Pathobiology and Molecular Profiling of Peripheral T-Cell Lymphomas

Laurence de Leval[1] and Philippe Gaulard[2]

[1]Pathology Department, University of Liège, Liège, Belgium; [2]Pathology Department and Inserm U841, Hôpital Henri Mondor, Créteil, France

Peripheral T-cell lymphomas (PTCLs) are a heterogeneous group of rare diseases, usually manifesting clinical aggressiveness. Although important novel insights into the pathobiology of nodal PTCL have been gained recently from molecular profiling studies and clinico-pathological analyses, the pathogenetic molecular lesions remain to be deciphered for most entities. Angioimmunoblastic T-cell lymphoma (AITL) comprises CD4$^+$ CXCL13$^+$ neoplastic cells displaying overlapping immunophenotypical and molecular features with normal follicular helper T cells. This derivation might account for the presence of a prominent non-neoplastic component in AITL tissues and the clinical manifestations of the disease reflective of an immunological dysfunction. ALK$^+$ anaplastic large cell lymphoma (ALCL), defined by ALK gene translocation with various gene partners, is composed of CD30$^+$ ALK$^+$ cells with a cytotoxic phenotype and usually carries a good prognosis. ALK$^-$ ALCL, now considered as a distinct disease entity, is morphologically and immunophenotypically similar to ALK$^+$ ALCL, except for ALK expression, but has distinctive molecular features. PTCL, not otherwise specified (PTCL, NOS), the largest PTCL category, which is derived from activated CD4$^+$ (or CD8$^+$) T cells, is markedly heterogeneous, including at the molecular level. Gene expression profiling approaches have identified novel biomarkers of potential therapeutic interest, and suggest the existence of molecularly distinct PTCL, NOS subgroups.

Malignancies derived from mature (post-thymic) T cells and NK cells, collectively referred to as peripheral T-cell lymphomas (PTCLs), encompass a variety of uncommon and rare diseases, altogether accounting for less than 15% of all non-Hodgkin lymphomas (NHL) worldwide.[1] There is an overall higher frequency of PTCL in Asia and Central/South America than in western countries, especially of those induced by the human T-lymphotropic virus-1 (HTLV1) and by the Epstein-Barr virus (EBV). Irrespective of their pathobiological heterogeneity, PTCLs are, with few exceptions, aggressive diseases with poor prognosis.

Principles of Classification

The WHO classification of PTCLs—like that of other hematological neoplasms—relies on a combination of morphologic, immunophenotypic, genetic and clinical features, and attempts to correlate disease entities with the normal cellular counterpart.[2] At variance with B-cell lymphomas where specific translocations have been instrumental in defining lymphoma entities, virtually no recurrent genetic or molecular lesions—with the notable exception of anaplastic lymphoma kinase (ALK) rearrangements in ALK-positive anaplastic large cell lymphoma (ALCL)—have been identified in PTCLs. Moreover, distinct T/NK-cell tumors have a broad range of cellular composition, distinct PTCLs diseases lack distinct immunophenotypic profiles, and there is significant morphologic and immunophenotypic overlap across different entities. Conversely, the clinical features and anatomic location of the disease are critical in defining lymphoma entities, and in the WHO classification PTCLs are listed according to their presentation as disseminated, predominantly extranodal or cutaneous, or predominantly nodal diseases. The list of entities according to the updated WHO classification is given in **Table 1**. With respect to the histogenetic derivation, the T-cell system is complex, with only few phenotypic and no genotypic markers of normal T-cell maturation, and numerous functional subsets. Thus, whereas most B-cell lymphomas are assigned to a normal cellular counterpart, the cellular origin of many PTCL entities remains more ambiguous (**Figure 1**; see Color Figures, page 502).

In addition to the rarity, diversity and heterogeneity of PTCLs hampering the collection of large cohorts of patients, the analysis of tumor samples is often contaminated by numerous reactive T cells, few cell lines are available and good animal models are lacking. Therefore, our understanding of the pathobiology of PTCLs remains far behind the accumulated knowledge on B-cell malignancies, and a comprehensive pathophysiological scheme is lacking for most entities. In recent years, few works have been devoted to the molecular profiling of T-cell neoplasms.[3-9] Overall, transcriptional profiling studies have evidenced distinct signatures in precursor T-lymphoblastic and mature T-cell lymphomas, supporting the notion of biologically unre-

lated categories of tumors,[3,5] and have provided some novel insights into the pathogenesis of PTCL subtypes. In this article we will highlight the contribution of these studies and other recent advances relevant to the pathobiology of PTCL entities. The discussion will be restricted to primarily nodal PTCLs which are the most commonly encountered and extensively studied.

