CHAPTER 1

Introduction to the Classification of Lymphoma

Kiel Lukes–Collins, and Working Formulation classifications

Since the first edition of this book, further clinical, morphologic and genetic research has continued to shed light on the different aspects of lymphoma. A significant revision of the classification was published in 2008 (Swerdlow *et al.*, 2008), which highlights the extensive advances that have been made over the decades in understanding these hematologic disorders. The prominent aspects of this approach will be considered, as well as the basis for the new recommendations. A review of some suggestions for further classifications of T cell lymphomas will also be detailed. The earliest classification schemes were based on architectural criteria; specifically, lymphomas were categorized in terms of those that assumed a diffuse versus a nodular growth pattern (Rappaport *et al.*, 1956; Lennert *et al.*, 1975; Lennert, 1978; Lennert and Feller, 1992). In the 1960s, the Rappaport classification scheme, prior to the advent of immunophenotyping, added a consideration of the cell type. In that classification scheme, the large lymphocytes were, not surprisingly, mistaken for histiocytes. Thus, for example, that scheme recognized a *diffuse histiocytic lymphoma*, which we now know to derive from lymphocytes and to be, most often, a diffuse large B cell lymphoma. With the use of immunophenotyping, and the recognition of the distinction between T and B lymphocytes and histiocytes, new approaches to lymphoma classification emerged. One such scheme, designated the Kiel classification (see Table 1.1), graded lymphoid neoplasms into low-grade versus high-grade lymphomas and attempted to relate the cell types identified in any particular lymphoma to their non-neoplastic counterparts in the benign lymph node (Gerard-Marchant *et al.*, 1974; Lennert *et al.*, 1975; Lennert, 1978, 1981; Stansfield *et al.*, 1988; Lennert and Feller, 1992). Popular in the Western hemisphere from the mid-1970s to the mid-1980s, the Lukes–Collins classification emphasized immunophenotypic profiling (Lukes and Collins, 1974).

In the early 1980s, the International Working Formulation categorized lymphoid neoplasms into low, intermediate, and high grade malignancies based on clinical aggressiveness in concert with light microscopic findings. The goal was to produce a categorization of hematologic malignancies regardless of site of origin that was clinically useful, yet had scientific merit and diagnostic reproducibility (the non-Hodgkin pathological classification project 1982). Although the Kiel classification presaged the Working Formulation, this newer classification scheme did not emphasize B and/or T cell ontogeny *per se*; this was in contradistinction to the updated Kiel classification (Table 1.2). Among the low-grade malignancies were small lymphocytic lymphoma, chronic lymphocytic leukemia, small cleaved follicular lymphoma, and follicular lymphoma of mixed cell type. The intermediate-grade tumors included malignant lymphoma of follicle center cell origin with a predominance of large cells, diffuse lymphoma of small cleaved cells, and diffuse lymphoma of mixed and/or cleaved or noncleaved large cell type. The high-grade tumors were the diffuse immunoblastic, lymphoblastic, and Burkitt's lymphoma. The cytomorphology

Table 1.1 Kiel classification of lymphomas

B cell	T cell
	Low grade
Lymphocytic	Lymphocytic
Chronic lymphocytic and prolymphocytic leukemia	Chronic lymphocytic and prolymphocytic leukemia
Hairy cell leukemia	Small, cerebriform cell
	Mycosis fungoides, Sézary syndrome
Lymphoplasmacytic/cytoid (LP immunocytoma)	Lymphoepithelioid (Lennert's lymphoma)
Plasmacytic	Angioimmunoblastic (AILD, LgX)
Centroblastic/centrocytic	
Follicular ±diffuse	T zone
Diffuse	
Centrocytic	Small cell (HTLV-1)
	High grade
Centroblastic	Pleomorphic, medium and large cell (HTLV-1 ±)
Immunoblastic	Immunoblastic (HTLV-1 ±)
Large cell anaplastic (Ki-1+)	Large cell anaplastic (Ki-1+)
Burkitt's lymphoma	
Lymphoblastic	Lymphoblastic

Source: Lennert, 1981. Reproduced with permission of Springer.

The Cutaneous Lymphoid Proliferations: A Comprehensive Textbook of Lymphocytic Infiltrates of the Skin, Second Edition. Cynthia M. Magro, A. Neil Crowson and Martin C. Mihm.

Table 1.2 Working Formulation

Low grade	**Malignant lymphoma, diffuse** Small lymphocytic Consistent with chronic lymphocytic leukemia; plasmacytoid
	Malignant lymphoma, follicular Predominantly small cleaved diffuse areas; sclerosis
	Malignant lymphoma, follicular Mixed, small cleaved and large cell diffuse areas; sclerosis
Intermediate grade	**Malignant lymphoma, follicular** Predominantly large cell Diffuse areas; sclerosis
	Malignant lymphoma, diffuse Small cleaved Sclerosis
	Malignant lymphoma, diffuse Mixed, small and large cell Sclerosis; epithelioid cell component
	Malignant lymphoma, diffuse Large cell Cleaved; noncleaved; sclerosis
High grade	**Malignant lymphoma** Large cell, immunoblastic Plasmacytoid; clear cell; polymorphous; epithelioid cell component
	Malignant lymphoma Lymphoblastic convoluted; nonconvoluted
	Malignant lymphoma Small noncleaved Burkitt's; follicular areas
Miscellaneous	Composite lymphoma Mycosis fungoides Histiocytic lymphoma Extramedullary plasmacytoma Unclassifiable Other

Source: Non-Hodgkin lymphoma pathologic classification project, 1982. Reproduced with permission of John Wiley & Sons.

Table 1.3 Revised European–American Lymphoma classification (REAL)

Precursor B cell neoplasm
Precursor B-lymphoblastic leukemia/lymphoma
Mature (peripheral) B cell neoplasms
B cell chronic lymphocytic leukemia/small lymphocytic lymphoma
B cell prolymphocytic leukemia
Lymphoplasmacytic lymphoma
Splenic marginal zone B cell lymphoma (+/–villous lymphocytes)
Hairy cell leukemia
Plasma cell myeloma/plasmacytoma
Extranodal marginal zone B cell lymphoma of mucosa-associated lymphoid tissue type
Nodal marginal zone lymphoma (+/–monocytoid B-cells)
Follicle center lymphoma, follicular,
Mantle cell lymphoma
Diffuse large cell B cell lymphoma
Mediastinal large B cell lymphoma
Primary effusion lymphoma
Burkitt's lymphoma/Burkitt's cell leukemia
T cell and natural killer cell neoplasms
Precursor T cell neoplasm
Precursor T lymphoblastic lymphoma/leukemia
Mature (peripheral) T cell and NK cell neoplasms
T cell prolymphocytic leukemia
T cell granular lymphocytic leukemia
Aggressive NK cell leukemia
Adult T cell lymphoma/leukemia (HTLV-1+)
Extranodal NK/T cell lymphoma, nasal type
Enteropathy-type T cell lymphoma
Hepatosplenic γ/δ T cell lymphoma
Mycosis fungoides/Sézary syndrome
Anaplastic large cell lymphoma, T/null cell, primary cutaneous type
Peripheral T cell lymphoma, not otherwise characterized
Angioimmunoblastic T cell lymphoma
Anaplastic large cell lymphoma, T/null cell, primary systemic type
Hodgkin lymphoma
Nodular lymphocyte predominance Hodgkin lymphoma
Classical Hodgkin lymphoma
Nodular sclerosis Hodgkin lymphoma
Lymphocyte-rich classical Hodgkin lymphoma
Mixed cellularity Hodgkin lymphoma
Lymphocyte depletion Hodgkin lymphoma

Source: Harris *et al.*, 2000. Reproduced with permission of Oxford University Press.

and architecture were clearly of cardinal importance and, in essence, took precedence over the cell of origin in this classification scheme.

By the mid-1990s there was sufficient data gleaned from immunohistochemistry, cytogenetics, and molecular techniques to better categorize these tumors as distinct clinical and pathological entities manifesting reproducible phenotypic, cytogenetic, and molecular features, all defining critical determinants in the clinical course and prognosis. To attempt to evaluate whether a new classification scheme could be devised, a panel of 19 hematopathologists from Europe and the United States met to evaluate the current classification systems to consider whether a synthesis of the prior efforts could be made into a more usable and practical device to aid pathologists and clinicians. The classifications under consideration were the Kiel classification (Gerard-Marchant *et al.*, 1974; Lennert *et al.*, 1975; Lennert, 1978, 1981; Stansfield *et al.*, 1988; Lennert and Feller, 1992), the Lukes–Collins classification (Lukes and Collins, 1974), and the Working Formulation (non-Hodgkin lymphoma pathologic classification project, 1982). What ultimately eventuated from this meeting was the Revised European–American Classification of Lymphoid Neoplasms (REAL classification) (see Table 1.3). It represented a synopsis of the existing hematologic literature, allowing categorization based on distinctive forms of hematopoietic and lymphoid malignancy separated on the basis of their peculiar clinical, light microscopic, phenotypic, molecular, and cytogenetic profiles (Harris *et al.*, 1994; Cogliatti and Schmid, 2002).

