CHAPTER ONE

INTRODUCTION TO THE CLASSIFICATION OF LYMPHOMA

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KIEL, LUKES–COLLINS, AND WORKING FORMULATION CLASSIFICATIONS

The classification of lymphoma has evolved over the last 30 years in light of advances in our understanding of biological behavior, of morphology, and of its clinical, immunophenotypic, and molecular correlates. 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 nonneoplastic 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's 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

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TABLE 1.1 **Kiel Classification of Lymphomas (Lennert 1981) (Musshoff K 1981)**

B Cell	T Cell
Low Grade	
Lymphocytic Chronic lymphocytic and prolymphocytic leukemia Hairy cell leukemia	Lymphocytic Chronic lymphocytic and prolymphocytic leukemia
	Small, cerebriform cell Mycosis fungoides, Sézary syndrome
Lymphoplasmacytic/cytoid (LP immunocytoma)	Lymphoepithelioid (Lennert's lymphoma)
Plasmacytic	Angioimmunoblastic (AILD, LgX)
Centroblastic/centrocytic Follicular ± diffuse Diffuse	T zone
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

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 cleared or noncleared large cell type. The high grade tumors were the diffuse immunoblastic lymphoblastic and Burkitt's lymphoma. The cytomorphology 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 (Lennert et al., 1975; Gerard-Marchant et al., 1974; 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's 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, AND 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

TABLE 1.2 Working Formulation (Cancer 1982)

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 Mycosis fungoides Histiocytic Extramedullary Plasmacytoma Unclassifiable Other

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 presently. 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 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).

TABLE 1.3 Revised European–American Lymphoma Classification (REAL) (Harris et al., 2000)

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's lymphoma
- Nodular lymphocyte predominance Hodgkin's lymphoma
- Classical Hodgkin's lymphoma
  - Nodular sclerosis Hodgkin's lymphoma
  - Lymphocyte-rich classical Hodgkin's lymphoma
  - Mixed cellularity Hodgkin's lymphoma
  - Lymphocyte depletion Hodgkin's lymphoma

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 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

TABLE 1.4 EORTC Classification for Primary Cutaneous Lymphomas (Willemze 1997)

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, $CD30^-$	
Immunoblastic, Pleomorphic	
Provisional	Provisional
Granulomatous slack skin	Intravascular large B-cell lymphoma
CTCL, pleomorphic small/medium-sized	Plasmacytoma
Subcutaneous panniculitis-like T-cell lymphoma	

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

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 cell 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 the years 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; 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. 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 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. An 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, refers to as an aggressive form of cutaneous T cell 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).

TABLE 1.5 WHO–EORTC Classification of Cutaneous Lymphomas (Willemze et al., 2005)

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/δ T 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)

SUMMARY

Tables 1.1–1.5 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.

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APPENDIX: DEFINITIONS OF KEY TERMS AND TECHNIQUES

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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 naive 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 some 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 periperhal 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 substantial reduction of this marker is characteristic for mycosis fungoides and primary cutaneous pleomorphic T cell lymphoma, it is diminished in most 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 naïve 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 CD4-negative. 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 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.

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

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

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.

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 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.

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.

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 (B-CLL), multiple myeloma, splenic marginal zone lymphoma, hairy cell leukemia, and mantle cell lymphoma.

BCL2: 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 mitochondria 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 folliculae center B cell origin.

Bcl-10: Apoptosis regulator B-cell lymphoma 10 (BCL10) may show aberrant nuclear expression in primary cutaneous marginal zone lymphomas associated with extracutaneous dissemination.

Myelomonocytic Markers

CD15 (C3D-1): Expressed by Reed–Sternberg cells and Hodgkin's cells along with a small subset of mature T and B cell lymphomas.

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

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 meylofibrosis. 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).

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.

CD123: The protein encoded by this gene is an interleukin-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 interleukin-3 (IL-3), colony stimulating factor 2 (CSF2/GM-CSF), and interleukin-5 (IL-5). The binding of this protein to IL-3 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 a X-Y pseudoautosomal region on chromosomes X or Y. It is positive in hematodermic neoplasm.

Activation/Proliferation Markers

CD25 (Tac, ACT-1): An activation marker that detects the α chain of the interleukin-2 receptor. The C25 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's disease.

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
CD99
Beta F1

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
CD30
mRNA κ/λ to ascertain light chain restriction
TdT
CD99

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

CD30 & lymphoproliferative disease:
CD4
CD8
CD30
granzyme
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′-GAACCCGCA-TGCTCTCCTT- 3′ and 5′-TCTGGATGATGCCCA-AGACA-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 $\geq$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

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

hematologic malignancies 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 approximate equal number of MYC signals with a somewhat random distribution of both probe signals are considered polysomy 8.

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.

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 *C-MYC* 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/BCL2 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.

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.