Angioimmunoblastic T-Cell Lymphoma

Angioimmunoblastic T-cell lymphoma (AITL), otherwise known as angioimmunoblastic lymphadenopathy with dysproteinemia, is one of the most common PTCL in Western countries, accounting for 25% to 30% of cases.[1]

AITL affects elderly adults at a median age of 60 years or higher.[10] In its full-blown expression, AITL is a systemic disease manifested by generalized peripheral lymphadenopathy, fever and weight loss. Up to half of the patients have skin rash, and arthralgias. Laboratory tests often disclose immunologic abnormalities including polyclonal hypergammaglobulinemia, and Coombs-positive hemolytic anemia. Many patients have concomitant extranodal disease, most frequently involving the spleen, bone marrow, skin, liver and lungs, and the disease is stage III or IV in more than 80% of cases. The course is variable, with occasional spontaneous remissions. The prognosis is dismal, with a median survival < 3 years in most studies. However, AITL is not always lethal; 30% of patients experiencing long-term survival.[10]

Table 1. WHO 2008 classification of mature T/NK-cell neoplasms.[2]

Leukemic or disseminated
T-cell prolymphocytic leukemia
T-cell large granular lymphocytic leukemia
Chronic lymphoproliferative disorders of NK cells*
Aggressive NK-cell leukemia
Adult T-cell lymphoma/leukemia (HTLV1-positive)
Systemic Epstein-Barr virus (EBV)–positive T-cell lymphoproliferative disorders of childhood
Extranodal
Extranodal NK/T-cell lymphoma, nasal type
Enteropathy-associated T-cell lymphoma
Hepatosplenic T-cell lymphoma
Extranodal—cutaneous
Mycosis fungoides
Sézary syndrome
Primary cutaneous $CD30^+$ lymphoproliferative disorders
Primary cutaneous anaplastic large cell lymphoma
Lymphomatoid papulosis
Subcutaneous panniculitis-like T-cell lymphoma
Primary cutaneous γδ T-cell lymphoma*
Primary cutaneous aggressive epidermotropic $CD8^+$ cytotoxic T-cell lymphoma*
Primary cutaneous small/medium $CD4^+$ T-cell lymphoma*
Nodal
Angioimmunoblastic T-cell lymphoma
Anaplastic large cell lymphoma, ALK-positive
Anaplastic large cell lymphoma, ALK-negative*
Peripheral T-cell lymphoma, not otherwise specified

* designates provisional entities

Pathological features

AITL has distinctive pathological features: (1) a diffuse polymorphous infiltrate including variable proportions of medium-sized neoplastic T cells with abundant clear cytoplasm, admixed with small lymphocytes, histiocytes or epithelioid cells, immunoblasts, eosinophils and plasma cells; (2) prominent arborizing blood vessels; (3) perivascular proliferation of follicular dendritic cells (FDCs); and (4) the presence of large B-cell blasts often infected by the EBV, which may morphologically mimic Reed-Sternberg cells. The neoplastic component is often less abundant than the reactive background, and may even comprise only a minority of the T-cell compartment.[11] There is a morphologic spectrum. Three architectural patterns have been described: with hyperplastic follicles (pattern I, seen infrequently), with depleted follicles or without follicles (patterns II and III). These patterns associated with increasing numbers of neoplastic cells, are thought to represent progressive stages of the disease.[12,13] According to the cell content, several variants have been recognized, including a variant rich in epithelioid cell clusters, which may be confused with the lymphoepithelioid variant of PTCL not otherwise specified (PTCL, NOS), and a variant rich in large cells. The large cells may be neoplastic T-cells, and/or represent an expansion of B-cell blasts. The cytological grading does not seem to impact the clinical outcome.[10,14]