WHO, REAL, EORTC, and the Combined WHO/EORTC classifications

The new WHO classification was a modest revision of the REAL classification, once again amalgamating reproducible clinical, light microscopic, phenotypic, molecular, and cytogenetic features into a coherent scheme (Jaffe *et al.*, 2001; Cogliatti and Schmid, 2002). The concept of a classification scheme based purely on morphology was now considered archaic. However, the WHO/REAL classification was deficient from the perspective of cutaneous hematologic dyscrasias, as will be alluded to presently (Cogliatti and Schmid, 2002) (Table 1.3). Hence, in 1997 the European Organization for the Research and Treatment of Cancer (EORTC) established a scheme for the classification of cutaneous lymphomas (see Table 1.4). This classification scheme was met with criticism for reasons that will be discussed. Among the distinct clinical and pathological entities that were recognized by the EORTC classification were mycosis fungoides, including specific variants, lymphomatoid papulosis, large cell CD30-positive lymphoma, large cell CD30-negative lymphoma, panniculitis-like T cell lymphoma, marginal zone B cell lymphoma, primary cutaneous follicle center cell lymphoma, primary cutaneous large B cell lymphoma of the leg, and primary cutaneous plasmacytoma (Willemze *et al.*, 1997) (Table 1.4). The main problem with this classification scheme was not the specific entities *per se* or even their purported clinical behavior. The difficulty

Table 1.4 EORTC Classification for Primary Cutaneous Lymphomas

Primary CTCL	Primary CBCL
Indolent	Indolent
MF	Follicle center cell lymphoma
MF + follicular mucinosis	
Pagetoid reticulosis	Immunocytoma (marginal zone B-cell lymphoma)
Large cell CTCL, CD30+	
Anaplastic, Immunoblastic	
Pleomorphic	Intermediate
Lymphomatoid papulosis	Large B-cell lymphoma of the leg
Aggressive	
SS	
Large cell CTCL, CD30−	Provisional
Immunoblastic, Pleomorphic	Intravascular large B-cell lymphoma
Provisional	Plasmacytoma
Granulomatous slack skin	
CTCL, pleomorphic small/medium-sized	
Subcutaneous panniculitis-like T-cell lymphoma	

CTCL, cutaneous T-cell lymphoma; CBCL, cutaneous B-cell lymphoma; MF, mycosis fungoides; SS, Sezary syndrome.

Source: Willemze et al., 1997. Reproduced with permission of American Society of Hematology.

was that there were a number of cutaneous hematologic dyscrasias that either were not included in this classification scheme or were phenotypically and biologically disparate, yet had to be forced into the same category. For example, both diffuse large B cell lymphomas of the trunk without features of follicle center cell origin and CD30-negative large cell T cell lymphoma would be categorized as CD30-negative large cell lymphomas. However, they are different from a prognostic perspective, the former being indolent and the latter being an aggressive form of lymphoma. Adult T cell leukemia lymphoma, nasal and extranodal NK/T cell lymphoma, nasal type, angioimmunoblastic T cell lymphoma, and T prolymphocytic leukemia commonly involve the skin as part of a disseminated lymphomatous process, yet they were not recognized in this classification scheme (Cogliatti and Schmid, 2002; Willemze *et al.*, 2005).

Those who were proponents of the updated WHO classification (i.e., the REAL classification) contended that the WHO scheme was superior to the EORTC classification of cutaneous lymphomas. However, in the REAL/WHO classification scheme, there was only recognition of a few distinctive forms of cutaneous lymphoma, namely, mycosis fungoides, Sézary syndrome, and panniculitis-like T cell lymphoma. All of the other lymphomas were in the context of disease not specifically involving the skin, albeit recognizing that the diagnostic terms rendered could certainly be applied to various cutaneous lymphomas, including anaplastic large cell lymphoma, peripheral T cell lymphoma, not otherwise specified, NK/T cell lymphoma, extranodal marginal zone lymphoma, follicular lymphoma, diffuse large B cell lymphoma, and extramedullary plasmacytoma. Furthermore, all of the systemic and/or extracutaneous lymphomas that commonly involved the skin, such as adult T cell leukemia lymphoma were recognized by the WHO (Harris *et al.*, 1994; Jaffe *et al.*, 2001). Thus, the advantage of this classification scheme was that it encompassed a much broader spectrum of hematologic diseases having the potential to involve the skin. The problem was the radical difference in prognosis between the various lymphomas at extracutaneous sites relative to their behavior when presenting as primary cutaneous neoplasms. Perhaps the best example of this is primary cutaneous follicle center lymphoma and primary cutaneous diffuse large cell B cell lymphoma, which can represent indolent forms of malignancy in the skin. The same potentially benign clinical course may apply to primary cutaneous anaplastic large cell lymphoma and localized peripheral T cell lymphoma in the skin, when dominated by small- and medium-sized lymphocytes.

To address the deficiencies in both the WHO and EORTC schemes as they apply to cutaneous hematologic disorders, a group of dermatologists and pathologists met in Lyon, France and Zurich, Switzerland in 2003 and 2004. The result was a publication that represents an amicable marriage, falling under the designation of the joint WHO–EORTC classification for cutaneous lymphomas (Jaffe *et al.*, 2001; Cogliatti and Schmid, 2002; Burg *et al.*, 2005; Slater, 2005; Willemze *et al.*, 2005) (see Table 1.5). The WHO–EORTC classification recognizes 10 types of cutaneous T cell lymphoma and 4 forms of cutaneous B cell lymphoma, with clinical outcomes for those neoplasms designated as primary cutaneous lymphomas being recognized as distinct and separate from their extracutaneous counterparts. For example, diffuse large B cell lymphoma of follicle center cell origin is an indolent lymphoma while the "leg" type is an intermediate-prognosis lymphoma. The WHO–EORTC classification scheme also recognizes hematodermic neoplasm, which is a nonlymphoid tumor; hematodermic neoplasm now falls under the designation of blastic plasmacytoid dendritic cell neoplasm. Furthermore, it does include systemic lymphomas that commonly involve the skin, such as adult T cell leukemia lymphoma and intravascular large B cell lymphoma. The main deficiencies are the failure to include certain lymphoid neoplasms that characteristically involve the skin, namely, primary cutaneous B cell lymphoblastic lymphoma, angioimmunoblastic lymphadenopathy, lymphomatoid granulomatosis, and T cell prolymphocytic leukemia. In addition, while it does consider folliculotropic mycosis fungoides, there is no mention of syringotropic mycosis fungoides. The scheme does not address primary cutaneous post-transplant lymphoproliferative

Table 1.5 WHO–EORTC Classification of Cutaneous Lymphomas

Cutaneous T cell and NK cell lymphomas
Mycosis fungoides
Mycosis fungoides variants and subtypes
Folliculotropic mycosis fungoides
Pagetoid reticulosis
Granulomatous slack skin
Sézary syndrome
Adult T cell leukemia/lymphoma
Primary cutaneous CD30+lymphoproliferative disorders
Primary cutaneous anaplastic large cell lymphoma
Lymphomatoid papulosis
Subcutaneous panniculitis-like T cell lymphoma
Extranodal NK/T cell lymphoma, nasal type
Primary cutaneous peripheral T cell lymphoma, unspecified
Primary cutaneous aggressive epidermotropic CD8+T cell lymphoma (provisional)
Cutaneous γ/δT cell lymphoma (provisional)
Primary cutaneous CD4+ small/medium sized pleomorphic T cell lymphoma (provisional)
Cutaneous B cell lymphomas
Primary cutaneous marginal zone B cell lymphoma
Primary cutaneous follicle center lymphoma
Primary cutaneous diffuse large B cell lymphoma, leg type
Primary cutaneous diffuse large B cell lymphoma, other
Intravascular large B cell lymphoma
Precursor hematologic neoplasm
CD4+/CD56+ hematodermic neoplasm (blastic NK cell lymphoma)

Source: Willemze *et al.*, 1997. Reproduced with permission of American Society of Hematology.

disease (PTLD) and methotrexate associated lymphoproliferative disease, although most of these in fact would fall in the category of diffuse large B cell lymphoma or anaplastic large cell lymphoma. As regards to PTLD, polymorphic variants and plasmacytic hyperplasia, however, would not be recognized. In contrast, the WHO considers these categories of iatrogenic dyscrasia (Jaffe *et al.*, 2001). Other Epstein–Barr virus (EBV)-related disorders, such as plasmablastic lymphoma and hydroa vacciniforme-like lesions are not considered. It does not recognize those primary cutaneous small/medium sized pleomorphic T cell lymphomas that are rarely of the CD8 subset and which are to be distinguished prognostically from primary cutaneous aggressive epidermotropic CD8-positive T cell lymphoma. The designation of peripheral T cell lymphoma, type unspecified, can denote an aggressive form of cutaneous T cell lymphoma, however. The more accurate designation is that of CD30 negative large T cell lymphoma and one could argue that the latter designation would be more apposite. While the new scheme does consider hematodermic neoplasm a tumor of monocytic derivation, there is no consideration of granulocytic sarcoma, the histiocytopathies, or mast cell disease. The endogenous T cell dyscrasias that may presage lymphoma such as syringolymphoid hyperplasia with alopecia, atypical lymphocytic lobular panniculitis, pigmented purpuric dermatosis, and pityriasis lichenoides are not part of the classification scheme. Despite these deficiencies, it is to date the most accurate classification scheme for the categorization of hematologic diseases expressed in the skin (Burg *et al.*, 2005; Willemze *et al.*, 2005).

