS irradiation is avoided in most protocols nowadays, it remains open to discussion whether it might be beneficial in B-AL or in patients with initial CNS involvement. It should probably be considered in patients with solid lesions in the brain or neuroaxis.

9.9.5 Role of Local Irradiation

Irradiation at the end of chemo immunotherapy is still part of many R-CHOP-based regimens for DLBCL. In most of the protocols for BL or B-AL, irradiation was not administered systematically. In the pediatric counterparts of short- intensive chemotherapy, no irradiation was offered – albeit higher doses of drugs with extra compartment activity such as HDMTX have been administered. Therefore, no conclusive data are available. Irradiation might have a role with specific types of extranodal involvement, which may not be reached sufficiently by chemotherapeutic drugs, such as involvement of brain, spine, extradural tumors, or bone involvement. Irradiation has probably also a role in patients with residual tumors at the end of chemotherapy. No other promising salvage chemotherapies are available. Whereas positron emission tomography (PET) can hardly be applied in the short intervals between treatment cycles, it could be used in order to address the question whether residual tumors at the end of treatment still contain active lymphoma cells, thereby applying the revised criteria for remission evaluation in lymphoma (Cheson et al. 2007; Delbeke et al. 2009).

9.9.6 Role of Hematopoietic Cell Transplantation in First Remission

In historic trials in the pre-rituximab era, the use of autologous or allogeneic transplantation was reported for a number of trials. Most of them are retrospective. It has to be considered that patients actually receiving a transplant are always a selected group of patients surviving until the time of transplantation, most probably in complete remission and in good general condition. Therefore, it can be assumed that many of the patients included in transplantation programs had already been cured by chemotherapy. Registry data clearly represent such selected patient groups. Thus, the EBMT reported in 70 patients with BL

an OS of 72 % after autologous transplantation in first remission (Sweetenham et al. 1996).

In a report from British Columbia, 43 patients with BL or B-AL were analyzed. Only 27 of 43 patients proceeded to SCT, which was autologous in the majority of cases. OS and EFS was 45 % and 42 % for all patients, whereas EFS for transplanted patients was 51 % (Song et al. 2006). These results are clearly inferior to intensive chemotherapy-based regimens, particularly in the rituximab era.

In another small series, autologous transplantation was suggested after two cycles of chemotherapy. In 27 patients with a median age 36 (15–64) years without CNS or extensive bone marrow involvement, OS was 81 % (van Imhoff et al. 2005). Again, these results are not superior to rituximab-based contemporary protocols.

Since no clear prognostic factors – besides response – can be identified in BL and B-AL, which could provide a risk-adapted indication for SCT in first CR, autologous and allogeneic transplantation have no role in patients responding to contemporary immunochemotherapy regimens.

9.9.7 Relapsed and Refractory Disease

In BL and B-AL relapses generally occur early during the first year after diagnosis and only few relapses are reported later. Outcome after failure to achieve a remission or relapse is poor. It is therefore essential to closely follow up for response during first-line treatment in order to change treatment strategy in patients not achieving CR after four cycles. In earlier studies, all patients with a PR eventually relapsed and died (Magrath et al. 1996; Divine et al. 2005). Outcome of relapsed and refractory BL and B-AL remains poor after rituximab-based regimens; relapses occur however rarely, which makes it difficult to conduct prospective trials for new regimens in relapsed/refractory BL or B-AL.

There is no standard treatment for relapsed/refractory BL or B-AL. In clinical practice usually new, alternative combination regimens are administered such as protocols developed for relapsed DLBCL as ICE or DHAP. Data on results are not available but according to clinical practice response rates and particularly response duration is poor. High-dose chemotherapy with autologous stem cell support may be utilized to achieve a CR and to gain time to prepare for an allogeneic transplantation. Two registry studies reported survival rates of 37 and 32 %, respectively for patients with autologous transplantation in chemotherapy responsive relapsed/refractory BL (Sweetenham et al. 1996; Appelbaum and Thomas 1983). Survival was only 7 % in 14 patients with autologous SCT in refractory status (Sweetenham et al. 1996).

Results of allogeneic SCT are scarce. The EBMT reported results of 71 patients with BL or B-AL with a median age of 23 years. Most of the patients were transplanted with advanced disease with sibling SCT. The overall survival was 37 % and status at transplant and age were relevant prognostic factors. Interestingly in a matched pair analysis comparing allogeneic and autologous transplant, no difference was observed for BL/B-AL (Peniket et al. 2003).

Results from a single institution are somewhat in contrast to these findings. 25 patients with a median age of 16 years received autologous and 13 patients with a median age of 13 years allogeneic SCT. OS was 23 % after autologous and 31 % after allogeneic SCT. Outcome was influenced by remission status at SCT and number of prior regimens.

Altogether, it remains inconclusive whether allogeneic SCT is a reasonable option in relapsed/refractory BL. According to published data in patients achieving a CR, 20–30 % of the patients may achieve long-term survival. It is one particular risk in diseases with rapid proliferation that due to rapidly upcoming relapses after SCT, there is no time to establish graft-versus-lymphoma effects. Therefore, a concept with autologous SCT for remission induction followed by allogeneic SCT may be an option of interest.

9.9.8 Management of Lymphoma Intermediate Between BL and DLBCL

After new results on molecular pathology of BL and DLBCL became available, three new categories of disease are discussed regarding optimal

treatment: c-MYC-positive DLBCL, double-hit DLBCL, and aggressive B-cell lymphoma with features intermediate between DLBCL and BL (BL-DLBCL). There is some evidence that patients with these disease subtypes have poorer outcome compared to DLBCL if treated with standard therapies such as R-CHOP (reviewed by Sweetenham 2012).

Burkitt-like lymphoma was often included into trials for BL, and the GMALL group reported similar outcome compared to BL (Hoelzer et al. 2007). Another trial showed a poorer outcome with survival rates of 22 % compared to 75 % for BL and Burkitt-like lymphoma if treated with CHOP compared to short intensive regimens (Nomura et al. 2008). Since the new WHO classification no longer identifies Burkitt-like lymphoma, the new entity of BL-DLBCL data is still rare, and to define optimal treatment will remain a challenge (Thomas et al. 2011).

It was reported that patients with *DLBCL with MYC* rearrangement have a poor outcome with survival rates around 30 % if treated with R-CHOP (Johnson et al. 2009; Barrans et al. 2010; Savage et al. 2009). In one study, the survival was 31 % for MYC-positive DLBCL compared to 66 % in MYC-negative cases (Savage et al. 2009). Similar results were reported from a population-based study were seen (Barrans et al. 2010).

Double-hit lymphoma also has a poor prognosis with median survival below 1.5 years if treated with conventional regimens (Johnson et al. 2009; Le Gouill et al. 2007; Lin and Medeiros 2007; Tomita et al. 2009).

It would be of interest to test whether outcome of these entities may be improved by therapy with short intensive regimens. First, preliminary data have been reported for the DA-EPOCH-R regimen. In a small series, MYC rearrangements were detected in 10 % of DLBCL cases. Survival of six positive patients was 83 % compared to 76 % in MYC-negative DLBCL (Dunleavy et al. 2011). Three groups have reported outcome of CODOX-M/IVAC in patient cohorts including patients with BL-DLBCL with survival rates ranging from 82 to 87 % (Corazzelli et al. 2012; Maruyama et al. 2010; Mohamedbhai et al. 2011).

9.9.9 Supportive Care

Given the rapid doubling time of BL, the rapid institution of chemotherapy is of utmost importance. Often, therapy needs to be instituted prior to the results of molecular studies typically used to confirm the diagnosis. Therefore, a high degree of suspicion is required along with surgical colleagues who can rapidly obtain tissue for review by an expert hematopathologist. As described above, therapy in general consists of dose-intense multi-agent chemotherapy regimens. Some protocols employ a single less intense pre-phase chemotherapy regimen to provide additional time in completing the initial workup as well as to further protect against tumor lysis syndrome and improve a patient's performance status.

Patients are at high risk for tumor lysis syndrome (TLS), occurring spontaneously or with treatment initiation. Release of cellular contents can result in hyperkalemia, hyperphosphatemia, hyperuricemia, and hypocalcemia with or without renal failure. Prevention and management of tumor lysis syndrome typically includes aggressive intravenous hydration and the recombinant urate oxidase, rasburicase (Coiffier et al. 2008). In the pediatric population, recombinant or nonrecombinant urate oxidase has demonstrated faster lowering of uric acid levels as well as a decrease in TLS, renal failure, and hemodialysis when compared to allopurinol (Cairo et al. 2007; Goldman et al. 2001).

Post-chemotherapy supportive care is critical given the dose intensity and subsequent side effects associated with standard treatment regimens. As such, close outpatient follow-up to monitor side effects and transfusion requirements is needed. Patients require appropriate inpatient resources and staff experienced in chemotherapy administration and their respective side effects. Most notably is the sophistication involved in the use of IV high-dose methotrexate. Patients require pretreatment hydration and alkalization, monitoring of urine pH, renal and hepatic function, rapid turnaround in methotrexate levels, and attention to leucovorin rescue. For patients developing delayed methotrexate clearance from impaired renal function, glucarpidase, recently approved by the US food and drug administration, can be considered. Additionally, granulocyte colony-stimulating factor support as

Table 9.4 Short intensive therapy in HIV-positive BL

Author	Year	Age (median)	*N*	CD4 (cells/ml)	HAART (%)	Regimen	CR (%)	OS (%)
Hoffmann et al.	2006	41	20	254	65	B-NHL 86	75	55
Oriol et al.	2005	41 (23–65)	18	420	53	B-NHL90	68	46
Oriol et al.	2008	39 (29–54)	19	58 %>200	100	B-NHL-2002+R	88	73
Wang et al.	2003	40 (19–61)	14	149	46	CODOX-M/IVAC	63	60
Rodrigo et al.	2012	45 (32–56)	14	375	93	CODOX-M/IVAC±R	n.r.	86
Barnes et al.	2011	46 (17–68)[a]	14	237	93	CODOX-M/IVAC±R	93	68
Galicier et al.	2007	40 (20–57)	63	239	79	LMB86 (Stage IV)	70	47
Costello et al.	2004	18–65	13	158	100	CHOP+HDAC+HDMTX	n.r.	60
Cortes et al.	2002	43 (32–55)	14	77	64	Hyper-CVAD	92	48
Sparano et al.	2010	43–44[a]	27	194–295	71	EPOCH+R	70	n.r.

[a]Total cohort

well as antibacterial, antiviral, and antifungal prophylaxis are routinely administered.

9.10 Management in HIV-Positive Patients

Survival of HIV-positive patients has been improved dramatically with the use of combination antiretroviral therapy (CART). A Spanish cohort study analyzed the outcome after different lymphomas in HIV-infected patients treated with CART. The only prognostic factors were IPI and histologic subtype underlining the assumption that in the CART era HIV-positive patients have the chance to obtain similar outcomes as HIV-negative patients if adequately treated (Miralles et al. 2007).

The lifetime incidence of HIV-positive patients to develop BL has been estimated at 10–20 % (Noy 2010). BL or Burkitt-like lymphoma accounts for 20–40 % of HIV-related lymphoma. Although the clinical situation of HIV-positive patients diagnosed with BL seems to be improved in the CART era, a considerable number of patients are still diagnosed with BL and HIV in parallel.

In a retrospective, multicentric cohort study, HIV-positive BL patients were treated either with CHOP ($N=31$) or with the GMALL B-NHL86 protocol with a MTX dose of 1.5 g/m^2 ($N=20$). Median CD4 count at diagnosis was 213/l and the majority of patients had stage IV disease (61 %). With GMALL protocol compared to CHOP, the CR rate was 75 % compared to 40 % and the survival 55 % versus 34 % after 2 years. Patients treated with GMALL protocol had a higher median CD4 count and more patients had CART. The use of the GMALL protocol, the absence of previous AIDS, and the absence of cerebral manifestations were favorable prognostic factors in multivariable analysis (Hoffmann et al. 2006). Another retrospective comparison of HIV-positive patients with BL or B-AL was reported by the PETHEMA group. 31 patients received CHOP and 44 patients intensive short therapies. Both groups were comparable regarding major risk factors and regarding the use of CART. CR rates were 32 % in CHOP versus 67 % with intensive therapy with survival rates of 27 % versus 57 %, respectively (Xicoy et al. 2011). Overall there is general agreement nowadays that HIV-positive patients with BL should be treated with short intensive regimens similar to those used for HIV-negative patients (Table 9.4).

An earlier large prospective study included 63 HIV-positive patients treated with the French LMB protocol between 1992 and 2006. The majority of patients started or maintained CART parallel to chemotherapy. The CR rate was 70 % and the treatment-related mortality 11 %. Neutropenia-associated sepsis was particularly observed in patients with poor performance status and severely compromised immune function. The overall survival was 47 %. CD4 counts below 200 and ECOG status of more than 2 were identified as poor prognostic features (Galicier et al. 2007). Overall results appeared to be inferior compared to the same regimen in HIV-negative patients with 67 % survival (Divine et al. 2005).

CODOX-M/IVAC was evaluated in a small single center trial in HIV-positive patients with BL. The OS of 60 % was comparable to HIV-negative patients treated with the same protocol (Wang et al. 2003). A retrospective analysis of the same regimen with HDMTX dose of 3 g/m^2 included 14 patients; 13 had received CART and 10 had received rituximab. PCP prophylaxis and G-CSF had been added. OS after 1 year was 86 % and all ten patients receiving CART, rituximab, and chemotherapy were alive (Rodrigo et al. 2012). In another retrospective analysis on CODOX-M/IVAC with or without rituximab, 14 HIV-positive patients were included. The CR rate of 93 % and the OS of 68 % were similar to HIV-negative patients (Barnes et al. 2011).

A modified *CHOP* regimen with HDMTX (8 g/m^2) and HDAC (8 g/m^2) with G-CSF support and CART therapy without rituximab yielded overall survival rates of 60 % in 13 patients. CD4 count, virus load, and previous HAART therapy did not affect outcome. Radiotherapy was scheduled in patients with residual tumor or initial tumor size >4 cm (Costello et al. 2004).

Results of the *GMALL* protocol B-NHL90 with 3 g/m^2 HDMTX without rituximab were reported by the PETHEMA group. The CR rate was 71 % in 14 HIV-positive compared to 77 % in HIV-negative patients; corresponding survival rates were 43 % versus 55 %, respectively. Outcome was significantly poorer in patients older than 60 years (Oriol et al. 2003). A follow-up of this study with 19 patients confirmed the results. Responders to CART, either before onset of chemotherapy or after ($N=7$) had a significantly better survival compared to non-responders or non-CART users ($N=11$) with 85 % versus 27 % (Oriol et al. 2005).

In the subsequent study based on the GMALL protocol B-NHL2002 with rituximab, 19 HIV-infected patients were included. The majority had already received CART before or started CART during chemotherapy. CR rates (84 % versus 88 %) and OS rates (73 % versus 82 %) were not significantly lower for HIV-positive compared to HIV-negative patients. Of note, HIV-positive patients developed significantly more episodes with grade III–IV mucositis or severe infections (Oriol et al. 2008).

The *hyper-CVAD* regimen yielded in 14 patients with BL or B-AL 92 % complete remissions and 48 % overall survival. Again, patients receiving CART in parallel to chemotherapy appeared to have a better survival. Toxicities including infectious complications appeared to be similar to those in patients without HIV infection (Cortes et al. 2002).

In order to elucidate the role of rituximab in HIV-associated lymphoma, a randomized study with the *EPOCH* regimen was conducted. Rituximab was given either before each chemotherapy cycle or weekly for six weeks after completion of chemotherapy. The CR rate was 73 % in the concurrent arm and 55 % in the sequential arm. Toxicity was comparable. One-third of the patients had BL, and no difference in terms of CR rate was detected comparing concurrent or sequential arm. Also no difference was observed regarding OS; the trial was however not adequately powered to answer this question (Sparano et al. 2010).

Still a number of questions are discussed regarding optimal treatment of HIV-positive patients with BL, e.g., whether doses of chemotherapy should be reduced, whether rituximab should be added, and whether CART should be given in parallel to chemotherapy (Dunleavy and Wilson 2012). Data of prospective trials are scarce. Therefore, some conclusion must be based on results in HIV-negative patients. Overall, short intensive treatment appears to be feasible in HIV-positive patients, and results are nearly comparable to HIV-negative patients. Rituximab improves efficacy in HIV-negative patients and allows to reduce chemotherapy doses. No data indicate increased toxicity in HIV-positive patients. Rituximab should therefore be added to protocols for HIV-positive patients. Finally, most trials indicate that parallel CART is feasible and may even contribute to better results. Possible interactions between antiretroviral drugs and chemotherapy should be considered. HIV-positive patients are at higher risk for treatment-associated complications such as mucositis and infections and need specific efforts in terms of supportive care.

9.11 Open Questions and Future Treatment Options

Treatment of BL and B-AL with short intensive regimens including rituximab is one of the impressive success stories in hematology. Nevertheless, many questions remain open to discussion and there is space for treatment optimization.

For BL it will be one important goal to identify patients with favorable prognosis for reduction of treatment intensity. Markers of disease progression such as stage and type of involvement can be considered as well as new methods such as evaluation of MRD. The feasibility of this approach was demonstrated not only for PCR of t(8;14) (Mussolin et al. 2011) but also for quantitative PCR of individual IgH rearrangements (Shiramizu et al. 2011).

Outcome of B-AL needs to be improved further, particularly in older patients. Patients with B-AL have prolonged cytopenias due to bone marrow involvement and are at higher risk for treatment-related toxicities such as mucositis or severe infections. In older patients the risk of CNS recurrence may be increased due to lower applicable doses of HDMTX. More intensive CNS prophylaxis including new drugs such as liposomal cytarabine could be considered for clinical trials.

Improvement of supportive care still remains a challenge in all short intensive protocols, which are associated with considerable toxicities particularly in older patients. One unsolved question is the prophylaxis of mucositis. The 24-h infusion time and the dose of methotrexate are correlated with this complication. However, shortened infusion time may be associated with poorer outcome at least in advanced disease (Woessmann et al. 2005). Mucositis does not only impair quality of life of patients but leads to treatment delays and increases the risk of severe infections. Several approaches such as the use of keratinocyte-growth factor (Schmidt et al. 2008) are under investigation.

The number of patients with residual tumors after end of treatment is not neglectable. In these cases, partial remission or unconfirmed CR are stated and in some patient's irradiation or other types of salvage therapy are initiated. It is important to improve remission evaluation by integration of PET in patients with residual tumors after end of treatment to identify patients who need salvage therapy compared to those in whom treatment can be stopped.

Overall, the number of patients with refractory or relapsed disease is fortunately small. However, outcome of these patients is very poor. Several drugs such as histone deacetylase inhibitors, DNA methyltransferase inhibitors, cyclin-dependent kinase inhibitors, and proteasome inhibitors and targeted therapies to c-MYC such as antisense molecules, new CD20 antibodies, and CD19 bispecific antibodies are of interest (reviewed in (Foon et al. 2012)). However, due to the rareness of the disease, logistic challenges for clinical trials are considerable and interest of pharmaceutical companies in this rare entity is limited. Probably studies with new drugs in DLBCL should include for exploratory reasons also BL patients.

One of the most interesting fields of research is the question whether treatment of new molecularly defined entities such as BL-DLBCL, MYC-positive DLBCL, or double-hit lymphoma may be improved with Burkitt-type regiments. It will be a challenge to install timely diagnostic characterization by morphology, immunohistochemistry, Ki67 fraction analysis, and MYC analysis and other molecular tests in all patients with DLBCL. Since this characterization takes time, after identification of the respective features treatment may be shifted after a first cycle of standard R-CHOP therapy.

Finally, it will remain a challenge to define effective and practicable treatment regimens for BL also for low-income countries.

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Primary Mediastinal Large B-Cell Lymphoma

10

Peter Johnson, Jan Delabie, Scott Rodig, and Maurizio Martelli

Contents

Pathology: Jan Delabie and Scott Rodig

P. Johnson (✉)
Cancer Research UK Centre,
University Hospital Southampton, Southampton, UK
e-mail: johnsonp@soton.ac.uk

J. Delabie, MD
Department of Pathology, Oslo University Hospital,
Oslo, Norway
e-mail: jandel@ous-hf.no

S. Rodig, MD
Department of Pathology,
Brigham and Women's Hospital,
Harvard Medical School, Boston, MA, USA
e-mail: srodig@partners.org

M. Martelli
Division of Hematology, Department of Cellular Biotechnologies and Hematology,
Sapienza University of Rome, Rome, Italy
e-mail: martelli@bce.uniroma1.it

10.1 Introduction

Primary mediastinal large B-cell lymphoma (PMBCL) was first described in the 1980s (Lichtenstein et al. 1980; Levitt et al. 1982). It is a relatively uncommon clinicopathologic entity specifically recognised in the WHO classification of lymphoid malignancies (Harris et al. 1994; Harris et al. 1999). This malignancy is characterised by aggressive and locally invasive behaviour. Although in some respects it resembles nodal diffuse large B-cell lymphoma (DLBCL), it has distinct epidemiologic, morphologic, immunophenotypic, and clinical features. This lymphoma is a DLBCL that arises in the thymus from a putative thymic peripheral B cell.

10.2 Epidemiology

Primary mediastinal large B-cell lymphoma constitutes 2–4 % of non-Hodgkin lymphoma (NHL) and 6–10 % of diffuse large B-cell lymphomas (DLBCL) (Levitt et al. 1982; Harris et al. 1994).

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It is found worldwide (Cazals-Hatem et al. 1996; Armitage and Weisenburger 1998). It is more common in young adults (median age 35–40 years) with a female predominance and originates in the mediastinum, where it frequently presents with features of local invasion. No particular genetic or environmental risk factors have been clearly identified.

10.3 Pathology and Biology

The diagnosis of PMBCL is based on the integration of morphologic, immunophenotypic, genetic, and clinical data, according to the WHO classification, with the differential diagnosis mainly includes classical Hodgkin lymphoma (cHL), mediastinal grey zone lymphoma (MGZL), and other DLBCL subtypes, from which it cannot be reliably distinguished in some cases.

10.3.1 Cell of Origin

It is postulated that PMBCL derives from the small subset of thymic B cells with asteroid shape located around the Hassall's corpuscles in the medullary thymus which share with PMBCL a CD10-,CD21-,CD23+-phenotype. The clinical presentation within the anterior mediastinum and the identification of normal thymic cells that express the MAL protein support this hypothesis (Copie-Bergman et al. 2002).

10.3.2 Histopathology

Primary mediastinal large B-cell lymphoma has distinct morphological and phenotypic features. It is typically associated with compartmentalising alveolar fibrosis in the vast majority of cases (Moller et al. 1986; Cazals-Hatem et al. 1996; Paulli et al. 1999); however, this can vary from case to case and from field to field within the same specimen. The fibrosis tends to surround groups of lymphomatous elements, producing compartmentalisation of the neoplastic growth. In cases when thick collagen bands enclose clusters of neoplastic cells, the sclerosis is readily appreciated on hematoxylin- and eosin-stained sections. Tumour cells are large and polymorphic with rather abundant clear cytoplasm, and nuclei may be lobulated with prominent eosinophilic nucleoli. Not infrequently, Reed-Sternberg-like cells may be seen. In such instances, careful immunohistochemical evaluation is warranted in order to exclude the diagnosis of cHL. In this regard, it should also be noted that "grey zone" borderline cases combining features of PMBCL and cHL or cases of composite PMBCL and cHL can rarely be encountered (Moller et al. 1986; Paulli et al. 1999; Barth et al. 2002; Traverse-Glehen et al. 2005).

10.3.3 Immunophenotype

On immunophenotypic analysis, despite generally lacking surface and cytoplasmic immunoglobulin (Ig), PMBCL expresses B-cell-related antigens such as CD19, CD20, CD22, CD79a (at times variable), and PAX5 as well as the leukocyte common antigen (CD45) (Moller et al. 1986; Barth et al. 2002; Pileri et al. 2003; Loddenkemper et al. 2004). CD30 staining is observed in the vast majority of cases (~80 %), although it is weaker and less homogeneous than in cHL and anaplastic large-cell lymphoma (Pileri et al. 2003). CD15 is occasionally present. Tumour cells are more frequently positive for IRF4 (75 %), BCL2 (55–80 %), and CD23 (70 %), whilst BCL6 expression is variable (45–100 %) and CD10 is more often negative (8–32 %) (de Leval et al. 2001; Pileri et al. 2003). Tumour cells are often MAL positive, as a consequence of MAL gene overexpression (Copie-Bergman et al. 1999). The latter is located on the long arm of chromosome 2 and encodes a protein thought to play a role in membrane trafficking and signalling (Millan and Alonso 1998), which might contribute to pathogenesis. Furthermore, PMBCL usually expresses BOB1, PU1, and OCT2, co-expresses TRAF1, and presents with nuclear REL (Copie-Bergman et al. 2002; Copie-Bergman et al. 2003; Pileri et al. 2003; Rodig et al. 2007) (Fig. 10.1).

10.3.4 Diagnostic Criteria

The main differential diagnoses are classical Hodgkin lymphoma and diffuse large B-cell lymphoma (Table 10.1). Classical Hodgkin lymphoma can be distinguished from PMBCL by histological features such as abundant infiltration with granulocytes and small sized lymphocytes as well as histiocytes in the former. In addition, classical Hodgkin lymphoma expresses CD15 and less often a full set of B-cell markers. The B-cell transcription factor PAX5 is only weakly expressed in Hodgkin lymphoma, in contrast to PMBCL. MAL has been reported to be specifically expressed in PMBCL but is rather a difficult marker to stain for in routine practice (Copie-Bergman et al. 2002). Some cases with either morphological features of PMBCL but immunophenotypical features of classical Hodgkin lymphoma or vice versa do not allow a final diagnosis and are classified as B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma and classical Hodgkin lymphoma, or the so-called mediastinal grey zone lymphoma (Traverse-Glehen et al. 2005). The differential diagnosis with diffuse large B-cell lymphoma, NOS is not always easy. The distinct morphological features of PMBCL, such as clear cell proliferation and sclerosis, may be difficult to evaluate on small biopsies, and there is a lack of well-defined diagnostic criteria that can be routinely applied. The expression of CD23 in PMBCL may be useful in that respect (Calaminici et al. 2004). Recently, it was also demonstrated that immunohistochemical

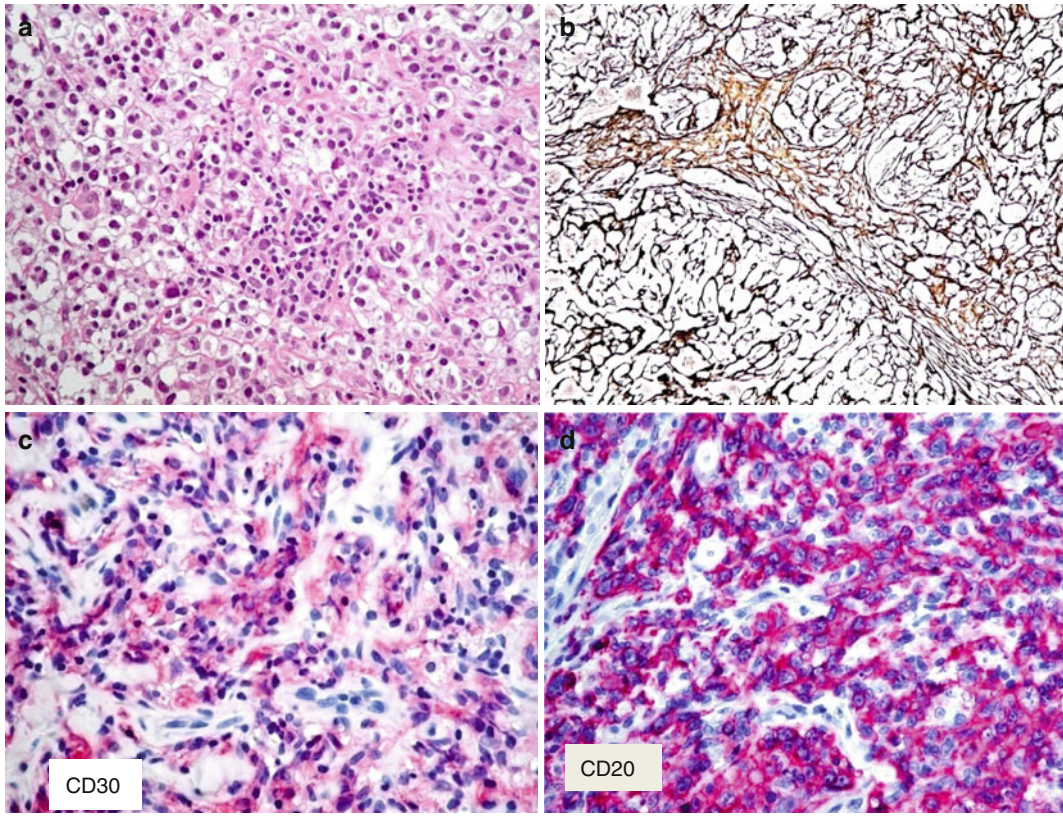

Fig. 10.1 Primary mediastinal B-cell lymphoma: (**a**) H&E staining, consists of the neoplastic large cells with clear cytoplasm, (**b**) fibrotic bands with compartmentalising alveolar fibrosis, (**c**) tumour cells express CD30 on their membrane, (**d**) typically shows CD20-positive cells, (**e**) tumour cells also show strong cytoplasmic staining for MAL antigen, (**f**) tumour cells are frequently BCL6 and IRF4 positive, (**g**) surface and cytoplasmic staining for immunoglobulin are mostly negative, and (**h**) the transcription factors OCT-2 and BOB-1 are usually expressed (Reprinted from Pileri et al. (2003). With the permission)

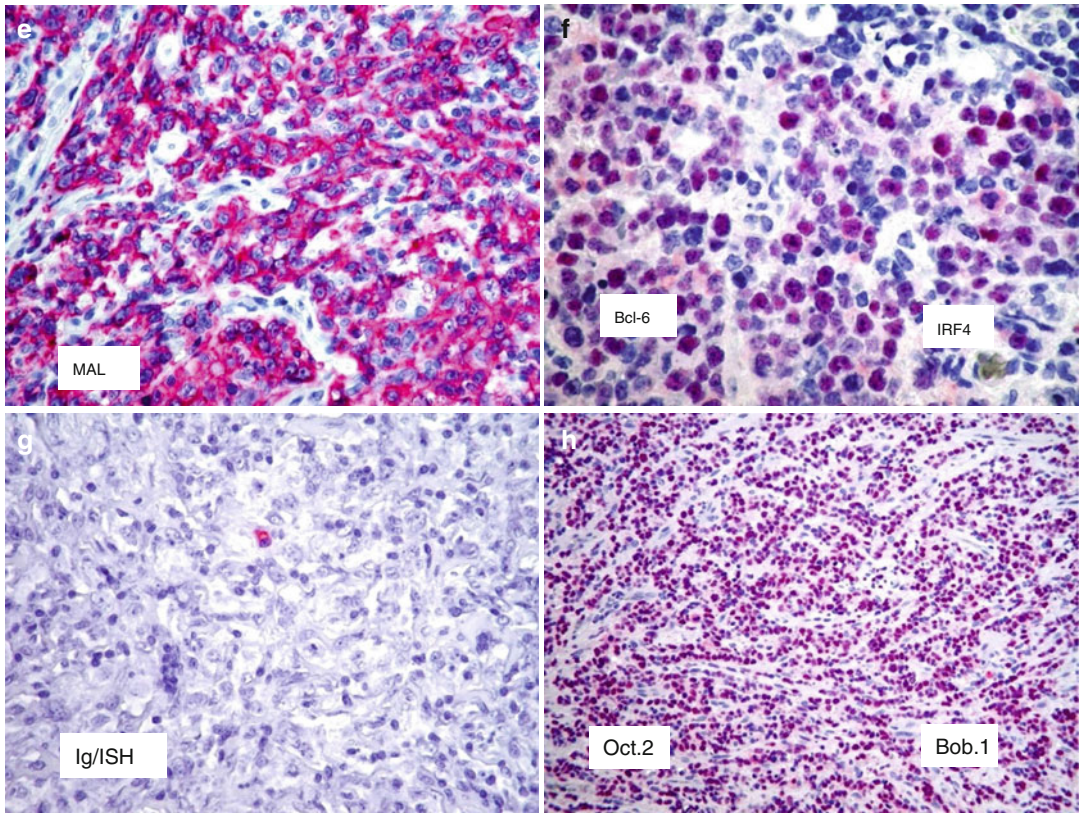

Fig. 10.1 (continued)

Table 10.1 Comparison of the pathological and immunophenotype features of primary mediastinal large B-cell lymphoma (PMBCL), diffuse large B-cell lymphoma (DLBCL), nodular sclerosis classical Hodgkin lymphoma (NScHD), and mediastinal grey zone lymphoma (MGZL)

Features	PMBCL	DLBCL	NScHL	MGZL
Morphology	Sheets of large cells; clear cells ; no inflammatory	Sheets of large cells with variable aspects	Lacunar Hodgkins Reed-Stenberg cells Inflammatory polymorphous infiltrate	Sheets of pleomorphic large cells; Lacunar Hodgkins Reed Stenberg cells; sparse inflammatory infiltrate
Sclerosis	70–100 % (alveolar, fine bands)	Absent	100 % (large bands)	Focal fibrous bands
CD45	Positive	Positive	Negative	Positive
CD30	Positive weak (70–80 %)	Rare (anaplastic variant)	Positive	Positive
CD15	Negative	Negative	Positive	Positive
CD20	Positive	Positive	Negative	Positive
CD79a	Positive	Positive	Usually negative	Positive
PAX-5	Positive	Positive	Weak positive	Positive frequently
Immunoglobulin	Negative	Positive	Negative	Negative
BOB-1	Positive	Positive	Negative	Positive frequently
OCT-2	Positive	Positive	Negative	Positive frequently
MAL expression	60–70 %	<10 %	<20 %	30–40 %

analysis of TNFAIP2, expressed by most cases of PMBCL and Hodgkin lymphoma but not by diffuse large B-cell lymphoma, NOS, may be useful for making a correct diagnosis (Kondratiev et al. 2011). Gene expression analysis allows for an improved distinction between PMBCL and diffuse large B-cell lymphoma, NOS, but can as yet not be used in clinical practice (Rosenwald et al. 2003).

10.3.5 Genetic Characteristics

Gene alterations are diverse, and copy number gains of REL, PDL1/PDL2, JAK2, and JMJD2C; chromosomal rearrangement of CIITA; mutations of SOCS1, STAT6, TNFAIP3, MYC, and TP53; or promotor hypermethylation of p16/INK may be seen (Steidl and Gascoyne 2011). The common consequences of these changes are activation of JAK-STAT signalling and NFkB pathways, resulting in increased cell proliferation and survival. In addition, downregulation of HLA class II molecules as well as overexpression of PD-1 ligands as a consequence of the genetic changes cited above may allow PMBCL to escape immune surveillance. Interestingly, gene expression studies have shown a remarkable overlap of highly expressed genes and gene changes between PMBCL and Hodgkin lymphoma (Rosenwald et al. 2003; Steidl and Gascoyne 2011). Not surprisingly, the differential diagnosis between PMBCL and mediastinal Hodgkin lymphoma can be difficult and virtually impossible in some cases (Traverse-Glehen et al. 2005). The latter cases are called B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma, and classical Hodgkin lymphoma, also known as "mediastinal grey zone lymphoma."

10.4 Clinical Presentation

10.4.1 Clinical Features

Primary mediastinal large B-cell lymphoma normally presents with a bulky tumour in the anterior mediastinum that is rapidly progressive and gives rise to local compressive effects including dyspnea, cough, dysphagia, and superior vena cava obstruction. Up to one-half of patients have symptoms and signs of superior vena cava syndrome, thoracic and neck vein distension, facial oedema, conjunctival swelling, and occasionally arm oedema. This results in relatively early presentation so that at diagnosis, most patients (around 80 %) have stage I or II disease. The mediastinal tumour is frequently bulky, being over 10 cm in two-thirds of patients, and infiltrating the lung, chest wall, pleura, and pericardium (Falini et al. 1995; Armitage and Weisenburger 1998). Pleural or pericardial effusions are present in one-third of cases (Lazzarino et al. 1997; Zinzani et al. 2001). Breast oedema is common, and hoarseness may reflect recurrent laryngeal nerve damage (Fig. 10.2). Despite the local invasiveness, distant spread is infrequent at the outset, and even spread to the supraclavicular nodes is unusual at presentation. Extranodal sites may, however, be involved, particularly in cases of disease recurrence, with an unusual propensity for involvement of the kidneys, adrenal glands, liver, and ovaries (Haioun et al. 1989; Lazzarino et al. 1993; Bishop et al. 1999). Systemic symptoms, mainly fever or weight loss, are present in a minority of cases. Bone marrow infiltration at presentation is rare, but elevated lactate dehydrogenase levels are observed in two-thirds of patients. MGZL shows similar clinical features but, compared to PMBCL, is more common in young men and more often has extranodal involvement (Table 10.2).

10.4.2 Diagnostic and Staging Procedures

The complete staging workup for PMBCL is the same as that routinely used for nodal lymphoma. It includes an accurate physical examination, complete hematologic and biochemical examinations, total body computerised tomography, and bone marrow biopsy. The staging system used is the standard Ann Arbor classification (Carbone et al. 1971). A diagnostic tissue sample can be obtained by mediastinoscopy, biopsy of the tumour mass through the supraclavicular fossa,

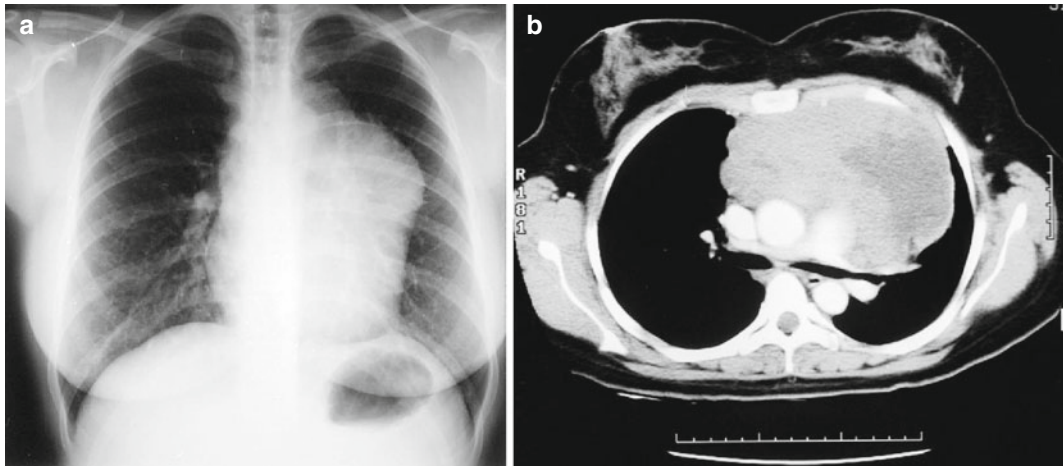

Fig. 10.2 CXR (**a**) and CT (**b**) scan from a female patient presenting with PMBCL. Note the large anterior mediastinal mass, with areas suggestive of central necrosis. Marked breast oedema is present

Table 10.2 Comparison of the clinical features of: primary mediastinal large B-cell lymphoma (PMBCL), diffuse large B-cell lymphoma (DLBCL), nodular sclerosis classical Hodgkin lymphoma (NScHD), and mediastinal grey zone lymphoma (MGZL)

Features	PMBCL	DLBCL	cHL	MGZL
Female/male ratio	2:1	1:1	1:1	1:2
Median age	35	55	28	35
Stage I–II	70–80 %	30 %	55 %	70–80 %
Mediastinal invol.	All	20 %	80 %	All
Extranodal sites	Uncommon	Common	Uncommon	Uncommon
Bone marrow	2 %	10–15 %	3 %	3 %
Elevated LDH	70–80 %	50 %	Rare	70–80 %
B symptoms	<20 %	50 %	40 %	40 %
Bulky disease	70–80 %	10–15 %	50 %	70–80 %

anterior mediastinotomy, or minithoracotomy. It is important to consider the anaesthetic risk for patients with critical airways narrowing by anterior mediastinal tumours: it may be preferable to obtain a needle core biopsy by a percutaneous route under local anaesthesia than to obtain a large biopsy but have a patient who cannot be extubated following the procedure because of airway compromise.

PMBCL shows almost universal avidity for [18F]-2-fluoro-2-deoxyglucose, making positron emission tomography (FDG-PET) an effective means to assess disease extent and to characterise residual masses at the completion of treatment. The extent of experience with this technique is, however, too limited to permit major changes to therapy based upon FDG-PET scans at present, pending the results of prospective trials.

10.4.3 Prognostic Factors

The utility of the International Prognostic Index (IPI) in PMBCL is limited by the age distribution of the disease and its usual confinement to the mediastinum. This is reflected in the observation that half of patients have low IPI scores at presentation (Abou-Elella et al. 1999). The age-adjusted IPI has similarly been reported to be of limited predictive value in PMBCL. This may reflect differences between studies, assigning patients as either stage IV or stage 2E when

contiguous extranodal sites such as the lung are involved (Todeschini et al. 2004; Hamlin et al. 2005; Savage et al. 2006). Elevated LDH to more than twice the upper limit of normal, age over 40, and performance status ≥2 all correlated with reduced survival in a population-based series from British Columbia (Savage et al. 2006), whilst in a large series from the International Extranodal Lymphoma Study Group (IELSG), male sex, poor performance status, and advanced-stage disease were significant negative predictors (Zinzani et al. 2002). Recent gene expression studies have suggested that low expression of major histocompatibility (MHC) class II genes correlate with a poor outcome (Roberts et al. 2006).

10.5 Treatment and Outcome

The first line of treatment and its outcome are critical in managing PMBCL. Therapy for recurrence or progressive disease is of strictly limited efficacy (Todeschini et al. 2004; Savage et al. 2006; Kuruvilla et al. 2008) making curative therapy at the first attempt even more important for this type of lymphoma. It is, however, important to strike an appropriate balance between the delivery of the highest possible cure fraction and minimising the long-term morbidity for this young population. A number of choices have to be made, including the initial chemotherapy/immunochemotherapy and whether there might be a benefit from high-dose therapy in first remission. The role of consolidation radiotherapy to the mediastinum is especially controversial.

10.5.1 Choice of Initial Treatment Regimen

There is broad agreement that for conventional DLBCL, the standard of care is the R-CHOP regimen. Prior to the introduction of rituximab, no advantage was demonstrated for the use of third-generation anthracycline-containing regimens over conventional CHOP for DLBCL in general (Fisher et al. 1993), but some retrospective series in PMBCL suggested that superior outcomes might be achieved with latter generation regimens (Todeschini et al. 2004). The largest series was from the IELSG, which reviewed the outcomes of 426 previously untreated patients with PMBCL (Zinzani et al. 2002). Most of the patients that were treated with a third-generation regimen received MACOP-B ($n=204$), the rest either VACOP-B ($n=34$) or ProMACE CytaBOM ($n=39$). Although the complete response rate was similar between the third-generation subgroup and those treated with conventional CHOP or CHOP-B, the relapse rate at 3 years was significantly lower in the third-generation group (12 % vs. 23 %; $P=0.02$), and the projected 10-year overall and progression-free survival were superior at 71 and 67 %, compared to 44 and 33 % ($P=0.0001$ and $P=0.0003$, respectively). The British Columbia group carried out a population-based retrospective analysis of 153 patients with PMBCL whose treatment was determined by era-specific guidelines (Savage et al. 2006). Between 1980 and 1992 MACOP-B or VACOP-B was used, switching to CHOP between 1992 and 2001 and then to rituximab with CHOP (R-CHOP) thereafter. The overall survival for the cohort was 75 % at 5 years, with the overall survival at 5 years being 87 % for those treated with MACOP-B/VACOP-B, significantly higher than the 71 % for those patients treated with CHOP ($P=0.048$). Comparison of the baseline characteristics, however, demonstrated a greater number of poor risk patients in the CHOP group. In the multivariate analysis for overall survival, the type of chemotherapy regimen showed a trend towards improved outcomes, but this was not statistically significant.

10.5.2 The Addition of Rituximab to Chemotherapy

It is generally accepted that the addition of rituximab to chemotherapy for PMBL yields superior results. The MiNT study compared the outcomes for 824 patients with low-risk large B-cell lymphoma randomised to receive CHOP-like chemotherapy with or without rituximab (Pfreundschuh et al. 2006), which included a subset of 87

patients with PMBCL. The addition of rituximab increased the CR rate from 54 to 80 % and the 3-year event-free survival from 52 to 78 % ($p=0.012$). The difference in overall survival did not reach statistical significance owing to the small number with PMBCL (3-year OS 78 vs. 89 %, $p=0.16$), but was of the same order as that seen for the whole trial (85 % vs. 93 %, $p<0.001$) (Rieger et al. 2011). The addition of rituximab to dose-adjusted EPOCH (etoposide, vincristine, doxorubicin, cyclophosphamide, and prednisone) in small numbers of patients with PMBL has also been reported as showing a favourable event-free ($P=0.036$) and overall survival ($P=0.023$) in a non-randomised comparison (Dunleavy et al. 2006). In a small series from Israel, the addition of rituximab appeared to improve progression-free survival, particularly in those patients receiving CHOP, whilst there was no difference in outcomes in a comparison between either a third-generation regimen with rituximab or CHOP with rituximab (R-VACOP-B vs. R-CHOP, 84 % and 74 %, respectively; $P=0.44$) (Avigdor et al. 2007). Overall, it appears likely that the use of rituximab removes the distinction between different chemotherapy regimens, and R-CHOP is now the most widely used for PMBCL, as it is for other types of large B-cell lymphoma.

10.5.3 Assessment of the Response to Initial Therapy

The presence of bulky masses at the time of diagnosis, together with the extensive fibrotic elements of PMBL, often results in a residual mediastinal mass being present at the completion of initial chemotherapy. It may be difficult to distinguish inert fibrous tissue from viable residual lymphoma on conventional cross-sectional imaging, and for this reason, functional imaging has been extensively investigated. The ^{67}gallium scan was found to have predictive value for PMBL in identifying patients at risk of relapse (Zinzani et al. 1999); however, it is time consuming to perform and has poor spatial resolution. The ^{18}Fluoro-Deoxy-Glucose Positron Emission Tomography (FDG-PET) scan has become the investigation of choice for residual masses in PMBCL, although there is some uncertainty about its positive predictive value in particular. A systematic review of FDG-PET studies has examined post-therapy response assessment in lymphoma (Terasawa et al. 2008). In the studies reporting evaluation of residual masses in aggressive NHL, the demonstrated sensitivity of PET ranged from 33 to 87 % and the specificity from 75 to 100 %. A prospective study of FDG-PET scanning in patients with PMBCL after 4 cycles of accelerated (14 days) R-CHOP performed at Memorial Sloan Kettering Cancer Center showed that among 14 patients with interim positive PET scans, none had viable lymphoma present on biopsy, and all remained in remission after completing consolidation R-ICE chemotherapy (Moskowitz et al. 2010). A prospective study of FDG-PET scanning 125 patients with PMBCL conducted by the IELSG yielded a relatively low rate of negative scans at under 50 % despite excellent clinical outcomes, albeit after the use of consolidation radiotherapy in 123 cases (Martelli et al. 2011). These data indicate that further evaluation is required before modifying planned therapy based upon FDG-PET evaluation alone in PMBCL. The false-positive rate in particular requires definition, although de-escalation of therapy based upon the finding of a negative FDG-PET scan is entering clinical practice and is the subject of a prospective randomised trial.

10.5.4 The Role of Consolidation Radiotherapy

Irradiation of the mediastinum is one of the most controversial aspects of the management of PMBCL. It is not attractive to administer radiation extensively to a group dominated by younger subjects, who may be put at increased risk of second malignancies, especially breast cancer and accelerated coronary artery disease. On the other hand, the chances of cure following recurrence of PMBCL are relatively poor, so that any approach which puts patients at increased risk of relapse is to be strenuously avoided. The best outcomes historically have been reported with regimens that

incorporated radiotherapy as part of the primary treatment (Todeschini et al. 2004; Mazzarotto et al. 2007; De Sanctis et al. 2008). It is clear from the IELSG series that many patients completing chemotherapy in PR may be converted to CR following radiotherapy (Zinzani et al. 2002), that radiotherapy may render active residual mediastinal masses ^{67}gallium negative (Zinzani et al. 1999), or result in long-term remission after a positive FDG-PET scan (Martelli et al. 2011). Univariate and multivariate analyses in two retrospective series have suggested that the use of radiotherapy was correlated with better event-free and overall survival (Todeschini et al. 2004; Rodriguez et al. 2008).

Those who would prefer to avoid irradiation of the mediastinum can however point to good results in studies that have used chemotherapy alone (Cazals-Hatem et al. 1996; Hamlin et al. 2005; Dunleavy et al. 2013; Massoud et al. 2008). In British Columbia, the introduction of routine radiotherapy to consolidate response after chemotherapy was not accompanied by any improvement in progression-free or overall survival, even for initially bulky disease (Savage et al. 2006). The study from Memorial Sloan Kettering Cancer Center which used radiotherapy in only 7 % of patients treated with the NHL-15 regimen (comprising intensified doxorubicin, vincristine, and cyclophosphamide) had excellent results, with overall survival of 84 % at a median follow-up of over 10 years (Hamlin et al. 2005). The results that have been reported with dose-adjusted EPOCH in combination with rituximab are also claimed to negate the need for irradiation (Dunleavy et al. 2013).

It is clear that further research is needed in order to determine the safety of omitting radiation in patients with non-FDG avid mediastinal masses at the completion of chemotherapy.

10.5.5 Intensification with High-Dose Therapy at First Remission

Before the widespread use of consolidation radiotherapy to the mediastinum, the results with PMBCL were thought to be inferior to those of other types of DLBCL, and this, together with the rarity of marrow involvement and the younger age of PMBCL patients, led to the testing of high-dose chemotherapy and peripheral blood progenitor rescue at first remission. The largest series reported comes from the GEL-TAMO registry (Rodriguez et al. 2008). Thirty-five patients in first CR, but considered at 'high-risk' of relapse, underwent high-dose therapy with variable conditioning regimens. At 4 years, the overall and progression-free survival were 84 and 81 %, respectively, similar to the results seen among 12 patients (8 in CR and 4 in PR) reported by Sehn et al. (1998). Just over half the patients in the GEL-TAMO series also received irradiation either before or after high-dose therapy, and this was one of the dominant variables associated with overall survival in multivariate analysis. In the IELSG analysis, a limited number of patients ($n=44$) underwent high-dose therapy which resulted in an estimated overall survival of 77 % at 10 years (Zinzani et al. 2002). In the Memorial Sloan Kettering experience high-dose therapy with progenitor cell rescue at first remission was not superior to dose-dense sequential therapy (Hamlin et al. 2005).

Taken overall, the results now obtained with R-CHOP and consolidation radiotherapy to the mediastinum appear favourable by comparison with the reports of high-dose consolidation, which is not widely used at first remission in other types of DLBCL. At present, there is no good evidence to support its use in this context for PMBCL.

The exception to this may be those patients whose lymphomas progress during primary therapy. These have a very poor outlook: of 14 patients in the British Columbia series, the majority were resistant to alternative chemotherapy regimens, and there were no long-term survivors (Savage et al. 2006). Sehn et al. however reported on 12 patients with refractory disease who at 5 years had a progression-free survival of 58 % following high-dose chemotherapy (Sehn et al. 1998). It is appropriate in this setting to test chemosensitivity to a second-line regimen prior to myeloablative treatment, proceed in those fit

enough to do so, and consolidate the response with involved field radiotherapy.

10.5.6 Treatment of Recurrent Disease

The probability of recurrence after successful initial therapy for PMBCL appears to be lower than that of DLBL in general, although this may reflect the earlier stage at presentation, the younger age, or possibly the biology of the disease. Most recurrences occur within the first year, and they are rare beyond two years from completion of therapy (Zinzani et al. 1999; Todeschini et al. 2004; Savage et al. 2006). Extranodal sites of recurrence are not uncommon, especially the kidneys and spleen, but spread to the central nervous system is highly unusual (Papageorgiou et al. 2012).

Second-line treatment strategies are similar to those used for DLBCL, attempting reinduction with non-cross-resistant agents, followed by consolidation with high-dose chemotherapy in those with a good response who remain fit enough. In general, the outcomes have been disappointing. In one series of 138 patients, all those who relapsed died from their lymphoma (Todeschini et al. 2004), although another series from the MD Anderson Cancer Center had 42 % long-term survivors (Popat et al. 1998). The general use of rituximab in first-line therapy has made recurrence less frequent but harder to manage successfully.

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CNS Lymphoma

11

Agnieszka Korfel, James Rubenstein, German Ott, and Eric D. Hsi

Contents

Pathology: German Ott and Eric D. Hsi

A. Korfel (✉)
Department of Hematology and Oncology, Charité Universitätsmedizin, Campus Benjamin Franklin, Berlin, Germany
e-mail: agnieszka.korfel@charite.de

J. Rubenstein
Division of Hematology and Oncology, Department of Medicine, University of California, San Francisco, CA, USA
e-mail: jamesr@medicine.ucsf.edu

G. Ott, MD
Department of Clinical Pathology, Robert-Bosch-Krankenhaus, Stuttgart, Germany
e-mail: german.ott@rbk.de

E.D. Hsi, MD
Department of Clinical Pathology, Cleveland Clinic, L11, 9500 Euclid Ave, Cleveland, OH 44195, USA
e-mail: hsie@ccf.org

11.1 Pathology

The great majority of primary PCNSL lymphomas (90 %) are diffuse large B-cell lymphomas (DLBCL), and in a recent series, all 75 cases of PCNSL were DLBCL (Gerstner and Batchelor 2010; Preusser et al. 2010). The other 10 % of cases are composed of rare occurrences of intravascular lymphomas, Burkitt lymphomas, and rare examples of peripheral T-cell lymphomas. Low-grade B-cell lymphomas such as lymphoplasmacytic lymphoma are extremely rare and seem to have a better prognosis (Figs. 11.1 and 11.2) (Jahnke et al. 2006a). MALT-type lymphomas typically involve the dura, mimicking meningioma, and are thought to arise from this structure but demonstrate features similar to MALT lymphomas at other sites (Tu et al. 2005).