The cellular origin of AITL

The neoplastic cells in AITL are mature αβ $CD4^+CD8^-$ T cells, sometimes with low or heterogeneous CD4 expression.[15] Importantly, several recent studies have provided convergent lines of evidence to indicate that AITL is a neoplasm derived from a peculiar subset of $CD4^+$ T cells normally found in reactive germinal centers (follicular helper T cells [TFH]). Normal TFH cells with a $CD4^+/CD57^+/CXCR5^+/CCR7^-$ immunophenotype are distributed in the light zone of germinal centers where they provide functional help to B cells by inducing expression of the activation-induced cytidine deaminase (AID) critical to the follicular B-cell differentiation.[16] The first hints to suggest a possible relationship to the germinal center were the documentation of BCL6[17] and CD10[12] expression in the majority of AITL cases. Both markers, typically associated with germinal center B cells, have also been documented in a subset of normal T cells with a follicular distribution.[18] Further steps were subsequent to the release of critical information gained from the study of normal T-cell subsets revealing the unique gene expression pattern of $CD4^+/CD57^+$ germinal center T cells.[16] The most highly up-regulated gene in TFH cells, the CXCL13 chemokine, was found

to be strikingly expressed in the majority of neoplastic cells in virtually all AITL cases.[18,19] Additional markers of normal TFH cells, including CXCR5, CD154, programmed death-1 (PD-1), a member of the CD28 costimulatory membrane receptor family, and SLAM-associated protein (SAP), a cytoplasmic adaptor protein, were demonstrated in AITL by immunohistochemistry.[20,21] The ontogenic derivation of AITL from TFH cells was further established, by the demonstration of similar molecular signatures, at a genome-wide level (**Figure 2**; see Color Figures, page 503).[6,8]

Pathogenesis of AITL

A tumor of follicular helper T cells

The cellular derivation of AITL from germinal center cells provides a rational model to explain several of the peculiar pathological and biological features inherent to this disease, i.e., the expansion of B cells, the intimate association with germinal centers in early disease stages and the striking proliferation of FDCs. Among the molecular mediators of TFH cells, CXCL13 probably plays a major role. This chemokine, critical in B-cell recruitment into germinal centers and for B-cell activation, likely promotes B-cell expansion, plasmacytic differentiation and hypergammaglobulinemia.

Tumor microenvironment: functional alterations in AITL

Non-neoplastic cells typically represent a quantitatively major component of AITL. Accordingly, by comparing the expression profiles of highly enriched tumor cell samples to tissues, nearly 90% of the AITL signature was contributed by non-neoplastic cells.[6] Clinically, the manifestations of the disease mostly reflect a deregulated immune and/or inflammatory response rather than direct complications of tumor growth,[10] supporting the concept of a paraneoplastic immunological dysfunction. Moreover, AITL patients have defective T-cell responses, linked to both quantitative and qualitative perturbations of T-cell subsets.[22] The complex pathways and networks and the mediators linking the various cellular non-neoplastic and neoplastic components are only partly deciphered. Lymphotoxin beta demonstrated in AITL tumor cells[23] and potentially released by B cells under CXCL13 stimulation might be involved in inducing FDC proliferation. Vascular endothelial growth factor (VEGF) is overexpressed in AITL and probably acts as a key mediator of the prominent vascularization observed in the disease.[6,8] By immunostaining, both neoplastic cells and endothelial cells are positive for both VEGF and its receptor, suggesting the possibility of some paracrine and/or autocrine loop.[8,24]

Infectious agents—viruses

EBV$^+$ cells are detected in most cases of AITL,[25] and it is now established that these EBV-infected cells are B cells, indicating the virus is unlikely to play a primary role in lymphomagenesis.[25,26] Zhou and colleagues recently found that higher EBV viral loads in tissue biopsies correlated with progression of histological patterns, and with B-cell clonality.[25] In the latter study, PCR showed the presence of HHV6B in almost half of the cases. The involvement of other herpesviruses, in particular HHV8, appears very unlikely.[25] Although viral infection/reactivation likely occurs as a consequence of the underlying immune dysfunction, EBV, and potentially also HHV6B, may through the modulation of cytokines, chemokines and membrane receptors play a role in the development of the tumor microenvironment, ultimately favoring disease progression. Interestingly, HHV6B also has immunosuppressive properties.