Since the 2006 WHO/EORTC classification of cutaneous lymphoma, further modifications have not been made of this classification scheme, although there are a number of emerging lymphoproliferative disorders, all of which we will consider in this latest edition of the book, including the new variants of lymphomatoid papulosis, indolent CD8 lymphoid proliferation, EBV+ lymphoproliferative disease of the elderly, indolent variants of gamma delta T cell lymphoma, and double-hit lymphoma. However, an important modification made by the International Society for Cutaneous Lymphoma/EORTC for the TNM classification of mycosis fungoides (MF) and Sézary syndrome was published in 2007 (Kim *et al.*, 2007). It was the advancement in the understanding of the pathophysiology, including the cytogenetic and molecular basis of MF/SS that emerged as the impetus for the revised TNM classification of MF/SS presented in Table 1.6. The basic principles are identical to those outlined in the 1979 classification scheme. In the revised classification scheme, T0, as defined by lesions that are clinically and or histopathologically suspicious for MF/SS no longer exists. Another modification reflects the designated T1 and T2 subscript as "a" for cases that are exclusively in the context of patch stage MF and "b" for cases that manifest a patch/plaque stage overlap. For skin, patch indicates any size skin lesion without significant elevation or induration. Presence/absence of hypo- or hyperpigmentation, scale, crusting, and/or poikiloderma is noted. A plaque indicates any size skin lesion that is elevated or indurated. Presence or absence of scale, crusting, and/or poikiloderma is noted. The percentage of the skin involved is another important staging determinant. In the 1979 classification, it was assumed that the palm represented 1% of the body surface area; however, the revised updated classification scheme indicates that the palm represents approximately 0.5% of the body surface area. Another methodology for calculating percentage of body surface involved addresses the percentage of the skin involved in 12 specific regions and then tabulates the cumulative percentages. In the revised classification scheme, ulceration does not define a criterion for warranting the designation of tumor stage MF. To qualify as tumor stage MF requires at least one tumor 1.5 cm in diameter. The total number of lesions, total volume of lesions, largest size lesion, and region of body involved is documented. Erythroderma qualifies as T4, independent of whether or not the biopsy shows neoplastic T cell infiltration. They isolate only two histologic features of prognostic significance, namely variants of MF showing folliculotropism, which are classified as representing either a T1 or T2 form of the disease. The second histologic feature is one of large cell transformation, defined as a biopsy specimen showing large cells (≥ 4 times the size of a small lymphocyte) in 25% or more of the dermal infiltrate. The large cells are then evaluated for expression of CD30, given the prognostic significance of cases showing CD30-positive large cell transformation versus cases of large cell transformation that are CD30 negative. The lymph node alterations range from dermatopathic lymphadenitis (N1) and collections of atypical lymphocytes (N2), to one of frank effacement of the lymph node (N3). Atypical lymphocytes may be small (6–10 μm) or large (> 11.5 μm) cells; the cells exhibit irregularly folded, hyperconvoluted nuclei. In the revised ISCL/EORTC classification, clonality in the lymph node in the absence of any histologic abnormalities does not alter the staging. Abnormal peripheral lymph node(s) indicates any palpable

Table 1.6 ISCL/EORTC revision to the classification of mycosis fungoides and Sézary syndrome

TNMB stages	
Skin	
T_1	Limited patches, papules, and/or plaques covering < 10% of the skin surface. May further stratify into T_{1a} (patch only) versus T_{1b} (plaque ± patch).
T_2	Patches, papules or plaques covering ≥ 10% of the skin surface. May further stratify into T_{2a} (patch only) versus T_{2b} (plaque ± patch).
T_3	One or more tumors (≥ 1-cm diameter)
T_4	Confluence of erythema covering ≥ 80% body surface area
Node	
N_0	No clinically abnormal peripheral lymph nodes; biopsy not required
N_1	Clinically abnormal peripheral lymph nodes; histopathology Dutch grade 1 or NCI LN_{0-2}
N_{1a}	Clone negative
N_{1b}	Clone positive
N_2	Clinically abnormal peripheral lymph nodes; histopathology Dutch grade 2 or NCI LN_3
N_{2a}	Clone negative
N_{2b}	Clone positive
N_3	Clinically abnormal peripheral lymph nodes; histopathology Dutch grades 3-4 or NCI LN_4; clone positive or negative
N_x	Clinically abnormal peripheral lymph nodes; no histologic confirmation
Visceral	
M_0	No visceral organ involvement
M_1	Visceral involvement (must have pathology confirmation and organ involved should be specified)
Blood	
B_0	Absence of significant blood involvement: ≤ 5% of peripheral blood lymphocytes are atypical (Sézary) cells
B_{0a}	Clone negative
B_{0b}	Clone positive
B1	Low blood tumor burden: > 5% of peripheral blood lymphocytes are atypical (Sézary) cells but does not meet the criteria of B_2
B_{1a}	Clone negative
B_{1b}	Clone positive
B2	High blood tumor burden: ≥ 1000/μL Sézary cells with positive clone

N, node; B, blood; T, tumor; M, metastatic; ISCL, International Society of Cutaneous Lymphoma; EORTC, European Organization for the Research and Treatment of Cancer.

peripheral node that on physical examination is firm, irregular, clustered, fixed or 1.5 cm or larger in diameter. Node groups examined on physical examination include cervical, supraclavicular, epitrochlear, axillary, and inguinal. Central nodes, which are not generally amenable to pathologic assessment, are not currently considered in the nodal classification unless used to establish N_3 histopathologically. Peripheral blood involvement has been recategorized whereby B0 represents 5% or less circulating Sézary cells, B_2 is now defined as a clonal rearrangement of the TCR in the blood and either 1.0 K/μL or more Sézary cells or one of two phenotypic criteria being T cells with CD4/CD8 of 10 or more, or an increase in circulating CD4+ T cells that show a loss of CD7 or CD26 representing 40% or 30%, respectively, of the peripheral blood CD4 T cells. B_1 is defined as more than 5% Sézary cells, but either less than 1.0 K/μL absolute Sézary cells or absence of a clonal rearrangement of the TCR, or both (Kim *et al.,* 2007).

In addition, the International Society of Cutaneous Lymphoma and the EORTC created a risk stratification for cutaneous lymphoma other than MF and Sézary syndrome. In this risk stratification scheme, they proposed a TNM classification for non-MF/SS cutaneous lymphomas, as summarized in Table 1.7. The authors emphasized the importance of a complete history/review of systems (e.g., +/− B-symptoms, organ-specific signs) and a thorough physical examination. Among the important laboratory values are a complete blood count with differential, and a comprehensive blood chemistry measurement, including lactate dehydrogenase (LDH). They recommend appropriate imaging studies, including the neck for evaluation of the cervical lymph nodes in cases showing significant head and neck involvement. Biopsies of suspicious extracutaneous sites are encouraged. They also suggest a bone marrow biopsy and aspirate should be performed in patients at risk of marrow involvement, especially in more aggressive forms of lymphoma, such as natural killer (NK)/T cell, aggressive CD8 + T cell and γ/δ T cell lymphoma and diffuse large B cell lymphoma, leg type). A bone marrow is not required in cases of indolent lymphoproliferative disease. A negative marrow involvement would further confirm that the skin involvement is primary and not secondary to a primary extracutaneous presentation. A lumbar puncture and spinal fluid assessment is recommended for patients with NK/T cell lymphoma (Kim *et al.,* 2007). A bone biopsy is recommended for all cases of diffuse large B cell lymphoma of leg type. Some physicians suggest a bone marrow assessment in cases of primary cutaneous follicle center lymphoma because of the reported incidence of bone marrow involvement in 10% of cases, which in turn is associated with an inferior survival. The international extranodal lymphoma study group emphasize three clinical parameters that are of prognostic value, namely elevated LDH, the presence of two or more lesions, and a cutaneous tumor that manifests a nodular morphology in the setting of primary cutaneous marginal zone lymphoma and primary cutaneous follicle center lymphoma.(Senff *et al.,* 2008)

The frequency and the clinical pathological spectrum of lymphomas of the skin diagnosed between the years of 2006 and 2013 at a major referral center in Austria, as categorized according to the two main recent classification schemes, namely the WHO/EORTC and the TNM ISCL/EORTC classifications, was recently published in 2015. Eighty-three percent of their cases fell into the cutaneous T cell lymphoma category with 60% of these cases being represented by mycosis fungoides, followed in decreasing order by CD-30positive lymphoproliferative disease, primary cutaneous CD4+ small/medium-sized pleomorphic T cell lymphoma, Sézary syndrome and subcutaneous panniculitis-like T cell lymphoma. Not surprisingly, the most common B cell lymphomas were marginal zone lymphoma, primary cutaneous follicle center lymphoma and diffuse large B cell lymphoma of leg type. Their experience in terms of disease frequency, clinical features, and prognosis mirrors most major academic centers. In their study they also found a male predominance, an increasing incidence of cutaneous lymphoma incidence with age, and a greater age of onset of B cell lymphoma in women compared to men (Eder *et al.*, 2015).

While there have not been any further updates of the 2006 EORTC-WHO classification of cutaneous lymphoma, the 4th edition of the WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues was published in 2008 by the International Agency for Research on Cancer (Swerdlow *et al.*, 2008; Jaffe *et al.,* 2009; Campo *et al.,* 2011). It was a modification of the earlier WHO classification of hematologic disorders based on the exact same philosophy as that which formulated the earlier WHO classification. In particular, hematologic disorders were considered as distinct clinicopathological entities where the combination of the clinical features, morphology, phenotypic profile, molecular features, and cytogenetics defined the entity with a precision that reflects the striking advances in our understanding of the genetic and epigenetic basis of disease. Compared to the earlier WHO classification of lymphoma, a far greater number of primary cutaneous lymphomas were recognized.