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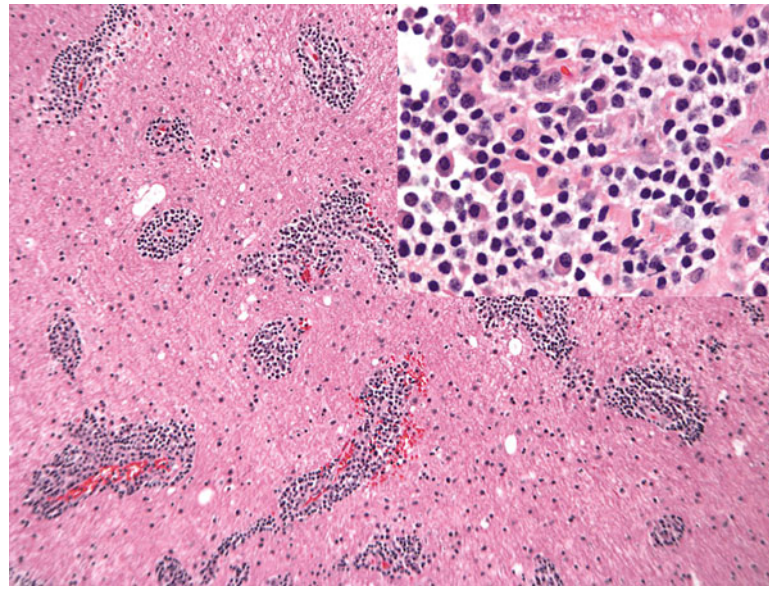

Fig. 11.1 A rare case of primary CNS lymphoplasmacytic lymphoma demonstrating a perivascular lymphoid infiltrate (10×) composed of lymphoplasmacytic cells (400×)

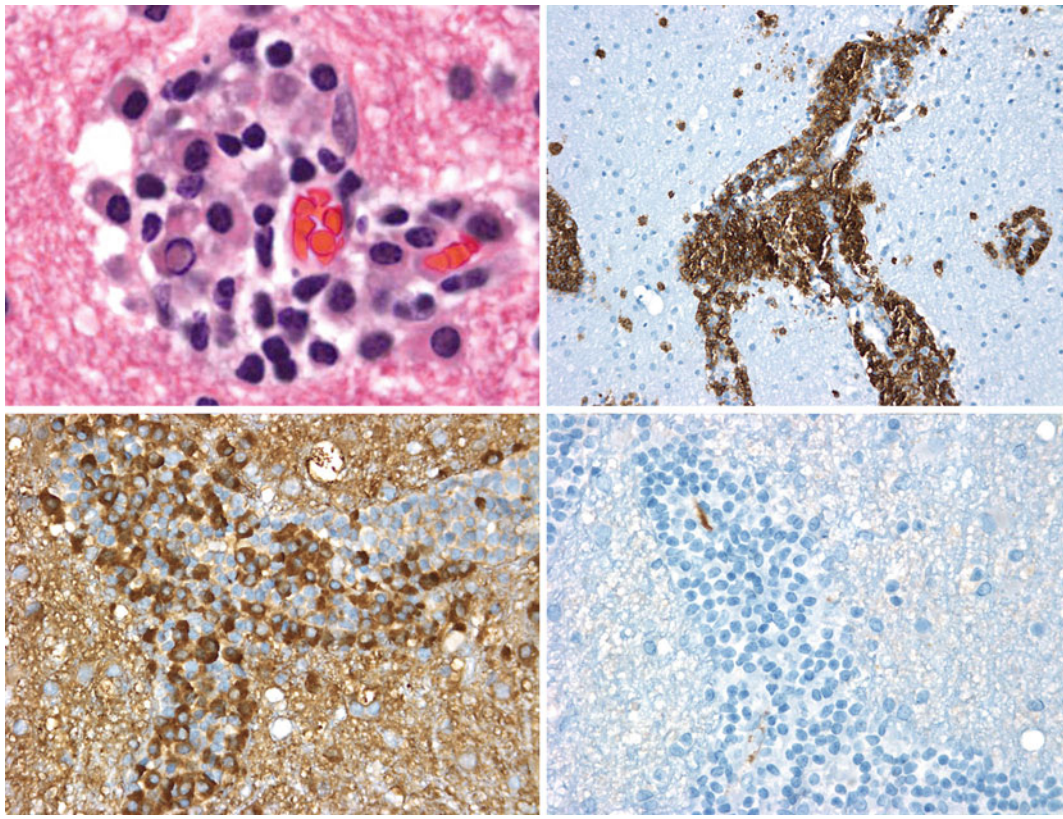

Fig. 11.2 Lymphoplasmacytic lymphoma from Fig. 11.1 showing the presence of a Dutcher body (*upper left*). The lymphomas expressed CD20 (*upper right*, 400×). Kappa (*lower left*) and lambda (*right*) light-chain staining showed kappa restriction (400×)

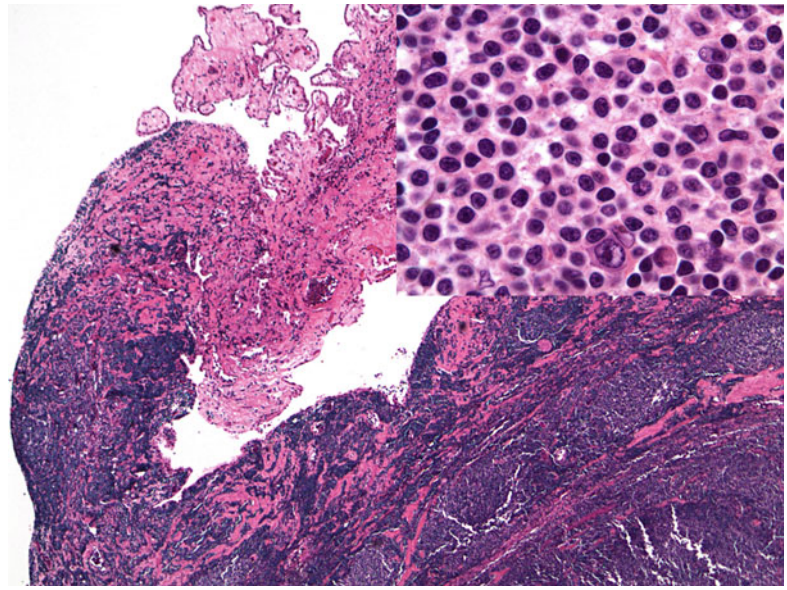

Fig. 11.3 A rare case of mucosa-associated lymphoid tissue (*MALT*)-type lymphoma arising from the ventricular choroid plexus (*upper left quadrant*) (20×). The *inset* (400×) shows the cytologic feature of the lymphomas, which was CD20+, CD5−, CD10−, and monoclonal

Rare cases have been reported in the brain parenchyma or ventricles (Kelley et al. 2005) (Fig. 11.3). Their pathologic features are similar to non-CNS sites; however, their detailed histopathologic features are not well characterized. A perivascular pattern is seen in lymphoplasmacytic lymphoma (Fig. 11.2), but many reported cases may not be primary PCNSL (Ly et al. 2011). Further discussion will be confined to the pathologic features of PCNSL DLBCL.

In PCNSL, many biopsies are now stereotactic biopsies and thus only a small amount of tissue is available for diagnosis. Although architecture is therefore limited, most cases will show a diffuse growth pattern consisting of intermediate-to-large cells with vesicular chromatin. A centroblastic appearance is most common with an immunoblastic appearance being seen in less than 10 % of cases (Preusser et al. 2010). Immunoblastic morphology is more frequently seen in the setting of HIV infection. Rare cases may demonstrate plasmablastic features (Urrego et al. 2011). Necrosis is often present and when vessels are represented, a propensity for tumor cells to be present in a perivascular location can be seen (Fig. 11.4) (Preusser et al. 2010).

Secondary CNS involvement is extremely rare in indolent lymphomas. It is observed in up to 6 % of aggressive non-Hodgkin's lymphomas and is more frequent in Burkitt lymphoma and lymphoblastic lymphoma (Herrlinger et al. 2009).

11.1.1 Immunophenotype

PCNS DLBCL expresses pan-B-cell antigens such as CD19 and CD20 as well as monotypic surface immunoglobulin light chains. CD10 is expressed in only a minority (<10 %) of cases, BCL6 in 60–80 %, and IRF4/MUM1 in 90 % (Preusser et al. 2010). Thus, a non-germinal B-cell phenotype is diagnosed in most cases (Fig. 11.5) (Preusser et al. 2010; Hattab et al. 2010; Hans et al. 2004). HLA molecules are often absent and likely related to genetic loss of the HLA locus at chromosome 6p21.3 (Booman et al. 2006; Riemersma et al. 2000). BCL6 expression has been found to be of prognostic relevance (favorable) in more than one study (Preusser et al. 2010; Braaten et al. 2003; Levy et al. 2008; Lin et al. 2006; Song et al. 2011), but others have found the opposite in the setting of high-dose methotrexate (HDMTX) and radiation (Momota et al. 2010).

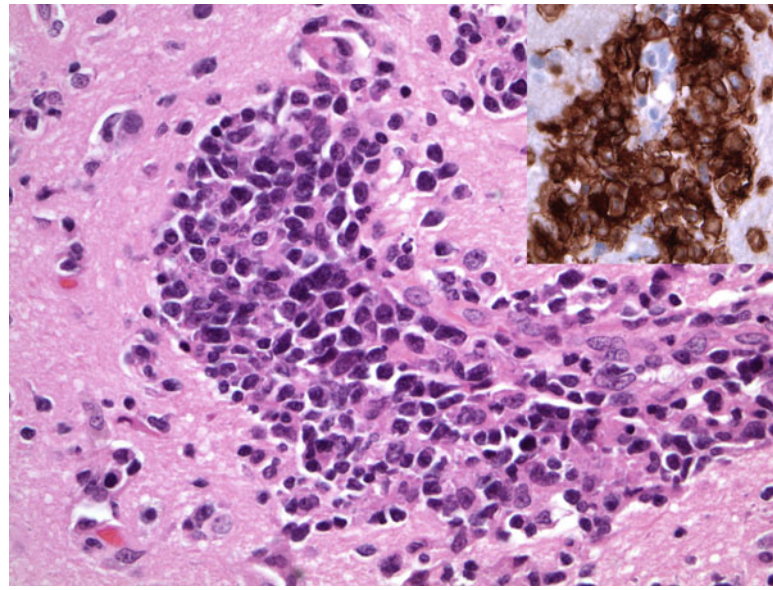

Fig. 11.4 PCNSL with the histology of diffuse large B-cell lymphoma showing a perivascular distribution (400×) and CD20 expression (*inset*, 400×)

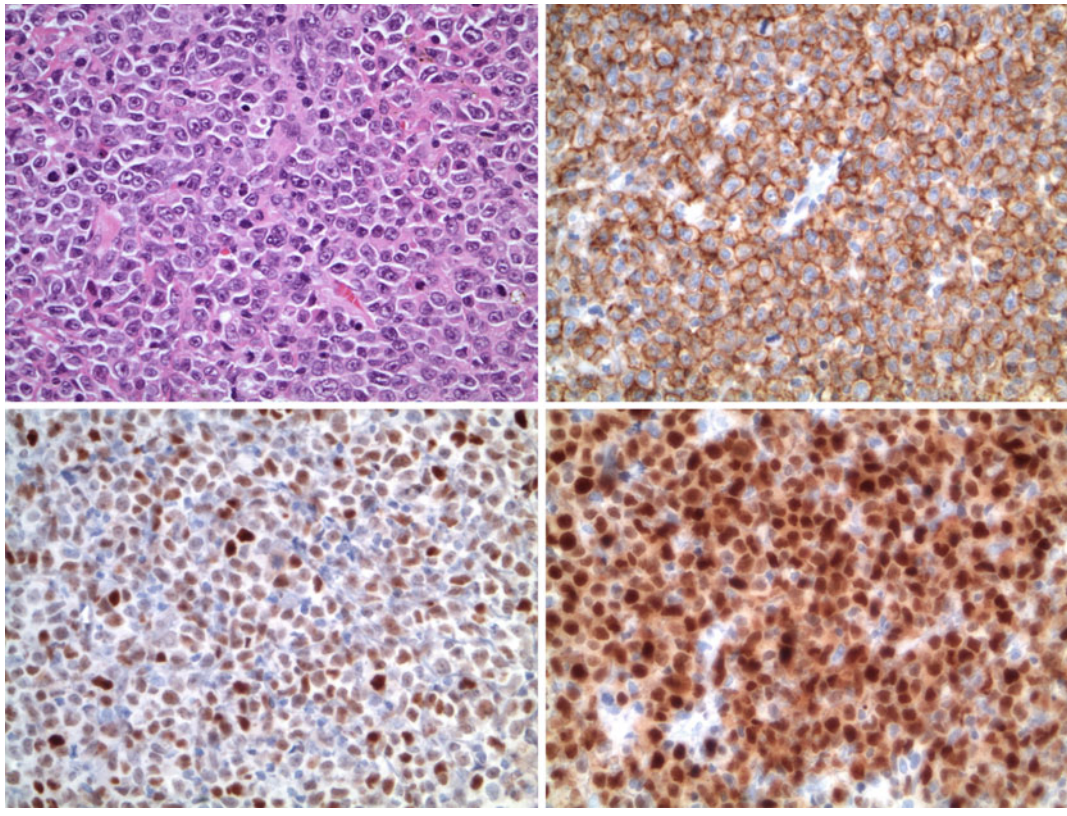

Fig. 11.5 PCNS diffuse large B-cell lymphoma with a non-germinal center B-cell immunophenotype. The hematoxylin and eosin stain shows a diffuse sheet of large lymphoid cells with prominent nucleoli that replaces the normal brain parenchyma (400×, *upper left*). The cells were positive for CD20 (*upper right*, 400×) and negative for CD10 (not shown) but expressed BCL6 (*lower left*, 400×) and MUM1 (*lower right*) and would be classified as non-germinal center B-cell phenotype according to the Hans classifier (Cady et al. 2008)

11.1.2 Genetics

Relatively little is known about the molecular genetics of PCNSL DLBCL, due to its rarity and lack of adequate tissue for such studies. Montesinos-Rongen et al. demonstrated that these tumors often show somatic hypermutation of the rearranged immunoglobulin genes and preferential use of the VH4-34 gene segment (Montesinos-Rongen et al. 1999). Del(6q)(q22) and *BCL6* translocation (usually partnered with *IGH@*) were reported in 45 and 17 % of cases, respectively, and appear to be associated with inferior survival in the setting of HDMTX therapy, whereas MYC translocations were found in 3 % of cases (Cady et al. 2008). EBV is usually absent in immunocompetent patients but is often present in cases involving immunocompromised patients (Preusser et al. 2010; Cavaliere et al. 2010; Knowles 2003). Gene expression studies have reported high-level expression of regulators of the unfolded protein response signaling pathway, *MYC*, and *PIM1* and have identified a potential role for IL-4/STAT6 signaling (Rubenstein et al. 2006). Pathway analysis revealed that PCNSL, as compared to non-CNS DLBCL, is characterized by differential expression of multiple extracellular matrix (ECM) and adhesion-related pathways. The most significantly upregulated gene was the ECM-related osteopontin (SPP1) (Tun et al. 2008). Differential expression of microRNAs (mRNAs) has been found between nodal and CNS DLBL (Fischer et al. 2011a). MiRNAs associated with the MYC pathway (*miR-17-5p*, *miR-20a*, *miR-9*), with blocking of terminal B-cell differentiation (*miR-9*, *miR-30b/c*), or with upregulation by inflammatory cytokines (*miR-155*) were upregulated in PCNSL, whereas the potential tumor suppressor MiRNAs such as *miR-199a*, *miR-214, miR-193b*, *and miR-145* were downregulated. Prompted by findings in nodal DLBCL related to potential activation of the NFκB pathway, activating mutations in *CARD11* and inactivating mutation of *TNFAIP3* have been studied, and mutations in the former have been found in approximately 10 % of cases while mutations in the latter are uncommon (Montesinos-Rongen et al. 2010; Rubenstein et al. 2013).

11.2 Differential Diagnosis

The differential diagnosis of CNSL includes inflammatory conditions such as sarcoidosis, cerebral vasculitis, or multiple sclerosis plaques but also infections such as tuberculoma or toxoplasmosis, particularly in immunosuppressed patients. Rare cases of DLBCL may present with lymphoma cells entirely *within* vessels, and these cases are best classified as intravascular large B-cell lymphoma. This uncommon variant of extranodal large B-cell lymphomas may occur in the CNS (Yegappan et al. 2001). Non-hematopoietic round cell neoplasms such as primitive neuroectodermal tumors, poorly differentiated or neuroendocrine carcinomas, melanoma, and primary brain tumors such as oligodendrogliomas can mimic lymphomas but are easily distinguished with immunohistochemistry. These tumors will all lack pan B-cell markers such as CD20 and CD79a.

11.3 Pathogenesis

A proposed mechanism for CNS tropism of the malignant B cell in PCNSL is one in which a clone of malignant B cells is selected via the upregulation of specific adhesion molecule(s) that facilitate homing to the CNS, and secondarily, the tumor cells proliferate and undergo secondary mutations in the absence of regulatory control by the immune system. In support of this is the demonstration that subclinical tumor-related clones are detectable in the blood and bone marrow of PCNSL patients, suggesting that the CNS microenvironment might promote a more aggressive phenotype (McCann et al. 2009; Jahnke et al. 2006b). However, to date, no differences in the expression of adhesion molecules have been identified between PCNSL and systemic lymphomas. Recently, CXCL13 (BCA-1), a B-cell-attracting chemokine, was determined to be expressed at significant levels in PCNSL tumors. Notably, CXCL13 is expressed in Helicobacter pylori-induced mucosa-associated lymphoid tissue as well as in gastric lymphoma (Mazzucchelli et al. 1999).

Similarly, expression of the chemokine stromal-derived factor-1 (SDF-1) has also been demonstrated by malignant B cells in PCNSL. Ectopic expression of these chemokines within the intraocular compartment and brain may contribute to lymphoma cell homing to the retina and CNS microenvironments (Smith et al. 2007; Fischer et al. 2009a).

11.4 Risk Factors

11.4.1 Risk Factors for PCNSL

Immunodeficiency is the only identified risk factor for development of PCNSL. However, PCNSL became very rare in HIV-infected persons since the introduction of HAART, reflecting the important role of immune system in the development of this disease.

11.4.2 Risk Factors for SCNSL

There is still a concern about the definition of risk group for CNS relapse in systemic lymphoma, since no study has been able to properly address this question. The existing risk models are based on clinical characteristics and have a low specificity and sensitivity implying a potential overtreatment in up to 70 % of patients deemed at high risk.

Current practice for prophylaxis varies widely, with involvement of particular sites such as paranasal sinuses, testes, orbital cavity, and bone marrow triggering prophylaxis at most centers. In the largest series of 1,693 elderly patients, a 6-year probability of CNS relapse tenfold higher was found for patients with testicular, orbit, and paranasal sinuses involvement as compared to other patients. Patients with testicular involvement had a 6-year probability of CNS relapse of 22.1 vs. 2.1 % in patients without testicular involvement ($p<0.001$). The probability of CNS failure at 6 years for patients with or without orbit and paranasal sinuses involvement was 33 % vs. 2 % ($p=0.02$) and 26 % vs. 2 % ($p>0.001$), respectively (Boehme et al. 2007).

Recently, a risk model was proposed based on an analysis of 1,222 elderly patients with DLBCL treated with CHOP without or with rituximab (R-CHOP). The group with involvement of more than one extranodal site, elevated LDH, and low ECOG performance status (4.8 % of patients treated with R-CHOP) showed a probability for CNS events at 2 years of 33.5 % as compared with 2.8 % in other patients given R-CHOP (Boehme et al. 2009).

New approaches to identify patients with systemic lymphoma at risk who should receive CNS prophylaxis are needed.

11.5 Clinical Presentation and Diagnostic Procedures

11.5.1 Symptoms of CNS Lymphoma

PCNSL most frequently presents with cognitive dysfunction, psychomotor slowing, disorientation and neurological focal symptoms, whereas cranial nerve palsies, seizures, cerebellar symptoms, and symptoms of elevated intracranial pressure are less frequent (<20 %). In patients with ocular involvement (see below), blurred vision and floaters are the most common symptoms.

SCNSL occurs after a median time of 6 months after first lymphoma diagnosis (Herrlinger et al. 2009). It may present as brain parenchyma lesions (approx. 40–80 %) with symptoms similar to those in PCNSL or as meningeal involvement. In most recent publications, 20–40 % of patients had simultaneous systemic disease (Boehme et al. 2007; Schmitz et al. 2012; Villa et al. 2010).

11.5.2 Diagnostic Procedures

Cranial MRI with contrast enhancement is the method of choice for further diagnostics and usually shows intense and homogenously enhancing lesions without necrosis and with a relatively small edema, typically localized in the periventricular space (Küker et al. 2005) (Fig. 11.6).

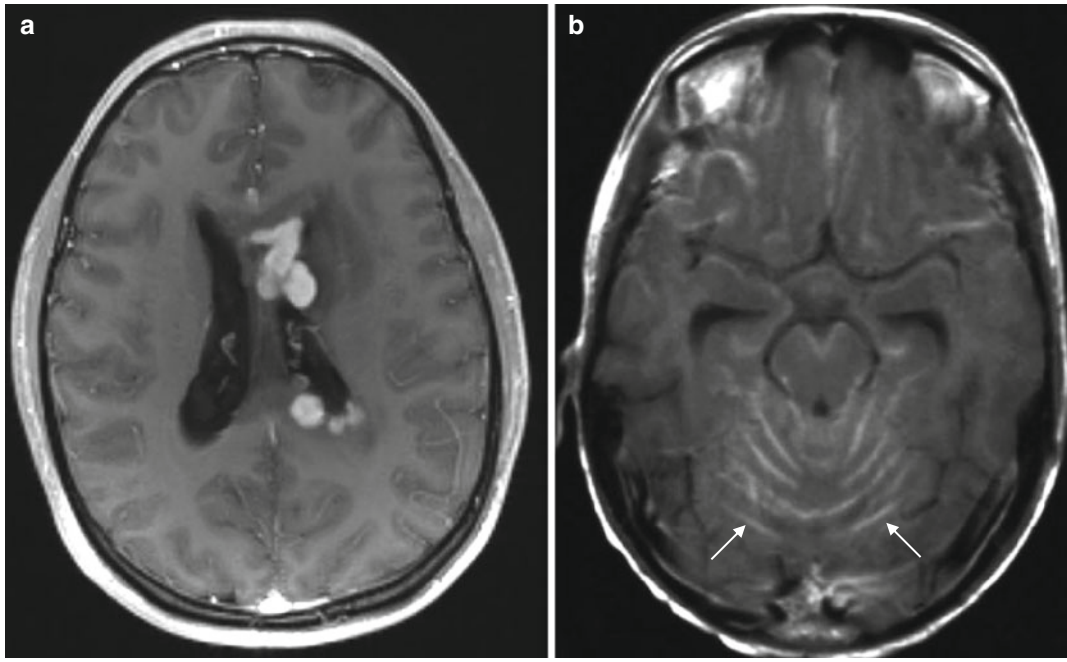

Fig. 11.6 PCNSL on MRI. (**a**) Parenchymatous lesion with a typical localization in the periventricular space, intense and homogenous contrast enhancement, and a relatively small edema and (**b**) contrast enhancement of the meninges indicating meningeal involvement

PCNSL most often presents as a solitary lesion, but multiple lesions may be detected in up to a third of the patients. Sometimes, contrast enhancement of the meninges indicating meningeal involvement can be seen (Fig. 11.6).

Diagnostic evaluation focusses on the establishment of the baseline extent of the disease and the exclusion of systemic lymphoma. According to the International PCNSL Collaborative Group (IPCG) (Abrey et al. 2005), staging examinations should include physical examination with palpation for enlarged lymph nodes as well as testicular examination in males; computed tomography of the neck, chest, abdomen, and pelvis; and bone marrow biopsy. Also, blood tests for HIV, complete blood cell count, basic metabolic profile, and lactate dehydrogenase level are recommended. Testicular ultrasonography should be considered in elderly males. Additionally, ophthalmologic examination and lumbar puncture (for cell count, protein and glucose measurement, cytology, and, facultatively, for flow cytometry studies and immunoglobulin heavy-chain gene rearrangement) should be performed.

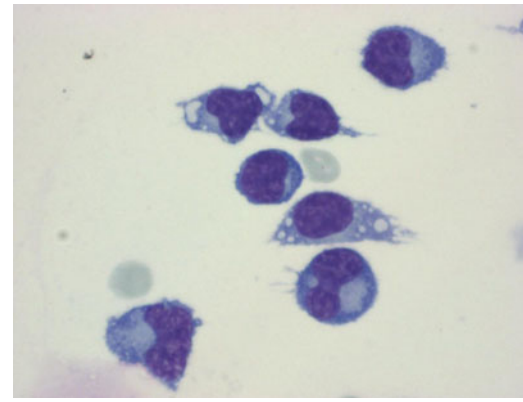

Fig. 11.7 Malignant lymphocytes in CSF

Making the diagnosis from the CSF is usually not possible since meningeal involvement can be found only in a minority of patients. Even using PCR for immunoglobulin heavy-chain gene rearrangement in addition to conventional CSF cytomorphology and MRI concurrent leptomeningeal involvement was seen in about 15 % of patients (Korfel et al. 2012) (Fig. 11.7). In systemic lymphoma, flow cytometry was reported to

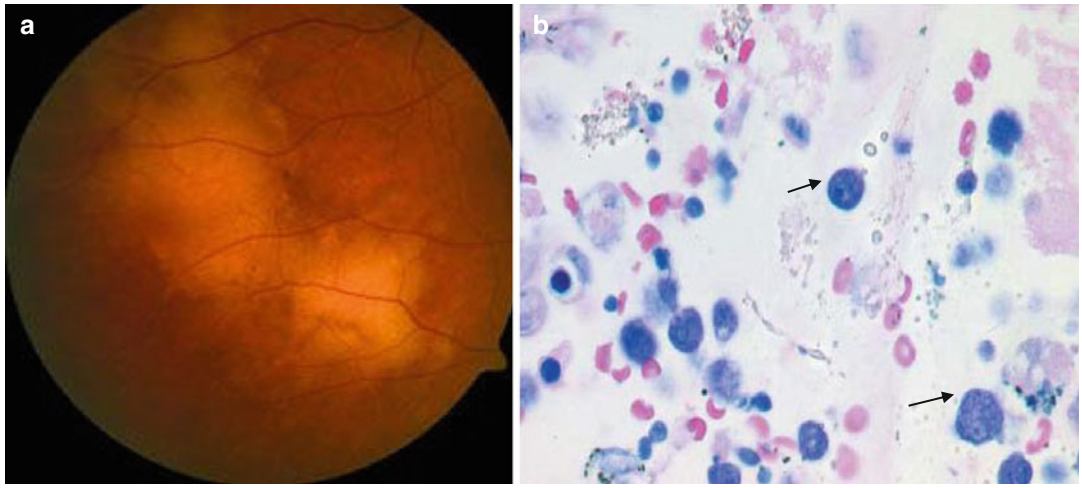

Fig. 11.8 Primary intraocular lymphoma: (**a**) subretinal infiltrates and (**b**) lymphoma cells in the vitreous

increase the diagnostic yield of cytologic examination of CSF alone from approximately 13 to 23 % (Schroers et al. 2010a; Collie and Hsi 2013). In the future, CSF multicolor flow cytometry as well as new CSF parameters such as free immunoglobulin light chains, miRNAs, or CXCL13 may become useful tools as noninvasive biomarker for the diagnosis of CNSL (Schroers et al. 2010b; Sancho et al. 2010; Baraniskin et al. 2011; Fischer et al. 2009b).

In the setting of HIV infection, examination of the CSF for EBV has been used to aid in the diagnosis of PCNSL in patients with suggestive radiographic findings without tissue biopsy (Ambinder et al. 2010; Cinque et al. 1993; De Luca et al. 1995).

Ocular involvement (retina, optic nerve, vitreous) is diagnosed in approx. 15 % of patients (Fig. 11.8) and may develop before, concurrent with, and after brain parenchyma manifestations. Typical clinical findings include vitreous cellular infiltration (lymphoma and inflammatory cells) and subretinal tumor cell infiltrates. Elevation of IL-10 levels in the ocular fluid and/or an IL-10:IL-6 ratio >1 is highly suggestive of ocular lymphoma; however, for diagnosis confirmation, vitrectomy or, at specialized centers, chorioretinal biopsy usually is required (Chan et al. 2011).

Diagnosis of PCNSL is usually established by stereotactic biopsy from a CNS lesion. Making the diagnosis can be significantly hampered by pretreatment with glucocorticoids; thus, they should be avoided prior to surgery whenever possible.

In systemic lymphoma, a search for CNS involvement should be considered in patients with more than one extranodal site, elevated serum LDH, and ECOG performance status >2.

11.6 Treatment

11.6.1 Treatment of PCNSL

11.6.1.1 Role of Surgery

Surgery alone is not a viable treatment option due the infiltrative nature of lymphoma and its multifocality. The current approach is to abandon tumor resection excepted for patients with uncontrollable neurological deterioration due to brain herniation. The role of surgery is currently limited to stereotactic guided biopsy for diagnosis establishment. However, data from the first randomized phase III trial (G-PCNSL-SG1) trial have challenged this view (Weller et al. 2012).

11.6.1.2 Role of Radiotherapy

Whole-brain radiotherapy (WBRT) produces complete remission (CR) in up to 90 % of patients, however, usually with a poor long-term

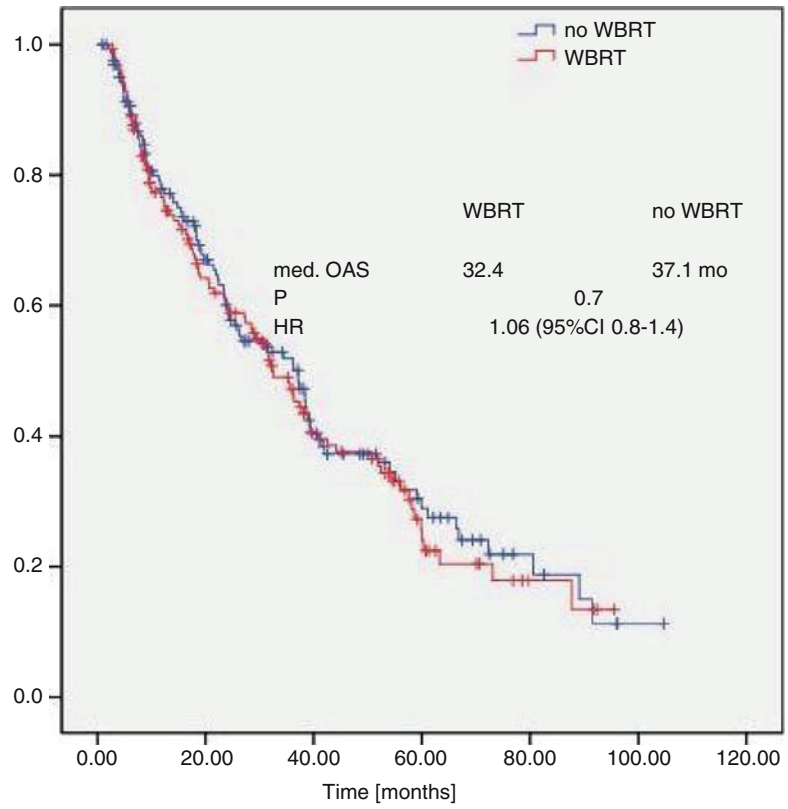

Fig. 11.9 Overall survival in the per protocol population in the G-PCNSL-SG1 trial with versus without whole-brain radiotherapy after high-dose methotrexate-based chemotherapy (Thiel et al. 2010)

disease control and median overall survival (OS) of only 12–16 months and a 5-year OS of 10–29 % (Nelson et al. 1992; Laperriere et al. 1997). In the latest 1990s, the combination of HDMTX-based chemotherapy followed by WBRT was established as standard therapy for PCNSL with CR rates between 69 and 87 % and median progression-free survival (PFS) of 24–40 months in phase II studies (Abrey et al. 2000; O'Brien et al. 2006; DeAngelis et al. 2002; Poortmans et al. 2003; Ferreri et al. 2006). Unfortunately, these improved long-term results were overshadowed by severe neurological impairment including dementia and death, particularly in older patients (Abrey et al. 1998; Gavrilovic et al. 2006). This led to the investigation of WBRT dose reduction or even radiotherapy withdrawal in patients with a CR after chemotherapy alone. It took until 2010 that a randomized phase III trial investigating the role of WBRT in the primary treatment of PCNSL was published. Here, no significant difference in OS (primary end point) was found when WBRT (45 Gy in 1.5 Gy fractions) was omitted from primary therapy (after HDMTX-based chemotherapy): OS in the per protocol population was 32.4 months with and 37.1 months without WBRT ($p = 0.7$; Fig. 11.9) (Thiel et al. 2010). However, a benefit for PFS was found (18.3 vs. 11.9 month, respectively, $p = 0.13$), which proved significant in subgroup analyses suggesting an important role of WBRT for disease control. This study has been criticized for the upfront randomization and the high number of protocol violations. Nevertheless, with a median patient age of 63 years, 24 % of patients >70 years, and 15 % of patients with KPS ≤ 40 %, this trial truly represented the "reality" of PCNSL management in the clinical routine. The results of this trial indicate that WBRT can be deleted from primary therapy of PCNSL.

When WBRT was used as salvage therapy, a response rate of 60–79 % and OS of 10.9–16 months were reported (Herrlinger et al. 2005;

Nguyen et al. 2003; Hottinger et al. 2007). Response rates to WBRT and survival were similar between refractory and recurrent patients (Hottinger et al. 2007).

11.6.1.3 Chemotherapy

Chemotherapy should be considered first-line treatment for all PCNSL patients able to receive it. Drugs for PCNSL treatment need to cross the blood–brain barrier (BBB) which is supported by the observation that WBRT + CHOP (cyclophosphamide, vincristine, doxorubicin prednisolone) regimen has proved no better than WBRT alone (Mead et al. 2000). HDMTX (>3 g/m^2) is the most important drug for treatment of PCNSL. With a short-time infusion (3–4 h), the majority of patients achieve cytotoxic levels in the CSF (Borsi and Moe 1987; Shapiro et al. 1975). In nonrandomized studies using chemotherapy alone, results comparable to those achieved with chemotherapy followed by WBRT were reported (Herrlinger et al. 2005; Batchelor et al. 2003; Hoang-Xuan et al. 2003; Pels et al. 2003; Juergens et al. 2010; Chamberlain and Johnston 2010). Higher response rates and probably longer disease control can be achieved when HDMTX is combined with other drugs. In the only randomized phase II trial with 79 patients comparing HDMTX monotherapy (3.5 g/m^2 every 3 weeks) to HDMTX+ high-dose cytarabine (HDAraC), a significantly improved outcome was observed with the combination with CR rate of 46 % vs. 18 % ($p=0.006$) and 3-year OS of 46 % vs. 32 % ($p=0.07$), respectively (Ferreri et al. 2009). Hematologic toxicity was higher in the combination arm. The problem with this study was the underdose in the monotherapy arm resulting in a very poor outcome.

In the G-PCNSL-SG1 trial, the addition of ifosfamide (1.5 g/m^2 over 3 days) to HDMTX introduced per amendment during the course of the study resulted in significantly improved CR rate of 42 % vs. 32 % and primary progression rate reduction of 15 % vs. 26 %. Not surprisingly, toxicity was higher with the combination, particularly in elderly patients (Thiel et al. 2010).

The best long-term results in PCNSL were reported with an intensive chemotherapy-only regimen including HDMTX (5 g/m^2), HDAra-C (3 g/m^2), vincristine, alkylating agents, and dexamethasone combined with intensive intraventricular chemotherapy (Pels et al. 2003). Median event-free survival (EFS) was 21 months and OS 50 months. A recent follow-up showed that 57 % of patients <60 years were alive after a median follow-up of 100 months without evidence of chemotherapy-related neurotoxicity (Juergens et al. 2010).

Intra-arterial infusion of MTX-based chemotherapy following osmotic blood–brain barrier disruption aiming at delivering higher drug concentrations to the tumor has been assessed by several groups. In the most recent multi-institutional analysis, the results were comparable or even better than with many conventional treatments with a 5-year PFS of 31 % and 7-year PFS of 25 %. However, the procedure can be associated with some acute toxicity and is presently available at specialized centers only (Angelov et al. 2009).

The role of the anti-CD20 monoclonal antibody rituximab in PCNSL is not defined. As a large protein, it has poor penetration into the CNS as measured by CSF levels (Rubenstein et al. 2003). The combination of rituximab and HDMTX-based chemotherapy proved feasible and active in small studies (Chamberlain and Johnston 2010; Shah et al. 2007; Wieduwilt et al. 2012); however, one study (Shah et al. 2007) suggested increased hematologic toxicity of the combination.

11.6.1.4 Salvage Therapy

Salvage treatment should be chosen based on patient's age, performance status, prior therapy, and duration of previous response. WBRT is a very effective salvage treatment with a response rate of >60 % and a median OS after relapse of 16 months, but with increased risk of neurotoxicity (Nguyen et al. 2003; Hottinger et al. 2007). Thus, delaying WBRT whenever possible and offering chemotherapy to patients with recurrent disease seems a reasonable option, particularly in those with good performance status and response to

previous chemotherapy. Patients with a long-term remission after HDMTX can be rechallenged with a good chance for a second long-term remission (Plotkin et al. 2004). In several small studies, responses and sometimes long-term control were reported for temozolomide alone or with rituximab (Reni et al. 2007; Enting et al. 2004), topotecan (Fischer et al. 2006), and ifosfamide- or etoposide-based combination chemotherapy.

Promising results at relapse were reported with high-dose chemotherapy followed by autologous stem-cell transplantation (ASCT). A regimen of thiotepa, busulfan, and cyclophosphamide followed by ASCT produced a 2-year OS of 45 % in 43 patients who failed HDMTX therapy (Soussain et al. 2008). However, this approach is only suitable for selected patients at rather young age and in overall good condition.

11.6.1.5 Intra CSF Therapy

There is currently no consensus on intra CSF treatment in PCNSL. Results of two German studies suggested that in PCNSL, the CSF represents a reservoir for tumor cells, and therefore, separate treatment may be beneficial. Very encouraging results of a polychemotherapy protocol including intensive intraventricular chemotherapy via Ommaya reservoir resulting in excellent long-term survival of young patients could not be confirmed in a second trial using the same regimen without intraventricular treatment (Pels et al. 2003, 2009). Rapid tumor recurrence observed in the second trial was attributed to the omission of intraventricular treatment. Activity of rituximab given intrathecally was demonstrated in a study with ten patients with SCNSL or PCNSL (Rubenstein et al. 2007), and this approach needs further assessment.

11.6.1.6 High-Dose Chemotherapy and Stem Cell Transplantation (HDCT-ASCT)

To date, only relatively small series and phase II trials have been published on HDCT-ASCT in PCNSL. In the first study, 28 patients received induction chemotherapy with HDMTX (3.5 g/m^2) and HD Ara-C (3 g/m^2 for 2 days) followed by BEAM as conditioning regimen before ASCT (Abrey et al. 2003). Only 50 % of the patients completed the therapy, and the median event-free survival was only 5.6 months. More promising results were reported in a German phase II study with 30 patients <65 years treated with induction chemotherapy including HDMTX (8 g/m^2), HD Ara-C (2×3 g/m^2), and thiotepa (40 mg/m^2) followed by a conditioning regimen with carmustine and thiotepa and ASCT. In the study, WBRT (45 Gy) was given to all patients as consolidation. With a median follow-up of 63 months, the 5-year OS was 69 % for all patients and 87 % for those completing HDCT-ASCT, respectively (Illerhaus et al. 2006).

Whether the discrepancies in effectiveness between BEAM and thiotepa-based conditioning regimens are related to the different capacity of these drugs to cross the BBB or have to be attributed to the efficacy of the specific agents is still unclear. The role of HDCT-ASCT in PCNSL remains to be defined and is currently being investigated.

11.6.1.7 Treatment of Elderly Patients

Approximately 50 % of all patients with PCNSL are aged ≥65 years. Age, beside performance status, is the most important prognostic factor in PCNSL (Abrey et al. 2006). Balancing treatment efficacy with toxicity is particularly challenging in the elderly. In a secondary analysis of the G-PCNSL-SG1 trial, the rate of complete and partial responses to HDMTX-based chemotherapy was lower (44 % vs. 57 %; $p=0.016$), death on therapy more frequent (18 % vs. 11 %; $p=0.027$), and PFS (4.0 vs. 7.7 months, $p=0.014$) and OS (12.5 vs. 26.2 months, $p<0.001$) inferior in the elderly (≥70 years). A striking difference between younger and elderly patients was the PFS of CR patients of 35.0 in the younger versus 16.1 in the elderly patients ($p=0.024$) (Roth et al. 2012). However, in more selected populations treated at highly specialized institutions, more favorable treatment results can be achieved, demonstrating that vigorous therapy comparable to that given to younger patients can be successfully given to some older patients (Ney et al. 2010).

11.6.2 Secondary CNS Lymphoma

11.6.2.1 Prophylaxis

Optimal regimen for prophylaxis of CNS relapse in systemic lymphoma has not been established thus far. Current data support the use of systemic CNS penetrating chemotherapy (e.g., HDMTX) rather than intrathecal prophylaxis (Korfel 2011). The addition of rituximab to the CHOP regimen was reported to prevent CNS dissemination of DLBCL in a retrospective German analysis of patients >60 years (Boehme et al. 2009) and in younger patients with a low age-adjusted International Prognostic Index (aaIPI) (Schmitz et al. 2012). This has not been confirmed for younger patients with higher aaIPI (Schmitz et al. 2012) and by a French analysis (Feugier et al. 2004).

11.6.2.2 Treatment

Data on therapy of SCNSL is very limited. With intrathecal chemotherapy and/or radiotherapy, the prognosis is very poor with a median survival of only a few months (Herrlinger et al. 2009). With HDMTX, longer survival can be observed as found in a retrospective multicenter analysis of 113 patients with isolated CNS relapse without systemic lymphoma (median age 61 years, 62 % treated with HDMTX, and 53 % with WBRT) (Doolittle et al. 2008). However, in another retrospective study, a median OS of only 7 months has been outlined in 23 patients with isolated CNS relapse, all of whom received an intensive HDMTX-based chemotherapy including intrathecal chemotherapy in 15 (Patrij et al. 2011).

After small retrospective analyses had revealed long-term remissions in some patients treated with HDCT-ASCT (Alvarnas et al. 2000; Kasamon et al. 2005; Williams et al. 1994; Jahnke et al. 2006c), the first prospective multicenter study to evaluate the feasibility and efficacy of HDCT-ASCT in patients with CNS relapse of aggressive systemic lymphoma has recently been conducted. The protocol included a sequential application of exclusively blood–brain barrier crossing cytostatics without radiotherapy (HDMTX, ifosfamide, HDAraC, followed by HDCT-ASCT with thiotepa, BCNU and etoposide) combined with liposomal cytarabine intrathecally. The results were very promising with 2-year time to treatment failure (primary end point) of 49 % for all patients and 58 % for patients completing HDCT-ASCT, suggesting that cure is possible in a substantial proportion of patients (Korfel et al. 2013).

11.7 Neurotoxicity

With improvements in survival, there is increasing concern regarding the incidence of late neurotoxicity associated with successful treatment of CNSL. The true risk of this complication has likely been underestimated since formal psychometric evaluations are not routinely performed and were not included in the vast majority of studies. Late neurotoxicity can be recognized by radiographic findings which indicate diffuse white-matter disease and cortical-subcortical atrophy with concordant findings at autopsy such as gliosis, thickening of small vessels, and demyelination (Fig. 11.10).

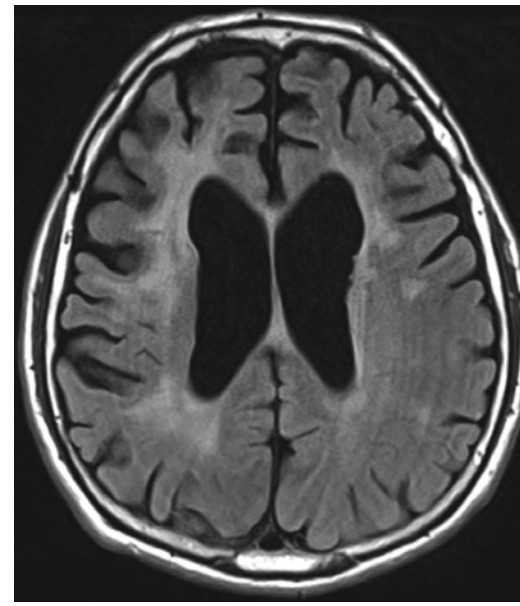

Fig. 11.10 Late neurotoxicity on MRI. T2 sequences show periventricular white-matter changes and brain atrophy

The risk of neurotoxicity increases with advanced age. In patients >60 years treated with WBRT, virtually all long-term survivors develop delayed neurotoxicity. In younger patients, late neurotoxicity was found in >20 % when evaluated clinically (Gavrilovic et al. 2006) and in 63 % when extensive neuropsychological assessment was used (Harder et al. 2004).

There is increasing recognition that radiotherapy is a primary mediator of neurotoxicity which is associated with progressive microvascular alterations and loss of oligodendrocyte progenitors. In a retrospective analysis of 185 patients, WBRT was the only factor associated with late neurotoxicity (evaluated by clinical examination only) in the multivariate setting (Omuro et al. 2005). In a most recent analysis of 80 long-term PCNSL survivors treated with different regimens with or without WBRT, those who received WBRT had significantly lower mean scores in attention/executive function, motor skills, and neuropsychological composite score compared to those treated with non-WBRT regimens. Moreover, on brain imaging, mean areas of total T2 abnormalities in the WBRT group were more than twice the mean of any other non-WBRT group. This was associated with poorer neuropsychological and QOL outcomes (Doolittle et al. 2012).

11.8 Future Directions

A better understanding of the pathogenesis and molecular biology of CNSL will help to improve current treatment strategies and develop novel therapeutic approaches. Because of the rarity of the disease, well-designed and adequately powered studies must be encouraged to allow for the collection of meaningful patient numbers within a reasonable time frame and to produce valid results. These trials would provide useful databases for translational research programs that may help to define particular patient populations at high risk for early relapse or the need for early treatment escalation. Standardized neuropsychological assessments should be included in all future trials whenever possible to help to determine cognitive alterations during the course of the disease more precisely and to allow the development of less toxic treatment.

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HIV-Associated Lymphomas

12

Kieron Dunleavy, German Ott, Eric D. Hsi, and Michele Spina

Contents

K. Dunleavy, MD (✉)
National Cancer Institute, Bethesda, MD, USA
e-mail: dunleavk@mail.nih.gov

G. Ott, MD
Department of Clinical Pathology,
Robert-Bosch-Krankenhaus, Stuttgart, Germany

E.D. Hsi, MD
Department of Clinical Pathology,
Cleveland Clinic, L11, 9500 Euclid Ave,
Cleveland, OH 44195, USA
e-mail: hsie@ccf.org

M. Spina, MD
Division of Medical Oncology A,
National Cancer Institute, Aviano, Italy
e-mail: mspina@cro.it

Pathology: German Ott and Eric D. Hsi

12.1 Definition

Different types of lymphoproliferations have been recognized in the setting of human immunodeficiency virus (HIV) infection. These lesions comprise HIV-related lymphoid hyperplasia of various kind and overt malignant lymphomas. Lymphomas arising in the setting of HIV infection have been subclassified based on their occurrence in immunocompromised individuals, in the setting of HIV exclusively, and those occurring in immunocompetent individuals as well (Raphael et al. 2008).

12.2 Pathogenesis

Chronic antigenic stimulation is believed to be the soil on which many HIV-associated lymphoproliferations and lymphomas do develop, and polyclonal hypergammaglobulinemia has been observed preceding overt malignant lymphoma.

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Obviously, Epstein-Barr virus (EBV) infection of the tumor cells, occurring in 20–100 % of malignant lymphoproliferations in HIV/AIDS, is a major factor. Clonal EBV genomes are found in the tumors, and EBV is believed to be associated with B-cell expansion and the acquisition of genetic alterations in the setting of impaired immune surveillance (Grulich et al. 2000; Shibata et al. 1991; Neri et al. 1991). Generally, infectious agents like EBV and HHV8 have been associated with particular cytokine secretion profiles of the tumor cells.

12.3 Pathology

Burkitt lymphoma (BL) constitutes 30–40 % of malignant lymphomas arising in HIV-infected individuals. Characteristically, BL occurs in younger persons and earlier in the disease course than DLBCL, with still higher CD4+ cell counts. As is the case with DLBCL, BL also presents in advanced stages in the setting of HIV, sometimes with extensive bone marrow infiltration and/or overt leukemia (Said 2011). Morphologically, a fraction of HIV+ BL has been recognized because of their greater pleomorphism of the tumor cells (Raphael et al. 1991; Davi et al. 1998) (Fig. 12.1a). A characteristic feature of HIV+ BL is the frequent occurrence of cases with plasmacytoid features and cytoplasmic immunoglobulin accumulation. This type of BL is virtually characteristic for HIV infection and has been closely linked to EBV infection. As do their counterparts occurring in immunocompetent individuals, AIDS-associated BL express B-cell antigens including CD10 and BCL6 and are negative for BCL2 and terminal deoxynucleotidyl transferase (TdT). EBV infection is observed in 40 % of classical BL, and in 70 % of plasmacytoid and pleomorphic variants, respectively (Fig. 12.1b).

Diffuse large B-cell lymphoma (DLBCL) accounts for roughly 25–30 % of malignant lymphomas in HIV-positive patients. In a certain contrast to DLBCL arising in immunocompetent individuals, HIV+ DLBCL frequently occurs at extranodal sites such as the CNS, the GI tract, and others. Histologically, DLBCL occurring in the setting of HIV resembles its counterparts in non-immunocompromised individuals ranging from centroblastic to immunoblastic types (Fig. 12.2a and b). IB lymphomas make up 20 % of HIV-associated DLBCL. There may be a higher frequency of cases with pleomorphic and HRS-like giant cells in this setting (Raphael et al. 2008). EBV infection of the tumor cells can be recognized in 30 % of cases, with 80 % of immunoblastic lymphomas harboring clonal EBV genomes (Subar et al. 1988; Ballerini et al. 1992; Shibata et al. 1993).

Classical Hodgkin lymphoma is the most common type of non-AIDS-defining lymphoma occurring in HIV-infected individuals (Spina

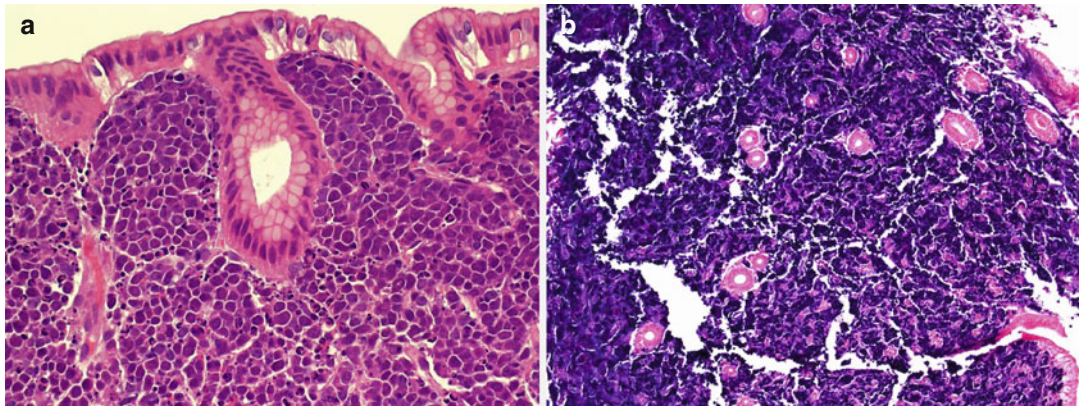

Fig. 12.1 *Burkitt lymphoma*. (**a**) In this example of a Burkitt lymphoma arising in the gastric wall of an HIV-positive patient, the tumor cells are slightly larger and more pleomorphic than in conventional BL (HE ×400). (**b**) EBER in situ hybridization discloses infection of the tumor cells with Epstein-Barr virus (EBER ×100)

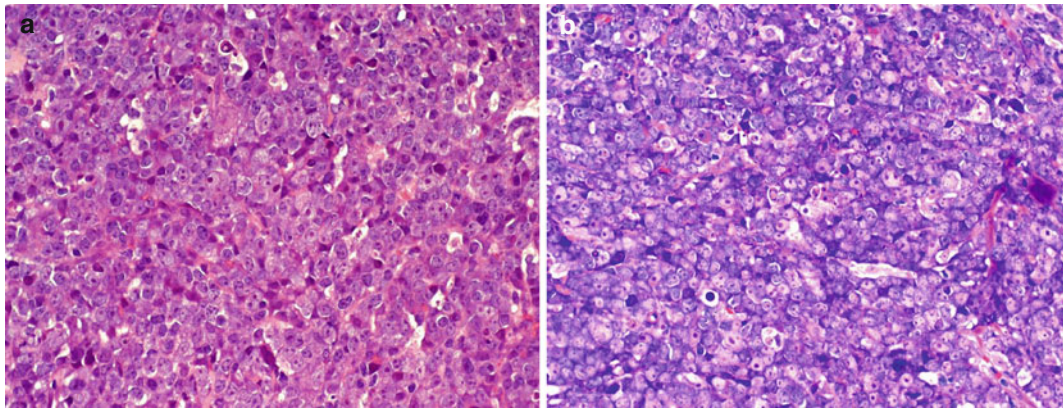

Fig. 12.2 (*EBV-negative*) *DLBCL with immunoblastic features*. (**a** and **b**) An EBV-negative DLBCL was diagnosed in a lymph node of a HIV+ patient. Giemsa staining discloses immunoblastic (-plasmablastic) differentiation of the tumor cells (**b**) (Giemsa ×400)

et al. 2000). Generally speaking, the disease presents more aggressively, with frequently higher clinical stages. Bone marrow infiltration is observed in 50 % of cases and, of note, can be present as the sole site of disease manifestation. Histologically, mixed cellularity and lymphocyte-depleted subtypes prevail, often with higher HRS cell numbers. HRS cells in HIV+ HL, consistently, are EBV infected. In contrast to HL occurring in immunocompetent individuals, the reactive background of the tumor cells is depleted of CD4 cells and enriched for CD8+ T cells (Said 2011).

Plasmablastic lymphoma (PBL) has been initially described as a characteristic HIV-related tumor occurring in the oral cavity (Delecluse et al. 1997). In HIV-infected individuals, there is a tendency of these neoplasias to manifest in the oropharyngeal region, although they have been described in a large number of other (primarily extranodal) sites. Morphologically, they are indistinguishable from PBL occurring in immunocompetent individuals (Teruya-Feldstein et al. 2004). Plasmablastic lymphomas display a cellular spectrum from immunoblasts to plasmablasts and by definition, do not express pan B-cell antigens. In contrast, they are usually, but not always, positive for plasma cell markers such as CD138 and IRF4/MUM1, with clonal light-chain expression present in some cases. Most cases are EBV associated, but LMP-1 and EBNA-2 may not be expressed requiring EBER in situ hybridization for its unequivocal demonstration.