Oncogenic alterations

The molecular alterations underlying the neoplastic transformation of TFH cells remain unknown. In that respect, genetic studies have provided fairly deceptive information.[27-30] Clonal aberrations are detected in up to 90% of the cases (reviewed in Dogan et al[31]). The most common recurrent events are trisomies of chromosomes 3, 5 and 21, gain of X, and loss of 6q.[29,31] Surprisingly, results obtained by matrix-based CGH analysis confirmed frequent aberrations but showed little overlap with classical cytogenetic data.[30] Chromosomal breakpoints affecting the T-cell receptor (TCR) gene loci appear to be extremely rare: only 1 out of 54 AITLs analyzed in two FISH-based studies had a TCR break, involving the TCR α/β locus.[27,28]

Mutations of *p53* are infrequent, and mutations in the 5' region of *BCL6* have not been detected.[32] A role for the c-maf transcription factor has been suggested, because its overexpression in transgenic mice induces the development of T-cell lymphomas, and high levels of c-maf have been detected in human AITL tissues.[33]

Clonal expansions of B cells

In addition to monoclonal TCR rearrangements which can be detected in > 95% of AITL cases, a clonal or oligoclonal expansion of B cells is also evidenced in up to one-third of patients,[34] reflecting the expansion of EBV-infected B-immunoblasts with a restricted repertoire.[26] Most EBV-infected cells carry hypermutated IG genes with destructive mutations, suggesting alternative pathways for their survival.[26] A subset of AITL patients go on to develop an EBV-associated B-cell lymphoproliferation, in most instances an EBV$^+$ diffuse large B-cell lymphoma.[13,35,36] Transformation into a large cell PTCL appears to be rare.[13] Occasionally, AITL may also be complicated by EBV B-cell or plasma cell neoplasms.[13,36] Irrespective of the EBV status, deregulated somatic hypermutation activity may play a role in the pathogenesis of the supervening B-cell lymphoproliferations.[26]

Anaplastic Large-Cell Lymphoma

The designation "anaplastic large cell lymphoma" (ALCL) originally applied to lymphomas composed of large anaplastic lymphoid cells, strongly positive for CD30 with a tendency for a sinusoidal and cohesive growth pattern, was later restricted to tumors of T-cell or null lineage, and two major clinical forms were recognized, according to presentation as a primary systemic versus primary cutaneous disease (reviewed in Stein et al[37]). Primary systemic ALCL shows one main peak of incidence in children and young adults, and another in later adulthood. It accounts for approximately 3% of adult NHL and 10% to 20% of childhood lymphomas, and represents about 12% of PTCLs.[38]

Patients with systemic ALCL usually present with lymphadenopathy, but involvement of extranodal sites, including the skin, bone and soft tissues, is frequent. Systemic symptoms are common, especially fever. More than half of the patients present with stage III or IV disease.

The majority of ALCLs (55% to 85% of cases) express a chimeric protein containing the cytoplasmic portion of anaplastic lymphoma kinase (ALK) as a consequence of a translocation involving the *ALK* gene, and these have an overall good response to therapy and good prognosis in both adults and children. A smaller subset of cases have comparable morphologic and phenotypic features to ALK-positive ALCL, but lack ALK protein expression and, importantly, have a less favorable prognosis. In the 2001 WHO classification, ALK^+ and ALK^- ALCLs were listed as two variants of the same disease entity. Since that time, however, several additional distinctive features have been evidenced that oppose ALK^+ and ALK^- ALCL, and in the 2008 WHO classification they are listed as separate disease entities.[2]

ALK-positive ALCL

ALK^+ ALCL represents a well-delineated entity, defined by a characteristic genetic alteration consisting of rearrangement of the *ALK* gene on chromosome 2p23. A variety of translocations involving the ALK gene have been reported (summarized in **Table 2A**). The most common is the t(2;5)(p23;q35), which fuses the *ALK* to the nucleophosmin gene (*NPM*) on chromosome 5. All translocations juxtapose the cytoplasmic catalytic domain of ALK to a partner protein, forming a chimeric fusion protein that induces constitutive activation of the tyrosine kinase ALK (reviewed in Pulford et al[39] and Amin and Lai[40]). The subcellular distribution of upregulated ALK as evidenced by immunohistochemistry depends on the type of translocation (**Table 2A**). *ALK* translocations are not entirely specific for ALCL,

Table 2A. Translocations and fusion proteins in alk-positive anaplastic large cell lymphoma.