In the category of mature B cell neoplasms, the two primary cutaneous forms of B cell lymphoma that are recognized in the new 2008 classification of hematologic dyscrasias are diffuse large b cell lymphoma of leg type and primary cutaneous follicle center lymphoma. In the category of mature T and NK cell neoplasms, mycosis fungoides, Sézary syndrome, primary cutaneous gamma delta T cell lymphoma, primary cutaneous CD30-positive T cell lymphoproliferative disease, primary cutaneous CD4+ small/medium sized pleomorphic T cell lymphoma and subcutaneous panniculitis-like T cell lymphoma are described. The variants of mycosis fungoides recognized include follicular MF, pagetoid reticulosis, and granulomatous slack skin. Each lymphoma is presented as a distinct clinical pathological entity with unique clinical and histologic features, a distinctive phenotypic, molecular and cytogenetic, and oncogenic gene profile. The evolution of the current classification to one of precision at the exact

Table 1.7 TNM Classification for lymphomas other than MF and SS

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 ≤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 at least three 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

MF, mycosis fungoides; SS, Sezary syndrome; T, tumor; N, node; M, metastatic.

Table 1.8 The 2008 WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues

MYELOPROLIFERATIVE NEOPLASMS
Chronic myelogenous leukaemia, BCR-ABL 1 positive
Chronic neutrophilic leukaemia
Polycythaemia vera
Primary myelofibrosis
Essential thrombocythaemia
Chronic eosinophilic leukaemia, NOS
Mastocytosis
  Cutaneous mastocytosis
  Systemic mastocytosis
  Mast cell leukaemia
  Mast cell sarcoma
  Extracutaneous mastocytoma
Myeloproliferative neoplasm, unclassifiable

MYELOID AND LYMPHOID NEOPLASMS WITH EOSINOPHILIA AND ABNORMALITIES OF PDGFRA, PDGFRB OR FGFR1
Myeloid and lymphoid neoplasms with PDGFRA rearrangement
Myeloid neoplasms with PDGFRB rearrangement
Myeloid and lymphoid neoplasms with FGFR1 abnormalities

MYELODYSPLASTIC/MYELOPROLIFERATIVE NEOPLASMS
Chronic myelomonocytic leukaemia
Atypical chronic myeloid leukaemia, BCR-ABL1 negative
Juvenile myelomonocytic leukaemia
Myelodysplastic/myeloproliferative neoplasm, unclassifiable
  Refractory anaemia with ring sideroblasts associated with marked thrombocytosis

MYELODYSPLASTIC SYNDROMES
Refractory cytopenia with unilineage dysplasia
  Refractory anaemia
  Refractory neutropenia
  Refractory thrombocytopenia
Refractory anaemia with ring sideroblasts
Refractory cytopenia with multilineage dysplasia
Refractory anaemia with excess blasts
Myelodysplastic syndrome, unclassifiable
Childhood myelodysplastic syndrome
  Refractory cytopenia of childhood

ACUTE MYELOID LEUKAEMIA (AML) AND RELATED PRECURSOR NEOPLASMS

AML with recurrent genetic abnormalities
AML with t(8;21)(q22;q22); RUNX1-RUNX1T1
AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22) CBFB-MYH11
  Acute promyelocytic leukaemia with t(15;17)(q22;q12); PML-RARA
AML with t9(;11)(q22;q23); MLLT3-MLL
AML with t(6;9)(p22;q34); DEK-NUP214
AML with inv (3)(q31q26.2) or t (3;3)(q31;q26.2); RPN1-ENV1
AML (megakaryoblastic) with t(1;22)(p13;q13); RBM15-MLK1
AML with mutated NPM1
AML with mutated CEBPA

AML with meylodysplasia-related changes

Therapy-related myeloid neoplasms

Acute myeloid leukaemia, NOS
AML with minimal differentiation
AML without maturation
AML with maturation
Acute myelomonocytic leukaemia
Acute monoblastic and monocytic leukaemia
Acute erythroid leukaemia
Acute megakaryoblastic leukaemia
Acute basophilic leukaemia
Acute panmyelosis with myelofibrosis

Myeloid sarcoma

Myeloid proliferations related to Down syndrome
Transient abnormal myelopoiesis
Myeloid leukaemia with associated Down syndrome

Blastic plasmacytoid dendritic cell neoplasm

ACUTE LEUKAEMIAS OF AMBIGUOUS LINEAGE
Acute undifferentiated leukaemia
Mixed phenotype acute leukaemia with t(9;22)(q34;q11.2); BCR-ABL1
Mixed phenotype acute leukaemia with t(v;11q23); MLL rearranged
Mixed phenotype acute leukaemia, B/myeloid, NOS
Mixed phenotype acute leukaemia, T/myeloid, NOS
Natural killer (NK) cell lymphoblastic leukaemia/lymphoma

PRECURSOR LYMPHOID NEOPLASMS

B lymphoblastic leukaemia/lymphoma
  B lymphoblastic leukaemia/lymphoma, NOS
  B lymphoblastic leukaemia/lymphoma with recurrent genetic abnormalities
  B lymphoblastic leukaemia/lymphoma with t(9;22)(q34;q11.2)BCR-ABL1
  B lymphoblastic leukaemia/lymphoma with t(v;11q23); MLL rearranged
  B lymphoblastic leukaemia/lymphoma with t(12;21)(p13;q22); TEL-AML1 (ETV6-RUNX1)
  B lymphoblastic leukaemia/lymphoma with hyperdiploidy
  B lymphoblastic leukaemia/lymphoma with hyperdiploidy (hypodiploid ALL)
  B lymphoblastic leukaemia/lymphoma with t(5;14)(q31;q32); IL3-IGH
  B lymphoblastic leukaemia/lymphoma with t(1;19)(q23;p13.3); E2A-PBX1 (TCF3-PBX1)

T lymphoblastic leukaemia/lymphoma

MATURE B-CELL NEOPLASMS
Chronic lymphocytic leukaemia/ small lymphocytic lymphoma
B-cell prolymphocytic leukaemia
Splenic marginal zone lymphoma
Hairy cell leukaemia
Splenic B-cell lymphoma/leukaemia, unclassifiable
  Splenic diffuse red pulp small B-cell lymphoma
  Hairy cell leukaemia-variant
Lymphoplasmacytic lymphoma
  Waldenström macroglobulinemia
Heavy chain diseases
  Alpha heavy chain disease
  Gamma heavy chain disease
  Mu heavy chain disease
Plasma cell myeloma
Solitary plasmacytoma of bone
Extraosseous plasmacytoma
Extranodal marginal zone lymphoma of mucosa-associated lympoid tissue (MALT lymphoma)
Nodal marginal zone lymphoma
  Paediatric nodal marginal zone lymphoma
Follicular lymphoma
  Paediatric follicular lymphoma
Primary cutaneous follicle center lymphoma
Mantle cell lymphoma
Diffuse large C-cell lymphoma (DLBCL), NOS
  T-cell/histiocyte rich large B-cell lymphoma
  Primary DLBCL of the CNS
  Primary cutaneous DLBCL, leg type
  EBV positive DLBCL of the elderly
DLBCL associated with chronic inflammation
Lymphomatoid granulomatosis
Primary mediastinal (thymic) large B-cell lymphoma
Intravascular large B-cell lymphoma
ALK positive large B-cell lymphoma
Plasmablastic lymphoma
Large B-cell lymphoma arising in HHV8-associated multicentric Castleman disease
Primary effusion lymphoma
Burkitt lymphoma
B-cell lymphomas, unclassifiable, with features intermediate between diffuse large B-cell lymphoma and Burkitt lymphoma
B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma and classical Hodgkin lymphoma

MATURE T-CELL AND NK-CELL NEOPLASMS
T-cell prolymphocytic leukaemia
T-cell large granular lymphocytic leukaemia
Chronic lymphoproliferative disorder of NK-cells
Aggressive NK cell leukaemia
Systemic EBV positive T-cell lymphoproliferative disease of childhood
Hydroa vacciniforme-like lymphoma
Adult T-cell leukaemia/lymphoma
Extranodal NK/T cell lymphoma, nasal type
Enteropathy-associated Tcell lymphoma
Hepatosplenic T-cell lymphoma
Subcutaneous panniculitis-like T-cell lymphoma
Mycosis fungoides
Sézary syndrome
Primary cutaneous CD30 positive T-cell lymphoproliferative disorders
  Lymphomatoid papulosis
  Primary cutaneous anaplastic large cell lymphoma
Primary cutaneous gamma-delta T-cell lymphoma
Primary cutaneous CD8 positive aggressive epidermotropic cytotoxic T-cell lymphoma
Primary cutaneous CD4 positive small/medium T-cell lymphoma
Peripheral T-cell lymphoma, NOS
Angioimmunoblastic T-cell lymphoma
Anaplastic large cell lymphoma, ALK positive
Anaplastic large cell lymphoma, ALK negative

HODGKIN LYMPHOMA
Nodular lymphocyte predominant Hodgkin lymphoma
Classical Hodgkin lymphoma
Nodular sclerosis classical Hodgkin lymphoma
Lymphocyte-rich classical Hodgkin lymphoma
Mixed cellularity classical Hodgkin lymphoma
Lymphocyte-depleted classical Hodgkin lymphoma

HISTIOCYTIC AND DENDRITIC CELL NEOPLASMS
Histiocytic sarcoma
Langerhans cell histiocytosis
Langerhans cell sarcoma
Interdigitating dendritic cell sarcoma
Follicular dendritic cell sarcoma
Fibroblastic reticular cell tumour
Indeterminate dendritic cell tumour
Disseminated juvenile xanthogranuloma

POST-TRANSPLANT LYMPHOPROLIFERATIVE DISORDERS (PTLD)
Early Lesions
  Plasmacytic hyperplasia
  Infectious mononucleosis-like PTLD
Polymorphic PTLD
Monomorphic PTLD (B- and T/NK-cell types)*
Classical Hodgkin lymphomas type PTLD*

NOS, not otherwise specified.

The italicized histologic types are provisional entities, for which the WHO Working Group felt there was insufficient evidence to recognize as distinct diseases at this time.