Other lymphoma types that have been associated with HIV infection, albeit occurring in smaller numbers, are *extranodal marginal zone lymphoma of MALT type* frequently occurring in the lungs of HIV+ children, *plasma cell myeloma*, and *mature T/NK cell lymphoma*. *T/NK cell lymphomas* occurring in the setting of HIV infection are uncommon but are increasingly encountered in HIV+-infected individuals. Most tumors are large-cell peripheral T cell lymphomas, NOS, but other lymphoma types such as Mycosis fungoides and anaplastic large-cell lymphomas (ALK+ and −) have been described (Said 2011).

In contrast to the aforementioned tumor types, *primary effusion lymphoma*, *extracavitary HHV8+ lymphoma*, and *AIDS-related polymorphic lymphoproliferative disorders* occur exclusively in the setting of HIV infection.

Primary effusion lymphoma (PEL) is a distinct tumor entity of the WHO classification invariably associated with immunodeficiency states, albeit of various nature (Said and Cesarman 2008). Most cases occur in HIV+ male homosexuals. The clinical hallmark of the disease is a malignant effusion of large blastic B cells in the pleura, the pericardium, or the peritoneal cavity (Fig. 12.3a). Extension into adjacent sites may occur, mostly with advanced disease. Human Herpes virus 8/Kaposi sarcoma virus

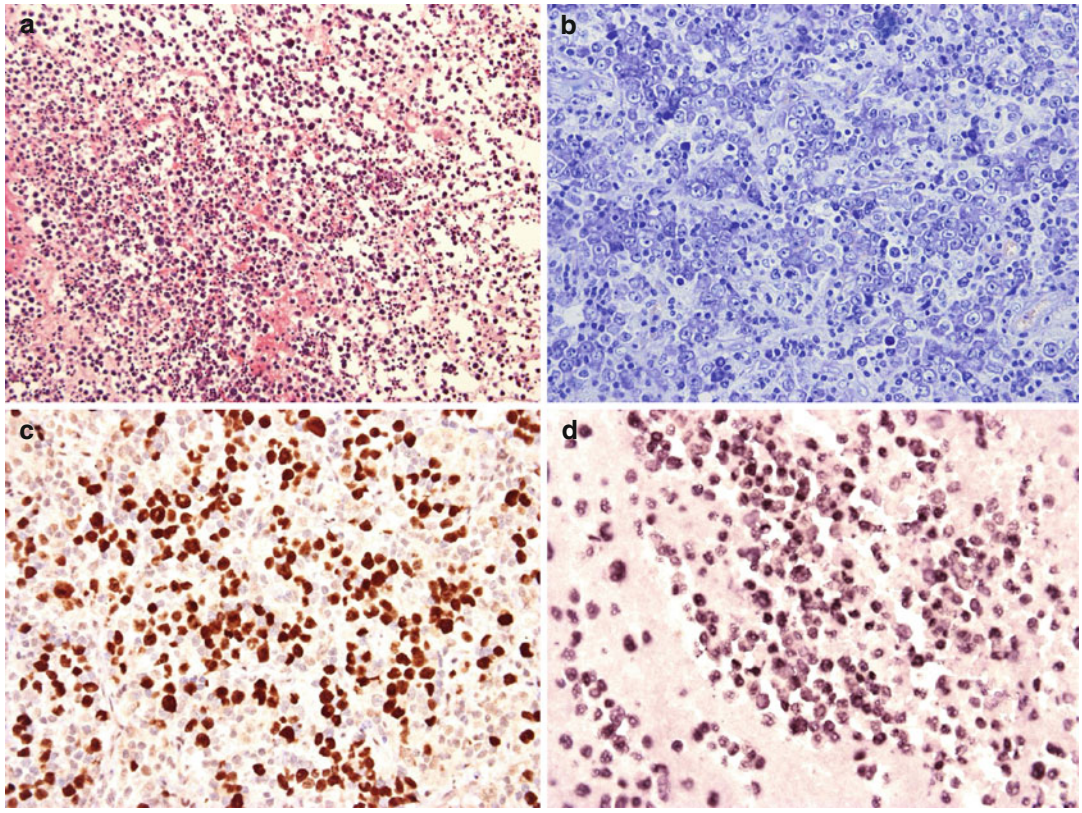

Fig. 12.3 *HHV8-associated primary effusion lymphoma*. (**a** and **b**) A cell block preparation from pleural fluid shows numerous large pleomorphic blasts (**a**, HE ×400). In this extracavitary HHV8+ solid variant, the immunoblastic/plasmablastic appearance of the tumor cells is well appreciated (**b**, Giemsa ×400). Latent HHV8 infection is demonstrated in this case using immunohistochemical detection of the LANA/HHV8 nuclear antigen (**c**, LANA ×400). In situ hybridization of PEL discloses infection of the tumor cells with EBV (**d**, EBER ×400)

(HHV8) infection is present in all cases and as such, a defining disease feature. HHV8 infection of the tumor cells can be demonstrated using immunohistochemistry (Fig. 12.3c). One third of patients will also have Kaposi sarcoma. Immunohistochemistry of PEL is characterized by the absence of B-cell antigen expression and the reactivity for plasma cell markers. Because of the occurrence of particular phenotypes such as CD30 and aberrant T cell marker expression, the clear-cut demonstration of HHV8 infection via immunohistochemistry is important. In the setting of HIV, in addition, most cases are EBV associated (Fig. 12.3d).

Cytologically, most PEL demonstrate immunoblastic/plasmablastic or anaplastic morphology (Fig. 12.3b), with some cases harboring more pleomorphic and HRS-like cells. *Extracavitary HHV8+ lymphoma* is a solid variant of PEL presenting as a localized tumor in the GI tract, the lungs, the skin, or the lymph nodes, among others, in the absence of effusions(Chadburn et al. 2004). In contrast to PEL, up to 25 % of cases have been reported to express B-cell associated antigens but most are reflecting terminal B-cell differentiation, as do PEL. As with PEL, there is coinfection of the tumor cells with HHV8 and EBV (Fig. 12.3b and c).

12.4 Molecular Genetics

Immunoglobulin heavy-chain and/or light-chain rearrangement can be demonstrated in most cases of HIV+ lymphomas. Rearrangements for *BCL6*

and *MYC* are described in a minor fraction of DLBCL. In AIDS-associated Burkitt lymphoma, the structure of *MYC* rearrangements is similar to its non-HIV counterpart. In addition, *TP53* mutations are relatively common (Ballerini et al. 1993). In contrast, PEL are negative for *BCL2*, *BCL6*, and *MYC* rearrangements, and no mutations in *RAS* or *TP53* have been detected. Aberrant *TCR* rearrangements can be found in PEL (Raphael et al. 2008).

12.5 Differential Diagnosis

For the pathologist, the single most important information to be provided to is that the patient has HIV infection, because many morphological features in BL and DLBCL are shared by the tumor infiltrates in immunodeficient as well as in immunocompetent settings. BL in immunocompromised individuals may be more pleomorphic or demonstrate plasmacytoid differentiation. EBV infection in AIDS-associated DLBCL may be suspected by greater cellular pleomorphism, angioinvasive and angiodestructive growth, and – at times extensive – necrosis. In general, plasmablastic morphology in DLBCL should prompt examination of EBV examination, and DLBCL diagnosis in an effusion should prompt analysis for HHV8 LANA protein expression.

12.6 Evaluation

Patients should have a comprehensive medical history with attention paid to signs and symptoms of lymphoma and a detailed HIV history including prior opportunistic infections and history of HIV resistance, immune function, HIV viral control, and antiretroviral treatment. The physical examination should include a careful assessment of lymph node regions, the liver, and spleen. Relevant laboratory studies include a complete blood count, chemistry profile with lactate dehydrogenase (LDH) and uric acid levels, CD4 cell count, and HIV viral load. HIV and hepatitis B and C serologies should be assessed. A bone marrow aspirate and biopsy should be performed at initial diagnosis as involvement by lymphoma is found in up to 20 % of cases. Patients with aggressive B-cell lymphomas should have a lumbar puncture for analysis of cerebrospinal fluid by flow cytometry and cytology to check for leptomeningeal lymphoma (Hegde et al. 2005).

Imaging studies should include computed tomography (CT) scanning of the chest, abdomen, and pelvis. Radiographic evaluation of the head should also be performed preferably by magnetic resonance imaging (MRI). Fluorodeoxyglucose positron emission tomography (FDG-PET) is useful in HIV-negative aggressive lymphomas, but its role in HIV-associated lymphomas is very poorly studied at this point in time. One of the greatest limitations in using PET is that interpretation can be confounded by inflammation from HIV-associated nodal reactive hyperplasia, lipodystrophy, and infections (Dunleavy et al. 2010a, b). Prior experience evaluating FDG-PET in HIV-associated lymphoma is limited to small retrospective series where most scans were not predictive of remission.

12.7 Prognostic Factors

The International Prognostic Index (IPI) is the standard prognostic assessment tool in HIV-negative DLBCL. Its applicability to HIV-associated DLBCL, however, is controversial. While in some studies using CHOP or R-CHOP, the IPI score has divided groups prognostically, this has not been the case with DA-EPOCH and in a recent study of short-course EPOCH-R (infusional etoposide, vincristine, and doxorubicin with prednisone, cyclophosphamide, and rituximab) in newly diagnosed HIV-associated DLBCL, the IPI did not predict PFS or OS (Dunleavy et al. 2010a; Ribera et al. 2008; Kaplan et al. 2005). The prognostic importance of CD4 cell count and immune function in HIV-associated DLBCL, neither of which are part of the IPI, are the most likely confounding variables. Patients with CD4 counts less than 100 cells/μL are at increased risk of serious opportunistic infections and death. Furthermore, as noted earlier, patients with severe immune suppression have a higher incidence of immunoblastic

subtypes, most of which are of ABC derivation, and a poor outcome compared to patients with preserved immunity, where the GCB subtype is more common (Dunleavy et al. 2010a). Although a recently reported study from the AIDS Malignancy Consortium (AMC) did not find an association between the cell of origin and outcome in HIV-associated DLBCL, their analysis was retrospective and included patients treated with a variety of different regimens (Sparano et al. 2010; Chadburn et al. 2009; Dunleavy and Wilson 2010). Involvement of the CNS, which is increased in HIV-associated aggressive B-cell lymphomas, also confers an adverse prognosis.

12.8 Treatment of HIV-Associated Lymphomas

The treatment of HIV-associated lymphoma has evolved over the last 30 years in line with improved control of HIV replication and preservation of immune function (Table 12.1).

In the pre-CART era, patients with HIV-associated lymphoma had poor outcomes with median survivals of 5–6 months. Because these outcomes were driven by both chemotherapy failure and infections, investigators have examined the effect of chemotherapy dose on survival. In one study, Kaplan and colleagues observed that higher doses of cyclophosphamide were associated with lower survival, suggesting that infections were a driving cause of death in these patients (Kaplan et al. 1989). In an attempt to reduce infectious deaths, the AIDS Malignancy Consortium (AMC) conducted a study of 192 untreated lymphoma patients randomly assigned to receive standard-dose m-BACOD (methotrexate, bleomycin, doxorubicin, cyclophosphamide, vincristine, and dexamethasone) with granulocyte macrophage colony-stimulating factor (GM-CSF) support or low-dose m-BACOD without GM-CSF in an effort to reduce the toxicity of chemotherapy (Kaplan et al. 1997). Compared to full-dose therapy, reduced-dose treatment had a similar response rate (52 % versus 41 %, respectively) and median survival (6.8 versus 7.7 months, respectively) but lower hematological toxicity. This led the authors to conclude that lower dose chemotherapy was preferable in HIV-associated lymphoma. One shortcoming of the study was that although the authors controlled for the absolute CD4 cell count in the survival analysis, they did not include enough patients with high CD4 counts and, ultimately, could not support a definitive recommendation for this group where the benefit of full-dose chemotherapy on cure of the lymphoma may outweigh the infectious risks (Little et al. 2003). Importantly, before completion of this trial, a randomized multicenter study in HIV-negative aggressive lymphoma showed CHOP to be equally effective as m-BACOD and less toxic (Fisher et al. 1993). The better therapeutic index of CHOP led to its acceptance as a standard for HIV-associated lymphoma (Lim and Levine 2005).

The introduction of CART some 15 years ago has had a dramatic effect on the outcome of HIV-associated lymphomas with increases in median survival. While the reasons are multifactorial, they can be ultimately attributed to salutary effects of CART on immune function. Patients with preserved immune function have a lower risk of infectious complications, thereby enabling optimal chemotherapy administration, and as noted earlier, a more favorable tumor biology (Little et al. 2003; Fisher et al. 1993; Lim and Levine 2005; Carbone and Gloghini 2005). Interestingly, in one study that looked at risk-adapted intensive chemotherapy in 485 patients with AIDS-related lymphoma (ARL), CART was significantly associated with survival while the dose-intensity of CHOP-based therapy was not (Mounier et al. 2006).

Although the benefit of rituximab is well established in HIV-negative DLBCL, its role in HIV-associated DLBCL has been controversial (Coiffier et al. 2002). This debate stems from an AMC randomized phase III study of CHOP ± rituximab in HIV-associated aggressive lymphomas that found rituximab was associated with significantly more infectious deaths but only a trend in improved tumor control; based on this, the authors concluded that rituximab does not improve the clinical outcome of HIV-associated DLBCL (Kaplan et al. 2005).

Table 12.1 Pivotal trials in human immunodeficiency virus (HIV)-associated lymphomas

Study	Study type	Study design	Results
Kaplan et al. (1997)	Prospective multicenter randomized phase III ($n=192$)	Randomization to standard-dose m-BACOD with GM-CSF versus low-dose m-BACOD without GM-CSF. No cART	Similar efficacy of both regimens but less hematological toxicity with low-dose m-BACOD
Ratner et al. (2001)	Prospective multicenter sequential phase II ($n=65$)	First 40 patients received modified-dose (m) CHOP (50 % cyclophosphamide and doxorubicin) and the next 25 patients received standard-dose CHOP. cART was administered	CR higher with full dose CHOP compared to mCHOP (48 % vs. 30 %). Authors concluded that concomitant cART was safe but unable to conclude superiority of one regimen over another
Sparano et al. (2004)	Prospective multicenter sequential phase II ($n=98$)	First 43 patients received didanosine and the next 55 patients received cART with CDE	At 2 years, FFS and OS were 36 % and 43 %. Patients receiving concomitant cART had better survival and less toxicity
Mounier et al. (2006)	Prospective multicenter phase III study	485 patients were randomly assigned to different CHOP-based chemotherapy regimens according to an HIV score that was based on performance status, prior AIDS and CD4 count	Though HIV score, IPI score and cART affected survival, the intensity of CHOP-based chemotherapy had no effect on survival
Little et al. (2003)	Prospective single center phase II ($n=39$)	All patients received EPOCH and G-CSF with cART suspension	CR was 74 %. At 53 months, DFS and OS were 92 and 60 %. Patients in CR achieved CD4 recovery and HIV control following treatment. Conclusion that EPOCH with cART suspension is feasible and highly effective
Kaplan et al. (2005)	Prospective multicenter randomized phase III ($n=150$)	Randomization (2:1) to R-CHOP versus CHOP with concomitant cART. Some patients received maintenance rituximab	CR rate higher with R-CHOP compared to CHOP (57.6 % vs. 47 %). Increased infectious deaths with R-CHOP mostly in patients with low CD4 counts. Conclusion that rituximab does not improve clinical outcome
Boue et al. (2006)	Prospective multicenter phase II ($n=61$)	All patients received R-CHOP	CR in 77% of patients. Estimated 2-year OS was 75%
Spina et al. (2005)	Retrospective analysis of 3 phase II trials	Pooled results from three trials of CDE with rituximab	CR rate was 70 %. At 2 years, FFS and OS were 59 % and 64 %. Conclusion that R-CDE is effective but rituximab may increase infections
Sparano et al. (2010)	Prospective multicenter phase II study	101 patients were randomized to receive either concurrent or sequential rituximab with DA-EPOCH	There was a superior outcome with concurrent rituximab and DA-EPOCH (CR rate 75 %) and this was considerably better when compared to the previous ANC results with CHOP +/– R
Dunleavy et al. (2010a)	Prospective single center phase II ($n=33$)	All patients received SC-EPOCH-RR with cART suspension	79 % of patients needed only three cycles of treatment. At 5-year follow-up, PFS and OS were 84 % and 68 %. Outcome was better for GCB versus non-GCB DLBCL (5-year PFS of 95 % versus 44 %).

GM-CSF granulocyte macrophage colony-stimulating factor, *G-CSF* granulocyte colony stimulating factor, *cART* combined anti-retroviral therapy, *CR* complete remission, *FFS* failure-free survival, *OS* overall survival, *DFS* disease-free survival, *m-BACOD*, methotrexate, bleomycin, doxorubicin, cyclophosphamide, vincristine and dexamethasone, *CHOP* cyclophosphamide, doxorubicin, vincristine and prednisone, *R* rituximab, *CDE* cyclophosphamide, doxorubicin, and etoposide, *EPOCH*, etoposide, prednisone, vincristine, cyclophosphamide and doxorubicin, *DA* dose adjusted, *SC* short-course

A retrospective analysis of 3 phase II trials from Italy, where patients received infusional cyclophosphamide, doxorubicin, and etoposide (CDE) with rituximab, also concluded that rituximab might increase infections (Spina et al. 2001, 2005). On closer evaluation of the AMC trial, however, the increased infectious deaths occurred primarily in patients with very low CD4 counts, and many patients received "maintenance" rituximab after chemotherapy, which has not been shown to be useful in HIV-negative DLBCL (Dunleavy et al. 2006).

Subsequent to the AMC study, a French group performed a phase II study of CHOP plus rituximab in HIV-associated NHL and the CR rate of 77 % and 2-year survival rate of 75 % suggested that rituximab was beneficial and could be given safely to this group of patients (Boue et al. 2006). To further address the controversy of rituximab, the AMC performed another randomized phase II study. At the time that this study was designed, the results of the EPOCH regimen in this population were very promising, and they randomized patients to receive concurrent versus sequential rituximab with EPOCH (etoposide, prednisone, vincristine, cyclophosphamide, and hydroxydaunorubicin) (Sparano et al. 2010; Chadburn et al. 2009; Dunleavy and Wilson 2010; Kaplan et al. 1989, 1997; Little et al. 2003). Importantly they found that concurrent rituximab was not associated with increased infectious deaths (Sparano et al. 2010; Chadburn et al. 2009; Dunleavy and Wilson 2010; Kaplan et al. 1989, 1997; Little et al. 2003). The study also examined if the CR rate with EPOCH-R was superior to CHOP ± rituximab, employing a predetermined retrospective analysis, and if concurrent versus sequential rituximab was more toxic and/or more effective. There was no difference in toxicity between the arms, and the authors rejected the null hypothesis of 50 % (associated with CHOP ± rituximab) in favor of 75 % CR for EPOCH with concurrent rituximab ($p=0.005$; power 0.89) (Sparano et al. 2010). Based on this study, we consider it very unwise to omit rituximab from upfront therapy in HIV-associated lymphoma.

While one group demonstrated good efficacy with R-CHOP in a multicenter setting, it is concerning that 15 % of enrolled patients were not evaluable for response due to early events or lacking clinical and radiological evaluations (Boue et al. 2006). Though the AMC's conclusions regarding EPOCH-R's superiority over R-CHOP are based on a historical comparison, the dramatic differential outcome with these two regimens in a similar patient population suggests that EPOCH-R may be a superior regimen in this population. Whether or not there are subgroups of patients with HIV-associated DLBCL who may do as well with R-CHOP is unknown at this time and has not been studied prospectively.

Following on from the initial promising results with EPOCH, a second-generation EPOCH regimen termed short-course EPOCH-RR was developed (Dunleavy et al. 2010a). This approach is designed to address the dual challenge of achieving excellent tumor control while preserving immune integrity. While it was previously demonstrated that 6 cycles of DA-EPOCH is highly effective (PFS and OS of 73 and 60 % at 53 months) in HIV-associated lymphoma, with 5 years follow-up, the PFS and OS of SC-EPOCH-RR are 84 % and 68 %, respectively, and 79 % of patients only required 3 treatment cycles (Dunleavy et al. 2010a, b; Ribera et al. 2008; Sparano et al. 2010; Chadburn et al. 2009; Dunleavy and Wilson 2010; Kaplan et al. 1989, 1997, 2005; Little et al. 2003). Interestingly, with this approach, the clinical prognostic characteristics that make up the IPI and the IPI itself do not predict PFS or OS. Only tumor histogenesis is associated with lymphoma-specific outcome with 95 % of GCB versus 44 % of non-GCB DLBCL progression free at 5 years. Although, both EBV positivity of the tumor and low CD4 count at diagnosis are significantly associated with an inferior overall survival, they are not associated with lymphoma-specific outcome.

12.8.1 Should CART Be Continued During Therapy?

The risks and benefits of continuing CART during curative chemotherapy of aggressive lymphomas have been variably interpreted. While many investigators rightly raise the concern that

uncontrolled HIV replication during chemotherapy will worsen immune function, they often do not consider the potentially adverse effects of CART on lymphoma-specific outcomes because they are difficult to quantify. One of the first trials to assess concurrent CART was a nonrandomized AMC study of dose-reduced and standard-dose CHOP (Ratner et al. 2001). A potentially important finding of the study comes from the pharmacokinetic (PK) analysis which showed that cyclophosphamide clearance was reduced 1.5-fold, but doxorubicin clearance was unchanged compared to historical results. While it is reassuring that the doxorubicin PK was unaffected, the reduced clearance of cyclophosphamide – an inactive prodrug – could likely result in a reduction of active metabolites and potentially compromise efficacy. In this study, CD4 counts increased significantly during therapy, and the mechanism for increased CD4 cell counts raises the concern that CART protects T cells from chemotherapy-induced cytotoxic stress, an effect that might occur in the lymphoma cells (Johnson and Parkin 1998; Phenix et al. 2000). Other groups however have suggested that CART can be safely administered with chemotherapy, and it has not been well prospectively studied and controversies abound (Sparano et al. 2004; Vaccher et al. 2001). In that respect, it is important to note that many newer antiretrovirals with fewer drug interactions (than those studied in the past) are now available.

12.9 HIV-Associated Burkitt Lymphoma

Though, following the advent of CART, there was a significant improvement in the outcome of HIV-associated DLBCL, this was not the case initially with HIV-associated Burkitt lymphoma, as reported in a retrospective series by Lim et al. (2005). This lack of improvement is likely explained by the widespread use of CHOP-based regimens, which have poor efficacy in BL (Dave et al. 2006; Bishop et al. 2000). While dose-intense regimens such as hyper-CVAD (hyperfractionated cyclophosphamide, vincristine, doxorubicin, and dexamethasone) and CODOX-M-IVAC, with or without rituximab, have shown encouraging results in HIV-negative BL, they have not been studied too extensively in HIV-associated BL. One of the concerns with CODOX-M-IVAC is treatment-related toxicity in this population (Barnes et al. 2011; Cortes et al. 2002). In an attempt to reduce this, the AMC recently presented the results of a feasibility and toxicity study for HIV-associated BL and atypical BL and reported good overall survival rates with 65 % of patients completing treatment as per protocol (Noy et al. 2009).

Burkitt lymphoma highlights the necessity to balance treatment efficacy and toxicity by optimizing the therapeutic index, especially in patients who are immune suppressed and/or elderly. Based on its excellent activity in highly proliferative DLBCL and its favorable toxicity profile, EPOCH-R was studied in untreated BL and was highly effective (Dunleavy et al. 2011). The AMC also included several patients with BL or Burkitt-like lymphoma in their study of concurrent versus sequential EPOCH-R and reported high response rates in this group (Sparano et al. 2010).

12.10 Approaches to Other HIV-Associated Lymphomas

12.10.1 Hodgkin Lymphoma

Similarly to that observed in HIV-NHL, one of the most peculiar features of HIV-HL is the widespread extent of disease at presentation and the frequency of systemic "B" symptoms that include fever, night sweats, and/or weight loss >10 % of the normal body weight. At the time of diagnosis, 70–96 % of the patients have "B" symptoms and 74–92 % have advanced-stage disease with frequent involvement of extranodal sites, the most common being bone marrow (40–50 %), liver (15–40 %), and spleen (around 20 %)(Tirelli et al. 1987, 1995; Andrieu et al. 1993). HIV-HL tends to develop as an earlier manifestation of HIV infection with higher median CD4+ cell counts, ranging from 275 to 306/μL (Tirelli et al. 1987, 1995; Andrieu et al. 1993). The widespread use of CART has resulted in substantial improvement

in the survival of patients with HIV infection and lymphomas. This is due to a reduced incidence of opportunistic infections, the improved ability to safely deliver more aggressive chemotherapy and to a pathobiologic shift towards less aggressive lymphomas compared to those in the pre-CART era (Tirelli et al. 1987, 1995; Andrieu et al. 1993; Rubio 1994; Vaccher et al. 2003).

Within the Italian Cooperative Group on AIDS and Tumors (GICAT), we have collected data on 290 patients with HIV-HL. Two hundred and eighty-one patients (87 %) were males, and the median age was 34 years (range 19–72 years), and 69 % of patients were intravenous drug users. The median CD4 cell count at diagnosis was 240/μL (range 4–1,100/μL), and 57 % of patients had a detectable HIV viral load.

MC was diagnosed in 53 % of cases, followed by NS in 24 % and LD in 14 %. Advanced stages of disease were observed in 79 % of patients and 76 % had B symptoms. The overall rate of extranodal involvement was 59 % with bone marrow, spleen and liver involved in 38 %, 30 %, and 17 % of patients, respectively. With a goal to evaluate the impact of CART on clinical presentation and outcome of our patients, we split the series into two subgroups: in the first group we included those patients who received CART within 6 months of the onset of HL (84 patients); in the second group we included those patients who had never received CART prior to the diagnosis of HL or had received CART more than 6 months before the diagnosis (206 patients). Briefly, compared to patients who had never received CART, patients who were on CART before the onset of HL were older, had fewer B symptoms, and had higher leukocyte and neutrophil count and a higher hemoglobin level. The following parameters were associated with a better overall survival (OS): NS subtype, the absence of extranodal involvement, the absence of B symptoms, and the prior use of CART. Interestingly, three parameters were associated with a better time to treatment failure: a normal alkaline phosphatase level, prior exposure to CART, and an international prognostic score less than 3 (Chimienti et al. 2008). A similar study was carried out within the Spanish group GESIDA where the authors compared the clinical characteristics and outcome of 104 patients with HIV-HL. Among these, 83 patients had previously received CART and 21 patients had not. No differences were observed between the groups at baseline, but the complete remission (CR) rate was significantly higher in the CART group (91 % versus 70 %, $p=0.023$). The median overall survival was not reached in CART group and was 39 months in non-CART group ($p=0.0089$); the median disease-free survival (DFS) was not reached in CART group and was 85 months in non-CART group ($p=0.129$). Factors independently associated with CR were a CD4 cell count >100 cells/μL and the use of CART; CR was the only factor independently associated with OS (Berenguer et al. 2008).

The optimal therapy for HIV-HL has not yet been defined. As most patients have advanced-stage disease, they are typically treated with combination chemotherapy regimens, but the CR rates remains lower than those of HL in HIV-negative patients – the median OS in patients with HIV-HL is approximately 1.5 years (Tirelli et al. 1987, 1995; Andrieu et al. 1993; Rubio 1994). Due to the low incidence of the disease, no randomized controlled trials have been conducted in this setting. However, several phase II studies have evaluated the feasibility and activity of different regimens. In a prospective trial, conducted within the GICAT between March 1989 and March 1992, 17 previously untreated patients with HIV-HL were treated with epirubicin, vinblastine, and bleomycin (EVB). Overall, CR was achieved in 53 % of the total group and lasted a median of 20 months. The median OS for the group was 11 months and the 2-year DFS was 55 % (Errante et al. 1994). In an attempt to improve upon these results, from 1993 to 1997, a second prospective trial consisting of full-dose EVB plus prednisone (EVBP regimen) and concomitant antiretroviral therapy (zidovudine or didanosine) was conducted. The results of this trial, which enrolled 35 patients, demonstrated a CR rate of 74 % and a 3-year OS and DFS of 32 % and 53 %, respectively (Errante et al. 1999). The AIDS Clinical Trials Group (ACTG) reported the results of a phase II study in 21 patients treated with ABVD chemotherapy for 4–6

cycles where there was primary use of G-CSF. Antiretroviral therapy was not administered. The CR rate, on an intent to treat analysis was 43 % with an overall objective response rate of 62 %. Median survival for all patients was 18 months (Levine et al. 2000). Similar data were reported in a small trial of only eight patients (Gastaldi et al. 2002). The widespread use of CART has made feasible the institution of more aggressive chemotherapeutic regimens. In another trial, we evaluated the Stanford V regimen, consisting of short-term chemotherapy (12 weeks) with adjuvant radiotherapy. From May 1997 to October 2001, 59 consecutive patients were treated in this prospective phase II study within the European Intergroup Study HL-HIV. The Stanford V regimen was well tolerated, and 69 % of patients completed treatment with no dose reduction or delayed chemotherapy administration. The most important dose-limiting toxicities were bone marrow toxicity and neurotoxicity. Eighty-one percent of patients achieved a CR, and after a median follow-up of 17 months, 33/59 (56 %) patients were alive and disease free. The estimated 5-year OS, DFS, and freedom from progression (FFP) were 59 %, 68 %, and 60 %, respectively. The probability of FFP was significantly higher ($p=0.002$) among patients with an international prognostic score (IPS) of ≤ 2 than in those with an IPS > 2, and the percentage of FFP at 2 years were 83 % and 41 %, respectively. Similarly, the probability of OS was significantly different ($p=0.0004$), and the percentage overall survival at 3 years was 76 % and 33 %, respectively, for IPS < 2 and IPS > 2 (Spina et al. 2002). Within the German group, the very intensive BEACOPP regimen was tested in 12 untreated patients with a 100 % of CR rate, but a high incidence of opportunistic infections was reported (Hartmann et al. 2003). Recently, the results of a large prospective phase II study using ABVD were published. Within a cooperative network in Spain, 62 patients with HIV-HL received the standard ABVD regimen plus CART. The scheduled six to eight ABVD cycles were completed in 82 % of cases. Six patients died during induction, 54 (87 %) achieved a CR, and two were treatment resistant. The 5-year OS and event-free survival (EFS) probabilities were 76 % and 71 %, respectively. An immunological response to CART had a positive impact on OS ($p=0.002$) and EFS ($p=0.001$)(Xicoy et al. 2007). Finally, within the GICAT, we have recently concluded the accrual of 71 patients in a prospective phase II study aiming to evaluate feasibility and activity of a novel regimen including epirubicin, bleomycin, vinorelbine, cyclophosphamide, and prednisone (VEBEP regimen). Seventy percent of patients had advanced stage disease and 45 % had an IPS > 2. The CR rate was 67 % and 2-year OS, DFS, TTF, and EFS were 69 %, 86 %, 59 %, and 52 %, respectively (Spina et al. 2008). The results of the largest prospective studies are showed in Table 12.2.

Because a large proportion of patients with HIV-HL progress or relapse, the use of high-dose chemotherapy and autologous stem cell transplantation (ASCT) has been investigated in this setting. Several data from different groups, including the GICAT, have demonstrated the feasibility of this approach that can be considered the gold standard in the salvage setting (Gabarre et al. 2004; Krishnan et al. 2005; Serrano et al. 2005; Re et al. 2003, 2009). Different conditioning regimens, that have or have not included total body irradiation, have been tested. Recently, the AIDS Malignancy Consortium (AMC) demonstrated in a multi-institutional trial that a regimen of a dose-reduced high-dose chemotherapy, which included cyclophosphamide, busulfan, and ASCT, is well tolerated and is associated with favorable DFS and OS probabilities for selected patients with HIV-associated NHL and HL (Spitzer et al. 2008).

12.10.2 Primary Central Nervous System Lymphoma

PCNSL typically presents in patients with severe immune suppression. Thus, it is not unexpected that since the advent of CART, its incidence has decreased dramatically. While the disease remains incurable in most patients, the duration of survival appears to have increased. Compared to HIV-negative patients, HIV-associated PCNSL

Table 12.2 Results of prospective studies in HIV-HL

Regimen	No. of patients	Stage III/IV	Response rate	Complete remission rate	Overall survival
EBV (Errante et al. 1994)	17	88 %	82 %	53 %	11 months
EBVP (Errante et al. 1999)	35	83 %	91 %	74 %	16 months
ABVD (Levine et al. 2000)	21	81 %	62 %	43 %	18 months
ABVD (Gastaldi et al. 2002)	8	75 %	100 %	100 %	43.5 months
Stanford V (Spina et al. 2002)	59	71 %	89 %	81 %	59 % at 5 years
BEACOPP (Hartmann et al. 2003)	12	92 %	100 %	100 %	75 % at 3 years
ABVD (Xicoy et al. 2007)	62	100 %	87 %	87 %	76 % at 5 years
VEBEP (Spina et al. 2008)	71	70 %	78 %	67 %	69 % at 2 years

EBV epirubicin, bleomycin and vinblastine, *EBVP*, EBV with prednisone, *ABVD* doxorubin, bleomycin, vinblastine and dacarbazine, *BEACOPP* bleomycin, etoposide, doxorubicin, cyclophosphamide, vincristine, procarbazine and prednisone, *VEBEP* epirubicin, bleomycin, vinorelbine, cyclophosphamide and prednisone

is typically EBV positive (Swerdlow et al. 2008). Patients frequently present with changes in mental status or focal neurological symptoms and, unlike HIV-negative PCNSL, they tend to present with multiple brain lesions. Because these patients are severely immune suppressed, intracranial opportunistic infections should always be considered in the differential diagnosis when evaluating intracranial lesions on imaging studies.

Unlike HIV-negative PCNSL, where high-dose methotrexate and, more recently, combination chemotherapy regimens are effective, total brain irradiation remains standard in HIV-associated PCNSL. While most studies in the pre-CART era report a median survival in the range of 3 months, survival over 1.5 years has been reported in patients who respond to CART and were treated with radiation (Hoffmann et al. 2001; Ling et al. 1994). The role of systemic therapy and rituximab remains undefined in this disease, although some studies are investigating these agents. Given the poor outcome with this disease with standard approaches, it is reasonable to refer patients for investigational studies or if unavailable, total brain radiation is reasonable.

12.10.3 Primary Effusion and Plasmablastic Lymphoma

The outcome of PEL is poor with standard treatment, and the median survival is in the range of 6 months (Boulanger et al. 2005). Unlike some other HIV-associated lymphomas, CART does not appear to have had a significant impact on survival. At this time, the optimal therapy for PEL remains to be defined, but regimens such as EPOCH and CDE may be beneficial. Other approaches such as high-dose methotrexate and parenteral zidovudine (AZT) with interferon alpha have been studied but have demonstrated limited efficacy (Boulanger et al. 2003; Ghosh et al. 2003). The prognosis of plasmablastic lymphoma in the setting of HIV has also been historically poor (Delecluse et al. 1997; Castillo et al. 2010). The impact of CART has not been well studied, but anecdotal reports suggest its prognosis may have improved since the introduction of CART (Lester et al. 2004). It is reasonable to consider regimens such as EPOCH or CDE for this disease. Newer agents like bortezomib and lenalidomide have been used anecdotally with some reports of activity and success (Bibas et al. 2010).

12.10.4 Relapsed Lymphoma

Relapsed lymphoma is associated with a poor prognosis, and median survivals tend to be less than 1 year. A recent Italian study prospectively evaluated high-dose therapy and stem cell transplantation in 50 patients with relapsed HIV-associated lymphoma (both HL and NHL) (Re et al. 2009). While the median overall survival of patients was 33 months, patients who had chemosensitive disease had a relatively favorable outcome and were disease free at 44 months follow-up. Given the significant improvements in HIV control and immune function, it is reasonable to approach relapsed HIV-associated lymphomas similarly to their HIV-negative counterparts and to pursue aggressive strategies if appropriate. Less aggressive strategies, such as ESHAP and CDE, have poor outcomes (Spina et al. 2001; Bi et al. 2001). The role of allogeneic transplantation has not been well evaluated at this time.

12.10.5 Future Directions

In summary, the treatment of HIV-associated lymphoma has significantly improved in recent years, and, nowadays, most patients with DLBCL and BL can be cured with approaches like EPOCH-R. For BL, other approaches like modified CODOX-M-IVAC are also being investigated. For HL, ABVD with antiretroviral continuation is a reasonable strategy, due to the long duration of therapy. For PCNSL and less common HIV-associated lymphomas, survival with standard approaches to date has been poor and experimental therapy should be considered.

The outcome of HIV-associated lymphoma has undergone significant improvement in recent years beginning with the widespread use of CART. Both DLBCL and BL are highly curable diseases for the most part. To further improve the outcome of these lymphomas, the challenge is to identify driver pathways and therapeutic targets. In this regard, approaches such as investigating modulation of the B-cell receptor cascade and NFκB transcription factor, which are involved in the pathobiology of ABC DLBCL, are interesting(Dunleavy et al. 2009; Davis et al. 2010). For GCB DLBCL and BL, current approaches have excellent efficacy with little room for improvement so that future studies should focus on further reducing treatment toxicity, particularly in highly immune suppressed patients. Advances in the therapeutics of poor prognostic diseases like HIV-associated PCNSL and PEL will likely come from improved understanding of their pathobiology.

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Non-MALT Marginal Zone Lymphoma

13

Catherine Thieblemont, Steven Bernstein, Scott Rodig, and Jan Delabie

Contents

Abbreviations

NMZL	Nodal marginal zone lymphoma
SMZL	Splenic marginal zone lymphoma

13.1 Introduction

MZLs 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

Pathology: Scott Rodig and Jan Delabie

C. Thieblemont, MD, PhD (✉)
Department of Hemato-Oncology,
AP-HP- Hopital Saint-Louis,
1, Avenue Claude Vellefaux,
F-75010 Paris, France

Laboratoire de pathologie,
Université Paris Diderot,
Sorbonne Paris Cité,
UMR-S 728, F-75010, Paris, France

INSERM, U728-, F-75010,
Paris, France
e-mail: catherine.thieblemont@sls.aphp.fr

S. Bernstein
Hematology, James P Wilmot Cancer Center,
Rochester, NY, USA

S. Rodig, MD
Department of Pathology,
Brigham and Women's Hospital,
Harvard Medical School, Boston, MA, USA
e-mail: srodig@partners.org

J. Delabie, MD
Department of Pathology,
Oslo University Hospital, Oslo, Norway
e-mail: jandel@ous-hf.no

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those lymphoid organs where an abundant influx of antigens is known to occur. The MZ is mainly developed in spleen and mucosa-associated lymphoid tissues, whereas it is rarely identifiable in lymph nodes (Weill et al. 2009). According to the sites involved and characteristic molecular findings, the International Lymphoma Study Group distinguished three distinct subtypes of MZL: (1) extranodal MZL of MALT type, (2) splenic MZL (with or without villous lymphocytes), (3) and nodal MZL (with or without monocytoid B cells) (Swerdow et al. 2008). 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 pathogenetic mechanisms, clinical features, and treatment of two of these lymphomas, the splenic MZL (SMZL) and the nodal MZL (NMZL).

13.2 Epidemiology: Role of Hepatitis C Virus in SMZL and NMZL

In adults, MZLs account for 5–17 % of all NHL depending on the series. Splenic and nodal MZLs represent 20 % and 10 % of MZL, respectively, and account for less than 2 % of NHL (Berger et al. 2000). Regarding NZML, several series include cases corresponding to nodal spread of extranodal marginal zone lymphoma or cases disseminated at diagnosis, with peripheral lymph nodes associated and/or extranodal or splenic involvement. Two-third of the NMZL cases of the Southwest Oncology Group study were described as "composite lymphomas" with concomitant follicular lymphoma, which might include follicular lymphomas with marginal zone differentiation (Fisher et al. 1995). The median age of occurrence for SMZL is 65 years (Oscier et al. 2005; Thieblemont et al. 2002) and between 50 and 62 years old for NMZL (Traverse-Glehen et al. 2006; Arcaini et al. 2007a, 2009a). 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. H*epatitis C virus* (HCV) has been highly correlated with lymphoplasmacytic immunocytoma, SMZL (Arcaini et al. 2004, 2006a), and with NMZL in some area such as in Italy (Arcaini et al. 2007a). In the setting of SMZL, the presence of HCV is of major relevance, due to its possible therapeutic implications (Hermine et al. 2002). 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 supporting the role of infectious agents on the pathogenesis of SMZL (Bates et al. 2001).

13.3 Physiopathology: A Post-germinal Center Origin

The precise pathogenesis of SMZL and NMZL is essentially unknown. The origin of SMZL and NMZL is a marginal zone memory B cell, claimed to derive in most cases from a post-germinal origin, as demonstrated by the study of somatic mutations in Ig heavy chain variable (IGHV) region genes (Arcaini et al. 2009b; Traverse-Glehen et al. 2005; Zibellini et al. 2010). However, a limited degree of heterogeneity in the mutational profile among SMZL has been described, one-third of the cases being non-mutated (Traverse-Glehen et al. 2005; Algara et al. 2002). On the other hand, these lymphomas exhibit a low frequency of somatic mutations involving some oncogenes (*bcl-6*, *PAX5*, *PIM1*, *RHO-H*) (Traverse-Glehen et al. 2007). These findings suggest a particular differentiation pathway that may occur without transit through the germinal center (Traverse-Glehen et al. 2005; Algara et al. 2002). Interestingly, a bias in the use of IGHV genes has been found, in SMZL and in NMZL suggesting antigenic selection (Traverse-Glehen et al. 2005; Zibellini et al. 2010).

In SMZL associated with HCV, it has been demonstrated that the E2 glycoprotein of HCV could interact with CD81 in the B cells and could be responsible for B-cell activation through the BCR, thereby leading to their increased proliferation (Morse et al. 2001). In murine models, MZLs have been described following chronic stimulation by HCV and have been associated with mutations of *FAS*, *AP12*/*ML*, and *p53* (Morse et al. 2001). A special form of SMZL related to HCV has been correlated with the presence of cryoglobulin (Saadoun et al. 2004). A decrease in lymphoproliferation following antiviral treatments reinforces the data suggesting a contribution of chronic antigenic stimulation to the pathophysiologic process of HCV-related MZL (Arcaini et al. 2004; Hermine et al. 2002).

13.4 Splenic Marginal Zone Lymphoma

13.4.1 Clinical Presentation

The hallmark of the clinical presentation of SMZL is massive splenomegaly. However, most 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 (Thieblemont et al. 2003). 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. Serum LDH level is usually normal in SMZL, but the β2-microglobulin level is increased. A considerable proportion of patients (10–40 % of cases) have a serum monoclonal paraprotein (M-component), mainly of the μ-subtype (IgM) (Thieblemont et al. 2003; Parry-Jones et al. 2003). Autoimmune 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.

HCV-associated SMZL is clinically indistinguishable from typical SMZL, except for the presence of HCV viral replication, and coexistence of liver disease and presence of cryoglobulinemia (Hermine et al. 2002).

13.4.2 Pathological Features

13.4.2.1 Morphology

The morphologic features of SMZL are based on the study of peripheral blood lymphocytes and bone marrow biopsies or, when appropriate, on the study of surgical specimens from splenectomy. Most cases of SMZL do not require splenectomy for the diagnosis, which can be accurately based on the study of the bone marrow and peripheral blood. Revised criteria for SMZL diagnosis have been recently published based on a collaborative effort (Matutes et al. 2008).

Histology of the spleen shows a micronodular infiltration by a polymorphic population of B cells, including small cells, marginal cells, and scattered immunoblasts of the white pulp, with a variable degree of red pulp involvement. A specific subgroup of SMZL with red pulp lymphoma involvement associated with numerous basophilic villous lymphocytes has been identified (Traverse-Glehen et al. 2008). Likewise, the blood infiltration is pleomorphic showing small lymphocytes, centrocyte-type lymphocytes, and villous lymphocytes. In the bone marrow, the involvement can be paratrabecular, nodular, or diffuse. Intrasinusoidal infiltration is highly typical of SMZL (Boveri et al. 2009).

The imunophenotypic 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 (Matutes et al. 2008).

13.4.2.2 Cytogenetic and Molecular Features

Cytogenetic analyses in SMZL demonstrate that complex chromosomal aberrations are common (80 % of cases with an abnormal karyotype). Complete or partial trisomy 3 is the most frequent cytogenetic abnormalities (85 % of patients) (Callet-Bauchu et al. 2005; Dierlamm et al. 1997; Sole et al. 2001; Salido et al. 2010). The abnormality considered typical of SMZL, reported in 40 % of cases, consists of deletion or translocation of chromosome 7q32. No tumor suppressor genes have been found in this region, and evidence supports that the deletion of a cluster of miRNAs located in this region could contribute to the deregulation of some of the key oncogenes in this disorder, such as TCL1. More rare translocations involving CDK6 and cyclin D3 with IgH have been identified in small subsets of cases (Corcoran et al. 1999).

Other chromosomal abnormalities reported at diagnosis include trisomy 18, trisomy 12, 17q isochrome, 13q14 deletion, and structural abnormalities of chromosome 1 (Salido et al. 2010). 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 morphologic, phenotypic, and cytogenetic features suggesting a diagnosis of mantle cell lymphoma (Cuneo et al. 2001; Jadayel et al. 1994; Oscier et al. 1993).

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 (Rinaldi et al. 2011). More specific abnormalities were described in SMZL such as del(7q31) and del(8p).

None of the abovementioned cytogenetic abnormalities, with the exception of 7q32 deletion, is considered typical of SMZL, but they may be helpful for the diagnosis, particularly for differential diagnosis with CLL, hairy cell leukemia, mantle cell lymphoma, follicular lymphoma, or lymphoplasmacytic lymphoma. Contrary to other MZLs, translocations involving the *MALT 1* gene are not found in SMZL. In terms of prognostic impact, only the association with del(17p) and del(8p) had a significant negative impact on the outcome of SMZLs.

Initial analyses of the mutational status of the IGHV genes have shown the presence of somatic hypermutation in most of cases (Algara et al. 2002). However, more recent studies have found an absence of somatic mutations in one-third of studied cases, possibly reflecting a relative degree of molecular heterogeneity of MZL. In addition, SMZL B cells express a biased repertoire with preferential usage of certain IGHV genes such as IGHV1-2, IGHV3-23, and IGHV4-34 (Arcaini et al. 2009b). This, along with the finding of some cases expressing BCR with quasi-identical IGHV sequences including the antigen-binding site, strongly suggests that antigen selection might contribute to the development of SMZL (Arcaini et al. 2009b; Traverse-Glehen et al. 2005). As mentioned above, antigen selection is also 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.

13.4.2.3 Genome-Wide Analysis

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 (Rinaldi et al. 2011; Kiel et al. 2012). This specific molecular signature includes genes involved in the signaling cascade of the AKT1 pathway (Kiel et al. 2012) but also the BCR signaling pathway, tumor necrosis factor (TNF) , and NF-κB targets (Rinaldi et al. 2011). To date, gene expression analysis is not routinely available and cannot be applied to routine diagnosis.

Recently whole-genome DNA sequencing (Kiel et al. 2012) and integrated whole-exome sequencing with genome-wide high-density single-nucleotide polymorphism (SNP) array data (Rossi et al. 2012) have demonstrated recurrent somatic gain-of-function mutations in

NOTCH2, a gene encoding a protein required for marginal zone B-cell development. This lesion was the most frequent lesion in SMZL accounting for 20–25 % of the cases, with a specific occurrence in SMZL among indolent B-cell lymphoproliferative disorders. In addition to NOTCH2, other modulators or members of the NOTCH pathway were shown to be recurrently targeted, including NOTCH1, SPEN, and DTX1, together with other signaling pathways normally involved in MZ B-cell development, suggesting that deregulation of MZ B-cell development pathways plays a role in the pathogenesis of ~60 % SMZL.

13.4.3 Prognostic Factors in SMZL: Biological and Clinical Parameters

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 (Bertoni and Zucca 2005). Clinical and biological prognostic factors have been identified by several investigators (Table 13.1). 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,

Table 13.1 Clinical and biological adverse prognostic factors in SMZL and NMZL

Author (year)	*n*	PFS	OS
SMZL			
Thieblemont et al. (2002)	81	Presence of M-component Presence of an immunological event	Beta2 microglobulin ≥3 mg/L Leukocytes ≥20 10^9/L Lymphocytosis ≥9 10^9/L Presence of M-component Presence of an immunological event
Ruiz-Ballesteros et al. (2005)		–	Expression of CD38 Unmutated Ig-VH gene status Expression of NF-κB-activated genes by GEP
Arcaini et al. (2006b)	309	–	Hemoglobin <12 g/dL Elevated LDH Albumin >3.5 g/dL
NMZL			
Camacho et al. (2003)	27	Survivin Caspase 3	Cyclin E
Petit et al. (2005)	12	–	Ki67 IRF4
Oh et al. (2006)	36	Age >60 Elevated LDH Hb <12 g/dL BM+ No anthracycline ECOG ≥2 Stage III/IV	B symptoms ECOG ≥2 Hb <12 g/dL BM+ Stage III/IV
Traverse-Glehen et al. (2006)	21	None	None
Arcaini et al. (2007a, b)	47	B symptoms Hb <12 g/dL	Age >60 Elevated LDH BM+ HCV+
Kojima et al. (2007)	65	–	Age >60

Table 13.2 Response to treatment in SMZL

Author (year)	*n*	Schedule	Status of disease	Response rate (%)	CR/CRu (%)	PR (%)	PFS (at *n* years)	OS (at *n* years)
Splenectomy alone								
Chacon et al. (2002)	29	–	First line	100	0	100	*	*
Thieblemont et al. (2003)	25	–	First line	100	0	100	71 % (2)	81 % (5)
Chemotherapy alone								
Lefrere et al. (2000)	10	Fludarabine	Relapsed	100	70	30	42 % (4.7).	50 % (5)
Cervetti et al. (2004)	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. (2006)	26	R once/W×4 or 8	First line	88	43	46	86 % (3)	95 % (3)
Kalpadakis et al. (2007)	16	R once/W×6	First line	100	79	11	92 % (2.1)	100 % (3)
Bennett et al. (2005)	14	R once/W×4	First line	78	57	21	60 % (6)	80 % (6)
Rituximab and chemotherapy								
Tsimberidou et al. (2004)	6	R-FMD or RFC	First line	83	34	50	100 % (3)	100 % (3)
Arcaini et al. (2004)	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, (*) not provided

LDH level greater than normal, and albumin level less than 3.5 g/dL) leading to a prognostic index (Arcaini et al. 2006b). 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). 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-κB-activated genes based on gene expression analysis (Ruiz-Ballesteros et al. 2005).

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 (Camacho et al. 2001). 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 (Thieblemont et al. 2002).

13.4.4 New Treatment 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 (Traverse-Glehen et al. 2006). The only exception to this management approach is in the setting of SMZL associated with active HCV infection. Antiviral therapy with pegylated interferon-α and ribavirin will lead to clearance of HCV RNA in 75 % of the patients and in concomitant clinical remission of the lymphoma (Vallisa et al. 2005) (Table 13.2).

When patients become symptomatic because of anemia, abdominal pain, or thrombocytopenia

Table 13.3 Median progression and overall survival in the published series of patients with NMZL

Author (year)	Number of patients (*n*)	Median progression (years)	Median OS (years)	5-year OS (%)
Armitage and Weisenburger (1998)	25	Nd	Nd	57
Nathwani et al. (1999b)	20	Nd	Nd	56
Berger et al. (2000)	37	Nd	Nd	55
Camacho et al. (2003)	22	Nd	Nd	79
Arcaini et al. (2004)	9	2.8	Not reached	Nd
Traverse-Glehen et al. (2006)	21	1.3	Nd	64
Oh et al. (2006)	36	1.3	5.5	82.7
Arcaini et al. (2007b)	47	2.6	Not reached	69
Kojima et al. (2007)	65	Nd	Nd	85

(Arcaini et al. 2006b), several treatment options may be proposed to the patient. Regarding cytopenia, the level of cytopenia to start treatment is not defined precisely in any retrospective studies, and, except for specific clinical trial, the decision of treatment should be taken on symptoms. As first option, splenectomy will rapidly improve performance status and correct anemia, thrombocytopenia, and neutropenia within 6 months after splenectomy (Thieblemont et al. 2003). 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 (Thieblemont et al. 2002). For patients who are unfit for splenectomy or unwilling to undergo surgery, systemic therapy may be effective (Table 13.3). Rituximab alone is reported to afford excellent response rate with a shorter PFS than that observed when rituximab is combined with cladribine or fludarabine or polychemotherapy (Arcaini et al. 2004; Thieblemont et al. 2003; Chacon et al. 2002; Kalpadakis et al. 2007; Lefrere et al. 2000; Tsimberidou et al. 2006; Bennett et al. 2005; Cervetti et al. 2004). Recently, bendamustine has emerged as a highly effective drug for NHL, including marginal zone lymphomas (Cheson et al. 2010). A European trial for the evaluation of combined rituximab and bendamustine for symptomatic SMZL patients has been recently opened (EudraCT number 2011-000880-28). For clinical trials 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 (Matutes et al. 2008).

13.5 Nodal Marginal Zone Lymphoma

13.5.1 Clinical Presentation

Given the rarity of this disease and the absence of clinical prospective trial, description of clinical features of NMZL is based on few reports with relatively small numbers of patients (Berger et al. 2000; Traverse-Glehen et al. 2006; Arcaini et al. 2004, 2007b; Armitage and Weisenburger 1998; Camacho et al. 2003; Nathwani et al. 1999a; Oh et al. 2006; Kojima et al. 2007). The median age is 50–64 years, and the sex ratio differs from one series to the next. The vast majority of patients present with disseminated peripheral, abdominal, and thoracic nodal involvement, with a good performance status and no B symptoms. Bone marrow involvement is usually less prominent than in SMZL observed in 19–62 % of the patients. Peripheral blood involvement is rare and cytopenias are rare. A serum M-component is unfrequently detected (10 % of the patients). Cryoglobulin may be present when associated with HCV infection (Arcaini et al. 2007a). HCV seroprevalence was reported in 24 % of patients

in a series from Italy, in 20 % of patients from Spain, and in 5 % from Korea. In contrast with the other MZL entities, there is no history of autoimmune disease in most patients with NMZL.

13.5.2 Pathological Features

13.5.2.1 Morphology

The morphologic features of NMZL are very heterogeneous in terms of both architecture and cytology (Traverse-Glehen et al. 2012). Different patterns of lymph node infiltration have been reported, including marginal zone-like/perifollicular, "inverse follicular," perisinusoidal, follicular via colonization of reactive follicles, and diffuse (Arcaini et al. 2009a). A combination of different patterns in a single case is a common finding. Unlike in MALT lymphoma and splenic MZL, the neoplastic population often contains a relatively high number (more than 20 %) of large blastic B cells, and the mitotic index is frequent elevated as well.