Translocation	Partner gene	Frequency, %	ALK staining pattern
t(2;5)	Nucleophosmin (NPM)	75	Cytoplasmic + nuclear + nucleolar
t(1;2)	Tropomyosin 3 (TPM3)	10-20	Cytoplasmic
t(2;3)	TRK fused gene (TFG)	2-5	Cytoplasmic
inv (2)	ATIC (Pur H gene)	2-5	Cytoplasmic
t(2;17)	Clathrin heavy chain (CLTC)	2-5	Cytoplasmic/granular
t(2;22)	Myosin heavy chain (MYH9)	rare	Cytoplasmic
t(2;17)	ALK lymphoma oligomerization partner on chromosome 17 (ALO17)	rare	Cytoplasmic
t(2;19)	Tropomyosin 4 (TPM4)	rare	Cytoplasmic
t(2;X)	Moesin (MSN)	rare	Membrane-associated

Table 2B. ALK-positive anaplastic large cell lymphoma: morphological variants.

	Common variant	Small cell variant	Lymphohistiocytic variant	Nature	Immuno-phenotype
Hallmark cells	Numerous	Scattered, perivascular, smaller than in the common variant		Neoplastic	$CD30^+$ EMA^+ ALK^+
Small cells	Absent	Numerous	Variable	Neoplastic	$CD30^{-/+}$ $EMA^{-/+}$ ALK^{+*}
Histiocytes	Variable	Variable	Prominent	Reactive	$CD68^+$

* ALK expression in the small cells is mostly restricted to the nucleus
Other morphological variants seen infrequently include : a neutrophilic-rich variant, a giant cell variant, a sarcomatoid variant.

since they are also occasionally encountered in a rare large B-cell lymphoma subtype, and in inflammatory myofibroblastic tumors (reviewed in Pulford et al[39]).

Pathological features

ALK+ ALCL comprises several morphologic variants (**Table 2B**), bearing no correlation to the genetic variants of the ALK translocation. All contain so-called "hallmark cells" typical of ALCL, characterized by an eccentric horseshoe-shaped nucleus and a prominent eosinophilic golgi region. The common variant (75%) comprises sheets of large cells, with numerous hallmark cells. In the small cell variant (5% to 10%), the neoplastic population comprises small lymphoid cells with irregular nuclei, and fewer larger hallmark cells, which tend to cluster around vessels. In the lymphohistiocytic variant (10%), closely related to the small cell variant, the neoplastic cells are scattered within a predominant population of reactive histiocytes. More than one variant may be seen in one biopsy (mixed variants), and relapses may reveal morphologic features different from those seen initially.

In addition to expression of CD30 and ALK, the vast majority of cases are also positive for EMA. The tumor cells exhibit an aberrant T-cell immunophenotype with defective expression of many T-cell antigens, and often an apparent "null" immunophenotype. By molecular analysis, however, ALCL can usually be shown to be of T-cell origin with monoclonal rearrangement of the TCR beta and gamma chains.[34] CD3 is negative in > 75% of cases, CD5 and CD7 are often lost as well, CD8 is usually negative, while CD2, CD4 and CD45 are positive in a significant proportion of cases. Most cases exhibit positivity for cytotoxic associated antigens (TIA-1, granzymeB, perforin) and accordingly ALCL is thought to derive from activated cytotoxic T cells. Intriguingly, the pattern of expression of chemokine/cytokine receptors in ALCL has features of a Th2 phenotype.[41]

Pathogenesis and molecular features

Numerous studies have proven that NPM-ALK is oncogenic (for review, see Amin and Lai[40] and Chiarle et al[42]). In vitro, constitutively activated ALK chimeras induce cellular transformation, enhance cell proliferation and survival, and lead to cytoskeletal rearrangements and changes in cell shape. Oncogenic ALK transformation is mediated by interaction with downstream molecules that engage intracellular signaling pathways, the most relevant and better characterized being the ERK pathway, the JAK3-STAT3 pathway, and the PI3K-Akt pathway. NPM-ALK transgenic mice have been established, which spontaneously develop precursor T-cell lymphomas, and in this *in vivo* model lymphomagenesis is dependent on STAT3. Interestingly, enforced expression of NPM-ALK in transgenic models also leads to B-cell transformation and B-cell lymphomas or plasma cell neoplasms.