*These lesions are classified according to the leukaemia or lymphomas to which they correspond, and are assigned the respective ICD-O code.

genomic level is a dichotomous contrast to the original nascent classification scheme, which recognized only cell size and architecture. A summary of the classification scheme is presented in Table 1.8.

Summary

Tables 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, and 1.7 summarize the classification schemes as they have evolved over time. It should be apparent to the reader that the most recent classification scheme is certainly apropos, but still not globally inclusive. Each of the conditions listed in the classification scheme are discussed in the ensuing chapters, emphasizing the approach that should be given to each hematologic dyscrasia. Specifically, the entities are presented in the context of an integration of clinical, light microscopic, phenotypic, molecular, and cytogenetic data, and, where appropriate, additional considerations are given regarding pathobiology. Each cutaneous disorder truly has its own fingerprint; in this regard we have considered many of the individual hematologic disorders in their own respective chapters and/or considered no more than a few entities in a given chapter to emphasize the truly distinctive nature of so many of these disorders. In addition, we consider other forms of lymphoid dyscrasia that commonly involve the skin, recognizing that they are rare conditions and are still not part of the WHO–EORTC classification scheme.

References

Burg G, Kempf W, Cozzio A, *et al*. WHO/EORTC classification of cutaneous lymphomas 2005: histological and molecular aspects. *J Cutan Pathol*. 2005; 32:647–674.

Cogliatti SB, Schmid U. Who is *WHO* and what was *REAL*? A review of the new WHO classification (2001) for malignant lymphomas. *Swiss Med Wkly*. 2002; 132:607–617.

Campo E1, Swerdlow SH, Harris NL, Pileri S, Stein H, Jaffe ES. The 2008 WHO classification of lymphoid neoplasms and beyond: evolving concepts and practical applications. *Blood*. 2011 May 12; 117(19):5019–5032.

Eder J, Kern A, Moser J, Kitzwögerer M, Sedivy R, Trautinger F. Frequency of primary cutaneous lymphoma variants in Austria: retrospective data from a dermatology referral centre between 2006 and 2013. *J Eur Acad Dermatol Venereol*. 2015 Jan 20. doi: 10.1111/jdv.12907. [Epub ahead of print] PubMed PMID: 25600184.

Gerard-Marchant R, Hamlin I, Lennert K, Rilke F, Stansfeld A, van Unnik J. Classification of non-Hodgkin lymphoma. *Lancet*. 1974; 2:406.

Harris NL, Jaffee ES, Stein H, *et al*. A revised European–American classification of lymphoid neoplasms: a proposal from the International Lymphoma Study Group. *Blood*. 1994; 84:1361–1392.

Harris NL, Jaffe ES, Diebold J, Flandrin G, Mullerhermelink HK, Vardiman J. Lymphoma classification – from controversy to consensus: the R.E.A.L. and WHO classification of lymphoid neoplasms. *Ann Oncol*. 2000; 11 Suppl 1:3–10.

Jaffe ES. The 2008 WHO classification of lymphomas: implications for clinical practice and translational research. *Hematology Am Soc Hematol Educ Program*. 2009:523-31

Jaffe ES, Harris NL, Stein H, Vardiman JW, eds. *World Health Organization of Tumors: Pathology and Genetics of Tumours of Hematopoietic and Lymphoid Tissues*. Lyons, France: IARC Press; 2001.

Kim YH, Willemze R, Pimpinelli N, *et al*. ISCL and the EORTC. TNM classification system for primary cutaneous lymphomas other than mycosis fungoides and Sezary syndrome: a proposal of the International Society for Cutaneous Lymphomas (ISCL) and the Cutaneous Lymphoma Task Force of the European Organization of Research and Treatment of Cancer (EORTC). *Blood*. 2007; 110(2):479–484.

Lennert K, *Malignant Lymphomas Other than Hodgkin Disease*. New York: Springer-Verlag; 1978.

Lennert KL. *Histopathology of the Non-Hodgkin Lymphomas: Based on the Keil Classification*. New York: Springer-Verlag; 1981.

Lennert K, Feller A. *Histopathology of Non-Hodgkin Lymphomas*, 2nd edn. New York: Springer-Verlag; 1992.

Lennert K, Mohri N, Stein H, Kaiserling E. The histopathology of malignant lymphoma. *Br J Haematol*. 1975; 31 Suppl:193.

Lukes R, Collins RL. Immunologic characterization of human malignant lymphoma. *Cancer*. 1974; 34:1488.

Non-Hodgkin Lymphoma Pathologic Classification Project. National Cancer Institute sponsored study of classification of non-Hodgkin lymphomas: summary and description of a Working Formulation for clinical usage. *Cancer*. 1982; 49:2112.

Quintanilla-Martinez L, Kumar S, Fend F, Reyes E, Teruya-Feldstein J, Kingma DW, Sorbara L, Raffeld M, Straus SE, Jaffe ES. Fulminant EBV(+) T-cell lymphoproliferative disorder following acute/chronic EBV infection: a distinct clinicopathologic syndrome. *Blood*. 2000; 96(2):443–451.

Rappaport W, Winter WJ, Hicks EB. Follicular lymphoma. A re-evaluation of its position in the scheme of malignant lymphoma based on a survey of 253 cases. *Cancer*. 1956; 9:792–821.

Senff NJ, Noordijk EM, Kim YH, *et al.;* European Organization for Research and Treatment of Cancer; International Society for Cutaneous Lymphoma. European Organization for Research and Treatment of Cancer and International Society for Cutaneous Lymphoma consensus recommendations for the management of cutaneous B-cell lymphomas. *Blood*. 2008; 112(5):1600–1609.

Slater DN. The new World Health Organization–European Organization for Research and Treatment of Cancer classification for cutaneous lymphomas: a practical marriage of two giants. *Br J Dermatol*. 2005; 153(5):874–880.

Stansfield A, Diebold J, Kapanci Y, *et al*. Update Kiel classification for lymphomas. *Lancet*. 1988; 1:292.

Swerdlow SH, Campo E, Harris NL, *et al*. *WHO Classification of Tumours of Haematopoietic and Lymhoid Tissues*, 4th edn. Lyon, France: IARC Press; 2008.

Willemze R, Jaffe ES, Burg G, *et al*. EORTC classification for primary cutaneous lymphomas: a proposal from the Cutaneous Lymphoma Study Group of the European Organization for Research and Treatment of Cancer. *Blood*. 1997; 90:354–371.

Willemze R, Jaffe ES, Burg G, *et al*. WHO–EORTC classification for cutaneous lymphomas. *Blood*. 2005; 105(10):3768–3785.

Appendix: Definitions of key terms and techniques

T cell antibodies

CD1a (T6, Leu6, OKT6, O10): An immature T cell antigen, found on cortical thymocytes and Langerhans cells, but not mature T cells.

CD2 (T11, Leu5, OKT11, MT910): A pan T cell antigen that corresponds to the sheep erythrocyte rosette receptor. It is present on all normal mature T cells.

CD3 (Leu4, T3, OKT3,SP7, PS1, Polyclonal): A pan-T cell antigen that is composed of five polypeptide chains covalently linked to the T cell receptor. All elements of the CD3/T cell receptor must be present for cell surface expression. Most anti-CD3 antibodies are directed toward the epsilon chain of the CD3/T cell receptor complex. The majority of mature T cells are CD3 positive. The CD3 antigen is first expressed in the cell cytoplasm and then on the surface. NK cells will manifest only cytoplasmic expression.

TCR-1, BF-1: They are antibodies that recognize the α/β heterodimer of the human T cell antigen receptor. It is expressed on normal mature peripheral blood T lymphocytes and on 50–70% of cortical thymocytes. The vast majority of T cell malignancies are derived from T cells of the $\alpha\beta$ subtype.

TCR-gamma 1: An antibody that recognizes the γ/δ heterodimer portion of human T cell antigen receptor. It is present on a minor subset of CD3-positive T cells in peripheral blood, thymus, spleen, and lymph node.

CD5 (T1, Leu1, OKT1, CD5/54/F6, 4C7): A pan T cell antigen present on the majority of thymocytes and mature peripheral blood T cells; a loss of CD5 expression in T cells is indicative of ensuing neoplasia. The CD5 antigen is present on a small subset of normal B cells representing naïve B cells with endogenous autoreactive features and which have been implicated in innate immunity. It is also expressed on neoplastic B cell lymphoma cells of chronic lymphocytic leukemia, small lymphocytic lymphoma, rare cases of marginal zone lymphoma, and mantle zone lymphoma.

CD43 (DF-T1): This T-cell-associated antigen is expressed by normal T cells, granulocytes, and a subset of plasma cells, but not normal B cells. CD43 expression by a B cell is a feature of B cell neoplasia. Primary cutaneous diffuse large B cell lymphomas, marginal zone lymphomas, and follicle center cell lymphomas can be CD43-positive.

CD7 (Leu9, DK24): A pan T cell marker that is expressed by the majority of peripheral T cells. The expression of CD7 is an event that occurs relatively early in T cell ontogeny prior to rearrangement of the TCR-β chain. The CD7 antigen is expressed by both mature and immature T cell neoplasms. The CD7 antigen may not be expressed by memory T cells manifesting selective homing to the skin. Although a substantial reduction of this marker is characteristic for mycosis fungoides can be seen in other forms of peripheral T cell lymphoma, it is also diminished in the prelymphomatous T cell dyscrasias and many reactive dermatoses, albeit to a lesser degree than in mycosis fungoides. There is variation in the intensity of staining based on the detection system.