13.5.2.2 Immunophenotype

NMZL cells express a similar pattern of antigens as SMZL. They are B cells expressing CD19+, CD20+, and CD79a+. Bcl-2 is positive in most of the cases. They usually express sIgM+−D/G+, cIg+−, and PAX5+. Negativity of CD5, CD10, CD23, and cyclin D1 is typical (Traverse-Glehen et al. 2012). The plasmacytic differentiation is usually associated with the expression of CD38, CD138, and MUM1(Camacho et al. 2003).

13.5.2.3 Cytogenetic and Molecular Features

Recurrent clonal abnormalities found in the other types of MZLs have been described (Rinaldi et al. 2011) and may help in contributing to the diagnosis by ruling out other small B-cell lymphomas. This include trisomy 3, trisomy 18, trisomy 7, trisomy 12, and del6q (Rinaldi et al. 2011). The most frequent abnormalities are gain of chromosome 3 (affecting *FOXP1* and *NFKBIZ*, and *BLC6*), occurring in 24 % of the cases, and 18q23 (affecting *NFATC1*). Deletion of 7q, which is frequently observed in SMZL, is not present in NMZL (Rinaldi et al. 2011).

Inactivation of the A20 genes (localized on 6q23) by either somatic mutation or deletion has been described in 33 % ($n=3$) of the 9 analyzed cases. This represents a common genetic aberration across all MZL subtypes that may contribute to lymphomagenesis via induction of constitutive nuclear factor kappa B (Novak et al. 2009).

The majority of NMZL cases (≥75 %) show somatic mutations of IGHV genes and a biased usage of IGHV4-34, or IGHV1-69 in case associated with HCV infection, evidence of antigen selection in most cases, but without ongoing mutations (Traverse-Glehen et al. 2005; Tierens et al. 1998; Conconi et al. 2001). VH1-69-encoded antibodies have been shown to be specific for the viral antigen E2. No difference in outcome between patients with mutations of IGHV genes and those without mutations has been described.

13.5.2.4 Genome-Wide Analysis

A comparative expression-profiling study has shown a set of markers to be differently expressed in NMZL compared to follicular lymphoma. These include myeloid cell nuclear differentiation antigen (MNDA) (Kanellis et al. 2009), especially expression in the three subtypes of MZL, but rarely in follicular lymphoma.

A recent gene and miRNA expression-profiling analysis has confirmed the differences between the signatures of NMZL and follicular lymphoma (Arribas et al. 2012). New markers were proposed that can be used to differentiate between NMZL and follicular lymphoma, including CHIT1, TGFBi, TAC1, miR-221, and miR-223 as markers for NMZL and miR-494 as a marker for follicular lymphoma.

13.5.3 Prognostic Factors in NMZL

Outcome of patients with NMZL is heterogeneous (Table 13.3). The average 5-year OS of NMZL is approximately 60–70 %, with an estimated 5-year event-free survival of about 30 % (Armitage and Weisenburger 1998). Complete

response to first-line treatment is seen in 50–60 % of cases. Relapse at extranodal sites is rare and occurs predominantly in nodes.

Given the small numbers of series and the heterogeneity of treatment in this retrospective analysis, no specific prognostic factors have been reported for this entity. A poor performance status at diagnosis was the only clinical parameter that significantly influences outcome. IPI and FLIPI both discriminate patients with high and low risk. Biological characteristics of the tumor cells have been reported to be significantly associated with survival, such as loss of survivin, active caspase 3, and overexpression of cyclin E (Camacho et al. 2001) (Table 13.1). Lack of expression of both MUM1/IRF4 and expression of Ki67 in less than 5 % of the cells has been shown to be associated with a better prognosis (Petit et al. 2005).

13.5.4 Treatment of NMZL

The treatment of NMZL is not standardized. Patients with truly localized disease may be considered for localized radiation therapy (Nathwani et al. 1999a, b). Patients with low tumor burden may be managed with a "wait-and-see" strategy. In advanced stage disease, immunochemotherapy (rituximab+polychemotherapy with or without anthracycline) is a relevant option. Among new drugs, bortezomib has demonstrated activity in NMZL (O'Connor et al. 2005). Veltuzumab, a humanized anti-CD20 antibody, has been reported in few cases of NMZL (Morschhauser et al. 2009). In relapsed young patients, high-dose therapy and autologous transplant could be considered (Traverse-Glehen et al. 2006).

Conclusion

SMZL and NMZL are considered distinct entities among NHLs, with definite clinical and morphologic characteristics. Although these two entities are 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.

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Mucosal-Associated Lymphoid Tissue (MALT) Lymphoma

14

Caron A. Jacobson, Luca Arcaini, Ann S. LaCasce, Jan Delabie, and Scott Rodig

Contents

Pathology: Jan Delabie and Scott Rodig

C.A. Jacobson, MD • A.S. LaCasce, MD
Dana-Farber Cancer Institute, Boston, MA, USA
e-mail: ann_lacasce@dfci.harvard.edu

L. Arcaini, MD (✉)
Division of Hematology Oncology, Department of Molecular Medicine, Fondazione IRCCS Policlinico San Matteo, University of Pavia, Pavia, Italy
e-mail: luca.arcaini@unipv.it

J. Delabie, MD
Department of Pathology,
Oslo University Hospital, Oslo, Norway
e-mail: jandel@ous-hf.no

S. Rodig, MD
Department of Pathology, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA
e-mail: srodig@partners.org

14.1 Epidemiology

MALT lymphomas account for approximately 5–8 % of all non-Hodgkin lymphomas, but represent 50–70 % of all marginal zone lymphomas (1997; Armitage and Weisenburger 1998; Thieblemont and Coiffier 2006). It is the third most common subtype of non-Hodgkin lymphoma after diffuse large B-cell lymphoma (DLBCL) and follicular lymphoma. The median age at diagnosis is 60 years, with a nearly equal incidence in men and women. Two-thirds of patients present with stage I or II disease, and one-third of patients have more advanced disease at diagnosis. The disease is rarely associated with systemic B symptoms of fever, night sweats, or weight loss, or bone marrow involvement. The majority of patients have a low or intermediate international prognostic index (IPI). MALT lymphomas, like other indolent non-Hodgkin lymphomas, can transform into a more aggressive lymphoma, but this occurs rarely. The most common transformation is into an activated B-cell-like DLBCL (Connor and Ashton-Key 2007).

Nearly half of all MALT lymphomas involve the gastric mucosa, where over 60 % are associated with *H. pylori* infection (Parsonnet et al. 1994). The incidence of *H. pylori* infection is high: in the United States, it is estimated to be 30 % overall and affects nearly 50 % of patients older than 50 years (Megraud 1993). The patient characteristics of gastric MALT lymphoma are similar to that of all MALT lymphomas: the median age at diagnosis is 57 years, with men and women equally affected,

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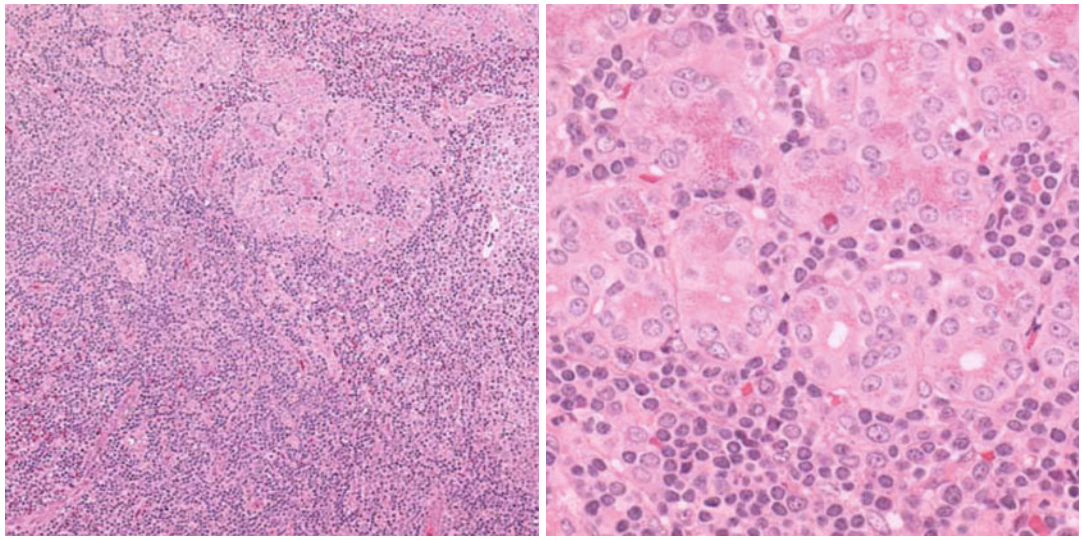

Fig. 14.1 Path slides to be chosen by pathologist

and bone marrow involvement, elevations in lactate dehydrogenase and/or β_2-microglobulin levels, or systemic B symptoms rarely occurring (Pinotti et al. 1997). While approximately 22 % of patient with MALT lymphoma will have bone marrow involvement at diagnosis, this number increases to 34 % during follow-up; the pattern of marrow involvement is predominantly nodular, and histologic examination is more sensitive than flow cytometry in these cases (Boveri et al. 2009). Marrow involvement is associated with other poor prognostic factors such as advanced stage, leukemic disease, B symptoms, more than one extranodal site of disease, splenomegaly, elevated β_2-microglobulin level, serum monoclonal component, and high IPI. Gastric MALT lymphomas are more likely to be localized at diagnosis than non-gastric MALT lymphomas, with early stage disease being reported in up to 88 % of patients in one series (Pinotti et al. 1997; Raderer et al. 2006). Approximately one-quarter of non-gastric MALT lymphoma patients are found to have concurrent involvement of the stomach at diagnosis (Pinotti et al. 1997; Raderer et al. 2006).

As mentioned previously, MALT lymphomas have been reported in any number of different tissues, but most commonly affect the gastrointestinal tract, ocular adnexa, thyroid gland, skin, lungs, and breasts. Nearly all lymphomas involving the ocular adnexa are MALT lymphomas, whereas lymphomas of the thyroid gland are predominantly DLBCL arising as a result of transformation from a low-grade component; pure MALT lymphomas of the thyroid gland are rare. Similarly, lymphomas of the breast, while making up a small proportion of primary breast malignancies, are typically aggressive lymphomas such as DLBCL or Burkitt lymphoma and MALT lymphoma in only 10–35 % of cases (Giardini et al. 1992; Hugh et al. 1990).

14.2 Pathology and Biology

MALT lymphomas are malignancies of B cells of the marginal B-cell compartment of mucosal-associated lymphoid tissue found outside the follicular mantle zone (Isaacson 1990). Histologically they are characterized by a monoclonal infiltrate of small- to medium-sized cells with abundant cytoplasm and irregular nuclear contours, although larger centroblast-like cells may be present (Fig. 14.1). An essential pathologic feature is the presence of lymphoepithelial lesions with invasion of mucosal glands and crypts by aggregates of lymphoma cells associated with destruction. They are surface Ig positive, as well as positive for B-cell markers (CD19, CD20, CD79a, and CD22) but are typically negative for CD5, CD10, CD23, and bcl-1 (cyclin D1) (Harris et al. 1994). Rarely,

MALT lymphomas are CD5 positive and this is associated with a worse prognosis; these lymphomas may have cytogenetic changes such as trisomy 3 and del7q (Batstone et al. 2003; Jaso et al. 2012; Wenzel et al. 2001). Distinguishing MALT lymphomas from benign reactive lymphoid infiltrates may be difficult; in this circumstance, light chain restriction by flow cytometry or immunoglobulin heavy chain gene rearrangement studies by polymerase chain reaction (PCR) can be helpful.

Other cytogenetic abnormalities that have been reported in MALT lymphomas include t(11;18), t(14;18), t(1;14), t(3;14), and trisomy 8. The translocation t(11;18) is the most common, occurring in 18–53 % of MALT lymphomas of any tissue and associated with low-grade histology (Auer et al. 1997; Ott et al. 1997). It results in the fusion of the apoptosis inhibitor 2 (*API2*) gene with the *MALT1* gene whose product increases nuclear factor-κB (NF-κB) transcriptional activation of a number of genes, including ones that promote proliferation and inhibit apoptosis (Dierlamm et al. 1999; Stoffel et al. 2004). The translocation is most common in gastric MALT lymphomas, but translocation t(14;18), which pairs the *MALT1* gene with the immunoglobulin heavy chain gene, has been described with increased frequency in MALT lymphomas of non-gastric sites (Dierlamm et al. 1999; Streubel et al. 2003). Translocation (1;14), on the other hand, is rarer overall but more frequent in gastric and pulmonary MALT lymphomas; it results in the overexpression of bcl-10 which also activates NF-κB and results in the transcription of genes that promote proliferation and inhibit apoptosis (Lucas et al. 2001; Willis et al. 1999). In the stomach, *H. pylori*-negative MALT lymphomas are enriched for the t(11;18) translocation; in addition *H. pylori*-negative gastric MALT lymphomas exhibit greater CpG island methylation which is presumed to result in the silencing of tumor suppressor genes and increased nuclear expression of bcl-10 and NF-κB (Kaneko et al. 2003; Liu et al. 2001; Ye et al. 2003; Yeh et al. 2005). The identification of a t(3;14) translocation in a MALT lymphoma of the thyroid prompted further analysis of additional thyroid, ocular adnexal, and cutaneous MALT lymphomas that had been known not to harbor a t(11;18), t(1;14), or t(14;18) translocation; the t(3;14) translocation was present in this cohort at an overall frequency of 10 % (Streubel et al. 2005). This translocation involves the *IGH* and *FOXP1* genes and is thought to activate NF-κB as well. This translocation was not found in additional series of over 200 patients, but strong nuclear FOX-P1 expression has been seen regardless of whether this translocation is present in approximately 30 % of patients with marginal zone lymphoma (Goatly et al. 2008; Haralambieva et al. 2006; Remstein et al. 2006). Single-nucleotide polymorphism (SNP) arrays of MALT, nodal marginal zone, and splenic marginal zone lymphomas revealed that MALT lymphomas are more often associated with gains at chromosomes 3p, 6p, and 18p and del(6q23) than the other subtypes of marginal zone lymphoma; del(7q31) and del(8p) were more frequent in splenic marginal zone lymphoma (Rinaldi et al. 2011). All marginal zone lymphoma subtypes were associated with gains in chromosomes 3q and 18q.

H. pylori-associated gastric MALT lymphomas illustrate the process of MALT lymphomagenesis. In the stomach, *H. pylori* infection results in the development of gastric mucosal lymphoid tissue resulting in chronic gastritis. Although only a small percentage of these patients will go on to develop MALT lymphoma, understanding who these patients are is under investigation. There is evidence that polymorphisms in certain genes involved in inflammation and immune reactions are associated with the risk of developing MALT lymphoma following *H. pylori* infection, including polymorphisms in the toll-like receptor 4 (*TLR4*) gene, as well as genes that play a role in antioxidant capacity including *IL1RN* and *GSTTI* (Hellmig et al. 2005; Rollinson et al. 2003). MALT lymphomas of the stomach are likely antigen driven in their early phases, and the dependency on antigenic stimulation may explain why these lymphomas are unlikely to disseminate to antigen-negative tissues for long periods of time. The malignant lymphocytes have undergone somatic hypermutation suggesting that they were selected for during a secondary immune response (Du et al. 1996; Qin et al. 1995). Furthermore, *H. pylori* strain-specific activated T cells stimulate proliferation of gastric MALT lymphoma cells in vitro (Hussell et al. 1996). Interestingly,

however, these clonal B cells do not produce antibodies that recognize *H. pylori* epitopes, suggesting that the antigen specificity of the immune reaction lies in the T-cell response leading to the ultimate clonal expansion of a B-cell population (Bende et al. 2005; Hussell et al. 1993). A substantial proportion of malignant B cells in MALT lymphomas express B-cell receptors with strong homology to rheumatoid factors, and this appears to be mutually exclusive with the presence of the t(11;18) translocation (Bende et al. 2005; Sakuma et al. 2007). This suggests that t(11;18)-negative MALT lymphomas are driven by stimulation of high-affinity B-cell receptors by antibody-antigen immune complexes and activated T cells, while t(11;18)-positive MALT lymphomas are not dependent on B-cell receptor signaling but instead are driven by constitutive activation of NF-κB. Interestingly and consistent with this model, t(11;18)-positive MALT lymphomas are enriched in patients without a history of autoimmune diseases (Wohrer et al. 2007).

14.3 Risk Factors and Disease Associations

Prolonged lymphoid proliferation, as is the case in settings of chronic inflammation and infection like *H. pylori* infections described above, is thought to result in the formation of a malignant B-cell clone that can develop into a MALT lymphoma. Inflammation is believed to result in the genesis of ectopic, organized lymphoid tissue in affected tissues as a result of the elaboration of certain cytokines and chemokines that recruit and B and T cells and facilitate the formation of germinal centers, germinal center reactions, and somatic hypermutation within activated B cells (Bende et al. 2009).

14.3.1 Chronic Inflammation

The most well-described conditions of chronic inflammation that have been associated with a risk of developing MALT lymphoma include systemic and organ-specific autoimmune diseases. In some series, up to 40 % of patients with MALT lymphoma have a history of autoimmune disease (Wohrer et al. 2007). Of these, Sjögren's syndrome and Hashimoto's thyroiditis have the strongest correlation with the development of MALT lymphomas of the salivary and thyroid glands, respectively (Ansell et al. 1999; Diss et al. 1995; Kassan et al. 1978; Pertovaara et al. 2001). Patients with Sjögren's syndrome, a condition associated with inflammation of the lacrimal and salivary glands leading to dry eyes and dry mouth, are 44 times more likely to develop non-Hodgkin lymphoma than the general population, and this risk appears to be increased further in patients with elevated β_2-microglobulin levels and evidence of reactive lymphadenopathy and/or splenomegaly (Kassan et al. 1978; Pertovaara et al. 2001). Biopsies of salivary and lacrimal glands from patients with Sjögren's syndrome are notable for CD4 and CD8 T cell and CD20 B-cell infiltrates with increased expression of T- and B-cell chemokines CXCL12, CXCL13, and CCL21; the degree of elevation of these chemokines directly correlates with the extent of lymphocytic infiltration and organization (Barone et al. 2005; Salomonsson et al. 2003). The result is a local inflammatory environment marked by high interferon-γ, interleukin-2, interleukin-10, and B-cell activating factor levels, all of which promote the transcription of genes involved in cell survival and proliferation and the ultimate development of a malignant B-cell clone (Fox et al. 1994; Groom et al. 2002). A similarly organized and prolific lymphocyte infiltrate with elaboration of a T-helper-1 cytokine profile can be seen in biopsy specimens from patients with Hashimoto's thyroiditis (Armengol et al. 2001; Del Prete et al. 1989). As a result, patients with Hashimoto's thyroiditis are 67 times more likely to develop lymphoma of the thyroid than the general population, though thyroid MALT is a very rare disease (Holm et al. 1985). Clonal B-cell populations have been found in patients with autoimmune thyroid disease without evidence of lymphoma, and this did not progress to overt lymphoma in three such patients with over a decade follow-up, however (Saxena et al. 2004). Other autoimmune diseases have been shown to be associated with an increased risk of lymphoma and specifically

marginal zone lymphoma, but to a lesser degree, including systemic lupus erythematosus which carries an eightfold increased risk of developing marginal zone lymphoma. Data regarding the risk of rheumatoid arthritis and lymphoma is conflicting, and a recent meta-analysis suggests that there is no significant association between the two diseases (Baecklund et al. 2006; Ekstrom Smedby et al. 2008; Smedby et al. 2006). Patients who develop MALT lymphomas in the context of autoimmune disease are more commonly female and of younger age, and the lymphomas are more often non-gastric and negative for the t(11;18) translocation or for trisomy 3; their prognosis appears to be similar to other MALT lymphoma patients (Wohrer et al. 2007).

14.3.2 Infection

As discussed previously, epidemiologic studies support a causal role of *H. pylori* infection in the development of MALT lymphomas, specifically of the stomach. In Northeastern Italy, where the incidence of *H. pylori* infections is high, there is an associated increased incidence of gastric lymphomas (Doglioni et al. 1992). Similarly, a case-controlled study documented an increased risk of gastric lymphoma in patients with *H. pylori* infection (Parsonnet et al. 1994). In addition, in some populations, the incidence of gastric MALT lymphoma and proportion of *H. pylori*-associated gastric MALT lymphoma is decreasing with increasing recognition and treatment of *H. pylori* infections (Luminari et al. 2010). As will be outlined in Sect. 14.5.1.1 of this chapter, the most compelling evidence for a causal association between *H. pylori* infection and gastric MALT lymphoma comes from the efficacy of *H. pylori* treatment in the treatment of these lymphomas (Bayerdorffer et al. 1995; Chen et al. 2005; Fischbach et al. 2004; Neubauer et al. 1997; Roggero et al. 1995; Stathis et al. 2009; Steinbach et al. 1999; Wotherspoon et al. 1993; Wundisch et al. 2005).

Infections with *C. psittaci, B. burgdorferi, C. jejuni*, and HCV have also been associated with an increased risk of MALT lymphoma in some populations. In a cohort of Italian patients with MALT lymphoma of the ocular adnexa, there was an increased frequency of *C. psittaci* in the tumor tissue and peripheral blood mononuclear cells than in healthy individuals (Ferreri et al. 2004). Seven of these patients were treated with doxycycline with eradication of the organism, and two of four evaluable patients had a documented tumor response. After this first report, many studies have been conducted showing high prevalence variations among different geographic regions (Chanudet et al. 2006; Mulder et al. 2006; Rosado et al. 2006). There are similar reports of an association between *B. burgdorferi* infection and cutaneous MALT lymphoma, *C. jejuni* infection and small bowel MALT lymphoma, and HCV infection and MALT lymphoma (Ascoli et al. 1998; Cerroni et al. 1997b; Lecuit et al. 2004; Luppi et al. 1996; Roggero et al. 2000; Zucca et al. 2000a, b). While an association between *B. burgdorferi* and primary cutaneous MALT lymphoma has been described in Europe, this has not been replicated in North American and Asian studies (Goodlad et al. 2000; Jelic and Filipovic-Ljeskovic 1999; Li et al. 2003; Wood et al. 2001). Immunoproliferative small intestinal disease is a lymphoma arising from small bowel MALT that is most commonly seen in the Middle and Far East, Mediterranean basin, and Africa (Lecuit et al. 2004). This geographic pattern of disease and the fact that reports of early stage disease response to antibiotics prompted a search for an infectious etiologic agent in its genesis. In seven patients who had responded to antimicrobial treatment, there was evidence of *C. jejuni* infection in four patients and *H. pylori* infection in no patients, thus establishing a potential link between *C. jejuni* infection and lymphomagenesis. The relative risk for patients with HCV infection to develop marginal zone lymphoma (particularly splenic and extranodal marginal zone lymphoma) is 2.5 times higher than the general population in a large intercontinental study (de Sanjose et al. 2008). Although this association is perhaps best described in patients with splenic marginal zone lymphoma, there is a high frequency of HCV infection in MALT lymphomas, and there have been reports of advanced MALT lymphomas of the salivary gland and intestines in patients with HCV infection that responded to treatment of the viral

infection (Arcaini et al. 2004, 2009; Kelaidi et al. 2004; Svoboda et al. 2005). There is an association between patients presenting with a subcutaneous subtype of MALT lymphoma and HCV infection as well, with some patients responding to antiviral therapy (Paulli et al. 2010). In patients with MALT lymphoma, the incidence of HCV infection has been reported to be 35–43 %, most frequently in lymphomas of the skin, salivary glands, and orbit (Arcaini et al. 2007, 2009). Interestingly, these lymphomas frequently harbor the classical t(14;18) translocation joining the genes for *BCL2* and the immunoglobulin heavy chain (Libra et al. 2004). The presence of HCV infection does not appear to influence the prognosis of the MALT lymphoma (Arcaini et al. 2007).

14.4 Clinical Presentation and Evaluation

14.4.1 Clinical Presentation

The clinical presentation of MALT lymphoma depends in large part on the site of disease. Gastric and intestinal MALT lymphomas may present with symptoms of dyspepsia and abdominal pain, sometimes with signs and symptoms of bowel obstruction but rarely with bleeding. These lymphomas are diagnosed on endoscopy with biopsies from multiple areas of endoscopically abnormal tissue as well as random sampling of macroscopically uninvolved mucosa. Lymphomas involving the small bowel may require a capsule video endoscopy for visualization. Involvement of the salivary and lacrimal glands, on the other hand, can result in Sjögren-like syndromes of dry eyes and mouth. MALT lymphomas involving the ocular adnexa typically present with painless conjunctival injection and photophobia, resembling allergic conjunctivitis. In the latter case, lesions are often bilateral and multifocal. Bronchus-associated lymphoid tissue (BALT) lymphoma involving the lungs and bronchi is a disease that most often affects older men (>60 years) (Fiche et al. 1995; Li et al. 1990). Unlike other MALT lymphomas, just over half of patients are symptomatic at diagnosis, with symptoms including cough, fever, and/or weight loss. This disease is often multifocal, spreading to other areas of the lungs and to other mucosal sites (Cordier et al. 1993). Other sites of disease often present with an obstructing mass. Some patients are diagnosed incidentally, either because of imaging studies or an exam of the eye or gastrointestinal track done for another reason or as part of an evaluation for a monoclonal gammopathy, which is present in approximately 25–35 % of MALT lymphoma patients; this feature is generally associated with plasmacytoid differentiation (Wohrer et al. 2004). B symptoms are rare in this disease (Armitage and Weisenburger 1998). Bone marrow involvement is present in a minority of patients, so cytopenias are rare, as is disease in the peripheral blood (Armitage and Weisenburger 1998).

14.4.2 Evaluation and Staging

The initial evaluation of a patient newly diagnosed with a MALT lymphoma should include a complete physical exam with attention to Waldeyer's ring, including an assessment of performance status with either the Karnofsky performance scale or Eastern Cooperative Oncology Group performance status assessment. Laboratory evaluation should include a complete blood count with differential, comprehensive metabolic panel and liver function tests, lactate dehydrogenase, and an assessment of complete hepatitis B virus markers in patients who may be treated with rituximab. HCV testing may also be performed given its association with MALT lymphoma; a human immunodeficiency virus test is advised. Additional laboratory studies to consider include a β_2-microglobulin, serum protein electrophoresis and immunofixation, and serum light chains. Staging is done with computed tomography (CT) scans of the chest, abdomen, and pelvis, as well as imaging of the neck, including the parotids and salivary glands, and orbits with CT or MRI. Although reports on the utility of positron emission tomography (PET) scans have been conflicting in this disease, with some demonstrating a lack of PET-avid signal in involved tissues and nodes and others suggesting

that PET scans result in the upstaging of the disease at diagnosis, current recommendations of an International Harmonization Project in 2007 recommend against the use of PET scanning in the initial evaluation of lymphomas that are not routinely PET avid, including marginal zone lymphoma, based on a lack of sufficient evidence supporting its use (Beal et al. 2005; Fueger et al. 2009; Hoffmann et al. 1999; Juweid et al. 2007). A bone marrow biopsy should be considered for patients with multifocal disease, and an evaluation of the gastric mucosa is reasonable for all patients with non-gastric MALT lymphoma given the documented high rate of gastric involvement in these patients (Raderer et al. 2006).

Gastric and intestinal MALT lymphomas are staged by the Lugano staging system for gastrointestinal lymphomas (Table 14.1) (Rohatiner et al. 1994). By this system, stage I disease is limited to the gastrointestinal track, whereas stage II disease involves extraintestinal lymph nodes within the abdomen, and stage IV disease involves supradiaphragmatic nodal or disseminated extranodal tissue; there is no stage III disease. Endoscopic ultrasound has allowed for an estimate of the depth of infiltration into the gastric wall, which correlates with the extent of lymph node involvement and prognosis (Steinbach et al. 1999). Although the majority are localized at diagnosis, these lymphomas can spread to other parts of the gastrointestinal tract and the splenic marginal zone, perhaps due to *H. pylori*-activated T-cell-induced overexpression of the mucosal-homing integrin $\alpha_4\beta_7$, whose ligand is expressed by these two tissues (Briskin et al. 1997; Du et al. 1997; Kraal et al. 1995). Staging for non-gastric MALT lymphoma is by the Ann Arbor staging system for lymphoma, which takes the extent of lymph node stations involvement as well as extranodal involvement into account when assigning a stage (Table 14.1) (Carbone et al. 1971; Lister et al. 1989).

Table 14.1 Staging MALT lymphoma

Ann Arbor staging for lymphoma	
Stage	*Description*
I	Involvement of a single lymph node region (I) or single extranodal site (IE)
II	Involvement of two or more lymph node regions or lymphatic structures on the same side of the diaphragm alone (II) or with involvement of limited, contiguous, extralymphatic organ or tissue (IIE)
III	Involvement of lymph node regions on both sides of the diaphragm (III), which may include the spleen (IIIS), or limited, contiguous, extralymphatic organ or tissue (IIIE), or both (IIIES)
IV	Diffuse or disseminated foci of involvement of one or more extralymphatic organs or tissues, with or without associated lymphatic involvement
All stages are further subdivided according to the absence (A) or presence (B) of systemic B symptoms including fevers, night sweats, and/or weight loss (>10 % of body weight over 6 months prior to diagnosis)	

Lugano staging system for gastric lymphomas		
Stage	*Description*	
I		Tumor confined to the gastrointestinal tract
	I_1	Infiltration limited to mucosa with or with submucosa
	I_2	Infiltration of muscularis propria, subserosa, or serosa
II		Tumor extending into the abdomen from a primary gastrointestinal site
	II_1	Local nodal extension
	II_2	Distant nodal extension (para-aortic, para-caval, pelvic, inguinal)
	IIE	Penetration of serosa to involve adjacent organs or tissues
IV		Disseminated extranodal disease or supradiaphragmatic involvement

14.4.3 Prognosis

Unlike for other non-Hodgkin lymphomas, there exists no specific prognostic scoring system for marginal zone or MALT lymphomas. The Follicular Lymphoma International Prognostic Index (FLIPI) was developed as a prognostic tool in follicular lymphoma and is often applied to other indolent lymphomas (Buske et al. 2006; Solal-Celigny et al. 2004). This index incorporates age (>60 years), Ann Arbor stage (III–IV), hemoglobin level (<12 g/dL), lactate dehydrogenase serum level (> upper limit of normal), and number of involved nodal areas (>4) into a 5-point scoring scale that corresponds to low (0–1 points), intermediate (2 points), and high (3 or more points) risk. A revised FLIPI has been

developed, the FLIPI2, based on the results of a prospective study of almost 1,000 patients with newly diagnosed follicular lymphoma who underwent therapy that incorporates age (>60 years), bone marrow involvement, hemoglobin level (<12 g/dL), longest diameter of the largest involved lymph node (>6 cm), and a serum β_2-microglobulin level (> upper limit of normal) into a 5-point scoring scale corresponding to low- (0 points), intermediate- (1–2 points), and high- (3 or more points) risk disease (Federico et al. 2009).

MALT lymphomas overall, however, have a good prognosis with a 5-year overall and failure-free survival of 81 % and 65 %, respectively (Nathwani et al. 1999). Patients with stage III or IV disease assessed before the era of rituximab and treated with cyclophosphamide, adriamycin, vincristine, and prednisone (CHOP) chemotherapy had a median overall survival of 5 years with median failure-free survival of 3 years (Fisher et al. 1995). In one retrospective series of a heterogeneous cohort of patients treated for MALT lymphoma, relapse rates approached 40 % at 4-year follow-up (Raderer et al. 2005). Non-gastric lymphomas had a higher rate of relapse than their gastric counterparts (48 vs. 22 %). In these patients, a high IPI and lymph node involvement has been associated with worse outcomes, whereas multiple mucosal sites were not (Thieblemont et al. 1997; Zucca et al. 2000a, b). Other series have shown that poor prognostic features in non-gastric MALT lymphomas include multiple MALT lymphoma sites, advanced stage disease, bone marrow and nodal involvement, and MALT lymphomas outside the skin and ocular adnexa (Arcaini et al. 2006). Primary cutaneous marginal zone lymphomas are particularly indolent and unlikely to disseminate with 5-year survival rates of 98–100 % (Hoefnagel et al. 2005). The presence of systemic symptoms in BALT lymphomas has been associated with a poorer prognosis (Cordier et al. 1993).

In gastric MALT lymphomas, the presence of a t(11;18) translocation is associated with an increased risk of disseminated disease, whereas trisomy 18 is associated with a risk of advanced stage disease in extragastric MALT lymphoma (Raderer et al. 2006). However, whereas t(11;18)-positive MALT lymphomas rarely are associated with additional clonal aberrations, a majority of t(11;18)-negative MALT lymphomas have allelic imbalances that are also present in gastric DLBCL (Starostik et al. 2000; Starostik et al. 2002). Thus, t(11;18)-positive lymphomas appear to be genetically stable and therefore at lower risk for transformation into a more aggressive DLBCL. Instead, certain genetic alterations appear to increase the risk of histologic transformation into an aggressive lymphoma including loss or deletion of *TP53*, hypermethylation or deletion of *CDKN2A*, and other chromosomal gains or losses (Du et al. 1995; Martinez-Delgado et al. 1997; Neumeister et al. 1997). Aberrant expression of CD5 detected by flow cytometry or immunohistochemistry is likewise associated with a more aggressive phenotype, as is high-grade histology (de Jong et al. 1997, 2000; Wenzel et al. 2001). A grading system for MALT lymphoma has been devised that assigns a letter grade (A to D) based on the number of blast or blast clusters seen histologically, ranging from <5 % blasts in clusters of up to ten cells to pure DLBCL without a low-grade component (de Jong et al. 1997). Gastric lymphomas that do not respond to *H. pylori* eradication are enriched for higher-grade histology, although many patients with high-grade histology do respond to antibiotic therapy (Bayerdorffer et al. 1995; Chen et al. 2001). In one series of 16 patients with stage I–II *H. pylori*-positive DLBCL of the stomach, half achieved a complete remission with antibiotic therapy alone, and another 13 % had a partial remission convert to a complete remission after the addition of rituximab monotherapy (Govi et al. 2011b). After 53-month follow-up, nine of the ten complete responders were still in remission. Grade similarly correlated with prognosis in a small number of patients with MALT lymphoma of the thyroid gland treated in the pre-rituximab era; all patients with low-grade lesions were alive at a median follow-up of 26 months, whereas patients with high-grade lesions appeared to do worse than patients with pure DLBCL of the thyroid gland with a low 5-year overall survival rate of 25 % owing to a higher rate of more advanced stage disease at diagnosis (Skacel et al. 2000).

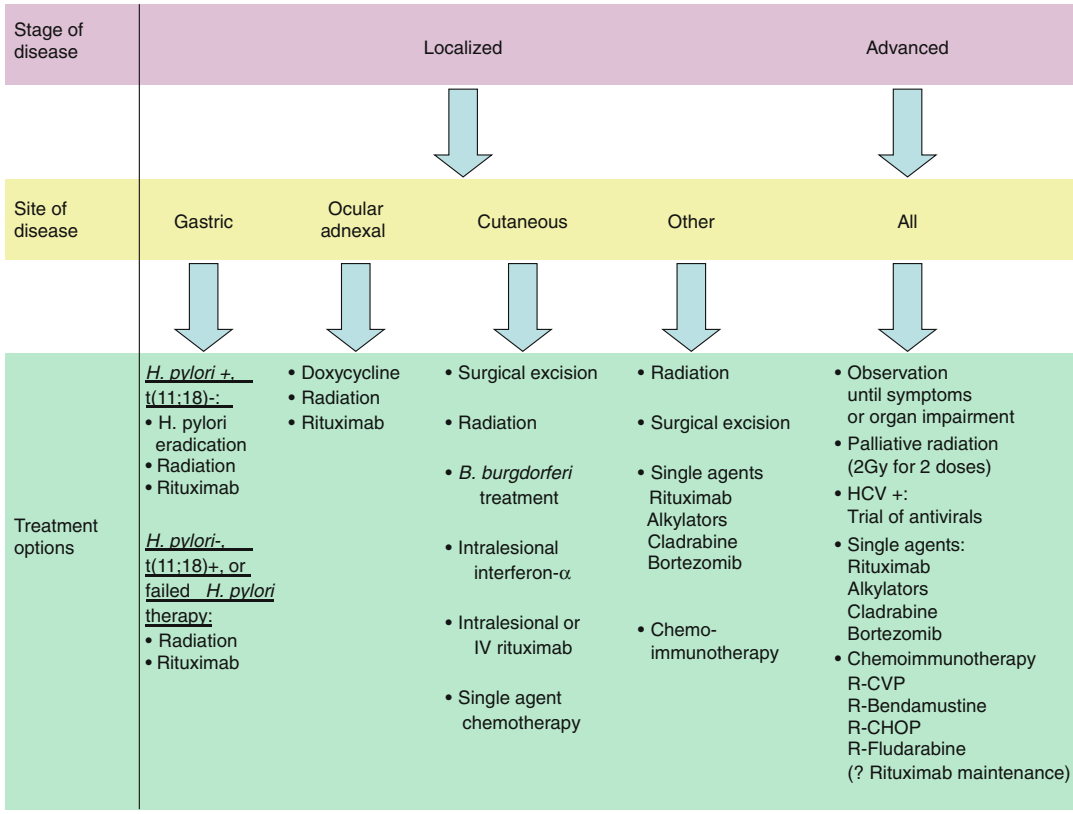

Fig. 14.2 Treatment algorithm for MALT lymphoma (Treatment options for localized and advanced stage MALT lymphoma. In general, the order of therapies in each category parallels the order preference for treatment in clinical practice. Abbreviations: *R-CVP* rituximab; cyclophosphamide; vincristine; prednisone, *R-CHOP* rituximab; cyclophosphamide; doxorubicin; vincristine; prednisone, *HCV* Hepatitis C virus)

14.5 Management

Management of MALT lymphoma depends both on stage and site of disease. As an indolent lymphoma with a long overall survival, close observation at diagnosis until the development of signs, symptoms, or organ function impairment as a result of the disease is appropriate for patients with more advanced stage disease. An exception is patients with advanced stage MALT lymphoma and concomitant HCV infection; a trial of anti-HCV antiviral therapy in these patients may result in regression of their lymphoma. For patients with early stage and localized disease, however, treatment with local therapies such as radiation and at times surgery, or treatment with antibiotics for *H. pylori*-positive gastric MALT lymphoma, has been associated with high response rates and durable responses, some of which may represent cures. Treatment of symptomatic or organ impairing relapsed, refractory, or advanced stage disease is similar to approaches used in follicular lymphoma with chemotherapy, immunotherapy, or chemoimmunotherapy. The general treatment strategies as well as the treatment strategies employed for specific situations will be outlined in detail in this section (Fig. 14.2).

14.5.1 Early Stage Gastric MALT Lymphoma

Early stage or localized MALT lymphoma of the stomach is an indolent disease that is often associated with *H. pylori* infection. Eradication of

H. pylori is effective treatment for many patients with good long-term disease control and overall survival and is recommended for most patients with *H. pylori*-positive gastric MALT lymphomas that do not harbor a t(11;18) translocation (Bayerdorffer et al. 1995; Chen et al. 2005; Fischbach et al. 2004; Neubauer et al. 1997; Roggero et al. 1995; Stathis et al. 2009; Steinbach et al. 1999; Wotherspoon et al. 1993; Wundisch et al. 2005). In patients with *H. pylori*-negative lymphomas, MALT lymphomas with a t(11;18) translocation, or lymphomas that fail to respond to *H. pylori* therapy, radiation therapy is the preferred treatment modality (Hitchcock et al. 2002; Schechter et al. 1998; Tomita et al. 2009; Tsang et al. 2001, 2003). Chemotherapy, immunotherapy, or chemoimmunotherapy is active in this disease but is generally reserved for patients with relapsed or refractory disease to antibiotic or radiation therapy or patients with more advanced stage or aggressive disease (Conconi et al. 2003, 2011; Hammel et al. 1995; Jager et al. 2002; Martinelli et al. 2005; Nakamura et al. 2005; Raderer et al. 2003).

14.5.1.1 Eradication of *H. pylori*

Based on the epidemiologic and preclinical data supporting a causal role of *H. pylori* infection in the pathogenesis of gastrointestinal MALT lymphoma, the effect of *H. pylori* therapies on these lymphomas was investigated. The first report of six patients treated as such was published in 1993: all six patients had complete eradication of their *H. pylori*, and all but one had complete regression of their lymphoma (Wotherspoon et al. 1993). A number of additional patient series have since been reported, with complete response rates ranging from 50 to 83 % and 7-year freedom from relapse rates approaching 78 % (Bayerdorffer et al. 1995; Chen et al. 2005; Fischbach et al. 2004; Neubauer et al. 1997; Roggero et al. 1995; Stathis et al. 2009; Steinbach et al. 1999; Wundisch et al. 2005). Perhaps the only randomized trial in MALT lymphoma, the LY03 trial, randomized patients to adjuvant chlorambucil versus observation following *H. pylori* treatment for early stage gastric MALT lymphoma (Hancock et al. 2009). This trial demonstrated no benefit of adjuvant chlorambucil over observation with respect to relapse rate, progression-free survival, or overall survival.

Modern *H. pylori* treatment regimens include a combination of two or three antibiotics, often clarithromycin and either amoxicillin or metronidazole, with a proton pump inhibitor, with or without bismuth salicylate for a total of 10–14 days; approximately 20 % of patients will require a second course of therapy for complete eradication of the organism. The median time from treatment to the histologic complete regression of the lymphoma ranges from 6 to 36 months. Posttreatment evaluation has not been systematically studied, but general recommendations include a urea breath test at 4–8 weeks after treatment to confirm eradication of the organism and once eradicated, endoscopic biopsies every 1–3 months until a histologic complete response is documented. Endoscopic surveillance is then every 6 months for up to 2 years or as indicated by symptoms.

Approximately 20–30 % of patients will not respond to *H. pylori* therapies within 12–18 months of treatment. Depth of invasion in one study correlated inversely with the likelihood of response, with only 42 % of patients with lymphomas extending to the muscularis, subserosa, or perigastric lymph nodes achieving a complete response to *H. pylori* treatment (Steinbach et al. 1999). Other studies have also shown that lymph node involvement was associated with a decreased response rate (Ruskone-Fourmestraux et al. 2001). The t(11;18) translocation predicts for poor response to *H. pylori*-directed therapies as well (Alpen et al. 2000). In addition, approximately 10–20 % of patients who do achieve a complete response will relapse (Neubauer et al. 1997; Stathis et al. 2009). Many of these relapses occur in the context of persistently negative *H. pylori* studies, pointing towards the development of a self-sustaining antigen-independent lymphoma clone in these patients (Fischbach et al. 2004; Neubauer et al. 1997). Molecular evidence of B-cell clonality often persists following *H. pylori*-directed therapies, and while the clinical significance of this is unknown, it does not appear to correlate with clinical relapse with reasonable follow-up (Bertoni et al. 2002;

Fischbach et al. 2002; Montalban et al. 2005; Thiede et al. 2001). There is evidence for clonal instability in this setting, with ongoing somatic hypermutation and antigen selection evidenced by immunoglobulin heavy chain sequencing (Thiede et al. 1998). The persistence of molecular evidence of disease does support the notion that eradication of *H. pylori* results in suppression but not elimination of the lymphoma clone. Longer follow-up, then, is necessary to determine if antimicrobial-directed therapy is a curative option in this disease.

14.5.1.2 Radiation Therapy

In the early 1990s, gastrectomy and surgical resection were the preferred treatment modality for patients with early stage gastric MALT lymphoma (Bozzetti et al. 1993). The issue with partial gastrectomy alone was mostly a high rate of relapse given the multifocal nature of this disease, and total gastrectomy was associated with a high degree of morbidity (Montalban et al. 1995). With the efficacy and tolerability of less invasive treatments, like *H. pylori* therapies and radiation, surgery plays a limited, if any, role in the treatment of MALT lymphoma. Radiation therapy, then, is the treatment of choice for patients with *H. pylori*-negative gastric MALT lymphoma, gastric MALT lymphomas with a t(11;18) translocation, or gastric MALT lymphomas that have failed an antimicrobial approach.

The first series of 17 patients treated with gastric MALT lymphoma treated with radiation therapy alone at a dose of 30 Gy over 4 weeks resulted in a complete remission rate of 100 % and 2-year event-free survival of 100 %, even in patients who had evidence of perigastric lymph node involvement (Schechter et al. 1998). The use of radiation for the treatment of early stage MALT lymphoma in series of patients involving multiple tissue types demonstrates excellent local control with local relapses observed in 0–5 % of patients and good long-term disease control with 5-year progression-free and overall survivals of 75–82 % and 93–97 %, respectively (Hitchcock et al. 2002; Tomita et al. 2009; Tsang et al. 2001, 2003). Radiation therapy to the stomach is well tolerated, and the risk of secondary malignancies is low.

14.5.1.3 Chemotherapy and Immunotherapy

The use of chemotherapy and/or immunotherapy in gastric MALT lymphoma is typically limited to patients who fail antimicrobial-directed therapies or for patients with locally advanced or advanced stage disease. For early and more advanced stage disease, agents that have been used and reported include single-agent therapy with alkylating agents such as chlorambucil or cyclophosphamide; purine analogues such as cladribine, bortezomib, and rituximab; and occasionally multi-agent anthracycline-based chemotherapy for younger patients with more aggressive disease.

The use of single-agent, continuous, low-dose oral chlorambucil or cyclophosphamide in 24 patients with early or advanced stage disease yielded complete response rates of 75 % and a relapse rate of 21 % during the 8-year follow-up (Hammel et al. 1995). Similar to resistance to *H. pylori* therapies, the t(11;18) translocation was associated with alkylator resistance in this study. While these outcomes appear to be inferior to those observed following radiation or *H. pylori* eradication, the use of single-agent oral alkylating agents as second-line treatment after a failure of *H. pylori* treatment was equivalent to radiation therapy in one study of small patient numbers (Nakamura et al. 2005). As mentioned above, the LY03 trial was a randomized trial of observation versus adjuvant chlorambucil in patients with localized gastric MALT lymphoma treated with *H. pylori* therapies and showed no benefit with the addition of systemic therapy, even in patients with molecular evidence of disease following eradication therapy (Bertoni et al. 2002; Hancock et al. 2009).

The purine analogue cladribine has been investigated as first-line therapy for patients with stage I–IV gastric and non-gastric MALT lymphoma. All 19 patients with gastric MALT lymphoma treated achieved a complete remission, including those with early stage disease that had failed *H. pylori* eradication, but three of these patients relapsed within 32 months (Jager et al. 2002). Although all seven patients with non-gastric MALT, all of whom had stage II–IV disease, had a disease response, only 43 % of them

had a complete response. This data suggests that cladribine is active in MALT lymphoma that is either refractory to initial therapy or more advanced stage at presentation.

The introduction of the highly efficacious anti-CD20 antibody rituximab for the treatment of mature B-cell malignancies has made this an attractive therapy for gastric MALT lymphomas, especially those that are *H. pylori* negative or refractory to *H. pylori* eradication. The first report of nine patients with advanced MALT lymphoma treated with single-agent rituximab was disappointing; however, with responses seen in five patients, three of whom achieved a complete response (Raderer et al. 2003). A larger, prospective cohort of 35 patients with stage I–IV MALT lymphoma (15 gastric, 10 non-gastric) who were either chemotherapy naïve or who had progressed following chemotherapy was treated with single-agent rituximab; the overall response rate in this population was 73 % and was better for chemotherapy naïve patients than for previously treated patients (87 vs. 45 %) (Conconi et al. 2003). Duration of response was short, however, with 36 % of responders progressing at a median of 10.5 months. A series of 27 patients with gastric MALT lymphoma that was either relapsed/refractory to, or not otherwise eligible for, *H. pylori* eradication was treated with rituximab with slightly more promising results: the overall response rate was 77 % with a complete response rate of 46 %, and only two patients had relapsed at a median follow-up of 33 months (Martinelli et al. 2005). Interestingly, this and cladribine are the first two treatment modalities for which the presence of the t(11;18) translocation did not predict for lack of response or relapse (Martinelli et al. 2005; Streubel et al. 2004). Rituximab, then, is an attractive option for patients who have localized disease relapse following *H. pylori* eradication and/or radiation therapy, or for whom either of these treatment modalities are not options. Additionally, it is an attractive and effective option for advanced disease as will be outlined later in this section. Combination chemoimmunotherapy has been investigated, and the combination of rituximab and fludarabine for patients with systemically untreated gastric and non-gastric MALT lymphoma of any stage does appear to improve response rates and response duration with response rates of 85–100 % and 2–3-year progression-free survival of 80–100 % (Brown et al. 2009; Salar et al. 2009). This comes at the expense of significantly greater hematologic and infectious toxicity, however, that is prohibitive in these patients. There is an ongoing phase III three arm randomized trial comparing rituximab alone, chlorambucil alone, and rituximab and chlorambucil in combination in patients with any stage MALT lymphoma having no prior therapy or following antibiotic or radiation therapy.

Most recently the proteasome inhibitor bortezomib, which is known to inhibit the NF-κB pathway, has been studied in relapsed or refractory MALT lymphoma based on the understanding of the importance of NF-κB signaling in the pathogenesis of these diseases (Conconi et al. 2011). Thirty-two patients with both gastric and non-gastric, early stage and advanced stage, and relapsed and refractory MALT lymphoma received bortezomib monotherapy with an overall response rate of 48 %; an additional 36 % of patients had stable disease after a median follow-up of 24 months. Other options for the treatment of relapsed or refractory disease mirror chemoimmunotherapy regimens used in follicular lymphoma and include combination therapies with rituximab and bendamustine or cyclophosphamide, vincristine, and prednisone (R-CVP) or anthracycline-containing regimens like rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP) for younger patients with more aggressive disease. These multi-agent regimens will be discussed in more detail in Sect. 14.5.3 but are recommended for more aggressive and advanced stages of disease.

14.5.2 Early Stage Non-gastric MALT Lymphoma

Treatment of early stage non-gastric MALT lymphomas follows many of the same paradigms as for early stage gastric MALT lymphomas in Sect. 14.5.1, except that there is no association between a particular infectious organism and these lymphomas that is as strong or penetrant as the

association between *H. pylori* and gastric MALT lymphoma. MALT lymphomas outside the stomach are also positive for *H. pylori,* in up to 45 % of cases in one series, but *H. pylori* treatment only led to regression of one colonic tumor in 16 such patients (Grunberger et al. 2006). Although antibiotic approaches against *B. burgdorferi* for cutaneous lymphomas and *C. jejuni* for intestinal lymphomas have been investigated and successful in some cases, the treatment of choice for most localized, early stage disease is radiation therapy (Hitchcock et al. 2002; Isobe et al. 2007; Tomita et al. 2009; Tsang et al. 2001, 2003). The use of doxycycline for treatment of *C. psittaci*-associated MALT lymphoma of the ocular adnexa has been resulted in high response rates in certain patient populations and is a reasonable upfront treatment option, in addition to radiation therapy (Govi et al. 2011a). For many of these lymphomas, doses of 24–30 Gy are sufficient and associated with minimal toxicity. One prospective series of 37 patients with stage IE non-gastric MALT lymphoma treated with a median of 30.6 Gy of radiation reported a 92 % complete response rate, with a 3-year progression-free and overall survival of 92 % and 100 %, respectively (Isobe et al. 2007). Many of the relapses involved the contralateral paired organ with nearly 100 % local control (Tsang et al. 2001, 2003). Surgical resection is appropriate for tumors that are not amenable to radiation and has been reported in patients with lymphomas involving the salivary glands, thyroid, skin, breasts, lung, genitourinary tract, and dura (Ambrosetti et al. 2004; Cerroni et al. 1997a; Ferraro et al. 2000; Gogas et al. 2002; Kees et al. 2005; Kempton et al. 1997; Zinzani et al. 2003). Surgery may be performed before the diagnosis of MALT lymphoma is known, and if complete excision is achieved, these patients should be observed without further treatment; if margins remain positive though, adjuvant radiation should be administered when feasible. Improving on outcomes following radiation therapy with adjuvant chemotherapy has not been beneficial: the addition of adjuvant anthracycline-based chemotherapy following radiation for stage IE orbital MALT lymphomas did not improve outcomes, whereas such adjuvant chemotherapy in stage III–IV non-gastric MALT lymphomas improved only the complete response rate but not progression-free survival (Aviles et al. 2006; Oh et al. 2007). Chemotherapy and/or immunotherapy approaches as were outlined for early stage gastric MALT lymphomas have been studied in non-gastric MALT lymphomas as well and are appropriate for cases of relapsed or refractory disease to local therapies like surgery or radiation, multifocal disease that is not amenable to local therapies, or more aggressive or advanced stage disease (Conconi et al. 2003, 2011; Hammel et al. 1995; Jager et al. 2002; Raderer et al. 2003). Although perhaps less effective for non-gastric compared to gastric MALT lymphomas, rituximab is a reasonable first option for some of these patients. Single-agent alkylating agents like oral cyclophosphamide or chlorambucil or purine analogues like cladribine have activity in these diseases as well (Conconi et al. 2003, 2011; Hammel et al. 1995; Jager et al. 2002). The response rates and duration of responses seen with these single agents can be low, making combination chemoimmunotherapy with regimens like rituximab and bendamustine or rituximab and cyclophosphamide, vincristine, and prednisone more appropriate for some patients. For patients who have no signs or symptoms related to their disease and who have either relapsed following local therapies or are not candidates for local therapies, observation with close follow-up is the preferred option (Ardeshna et al. 2003). The remainder of this section will outline the approaches that have been investigated for MALT lymphomas of specific disease sites: ocular adnexa, thyroid, breast, skin, lung, dura, and genitourinary tract.

MALT lymphoma of the ocular adnexa is primarily treated with radiation therapy. When 50 patients with localized disease were treated with radiation therapy, the response rate was 92 % with 52 % of patients achieving a complete response, and the 5-year overall survival was 91 % (Uno et al. 2003). Radiation to this area is complicated by a slightly increased risk of premature cataract development, and at higher doses of radiation, a few patients developed remote retinopathy or retinal bleeding, some with decreased visual acuity. No severe, late lacrimal complications arose in this cohort of patients. In the event of lacrimal gland involvement, however, low-dose radiation to the lacrimal gland is effective with minimal

toxicity (Agulnik et al. 2001). Local injection of interferon-α has been studied in small groups of patients with high response rates and good long-term local disease control (Blasi et al. 2001). Given the association described by some groups between *C. psittaci* infection and MALT lymphoma in this area, antibiotic therapy with doxycycline has been studied (Ferreri et al. 2005; Govi et al. 2011a). In a pilot study, nine patients, over half of whom had relapsed or refractory disease, were treated with doxycycline for 3 weeks with a response rate of 44 % (Ferreri et al. 2005). A prospective international phase II trial has enrolled 54 patients, 34 of whom have a new diagnosis of stage IE MALT lymphoma of the ocular adnexa and 20 of whom have other malignant and non-malignant lesions of the eye (Govi et al. 2011a). *C. psittaci* was found in 86 % of the MALT lymphoma biopsies compared with 57 % of the other ocular lesions, and all of the *C. psittaci* infections were detectable by either conjunctival swabs or in peripheral blood mononuclear cells at presentation. Patients with MALT lymphoma received 3 weeks of doxycycline and were monitored for bacterial eradication using conjunctival swabs or analysis of peripheral blood mononuclear cells with a rate of bacterial eradication of 50 %. The response rate of single-agent doxycycline for MALT lymphoma of the ocular adnexa was 83 %, with two-thirds of patients having partial responses. The 2-year progression-free survival was 55 %. Both response rate and progression-free survival trended towards improvement in patients who achieved organism eradication.