Secondary genetic alterations are common according to a recent CGH-based study,[43] with a similar profile in the morphologic variants and irrespective of the type of the translocation partner. Among several aberrations identified, gain of 17p (including a gain of *TP53*, but with no apparent correlation with protein expression) and loss of 4q seem to be the most characteristic of ALK+ ALCL.

Gene expression profiling studies have revealed peculiar molecular features of ALK+ ALCL in comparison to ALK– ALCL.[44,45] Lamant and colleagues analyzed 25 ALK+ and 7 ALK– samples[45] and found 117 genes defining the ALK+ ALCL signature. Among the most discriminant genes were *BCL6*, *CEBPB* (CAAT/enhancer binding protein beta) and *SERPINA1*. Expression of CEBPB has indeed been validated at the protein level in most ALK+ ALCL and appears to be a critical ALK-regulated target gene necessary to induce cell transformation and sustain the growth and survival of ALK+ ALCL cells *in vitro*.[46,47] SerpinA1 is also a plasma serine protease inhibitor regulated by ALK and may have an invasion-promoting effect in ALK+ ALCL.[48]

ALK-negative ALCL

Overall, ALK– ALCL has comparable morphologic and immunophenotypic features to ALK+ ALCL, except for lack of ALK expression. The expression of pan–T-cell antigens tends to be more frequent in ALK– ALCL, whereas the expression of cytotoxic markers and of EMA tends to be less frequent than in ALK+ ALCL.[38] As the morphological variants of ALCL cannot reliably be identified without ALK expression, ALK– ALCL comprises exclusively cases with classical morphology. ALK gene rearrangements are also absent, and the oncogenic alterations are unknown. There is no association with EBV infection. Chromosomal aberrations are detected by genomic profiling in about two-thirds of the cases and differ from the secondary aberrations seen in ALK+ ALCL.[43] Transcriptional profiling has not provided a meaningful insight into the pathobiological mechanisms of this lymphoma.[45]

ALK– ALCL tends to occur in older individuals, extranodal involvement is less common, and the clinical course and prognosis are worse in comparison to patients with ALK+ tumors, but more favorable when compared to PTCL, NOS patients.[38]

Peripheral T-Cell Lymphoma, Not Otherwise Specified

PTCL, NOS is an exclusion diagnosis for cases not fulfilling the criteria for one of the "specific" PTCL types. As such, it is the most common and also most heterogeneous category of PTCL. So far, the numerous attempts to individualize meaningful variants of PTCL, NOS have failed

to identify clinically relevant subtypes. Presentation is usually nodal but any site can be affected and extranodal involvement is common. The median age of patients is in the seventh decade, and 65% of the patients have stage IV disease. Blood eosinophilia, pruritis and hemophagocytic syndromes may occur. The clinical course is aggressive, with frequent relapses, and poor overall outcome (20% to 30% 5-year survival).[49]

PTCL, NOS typically contain a mixture of small and large atypical cells. The presence of cells with clear cytoplasm, increased vascularization and eosinophilia are frequent features. T-cell-associated antigens are variably expressed with frequent loss of CD7 or, more rarely, of CD3, CD5 and/or CD2. Most cases are $CD4^+CD8^-$ and are usually non-cytotoxic. EBV is detected in a variable proportion of tumor cells in a small subset of cases.

Morphologic variants

Several variants of PTCL, NOS have been described according to distinctive morphologic features, but without clear translation into distinct clinico-pathological entities. The lymphoepithelioid variant of PTCL, NOS (Lennert's lymphoma) has a characteristic background made up of numerous epithelioid histiocytes and consists of small cytotoxic T-cells, most commonly $CD8^+$.[50]

The follicular variant of PTCL, NOS named after a pattern of growth intimately related to follicular structures, has been recently described. It comprises cases with a truly follicular pattern, mimicking follicular lymphoma,[51] T-cell lymphomas with a perifollicular growth pattern,[52] or involving expanded mantle zones.[53] In view of the TFH phenotype, the possible relationship to AITL is questionable.[51] A novel recurrent chromosomal translocation t(5;9)(q33;q22) involving ITK and SYK tyrosine kinase has been described in association with follicular PTCLs,[54] but is otherwise rarely incriminated in non-follicular PTCLs, which may support the concept of a distinct subtype. Interestingly, however, overexpression and activation of SYK have been recently reported as a feature common to most PTCLs, which potentially represents a novel therapeutic target.[55]