CD62L (LECAM-1, LAM-1, MEL-14): CD62L is part of the family of selectins that comprises three subcategories: L-selectin, E-selectin, and P-selectin designated as CD62L, CD62E, and CD62P, respectively. All of the selectins exhibit a similar glycan contributing to their adhesion function and participating in the interactions between inflammatory cells and endothelium. CD62L is expressed on blood monocytes, blood neutrophils, subsets of natural killer cells, and T and B lymphocytes, including those of näive phenotype. Virgin T cells in human peripheral blood uniformly express CD62L, whereas among the memory/effector population, the three predominant subsets are CD62L+/CLA+, CD62L+/CLA−, and CD62L−/CLA−.

CD4 (Leu3a, OKT4, MT310): A helper/inducer cell antigen. It is expressed by the majority of peripheral blood T cells and 80–90% of cortical thymocytes. Cortical thymocytes that are CD4-positive usually coexpress CD8. The majority of T cell neoplasms are of the CD4 subset. $\gamma\delta$ T cells and NK cells are CD4negative. CD4 is also expressed by monocytes including, in the context of histiocytic proliferative disorders, myelomonocytic dyscrasias and hematodermic neoplasm.

CD8 (Leu 2a, C8/144B): A suppressor/cytotoxic cell antigen. The CD8 antigen is a 32 kilodalton heterodimeric protein that is expressed by approximately 30% of peripheral blood mononuclear cells and 60–85% of cortical thymocytes (P/F). Cortical thymocytes coexpress CD4. $\gamma\delta$ Cells are frequently CD8-negative. A small percentage of peripheral T cell lymphomas are of the CD8 subset, such as primary cutaneous CD8-positive epidermotropic cytotoxic T cell lymphoma, some $\gamma\delta$ T cell lymphomas, and panniculitis-like T cell lymphoma. Rarely, classic lesions of cutaneous T cell lymphoma (i.e., mycosis fungoides) will be CD8-positive. CD8 cells may be suppressive or cytotoxic in nature. The latter express cytotoxic proteins such as TIA and granzyme.

CD26: The protein encoded by the *DPP4* gene is an antigenic enzyme expressed on the surface of most cell types and is associated with immune regulation, signal transduction and apoptosis. It is an intrinsic membrane glycoprotein and a serine exopeptidase that cleaves X-proline dipeptides from the N-terminus of polypeptides. The neoplastic cells of Sézary syndrome do not express CD26 and hence this particular marker is of value in the assessment of the peripheral blood in patients who are suspected as having Sézary syndrome.

CD52 (VTH34.5, Campath-1G): Expressed in lymphocytes, monocytes, eosinophils, thymocytes, and macrophages. It is expressed on most B and T cell lymphoid-derived malignancies; expression on myeloma cells is variable.

Cutaneous Lymphocyte Antigen (HECA-452): Expressed in memory T lymphocytes with preferential homing proportion to the skin endothelial cells and epithelial cells.

Fox P3 (236A/F7): Constitutive high expression of FOXP3 mRNA has been shown in CD4+CD25+ regulatory T cells (Treg cells), and ectopic expression of FOXP3 in CD4+CD25− cells imparts a Treg phenotype in these cells.

TCL1 oncogene: The TCL1 locus on the chromosome 14q32.1 is associated with the development of leukemia when there is a translocation and or an inversion resulting in juxtaposition to various regulating elements of the T cell receptor. Tcl1 positivity is observed amidst the neoplastic cells in blastic plasmacytoid dendritic cell neoplasm, adult T cell leukemia, and T cell prolymphocytic leukemia.

NFATc : Calcineurin/Nuclear factor of activated T cells (NFAT) signaling plays a critical role in peripheral T-cell activation following TCR engagement. In resting cells, inactive NFAT transcription factors are located in the cytoplasm. Pathway activation leads to NFAT dephosphorylation, nuclear translocation, and activation of its transcriptional targets. In reactive lymphocytic infiltrates and early lesions of mycosis fungoides, the expression of NFAT is primarily confined to the cytoplasm. With advanced mycosis fungoides and or other forms of cutaneous T cell lymphoma, such as peripheral T cell lymphoma, type unspecified, there is acquisition of nuclear expression of NFAT within the nucleus. Of particular relevance is the finding that the catalytic domain of PLCG1 is frequently

mutated in tumoral samples of cutaneous T cell lymphoma and is associated with the nuclear expression of NFAT.

PD-1: Programmed death-1 (PD-1/CD279) cell surface protein, an inhibitory member of the CD28 costimulatory receptor superfamily, is expressed mainly in the subset of B cells, NK T cells, activated monocytes dendritic cells, activated T lymphocytes, and follicular helper T cells. The PD-1 pathway exerts its function through inhibiting TCR-mediated T cell proliferation and cytokine secretion, via its two ligands PD-L1 (B7-HICD274), and PD-L2 (B7-DC/CD273). PD1 is expressed in certain T cell malignancies of putative follicular helper T cell origin, including angioimmunoblastic lymphoma, primary cutaneous CD4+ small/medium-sized pleomorphic T cell lymphoma, and peripheral T cell lymphoma with a follicular pattern. In addition, in Sézary syndrome, the neoplastic cell populace is characteristically PD1 positive.

TOX: Thymocyte selection-associated high-mobility group box factor (TOX) is another critical regulator of early T-cell development, specifically during the transition from CD4+ CD8+ precursors to CD4+ T cells. However, upon completion of this process, it is tightly suppressed and mature CD4+ cells do not have significant TOX expression, except follicular helper T cells. There is significant upregulation of nuclear TOX expression in the neoplastic epidermotropic T cells of mycosis fungoides. Nuclear expression of TOX is not an absolute criterion of malignancy as it can be seen in reactive lymphocytes, although the extent and intensity of intraepidermal and dermal nuclear TOX expression amidst T cells is less in reactive inflammatory dermatoses. Since TOX is upregulated in follicular helper T cells, it is common to see very strong expression of TOX in cases of primary cutaneous CD4+ small/medium-sized pleomorphic T-cell lymphoma.

Plasma cell markers

CD138 (MI15): CD138/syndecan-1 protein backbone is a single chain molecule of 30.5 kDa. Five putative GAG attachment sites exist in the extracellular domain. GAG fine structure appears to reflect the cellular source of the syndecan. Expression of CD138 in human hematopoietic cells is restricted to plasma cells in normal bone marrow. Early B cell precursors in human bone marrow are CD138 negative. CD138 is also expressed in endothelial cells, fibroblasts, keratinocytes, and normal hepatocytes.

Natural killer cell-associated markers

CD16 (DJ130c): A natural killer cell and myelomonocytic antigen. It is expressed by all resting natural killer cells, neutrophils, and activated macrophages. It is also the antibody receptor for antibody dependent cellular cytotoxicity.

CD56 (MOC1, T199, C5.9): A natural killer cell antigen. This antigen is expressed by all resting and activated natural killer cells, a subset of cytotoxic T cells that mediates non-major histocompatibility complex (non-MHC) restricted cytotoxicity, and dendritic monocytes. However, it is expressed by other cell types including CD T cells and plasmacytoid dendritic cells, and myeloid leukemic cells can express CD56.

Cytotoxic protein markers

TIA
Perforin
Granzyme

B cell markers

The immunoglobulin light chains are the most reliable way of distinguishing a malignant B cell process from a reactive one (restricted light chain expression).

CD10 (CALLA): This B cell antigen was originally thought to be a tumor-specific marker expressed by neoplastic cells of acute lymphoblastic leukemia. The CD10 antigen can be expressed by follicular lymphomas, B cell lymphoblastic lymphomas, normal T cells undergoing apoptosis and certain T cell malignancies namely in the context of angioimmunoblastic lymphadenopathy.

CD19 (HD37): The CD19 antigen is expressed initially at the time of immunoglobulin heavy chain gene rearrangement. Anti-CD19 antibodies stain almost all cases of non-T cell acute lymphoblastic leukemia, as well as mature B cell leukemias and lymphomas. Restricted to use in flow cytometry or frozen tissues.

CD20 (B1, L26, Leu16): A pan B cell antigen that is expressed at the time of light chain gene rearrangement. Anti-CD20 antibodies react with 50% of immature B cell lymphoblastic leukemia cells. CD20 is not expressed by plasma cells. It can occasionally be expressed by neoplastic T cells and there is also a population of normal T cells that weakly expresses CD20.

CD22 (4 KB128, To15): A pan B cell antigen that is very similar to the CD20 antigen.

Bcl-1: Bcl-1/cyclin D1 belongs to the G1 cyclins and plays a key role in cell cycle regulation during the G1/S transition by cooperating with cyclin-dependent kinases (CDKs). Its overexpression may lead to growth advantage for tumor cells by way of enhanced cell cycle progression, and it has been reported in various human cancers, for example, esophageal, breast, and bladder carcinomas. Among hematolymphoid malignancies, cyclin D1 overexpression resulting from translocational activation has also been recognized in a subset of B chronic lymphocytic leukemia (BCLL), multiple myeloma, splenic marginal zone lymphoma, hairy cell leukemia, and mantle cell lymphoma.

Bcl-2: The bcl-2 family of proteins (bcl-2, bcl-w, bcl-x_L, bcl-2 related protein A1, etc.) regulates outer mitochondrial membrane permeability. Bcl-2, bcl-w, bcl-x_L, and bcl-2 related protein A1 are antiapoptotic members that prevent release of cytochrome *c* from the mitochondrial intermembrane space into the cytosol. Bcl-2 and bcl-x_L are present on the outer mitochondrial membrane and are also found on other membranes in some cell types. Bcl-w is required for normal sperm maturation. In the context of its value in lymphoid infiltrates, it is ubiquitously expressed by small mature lymphocytes. Normal germinal center cells are bcl-2 negative. In contrast, neoplastic germinal center cells can be bcl-2 positive and are typically positive in nodal follicular lymphoma. In primary cutaneous diffuse large cell lymphomas, bcl-2 expression is an adverse prognostic variable.