For salivary gland tumors specifically, there is no definitive optimal therapy. Options include surgical resection, radiation, and chemoimmunotherapy, all of which were equivalent with respect to outcomes in 35 patients with stage I–IV MALT lymphoma of the salivary glands (5-year progression-free and overall survival 65 and 85 %, respectively) (Ambrosetti et al. 2004). Although an indolent disease in most cases that does quite well with local therapy, these patients have been included in the trials of single-agent chemotherapy and immunotherapy with rituximab outlined above in the section on early stage gastric MALT lymphomas, and these remain reasonable options for relapsed/refractory or more advanced disease (Conconi et al 2003, 2011; Jager et al 2002; Raderer et al 2003). A similar treatment approach is applicable to MALT lymphomas of the thyroid gland where surgery and/or radiation is preferred with systemic therapies reserved for more advanced disease (Gogas et al 2002). There has been one case report of a MALT lymphoma of the thyroid responding to *H. pylori-directed* therapy despite the fact that the tumor was not positive for *H. pylori* (Arima and Tsudo 2003). MALT lymphomas of the breast and skin are very indolent and can be treated with surgical excision, radiation, or observation (Cerroni et al 1997a; Mattia et al 1993).

Primary cutaneous marginal zone lymphoma is a distinct diagnosis that is made once; complete staging reveals no other sites of disease. As discussed previously, there have been variable associations between this disease and *B. burgdorferi* infection (Goodlad et al 2000; Jelic and Filipovic-Ljeskovic 1999; Li et al 2003; Wood et al 2001). There has been one case report of regression of a cutaneous lymphomatous lesion with treatment of *B. burgdorferi* (Cerroni et al. 1997b). The largest series of patients details the treatment and outcomes of 288 patients: 173 treated with radiation (complete response 99 %, relapse 46 %), 75 with surgical excision (complete response 99 %, relapse 43 %), 8 with intralesional interferon-α (complete response 100 %, relapse 25 %), 9 with intralesional rituximab (complete response 89 %, relapse 62 %), 3 with systemic rituximab (complete response 67 %, relapse 50 %), 14 with single-agent chlorambucil (complete response 64 %, relapse 33 %), 14 with antibiotics against *B. burgdorferi* (complete response 43 %, relapse 20 %), and 33 with combination chemotherapy (complete response 85 %, relapse 57 %) (Senff et al. 2008). The vast majority of relapses were in the skin alone.

As with cutaneous disease, treatment of bronchus-associated lymphoid tissue (BALT) lymphoma depends on the extent of disease. Localized disease is typically treated with surgical resection, low-dose radiation therapy, or sometimes chemoimmunotherapy, while chemoimmunotherapy is the treatment of choice

for multifocal disease (Ali et al. 2003; Fiche et al. 1995; Girinsky et al. 2012). Surgical resection for localized disease was examined a series of 48 patients with lymphoma of the lung, 35 of whom had BALT lymphoma (Ferraro et al. 2000). Complete resection was possible in 40 % of patients, and this treatment modality was associated with a 5- and 10-year overall survival of 68 % and 53 %, respectively; the use of adjuvant chemotherapy has not improved outcomes in these patients (Ahmed et al. 2004). A single-center experience of surgery or chemotherapy alone or a combined modality approach in 12 patients yielded a 6-year overall survival of 100 % (Zinzani et al. 2003). Outside of these series of patients, there have been case reports of patients responding to chemotherapy regimens including alkylating agents, anthracyclines, and rituximab (Ahmed et al. 2004; Chong et al. 2005; Kees et al. 2005).

MALT lymphoma of the dura is a rare entity that typically presents with focal neurologic findings and a mass on imaging consistent with a meningioma. There have been no reports of disease recurrence following surgical excision (Cohen et al. 2006). Local treatment with surgery and/or radiation of MALT lymphomas of the bladder and kidney has a similarly excellent prognosis (Kempton et al. 1997).

14.5.3 Advanced Stage MALT Lymphoma

For more advanced stage disease, or early stage disease that is not amenable to antibiotic or local therapies, that is asymptomatic and not causing organ dysfunction, observation is recommended based on studies that have demonstrated no improvement in survival with treatment at diagnosis compared to at the time of symptomatic or organ impairing disease progression (Ardeshna et al. 2003). Indications for treatment include systemic symptoms such as fevers, night sweats, fatigue, and/or weight loss, pain or obstructive symptoms from tumor growth, organ-specific symptoms based on the location of the tumor, and/or cytopenias. For localized symptoms in advanced stage disease, palliative radiation rather than systemic therapy is an option; 2Gy of radiation for 2 doses results in an objective response rate of 89 % lasting up to 3 years for some patients (Ganem et al. 1994). Patients with concomitant HCV infection may derive benefit from antiviral therapy of their HCV, and this is a reasonable approach in the asymptomatic patient (Kelaidi et al. 2004; Paulli et al. 2010; Svoboda et al. 2005). Patients with transformation to DLBCL should be treated similarly to patients with de novo DLBCL.

As has been outlined previously in Sects. 14.5.1 and 14.5.2 of this chapter, single-agent cyclophosphamide, chlorambucil, cladribine, rituximab, and bortezomib all have been shown to have activity in MALT lymphomas of all stages and of all disease sites and are reasonable treatment options for patients with less aggressive disease (Conconi et al. 2003, 2011; Hammel et al. 1995; Jager et al. 2002; Martinelli et al. 2005; Nakamura et al. 2005; Raderer et al. 2003; Streubel et al. 2004). For more aggressive disease or disease that does not respond adequately to single-agent approaches, combination chemotherapy regimens used in other indolent non-Hodgkin lymphomas, specifically follicular lymphoma, are often employed as many of the studies of these regimens included some marginal zone lymphoma patients. These include R-CVP, rituximab and bendamustine, and R-CHOP. While R-CHOP and R-CVP have not been compared directly in a randomized fashion, historical outcomes suggest that while R-CHOP results in a higher response rate and a longer duration of response, the two are equivalent in terms of overall survival (Czuczman et al. 2004; Hiddemann et al. 2005; Marcus et al. 2005, 2008). Most recently rituximab and bendamustine were compared to R-CHOP in a multicenter randomized controlled trial of previously untreated indolent lymphoma, predominantly follicular and indolent mantle cell lymphoma patients, and was found to be superior with respect to complete response rate, event-free and progression-free survival, and time to next treatment with no difference in overall survival (Rummel et al. 2009). In addition, rituximab and bendamustine were better tolerated. Based on

these results, the regimen of rituximab and bendamustine is increasingly being used in the upfront setting of advanced stage indolent lymphomas, including marginal zone lymphomas, which are not candidates for the single-agent therapies outlined above.

There has been interest in using maintenance rituximab following induction chemoimmunotherapy to prolong remissions and perhaps impact overall survival. The ECOG1496 study was a randomized trial comparing observation to maintenance rituximab following treatment with CVP and showed a significant improvement in magnitude of response and progression-free survival with a trend towards improved overall survival at 3-year follow-up (Hochster et al. 2009). Unfortunately, this trial included only ten patients with marginal zone lymphoma. Although the PRIMA trial did not include patients with marginal zone lymphoma, it randomized patients with predominantly untreated follicular lymphoma to rituximab maintenance versus observation following induction chemotherapy and revealed a progression-free survival benefit with rituximab maintenance (Salles et al. 2011). Additionally, the addition of maintenance rituximab after single-agent rituximab induction therapy for previously treated and untreated patients with follicular lymphoma who did not progress during induction on the SAKK 35/98 trial resulted in an improvement in event-free survival compared with observation (Martinelli et al. 2010). More recently, however, results of the RESORT trial were reported and showed no benefit of maintenance rituximab over observation in patients with previously untreated, low-bulk, follicular lymphoma, many of whom would have been otherwise observed (Kahl et al. 2011). From these studies, maintenance rituximab is a reasonable option for patients with follicular lymphoma, and perhaps other indolent non-Hodgkin lymphomas like MALT lymphoma, following induction chemotherapy with R-CVP, R-CHOP, and rituximab alone, though maintenance may not impact on overall survival. It should be stressed, however, that maintenance rituximab has not been studied in MALT lymphoma specifically.

For patients with refractory disease or multiple relapsed disease, radioimmunotherapy with drugs such as ibritumomab, high-dose chemotherapy with autologous stem cell transplantation (HDC-ASCT), and allogeneic stem cell transplantation remain options to achieve disease control. A series of 14 relapsed and/or refractory marginal zone lymphoma patients treated with HDC-ASCT was recently reported; these patients had a median failure-free survival of 9 years and a median overall survival of 10 years with relapses seen in only two patients (Li et al. 2011). Similarly, the use of reduced intensity conditioning allogeneic stem cell transplantation for patients with relapsed, advanced stage indolent non-Hodgkin lymphoma has resulted in 3-year event-free and overall survival of 55–75 % and 64–81 %, respectively (Armand et al. 2008; Shea et al. 2011). Finally, drugs that target different epitopes of the CD20 molecule, PI3 kinase, bcl-2, and Bruton's tyrosine kinase are being investigated in a variety of relapsed B-cell malignancies, including marginal zone lymphoma.

14.6 Summary and Conclusions

MALT lymphomas are rare indolent non-Hodgkin B-cell lymphomas that often arise in the setting of chronic inflammation and infection. They are by definition extranodal and can involve ectopic mucosal-associated lymphoid tissue of a variety of organs, most commonly the stomach. Although advanced stage disease can be managed expectantly with close observation, reserving treatment for the development of symptoms or organ impairment as a result of disease progression, the majority of patients have limited stage disease at diagnosis and are candidates for antibiotics in some cases such as gastric, ocular adnexal, and cutaneous MALT lymphoma or local therapy with surgery or radiation. These latter approaches have been associated with excellent long-term disease control and survival and are potentially curable in a proportion of patients. For more advanced or relapsed/refractory disease, approaches used in follicular lymphoma, which is a significantly more common disease, are employed. Newer drugs that target signaling pathways known to be important

in B-cell proliferation and survival, including but not limited to PI3 kinase, Bruton's tyrosine kinase, and bcl-2, are currently being investigated in these and other B-cell malignancies.

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Mantle Cell Lymphoma

15

Michael E. Williams, L. Kyle Brett, Martin Dreyling, German Ott, and Eric D. Hsi

Contents

Pathology: German Ott and Eric D. Hsi

M.E. Williams, MD, ScM (✉)
Division of Hematology/Oncology and Cancer Center, University of Virginia Health System, Jefferson Park Avenue, Charlottesville, VA 22908, USA
e-mail: mew4p@virginia.edu

L.K. Brett, MD
Division of Hematology/Oncology and Cancer Center, University of Virginia Health System, Charlottesville, VA, USA
e-mail: kyle.brett@virginia.edu

M. Dreyling, MD
Medizinische Klinik und Poliklinik III, Klinikum Großhadern, Universität München, Marchioninistr. 15, 81377 München, Germany
e-mail: martin.dreyling@med.uni-muenchen.de

G. Ott, MD
Department of Clinical Pathology, Robert-Bosch-Krankenhaus, Stuttgart, Germany
e-mail: german.ott@rbk.de

E.D. Hsi, MD
Department of Clinical Pathology, Cleveland Clinic, L11, 9500 Euclid Ave, Cleveland, OH 44195, USA
e-mail: hsie@ccf.org

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15.1 Introduction

Mantle cell lymphoma (MCL) is a unique subtype of non-Hodgkin lymphoma (NHL) characterized by the chromosomal translocation t(11;14)(q13;q32) and nuclear cyclin D1 overexpression (Swerdlow and Williams 2002; Williams et al. 1993a, 1995; Perez-Galan et al. 2011). Based on the recognition of characteristic morphologies, phenotype, and the t(11;14), the term "mantle cell lymphoma" was adopted in 1992 to reflect the apparent derivation from mantle zone B cells (Dreyling and Hiddemann 2009).

MCL comprises approximately 4–6 % of all non-Hodgkin's lymphomas, with a preponderance of older males relative to other lymphoma subtypes. The male–female ratio is 2–3:1 and median age at presentation is 65 years (Swerdlow and Williams 2002; Williams et al. 1993a, 1995; Perez-Galan et al. 2011). No specific etiologic factors have been identified for this disease. As with lymphoproliferative disorders in general, an increased risk of other lymphoid neoplasms is recognized among first-degree relatives of MCL patients, although MCL occurrence among multiple family members is quite rare.

Most patients present with advanced stage disease and may pursue either an indolent, steadily progressive, or an aggressive clinical course. No standard curative therapy exists aside from allogeneic transplantation. However, response durations and overall survival have recently been improved through the use of immunochemotherapy regimens with consolidative autologous stem cell transplantation in younger patients and maintenance rituximab in older patients. Knowledge of effective combination regimens, maintenance approaches, and novel targeted agents are also improving outcomes.

15.2 Clinical Presentation

Over 90 % of MCL patients present with stage III–IV disease (Romaguera et al. 2003). In some patients, disease is largely non-nodal and confined to the blood, bone marrow, and spleen; these individuals may experience a more indolent clinical course than those with predominantly nodal disease (Table 15.1). The gastrointestinal tract is the most common extranodal site of disease. Though the affinity of MCL for the GI tract is not yet fully understood, subclinical involvement of the gastric or colonic mucosa has been reported in most (Romaguera et al. 2003). Less frequently, MCL can involve the genitourinary, pulmonary, head and neck, or periorbital sites. Central nervous system involvement, either parenchymal or leptomeningeal, is unusual at presentation but subsequently may develop in association with disease progression and in the presence of the blastoid variant, elevated LDH, high Mantle Cell Lymphoma International Prognostic Index (MIPI) score, and poor performance status (Conconi et al. 2013; Cheah et al. 2013). As overall survival improves following more effective systemic therapies, the incidence of CNS relapse may increase over time. As a result, a role for CNS prophylaxis may emerge.

As is true for most cancers, the natural history and time course from initial transforming event to

Table 15.1 Prognostic markers in mantle cell lymphoma

	Better prognosis	Poorer prognosis
MIPI[a] score (Geisler et al. 2010)	Low	Intermediate or high
MIPI-b[b] score (Schaffel et al. 2010)	Low	Intermediate or high
Posttreatment MRD[c] (Pott et al. 2010a)	Negative	Positive
Ki-67 index (Determann et al. 2008)	<10 %	>30 %
Proliferation signature (Rosenwald et al. 2003)	Low	High
Sox11 (Wang et al. 2008; Ondrejka et al. 2011b; Vegliante et al. 2013)	Negative	Positive
p53	Wild type (WT)	Mutated

[a]Mantle cell lymphoma international prognostic index
[b]Mantle cell lymphoma international prognostic index-biologic
[c]Minimal residual disease (MRD)

clinical presentation is unknown. A series of MCL patients have been shown retrospectively to have harbored occult MCL as much as 7–15 years prior to a clinical diagnosis of MCL (Racke et al. 2010). This and other studies of incidentally identified in situ MCL support the notion that MCL has a long preclinical latency (Adam et al. 2012).

15.3 Pathology

15.3.1 Definition and Clinical Presentation

Mantle cell lymphoma represents approximately 6 % of non-Hodgkin lymphomas (Anonymous 1997). It presents in adults with a median age of 63 years and a male predominance of approximately 2.3:1 (Argatoff et al. 1997). Patients usually present with clinical stage IV disease (70 % with stage IV), with generalized adenopathy and bone marrow involvement. Hepatosplenomegaly is also frequent (30–60 %). B symptoms may occur in up to 50 % of cases (Camp et al. 2011). Blood involvement is seen in the majority of patients and occurs in up to 75 % of patients at diagnosis by conventional morphology. When sensitive flow cytometric methods are used, this number increases to over 90 % (Ferrer et al. 2007).

In addition to this common presentation, some clinical variants are also recognized. Gastrointestinal involvement with presentation as multiple lymphoid polyps in the small and large intestine is termed multiple lymphomatous polyposis (MLP). These patients often present with abdominal pain (O'Briain et al. 1989). It should be noted that other lymphomas such as low-grade follicular lymphoma may have similar presentation. Thus, pathologic confirmation of the diagnosis is required when a clinical picture of MLP is encountered.

A non-nodal, leukemic variant has been described that appears to have an indolent clinical course and should be distinguished from the typical mantle cell lymphoma with blood involvement (Royo et al. 2012; Ondrejka et al. 2011a; Orchard et al. 2003). These patients generally present with a variable degree of lymphocytosis with or without splenomegaly and appear to have an indolent course, some not requiring therapy for a prolonged period of time. The clinical, pathologic, and genetic features of this variant are the subject of ongoing investigation.

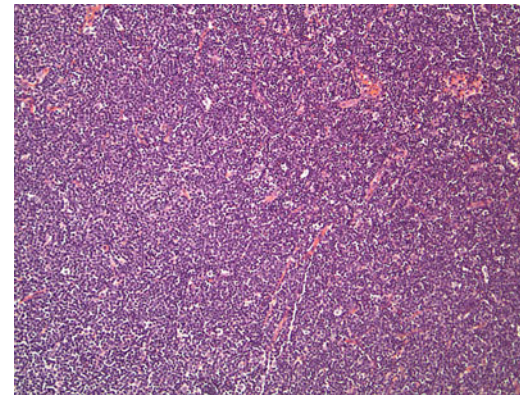

Fig. 15.1 Mantle cell lymphoma. A diffuse infiltrate of monotonous small lymphocytes (hematoxylin and eosin, 100x)

15.3.2 Histopathology

Lymph node involvement may manifest in diffuse, nodular, or mantle zone patterns. Mixtures of patterns can be seen. In a large series, 80.5 % of cases showed a diffuse growth pattern, 18.1 % had a nodular pattern, and a prominent mantle zone pattern was seen in only 1.4 % of cases (Tiemann et al. 2005). The infiltrate is typically very monotonous, without admixed large, transformed cells or paraimmunoblasts. However, scattered individual epithelioid histiocytes, which do not form granulomas, are characteristically distributed throughout (Figs. 15.1 and 15.2) the infiltrate. Hyalinized vessels are also sometimes seen. Follicular dendritic cell (FDC) networks may present but may be disrupted and poorly formed. In some examples, well-formed residual FDC networks are seen in the center of lymphomatous nodules or in the mantle zone pattern. These are best seen with immunostains for FDCs such as CD21 or CD23 (Tiemann et al. 2005; Schrader et al. 2006; Weisenburger et al. 1987;

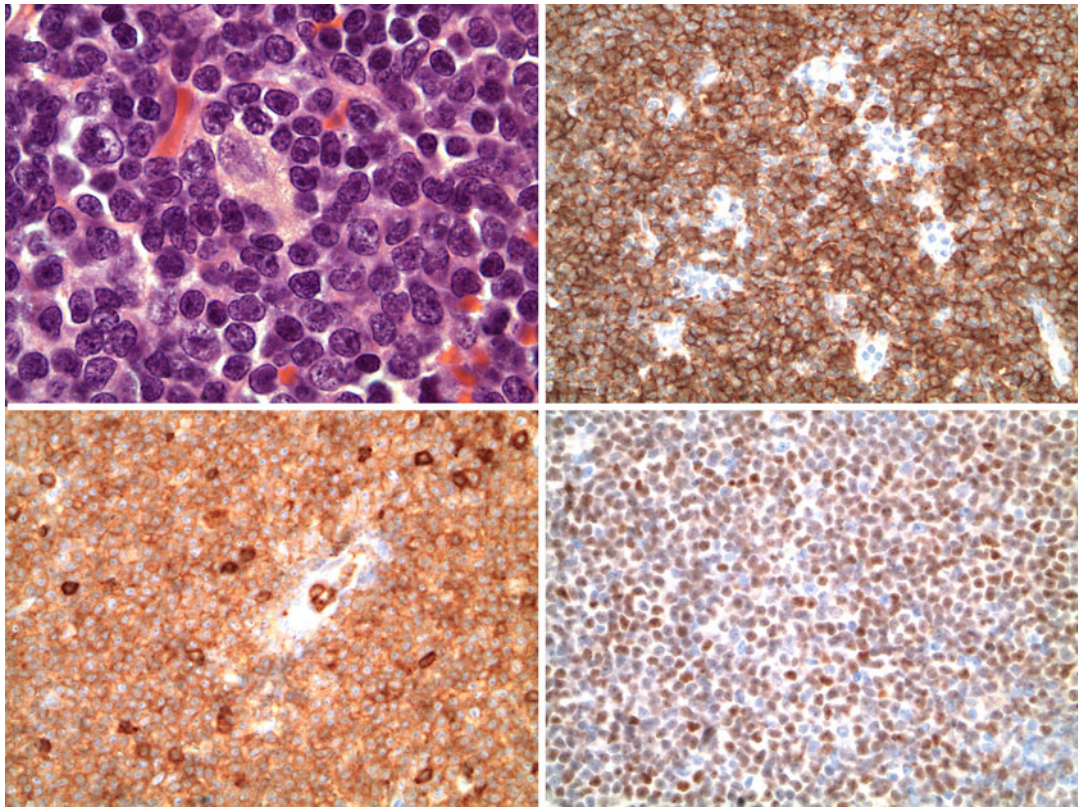

Fig. 15.2 Mantle cell lymphoma. The *upper left* panel shows the cytologic features of the common type of mantle cell lymphoma. The cells are small with mature, condensed chromatin with slight nuclear irregularities. An epithelioid histiocyte is present in the middle of the field (hematoxylin and eosin, 1000x). Immunostains show the cells express CD20 (*upper right*, 400x), CD5 (*lower left*, 400x), and cyclin D1 (*lower right*, 400x)

Jaffe et al. 1987; Lardelli et al. 1990). In contrast to other mature B-cell lymphomas of small lymphocytes in which mitotic figures are quite rare, mitotic figures are often readily seen in mantle cell lymphoma. While the pattern and cytologic features tend to be stable in an individual patient, progression from mantle zone to nodular and nodular to diffuse patterns may be seen.

Several cytologic variants are recognized. In the common classical type, the cells are small with mature, condensed chromatin and slightly irregular nuclear borders, resembling centrocytic cells. A small cell variant also exists (<5 % of cases) in which the cells are round and more closely resemble small lymphocytic lymphoma; however, paraimmunoblasts or prolymphocytes are not present (Tiemann et al. 2005). The blastoid variant has been divided into the pleomorphic type that resembles diffuse large B-cell lymphoma and a lymphoblastoid type that resembles lymphoblastic lymphoma (Fig. 15.3). In these types, mitotic figures are numerous (over 50/10 high-power fields) compared to the common type (usually <20/10 high-power fields) (Ott et al. 1997). The blastoid variants are typically seen at diagnosis rather than progression or "transformation" events, but such events have been reported and appear clonally related (Laszlo and Matolcsy 1999). Rare cases of mantle cell lymphoma may have plasmacytic differentiation that appears to be clonally related or a monocytoid B-cell appearance (Visco et al. 2013; Swerdlow et al. 1996).

Recently, a so-called "in situ" form of mantle cell lymphoma has been recognized in which the lymphoma cells are confined to non-expanded mantle zones with overall intact and non-altered lymph node architecture (Nodit et al. 2003). It is

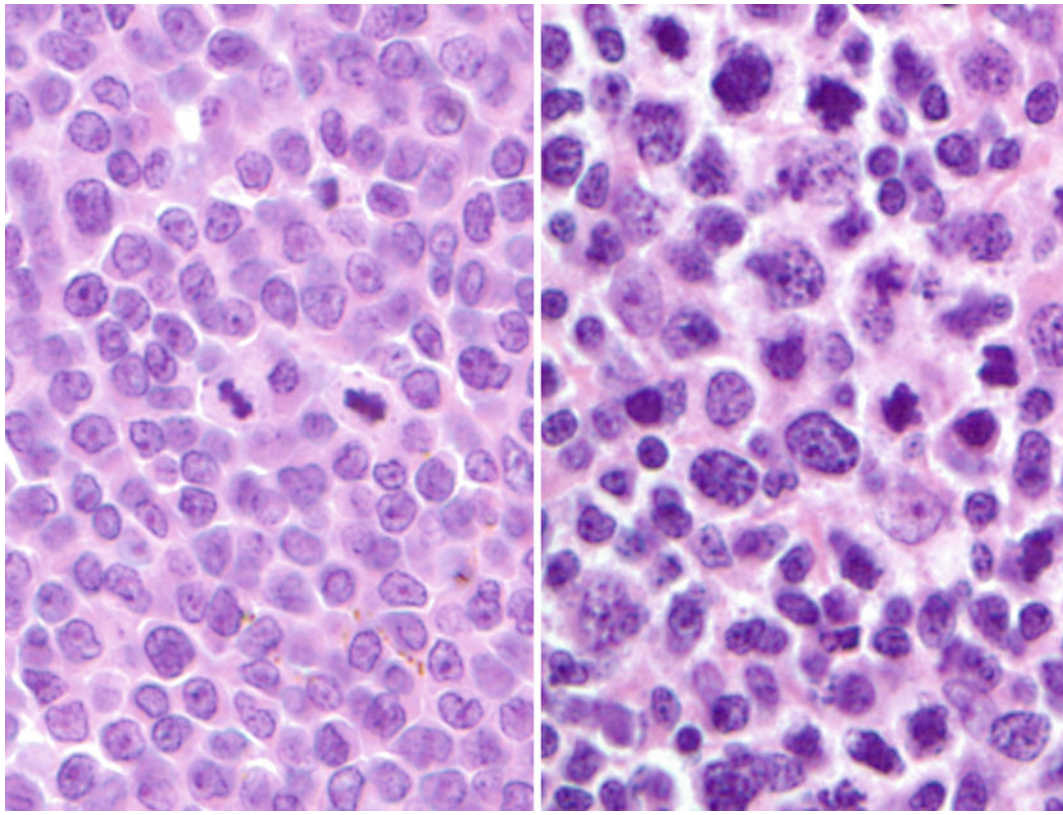

Fig. 15.3 Blastoid variant of mantle cell lymphoma. The *left panel* shows the lymphoblastoid type and the *right panel* illustrates the pleomorphic variant

often an incidental finding or found when prior biopsy tissue (often years prior) is examined in a patient subsequently diagnosed with mantle cell lymphoma. These cases deserve further study but many appear indolent and may not progress to overt lymphoma (Carbone and Santoro 2011; Carvajal-Cuenca et al. 2012). A leukemic counterpart may also exist in which circulating mantle cell lymphoma cells can be found in the absence of lymphadenopathy, with our without splenomegaly. Such cases show sparse interstitial bone marrow infiltration, simple karyotype, and propensity for kappa light chain expression by flow cytometry (Ondrejka et al. 2011a).

Bone marrow involvement can be seen histopathologically in over 90 % of cases and can take the form of nodular, interstitial, paratrabecular, and diffuse infiltrates, in order of decreasing frequency (Cohen et al. 1998). Blood involvement, as noted above, is quite common, and blastoid/pleomorphic variants also can be seen in blood as well. Cases previously diagnosed as B-prolymphocytic leukemia with t(11;14) are now considered leukemic variants of mantle cell leukemia (Schlette et al. 2001; Wong et al. 2002).

15.3.3 Immunohistochemistry

Mantle cell lymphoma has the distinctive immunophenotype of a CD5+ mature B cell expressing CD19, CD20, CD22, CD79a, PAX5, and monotypic surface immunoglobulin light chain with a lambda predominance. IgM and IgD are expressed. CD10 and CD23, typically strongly expressed in follicular lymphoma and chronic lymphocytic leukemia, respectively, are usually not expressed in mantle cell lymphoma, but some cases may variably be positive, thus limiting their utility unless one is familiar with detailed

expression patterns (Asplund et al. 2005; Gong et al. 2001). Cyclin D1 is aberrantly expressed as a result of the t(11;14)(q13;q32) translocation and is a sensitive and specific marker for the diagnosis of mantle cell lymphoma, particularly with newer paraffin-reactive rabbit monoclonal antibodies to cyclin D1 (Fig. 15.2) are used (de Leon et al. 1998; Cheuk et al. 2004). Ki-67(MIB1) staining shows a variable proliferative index, and increased proliferative fraction (>30–35 %) has been associated with poor prognosis in patients treated with modern therapy (Determann et al. 2008; Hoster et al. 2008a; Hsi et al. 2008; Klapper et al. 2009). This correlates with the importance of the proliferative gene signature in mantle cell lymphoma.

Rare cases of cyclin D1-negative mantle cell lymphoma exist, comprising less than 5 % of mantle cell lymphoma cases (Fu et al. 2005). *SOX11*, a gene recently found to be upregulated in mantle cell lymphoma, is a useful immunohistochemical marker to identify such cases, but its widespread use has been hampered by lack of reliable reagents (Dictor et al. 2009; Mozos et al. 2009). However, a new commercially available monoclonal antibody has been developed that is promising (ED Hsi, personal observation).

15.3.4 Molecular Genetics

The hallmark of mantle cell lymphoma is the t(11;14)(11q13;q32) involving *CCND1* and *IGH@* and resulting in overexpression of cyclin D1 (Williams et al. 1992, 1993b). This is the primary genomic alteration, present in almost all (>95 %) cases. Diagnostically, this can be detected by in situ hybridization in paraffin tissues, or the gene product can be seen by immunohistochemistry. Rare cases of variant immunoglobulin light chain partner genes exist. The existence of cyclin D1-negative mantle cell lymphoma has been proven, as noted above, but it is a rare occurrence and appears to be associated with abnormalities/overexpression of cyclin D2 or D3 (Fu et al. 2005; Dictor et al. 2009; Mozos et al. 2009).

Extensive work has been done to understand the detailed molecular genetic diversity of mantle cell lymphoma. Evaluation of the *IGH@* gene and somatic hypermutational analysis have shown that approximately 70 % of cases show at least some degree of somatic hypermutation of the *IGHV* gene segments with biased usage *IGHV3-21*, *IGHV4-34*, *IGHV1-8*, and *IGHV3-23* genes accounting for 46.3 % of cases, suggesting that the cell or origin may, at least in some cases, be a post-germinal center, antigen-driven cell as opposed to a naive B cell (Agathangelidis et al. 2011; Hadzidimitriou et al. 2011). Gene expression profiling of mantle cell lymphoma has demonstrated key signatures such as a cell proliferation signature, which was shown to have prognostic significance, and high proliferation has been incorporated into the Mantle Cell Lymphoma International Prognostic Index (MIPI) (Hoster et al. 2008a; Rosenwald et al. 2003).

The genetic complexity of mantle cell lymphoma has been addressed by multiple methods, and numerous secondary abnormalities have been identified. Although detailed enumeration of these findings are beyond the scope of this chapter, some of the more common alterations (potential genes of interest) include loss of 1p, 6q, 9p (*CDKN2A*), 11q (*ATM*), 13q (miR-17-92 cluster), and 17p (*TP53*) and gains of 3q, 8q (*MYC*), 10p (*BMI1*), 12q (*CDK4*), 15q, and 18q (*BCL2*) (Halldorsdottir et al. 2011; Jarosova et al. 2004; Schraders et al. 2005; Bea et al. 1999; Monni et al. 1998; Bentz et al. 2000; Kohlhammer et al. 2004; Martinez-Climent et al. 2001; Allen et al. 2002; Salaverria et al. 2007; Royo et al. 2011). Pathways associated with genes in these regions may present important in the pathogenesis and progression of mantle cell lymphoma. These include cell cycle (*INK4A/CDK4/RB1*, *ARF/MDM2/TP53*, *BMI1*, *CDKN2B*, *CDKN2C*), DNA damage response (*ATM*, *CHK1*, *CHK2*), cell survival (*BCL2*, NFkB pathway), Hippo pathway signaling (*MOBKL2B*, *MOBKL2A*, *LATS1*, *LATS2*), and microtubule-associated proteins (*MAP6*) (Royo et al. 2011).

Application of next generation sequencing has identified recurrent *NOTCH1* mutations in approximately 12 % of cases of MCL, clustered near the PEST domain and is similar to the PEST domain mutation seen in T-acute lymphoblastic leukemia. In keeping with a presumed tumor-promoting role, inhibition of NOTCH1 in mutated MCL cell

Table 15.2 Immunophenotypic profile of small B-cell lymphomas/leukemias

	CD19	CD20	CD5	CD10	CD23	BCL6	Cyclin D1	Sox11	LEF1	pERK
CLL/SLL	+	+	+	–	+	–	–	–	+	–
MCL	+	+	+	–	–/+^{weak}	–	+	+	–	–
FL	+	+	–	+	–	+	–	–	–	–
MZL	+	+	–	–	–	–	–	–	–	–
LPL	+	+	–	–	–	–	–	–	–	–
HCL	+	+	–	–/+^{weak}	–	–	+^{weak}	–	–	+

CLL/SLL small lymphocytic lymphoma/chronic lymphocytic leukemia, *MCL* mantle cell lymphoma, *FL* follicular lymphoma, *MZL* marginal zone lymphoma, *LPL* lymphoplasmacytic lymphoma, *HCL* hairy cell leukemia

lines decreased proliferation, and in clinical samples, *NOTCH1* mutation appears to be associated with shorter progression-free and overall survival (Kridel et al. 2012).

15.3.5 Differential Diagnosis

The differential diagnosis of mantle cell lymphoma includes other small B-cell lymphomas. Immunophenotyping is critical (Table 15.2) since the profiles are different. The most difficult differential is with small lymphocytic lymphoma (SLL), particularly when only small biopsies are available such as needle core biopsies or endoscopic biopsy. In particular, the small cell variant of mantle cell lymphoma may closely mimic SLL because of the predominance of round nuclei. However, SLL will show paraimmunoblasts/prolymphocytes that often cluster to form proliferation centers, which are not seen in mantle cell lymphoma. Additionally, the epithelioid histiocytes seen in mantle cell lymphoma are absent in SLL. The presence of CD5 in both lymphomas further complicates the distinction, but expression of cyclin D1 and absence of LEF1 (Table 15.2) make accurate diagnosis possible (Tandon et al. 2011).

Follicular lymphoma can enter the differential diagnosis in nodular variants of MCL, but the cytologic features of true centrocytes with highly convoluted, angulated nuclei in follicular lymphoma along with admixed centroblasts usually allow distinction between the two. Furthermore, expression of cyclin D1 and lack of the *IGH@/BCL2* fusion as well as differences in immunophenotype help consolidate the diagnosis.

Marginal zone lymphoma and lymphoplasmacytic lymphoma are both usually CD5-negative and CD10-negative. The cytologic features of marginal zone cells differ from most cases of mantle cell lymphoma, although rare cases of mantle cell lymphoma may have marginal zone cytology with more abundant cytoplasm than normally seen. Again, expression of cyclin D1 in mantle cell lymphoma and lack of CD5 (usually) in marginal zone lymphomas will allow correct diagnosis. Lymphoplasmacytic lymphoma (LPL) shows plasmacytoid differentiation that can be also be seen by cytoplasmic immunoglobulin light chain restriction using immunohistochemistry. These latter features are distinctly unusual in mantle cell lymphoma. Again, LPL is also negative for cyclin D1. Hairy cell leukemia (HCL) usually does not enter the differential diagnosis due to distinctive morphology of HCL and the bone marrow/blood-based presentation; however, it is included for completeness due to the weak cyclin D1 expression that is common in HCL (Table 15.2). Phospho-ERK$^{THR202/TYR204}$ is expressed in essentially all cases of HCL in bone marrow sections but is not seen in other small B-cell lymphomas lacking *BRAF V600E*, likely as a consequence of this kinase-activating mutation (Tiacci et al. 2011; Warden et al. 2012).

15.4 Molecular Pathogenesis

MCL has proven to be a useful model of neoplastic pathogenesis, especially as relates to alterations in cell cycle machinery and the response

to DNA damage. The overexpression of cyclin D1 dysregulates the G1/S-phase transition of the cell cycle. Cyclin D1 complexes with cyclin-dependent kinase-4 (CDK4) and −6 (CDK6) which in turn phosphorylate the retinoblastoma protein (Rb), leading to cell cycle progression (Zhao et al. 2010). Cyclin D1/CDK complexes also sequester the CDK inhibitors $p27^{kip1}$ and p21 to further promote G1 to S-phase progression (Quintanilla-Martinez et al. 2003).

The DNA damage response pathway is altered in MCL via loss-of-function mutations. Examples include hemizygous deletion of the chromosomal region 11q22-23 affecting the ataxia-telangiectasia mutated (*ATM*) gene, often in association with mutation of the remaining ATM allele (Fang et al. 2003). ATM encodes a kinase that belongs to the PI3 kinase-related superfamily and plays a pivotal role in the cellular response to DNA damage. The tumor suppressor gene p53 is inactivated in approximately 30 % of MCL cases with blastoid morphology and with high proliferation rates. Loss-of-function mutations affecting the 17p13/p53 or 9p21/CDKN2A/Hippo signaling loci, as well as 3q gain or deletion 13q14, have been associated with poorer survival in MCL.

The role of microRNA aberrations in MCL pathogenesis has also been recognized. Examples include the loss of expression of miR-29 family members and overexpression of the miR-17-29 cluster, which have been associated with a more aggressive clinical courses and poorer outcome (Zhao et al. 2010). Of interest, truncation of the 3′ untranslated region of cyclin D1 mRNA, itself a marker of poorer prognosis in MCL, leads to loss of miR-16-1 binding sites which in turn impairs normal cell cycle regulation.

The transcription factor SOX11 is constitutively expressed in most MCL, including cyclin D1-negative variants. Recent investigation has shown that its expression blocks normal B-cell differentiation via PAX5 modulation and serves an oncogenic function (Vegliante et al. 2013). Additionally, identification of recurring mutations in the NOTCH1 and Hippo pathways is shedding light on MCL pathogenesis (Hartmann et al. 2010; Kridel et al. 2012).

15.5 Staging

The majority of patients present with advanced stage, symptomatic disease. All patients should have a thorough history and physical examination with attention to potential extranodal disease common to MCL including the GI tract and soft tissue sites. Routine blood counts and chemistry profiles plus LDH are necessary, as are CT and/or PET/CT scans and bone marrow aspirate and biopsy (Brepoels et al. 2008). Bone marrow analysis should include flow cytometry and cytogenetics with fluorescent in situ hybridization (FISH) for the t(11;14). Colonoscopy and esophagogastroduodenoscopy should be considered in the staging evaluation if there is evidence of gastrointestinal bleeding or abdominal symptoms. Leukemic involvement can be confirmed in nearly all patients at diagnosis using flow cytometric and molecular detection assays, although only about 25 % of patients have overt lymphocytosis. In the latter event, peripheral blood flow cytometry and FISH analysis may replace the need for marrow biopsy.

15.6 Prognostic Factors

While the majority of MCL patients ultimately follow a steadily progressive clinical course and require therapy at diagnosis or shortly thereafter, up to 25 % of patients have a slow pace of disease typical of indolent B-cell lymphomas and may defer anti-lymphoma therapy for 6–12 months or more (Ondrejka et al. 2011b). As we better understand the molecular and cellular pathogenesis of this heterogeneous disease, we are able to approach each patient with individualized prognosis and risk-adapted treatment (Table 15.2).

15.6.1 Phenotypic and Molecular Markers

The proliferation rate was identified as the most important prognostic factor in studies supporting a two-step model with initial inhibition of apoptosis pathways and secondary cell cycle alteration. These findings are extended by gene expression profiling

of MCL by Rosenwald and colleagues that provided a quantitative measurement of tumor cell proliferation, termed "proliferation signature," allowing for the definition of prognostic subgroups that differ in their median survival by more than 5 years (see above Rosenwald et al. 2003). Accordingly, the clinical application of Ki-67 immunostaining has been confirmed as a major prognostic factor in the vast majorities of studies (Determann et al. 2008). Immunohistochemical staining of paraffin-embedded MCL samples for Ki-67 expression, a marker of cellular proliferation, correlates inversely with survival (Determann et al. 2008). Significant differences in overall survival were shown for MCL patients treated with CHOP or R-CHOP stratified by fewer than 10, 10–29, and 30 % or more Ki-67-positive tumor cells, suggesting that the Ki-67 index may be a useful surrogate for the molecular profile proliferation index (Fig. 15.2).

15.6.2 Morphologic Subtype

Most patients show a diffuse nodal effacement, although nodular or mantle zone patterns are well recognized and often correlate with a more indolent pace of disease. A blastoid cell type may be present at diagnosis or may develop with disease progression and usually portends a survival averaging 1–2 years as compared with the non-blastoid MCL survival of more than 5 years.

Markers of "indolent MCL" have been characterized in a cohort of MCL patients for whom therapy was not required for months or years (Espinet et al. 2010). These include the presence of mutated immunoglobulin heavy variable chain genes (IgVH), a lack of p53 mutations, limited secondary genetic aberrations, lack of SOX11 expression, and a unique 13-gene molecular expression array signature (Navarro et al. 2009; Wang et al. 2008).

15.6.3 Clinical Prognostic Factors

Patients with predominantly peripheral blood, bone marrow, and splenic involvement without significant lymphadenopathy at presentation often experience an indolent clinical course; these cases may be misdiagnosed as chronic lymphocytic leukemia if FISH for the t(11;14) and CLL markers (e.g., del 13q, trisomy 12) are not determined.

The Mantle Cell Lymphoma International Prognostic Index (MIPI) has been validated in the context of several therapeutic regimens as a highly useful tool that incorporates clinical and laboratory parameters: patient age, ECOG (Eastern Cooperative Oncology Group) performance status, total leukocyte count, and serum lactate dehydrogenase (LDH) (Geisler et al. 2010). A biologic MIPI ($MIPI_b$) further incorporates data for Ki-67 staining, described above, to provide an index with improved predictive power – patients with low-risk scores had median overall survival rates following induction chemotherapy of more than 6 years, whereas high-risk patients had median survivals of only 3 years (Fig. 15.4) (Geisler et al. 2010; Schaffel et al. 2010).

15.6.4 Minimal Residual Disease

Using sensitive RQ-PCR assays for clonal IgVH and t(11;14) breakpoints using patient-specific probes, one can determine the presence of low-level minimal residual disease (MRD) in the peripheral blood and bone marrow (Pott et al. 2010a). Achieving an MRD-negative molecular remission has been associated with prolonged clinical remission both in younger patients undergoing intensive induction chemotherapy with autologous stem cell transplant consolidation as well as in older patients receiving lower-intensity immunochemotherapy (Liu et al. 2012). In these trials, 90 % of patients had a detectable molecular marker, and MRD could be assayed serially during and after therapy. The achievement of MRD negativity correlated with significantly longer response duration.

15.7 Initial Therapy

Increasing numbers of effective agents and regimens have evolved for MCL therapy in recent years, improving the outcomes for most patients.

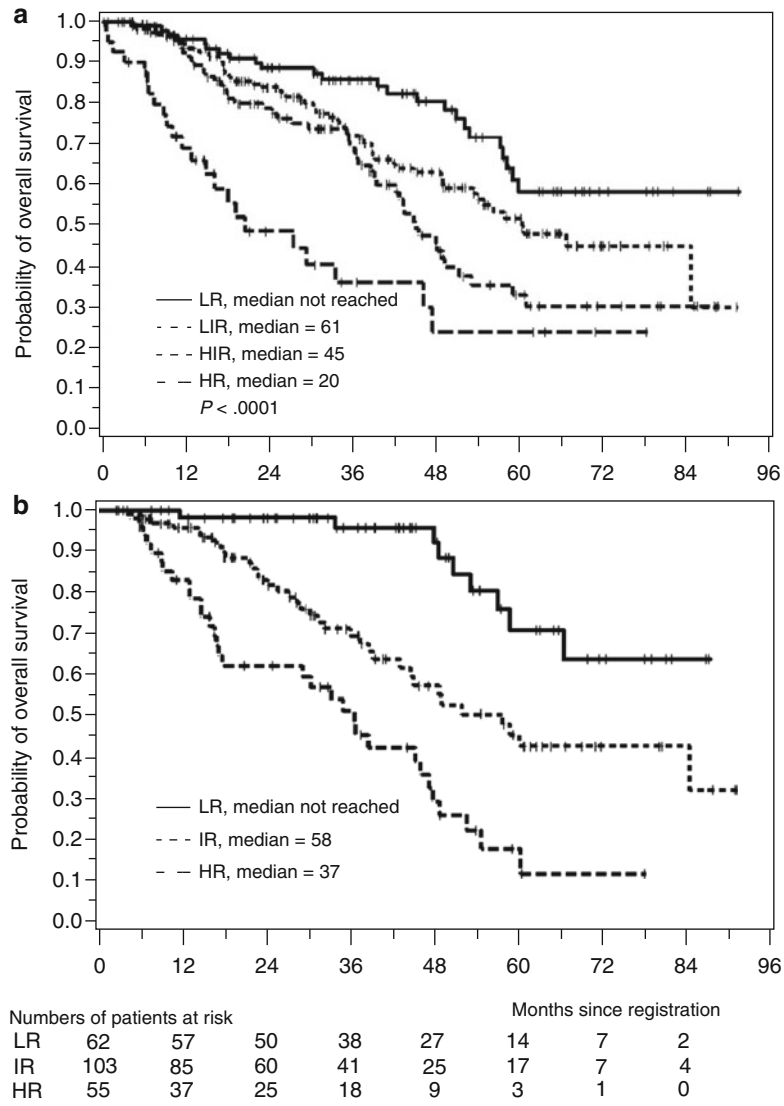

Fig. 15.4 Mantle Cell Lymphoma International Prognostic Index (MIPI) (**a**) and combined biologic index (MIPIb) (**b**) predicts overall survival (Hoster et al. 2008a)

However, durable remissions remain a challenge for most patients. Given its clinical and biologic heterogeneity, MCL is one of the most difficult to manage, with a median survival of only 5–6 years and a high incidence of chemotherapy refractoriness (Anonymous 1997). Most cases of MCL present with symptomatic, advanced stage disease. Nonetheless, about 20 % of patients present with slow-paced and low-volume disease and may be considered for cautious "watchful waiting" (Martin et al. 2009), as frequently employed for follicular and other indolent NHL subtypes (Table 15.1).

The following sections will focus on emerging phase III data and on promising novel agents that are rapidly changing treatment approaches.

15.8 Limited Stage

A rare patient will present with limited stage disease. In a retrospective study of 17 patients with stage I–II MCL, 5-year progression-free and overall survival was 68 and 71 % after involved field radiotherapy, respectively, either alone or in

Table 15.3 Selected frontline therapies in MCL

Phase	*n*	Disease status	Regimen	OR(CR)	PFS	OS	Author
III	497	First line	R-CHOP R-CHOP/R-DHAP f/b ASCT	90 % (36 %) 95 % (54 %)	49 m 84 m	82 m NR	Hermine et al., ASH (2012), #141, MCL Younger
III	48 45	First line	R-CHOP BR	95 % (35 %) 89 % (32 %)	22 m 33 m	Median NR –	Rummel et al., Blood (2009)
III	436	First line, NHL or MCL	BR R-CVP or R-CHOP	94 % (51 %) 84 % (24 %)	na –	na –	Flinn et al., ASH (2012), #902, Bright
III	532	First line, elderly	RCHOP → IFN·α RCHOP → MR R·FC → IFN·α R·FC → MR	86 % (34 %) – 78 % (40 %) –	29 % 4 year 58 % 4 year – –	63 % 4 year 87 % 4 year 47 % 4 year –	Kluin-Nelemans et al., NEJM (2012)
II	30	First line	VcR-CVAD + MR	(77 %)	63 % 3 year	86 % 3 year	Chang et al., Br J Haematol (2011)
II	75	First line	VcR-hyperCVAD + MR VcR-hyperCVAD	97 % (68 %) –	73 % 3 year 74 % 3 year	88 % 3 year 88 % 3 year	Kahl et al., ASH (2012) #153, ECOG (E1405)
II	40	First line Relapse	R-BAC R-BAC	100 % (95 %) 100 % (80 %)	95 % 2 year 70 % 2 year	na –	Visco et al., JCO (2013)

Abbreviations: *n* number of patients, *CR* complete remission, *OR* overall response, *PFS* progression-free survival, *OS* overall survival, *na*, not available, *NR* not reached
R-CHOP rituximab, cyclophosphamide, doxorubicin, vincristine, prednisone, *R-DHAP* rituximab, dexamethasone, cisplatin, cytarabine, *ASCT* autologous stem cell transplant, *BR* bendamustine, rituximab, *VcR-hyperCVAD* bortezomib, rituximab, cyclophosphamide, vincristine, adriamycin, dexamethasone, *R-FC* rituximab, fludarabine, cyclophosphamide, *MR* maintenance rituximab, *IFN-α* interferon alpha, *R-BAC* rituximab, bendamustine, cytarabine

combination with conventional chemotherapy (Leitch et al. 2003). On the other hand, median PFS in such patients was below 1 year in a phase III trial of the German lymphoma study group after radiation only. Optimal management of these patients, and those found incidentally to have MCL *in situ*, is not established.

15.9 Aggressive Upfront Therapy

15.9.1 Monoclonal Antibody and Combined Immunochemotherapy

In the modern era of MCL treatment, combined immunochemotherapy forms the backbone for first-line treatment (Table 15.3). A randomized phase III trial confirmed earlier studies showing that the addition of rituximab resulted in a superior overall response rate of 94 vs. 75 % with CHOP alone; CR rates were also improved (34 vs. 7 %), with a doubling of PFS from 14 to 28 months (Hoster et al. 2008b). Another randomized trial compared combined rituximab immunochemotherapy to chemotherapy only with the MCP regimen (mitoxantrone, chlorambucil, prednisone) and also showed a trend towards higher complete and overall response rates in the experimental arm, but the study was underpowered to detect a significant difference (Herold et al. 2004). A meta-analysis of R-chemotherapy in MCL demonstrated a benefit in overall survival, although the studies were statistically heterogeneous (Schulz et al. 2007). The observation of constant relapses in MCL patients after induction immunochemotherapy has led to the use of maintenance or consolidation strategies to translate high initial response rates into improved long-term survival.

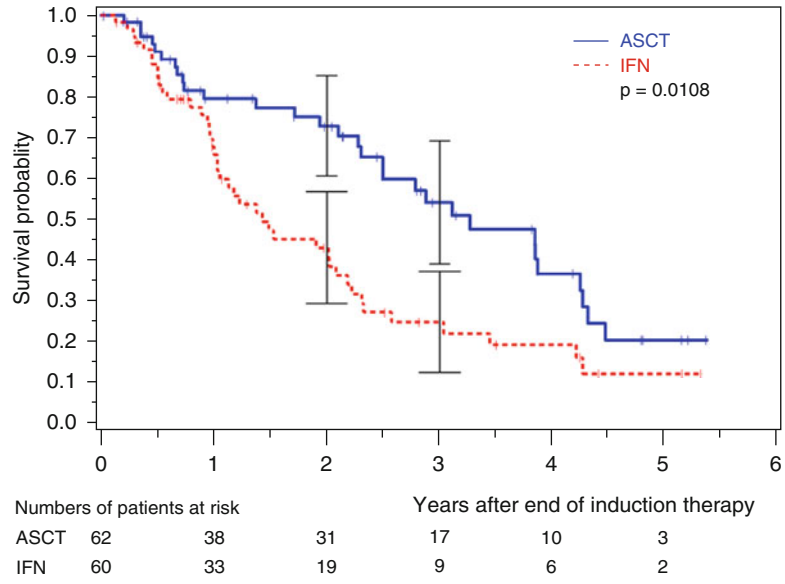

Fig. 15.5 Progression-free survival after high-dose radiochemotherapy followed by autologous stem cell transplantation (ASCT) or interferon-α (IFN-α) maintenance in MCL (Dreyling et al. 2005)

15.9.2 Dose-Intensified Regimens

Various studies on the efficacy of high-dose cytarabine (Ara-C) have established an important role for this agent in MCL induction therapy. Lefrere et al. observed a CR rate of <10 % in previously untreated patients with MCL following R-CHOP therapy which was converted into an impressive 84 % complete remissions after four additional cycles of the high-dose cytarabine-containing R-DHAP regimen (Lefrere et al. 2004).

Another dose-intensified approach utilized rituximab plus hyper-CVAD (hyper-fractionated cyclophosphamide, dexamethasone plus 24-h infusional vincristine, and doxorubicin) alternating with high-dose methotrexate/cytarabine. This single-institution phase II trial achieved impressive response rates (ORR 97 %, CR 87 %) and, importantly, prolonged remissions (3-year failure-free survival 64 %) similar to the sequential dose-intensified approach with myeloablative consolidation (Romaguera et al. 2010a). However, toxicity was significant: 29 % of patients could not complete scheduled treatment, and there was a therapy-associated mortality of 8 %, including infectious complications as well as secondary myelodysplasia and/or acute myelogenous leukemia in four of 97 patients (Romaguera et al. 2010b). A subsequent multicenter study also demonstrated high degrees of myelosuppression and toxicity-related deaths and poorer outcomes with this regimen in a more general population (Merli et al. 2012). Due to toxicity, many MCL patients are not candidates for this intensive therapy due to age or comorbid disease; therefore, modifications of the regimen are under study.

15.9.3 Sequential Dose Intensification and Autologous Transplantation

In young patients who are able to tolerate aggressive therapy, autologous transplantation (ASCT) is one method of consolidation after induction therapy (Dreyling et al. 2005; LaCasce et al. 2012; Lefrere et al. 2004). After several phase II studies demonstrated encouraging results, a prospective randomized trial of more than 200 patients with previously untreated MCL was undertaken. Results demonstrated that the addition of myeloablative consolidation and ASCT after initial CHOP induction to conventional chemotherapy conferred impressive improvement of complete remission (81 % vs. 37 %) and significantly longer progression-free survival (PFS) rates (median PFS: 39 vs. 17 months) than CHOP plus IFN-α (Fig. 15.5) (Dreyling et al. 2005). In subgroup analysis, the benefit of myeloablative consolidation was found to be strongest in patients with

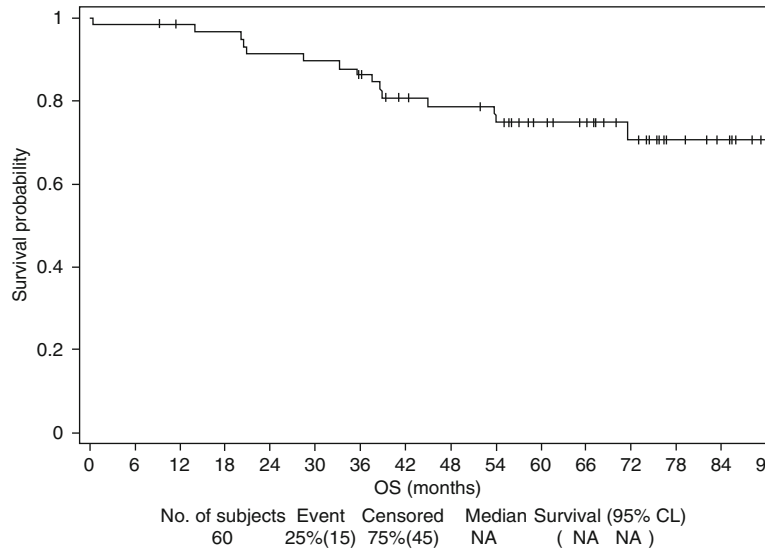

Fig. 15.6 Overall survival after R-CHOP alternating with R-DHAP followed by autologous stem cell transplantation in MCL (Delarue et al. 2013)

low and intermediate IPI scores as well as those with CR or PR after chemotherapy induction. After extended follow-up, a significant improvement of overall survival has been observed ($p=0.037$). Thus, myeloablative radiochemotherapy followed by ASCT represents one of the standard therapeutic approaches in first-line treatment of younger MCL patients (age <65 years). However, even after such a dose-intensified consolidation, a majority of patients with MCL eventually relapse.