Subsets defined according to immunological features

There have been several attempts to subclassify PTCL, NOS according to immunological features (CD4 versus CD8 subsets, Th1 versus Th2 helper function, cytotoxic phenotype, differentiation stage); these approaches, however, are technically challenging and hampered by the difficulty in assessing marker expression in the tumor cells frequently admixed with a benign immunologically reactive component, and so far have not emerged as providing clinically meaningful groups.[56,57]

Genetic features

In PTCLs, NOS complex karyotypes are common, especially in cases with larger cells. The genetic imbalances observed in PTCL, NOS differ from those observed in AITL and ALCL. By CGH,[29,30,58] recurrent chromosomal gains have been observed in chromosomes 7q (targeting cyclin-dependent kinase 6),[59] 8q (involving the *MYC* locus),[30] 17q and 22q, and recurrent losses in several chromosomes. In one study, deletions in chromosomes 5q, 10q and 12q were associated with a better prognosis.[58]

Insights gained from gene expression profiling studies

Gene expression profiling has now been applied to several small series of PTCL, NOS.[4-7,9] Although the results are somewhat difficult to interpret and compare because of different hybridization platforms and analytical tools, they have been providing some hints for better understanding PTCL, NOS. As expected, PTCLs, NOS are also heterogeneous at the molecular level. Compared to normal T cells, PTCLs, NOS appear to be most closely related to either $CD4^+$ or $CD8^+$ activated T cells and are characterized by deregulation of genes related to proliferation, apoptosis, cell adhesion, and matrix remodeling, which is similar to many other malignancies.[7] Importantly, gene expression profiling has identified overexpression of the PDGFRα a in PTCL, NOS, which was confirmed by immunohistochemistry and might have therapeutic implications given the potential sensitivity of this tyrosine kinase to imatinib.[7,60]

Gene profiling has identified distinct gene clusters, reflecting biological features related to the neoplastic cells and/or the reactive cell populations, that may be relevant to the delineation of molecular subgroups. Classification according to differential expression of NF-κB pathway genes may be clinically useful, since overexpression of this pathway was found to correlate with a better outcome.[4] Conversely, overexpression of a proliferation signature correlated with an adverse prognosis and appears to be inversely related to signature clusters related to inflammatory response and reflective of an abundant reactive background in the tumor.[5,9]

It should also be highlighted that clustering of PTCLs according to their molecular profiles variably correlated with the pathological classification, indicating some overlap between PTCL, NOS, AITL and ALCL, which may reflect the influence of non-neoplastic elements or the existence of common tumor-associated pathways.[7] More specifically, traces of the TFH signature have been identified amongst $CD30^-$ PTCL, NOS, suggesting that the AITL spectrum may be wider than suspected, while conversely molecular similarities are observed between $CD30^+$ PTCL, NOS and ALK^- ALCL (**Figure 3**; see Color Figures, page 503).[61]

Acknowledgments

Laurence de Leval is a senior research associate of the Belgian National Fund for Scientific Research (F.N.R.S.).

Disclosures

Conflict-of-interest disclosure: The author declares no competing financial interests.

Off-label drug use: None disclosed.

Correspondence

Laurence de Leval, MD PhD, Département de Pathologie, CHU Sart Tilman, Tour de Pathologie, B23, +1, B- 4000 Liège, Belgium; Phone: +32 4 3662405; Fax: +32 4 3662919; e-mail: L.deLeval@ulg.ac.be