Bcl-6: Bcl-6 protein is expressed in B cell lymphomas of follicle center B cell origin.

Bcl-10: Apoptosis regulator B cell lymphoma 10 (bcl-10) may show aberrant nuclear expression in primary cutaneous marginal zone lymphomas associated with extracutaneous dissemination.

CD79a: CD79a is expressed during all phases of B cell ontogeny and in this regard, CD79a is positive in B cells in both early- and late-stage B cell ontogeny. It is expressed prior to the expression of CD20 and is retained in the postgerminal B cell after CD20 is no longer expressed. CD79a is involved in B cell receptor development whereby a genetic deletion of CD79a can prevent and halt B cell development. Since CD79a is expressed at all stages of B cell ontogeny, it is a valuable marker in concert with CD20; a decrement in the expression of CD79a would potentially signify B cell neoplasia.

PAX5: The PAX5 gene is a transcription factor that exhibits a highly conserved DNA binding motif that defines an important factor in the early development of B cells. It has been postulated that dysregulation of the PAX5 gene contributes to lymphomagenesis. It is expressed in mature B cells including Hodgkin lymphoma. There are rare cases of its expression in anaplastic large cell lymphoma.

Myelomonocytic markers including dendritic cell markers

CD15 (C3D-1): It is normally expressed on neutrophils and most forms of nonlymphoid acute leukemia. It is aberrantly expressed by Reed–Sternberg cells of Hodgkin lymphoma along with chronic lymphocytic leukemia and lymphoblastic lymphoma.

CD68 (PGM1, KP1): This antigen is found on monocytes, granulocytes, mast cells, and macrophages.

CD34 (QBEnd10): The CD34 antigen is a single-chain transmembrane glycoprotein that is associated with human hematopoietic progenitor cells. It is present on immature hematopoietic precursor cells and TdT-positive B cells and T lymphoid precursors. CD34 expression decreases as these hematopoietic precursors undergo progressive maturation. CD34 myeloid progenitors can differentiate into two major myeloid subsets in the skin: Langerhans cells and dermal interstitial dendrocytes. While these mature antigen-presenting cells are CD34 negative, the dermal dendritic and Langerhans cell precursors manifest a CD34+ CD14+ CD116+ phenotype. The quantity of CD34+ progenitor cells in the marrow is closely associated with advancement of disease in patients with chronic idiopathic myelofibrosis. Expectedly, patients with myelofibrosis can develop paraneoplastic Sweet's-like reactions whereby the presence of CD34+ cells in the infiltrate could be a harbinger of a more accelerated clinical course (personal observations). CD34+ hematopoietic stem cells are the source of dermal fibrocytes involved in wound healing and representing the implicated fibrogenic cell of nephrogenic systemic fibrosis.

CD43: CD43 antigen is expressed by T cell lymphomas and about 30% of B cell lymphomas. CD43 is expressed on the membrane and in the cytoplasm of T cells and cells of myeloid lineage, including monocytes. CD43 expression by a B cell is a phenotypic aberration indicative of B cell neoplasia.

CD123: The protein encoded by this gene is an interleukin-3 (IL-3)-specific subunit of a heterodimeric cytokine receptor. The receptor is composed of a ligand-specific α subunit and a signal transducing β subunit shared by the receptors for IL-3, colony stimulating factor 2 (CSF2/GM-CSF), and interleukin-5 (IL-5). The binding of this protein to IL3 depends on the β subunit. The β subunit is activated by the ligand binding and is required for the biological activities of IL-3. This gene and the gene encoding the colony-stimulating factor 2 receptor α chain (CSF2RA) form a cytokine receptor gene cluster in an X–Y pseudoautosomal region on chromosomes X or Y. It is positive in acute myelogenous leukemia and blastic plasmacytoid dendritic cell tumor.

CD83: This protein is a member of the Ig superfamily manifesting expression on mature dendritic cells of all types, including plasmacytoid dendritic cells and Langerhans cells.

CD11c: CD11c transmembrane protein expressed at high levels on dendritic cells and monocytes that are likely destined to become dendritic cells. It is also positive on hairy cell leukemia cells and chronic lymphocytic leukemia cells.

MXA: MXA is a surrogate marker for the type-I-rich microenvironment. It is expressed in plasmacytoid dendritic cells and hence can be expressed in neoplastic cells of the blastic plasmacytoid dendritic cell tumor. In addition, myeloid dendritic cells can express MXA. including in the context of a neoplastic counterpart characteristic of clonal myeloid dendritic cell dyscrasia, a marker of chronic myeloproliferative disease (i.e. myelofibrosis, chronic myelodysplastic syndrome, myelomonocytic leukemia)

Lysozyme: Lysozyme is also referred to as muramidase. It is a hydrolytic glycosidase with potential antibacterial properties. It is found in high concentrations in various bodily secretions and is present at high levels in egg whites. Lysozyme is expressed in macrophages and neutrophils. It is also expressed by earlier precursor cells of myelomonocytic derivation and hence is positive in myeloid, monocytic and myelomonocytic acute leukemias.

CD163: CD163 is a scavenger receptor for the hemoglobin haptoglobin complex and is expressed in macrophages. Certain terminally differentiated monocytes with dendritic cell properties may not be positive for CD163; for example, Langerhans cells do not express CD163. Acute myeloid leukemia with monocytic differentiation can, however, exhibit positivity for CD163.

Langerin: Langerin is a transmembrane receptor specific for Langerhans cells, manifesting localization to the Birbeck granule, where it plays a role in the internalization of antigen prior to antigen presentation to T cells. It is not expressed on indeterminate cells en route to the lymph node, but rather is expressed on immature Langerhans cells, which reside in the epidermis.

CD14. This molecule functions as a toll receptor and is a marker of terminally differentiated monocytes that are likely destined to become dendritic cells. It performs a critical function in the detection of bacterial lipopolysaccharide. While the dominant expression is by macrophages and other related mature monocytes, there is weak expression amidst neutrophils. The differentiation of the CD14 positive monocyte into a myeloid dendritic cell and other dendritic cell types occurs in the setting of a cytokine milieu rich in interleukin 4 and granlocyte macrophage colony-stimulating factor.

CD117: Mast/stem cell growth factor receptor(SCFR), also known asproto-oncogene c-CD117 falls under the alternative designations of tyrosine protein kinase and is a receptor tyrosine kinase protein that is encoded by the KIT gene. It is expressed in mast cells and in melanocytes, but it is also expressed by hematopoietic stem cell precursors. This latter cell type is normally present at very low levels in the peripheral blood; however, certain agents, such as granulocyte colony-stimulating factor can lead to mobilization to the peripheral blood and extramedullary organ sites. CD117 is a proto-oncogene that is overexpressed in myeloid leukemias and of course is extensively positive in benign and neoplastic mast cell infiltrates.

Myeloperoxidase: Myeloperoxidase is a peroxidase enzyme that is abundantly expressed in neutrophils at high levels. Over and above its expression in mature granulocytes, is its positivity in neutrophil precursors. In this regard it is expressed in the setting of myeloid leukemia. Myeloperoxidase is also expressed in activated macrophages and therefore can be found in certain histiocyte-rich inflammatory conditions, such as Kikuchi's disease, and in the setting of histiocytoid Sweet's syndrome.

Follicular dendritic cell markers

CD21: CD21 also falls under the designation of the C3d receptor and Epstein Barr virus receptor. It is expressed on all mature B cells and follicular dendritic cells. It forms a complex with CD19 and CD81 defining the coreceptor B complex. It interacts with antigen and

optimizes the B cell response to antigen. CD21 is of value in the assessment of the follicular dendritic network in B cell proliferations, as significant disruption of the orderly follicular dendritic network in a germinal center is a feature of follicle center lymphoma and marginal zone lymphoma.

CD23: While there is no literature precedent on either the expression of CD23 in lesions of primary cutaneous B cell lymphoma, CD23 expression in non-neoplastic lymphoid cells is well described, occurring in naïve B cells, monocytes and follicular dendritic cells. In human tonsillar tissue, CD23 is a precentroblast marker; it is expressed on naïve B cells both in the mantle zone and early germinal center phase. It is upregulated in the early stages of B cell activation by interleukin 4 and functions as an IgE receptor and lymphocyte growth factor. CD23 also plays a role in the augmentation of B cell proliferation and of antigen presentation. Human B lymphocytes induced from a resting state to one of blastic transformation demonstrate CD23 expression.

CD35: CD35 also falls under the designation of Complement receptor type 1 (CR1) representing a glycoprotein found on erythrocytes, leukocytes, glomerular podocytes, hyalocytes, and splenic follicular dendritic cells. The protein is important in the mediation of interactions between effector cells and immune complexes containing activated complement. It plays a critical role in the removal of complement opsonized immune complexes. It is a negative regulator of the complement cascade, resulting in inhibition of both the classic and alternative pathways.

Activation/proliferation markers

CD25 (Tac, ACT-1): An activation marker that detects the α chain of the interleukin-2 receptor. The CD25 antigen is a 55 kilodalton glycoprotein that is expressed by activated B and T lymphocytes and weakly by histiocytes. The CD25 antigen is strongly expressed by cutaneous T cell neoplasms undergoing transformation. The CD25 antigen is also expressed by the Reed–Sternberg cells of Hodgkin lymphoma.

CD30 (Ber-H2, Ki-1): An antigen (glycoprotein) associated with activation of hematopoietic cells of B, T, and monocyte origin.