Other groups have reported high-dose cytarabine-containing induction with rituximab followed by ASCT in phase II trials ($n=160$ and 113, respectively) (Gressin et al. 2010; Geisler et al. 2008). In a historical comparison, this combined approach appeared superior to the previous study generation of CHOP-based induction and myeloablative consolidation only. The European MCL Network confirmed the benefit of the addition of Ara-C in a large international phase III trial comparing R-CHOP for 6 cycles followed by radiochemotherapy conditioning and ASCT consolidation versus induction with alternating courses of R-CHOP with R-DHAP for 3 cycles each, followed by a high-dose cytarabine-containing radiochemotherapy conditioning regimen with subsequent ASCT (Pott et al. 2010b; Hermine et al. 2010; Delarue et al. 2013). Among the 497 previously untreated MCL patients enrolled, significantly higher CR/CRu rates were observed with the Ara-C-containing regimen (54 % vs. 36 %, $p=0.0003$) as well as a longer time to treatment failure (88 m vs. 46 m, $p=0.0382$) and overall survival (NR vs. 82 m, $p=0.045$, Fig. 15.6). There was no clinically significant difference in hematologic toxicity between the two arms. This study established a new standard of care in younger MCL patients. The recent LyMa trial tested the efficacy of eliminating R-CHOP from induction and demonstrated high CR/CRu rates with 4 cycles of R-DHAP alone followed by ASCT; however, longer follow-up is needed to verify the PFS and OS results in this study population (LeGouill et al. 2012). Analyses to date suggest that MIPI low- and intermediate-risk appear to derive the greatest benefit from these dose-intensive approaches. An induction and conditioning regimen that incorporates high-dose cytarabine prior to autologous transplantation is favored based upon present phase II and phase III data

15.9.4 Allogeneic Stem Cell Transplantation

Allogeneic bone marrow or stem cell transplantation is still the only established curative approach in patients with advanced stage MCL. A graft-versus-lymphoma effect has been suggested to induce long-lasting complete remissions even in

patients with relapsed or refractory MCL. However, transplantation-related mortality is high, and graft versus host disease and infectious complications are common in this older patient population.

Two phase II studies applying a dose-reduced conditioning reported more encouraging survival rates in less intensively pretreated patients (Tam et al. 2009). With a reduced-intensity conditioning regimen (fludarabine and 2 gray total body irradiation), disease-free and overall survival in 33 patients with relapsed and refractory MCL was 60 % and 65 %, respectively, with non-relapse mortality of 24 % at 2 years. Thus, despite promising results, allogeneic transplantation should be applied only in relapsed disease or selected high-risk patients not appropriately responding to dose-intensified first-line therapy.

15.9.5 Bortezomib

Bortezomib, an approved agent for treating relapsed MCL, targets the ubiquitin-proteasome pathway and is thought to provide therapeutic efficacy via effects on multiple cellular mechanisms in lymphoid neoplasms. Bortezomib has been investigated in combination with R-hyper-CVAD followed by maintenance rituximab in 75 de novo MCL patients (Chang et al. 2011; Kahl et al. 2012). In this study, 3-year PFS was found to be 73 %, with 3-year OS of 88 %. Bortezomib has also been demonstrated to have efficacy when given in combination with R-CHOP in upfront therapy in a phase II trial (Ruan et al. 2011). The efficacy of adding bortezomib in upfront treatment is currently being evaluated in randomized trials.

15.10 Treatment of Elderly Patients or Patients with Significant Comorbidities

Dose-intensive therapy and stem cell transplant should be reserved for patients who are relatively young with a good performance status or minimal comorbid disease. There are many therapeutic options that confer a smaller risk of significant toxicity and are better tolerated. Rituximab in combination with traditional chemotherapy such as CHOP or bendamustine is efficacious. Radioimmunotherapy and maintenance rituximab can also be considered in elderly or transplant-ineligible populations.

15.10.1 Immunochemotherapy

In a recent phase III study of the European MCL Network, R-FC (fludarabine, cyclophosphamide) and R-CHOP were compared in 560 older patients with de novo MCL (Kluin-Nelemans et al. 2012). Interestingly, this study demonstrated a significantly shorter overall survival with the R-FC regimen (47 % vs. 62 %, $p = 0.005$). Given findings of equivalent efficacy with superior toxicity profile and overall survival, R-CHOP is preferred over purine analog-containing regimens in the upfront treatment of older patients with MCL.

15.10.2 Bendamustine

Bendamustine is a novel "hybrid" cytotoxic agent composed of a benzimidazole ring with an attached nitrogen mustard moiety, which acts primarily as a bifunctional alkylating agent but is not cross-resistant with other alkylators. It has single-agent activity in a variety of hematologic neoplasms including NHL, multiple myeloma, and chronic lymphocytic leukemia. Bendamustine with rituximab was compared to the R-CHOP regimen in a phase III study in patients with newly diagnosed indolent NHL or MCL (Rummel et al. 2010, 2013). 549 patients were randomized, including 94 patients with MCL. The study found significant improvement in PFS with BR in all NHL patients (69.5 m vs. 31.2 m, $p < 0.001$), although OS did not differ between the two groups. An additional phase III study evaluated BR versus either R-CHOP or R-CVP as first-line therapy in NHL and MCL (Flinn et al. 2012). In early follow-up analysis, this study corroborated previously published results, demonstrating a significantly higher CR rate in the BR group. In each of these studies, outcomes were improved with BR as compared with

R-CHOP in newly diagnosed MCL. Thus, particularly in elderly patients not qualifying for subsequent dose intensification, the BR regimen represents one of the new standard approaches.

BR has also been explored in combination with cytarabine in a recent phase II trial of 40 patients with upfront or relapsed MCL. In upfront and relapsed patients, ORR was found to be 100 and 80 %, with a 2-year PFS of 95 and 70 %, respectively (Visco et al. 2013). Although this data needs confirmation in an ongoing phase III clinical trial, this regimen is appealing as it incorporates cytarabine, a highly active cytotoxic agent, with BR, a well-tolerated and efficacious regimen in MCL.

15.10.3 Rituximab

Despite high CD20 expression in MCL, rituximab monotherapy achieves only moderate response rates of 20–35 %. Thus, antibody monotherapy should be applied only in low tumor burden patients with contraindications for systemic chemotherapy.

Given continuous relapses in most patients, effective consolidation and maintenance strategies have been investigated. Interestingly, a subgroup analysis of a randomized trial in relapsed malignant lymphoma revealed some benefit of rituximab maintenance even after a rituximab-containing induction in a limited number of patients with relapsed MCL. The R-CHOP vs. R-FC trial, discussed above, confirmed the efficacy of regular post-induction antibody application in MCL (Forstpointner et al. 2006). The 316 older, transplant-ineligible MCL patients with a response to upfront therapy with either R-FC or R-CHOP were assigned to maintenance therapy with either standard-dose IFN-α or single-dose rituximab every 2 months. Rituximab maintenance reduced the risk of disease recurrence (58 % remission at 4 years vs. 29 %, $p=0.01$) and increased 4-year overall survival (87 vs. 63 %, $p=0.005$, Fig. 15.7) (Kluin-Nelemans et al. 2012). These results support R-CHOP followed by rituximab maintenance as a preferred upfront regimen for older and transplant-ineligible patients with MCL.

15.11 Management of Relapsed Disease

While newer immunochemotherapy regimens and ASCT have increased the objective response rate to initial therapy, MCL will ultimately relapse. There remains a need to improve the treatment options available for relapsed disease, with the last 5 years providing promising advancements in this regard. Since the management of therapeutic sequencing necessarily depends on the initial treatment administered, it is difficult to compare published data in the relapsed setting.

In younger, fit patients relapsing after dose-intensified regimens, an initial reduction of tumor load via chemo- or immunochemotherapy and subsequent allogeneic transplantation should always be considered as a potentially curative option.

In elderly patients (>65 years) with relapsed MCL, treatment must be selected considering patient comorbidity and mechanisms of prior therapies. Even in the setting of failure following R-chemo regimen, the addition of rituximab to salvage therapies may be reasonable especially if a remission of at least 6–9 months was achieved with prior treatment.

Thus, these patients may be considered for bendamustine alone or in combination with rituximab. In medically fit patients, dose intensification with SCT, if not applied in first line, may be re-discussed.

Alternatively, the proteasome inhibitor bortezomib (in USA) and temsirolimus (in EU) have been approved for second-line therapy in patients relapsing after immunochemotherapy. Bortezomib should be combined with dexamethasone, with or without rituximab. Given the lack of standards of care for treatment of newly diagnosed and relapsed disease, clinical trial participation is always recommended whenever possible. Selected options for the treatment of relapsed MCL are detailed as follows.

15.11.1 Bendamustine

Bendamustine, detailed above, is a hybrid cytotoxic agent with both alkylator and possibly antimetabolite properties. In a German study of

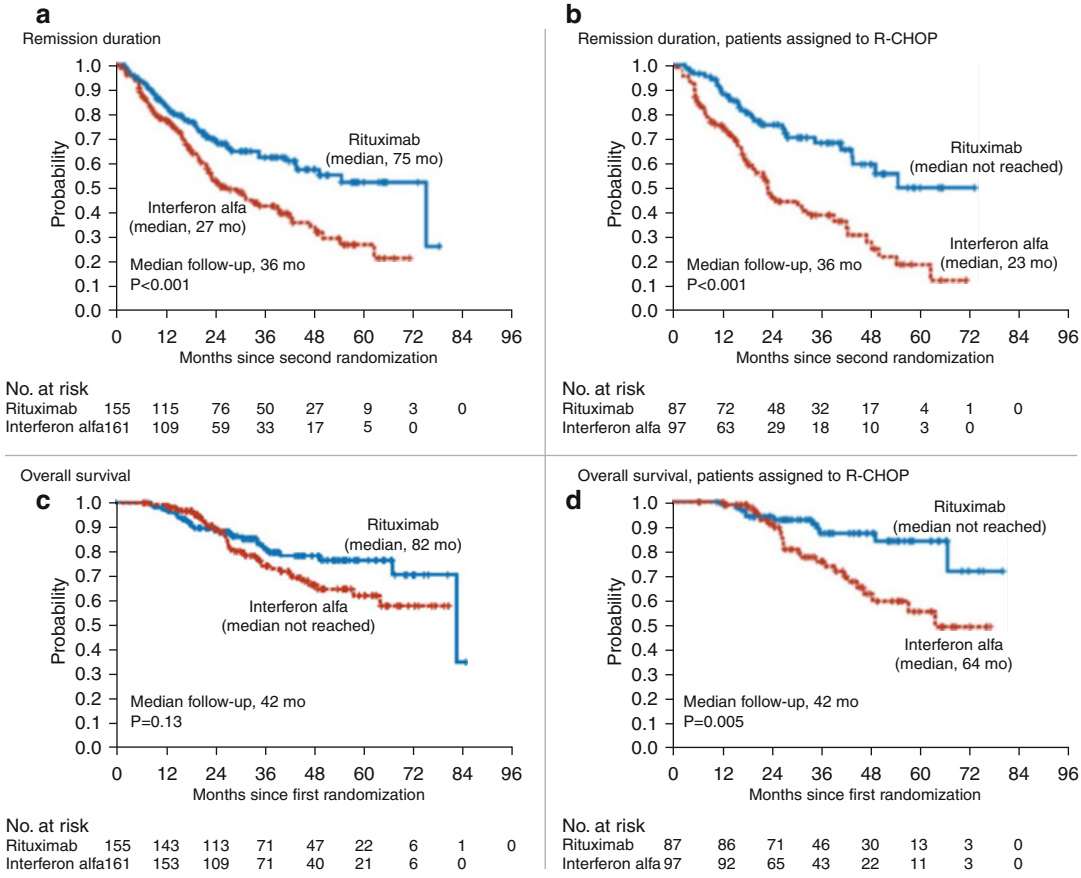

Fig. 15.7 Remission duration and overall survival with maintenance rituximab vs. IFN-α after R-CHOP or R-FC in elderly patients with MCL (Kluin-Nelemans et al. 2012)

bendamustine plus rituximab (BR) in 16 patients with relapsed MCL, including seven refractory to prior therapy, the median progression-free survival was 18 months (Robinson et al. 2008). Grade 3 leukopenia was observed in 16 % of all NHL patients treated in this study, but otherwise the non-hematologic toxicity was mild. A US phase II trial of BR in 12 relapsed MCL patients showed objective responses in 11 patients with a median duration of response of 19 months (Rummel et al. 2010). Preliminary results of a phase III trial of BR versus fludarabine plus rituximab in patients with relapsed indolent and MCL showed significant benefit in ORR and CR for BR, with similar toxicity profiles.

Bendamustine can carry a risk of prolonged myelosuppression, especially in patients who have received prior alkylator therapy. In such situations, the recommendation is to dose-reduce bendamustine to minimize risk of severe toxicity or to consider alternative agents.

15.11.2 Bortezomib

Pooled data from two phase II studies demonstrated a 44 % overall response rate with 18 % complete responses; responding patients showed a median time to next treatment of 14 months (Goy et al. 2008). Bortezomib is being tested in multicenter studies in combination with immunochemotherapy (Chang et al. 2011; Kahl et al. 2012). Bortezomib can be administered safely to patients with severe renal insufficiency but is associated with peripheral neuropathy in many patients which may become dose-limiting, necessitating dose modification and

vigilant monitoring when given in combination with vincristine-containing regimens. Reactivation of herpes zoster is also frequently observed in patients treated with bortezomib, prompting consideration of antiviral prophylaxis during the course of therapy.

15.11.3 mTOR Inhibitors

The mammalian target of rapamycin (mTOR) is a downstream signaling molecule in the phosphatidylinositol-3 kinase (PI3K)/AKT pathway that serves a critical role in regulating mRNA translation, including a potential ability to interrupt cyclin D1-dependent pathways. Temsirolimus, a derivative of rapamycin, has been shown in two phase II single-agent trials in relapsed MCL to confer a 40 % ORR. A phase III comparison of temsirolimus versus investigators' choice of therapy found superior ORR and PFS with temsirolimus, in a heavily pretreated a patient population (Hess et al. 2009; Ansell et al. 2008). Temsirolimus is being investigated in frontline combination regimens and, like the related mTOR inhibitor everolimus, as consolidation or maintenance therapy for MCL high-risk aggressive large cell lymphomas. Everolimus has also shown single-agent efficacy in relapsed or refractory MCL, including patients who are refractory to bortezomib (Renner et al. 2012).

15.11.4 Radioimmunotherapy (RIT)

RIT delivers a targeted radiotherapeutic, ^{90}yttrium or ^{131}iodine, via an anti-CD20 murine monoclonal antibody. Administration of RIT is often precluded in the presence of significant bone marrow positivity in MCL but is being explored as part of consolidation or conditioning regimens prior to ASCT.

15.12 Novel Therapeutic Approaches

While MCL responds well in most cases to initial therapy, most patients relapse within 1–5 years even after induction therapy and/or autologous stem cell transplant consolidation. Second-line regimens can show high therapeutic activity, although the durability of these responses is often short-lived. Fortunately, an increasing number of novel agents in development show clinical activity in the relapsed and refractory setting. These may be targeted to the dysregulated cell cycle elements characteristic of this disease or to other growth and proliferation or apoptosis pathways. Some of these agents are already being incorporated into frontline regimens, either as a component of combination therapy or as maintenance or consolidation strategies (Table 15.4). Here, we summarize some of the most promising agents currently undergoing clinical testing.

15.12.1 Immunomodulatory Drugs (IMiDs)

Lenalidomide is highly active in multiple myeloma and chronic lymphocytic leukemia and acts via direct antiproliferative activity, downregulation of tumor cell/stromal cell interactions with disruption of essential cytokine loops, immunomodulatory and anti-angiogenic effects. Among heavily pretreated MCL patients, partial and complete responses were observed with single-agent oral lenalidomide. These encouraging findings led to multiple international phase II trials of lenalidomide in relapsed/refractory MCL. The EMERGE study found an ORR of 28 %, with a median PFS of 4.0 m and median OS of 19 m in 134 heavily pretreated patients who had failed bortezomib (Goy et al. 2013). In the NHL-003 study, 35 % of 57 relapsed MCL patients responded, with a median PFS of 5.7 months (Zinzani et al. 2012; Reeder et al. 2009). Toxicity in both studies was predominantly reversible myelosuppression. Responses have been observed in patients relapsing after stem cell transplantation, including CRs.

Additional studies are in progress among patients failing R-chemo and bortezomib. Preclinical data demonstrating synergy with rituximab has led to current testing of so-called "R^2" regimens in CLL and indolent lymphoma, which may also find utility as induction or maintenance in MCL. A phase I/II study of lenalidomide and rituximab in combination was able to demonstrate an

Table 15.4 Selected novel therapies in relapsed/refractory MCL

Target	Drug	Phase	*n*	Disease status	OR(CR)	PFS	OS	Author/comments
Angiogenesis, microenvironment, cytokines	Lenalidomide	II	134	R/R	28 %	4 m	19 m	Goy et al., JCO (2013)
	Lenalidomide	II	57	R/R	25 %	8.8 m	NR	Zinzani et al., Blood (2012)
Bruton's tyrosine kinase	Ibrutinib (PCI-32765)	II	115	R/R	66 %	52 % 12 m	67 % 12 m	Wang et al., NEJM (2013)
	Ibrutinib (PCI-32765) + BR	I	11	R/R NHL (3 MCL)	38 % (66 % in MCL)	na	na	Blum et al., Blood (2012)
PI3 kinase	Idelalisib (CAL-101, GS 1101)	I	55	R/R NHL (18 MCL)	66 %	na	na	Kahl et al., Blood (2012)
HDAC	Abexinostat (PCI-24781)	II	30	R/R NHL (14 MCL)	27 % in MCL	4 m	na	Evens et al., Blood (2012)
BCL2	ABT-199	I	17	R/R NHL (6 MCL)	100 % in MCL	na	na	Davids et al., Blood (2012)

R/R relapsed/refractory

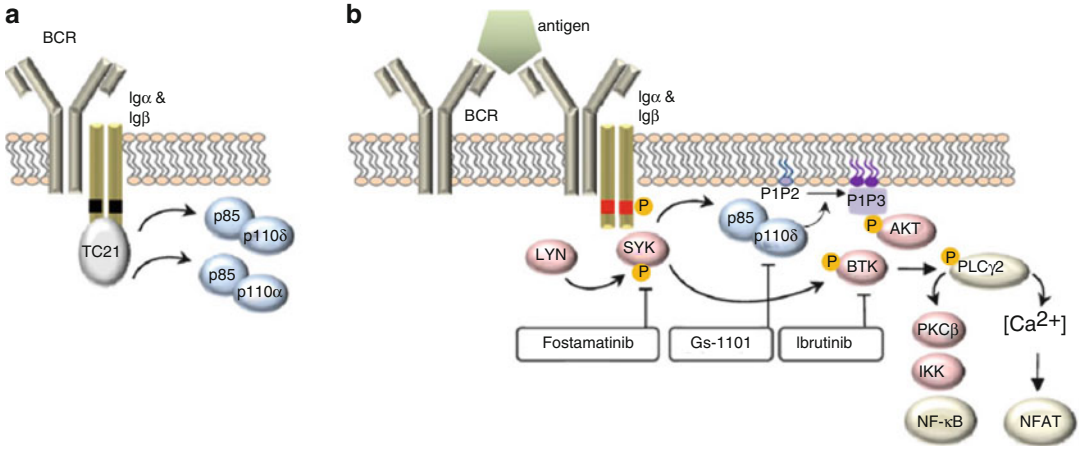

Fig. 15.8 Potential therapeutic targets within the B-cell receptor pathway (Weistner 2012). Upstream events in BCR signaling. (A) BCR signaling in the absence of antigen binding provides a tonic survival signal dependent on PI3K. In this model, the Ras GTPAse TC21 binds to non-phosphorylated tyrosine motifs (black boxes) in Igα and Igβ and activates PI3K-dependent survival signals. PI3Kα and PI3Kδ assume redundant functions in this pathway. (B) BCR signaling in response to antigen binding induces LYN- and SYK dependent phosphorylation (phosphorylation denoted by "P" in orange circle) of tyrosine motifs (red boxes) on CD79A and CD79B. A number of protein kinases (red symbols) and the lipid kinase PI3Kδ (blue symbol) transmit survival, cell growth, and proliferation signals and regulate cell migration. The transcription factors NF-κB and NFAT are important regulators of BCR-induced gene expression changes. Small-molecule inhibitors of select kinases in the BCR pathway that have demonstrated significant clinical activity are indicated

ORR of 56 % with an 11 month PFS and tolerable toxicity profile in 44 patients with relapsed/refractory MCL (Wang et al. 2012).

15.12.2 B-Cell Receptor (BCR) Pathway Inhibitors

In normally functioning B cells, antigen stimulation triggers dimerization of the BCR, a transmembrane antibody complex. Subsequently, a complex downstream signaling kinase cascade is activated, ultimately resulting in B-cell maturation, proliferation, and survival (Fig. 15.8). BCR can also be tonically activated in an antigen-independent fashion via the PI3K pathway. Once antigen stimulation has occurred, the BCR dimerizes, resulting in phosphorylation of tyrosine kinases LYN and SYK and subsequent activation of a phosphorylation cascade of multiple other tyrosine kinases including Bruton's tyrosine kinase (BTK), PI3Kδ, and PLCγ, all of which have been shown to be critical to BCR pathway activation (Weistner 2012). This pathway is thought to be constitutively activated in many B-cell lymphomas, a concept that has been validated in animal knockdown models of critical components of the BCR pathway in the activated B-cell-line subtype of diffuse large B-cell lymphoma. Signal transduction via this pathway has been therapeutically targeted at several downstream points in phase I–II studies with oral small molecule inhibitors in mantle cell and other lymphomas.

BTK is a critical mediator of BCR pathway signaling pathogenesis and thus a logical therapeutic target. This process is illustrated in the heritable disease Bruton's agammaglobulinemia which results from an X-linked deficiency in BTK. Ibrutinib (PCI-32765), an orally bioavailable BTK inhibitor, has shown significant promise in early studies. An international phase II study of ibrutinib in 115 patients with previously treated MCL demonstrated an ORR of 66 %, regardless of prior bortezomib exposure (Advani et al. 2013; Wang et al. 2013). This drug was well tolerated with only rare grade 3 or higher adverse events, most

commonly low-grade diarrhea and fatigue. A phase I trial of ibrutinib in combination with bendamustine and rituximab showed promising efficacy and safety profiles (Blum et al. 2012). Ibrutinib has also been shown to inhibit CXCR4, leading to mobilization of lymphoma cells into the peripheral blood and explaining the transient treatment-related lymphocytosis that results in many patients upon starting initial therapy (de Rooij et al. 2012). Phase III trials are ongoing to evaluate the efficacy ibrutinib, both alone and in combination, in MCL as well as other B-cell malignancies.

Additionally within the BCR pathway, PI3K and AKT are frequently activated in MCL (Fig. 15.8); the delta isoform of PI3K is expressed in over 90 % of B-cell lymphomas, providing a logical therapeutic target for small molecule inhibitors. Idelalisib (GS-1101, CAL-101) is an oral PI3K inhibitor shown to have significant activity in phase I trials of relapsed or refractory CLL, non-Hodgkin lymphoma, and MCL (Kahl et al. 2010). Studies are ongoing to evaluate its efficacy as single-agent therapy and in combination.

Other BCR pathway inhibitors are also in development for numerous B-cell malignancies, including the SYK inhibitor fostamatinib, as well as the PKCβ inhibitor enzastaurin. Thus, BCR pathway inhibition represents an exciting new approach for the treatment of B-cell malignancies.

15.12.3 HDAC Inhibitors

Histone deacetylase (HDAC) regulates oncogenesis via preferential selection of transcription of oncogenes over tumor suppressor genes. This transcriptional regulatory mechanism has been found to be altered in most cancers, including lymphoma. Cyclin D1 protein levels as well as PI3K/AKT pathway can be downregulated in MCL cells in vitro by treatment with vorinostat, an HDAC inhibitor currently registered for treatment of cutaneous T-cell lymphoma. Preliminary studies of vorinostat have shown clinical responses in MCL, with further trials of this and other HDAC inhibitors in progress (Kirschbaum et al. 2011). Novel combinations with cytotoxic agents and bortezomib also are being pursued. Abexinostat (PCI-24781) is another HDAC inhibitor shown to confer a 27 % ORR in 14 relapsed/refractory MCL patients in a recent phase II trial (Evens et al. 2012).

15.12.4 Cell Cycle Inhibitors

All cases of MCL exhibit cell cycle dysregulation via cyclin D1, D2, or D3 overexpression, although it has been difficult to effectively target this pathway. Flavopiridol is a synthetic flavone which downregulates cyclin D1 and cyclin D3 and competitively inhibits cyclin-dependent kinases CDK4/CDK6. Early studies have shown only modest response rates in MCL, although pharmacokinetically driven schedules have shown higher response rates in CLL which may enable the development of better approaches for MCL (Lin et al. 2010). Directly targeting CDK4/CDK6 theoretically circumvents the upregulation of cyclin D2 or D3 which may follow cyclin D1 inhibition. PD-0332991 is one such selective CDK4/6 inhibitor which has shown activity in relapsed MCL, with an 18 % ORR in early study results (Leonard et al. 2012). PD-0223991 is also being tested in combination with bortezomib.

15.12.5 BCL2 Inhibitors/BH3 Mimetics

The regulation of cell death pathways is highly complex and consists of both pro-survival and pro-apoptotic proteins, the latter characterized by the presence of a BH3 domain. As pro-survival proteins such as BCL2 are strongly expressed in MCL, a current therapeutic strategy is to utilize agents that mimic the BH3-only proteins which will in turn promote apoptosis. Several BH3 mimetics are in clinical trial, including ABT-199, obatoclax (GX 15–070), and navitoclax (ABT-263) (Davids et al. 2012). A recent phase I study demonstrated that ABT-199 is both active and well tolerated in patients with relapsed NHL (Paoluzzi et al. 2008).

15.13 Future Directions

Mantle cell lymphoma, characterized by a highly variable clinical course, poor duration of response to therapy, and poor long-term survival, has historically proven to be one of the most challenging types of lymphoma to manage. Recent advances in our ability to prognosticate outcomes using the MIPI score, minimal residual disease, Ki-67, and the proliferative index are improving our ability to appropriately triage patients to more aggressive and targeted therapeutic options. The incorporation of immunotherapeutics exemplified by rituximab in combination with chemotherapy as well as in the maintenance setting has changed the face of treatment for MCL.

Advances in treatment modalities for young, fit patients have incorporated dose-intensive therapy plus autologous stem cell transplant consolidation up front. In elderly and infirm patients, the advent of bendamustine has proven efficacious. Novel agents targeted to disease-specific pathway alterations are likely to be practice changing in the years ahead. Although challenging, MCL has proven to be a model for cancer pathogenesis and cell cycle dysregulation, apoptosis and cell signaling pathways, with anticipation that advancements in the management of MCL may very well prove useful for other hematologic and non-hematologic malignancies.

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Waldenström's Macroglobulinemia

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Véronique Leblond, Giampaolo Merlini,
Steven P. Treon, Scott Rodig, and Jan Delabie

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Pathology: Scott Rodig and Jan Delabie

V. Leblond (✉)
Department of Hematology Pitié Salpêtrière Hospital, Pierre et Marie Curie University, UPMC, GRC11-GRECHY, 75013 Paris, France
e-mail: veronique.leblond@psl.aphp.fr

G. Merlini
Department of Molecular Medicine, University of Pavia, Pavia, Italy

Biotechnology Research Laboratories, University Hospital Policlinico San Matteo, Pavia, Italy

S.P. Treon
Bing Center for Waldenstrom's Macroglobulinemia, Dana Farber Cancer Institute, Harvard Medical School, Boston, MA, USA
e-mail: steven_treon@dfci.harvard.edu

S. Rodig, MD
Department of Pathology, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA
e-mail: srodig@partners.org

J. Delabie, MD
Department of Pathology, Oslo University Hospital, Oslo, Norway
e-mail: jandel@ous-hf.no

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16.1 Introduction

The diagnostic concept of Waldenström's macroglobulinemia (WM) has changed dramatically since Jan Waldenström originally reported two patients with a syndrome of oronasal bleeding, lymphadenopathy, an elevated sedimentation rate, hyperviscosity, normal bone films, cytopenias, and a predominant bone marrow infiltrate (Waldenström 1944). The second international workshop on WM attempted to refine the working definition of the disease within the context of a LPL (Owen et al. 2003a). 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. This condition is considered to correspond to the lymphoplasmacytic lymphoma (LPL) as defined by the World Health Organization classification system (Swerdlow et al. 2008). Most cases of LPL are WM, with less than 5 % of cases made up of IgA, IgG, and nonsecreting LPL.

16.2 Epidemiology

WM is an uncommon disease, accounting for 1–2 % of hematological neoplasm, with a reported age-adjusted incidence rate of 3.4 per million among males and 1.7 per million among females in the USA and a geometrical increase with age (Groves et al. 1998). The median age is 63–68 years with a male predominance. The incidence rate for WM is higher among Caucasians, with African descendants representing only 5 % of all patients. The etiology of WM remains unknown. However, genetic factors appear to be important to the pathogenesis of WM, with numerous reports of familiar clustering of individuals with WM alone and with other B-cell lymphoproliferative diseases (Renier et al. 1989; Treon et al. 2006; Kristinsson et al. 2008; McMaster et al. 2007; Ogmundsdottir et al. 1999). Familial predisposition is common in WM as up to 20 % of WM patients have a first-degree relative with either a WM or a closely related B–cell disorders (Treon et al. 2006). 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 (Ogmundsdottir et al. 1999, 2011). An increased risk of solid tumors has been reported in WM patients analogous to observations in forms of indolent lymphoproliferative disorders (Morel et al. 2000; García-Sanz et al. 2001; Hanzis et al. 2011). The Italian group recently reported an increased incidence of second cancers in a retrospective study of WM patients either untreated or treated with alkylating agents with a cumulative incidence of solid cancers of 12 % at 10 years and 17 % at 15 years (Varettoni et al. 2011). The Surveillance, Epidemiology and End Results program (SEER multiple primary data base) yielded 1,618 WM patients for analysis with population and age-matched controls. The data were consistent with Italian data regarding the increase risk of acute leukemia and non-Hodgkin lymphoma but did not support an increased risk of brain cancer. However, the larger SEER sample yielded evidence that there was an increased risk of myeloma, melanoma, and cancers of colon, uterus, lung, and kidney (Ojha and Thertulien 2012). The greatest risk factor for the development of WM is having an MGUS. These patients have 46 times greater risk of developing WM than the general population (Kyle et al. 2002).

The role of environmental factors in WM remains to be clarified, an etiological role for hepatitis C virus (HCV) infection has been suggested, though in one study no association could

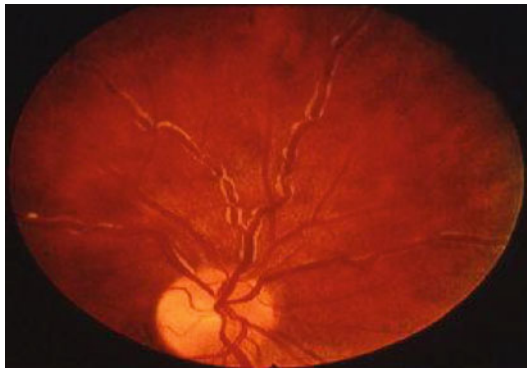

Fig. 16.1 Fundoscopic examination of a patient with Waldenström's macroglobulinemia demonstrating hyperviscosity-related changes including dilated retinal vessels, peripheral hemorrhages, and "venous sausaging" (Courtesy of Marvin Stone M.D.)

be established using both serological and molecular diagnostic studies for HCV infection in a hundred consecutive WM patients (Silvestri et al. 1996; Leleu et al. 2007a).

16.3 Biology

16.3.1 Morphology

The neoplastic lymphoid cells of LPL show a spectrum of appearances including small, mature lymphoid cells with scant cytoplasm, cells with more abundant cytoplasm and eccentrically placed nuclei (lymphoplasmacytoid cells), and fully differentiated, mature plasma cells (Fig. 16.1). Tumor cells of LPL colonize the bone marrow, where they form nodular aggregates that may be paratrabecular, and lymph nodes, where they colonize interfollicular spaces, frequently with preserved, dilated sinuses. Tumor cells can show PAS+ intranuclear inclusions (Dutcher bodies) or cytoplasmic inclusions (Mott cells). Mast cells are frequently intermixed with the neoplastic cells, and morphological evidence of immunoglobulin secretion, such as amyloid deposition, can occasionally be seen. Involvement of the liver, spleen, and peripheral blood can occur (Owen et al. 2003a). Involvement of the central nervous system is a rare but well-recognized phenomenon (Bing–Neel syndrome) (Fintelmann et al. 2009).

16.3.2 Immunophenotype

The lymphoid component of the tumor is positive for mature B-cell antigens, such as CD19, CD20, and CD79a, and expresses monotypic surface immunoglobulin light chain. These cells are most often negative for CD5 and CD10. The plasmacytic component of the tumor is positive for plasma cell antigens such as CD38 and CD138 and expresses monotypic cytoplasmic immunoglobulin light chain. In contrast to most cases of multiple myeloma, the neoplastic plasma cells of LPL are negative for CD56 (Leo et al. 1992; San Miguel et al. 2003).

16.3.3 Genetics

There are no chromosomal translocations associated with LPL, but a subset of cases show loss of chromosome 6q (Chang et al. 2007). Recently mutations in MYD88 have been discovered in the majority of LPLs – a finding that might prove useful for distinguishing LPL from marginal zone lymphoma (Treon et al. 2012).

16.3.4 Differential Diagnosis

Distinguishing LPL from marginal zone lymphoma (MZL) with plasmacytic differentiation can be difficult based on morphology and immunophenotype alone (Owen et al. 2003a). However, the systemic nature of the disease and the marked serum IgM paraprotein can usually rule out the latter diagnosis. Cases of LPL with a predominantly lymphocytic component often raise the diagnostic possibility of chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL). However, LPL lacks the characteristic proliferation centers and expression of CD5 typical for CLL/SLL. Finally, LPL with extensive plasma cell differentiation can raise the possibility of a plasma cell neoplasm. The demonstration of monotypic surface immunoglobulin expression on the B-cell population and the predilection of LPL for lymphoid tissues facilitate this distinction.

Table 16.1 Physicochemical and immunological properties of the monoclonal IgM protein in Waldenström'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

16.4 Clinical Features

It should be noted that most patients with WM will have limited and nonspecific symptoms at diagnosis, such as fatigue and malaise. Unlike most 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 16.1, 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 (Merlini et al. 1986; Farhangi and Merlini 1986; Marmont and Merlini 1991).

16.4.1 Morbidity Mediated by the Physicochemical Properties of IgM

16.4.1.1 Hyperviscosity Syndrome

Blood hyperviscosity is the most distinguished feature of WM but is only observed in less than 15 % of patients at diagnosis, effected by increased serum IgM levels leading to hyperviscosity-related complications (Gertz and Kyle 1995). The mechanisms behind the marked increase in the resistance to blood flow and the resulting impaired transit through the microcirculatory system are rather complex (Gertz and Kyle 1995; Mackenzie and Babcock 1975; Kwaan and Bongu 1999). 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

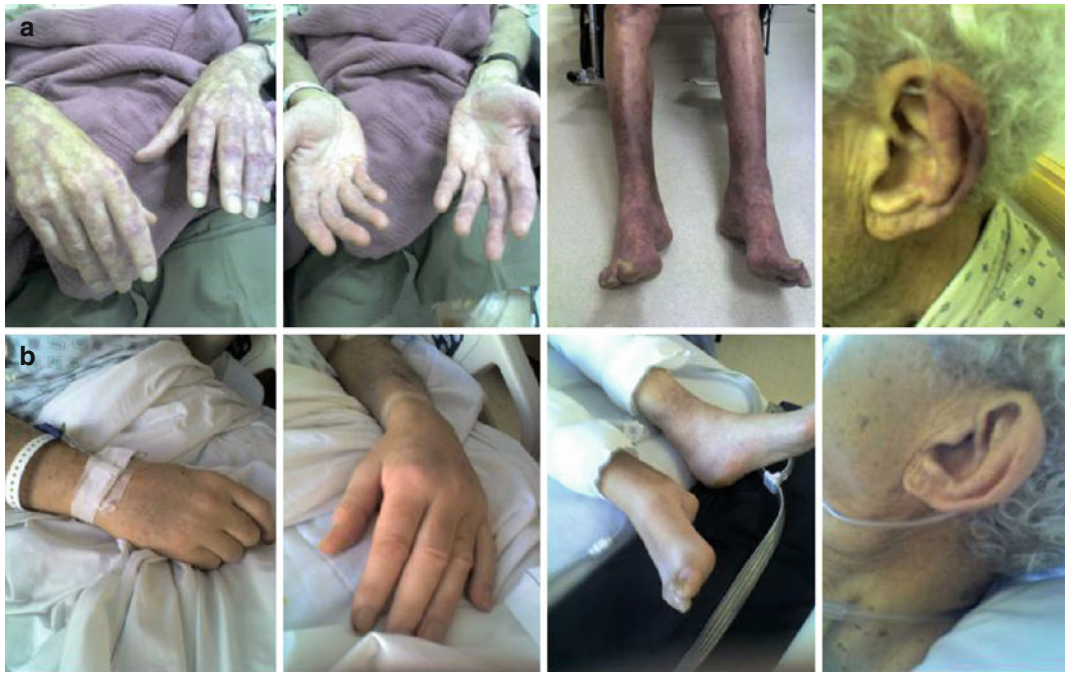

Fig. 16.2 Cryoglobulinemia manifesting with severe acrocyanosis in a patient with Waldenström's macroglobulinemia before (**a**) and following warming and plasmapheresis (**b**)

induce erythrocyte aggregation. 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 (Singh et al. 1993). Clinical manifestations are related to circulatory disturbances that can be best appreciated by ophthalmoscopy, which shows distended and tortuous retinal veins, exudates such as cotton-wool spots, hemorrhages, and papilledema (Menke et al. 2006) (Fig. 16.1). Symptoms usually occur when the monoclonal IgM concentration exceeds 50 g/L or when serum viscosity is >4.0 centipoises (cp) (corresponding to a serum IgM level of at least 30 g/L), but there is a great individual variability, with some patients showing no evidence of hyperviscosity even at 10 cp (Mackenzie and Babcock 1975). 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. Red cell transfusions should therefore be used with caution and sometimes in conjunction with pretransfusion plasmapheresis.

16.4.1.2 Type I Cryoglobulinemia

In up to 20 % of patients, monoclonal IgM may have tendency to precipitate upon cooling and, can thus behave as a type I cryoglobulin, but it is symptomatic in 5 % or less of the cases (Merlini et al. 2003). 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 (Fig. 16.2), malleolar ulcers, purpura, and cold urticaria. Renal manifestations may occur but are infrequent.

16.4.1.3 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 (Whittaker et al. 1996). Amorphous IgM deposits in the dermis determine the so-called IgM storage papules on the extensor surface of the extremities – macroglobulinemia cutis (Daoud et al. 1999). Deposition of monoclonal IgM in the lamina propria and/or submucosa of the intestine may be associated with diarrhea, malabsorption, and gastrointestinal bleeding (Gad et al. 1995; Case records of the Massachusetts General Hospital 1990). 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 (Isaac and Herrera 2002). 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 (Morel-Maroger et al. 1970). 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 (Gertz et al. 1993). In a large series of patients from the Mayo Clinic, amyloidosis develops in 2 % of patients with monoclonal IgM, among those 21 % had WM. Clinical expression and prognosis are similar to those of other AL patients with involvement of heart (44 %), kidneys (32 %), liver (14 %), lungs (10 %), peripheral/autonomic nerves (38 %), and soft tissues (18 %). In a French series of 72 patients, a peculiar pattern of relatively frequent lymph node (31 %) and lung (17 %) involvement was noted in patients with systemic AL amyloidosis (Terrier et al. 2008).

16.4.1.4 Interaction with Circulating Proteins

Monoclonal protein can interact with circulating proteins, including several coagulation factors, mainly factor VIII Willebrand and fibrinogen, and may cause prolonged clotting times. The macroglobulin can coat platelets, may impair their adhesion and aggregation, and may result in prolonged bleeding time (Farhangi and Merlini 1986).

16.4.2 Morbidity Mediated by the Immunological Effects of IgM

16.4.2.1 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 (reviewed in Stone and Pascual 2010).

16.4.2.2 Type II Cryoglobulinemia

In type II or mixed cryoglobulins, monoclonal IgM is an autoantibody to the Fc portion of polyclonal IgG. They are rheumatoid factor positive and often present at a high titer. The cryoprecipitating phenomenon is caused by the immune complex, as separation of the reactants yields clear solution. The manifestations are the same as previously described in type I. Renal manifestation particularly proliferative glomerulonephritis can be observed. Hepatitis C infection must be researched (Stone et al. 2005).

16.4.2.3 IgM-Related Neuropathy

The presence of peripheral neuropathy has been estimated to range from 5 to 38 % in WM patients (Dellagi et al. 1983; Nobile-Orazio et al. 1987; Nemni et al. 1994; Ropper and Gorson 1998; Treon et al. 2010). The nerve damage is mediated by diverse pathogenetic mechanisms: IgM antibody activity toward nerve constituents causing demyelinating polyneuropathies; endoneurial granulofibrillar deposits of IgM without antibody activity, associated with axonal polyneuropathy; and occasionally by tubular deposits in the endoneurium associated with IgM cryoglobulin and, rarely, by amyloid deposits or by neoplastic cell infiltration of nerve structures (Vital 2001). 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 (Latov et al. 1981; Chassande et al. 1998; Weiss et al. 1999). 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 (Nobile-Orazio et al. 1987; Latov et al. 1988). 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 (Dalakas and Quarles 1996; Eurelings et al. 2001). 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 (CANOMAD). The disialosyl epitope is also present on red blood cell glycophorins, thereby accounting for the red cell cold agglutinin activity of anti-Pr2 specificity (Ilyas et al. 1985; Willison et al. 2001). 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 (Lopate et al. 2002). 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 (Jacobs et al. 1997). 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 (Nobile-Orazio et al. 1994). Motor neuron disease has been reported in patients with WM and monoclonal IgM with anti-GM1 and sulfoglucuronyl paragloboside activity (Gordon et al. 1997).

However, neuropathy in Waldenström's macroglobulinemia (WM) is very heterogeneous. Neuropathy can be related to specific properties of the circulating IgM, leading to cryoglobulinemic or amyloid neuropathy or to neuropathy with endoneurial IgM deposits (Dellagi et al. 1983; Dimopoulos et al. 2000; Baehring et al. 2008). Neuropathy associated with tumoral infiltration, though rare, has also been described (Vital et al. 1982). For the neurologist and hematologist, diagnosing WM neuropathies is challenging because of their heterogeneous presentation. Yet it is crucially important to identify the mechanism involved in order to adapt the therapeutic strategy (Viala et al. 2012).

16.4.2.4 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 (Crisp and Pruzanski 1982) and is associated with cold agglutinin titers >1:1000 in most cases. The monoclonal component is usually an IgMκ and reacts most commonly with I/i antigens, with complement fixation and activation (Pruzanski and Shumak 1977a, b). The VH4-21 gene segment is necessary to encode anti-I specificity (Pascual et al. 1992). Many cold agglutinins have a high thermal amplitude so agglutination occurs in the 30–35 °C range. 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) and foreign ligands have also been reported.

16.4.3 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 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 % (Rausch and Herion 1980; Fadil and Taylor 1998; Kyrtsonis et al. 2001). Malabsorption, diarrhea, bleeding, or obstruction may indicate involvement of the gastrointestinal tract at the level of the stomach, duodenum, or small intestine (Kaila et al. 1996; Yasui et al. 1997; Rosenthal et al. 1998; Recine et al. 2001). 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 (Mascaro et al. 1982). 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 (Schnitzler et al. 1974), 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 (Roux et al. 1996). The neoplastic cells can infiltrate the periorbital structures, lacrimal gland, and retro-orbital lymphoid tissues, resulting in ocular nerve palsies (Orellana and Friedman 1981; Ettl et al. 1992). 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 Malkani et al. 2010).

16.5 Laboratory Investigations and Findings

16.5.1 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 (McMullin et al. 1995). Leukocyte and platelet counts are usually within the reference range at presentation, although patients may occasionally present with severe thrombocytopenia. 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.

16.5.2 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 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 (Hunter et al. 2010; Treon et al. 2008a).

16.5.3 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, the appearance of peripheral and mid-peripheral dot and blot-like hemorrhages in the retina, which are best appreciated with indirect ophthalmoscopy and scleral depression (Menke et al. 2006). 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.

16.6 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 (Morel et al. 2000, 2009; Gobbi et al. 1994; Dhodapkar et al. 2001; Kyle et al. 2003; Dimopoulos et al. 2004; Anagnostopoulos et al. 2006a). Most studies have focused on overall survival from diagnosis to last follow-up, but others have analyzed survival after initiation of treatment in patients with symptomatic WM (Gobbi et al. 1994; Dhodapkar et al. 2001). Indeed, a high proportion of patients die from unrelated causes, because of their advanced age at diagnosis (Morel et al. 2000; García-Sanz et al. 2001; Gobbi et al. 1994). As previously underlines in epidemiology section, some series have shown a high incidence of cancer. The vital prognostic value of events during follow-up is unknown. Preliminary results pointed to the high incidence of long-lasting monoclonal component during the course of WM and the low frequency (6 %) of patients who experienced a rapid rise of the monoclonal component (Stalnikiewicz et al. 2003). These results suggested a heterogeneous disease course.

Age is consistently an important prognostic factor (>60–70 years) (Morel et al. 2000; Gobbi et al. 1994; Kyle et al. 2003; Morel et al. 2009), though 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 (Morel et al. 2000, 2009; Gobbi et al. 1994; Dhodapkar et al. 2001). Cytopenias have also been regularly identified as a significant predictor of survival. The number of cytopenias in a given patient may predict survival (Morel et al. 2000). Serum albumin levels have correlated with survival in WM patients in certain but not all studies using multivariate analyses (Morel et al. 2000; Dimopoulos et al. 2004). High serum beta-2 microglobulin (>3–3.5 g/dL) levels (Dhodapkar et al. 2001; Dimopoulos et al. 2004; Morel et al. 2009), high serum IgM M-protein (>7 g/dL) (Morel et al. 2009), low serum IgM M-protein (<4 g/dL) (Dimopoulos et al. 2004), the presence of cryoglobulins (Gobbi et al. 1994), and the presence of a familial disease background (Treon 2011) have also been reported to confer adverse outcomes. The presence of 6q deletion as an adverse marker remains controversial (Ocio et al. 2007;

Table 16.2 Prognostic scoring systems in Waldenström's macroglobulinemia

Study	Adverse prognostic factors	Number of groups	Survival
Gobbi et al. (1994)	Hb < 9 g/dL Age >70 year Weight loss Cryoglobulinemia	0–1 prognostic factors 2–4 prognostic factors	Median: 48 month Median: 80 month
Morel et al. (2000)	Age ≥ 65 year Albumin < 4 g/dL Number of cytopenias: Hb < 12 g/dL Platelets < 150×10^9/L Wbc < 4×10^9/L	0–1 prognostic factors 2 prognostic factors 3–4 prognostic factors	5 year: 87 % 5 year: 62 % 5 year: 25 %
Dhodapkar et al. (2001)	β_2M ≥ 3 g/dL Hb < 12 g/dL IgM < 4 g/dL	β_2M < 3 mg/dL + Hb ≥ 12 g/dL β_2M < 3 mg/dL + Hb < 12 g/dL β_2M ≥ 3 mg/dL + IgM ≥ 4 g/dL β_2M ≥ 3 mg/dL + IgM < 4 g/dL	5 year: 87 % 5 year: 63 % 5 year: 53 % 5 year: 21 %
Application of International Staging System Criteria for Myeloma to WM Dimopoulos et al. (2004)	Albumin ≤3.5 g/dL β_2M ≥ 3.5 mg/L	Albumin ≥ 3.5 g/dL + β_2M < 3.5 mg/dL Albumin ≤ 3.5 g/dL + β_2M < 3.5 or β_2M 3.5–5.5 mg/dL β_2M > 5.5 mg/dL	Median: NR Median: 116 month Median: 54 month
International Prognostic Scoring System for WM Morel et al. (2009)	Age > 65 year Hb < 11.5 g/dL Platelets < 100×10^9/L β_2M > 3 mg/L IgM > 7 g/dL	0–1 prognostic factors* 2 prognostic factors** 3–5 prognostic factors *excluding age ** or age >65	5 year: 87 % 5 year: 68 % 5 year: 36 %

Nguyen-Khac et al. 2013; Chang et al. 2009). A few prognostic scoring systems have been proposed, and the International Prognostic Scoring System is the most validated (Table 16.2).

16.7 Treatment of Waldenström's Macroglobulinemia

16.7.1 Treatment Indications

Consensus guidelines on indications for treatment initiation were formulated as part of the 2nd International Workshop on Waldenström's macroglobulinemia (Kyle et al. 2003). Initiation of therapy should not be based on the IgM levels since this may not correlate with either disease burden or symptomatic status (Treon and How 2009; Dimopoulos et al. 2009). 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.

16.7.2 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 (Treon and How 2009; Dimopoulos et al. 2009). 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.

16.7.2.1 Plasmapheresis

Because 80 % of IgM is intravascular, plasmapheresis, conducted with a continuous blood flow separator with albumin and saline replacement, is very effective in reducing rapidly the amount of circulating IgM. Plasmapheresis is indicated for the treatment of patients who present with or develop symptomatic hyperviscosity. Even small reductions of serum IgM concentration with plasmapheresis can reduce significantly serum viscosity and can lead to resolution of hyperviscosity-related symptoms. Reductions of IgM by an average of 35 % resulted in a decrease of plasma viscosity from 5 to 2.1 (Kaplan 2001). In most patients with symptomatic hyperviscosity, concomitant administration of systemic treatment is required in order to suppress the underlying malignant process. However, some patients with predominant symptoms of hyperviscosity have been effectively managed for several years with plasmapheresis alone. This strategy may be also considered in patients who fail systemic treatment and who suffer primarily of hyperviscosity. Intensive plasmapheresis has also been used successfully in some patients with an IgM-related disorder such as peripheral neuropathy, cryoglobulinemia, and cold agglutinin disease. In such patients, a series of plasmapheresis may reduce the monoclonal protein, provide an opportunity for symptomatic improvement, and justify the subsequent administration of systemic therapy to achieve long-term control.

16.7.2.2 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) and an intermittent schedule. Kyle et al. (2000) 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). Approximately 50 % will achieve a response, but complete responses are uncommon. The use of steroids in combination with alkylator therapy has also been explored and has not been shown to affect response rate or overall survival but may be of benefit when WM is associated with autoimmune phenomena (Dimopoulos and Alexanian 1994).

Non-chlorambucil-based alkylator regimens employing melphalan and cyclophosphamide in combination with steroids have also been examined by Petrucci et al. (1989) and Case et al. (1991) producing slightly higher overall response rates and response durations, although the benefit of these more complex regimens over chlorambucil remains to be demonstrated. 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.

In a randomized study comparing the efficacy of fludarabine to that of chlorambucil, the response rate of 171 patients treated with chlorambucil was 36 % and the relapse-free survival time was 21.3 months with a response duration of 34.6 months. A higher cumulative incidence of second malignancies with a 6-year cumulative incidence of 3.7 % in the fludarabine arm and 20.6 % in the chlorambucil arm ($p=0.001$) was observed in patients treated with chlorambucil (Leblond et al. 2013).

16.7.2.3 Nucleoside Analogues

Both cladribine and fludarabine have been extensively evaluated in untreated as well as previously treated WM patients (Dimopoulos et al. 1993, 1994a, b, 1995; Delannoy et al. 1994; Fridrik et al. 1997; Liu et al. 1998; Hellmann et al. 1999; Betticher et al. 1997; Foran et al. 1999; Thalhammer-Scherrer et al. 2000; Zinzani et al. 1995; Leblond et al. 1998, 2001; Lewandowski et al. 2002). 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 % (Dimopoulos et al. 1994a, 1995; Delannoy et al. 1994; Fridrik et al. 1997; Liu et al. 1998; Hellmann et al. 1999; Betticher et al. 1997). 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 to 40 %, respectively (Dhodapkar et al. 2001; Dimopoulos et al. 1993; Foran et al. 1999; Thalhammer-Scherrer et al. 2000; Zinzani et al. 1995; Leblond et al. 1998, 2001), which are on par with the response data for cladribine. In a large randomized study in 168 untreated patients, the fludarabine response rate was 46 %, the relapse-free survival time 38.5 months, and the response duration was 50.1 months (Leblond et al. 2013).

Median time to achievement of response for fludarabine was also on par with cladribine at 3–6 months but took more than 6 months and more than 1 year in respectively 17 % and 5 % of responders in a large phase II study (Dhodapkar et al. 2001). In general, response rates and durations of responses have been greater for patients receiving nucleoside analogues as first-line agents.

Purine analogues (both fludarabine and 2 CDA) are effective in patients who are primary resistant or relapse after alkylating agents. Several phase II studies of purine analogues have involved patients who had received prior therapy (usually alkylating agents). The response rates varied from 14 to 78 %. Fludarabine induces responses in about one-third of patients who were resistant to a previous treatment and is highest in patients who are still sensitive to their primary therapy (Dimopoulos et al. 1993; Leblond et al. 2001).

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. 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. The principal toxicity of purine analogues is myelosuppression. For patients in whom high-dose chemotherapy and autologous stem cell transplantation are being considered, nucleoside analogues must be used with precaution, as several published data have shown that stem cell collection can be unsuccessful after fludarabine-containing regimens. The use of stem cell-damaging agents thus has to be reconsidered when the therapeutic strategy includes high-dose therapy and autologous stem cell transplantation (Thomas et al. 2008). The long-term safety of nucleoside analogues in WM was examined by Leleu et al. (2009a) 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 meta-analysis by Leleu et al. (2009b) 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. These results were not confirmed in a large randomized study comparing the efficacy of fludarabine alone to that of chlorambucil with a 6-year cumulative incidence of disease transformation

of 7.7 % in the fludarabine arm versus 11.1 % in the chlorambucil arm. Three MDS/AMLs were observed during the follow-up, all cases in the chlorambucil arm (Leblond et al. 2013). However, there is some evidence to suggest that this complication may be more frequent in patients treated fludarabine-alkylator combinations than with fludarabine monotherapy (Carney et al. 2010; Smith et al. 2011).

16.7.2.4 Monoclonal Antibodies

Rituximab is a chimeric monoclonal antibody which targets CD20, a widely expressed antigen on lymphoplasmacytic cells in WM (Treon et al. 2003). 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 (Treon et al. 2001; Gertz et al. 2004). 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 (Gertz et al. 2004). 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 (Dimopoulos et al. 2002; Treon et al. 2005a).

In many WM patients, a transient increase of serum IgM (IgM flare) may be noted immediately following initiation of rituximab treatment (Donnelly et al. 2001; Treon et al. 2004; Ghobrial et al. 2004). The IgM flare may be related to release of interleukin-6 by bystander immune in response to binding of rituximab to FcγRIIA receptors and also occurs in response to intravenous immunoglobulin administration in WM patients (Yang et al. 2010). 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 >3.5 cp 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. 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 (Treon et al. 2005a). 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 (Dimopoulos et al. 2002; Treon et al. 2005a). An 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/L M-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 (Dimopoulos et al. 2005a).

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 Fc 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 (Treon et al. 2005b). 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 FcγRIIIa-158 (Treon et al. 2011a).

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 (Furman et al. 2011). Responses were higher among rituximab-naïve patients. An IgM flare with symptomatic hyperviscosity was also observed in two patients in this series who required plasmapheresis.

The activity of alemtuzumab has also been investigated in WM patients given the broad expression of CD52 (Treon et al. 2003). 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 (Treon et al. 2011b). 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 three deaths. Long-term follow-up revealed late-onset idiopathic thrombocytopenia in four 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. (2003b) who reported their preliminary experience in a small series of heavily pretreated WM patients.

16.7.2.5 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 (Treon et al. 2007). All but one patient had relapsed/or refractory disease. The overall response rate was 85 %, with 10 and 13 patients achieving a minor (<25 % decrease in IgM) and major (<50 % decrease in IgM) response. 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. (2007) treated 27 patients with both untreated (44 %) and previously treated (56 %) disease. Patients in this study received bortezomib utilizing the standard schedule until they either demonstrated progressive disease or two 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 patients, 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. (2005b) observed major responses in 6 of 10 (60 %) previously treated WM patients. The combination of bortezomib with steroids and/or rituximab has also been investigated and is discussed below.

16.7.2.6 Immunomodulatory Agents

Thalidomide as monotherapy and in combination with dexamethasone and/or clarithromycin has been examined in WM. Dimopoulos et al. (2001) 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 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 (Coleman et al. 2003). However, in a follow-up study by Dimopoulos et al. (2003) using a higher thalidomide dose (200 mg orally daily) along with dexamethasone (40 mg 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.

16.7.2.7 Bendamustine

Bendamustine is a recently approved agent for the treatment of relapsed/refractory indolent non-Hodgkin lymphoma (NHL). Bendamustine has structural similarities to both alkylating agents and purine analogues (Cheson and Rummel 2009). Bendamustine in combination with rituximab has been investigated in both previously untreated and relapsed/refractory WM patients and is discussed below.

16.7.2.8 Combination Strategies

Because rituximab is an active and a non-myelosuppressive 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 (Buske et al. 2009). 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. (2007) 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.

Combination therapy with nucleoside analogues has been investigated as both first-line and salvage therapy in WM. Laszlo et al. (2010) 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 (Treon et al. 2009a). 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 six patients in this series.

The addition of alkylating agents to nucleoside analogues has also been explored in WM. Weber et al. (2003a) 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. The combination of fludarabine plus cyclophosphamide (FC) was also evaluated by Tamburini et al. (2005) 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 was the development of acute leukemia in two 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.

Weber et al. (2003a) 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.

Tedeschi et al. (2012) recently completed a multicenter study on with 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. Similar results were observed in 62 patients treated by rituximab at 375 mg/m^2 on day 1, fludarabine at 40 mg/m^2 orally on D1-D3, and cyclophosphamide at 250 mg/m^2 orally on D1–D3. In this retrospective study, the overall response rate was 85.5 %, with 30 % of patients attaining a major remission and a complete response. Prolonged cytopenia was observed in a third of patients. With a median follow-up of 45 months, the median progression-free survival for this study has not been reached, and the PFS rate was 65 % at 60 months (Compain et al. 2010).

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 (Treon et al. 2009b). 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 % (Ghobrial et al. 2010a; Agathocleous et al. 2010; Dimopoulos et al. 2010). A lower incidence of peripheral neuropathy was observed in two studies using once-a-week bortezomib. 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 (Treon et al. 2008b). 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 (Treon et al. 2008c). 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 a 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. (2013) in the frontline therapy of WM. As part of a randomized study, patients received six 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. (2011c). 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.

16.7.2.9 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 (Treon et al. 2011d). 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 survival (>120 vs. 116 months) were longer in patients who received maintenance rituximab.

These results must be confirmed in randomized trials.

16.8 Novel Agents

Novel therapeutic agents that have demonstrated efficacy in WM include perifosine, enzastaurin, everolimus, and histone deacetylases inhibitors (reviewed in Issa et al. 2011).

16.8.1 Perifosine

Perifosine is a novel AkT inhibitor that belongs to a class of lipid-related compounds called alkyphospholipids (Hideshima et al. 2006). A phase II clinical trial was conducted in 37 patients. Of the patients, 11 % achieved a PR and MR was observed in 24 % of the patients. Stable disease occurred in 54 % of the patients; PFS was 12.6 months (Ghobrial et al. 2010b).

16.8.2 Enzastaurin

Enzastaurin is an oral serine/threonine kinase inhibitor that targets the protein kinase C and PI3K/AKT pathways and had demonstrated activity in preclinical models of WM (Moreau et al. 2007). A multicenter trial was conducted in 42 patients (Ghobrial et al. 2012). Patients were treated with 1–5 prior regimens and received oral enzastaurin 250 mg twice daily (500 mg total) after a loading dose (day 1, cycle 1) of 375 mg 3 times daily (1,125 mg total) for 8 cycles of 28 days each or until progressive disease. The objective response rate (RR) was 38.1 % (2 partial and 14 minor responses). One patient had grade 3

leukopenia, and one patient died during the study from septic shock; both events were considered drug related. A statistically significant association between RR and interleukin 15 (IL-15) was observed, suggesting that higher concentration levels of IL-15 may be associated with better response. Enzastaurin was active and well tolerated in previously treated patients with WM, and these results warrant further investigation of enzastaurin for the treatment of WM.

16.8.3 Everolimus (RAD 001)

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 (Hatjiharissi et al. 2007; Leleu et al. 2007b).

Fifty patients with a median of 3 prior therapies were treated with everolimus in a joint Dana Farber/Mayo Clinic study (Ghobrial et al. 2010c). 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 (Treon et al. 2011e). 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.

16.8.4 Panobinostat

Preclinical studies have demonstrated that primary WM cells exhibit a higher level of histone deacetylases (HDACs), thus providing the rational for testing HDAC inhibitors. The activity of panobinostat was demonstrated in vitro in tumor cells and cell lines (Roccaro et al. 2010). In a phase II study enrolling 27 previously treated patients, the ORR was 60 % (PR: 24 %, MR: 36 %). Main toxicity was hematological with grades 3–4 anemia, neutropenia, and thrombocytopenia in 15 %, 26 %, and 52 %, respectively (Ghobrial et al. 2010d).

16.9 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. (1999) reported their initial experience of high-dose chemotherapy and autologous stem cell transplant, which has more recently been updated by Munshi and Barlogie (2003). 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. All eight patients responded, with 7 of 8 patients achieving a major response and one patient achieving a complete response with durations of response ranging from 5+ to 77+ months. Dreger et al. (1999) 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. (2001) wherein WM patients received various preparative regimens and demonstrated event-free survivals of 26+, 31, and 108+ months. Tournilhac et al. (2003) 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. Anagnostopoulos et al. (2006b) 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 2 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. Non-relapse mortality however, was 40 % in the allogeneic group and 11 % in the autologous group in this series.

Garnier et al. (2010) reported on the outcome of 24 high-risk WM patients who underwent allogeneic transplantation in the French registry (myeloablative 12, reduced-intensity: 13). The overall response rate was 92 %. With a median of follow-up of 64 months, 5-year overall survival and progression-free survival were respectively 67 % and 58 %. Only one of the six relapses occurred more than 3 years post-transplant.

Kyriakou et al. (2010a, b) 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. Non-relapse 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 et al. (2010b). 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. Non-relapse 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.

16.10 Response Criteria in Waldenström's Macroglobulinemia

As part of the International Workshops on WM, consensus panels developed guidelines for uniform response criteria in WM (Weber et al. 2003b; Kimby et al. 2006; Owen et al. 2013). 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 6th 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 (Treon et al. 2009a, b, 2011a, f; Kyriakou et al. 2010a). In distinction, the term major response is used to denote a response of ≥50 % in serum IgM levels and includes partial

Table 16.3 Summary of updated response criteria adopted at the 6th international workshop on Waldenström's macroglobulinemia (Owen et al. 2013)

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

or better responses (Kimby et al. 2006; Owen et al. 2013). Response categories and criteria for progressive disease in WM based on consensus recommendations are summarized in Table 16.3.

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 (Donnelly et al. 2001; Treon et al. 2004, 2007, 2011e; Ghobrial et al. 2004, 2010c). 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 (Donnelly et al. 2001; Treon et al. 2004, 2008b, c; Ghobrial et al. 2004), whereas bortezomib and everolimus can suppress IgM levels independent of tumor cell killing in certain patients (Treon et al. 2007, 2011e; Ghobrial et al. 2010c). Moreover, Varghese et al. (2009) 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 be considered in order to clarify the patient's underlying disease burden.

16.11 Treatment Strategies

The four main agents for systemic primary treatment of patients with WM include alkylating agents (chlorambucil, cyclophosphamide), nucleoside analogues (fludarabine, cladribine), bortezomib, and the monoclonal anti-CD20 antibody rituximab. Data from prospective randomized trial support the use of fludarabine over chlorambucil as single agent (Leblond et al. 2013). These agents have advantages and disadvantages which are shown in Table 16.4

Combination of these drugs with rituximab seems to increase the ORR and the duration of the response, but randomized studies are needed to choose the best combination. Outside a clinical

Table 16.4 Primary treatment of WM: advantages and disadvantages of four main agents

	Response (%)	Time to response (months)	Duration of treatment (months)	Cost	Myelosuppression	Opportunistic infections	Stem cell toxic	Miscella neous
Chlorambucil	50	>6	12–24	Low	Moderate	No	Yes	Secondary malignancies
Nucleoside analogues	40–80	1.5–5	2–6	Average	Significant	Yes	Yes	MDS/AML
Rituximab	40	3–5	1	High	None	No	No	IgM flare less active when high peaks
Bortezomib	60–80	1–2	4	High	Moderate	No	No	Neuropathy

trial, several factors should be taken into account in choosing the most appropriate primary treatment. These include the age of the patient and possible comorbid diseases, the presence of cytopenias and especially thrombocytopenia, the presence of symptoms and signs indicative of hyperviscosity, the need for rapid disease control due to severe symptoms, significant splenomegaly or lymphadenopathy, symptomatic peripheral neuropathy, and the candidacy for autologous stem cell transplantation. Based on those data, some suggestions could be made:

1. For patients who present with symptoms and signs of hyperviscosity, plasma exchange should precede any systemic treatment.
2. Patients who are not (and will not be candidates) for high-dose therapy, all four main primary treatments could be used.
3. For patients who are candidates for high-dose therapy (or may be candidates at some point of their disease), every effort should be made to avoid exposure to nucleoside analogues. If these agents seem necessary, a limited exposure is indicated before stem cells are collected.

For patients with refractory or relapsing disease, the use of alternate first-line agent is reasonable. For patients who are resistant to alkylating agents, a nucleoside analog and/or rituximab will be effective in 30–40 % of cases. If those patients are considered for high-dose therapy, rituximab would be preferable unless stem cells have been previously collected. For patients relapsing from unmaintained remission, the readministration of the same agent has a high likelihood of activity. For patients who develop resistance to all four classes of agents, few valid options are available. They can benefit of other monoclonal antibodies (new anti CD20, alemtuzumab) or bendamustine. Such patients are best served when treated within a context of a phase II trial. Every effort should be made to collect blood stem cells and to proceed to high-dose therapy, but this is usually not possible. In the future targeting MYD88 signaling might be a novel approach to impair WM growth.

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B-Cell Prolymphocytic Leukemia (B-PLL) and T-Cell Prolymphocytic Leukemia (T-PLL)

17

German Ott, Eric D. Hsi, John F. Seymour, and Georg Hopfinger

Contents

Pathology: German Ott, Eric D. Hsi

G. Ott, MD (✉)
Department of Clinical Pathology,
Robert-Bosch-Krankenhaus, Stuttgart, Germany
e-mail: german.ott@rbk.de

E.D. Hsi, MD
Department of Clinical Pathology,
Cleveland Clinic, L11, 9500 Euclid Ave,
Cleveland, OH 44195, USA
e-mail: hsie@ccf.org

J.F. Seymour
Division of Cancer Medicine,
Department of Haematology, Peter MacCallum
Cancer Centre, Locked Bag 1, A'Beckett St,
Melbourne, VIC 8006, Australia
e-mail: john.seymour@petermac.org

G. Hopfinger
3rd Department of Internal Medicine with
Hematology, Medical Oncology, Hemostaseology,
Infectious Disease, Rheumatology, Oncologic Center,
Laboratory for Immunological and Molecular
Cancer Research, Salzburger Landeskliniken,
Paracelsus Medical University, Salzburg, Austria
e-mail: g.hopfinger@salk.at

17.1 Definition of B-PLL and T-PLL

B-cell prolymphocytic leukemia (B-PLL) is a leukemic disorder, in which B-prolymphocytes by definition have to comprise more than 55 % of lymphoid cells in the peripheral blood (Galton et al. 1974). It is a rare disease constituting less than 1 % of lymphocytic leukemia. Most patients are over 60 years of age. B-PLL affects the bone marrow, the spleen, and the peripheral blood. Splenomegaly is usually massive, and lymphocyte counts are high ($>100\times10^9$/L) (see Fig. 17.1). In contrast, there is no significant lymphadenopathy. By definition, progressed stages of B-CLL with increased numbers of prolymphocytes are excluded (Campo et al. 2008). T-cell prolymphocytic leukemia (T-PLL), which constitutes roughly 3 % of T-cell lymphomas, is regarded an aggressive disease with a similar clinical presentation as B-PLL; however, skin infiltrations are encountered in one fourth of T-PLL patients. An increased frequency of T-PLL has been demonstrated in ataxia telangiectasia (AT) patients (Catovsky 1982).

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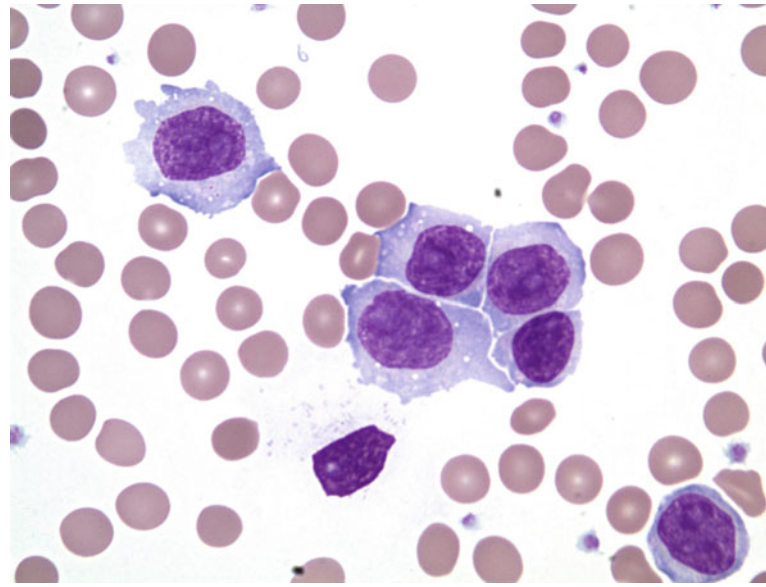

Fig. 17.1 Blood smear of a B-cell prolymphocytic leukemia. An 84-year-old man presented with abdominal pain to the emergency room with a WBC of 140,000 and splenomegaly. There is marked lymphocytosis with a population of medium-sized lymphocytes with nucleoli. FISH and immunohistochemistry (on bone marrow) for cyclin D1 was negative

17.2 Pathology of B-PLL and T-PLL

In B-PLL, in the peripheral blood, >55 % of the lymphocytes are prolymphocytes. They have a small rim of slightly basophilic cytoplasm, round even nuclei with a moderately condensed cytoplasm, and, usually, a single central nucleolus. T-PLL cells are medium-sized lymphocytes with deeply basophilic cytoplasm without granules and oval or irregular nuclei with deep indentations (Pawson et al. 1997). In the bone marrow, the infiltration pattern of both B-PLL and T-PLL may be interstitial, nodular, diffuse, or patchy. In the spleen, the white pulp nodules are enlarged in B-PLL, often producing a multinodular, at times coalescing, architecture. Red pulp sinusoids are variably expanded by prolymphocytes (Galton et al. 1974; Lampert et al. 1980; Ruchlemer et al. 2004). In T-PLL, the infiltration is mainly found in the red pulp (Osuji et al. 2005). Lymph nodes in B-PLL may show – rarely – patchy or vaguely nodular or diffuse infiltrates. In those cases, the picture may be similar to cases of prolymphocyte-rich B-CLL, but pseudofollicles are absent. In contrast, in T-PLL, the infiltration of lymph nodes is frequent, and infiltrates are centered in the interfollicular area, sometimes sparing residual germinal centers. One characteristic feature of T-PLL is the presence of arborizing high-endothelial venules that are transmigrated by lymphoma cells. The liver may show portal and sinusoidal infiltrates in both B-PLL and T-PLL. Skin infiltrations in T-PLL are usually confined to the dermis (Matutes et al. 1991).

For the pathologist, the prolymphocytes in B-PLL, in tissue sections, may be more similar to the paraimmunoblast as defined in the Kiel classification of lymphomas (Lennert 1992), and hence, differentiation from progressed (paraimmunoblast-rich or prolymphocyte-rich) stages of B-CLL may prove difficult. However, in contrast to B-CLL and B-CLL/PL, proliferation centers are absent. Presence of the t(11;14)(q13;q32) chromosomal translocation excludes the diagnosis of B-PLL (Dunphy and Perkins 2001; Ruchlemer et al. 2004; Singleton et al. 1999).

17.3 Immunophenotype of B-PLL and T-PLL

Prolymphocytes in B-PLL express pan-B-cell-associated antigens CD19, CD20, CD22, CD79A, PAX5, and FMC7 and strongly express surface IgM ± IgD. Roughly 50 % of cases will show positivity for CD5, while CD23 is rarely expressed. CyclinD1, CD10, and BCL6 are negative (Campo

et al. 2008; Hercher et al. 2001). T-PLL cells express pan-T-cell markers such as CD3, CD2, CD5, and CD7, and most cases are CD4+. However, both CD4- CD8+ and CD4+ CD8+ cases exist. TCRαβ and TCL1 proteins are usually positive, and NK markers are not expressed (Matutes et al. 1991).

17.4 Molecular Genetics of B-PLL and T-PLL

Immunoglobulin heavy chain (IGH) genes in B-PLL are clonally rearranged. B-PLL is a lymphoid neoplasia apparently deriving from both mutated and unmutated progenitor cells. Therefore, mutated IGHV genes are encountered in roughly 50 % of cases. Most B-PLL cases have been reported to use members of the VH3 and VH4 gene families, respectively (Davi et al. 1996; Del Giudice et al. 2006a, b). The t(11;14) chromosome translocation had been described in 20 % of cases that were previously thought to represent B-PLL owing to the prolymphocytic appearance of the tumor cells. To date, however, these lymphomas have been recognized as mantle cell lymphomas (MCL) with "prolymphocytic" features (Dunphy and Perkins 2001; Wong et al. 2002). Ninety of T-PLL cases usually demonstrate clonal chromosomal aberrations involving inversions of the TCL1 in 14q32 or MTCP1 in Xq28 loci with the TCRA/D locus in 14q11 (inv14q11q32) (Pekarsky et al. 1999; Stern et al. 1993). T-PLL is among those lymphoid tumors with the highest number of secondary chromosomal aberrations (Nowak et al. 2009), in virtually all sporadic cases also targeting the ATM gene in 11q21-q23 that usually shows biallelic inactivation by missense mutations (Stilgenbauer et al. 1997). Trisomies of chromosome 8 or 8q are also common.

17.5 Differential Diagnosis of B-PLL and T-PLL

The diagnosis of B-PLL warrants the exclusion of morphologically and clinically related entities, such as B-CLL with increased prolymphocytes, leukemic MCL, splenic marginal zone lymphoma (SMZL), hairy cell leukemia-variant (HCLv), and T-cell prolymphocytic leukemia (Viswanatha et al. 2012). In some cases, the distinction from "paraimmunoblastic" diffuse large B-cell lymphomas may equally prove difficult. While B-CLL, DLBCL, and T-PLL may be readily distinguished by their clinical presentation and/or immunophenotype, splenomegalic and leukemic forms of MCL have to be excluded by CyclinD1 staining and/or FISH for the t(11;14). The unequivocal exclusion of entities like SMZL and HCLv requires attention, because both the clinical presentation and the cytological presentation of the tumor cells may be similar. In general, however, the tumor cells in SMZL are more polymorphic with "villous" lymphocytes apparent, and HCLv is excluded by its distinct antigen expression profile including CD103 (Matutes et al. 2003).

The differentiation of T-PLL from other leukemic T-cell lymphomas may be challenging because of similarities in morphology and immunophenotype to other leukemic lymphomas such as hepatosplenic T-cell lymphoma, Sezary syndrome, or T-LGL. T-PLL, in contrast to the other entities, does show protein expression of TCL1 and CD26. Ultimately, however, the diagnosis must be confirmed by demonstration of the characteristic genetic profile of the disease including rearrangements of TCL1, MTCP1, trisomy 8 or 8q, and ATM inactivation.

17.6 Prognosis and Therapy of B-PLL

B-PLL initially described as a variant of CLL, is now recognized as a distinct clinicopathological entity in the current WHO classification, typically with an aggressive clinical course and a median overall survival of 3–4 years (Swerdlow et al. 2008). Historical series had included a substantial proportion of patients with t(11;14), but these are now classified as leukemic-phase MCL.

17.6.1 Conventional Therapy of B-PLL

The clinical course of B-PLL is usually aggressive; however, in some series, an initially indolent disease has been seen in up to 25 % of cases (Shvidel et al. 1999). Analogous to CLL, alkylation agents such as chlorambucil or even alkylating agent-based poly-chemotherapy, e.g., CHOP, have been used in B-PLL, but efficacy is only modest with response rates < 35 % (Sibbald and Catovsky 1979). Purine analogues such as fludarabine, cladribine, or pentostatin either alone or in combination with cyclophosphamide induce an overall response rate (ORR) of 35–50 %. However, the duration of response is usually less than 2 years (Herold et al. 2012; Rondelli et al. 1997; Saven et al. 1997).

Anecdotal series report on single-agent activity of the anti-CD20 monoclonal antibody rituximab (Mourad et al. 2004; Vartholomatos et al. 1999), and this observation has led to routine usage of combination chemoimmunotherapy, e.g., either fludarabine or bendamustine with an anthracycline (mitoxantrone or epirubicin) and rituximab in mixed populations including of CLL and B-PLL (Chow et al. 2011; Vartholomatos et al. 1999; Weide et al. 2004).

Although there is clear evidence for the benefit of adding rituximab to fludarabine-based chemotherapy in CLL (Hallek et al. 2010), there is no such data available in B-PLL. In order to better define the role of rituximab in combination with chemotherapy (e.g., fludarabine and cyclophosphamide (R-FC)), a prospective multicenter trial including only patients with chemotherapy naive B-PLL was started within the German CLL Study Group; however, recruitment was low, and therefore, the trial was stopped early. Complex cytogenetic aberrations are usually present in B-PLL, in particular about 50 % of patients with B-PLL display a TP53, which may explain the often refractory disease after conventional therapy (Dearden 2012; Lens et al. 2000; Put et al. 2012). There are also single cases reporting the activity of the anti-CDC52 antibody alemtuzumab in B-PLL (Bowen et al. 1997; Chaar and Petruska 2007; Dearden 2012; Lens et al. 2000; Put et al. 2012). Splenectomy is recommended only in a palliative setting but can provide good symptomatic relief (Matutes 2012). Central nervous system involvement has been reported and is usually a fatal event (Pamuk et al. 2009).

17.6.2 High-Dose Therapy of B-PLL

So far, again only case reports or small retrospective series are available, demonstrating substantial impact of either ASCT (Shvidel et al. 2000) or allogeneic transplant in selected B-PLL patients' (Castagna et al. 2005) registry data of the International Bone Marrow Transplant Registry. Concerning the latter publication, however, it must be noted that B- and T-PLL were not described separately within the overall group of 47 patients. However, no difference was found with regard to B-cell versus T-cell PLL, neither was there found a difference in a 2-year overall survival (OS) between patients with either acute or chronic Graft-versus-Host-Disease (GVHD); median PFS was 3.5 months for the patients with B-PLL ($n = 11$) with ~30 % remaining alive and relapse-free beyond 12 months (Kalaycio et al. 2010).

17.7 Prognosis and Therapy of T-PLL

In most patients, T-PLL shows an initially aggressive course and often chemo-refractory behavior to conventional cytotoxic agents resulting early relapses within 1 year (Herling et al. 2004; Herling et al. 2008; Matutes 1998; Matutes et al. 1991).

Some anecdotal reports and small series have described apparently initially indolent cases of T-PLL either associated with a complex karyotype (Soma et al. 2002) or a specific immunophenotype with a CD45RO+/CD45RA- phenotype (Garand et al. 1998). Despite this initial indolent phase with a median of 33 months until requiring treatment, most patients then display an aggressive course and an outcome that is nearly as poor as for patients showing a primarily aggressive course (Garand et al. 1998).

17.7.1 Conventional Therapy of T-PLL

Typically, T-PLL shows a marked resistance to conventional chemotherapy, as only 30–45 % of patients will achieve a response when alkylating agents such as chlorambucil or polychemotherapy based on alkylating agents or regimens such as CHOP (cyclophosphamide, doxorubicin, vincristine, prednisone) or VAPEC-B (vincristine, doxorubicin, prednisone, etoposide, cyclophosphamide, bleomycin) and no complete remissions were reported (Dearden et al. 2001).

These disappointing response rates are somewhat improved by the use of purine nucleoside analogues such as fludarabine or in particular by pentostatin (2′-deoxycoformycin), a potent inhibitor of adenosine deaminase. Pentostatin is usually administered at a dosage of 4 mg/m^2 i.v. every week leading to an ORR of 8–45 % including CR 9 % (Mercieca et al. 1994). Observed toxicity was usually low, but includes nausea/vomiting and modest hematologic toxicity.

More recently, response rates have been dramatically improved with the introduction of alemtuzumab, a humanized monoclonal antibody against the pan-lymphocyte antigen CD52, acting via cell death induction, complement activation, antibody-dependent cytotoxicity, and apoptosis (Dyer et al. 1989; Ginaldi et al. 1998; Greenwood et al. 1993; Heit et al. 1986; Rowan et al. 1998). So far, alemtuzumab is the most active single drug in T-PLL demonstrating response rates of 50–75 % in patients with relapsed or refractory disease.

Initially, alemtuzumab was investigated in a single-center retrospective analysis including 15 patients with relapsed T-PLL resulting in encouraging overall response of 73 % (CR 60 %). However, the median PFS was only 8 months (Pawson et al. 1997).

In a larger multicenter, prospective trial, 39 patients with relapsed T-PLL were treated with alemtuzumab resulting in an ORR of 76 % (CR 60 %) (Dearden et al. 2001). This impressive level of activity was confirmed when evaluating a compassionate use program with 79 patients with an ORR of 50 % (CR 38 %); however, long-term outcome again was poor, with a median PFS of 4.5 months (range, 0.1–45.4 months), and the median OS was only 7.5 months (Keating et al. 2002). Notably, the series of Keating et al. included four chemo-naive patients, with three who achieved a response.

However, given these encouraging response rates, alemtuzumab was then investigated as first-line therapy. In a recent report, alemtuzumab was administered to 32 patients using the i.v. route resulting in an OOR of 91 % including a very high CR rate of 81 %. Furthermore, this study emphasizes the importance of route of administration as in nine patients alemtuzumab was given subcutaneously resulting in an ORR of only 32 %, which compared unfavorably to the i.v. route (Dearden et al. 2011). A 12-month PFS rate of 67 % and an OS of 37 % at 48 months were described for the i.v. cohort. In analogy to other malignant lymphatic neoplasms, combination of monoclonal antibodies with chemotherapy would seem to be worth to be investigated, e.g., bendamustine, a drug widely used in non-Hodgkin lymphoma, recently showed activity in T-cell lymphoma alone and in T-PLL in combination with alemtuzumab (Yong et al. 2012).

However, in the clinical setting, a combination of alemtuzumab and pentostatin in 13 pretreated patients with T-PLL showed no further improvement compared with alemtuzumab alone (ORR 69 %, CR 62 %) (Ravandi et al. 2009). In a multicenter prospective trial of the German CLL Study Group, 25 patients (16 chemo-naive, 9 pretreated) received a fludarabine-based induction chemotherapy (FMC: fludarabine phosphate 25 mg/m^2/day (i.v.) on days 1–3, mitoxantrone 8 mg/m^2/day i.v. on day 1, and cyclophosphamide 200 mg/m^2/d i.v. on days 1–3 to be repeated on day 28) followed by an alemtuzumab consolidation. After a run-in phase, alemtuzumab was administered 30 mg i.v. three times weekly as consolidation for a maximum of 12 weeks. Four of 25 patients received only FMC, and 21 received FMC followed by alemtuzumab. Hematologic toxicities were the most frequent grade 3/4 side effects seen with FMC-A. Neutropenia represented

Table 17.1 Published series on systematic treatment evaluations in T-PLL (Adopted from Hopfinger et al. 2013)

Reference	Regimen	Disease status	Trial details	*n*	CR (%)	PR (%)	Median PFS (months)	Median OS (months)
Mercieca et al. (1994)	Pentostatin	Pretreated	Single-center retrospective	56	9	36	6	9
Pawson et al. (1997)	Alemtuzumab i.v.	Pretreated	Single-center retrospective	15	60	13	6	8
Dearden et al. (2001)	Alemtuzumab i.v.	Pretreated	Multicenter prospective	39	60	16	7	10
Keating et al. (2002)	Alemtuzumab i.v.	Pretreated (4 untreated)	Multicenter retrospective	76	38	12	4.5	7.5
Ravandi et al. (2009)	Pentostatin + alemtuzumab i.v.	Pretreated	Single-center prospective	13	62	8	7.8	10.2
Dearden et al. (2011)	Alemtuzumab i.v. Alemtuzumab s.c.	Untreated Untreated	Single-center prospective	32 9	81 33	10 0	(67 %)[a] (67 %)[a]	(37 %)[a] (33 %)[a]
Hopfinger et al. (2013)	FMC then alemtuzumab i.v.	9 pretreated 16 untreated	Multicenter prospective	25	46	46	11.5	17.1

[a]For PFS at 12 months; for OS at 48 months

10 % of the cumulative overall grade 3/4 events after FMC and 16 % after alemtuzumab. There were also 13 cases of CMV reactivation (62 % of patients) during the alemtuzumab phase. The ORR was 92 % (CR 46 %) which is similar to reports using alemtuzumab alone. However, the median PFS and OS were 11.5 and 17.1 months, respectively, suggesting benefit from the sequential therapy on long-term outcome although no plateau in survival was seen (Hopfinger et al. 2013). For an overview on conventional therapy, see Table 17.1.

In conclusion, typically outcomes after second or salvage therapies are very poor, and it is extremely rare to attain durable responses to such conventional salvage therapy.

17.7.2 High-Dose Therapy of T-PLL

Several strategies have been pursued in order to improve the dismal outcome of T-PLL by introducing high-dose therapy followed by either autologous stem cell transplantation (ASCT) or allogeneic transplant in T-PLL; see Table 17.2.

Initial small series were published reporting the feasibility of this approach in T-PLL (Collins et al. 1998; Curtin and Schwarer 2005; de Lavallade et al. 2006; Dearden et al. 2001; Garderet et al. 2001; Okamura et al. 2005; Tanimoto et al. 2005).

A larger series from the International Bone Marrow Transplant Registry (IBMTR) of 47 patients with either B-PLL or T-PLL included 21 patients with T-PLL receiving allogeneic transplant. The caveat must be expressed that no central review either of histology or immunophenotype was performed. The median PFS for the T-PLL cohort was 5.1 months, and treatment-related mortality (TRM) was 28 % for the whole group ($n=47$). The likelihood of remaining alive and disease-free beyond 1 year was only 33 %. With a short median follow-up of 13 months in this mixed population, this study demonstrates that long-term PFS and potential cure is only achieved in a minority of selected cases (Kalaycio et al. 2010). As data did not provide detailed information on long-term remissions, it only can be speculated from the IBMRT series and single case reports that there is a graft-versus-leukemia effect (Kalaycio et al. 2010; Kruspe et al. 2007).

A recent retrospective report described the aggregated multicenter experience of patients with T-PLL who had received either ASCT ($n=15$) or allogeneic ($n=13$) transplant following alemtuzumab induction (Krishnan et al. 2010). The TRM was 7 % in ASCT and 31 % in allogeneic group, which must be considered

Table 17.2 Published series on autologous/allogeneic transplant in T-PLL

Reference	Regimen	Disease status at Tx	Source	*n*	TRM (%)	PFS (months)	OS (months)
Collins et al. (1998)	Cy/TBI	PD	BM, sibling	1	–	36 +	36 +
Garderet et al. (2001)	Bu/Cy/ATG (nonablative)	PR	BM, sibling	1	–	2.8	5.2
Dearden et al. (2001)	Cy/TBI FDR/Mel/ alemtuzumab	PR 2 1st CR 2	Sibling	4	25	n.a.	2+,3,16+, 24+
Murase et al. (2003)	Bu/Cy	PR	MUD	1	–	n.a	16+
Tanimoto et al. (2005)	Cy/TBI	PD	UCB	1	–	n.a.	9+
de Lavallade et al. (2006)	RIC F/Bu/ATG	PR	Auto	1	–	n.a.	38 months
Kalaycio et al. (2010)	Bu/TBI various	Various	PB and BM	21	28	5,1	(11,2 months)[a]
Krishnan et al. (2010)	Mel or Cy/TBI various	PR	Auto Allo	15 13	7 31	28 24	52 33
Wiktor-Jedrzejczak et al. (2012)	TBI/chemo 22 Chemo alone18 NE 1	CR 11 PR 11 SD/PD 13 NE 5	Sibling 21 MUD 20	41	41	(19 %)[c]	(21 %)[b]

MUD matched unrelated donor, *TBI* total body irradiation, *Bu* busulfan, *FDR* fludarabine, *Mel* melphalan, *Cy* cyclophosphamide, *Auto* autologous stem cell transplantation, *allo* allogeneic stem cell transplantation
[a]Not separately for B-PLL and T-PLL
[b]For OS at 5 years
[c]For PFS and OS at 3 years

in the evaluation of the clinical outcome, as surprisingly the median disease-free survival (DFS) was similar (28 and 24 months) in both groups. With respect to long-term outcome, five patients receiving ASCT and five receiving allogeneic transplant were alive and disease-free at the time of the report. The median duration of their ongoing remissions from the date of transplant were 37 months (range 25–110) for the ASCT group and 81 months (range 8–115) for the allogeneic group, respectively. Treatment-related mortality (TRM) in the allogeneic cohort was classified as early in two cases and delayed in two; all patients had received full-intensity conditioning: fungal infection and multiorgan failure gut GvHD, pseudomonas sepsis, and EBV-associated posttransplant lymphoproliferative disease.

More recently, another retrospective analysis of 41 patients receiving allogeneic transplant for T-PLL was published by the EBMT, including 13 patients from the aforementioned British series (Wiktor-Jedrzejczak et al. 2012). The median age in this cohort was 51 years (24–71 years), and the median time from diagnosis to transplant was 12 months (4–58 months). Median relapse-free survival (RFS) was 10 months, and median overall survival (OS) was 12 months, resulting in a 3-year RFS rate of 19 % and a 3-year OS rate of 21 %. Of all relapses seen, 71 % were manifested within the first 12 months posttransplant, and 94 % within the first 3 years, with just one late relapse at 6 years. In multivariate analysis, the only prognostic factors favorably affecting event-free survival (EFS) were the use of total body irradiation (TBI; $p=0.034$) and interval of diagnosis and transplant <1 year ($p=0.05$); no such factors were evident when analyzed for OS. In conclusion, allogeneic stem cell transplantation might be curative in a small group of selected patients.

Using reduced conditioning regimen (RIC) might be a possible approach as outcome is described as similar after intensive or reduced conditioning, but follow-up is short, and the one late relapse reported at 6 years was following an RIC allograft (Kalaycio et al. 2010; Krishnan et al. 2010; Wiktor-Jedrzejczak et al. 2012).

17.7.3 New Drugs in T-PLL

There are only in vitro data with the proteasome inhibitor bortezomib available, reflecting a possible role of inhibition of NfkB in T-PLL (Ozpuyan et al. 2007); however, no clinical trial data are available yet. Reports have described that ATM mutation plays an important role in tumor genesis of T-PLL; as ATM mutant cells show impaired DNA double-strand repair, poly-ADP ribose polymerase (PARP) inhibition might be efficient in ATM-deleted and ATM-mutated tumors. The PARP inhibitor olaparib was only investigated in ATM-deficient CLL cell lines and mouse model so far (Weston et al. 2010). In peripheral T-cell lymphoma (PTCL), a variety of new drugs with different modes of action became available more recently, e.g., the folate-antagonist pralatrexate or histone deacetylase inhibitors, e.g., romidepsin. Given an ORR of approximately 30 % in relapsed or refractory nodal, PTCL with single-agent (Coiffier et al. 2012; O'Connor et al. 2011) activity of these new agents have to be explored in T-PLL.

17.8 Discussion

So far only a few prospective trials have been published on either B-PLL or T-PLL, which is mostly due to low incidence of these diseases. Therefore, information on the efficacy of protocols is often only available from small and/or retrospective series.

For both entities, the discouraging results for conventional chemotherapy with alkylating agents or multi-agent therapy such as CHOP as initial therapy have led to the assessment of alternative approaches.

For B-PLL, encouraging results were observed with alemtuzumab, but this approach might be reserved to a limited group of patients, perhaps those with proven TP53 mutations, due to side effects. Exploration of other molecular factors as BRAF V600E became of interest as this mutation is also occasionally found in B-PLL (Langabeer et al. 2012) and BRAF inhibitors, e.g., vemurafenib became available in other malignancies and have shown proof-of-principle activity in hairy-cell leukemia carrying the BRAF V600E mutation (Dietrich et al. 2012).

In T-PLL, even combination of pentostatin with alemtuzumab resulted in no further improvement compared to alemtuzumab alone (Ravandi et al. 2009; Dearden et al. 2011); therefore, this combination cannot currently be recommended as first-line therapy.

The emerging role of anti-CD52 antibody alemtuzumab has already been proven several times with ORR from 50 to 76 % in pretreated patients (Dearden et al. 2001; Keating et al. 2002) or as first-line therapy with ORR of 91 % (Dearden et al. 2011). When using alemtuzumab, the route of administration seems crucial, as a subcutaneous administration led to a dismal response when compared to intravenous administration (Dearden et al. 2011); therefore, intravenous alemtuzumab is the preferred therapy. An explanation for this phenomenon remains unclear, as alemtuzumab s.c. was successfully used in CLL (Stilgenbauer et al. 2009). A possible explanation might be the more aggressive course of disease in T-PLL and the slower increase to achieve a peak antibody levels (Dearden et al. 2011; Hale et al. 2004). When alemtuzumab is given as consolidation after a fludarabine-based induction therapy (FMC: fludarabine, cyclophosphamide, and mitoxantrone), an ORR of 92 % (CR 46 %) after completion of combination therapy was reached, which is in the range of that with alemtuzumab alone (Hopfinger et al. 2013). However, the ORR after FMC alone was 68 % (24 % CR), which is one of the highest response rates after chemotherapy alone; therefore, FMC might serve as chemo-backbone to improve response rates in T-PLL in clinical trials as alemtuzumab alone is known not to be very active in nodal disease, and in T-PLL, enlarged lymph nodes were found in 60 % and bulky tumor in 12 % of patients (Hopfinger et al. 2013).

Despite high response rates, achieving long-term survival seems the critical point, as no trial showed a plateau after conventional therapy thus far. To circumvent this issue, strategies to maintain response are warranted in addition to

attempts to improve response induction. One strategy might be the exploration of a maintenance therapy; currently, a prospective, multicenter trial is running with FMC and simultaneous alemtuzumab as induction followed by alemtuzumab maintenance therapy (T-PLL 2 trial of the German CLL Study Group, www.dcllsg.de).

Further, an obvious improvement in long-term survival was achieved by using high-dose therapy followed by stem cell transplant; however, only small retrospective series were published. In a single-center series of 28 patients (ASCT 15 or allogeneic 13), median OS was 52 months for the autologous group and 33 months for the allogeneic group, which might be correlated to treatment-related mortality (7 % for ASCT, 31 % for allogeneic); relapse rate was in favor for allogeneic transplant (60 % ASCT vs. 33 % allogeneic). In a retrospective EBMT analysis, a 3-year relapse-free survival (RFS) and OS were 19 and 21 % (Wiktor-Jedrzejczak et al. 2012); lower survival rate might be influenced as only 11/41 patients were in CR at time of transplantation. In conclusion, treatment of T-PLL remains challenging in terms of disease control and long-term survival. Over 90 % of patients respond to alemtuzumab alone or sequential therapy (FMC followed by alemtuzumab); however, most patients relapse within 1 year or frequently fail to respond to a salvage therapy. New strategies to maintain response are urgently needed.

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Nodular Lymphocyte-Predominant Hodgkin Lymphoma

18

Dennis A. Eichenauer, Ranjana H. Advani, Andreas Engert, Jan Delabie, and Scott Rodig

Contents

Pathology: Jan Delabie and Scott Rodig

D.A. Eichenauer • A. Engert (✉)
First Department of Internal Medicine, University Hospital Cologne, Kerpener Str. 62, Cologne D-50937, Germany

German Hodgkin Study Group, University Hospital Cologne, Cologne, Germany
e-mail: a.engert@uni-koeln.de

R.H. Advani
Division of Oncology, Department of Medicine, Stanford University Medical Center, Stanford, CA, USA
e-mail: radvani@stanford.edu

J. Delabie, MD
Department of Pathology, Oslo University Hospital, Oslo, Norway
e-mail: jandel@ous-hf.no

S. Rodig, MD
Department of Pathology, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA
e-mail: srodig@partners.org

18.1 Introduction

Nodular lymphocyte-predominant Hodgkin lymphoma (NLPHL) was first described in 1944 by Jackson and Parker as nodular paragranuloma (Jackson and Parker 1944). Other synonyms used were lymphocytic-predominant Hodgkin disease and lymphocytic- and histiocytic-predominant Hodgkin disease. NLPHL represents about 5 % of all Hodgkin lymphoma (HL) cases and has an estimated incidence of 1.5 newly diagnosed cases per 1,000,000 people per year (Diehl et al. 1999). In this chapter we provide an overview of pathology, clinical characteristics, risk factors, and treatment of NLPHL.

18.2 Pathology of NLPHL

In NLPHL, the normal lymph node architecture is completely effaced and replaced by large nodules (Fig. 18.1). Rarely, internodular banded sclerosis is seen as in nodular sclerosis classical HL (cHL). Remnants of normal lymph node parenchyma may be noted in the periphery of the lymph nodes. Occasionally, part of the lymph node may

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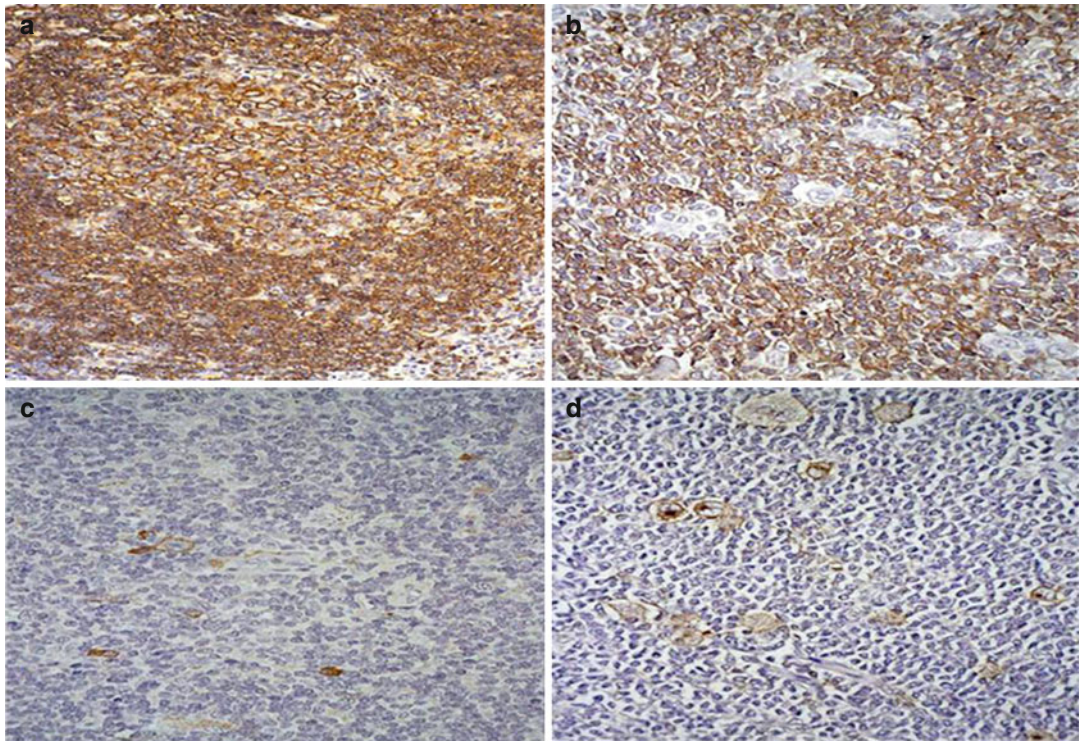

Fig. 18.1 CD20 (*above*) and CD30 (*below*) staining in NLPHL and cHL (lymphocyte-rich subtype)

show progressively transformed germinal centers. Variants with a partly diffuse architecture mimicking T-cell-rich B-cell lymphoma (TCRBCL) are also recognized and are associated with disease recurrence (Fan et al. 2003).

Typically, the nodules in NLPHL show dispersed large cells, previously known as lymphocytic and histiocytic (L&H) cells after the descriptive name of this lymphoma according to the Lukes and Butler classification of 1966, but now referred to as lymphocyte-predominant (LP) cells after the current name of the lymphoma. LP cells are large cells with polylobulated nuclei with moderately large eosinophilic nucleoli. The cells appear singly in a background with predominantly small lymphocytes, hence the name of the lymphoma. Not infrequently small collections of epithelioid histiocytes are also present. These may occasionally be located at the periphery of the nodular infiltrates. In some cases, the lymphocytes are slightly larger with somewhat irregular nuclei and clear cytoplasm (Sohani et al. 2011).

18.2.1 Immunophenotype

LP cells typically express the pan-leukocyte marker CD45 and B-cell surface antigens such as CD20 and CD79a, the epithelial membrane antigen (EMA), as well as the B-cell transcription factor PAX5 (Table 18.1). The latter is usually higher expressed than in Hodgkin and Reed-Sternberg (H-RS) cells of cHL. In addition markers of germinal center cells such as BCL6 and centerin are usually expressed. CD30, a marker usually expressed by H-RS cells, is not frequently expressed. CD15 is rarely expressed, if at all. Expression of CD15 and CD30 should question the diagnosis of NLPHL and favors a diagnosis of lymphocyte-rich cHL. Epstein-Barr virus (EBV) cannot be demonstrated in LP cells. Importantly, the majority of small lymphocytes within the nodules are B lymphocytes and lymphocytes in the immediate vicinity of LP cells are T lymphocytes. Typically, many of the T lymphocytes express CD57 and PD-1, markers of intrafollicular T cells. In rare cases of NLPHL with

Table 18.1 Immunophenotype of NLPHL, cHL, and TCRBCL

	NLPHL	cHL	TCRBCL
CD15	–	+	–
CD20	+	+/–	+
CD30	–	+	–
CD45	+	–	+
EMA	+	–	+/–

diffuse areas, most small lymphocytes in the background are T lymphocytes (Fan et al. 2003).

18.2.2 Genetics

Classic cytogenetics and comparative genomic hybridization of NLPHL have revealed mostly complex aberrant karyotypes suggestive of genomic instability. It has taken a long time to unravel more specific genetic changes in NLPHL, largely due to the scarcity of the lymphoma cells in the samples and to the difficulty of establishing tumor cell lines representative of the lymphoma. Sensitive single-cell analyses have demonstrated that LP cells show clonal and productive immunoglobulin gene rearrangements and have features of germinal center B cells (Braeuninger et al. 1997). About 50 % of cases show BCL6 gene translocation. Also inactivating mutations of SOCS1, a negative regulator of cytokine signaling through the JAK/STAT pathway, have been demonstrated. The latter explains, at least in part, why the JAK/STAT pathway is constitutively activated in LP cells (Schmitz et al. 2009). Also, gene expression analysis has revealed constitutive activation of the NF-kB pathways in LP cells (Brune et al. 2008).

18.2.3 Differential Diagnosis

The main differential diagnoses are reactive lymphadenopathy with progressive transformation of germinal centers, lymphocyte-rich cHL, nodular small B-cell lymphomas such as follicular lymphoma and mantle cell lymphoma, TCRBCL, and, in some cases with a larger number or clusters of LP cells, diffuse large B-cell lymphoma. Briefly, progressive transformation of germinal centers contains small foci with centroblasts in a larger nodule containing many small B cells, but no LP cells. Lymphocyte-rich cHL may mimic NLPHL, but H-RS cells are typically located at the periphery of the nodules and express CD30 as well as CD15. Follicular lymphoma and mantle cell lymphoma have, apart from their distinct cytological characteristics, typical immunophenotypes that differ from the mantle zone immunophenotype of most of the reactive B cells in NLPHL. TCRBCL is either diffuse or vaguely nodular. When nodular, nodules are typically smaller than those observed in NLPHL. Also, the malignant cells are typically smaller, although they may resemble LP cells. Most characteristically, small B cells are absent and most of the infiltrating cells are T cells and histiocytes. Diffuse areas in NLPHL may resemble TCRBCL. However, the presence of focal areas typical of NLPHL allows a correct diagnosis of NLPHL (Fan et al. 2003). NLPHL may rarely contain sheets of LP cells. However, its nodular architecture as well as the presence of histologic features in other areas of the biopsy that are typical of NLPHL distinguishes NLPHL with large numbers of LP cells from diffuse large B-cell lymphoma.

18.3 Clinical Characteristics

The most comprehensive data on patient characteristics and clinical course of patients with NLPHL are from a large retrospective analysis performed by the German Hodgkin Study Group (GHSG). Three hundred and ninety-four confirmed cases of NLPHL were compared with 7,904 cHL patients. The median age for NLPHL patients was 37 years and 75 % were male (Nogova et al. 2008). The majority (63 %) of newly diagnosed NLPHL patients had early-stage favorable, 16 % early-stage unfavorable, and 21 % advanced-stage disease. In contrast, patients with cHL more often had early-stage unfavorable (39 %) or advanced-stage (39 %) disease. In NLPHL, peripheral lymph node sites, particularly cervical and inguinal areas, were most often affected, and in contrast to cHL, fewer patients had B symptoms (9 % vs. 40 %)

Table 18.2 Characteristics of NLPHL and cHL patients

	NLPHL ($n=394$)	cHL ($n=7{,}904$)
Age (median)	37	33
Male gender (%)	75	56
B symptoms (%)	9	40
Early favorable stages (%)	63	22
Early unfavorable stages (%)	16	39
Advanced stages (%)	21	39

Adopted from Nogova et al. (2008)

(Table 18.2). Classical clinical risk factors were less common in NLPHL: three nodal areas (28 % vs. 55 %), elevated erythrocyte sedimentation rate (ESR) (4 % vs. 45 %), mediastinal bulk of more than one-third of the maximum thoracic width (31 % vs. 55 %), extranodal involvement (6 % vs. 14 %), and elevated lactate dehydrogenase (LDH) (16 % vs. 32 %).

Risk factors associated with poor outcomes in terms of freedom from treatment failure (FFTF) in the GHSG analysis were advanced stage ($p=0.0092$), hemoglobin <10.5 g/dl ($p=0.0171$), and lymphopenia (<8 % of white cell count; $p=0.01$). For overall survival (OS), hemoglobin <10.5 g/dl ($p=0.0014$), age ≥45 years ($p=0.0125$), and advanced stage ($p=0.0153$) were negative prognostic factors. Another long-term observation study from Jackson and colleagues which included 88 NLPHL patients with a median follow-up of 13 years identified stage at diagnosis, low albumin, presence of B symptoms, and poor initial response to treatment as factors associated with an inferior OS (Jackson et al. 2010).

18.4 Transformation to Non-Hodgkin Lymphoma

It is well recognized that NLPHL tends to transform into aggressive non-Hodgkin lymphoma (NHL) even 15–20 years after the initial diagnosis, and TCRBCL is the most common histology at transformation. Recently, two reports addressing this issue have been published. A registry-based analysis from France included 195 patients initially diagnosed with NLPHL between 1973 and 2003. For a variety of reasons, such as histological review of diagnosis, 31 patients were excluded so that the final analysis included 164 NLPHL patients. Sixty-six patients had recurrence of lymphoma of which 19 presented with histological transformation into aggressive NHL at a median of 4.7 years after initial diagnosis. Survival of these patients was inferior as compared to patients with NLPHL histology at the time of relapse (Biasoli et al. 2010).