CD71 (Ber-T9): An activation antigen that defines the transferrin receptor. It is expressed on activated T cells, bone marrow blasts, normal histiocytes, and intermediate- and higher-grade lymphomas, the Reed–Sternberg and Hodgkin cells of Hodgkin lymphoma, and other nonhematopoietic rapidly growing neoplasms.

HLA-DR: Expressed normally on B lymphocytes; however, HLA-DR is negative on quiescent T lymphocytes. It is expressed on activated T lymphocytes.

Ki-67 (MIB-1): The Ki-67 antibody detects a nuclear-associated antigen that is expressed by proliferating, but not resting cells. Ki-67 staining correlates with morphologic grade, whereby a higher number of staining cells are associated with a poor survival.

Panels on paraffin-embedded tissue

T cell:
- CD2
- CD3
- CD43
- CD5
- CD7
- CD62L
- CD8
- CD4
- CD30
- TdT
- Beta F1
- NFATc1
- TOX

CD52: clone, YTH34.5 or Campath-1G; concentration, 1:500
Fox P3: clone, 236A/E7; concentration, 1:100 CLA clone, HECA-452; concentration, 1:25
B cell:
- CD20
- CD79
- CD21
- CD23
- CD10
- CD5
- CD43
- Cyclin D1
- Bcl-1
- Bcl-2
- Bcl-6
- Oct-2
- Mum-1
- CD30
- mRNA κ/λ to ascertain light chain restriction
- TdT
- PAX5

Cytotoxic markers:
- TIA
- Perforin
- Granzyme

Plasma cell markers:
- mRNA κ/λ
- CD138

Natural killer cell:
- CD56
- CD16

Myeloid:
- CD34
- CD43
- CD68
- Leder (Chloroacetate esterase) histochemical stain
- TdT
- CD99
- CD15

Hodgkin specific:
- CD15
- CD40 clone, 11E9; concentration, 1:10
- Fascin clone, 55K-2; concentration, 1:500
- CD30
- CD45 Ro
- PAX5

CD30+ lymphoproliferative disease:
- CD2
- CD3
- CD4
- CD5
- CD8
- CD30
- TIA
- granzyme
- epithelial membrane antigen
- anaplastic lymphoma kinase
- clusterin

Special techniques

Reverse transcriptase in situ hybridization assays

Epstein–Barr virus-associated latent small nuclear RNA (EBER): EBER-1 and EBER-2, present in both the productive and various forms of latent EBV infection. We employ EBER rather than LMP-1 since EBER is present in both the latent and lytic phases of infection while LMP-1 is typically not present in the lytic stage. EBER-1 and EBER-2 are present in much higher copy numbers than LMP-1, potentially providing us with higher sensitivity than testing LMP-1 protein.

Viral thymidine kinase (vTK assay): EBV thymidine kinase detected with the probes 5′-GAACCCGCATGCTCTCCTT-3′ and 5′-TCTGGATGATGCCCAAGACA-3′, respectively, detects lytic infection.

HHV8: Detection of HHV8 RNA is accomplished using primers specific for the T0.7 viral message, which is expressed in latent and active infection.

Fluorescent in-situ hybridization (FISH)

MYC amplification *and translocation, and trisomy 8*: For *MYC* amplification, a ratio of the total number of MYC signals to the total number of CEP8 signals, in at least 60 interphase nuclei with nonoverlapping nuclei in the tumor cells, is determined. Cells with no signals or with signals of only one color are disregarded. Tumor cells displaying at least two centromeric chromosome 8 signals and multiple MYC signals, with a MYC/CEP8 ratio ≥2, are considered consistent with amplification of the *MYC* gene. Overamplification of *C-MYC* is not associated with any particular hematologic malignancy, but would only be expected in those with a more aggressive course and would not be a feature of a benign lymphoid cell population. Tumor cells displaying multiple centromeric chromosome 8 signals and an approximately equal number of MYC signals with a somewhat random distribution of both probe signals are considered polysomy 8.

Summary of antibodies, clones, and dilutions

Antibody	Clone	Ig class	Dilutions	Pretreatment incubation	Primary AB	Manufacturer
CD62L	9H6	IgG2a, kappa	1:50	EDTA	30 minutes	Vision Biosystems, Norwell, MA; Novacastra
CD7	CD7-272	IgG1	1:50	EDTA	30 minutes	Vision Biosystems; Novacastra
CD7	C BC.37	IgG2b	1:80	Citra Plus	30 minutes	DakoCytomation, Carpinteria, CA
CD3	PS1	IgG2a	1:400	EDTA	30 minutes	Vision Biosystems; Novacastra

ALK-1 breakapart probe: The LSI ALK (anaplastic lymphoma kinase) dual color, breakapart rearrangement probe contains two differently labeled probes on opposite sides of the breakpoint of the *ALK* gene. This region is involved in the vast majority of breakpoints for known 2p23 rearrangements that occur in t(2;5) and its variants. The translocation (2;5)(p23;q35) is identified in approximately 50% of cases of anaplastic large cell lymphoma (noncutaneous). The absence of the translocation (2;5)(p23;q35) does not exclude the diagnosis of anaplastic large cell lymphoma and in primary cutaneous anaplastic large cell lymphoma it is primarily not seen.

Interferon regulatory factor 4-breakapart dual color probes: Translocations involving the multiple myeloma oncogene-1/interferon regulatory factor-4 (*IRF4*) locus on 6p25 in primary cutaneous anaplastic large cell lymphoma and a subset of lymphomatoid papulosis cases. The 5′ *IRF4* CTD-2308G5 probe is labelled with Cyanine3 (R for red) and the 3′ *IRF4* RP11-164H16 probe with SpectrumGreen (G for green). After hybridization of 5′ and 3′ *IRF4* probes, the normal diploid pattern is one of two fusion signals (2F); a chromosomal break point at the vicinity of *IRF4* is associated with 1F-1R-1G pattern (1F-1 split), defining a translocation in this area of the genome.

MYC breakapart probe: The LSI MYC dual color, breakapart rearrangement probe is a mixture of two probes that hybridize to opposite sides of the region located 3′ of *MYC*. This region is involved in the vast majority of breakpoints for t(8;22)(q24;q11) and t(2;8)(p11;q24). Translocation involving the *CMYC* gene can be expected to occur in the vast majority (>90%) of Burkitt's lymphoma and atypical Burkitt's lymphoma.

MYC IgH fusion probe: The LSI IGH/MYC, CEP 8 tricolor, dual-fusion translocation probe is designed to detect the juxtaposition of immunoglobulin heavy chain (*IGH*) locus and *MYC* gene region sequences. The IGH probe contains sequences homologous to essentially the entire *IGH* locus, as well as sequences extending about 300 kb beyond the 3′ end of the *IGH* locus. The large MYC probe extends approximately 400 kb upstream of *MYC* and about 350 kb 3′ beyond *MYC*. A cell harboring the reciprocal t(8;14) with the 8q24 breakpoint well within the MYC probe target is expected to produce a pattern of one orange, one green, two orange/green fusions, and two aqua signals. Translocation involving the *C-MYC* gene can be expected to occur in the vast majority (>90%) of Burkitt's lymphoma and atypical Burkitt's lymphoma.

Bcl-2 IgH fusion probe: The LSI IGH/bcl-2 dual-color, dual-fusion translocation probe (Vysis) is designed to detect the juxtaposition of immunoglobulin heavy chain (*IGH*) locus and *bcl* gene sequences. It is detected in most lymphomas harboring a t(14;18).

Cyclin D1 IgH fusion probe: The LSI IGH/CCND1 dual-color, dual-fusion XT translocation probe (Vysis) is designed to detect the juxtaposition of immunoglobulin heavy chain (*IGH*) locus and *CCND1* gene sequences. It will detect most t(11;14)-bearing cells and is therefore seen in the majority of mantle cell lymphomas.

MALT1 breakapart probe: The LSI MALT1 dual-color, breakapart rearrangement probe consists of a mixture two FISH DNA probes. The first probe, a 460 kb probe labeled in SpectrumOrange™, flanks the 5′ side of the *MALT1* gene. The second probe, a 660 kb probe labeled in SpectrumGreen™, flanks the 3′ side of the *MALT1* gene. It will detect cells with t(18q21) and/or aneuploidy of chromosome 18. Translocation involving the *MALT1* gene can be expected to occur in approximately 25–50% of extranodal marginal zone lymphomas, but is quite uncommon in nodal-based marginal zone lymphoma and primary cutaneous marginal zone lymphoma.

MALT1 IgH fusion probe: The LSI IGH/MALT1 dual-color, dual-fusion translocation probe is composed of a mixture of a 1.5 Mb SpectrumGreen™ labeled IGH probe and a 670 kb SpectrumOrange™ labeled MALT1 probe. The IGH probe contains sequences homologous to essentially the entire *IGH* locus, as well as sequences extending about 300 kb beyond the 3′ end of the *IGH* locus. The LSI MALT1 probe contains sequences that extend from a point telomeric to the *D18S531* locus, through the *MALT1* and *HAK* genes, and end proximally at a point centromeric to the *HAK* locus. This probe is useful in identifying the *IGH/MALT1* t(14;18)(q32;q21) translocation.

API2 MALT1 fusion probe: The LSI API2/MALT1 dual-color, dual-fusion translocation probe is composed of a mixture of a SpectrumGreen™ labeled IGH probe and a SpectrumOrange™ labeled MALT1 probe. This probe is useful in identifying the *API2/MALT1* t(11;18)(q21;q21) translocation. It will detect cells with a t(11;18)(q21;q21) translocation.