A second report from the British Columbia Cancer Agency (BCCA) reported that 13 of 95 NLPHL cases had transformed into aggressive NHL at a median of 8.1 years. The actuarial risks for the development of transformed lymphoma after initial diagnosis of NLPHL were 5, 7, 15, 31, and 36 % after 5, 10, 15, 20, and 25 years, respectively, with one cluster of early transformation less than 3 years after initial lymphoma diagnosis (5/13) and another cluster of late transformation occurring 10–25 years (7/13) after initial lymphoma diagnosis. Transformation was more likely in patients with initial splenic involvement ($p=0.006$). In this series, prognosis after diagnosis of aggressive NHL was also worse than expected after NLPHL relapse. With multi-agent chemotherapy mostly followed by high-dose chemotherapy and autologous stem cell transplantation (ASCT), 10-year estimates for progression-free survival (PFS) and OS were 52 and 62 % at a median follow-up of 8.1 years after transformation (Al-Mansour et al. 2010). Collectively, the transformation rates reported in the analyses mentioned above underscore the importance of long-term follow-up of patients with NLPHL and the necessity of a rebiopsy at relapse.

18.5 Treatment

Traditionally, NLPHL patients are treated according to standard HL protocols and excellent prognosis has been reported. In a retrospective review by the European Task Force on Lymphoma (ETFL), 219 stage I/II NLPHL patients were treated with standard HL radiation therapy (RT) and/or chemotherapy protocols resulting in an excellent OS of >90 % (Diehl et al. 1999). A comprehensive analysis from Nogová and colleagues reported that FFTF and OS rates of NLPHL

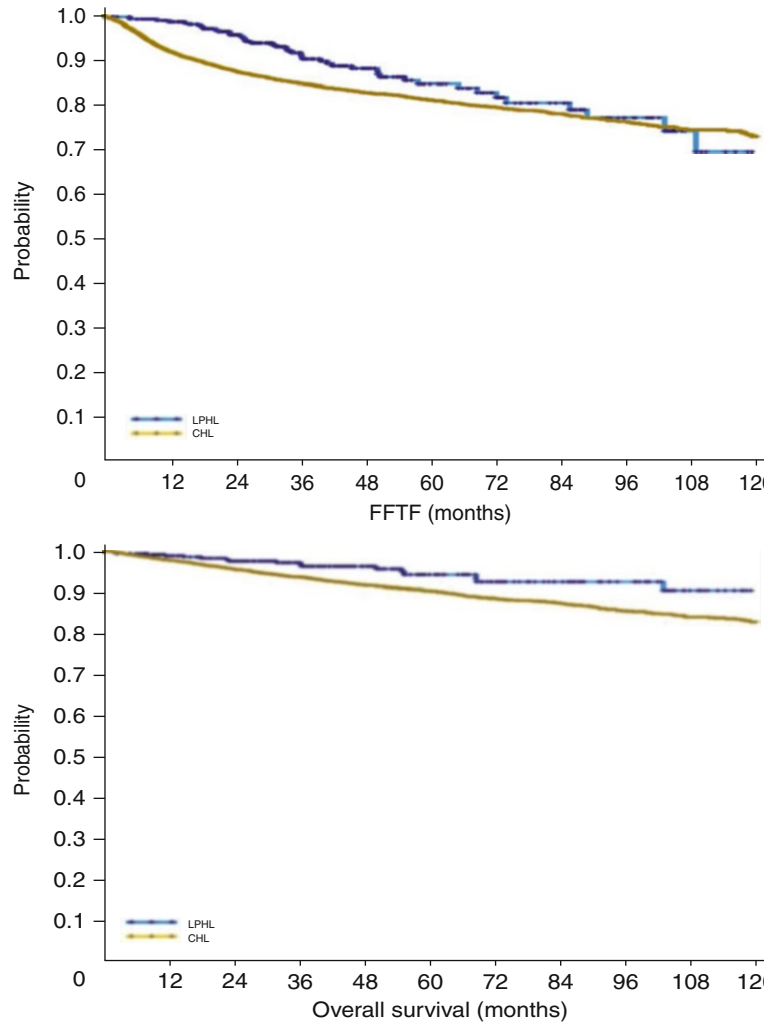

Fig. 18.2 FFTF (*above*) and OS (*below*) in NLPHL and cHL patients (Adopted from Nogova et al. 2008). *Legend*: *Blue curves*, NLPHL; *yellow curves*, cHL

patients were significantly superior as compared to cHL patients treated with similar protocols (FFTF: 88 % vs. 82 %, $p=0.093$; OS: 96 % vs. 92 %, $p=0.016$) (Fig. 18.2). However, in accordance with the indolent clinical behavior of NLPHL, late relapses were more frequent than in cHL particularly among patients initially diagnosed with advanced disease. Since most of these relapses were successfully salvaged, overall death from NLPHL was less common than death from secondary malignancies or other causes (Tsai and Mauch 2007). Due to the disproportion between lymphoma-related deaths and treatment-related deaths, current treatment approaches for NLPHL focus on minimizing therapy-related late effects and optimizing efficacy. Several groups have evaluated less toxic strategies including observation, involved-field RT (IF-RT), and more recently the anti-CD20 monoclonal antibody rituximab.

18.5.1 Treatment of Early Favorable Stages

Patients with early favorable NLPHL have an excellent outcome when treated with standard HL approaches. For most NLPHL patients in early favorable stages, RT has been the mainstay of treatment. According to GHSG data published in a large comprehensive analysis, FFTF at a median follow-up of 50 months was 93 % for this subgroup of patients (Nogova et al. 2008).

The Australasian Radiation Oncology Lymphoma Group performed a retrospective analysis including 202 stage I/II patients treated with RT alone between 1969 and 1995. Radiation fields included full mantle field, modified mantle field, inverted-Y field, modified inverted-Y field, and total lymph node irradiation. The median RT dose applied was 36 Gy. At a median follow-up of 15 years, the estimated 15-year PFS rate was 82 %; the estimated OS rate was 83 %. Among the 17 % of patients who had died after 15 years, only 3 % died from NLPHL, while 2 % died from secondary NHL, 2 % from in-field secondary solid tumors, 4 % from cardiac and respiratory reasons, and 6 % from other causes (Wirth et al. 2005).

The GHSG retrospectively analyzed 131 NLPHL patients diagnosed with stage IA disease without clinical risk factors. Patients received either extended-field RT (EF-RT) ($n=45$), IF-RT ($n=45$), or combined-modality treatment ($n=41$). Overall, 99 % of patients achieved a complete remission (CR) and 5 % relapsed (9 % after EF-RT and 2 % each after IF-RT and combined-modality treatment). The OS rate at 24 months irrespective of the treatment modality applied was 100 %; however, with longer follow-up it dropped slightly to 94 % for EF-RT (median follow-up = 78 months) and 96 % for combined-modality treatment (median follow-up = 40 months). In patients treated with EF-RT or IF-RT, acute toxicity was mild with grade III toxicity in only 2.2 % of patients and no grade IV toxicity. In contrast, 39 % of patients experienced grade III toxicity and 9.8 % grade IV toxicity when treated with combined-modality treatment (Nogova et al. 2005). Due to the natural clinical behavior of NLPHL, mature follow-up will be required to assess long-term efficacy as well as toxicity.

Chen and colleagues recently published long-term data on the clinical outcome of early-stage NLPHL patients treated at a single institution. 113 patients diagnosed with stage I/II NLPHL between 1970 and 2005 with a median follow-up of 136 months were included in the analysis (Chen et al. 2010). Ninety-three patients were treated with RT alone (limited field = 22 %, regional field = 31 %, EF-RT = 41 %), 13 with combined-modality approaches, and seven with chemotherapy alone. Patients treated with RT alone had an excellent clinical outcome with 5-, 10-, and 15-year PFS rates of 95, 89, and 76 % and 86, 72, and 50 % for patients diagnosed with stage I and II, respectively. While the PFS for patients with stage II disease was significantly worse than for stage I patients ($p<0.006$), OS rates were not significantly different ($p=0.53$) due to successful salvage therapy. Relapses observed after initial RT were by and large late, occurring more than 10 years after first-line treatment. Secondary solid tumors represented the main cause of death. It is important to note that most patients considered for this analysis, as well as those included in the Australasian report, were treated with outdated RT doses and fields. Recent data suggest that contemporary RT techniques, such as IF-RT or involved node RT (IN-RT), likely have a lower risk of secondary solid tumors (De Bruin et al. 2009). Currently, on the basis of the above studies reporting similar results for IF-RT compared with EF-RT, cooperative study groups such as the European Organisation for Research and Treatment of Cancer (EORTC), the GHSG, and the US National Cancer Center Network (NCCN) recommend IF-RT alone for the treatment of stage IA NLPHL.

A French phase II study has evaluated surgical resection alone in pediatric patients aged 4–16 years. Of 27 patients reported, 13 had surgical lymphadenectomy only, while 14 received additional treatment consisting of combined-modality treatment ($n=10$), chemotherapy alone ($n=3$), or IF-RT ($n=1$). At a median follow-up of 70 months, although the event-free survival (EFS) was significantly better for patients who received additional treatment (90 % vs. 42 %, $p=0.04$), there was no difference in OS (100 %). Patients with a residual mass after diagnostic lymphadenectomy had a higher probability for relapse when receiving no additional therapy than those in CR after surgical lymphadenectomy (Pellegrino et al. 2003).

The European Network Group on Pediatric Hodgkin Lymphoma (EuroNet-PHL) has also retrospectively analyzed resection only in limited-stage NLPHL. From a total of 58 children aged 4–17 years, 51 had CR after diagnostic lymph

Table 18.3 Rituximab for the treatment of NLPHL

Disease status	Stages included	Rituximab schedule	*n*	Response rate	PFS	References
Untreated	IA without RF	Standard	28	100 %	81.4 % at 36 m	Eichenauer et al. (2011)
Untreated	All stages	Standard/ extended	*S* = 10 *E* = 9	100 %	Median PFS: *S* = 50 m *E* = 67 m	Advani et al. (2011)
Untreated/relapsed	All stages	Standard	*U* = 12 *R* = 10	100 %	Median PFS: 10.2 m	Ekstrand et al. (2003)
Relapsed	All stages	Standard	15	94 %	Median PFS: 33 m	Schulz et al. (2008)

Standard schedule, Rituximab at 375 mg/m^2 for 4 consecutive weeks; extended schedule, standard schedule plus four rituximab doses every 6 months for 2 years
RF risk factor, *S* standard, *E* extended, U untreated, *R* relapsed, *PFS* progression-free survival, *m* months

node resection, while seven had residual lymphoma. At a median follow-up of 43 months, PFS rates were 57 % at 50 months for the entire group and 67 % at 26 months for patients in CR after surgery. All patients with incomplete resection eventually relapsed after a median of 17 months with no impact on OS (100 %). Collectively, these data suggest that watch and wait might be an option in carefully selected stage I patients in CR after diagnostic lymphadenectomy but cannot be routinely recommended in clinical practice.

Since consistent CD20 expression represents a hallmark of NLPHL, studies have prospectively evaluated the anti-CD20 antibody rituximab (Table 18.3). In a study conducted by the GHSG, 28 patients with stage IA disease without clinical risk factors received four weekly standard doses (375 mg/m^2) of rituximab. The recently published final analysis showed an impressive overall response rate (ORR) of 100 %. However, at a median follow-up of 43 months, 25 % of patients relapsed suggesting that rituximab alone appears to be less effective than standard RT (Eichenauer et al. 2011).

Another study from the Stanford group included 13 previously untreated early-stage NLPHL patients (six stage I and seven stage II patients) who received rituximab as single agent. While the response rate was 100 %, the relapse rate was similar to the GHSG analysis (Advani et al. 2011). Cumulatively, these studies suggest that rituximab alone cannot be recommended as first-line therapy for the majority of newly diagnosed NLPHL patients with early favorable stages.

Savage and colleagues recently published a retrospective analysis comparing the outcome of 32 early-stage NLPHL patients treated with RT alone between 1966 and 1993 with the outcome of 56 patients treated with two cycles of ABVD (adriamycin, bleomycin, vinblastine, dacarbazine) or ABVD-like chemotherapy followed by RT between 1993 and 2009. At 10 years, PFS and OS rates for patients treated with RT alone were 65 and 84 %, respectively, while patients who received combined-modality treatment had PFS and OS rates of 91 and 93 %, respectively, suggesting that combined-modality treatment may be superior to RT alone (Savage et al. 2011). However, these findings have to be interpreted with caution as the patients considered were treated over four decades (1966–2009) and other factors besides the treatment modality could have had significant impact on the outcome. For example, supportive care may have varied considerably between individual patients. In addition, the combined-modality treatment group had a much shorter follow-up (5.7 years) than the RT alone group (18.6 years). As relapses in NLPHL occur late, the inferior outcome of patients treated with RT alone may thus simply relate to the longer follow-up as compared to the combined-modality group.

In summary, there is consensus that IF-RT at 30 Gy alone is standard of care for patients with newly diagnosed stage IA NLPHL since clinical outcome is excellent. The major goal for the future consists in a reduction of radiation doses and fields.

However, first attempts using 2×2 Gy IF-RT led to relapses in five of nine patients (Haas et al. 2009). For patients with stage IB-IIB disease, the GHSG and the EORTC recommend combined-modality treatment, while the NCCN guidelines recommend RT only for stage IIA disease and combined-modality therapy for stage IB/IIB patients. Although treatment of NLPHL with rituximab leads to impressive response rates, relapse is more common than after RT or combined-modality approaches; therefore, rituximab alone cannot be routinely recommended in clinical practice. The question whether chemotherapy alone might be an option in patients with early favorable NLPHL has not been addressed to date.

18.5.2 Treatment of Early Unfavorable Stages

To date, patients with NLPHL with early unfavorable stages have been treated with combined-modality approaches developed for cHL with comparable outcomes. A retrospective analysis by the GHSG reported FFTF rates at 50 months of 87 % for NLPHL and 85 % for cHL patients (Nogova et al. 2008). Since only a minority of NLPHL patients are diagnosed in early unfavorable stages, prospective data for this subset are lacking. It may be reasonable to consider the addition of rituximab to chemotherapy; however, no definitive data are available.

18.5.3 Treatment of Advanced Stages

As with early unfavorable stages, patients with advanced NLPHL are often treated according to cHL chemotherapy protocols, such as ABVD and escalated BEACOPP (bleomycin, etoposide, adriamycin, cyclophosphamide, vincristine, procarbazine, prednisone). The largest series supporting the use of regimens similar to those used in cHL comes from the GHSG. A comprehensive retrospective analysis including 394 NLPHL and 7,904 cHL patients noted similar 50-month FFTF rates of 77 and 75 % for NLPHL and cHL patients, respectively, when treated with standard protocols (Nogova et al. 2008). Chemotherapy in this report consisted of COPP (cyclophosphamide, vincristine, procarbazine, prednisone)/ABVD, COPP/ABV/IMEP (ifosfamide/methotrexate/etoposide/prednisone), $BEACOPP_{baseline}$, and escalated BEACOPP. It is important to note that these regimens contain significantly higher doses of alkylating agents than ABVD alone and may therefore be equally effective for cHL and NLPHL. This theory is supported by another retrospective analysis of 37 patients with advanced NLPHL by the Cancer and Leukemia Group B (CALGB) (Canellos and Mauch 2010). Patients were treated with MOPP (mechlorethamine, vincristine, procarbazine, prednisone) and MOPP/ABVD or ABVD/EVA (etoposide, vinblastine, adriamycin). The major finding of this report was a relapse rate of 75 % among patients treated with ABVD/EVA but only 32 % among patients treated with MOPP or MOPP/ABVD suggesting that an alkylator-based chemotherapy may be more suitable for the treatment of NLPHL, at least in advanced stages.

There are also retrospective data on the use of R-CHOP (rituximab, cyclophosphamide, adriamycin, vincristine, prednisone) in advanced NLPHL reporting response rates up to 100 % (Fanale et al. 2010). It is speculative to consider this approach as an option particularly for patients with splenic involvement and abdominal disease due to an increased risk for transformation into aggressive NHL observed in this patient group (Al-Mansour et al. 2010; Advani et al. 2011).

The use of rituximab as single agent has also been evaluated in patients with advanced-stage disease and cannot be recommended due to the increased relapse rate in comparison with other treatment approaches (Advani et al. 2011; Ekstrand et al. 2003). The role of RT in advanced NLPHL is currently undefined and needs to be addressed in future trials.

18.5.4 Treatment of Relapsed NLPHL

The majority of NLPHL patients diagnosed with early favorable disease are cured with first-line treatment. In contrast, patients with advanced

disease have an increased propensity to develop late relapses when compared with cHL, and the standard treatment for relapsed NLPHL is largely undefined (Nogova et al. 2008; Biasoli et al. 2010; Chera et al. 2007).

Studies have prospectively evaluated rituximab in the treatment of relapsed NLPHL (Table 18.3). The Stanford group conducted a phase II study including a total of 22 patients with either newly diagnosed or relapsed NLPHL. Patients received four weekly doses of rituximab at the standard dose of 375 mg/m^2 with an ORR of 100 %. However, at a median follow-up of 13 months, nine patients had relapsed with an estimated median PFS of 10.2 months (Ekstrand et al. 2003). The study was subsequently modified and responding patients received rituximab maintenance (four weekly standard doses every 6 months for 2 years) (Horning et al. 2007). At a median follow-up of 30 months for patients receiving extended rituximab treatment, the median freedom from progression (FFP) was not reached and FFP at 30 months was 88 %.

A similar study was conducted by the GHSG. Fifteen patients with relapsed NLPHL received four weekly standard doses of rituximab. All but one patient responded to treatment. After a median observation of 63 months, the median time to progression was 33 months; the median OS was not reached (Schulz et al. 2008). While both studies report an excellent ORR, the longer time to progression among patients treated within the GHSG trial might relate to several factors, such as variable inclusion criteria, prior treatments, and follow-up schedule.

Data on the use of the standard of care in relapsed cHL consisting of high-dose chemotherapy followed by ASCT for relapsed NLPHL are scarce. A British analysis of eight patients treated with this approach at first or second relapse reported recurrent disease in five cases suggesting that the effectiveness in NLPHL may not be the same as in cHL (Jackson et al. 2010). Given that there is no clear advantage for high-dose chemotherapy followed by ASCT, rituximab appears to be a reasonable choice for relapsed NLPHL due to the high ORR and the excellent tolerability. Other treatment modalities such as localized RT, conventional chemotherapy, or combined-modality approaches may also be options although no large series on these strategies have been published to date.

In summary, great progress has been made in understanding the biology of NLPHL including similarities and differences as compared with cHL. Optimal therapy however remains a challenge and international cooperation is necessary to address the ongoing controversies in clinical management.

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Primary Cutaneous B-Cell Lymphomas

19

Sima Rozati, Reinhard Dummer, Matthew A. Lunning, Steven Horwitz, German Ott, and Eric D. Hsi

Contents

Pathology: Eric D. Hsi and German Ott

S. Rozati, MD (✉) • R. Dummer, MD
Department of Dermatology, University of Zurich Hospital, Zurich, Switzerland
e-mail: sima.rozati@usz.ch; reinhard.dummer@usz.ch

M.A. Lunning, DO
Internal Medicine, University of Nebraska Medical Center, Omaha, NE 68198, USA

S. Horwitz, MD
Memorial Sloan-Kettering Cancer Center, New York, NY, USA
e-mail: horwitzs@mskcc.org

G. Ott, MD
Department of Clinical Pathology, Robert-Bosch-Krankenhaus, Stuttgart, Germany
e-mail: german.ott@rbk.de

E.D. Hsi, MD
Department of Clinical Pathology, Cleveland Clinic, L11, 9500 Euclid Ave, Cleveland, OH 44195, USA
e-mail: hsie@ccf.org

19.1 Introduction and Epidemiology

Cutaneous lymphomas (CL) are a heterogeneous group of neoplasias that are characterized by an accumulation of mononuclear, mostly lymphocytic cells in the skin (Burg et al. 2006). Cutaneous lymphomas are the second most prevalent extranodal non-Hodgkin lymphomas (after gastrointestinal), representing approximately 19 % of extranodal non-Hodgkin lymphomas. Primary cutaneous B-cell lymphomas represent less than one third of cutaneous lymphomas (Willemze et al. 2005; Bradford et al. 2009). Distinguishing between low-grade CBCL and reactive B-cell pseudolymphomas can be quite difficult; even clonality studies cannot with certainty separate the two entities (Dummer et al. 2008).

The World Health Organization-European Organization for Research and Treatment of Cancer (WHO-EORTC) has categorized primary cutaneous B-cell lymphomas to three

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main subtypes: primary cutaneous follicle center lymphoma(PCFCL), primary cutaneous marginal zone lymphoma(PCMZL), and primary cutaneous diffuse large B-cell lymphoma (DLBCL) ("leg type" and "others") (Willemze et al. 2005). The relevance to distinguish these subtypes is in the different treatment options as well as different prognosis (Dummer et al. 2007). MZL and PCFCL are characterized as indolent disease with 99–95 % 5-year survival in comparison to DLBCL, leg type that has a more aggressive nature and less than a 50 % 5-year survival (Senff et al. 2007). The diagnosis of primary cutaneous B-cell lymphoma is only established when the complete staging is negative after the initial clinical and histopathological diagnosis.

19.2 Staging

The Ann Arbor system, first introduced as a staging system for Hodgkin disease in 1971, is the most widely used staging system for lymphoma. However, the Ann Arbor system has limited prognostic value when evaluating patients with extranodal lymphomas such as the cutaneous lymphomas (Rosenberg 1977). Therefore, in 2007, the International Society for Cutaneous Lymphoma and European Organization of Research and Treatment of Cancer (ISCL/EORTC) proposed anatomical classification of primary cutaneous lymphoma other than Mycosis fungoides (MF) and Sézary syndrome (SS) for documentation of disease extent and not necessarily as a prognostic guide (Table 19.1, Kim et al. 2007). ISCL/EORTC recommends complete staging at the time of initial diagnosis including a thorough history and physical exam; laboratory studies including complete blood count, comprehensive blood chemistry, and lactate dehydrogenase(LDH) level; and obtaining appropriate images (CT or PET-CT) of at least the chest, abdomen, and pelvis. Bone marrow biopsy is not needed in indolent CBCL (i.e., PCMZL) but is required in clinically intermediate to aggressive cutaneous B-cell lymphoma (Kim et al. 2007).

Table 19.1 ISCL/EORTC proposal on TNM classification of cutaneous lymphoma other than MF/SS (Kim et al. 2007)

T
T1: Solitary skin involvement
T1a: A solitary lesion <5 cm diameter
T1b: A solitary >5 cm diameter
T2: Regional skin involvement: multiple lesions limited to one body region or two contiguous body regions*
T2a: All-disease encompassing in a <15-cm-diameter circular area
T2b: All-disease encompassing in a >15- and <30-cm-diameter circular area
T2c: All-disease encompassing in a >30-cm-diameter circular area
T3: Generalized skin involvement
T3a: Multiple lesions involving two noncontiguous body regions
T3b: Multiple lesions involving >3 body regions
N
N0: No clinical or pathologic lymph node involvement
N1: Involvement of one peripheral lymph node region† that drains an area of current or prior skin involvement
N2: Involvement of two or more peripheral lymph node regions† or involvement of any lymph node region that does not drain an area of current or prior skin involvement
N3: Involvement of central lymph nodes
M
M0: No evidence of extracutaneous non-lymph node disease
M1: Extracutaneous non-lymph node disease present

*Definition of body regions (see Fig. 19.1): Head and neck: inferior border—superior border of clavicles, T1 spinous process. Chest: superior border—superior border of clavicles; inferior border—inferior margin of rib cage; lateral borders—midaxillary lines, glenohumeral joints (inclusive of axillae). Abdomen/genital: superior border—inferior margin of rib cage; inferior border—inguinal folds, anterior perineum; lateral borders—mid-axillary lines. Upper back: superior border—T1 spinous process; inferior border—inferior margin of rib cage; lateral borders—mid-axillary lines. Lower back/buttocks: superior border—inferior margin of rib cage; inferior border—inferior gluteal fold, anterior perineum (inclusive of perineum); lateral borders—midaxillary lines. Each upper arm: superior borders—glenohumeral joints (exclusive of axillae); inferior borders—ulnar/radial-humeral (elbow) joint. Each lower arm/hand: superior borders—ulnar/radial-humeral (elbow) joint. Each upper leg (thigh): superior borders—inguinal folds, inferior gluteal folds; inferior borders—mid-patellae, midpopliteal fossae. Each lower leg/foot: superior borders—mid-patellae, mid-popliteal fossae

†Definition of lymph node regions is consistent with the Ann Arbor system: Peripheral sites: antecubital, cervical, supraclavicular, axillary, inguinal-femoral, and popliteal. Central sites: mediastinal, pulmonary hilar, paraortic, iliac

19.3 Definitions and Clinical Features

19.3.1 Primary Cutaneous Marginal Zone Lymphomas (PCMZL)

Primary cutaneous marginal zone lymphomas of MALT type (PCMZL), previously known as primary cutaneous immunocytomas, comprises 24 % of all primary cutaneous B-cell lymphomas (Senff et al. 2007). They present as single or multiple lesions, with multifocal lesions being more common (72 %) (Hoefnagel et al. 2005a). MZL affects most commonly adults between third and fifth decade of life with a male to female ratio of 2.1. MZL presents as red to violaceous infiltrated cutaneous and subcutaneous plaques or multifocal nodules with a diameter of less than 2 cm (Golling et al. 2008). Over half of patients (55 %) have lesions on the trunk with upper and lower extremities being involved in 37 and 27 % of patients, respectively. The head and neck are involved in 14 % of patients. The tumors display slow growth and usually do not ulcerate. Biopsies should be deep in order to reflect the extent of the infiltrate. In Europe there have been cases of CMZL associated with Borrelia burgdorferi infection (Hoefnagel et al. 2005a; Aberer et al. 2011). An association of primary cutaneous MALT lymphomas with infectious etiologies (similar to *H. pylori* and gastric MALT lymphomas) has been postulated for many years, and *Borrelia burgdorferi* has been suggested and found in a fraction of cases in some series; however, recent reports are contradictory (Cerroni et al. 1997; Goodlad et al. 2000a; Ponzoni et al. 2011; Wood et al. 2001). Although still debatable, it has been suggested that MZL is associated with chronic inflammatory process or infections (Zendri et al. 2005; May et al. 2005). *H pylori* infection is often found in MALT lymphomas of the gastric mucosa and intestine, and remission has been induced by treatment of the underlying infection. This may also hold true for a subset of patients with cutaneous B-cell lymphomas (Bogle et al. 2005).

19.3.2 Primary Cutaneous Follicle Center Lymphoma (PCFCL)

PCFCL is the most common type of primary cutaneous B-cell lymphomas, making up 57 % of cases in a recent large review. The median age at diagnosis is 58 years with a male/female ratio of 1.8 (Senff et al. 2007). It presents as an erythematous papule, plaque, or nodule most commonly located on the trunk or head/neck. Lesions may be single or multiple but are localized when multiple. It is only rarely seen on the upper (2.3 % of patients) or lower (6.4 % of patients) extremities. The latter is at times difficult to differentiate from DLBCL, LT.

19.3.3 Primary Cutaneous Diffuse Large B-Cell Lymphoma, Leg Type (PCDLBL-LT)

PCDLBCL-LT comprises approximately 20 % of primary cutaneous B-cell lymphomas and has several distinctive clinical features. Compared to the above two types, this lymphoma occurs in an older population (median age 78 years) and has a striking female predominance (M:F ratio of 0.5). As the name implies, it presents most commonly (88 % of patients) on the lower extremity. However, it can occur at other sites including the head/neck, trunk, and upper extremities in 5–12 % of patients (Willemze et al. 2005; Senff et al. 2007). Clinically it presents as a nodule, either singly or as multiple regional lesions. Multifocal disease is seen in 20 % of cases. Uncharacteristic to other cutaneous lymphomas, PCDLBCL-LT will often disseminate to nodal and visceral site, which likely portends a transition in its already aggressive behavior (Grange et al. 2007; Vermeer et al. 1996).

19.4 Pathology

PCMZL manifests as a mid-dermal lymphoid infiltrate that can extend into the superficial dermis and deep dermis/subcutis. As in other marginal zone lymphomas, reactive germinal centers are usually

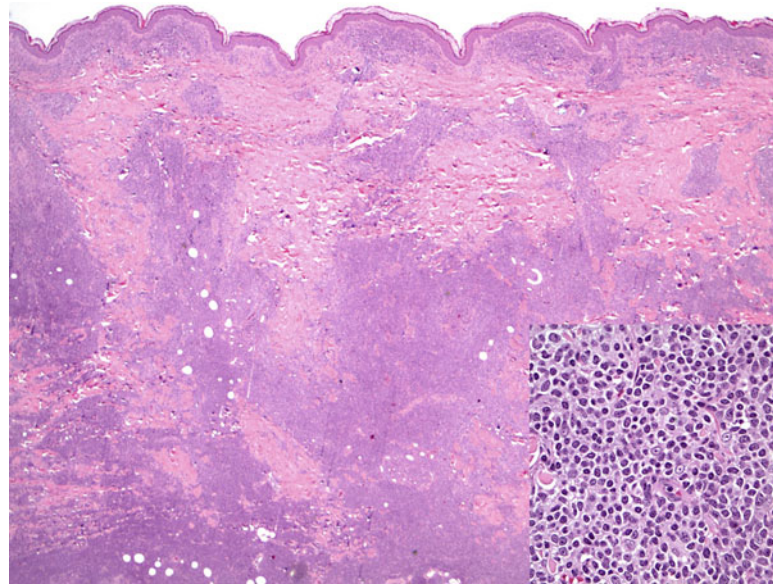

Fig. 19.1 Primary cutaneous marginal zone lymphoma. A dense lymphoid infiltrate is seen (hematoxylin and eosin, H&E, 20×). The inset shows the cytologic features of marginal zone cells (H&E 400×)

present but may become colonized and eventually obliterated. The cells are small with condensed chromatin, slight nuclear irregularities, and moderate-to-abundant amounts of pale cytoplasm. These marginal zone cells are often centered on the residual lymphoid follicles and expand into the surrounding dermis (Fig. 19.1). Admixed plasma cells may be present. These can represent plasmacytic differentiation of the lymphoma or an inflammatory component. Dutcher bodies, if present, would favor the former and investigation of the plasma cell component by immunohistochemistry or in situ hybridization for kappa and lambda immunoglobulin light chain expression is advisable. Although lymphoepithelial lesions (destructive infiltration of epithelium by clusters of lymphoma cells) are common in MALT-type lymphomas at sites such as stomach or salivary glands, they are not frequently seen in PCMZLs. When present they are usually seen in hair follicle epithelium. Some cases may have an extensive reactive, nonneoplastic lymphoid infiltrate and may be the type composed of class-switched PCMZL (Edinger et al. 2010). An eosinophilic infiltrate has been described in cases originating in Asia (Takino et al. 2008).

PCFCL has a heterogeneous appearance. The common features are a dense dermal lymphoid infiltrate of follicle center cells (varying proportions centrocytes and centroblasts). The architecture may be follicular, follicular and diffuse, or completely diffuse. Follicles may have attenuated or absent mantle zones, are not polarized, and lack tingible body macrophages. In diffuse examples that are composed of predominantly centroblastic cells, the histopathology may be that of a diffuse large B-cell lymphoma in other sites; however, the appropriate diagnosis given the anatomic site is still PCFCL (Fig. 19.2) (Cerroni and Kerl 2001a, b; Goodlad et al. 2002; Mirza et al. 2002). Thus, this lymphoma is defined more by the type of cells (follicle center cells) rather than architecture.

PCDLBL-LT shows a diffuse architecture and is entirely composed of large immunoblastic or centroblastic cells with round nuclear contours and variably prominent nucleoli (Fig. 19.3) (Vermeer et al. 1996). The dominant round cell morphology contrasts with PCFCL. Mitotic figures are easily found and characteristically there are few infiltrating small reactive lymphocytes.

19.5 Immunophenotype

The immunophenotype of PCMZL is CD19+, CD20+, CD5−, and CD10− with monotypic immunoglobulin light chains often demonstrable in paraffin sections (Fig. 19.4). The cells are often class switched (IgG, IgA, IgE), unlike other

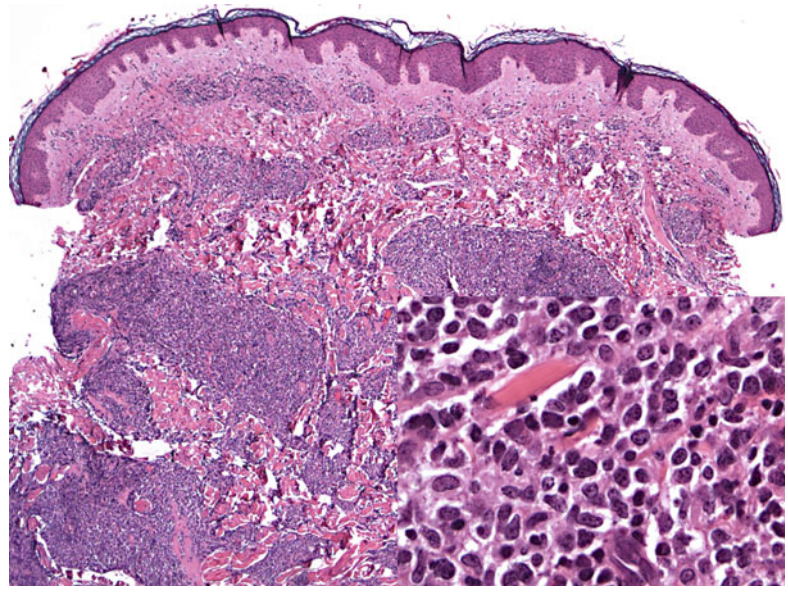

Fig. 19.2 Primary cutaneous follicle center lymphoma. A dense dermal lymphoid infiltrate is present (Fig. 19.2, H&E 400×) and is composed of follicle center-type cells (*Inset*, H&E 400×)

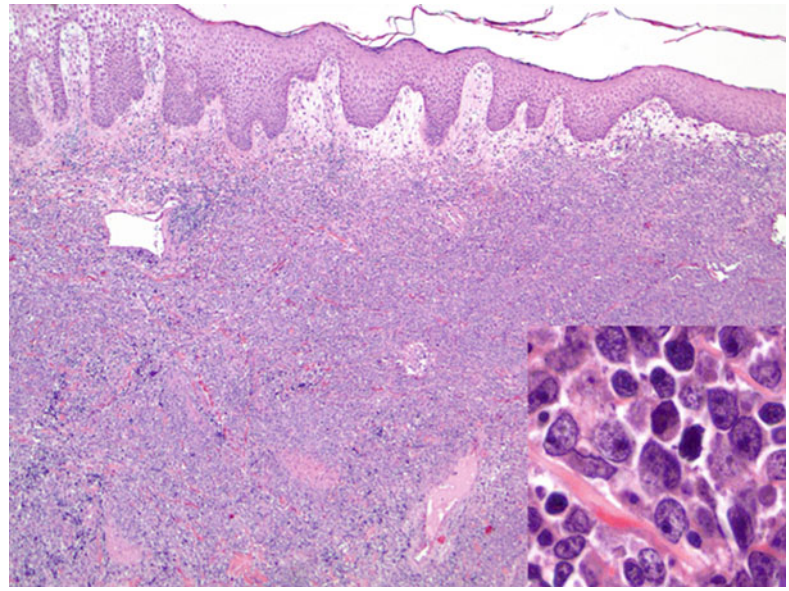

Fig. 19.3 Diffuse large B-cell lymphoma, leg type. A dense dermal lymphoid infiltrate is present and is composed of monotonous round large cells

types of extranodal marginal zone B-cell lymphomas. BCL2 is expressed in most cases (Edinger et al. 2010; Servitje et al. 2002; Cho-Vega et al. 2006). The characteristic presence of reactive follicles can be highlighted by a CD21 stain that marks follicular dendritic cells.

PCFCL expresses pan-B-cell markers CD19 and CD20 but also coexpresses BCL6. Other germinal center B-cell markers are also often expressed including CD10 and HGAL (Xie et al. 2008). Unlike nodal follicular lymphoma, in which BCL2 expression is a hallmark that reflects a t(14;18)(q32;q21), PCFCL is characteristically negative for BCL2 (Fig. 19.5). However, in examples that are follicular, especially when predominantly small cleaved cells, BCL2 is expressed in approximately 40 % of cases (Mirza et al. 2002; Xie et al. 2008).

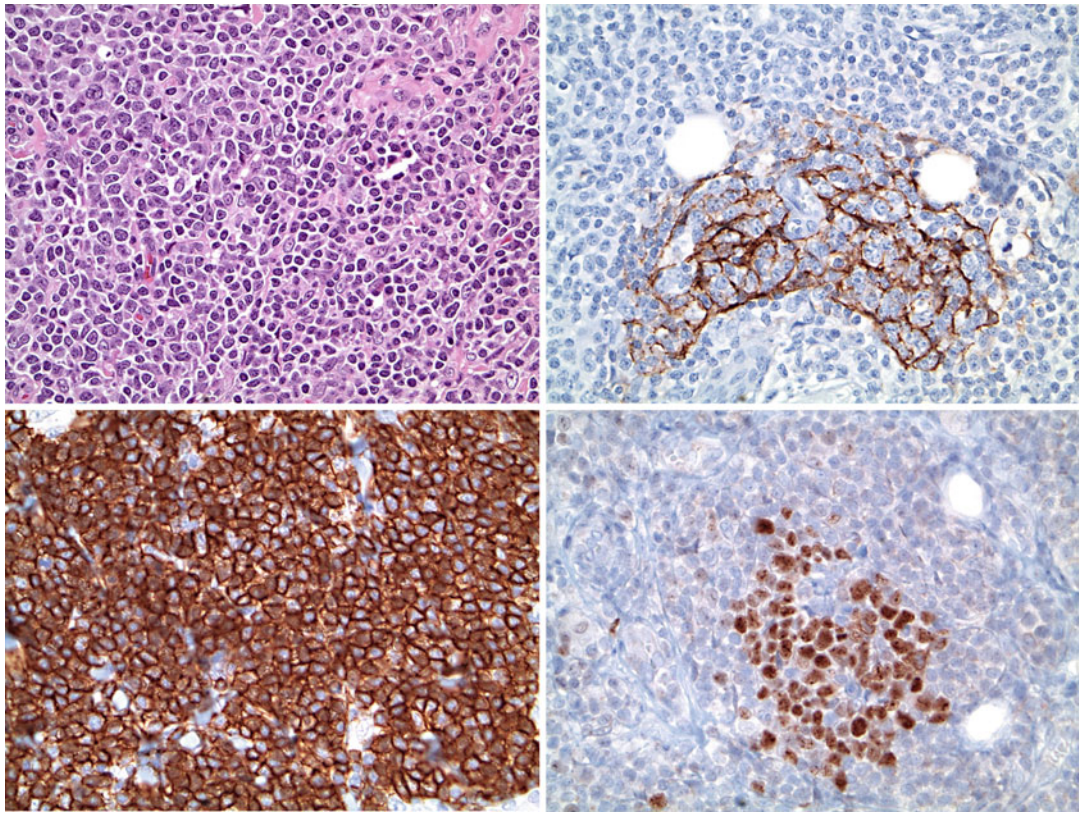

Fig. 19.4 Primary cutaneous marginal zone lymphoma. The *upper left panel* shows a remnant germinal center (*upper left*) with clusters of centroblastic cells. The marginal zone component extends into the surrounding dermis. A CD21 stain (*upper right*) showed one of many remnant follicular dendritic cell network. CD20 is expressed (*lower left*) and a BCL6 stain (*lower left*) shows that residual germinal center B cells are present but undergoing colonization by the neoplastic cells

PCDLBL-LT also expresses CD19 and CD20. Unlike PCFCL, expression of BCL2 is the rule and the post-germinal center B-cell maker MUM1 is usually expressed. BCL6 is expressed by most cases, but CD10 is not (Fig. 19.6) (Xie et al. 2008; Geelen et al. 1998; Grange et al. 2004; Hoefnagel et al. 2003; Sundram et al. 2005).

19.6 Molecular Genetics

Application of modern PCR-based methods for determining monoclonality in formalin-fixed tissue has greatly increased our ability to diagnose cutaneous lymphomas. At least 85 % of cases demonstrate monoclonality(Morales et al. 2008). However, since monoclonality can be seen rarely in reactive processes, interpretation in the context of histopathologic and immunophenotypic findings is essential (Morales et al. 2008; Fujiwara et al. 2013; Nihal et al. 2000). t(11;18)(q21;q21) involving *API2-MALT1* and t(3;14)(p14;q32) involving *FOXP1* and *IGH@* are seen in less than 10 % of cases. The t(14;18)(q32;q21) also involving *IGH@* and *MALT1* is present in less than 15 % of cases (Cho-Vega et al. 2006; Streubel et al. 2004). Molecular studies looking for a causative microorganism similar to *H. pylori* in gastric marginal zone lymphomas have raised the possibility of *Borrelia burgdorferi*; however, detection of this organism has not been consistent and a potential role has not been established (Ponzoni et al. 2011; Cho-Vega et al. 2006; Goodlad et al. 2000b; Roggero et al. 2000). The

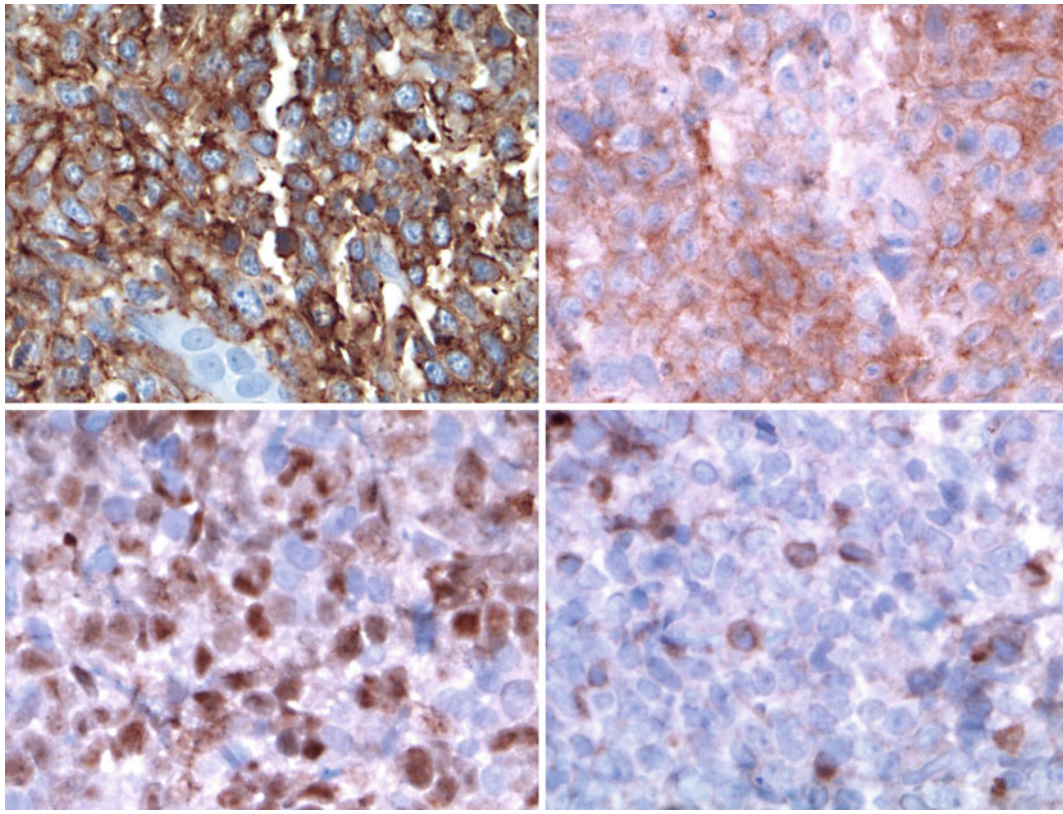

Fig. 19.5 Primary cutaneous follicle center lymphoma immunohistochemistry. The cells express CD20 (*upper left*), CD10 (*upper right*), and BCL6 (*lower left*) but are negative for BCL2 (*lower right*)

IGH@-BCL2 translocation typically seen in nodal follicular lymphoma can be seen in 0–40 % of cases of PCFCL with a follicular growth pattern (Mirza et al. 2002; Streubel et al. 2006; Cerroni et al. 2000). Variation may be related to technique (Streubel et al. 2006). Gene expression profiling studies have shown that the profile resembles germinal center-like diffuse large B-cell lymphomas (Hoefnagel et al. 2005b).

PCDLBL-LT lacks translocations seen in MALT-type lymphomas or follicular lymphoma. However, translocations of *BCL6, MYC*, and *IGH@* and amplification of BCL2 are commonly seen. Deletion in the region of cell cycle inhibitors *CDKN2A* and *CDK2NB* (chromosome 9p21.3) or promoter methylation is frequent and associated with poor outcome (Dijkman et al. 2006; Hallermann et al. 2004). Gene expression profiling shows a distinct profile from PCFCL and similarity to activated B-cell type of diffuse large B-cell lymphoma (Hoefnagel et al. 2005b).

19.7 Differential Diagnosis

The differential diagnosis of PCMZL is often a form of cutaneous lymphoid hyperplasia (CLH) that is B-cell rich due to the presence of reactive follicles. The follicles in CLH should contain preserved mantle zones and the overall immune architecture is preserved, with distinct B- and T-cell areas. Expansion of B cells with a marginal zone appearance away from follicles and demonstration of monotypic plasma cells or monoclonality by molecular methods strongly support lymphoma. As noted above, monoclonality by PCR-based methods can be seen in reactive conditions. In difficult cases, demonstration of the

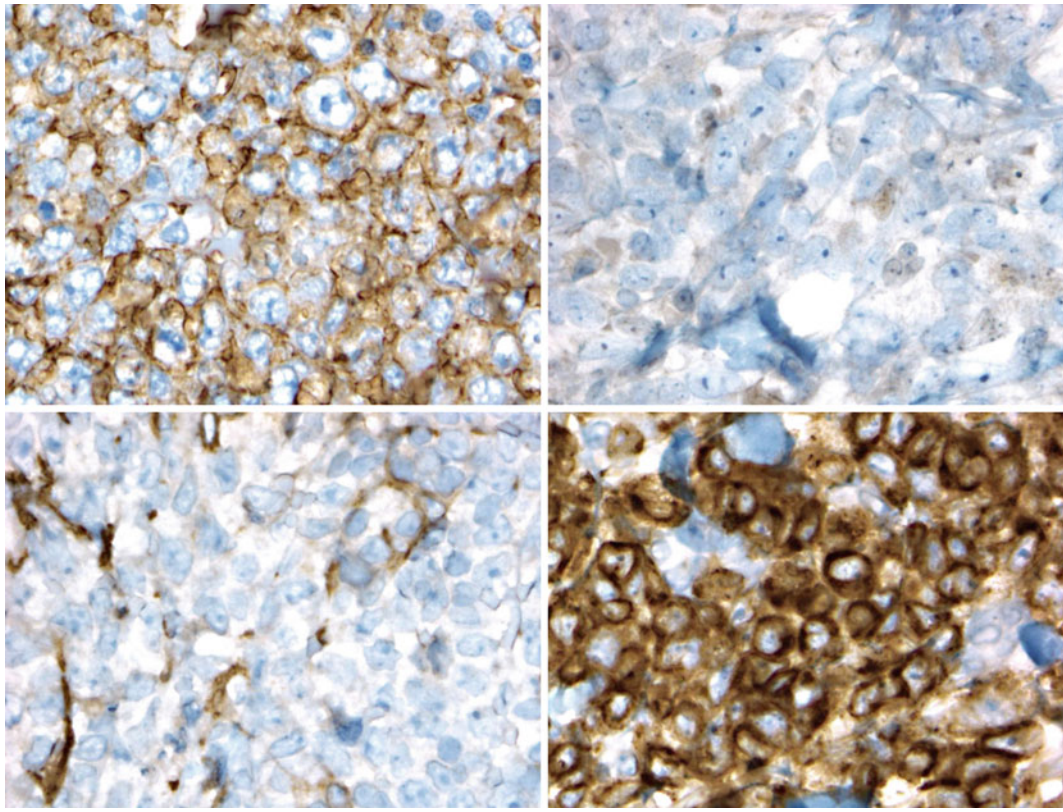

Fig. 19.6 Diffuse large B-cell lymphoma, leg type. Immunohistochemistry shows that the cells express CD20 (*upper left*) and BCL2 (*lower right*) but negative for BCL6 (*lower right*) and CD10

same clone in another lesion or subsequent lesion (identical clone separated in time or space) can help confirm a diagnosis of lymphoma. MZL is at times difficult to differentiate from pseudolymphomas, although increased general awareness between dermatologist and establishment of new markers have facilitated a more accurate diagnosis (Jenni et al. 2011). Skin manifestation of B-CLL can precede its systemic presentation by weeks to months (Cerroni et al. 1996); therefore, the appropriate diagnosis is important since the treatments are completely different. The detection of an immunoglobulin light chain restriction and immunohistochemical staining for CD5, CD23, and CD43 can be helpful in the differentiation of these two entities (Levin et al. 2012). The plasma cell-rich variants of MZL can resemble skin infiltrates by a plasma cell myeloma, but the latter entities can be recognized by adequate staging (Kempf et al. 2012).

PCFCL with a follicular pattern can be differentiated from cutaneous follicular hyperplasia by presence of monomorphous follicles, lack of polarization, and absence of tingible body macrophages in PCFCL. Diffuse forms of PCFCL are usually composed predominantly of large cells, and the diffuse infiltrative pattern of B cells makes CLH unlikely. Differentiation of a diffuse type of PCFCL composed of large cells from PCDLBCL-LT is done on clinical grounds (propensity for the leg of older women), morphology (sheets of centroblasts and immunoblasts), and immunophenotype (B cells that usually express MUM1 and BCL2).

Of course, clinical correlation and staging is required to confirm that the lymphomas represent primary cutaneous disease. It should be noted that bone marrow involvement can be found in up to 11 % of patients with follicle center lymphoma presenting in skin, arguing for routine bone marrow staging studies in these patients (Senff et al. 2008a).

19.8 Prognosis

The 5-year survival rate of 90–95 % for indolent cutaneous BCL is indicative of excellent prognosis. Although cutaneous relapses occur, dissemination to other organs is rare (Cerroni et al. 2000; Garcia et al. 1986).

PCDLBCL-LT is the most aggressive PCBCL and not surprisingly harbors the worst prognosis. The reported 5-year overall survival is approximately 50 % (Senff et al. 2007). Grange et al. attempted to identify characteristics of PCDLBCL-LT that may denote a more aggressive clinical course. They reported the presence of multiple skin lesions and location of the lesion on the leg as the two features with the most negative prognostic value (Grange et al. 2007). Interestingly, patients with tumors on the leg had a 3-year disease-specific survival of 43 %, while those with lesion not on the legs had a 77 % 3-year disease free survival.

19.9 Treatment

The standard treatment for indolent cutaneous B-cell lymphoma (MZL and FCL) depends on the number and size of the lesions as summarized in Table 19.1. Although there is no strong support in the literature for "watch *and* wait," it is recommended by the National Comprehensive Cancer Network (NCCN) guidelines (NCCN guidelines) and practiced by some experts for multifocal lesions or extensive disease.

Total excision and local radiotherapy is commonly considered as first-line therapy especially for solitary lesions. Recent studies have shown treatment with 20–54 Gy radiation could result in 99 % complete response rate, but the relapse rate in these studies widely varied (Senff et al. 2008b). Neelis et al. used low dose (2×4 Gy) in 18 indolent CBCL patients with 72 % complete response rate (Neelis et al. 2009). Low-dose local radiation has considerably less side effects, and moreover, it provides the possibility of repeating radiation when there is evidence of relapse.

In small studies, intralesional interferon-α (Cozzio et al. 2006), intralesional adenovirus-interferon-γ (Audigé et al. 2006), intralesional steroids (Perry et al. 2010; Burg et al. 1994; Wong and Weller 1998), and intralesional rituximab (Heinzerling et al. 2000a; Kyrtsonis et al. 2006) have been administered successfully with an acceptable relapse rate. Systemic rituximab monotherapy is often administered when there is multifocal disease or other therapies are contraindicated or unwanted (Fink-Puches et al. 2005; Gitelson et al. 2006; Heinzerling et al. 2000b). Topical imiquimod, an immune response modulator, is an option in certain cases (Farkas et al. 2009; Coors et al. 2006).

In cases with high suspicion for an infectious trigger such as Borrelia or H. pylori, appropriate antibiotic therapy can be attempted as first-line therapy (Bogle et al. 2005; Grange et al. 2002; Hofbauer et al. 2001; Kutting et al. 1997). Systemic mono- or multiagent chemotherapy such as chlorambucil (Hoefnagel et al. 2005c) or CHOP-like regimens are only considered in cases of extensive disease or failed prior therapies (Senff et al. 2008b).

The treatment of PCDLBCL-LT is extrapolated from the diffuse large B-cell lymphoma, the most common systemic non-Hodgkin lymphoma. Therefore, if manageable, immunotherapy with rituximab plus multiagent anthracycline-based chemotherapy is recommended (Grange et al. 2007; Senff et al. 2008c). Localized radiation therapy to a solitary lesion or grouped lesions has generally fallen out of favor given the significant risk of either cutaneous and/or systemic relapse. The most common regimens used in the up-front management of PCDLBCL-LT are R-CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone) or the infusional regimen, dose-adjusted R-EPOCH (cyclophosphamide, doxorubicin vincristine, etoposide, and prednisone). Commonly, full-course therapy with 6–8 cycles are used as there is a lack of evidence for "short-course" combined modality therapy in PCDLBCL-LT despite its use extensively in localized DLBCL (Persky et al. 2008). Rituximab monotherapy for PCDLBCL-LT is thought to be inferior therapy, however remains an option for those unable or intolerant of multiagent chemotherapy (Fenot et al. 2010). The role of consolidative radiation therapy despite full-course therapy in localized presentations remains

controversial although an option. To date, there are no randomized studies to provide guidance. For the patients who experience a relapse of PCDLBCL-LT despite initial multiagent chemotherapy, it can be considered for second-line therapies with intent to perform high-dose therapy with autologous stem cell recue.

19.10 Follow-Up

The follow-up is tailored to patient's needs and extend of disease. However, indolent CBCL patients with inactive disease have usually 6–12 months clinical evaluations; whereas patients under therapy should be seen every 4–6 weeks to access therapeutic response.

The follow-up of PCDLBCL-LT is more characteristic of DLBCL rather than skin-directed surveillance. Therefore, routine physical exam, laboratory evaluation, and a discussion regarding radiographic surveillance for nodal or extranodal recurrence are reasonable but remain individualized. Coordinated follow-ups with a dermatologist and medical oncologist often occur on an every-3-month basis for the first 2 years following completion of therapy then every 6 months thereafter until 5 years.

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