References

1. Rudiger T, Weisenburger DD, Anderson JR, et al. Peripheral T-cell lymphoma (excluding anaplastic large-cell lymphoma): results from the Non-Hodgkin's Lymphoma Classification Project. Ann Oncol. 2002;13:140-149.
2. Swerdlow S, Campo E, Harris N, et al. Pathology and genetics. Tumours of haematopoietic and lymphoid tissues. Lyon: IARC Press; 2008, in press.
3. Martinez-Delgado B, Melendez B, Cuadros M, et al. Expression profiling of T-cell lymphomas differentiates peripheral and lymphoblastic lymphomas and defines survival related genes. Clin Cancer Res. 2004;10:4971-4982.
4. Martinez-Delgado B, Cuadros M, Honrado E, et al. Differential expression of NF-kappaB pathway genes among peripheral T-cell lymphomas. Leukemia. 2005;19:2254-2263.
5. Ballester B, Ramuz O, Gisselbrecht C, et al. Gene expression profiling identifies molecular subgroups among nodal peripheral T-cell lymphomas. Oncogene. 2006;25:1560-1570.
6. de Leval L, Rickman DS, Thielen C, et al. The gene expression profile of nodal peripheral T-cell lymphoma demonstrates a molecular link between angioimmunoblastic T-cell lymphoma (AITL) and follicular helper T (TFH) cells. Blood. 2007;109:4952-4963.
7. Piccaluga PP, Agostinelli C, Califano A, et al. Gene expression analysis of peripheral T cell lymphoma, unspecified, reveals distinct profiles and new potential therapeutic targets. J Clin Invest. 2007;117:823-834.
8. Piccaluga PP, Agostinelli C, Califano A, et al. Gene expression analysis of angioimmunoblastic lymphoma indicates derivation from T follicular helper cells and vascular endothelial growth factor deregulation. Cancer Res. 2007;67:10703-10710.
9. Cuadros M, Dave SS, Jaffe ES, et al. Identification of a proliferation signature related to survival in nodal peripheral T-cell lymphomas. J Clin Oncol. 2007;25:3321-3329.
10. Mourad N, Mounier N, Briere J, et al. Clinical, biologic, and pathologic features in 157 patients with angioimmunoblastic T-cell lymphoma treated within the Groupe d'Etude des Lymphomes de l'Adulte (GELA) trials. Blood. 2008;111:4463-4470.
11. Willenbrock K, Renne C, Gaulard P, Hansmann ML. In angioimmunoblastic T-cell lymphoma, neoplastic T cells may be a minor cell population. A molecular single-cell and immunohistochemical study. Virchows Arch. 2005;446:15-20.
12. Attygalle A, Al-Jehani R, Diss TC, et al. Neoplastic T cells in angioimmunoblastic T-cell lymphoma express CD10. Blood. 2002;99:627-633.
13. Attygalle AD, Kyriakou C, Dupuis J, et al. Histologic evolution of angioimmunoblastic T-cell lymphoma in consecutive biopsies: clinical correlation and insights into natural history and disease progression. Am J Surg Pathol. 2007;31:1077-1088.
14. Lome-Maldonado C, Canioni D, Hermine O, et al. Angioimmunoblastic T cell lymphoma (AILD-TL) rich in large B cells and associated with Epstein-Barr virus infection. A different subtype of AILD-TL? Leukemia. 2002;16:2134-2141.
15. Lee SS, Rudiger T, Odenwald T, Roth S, Starostik P, Muller-Hermelink HK. Angioimmunoblastic T cell lymphoma is derived from mature T-helper cells with varying expression and loss of detectable CD4. Int J Cancer. 2003;103:12-20.
16. Vinuesa CG, Tangye SG, Moser B, Mackay CR. Follicular B helper T cells in antibody responses and autoimmunity. Nat Rev Immunol. 2005;5:853-865.
17. Ree HJ, Kadin ME, Kikuchi M, Ko YH, Suzumiya J, Go JH. Bcl-6 expression in reactive follicular hyperplasia, follicular lymphoma, and angioimmunoblastic T-cell lymphoma with hyperplastic germinal centers: heterogeneity of intrafollicular T-cells and their altered distribution in the pathogenesis of angioimmunoblastic T-cell lymphoma. Hum Pathol. 1999;30:403-411.
18. Dupuis J, Boye K, Martin N, et al. Expression of CXCL13 by neoplastic cells in angioimmunoblastic T-cell lymphoma (AITL): a new diagnostic marker providing evidence that AITL derives from follicular helper T cells. Am J Surg Pathol. 2006;30:490-494.
19. Grogg KL, Attygalle AD, Macon WR, Remstein ED, Kurtin PJ, Dogan A. Angioimmunoblastic T-cell lymphoma: a neoplasm of germinal-center T-helper cells? Blood. 2005;106:1501-1502.
20. Roncador G, Garcia Verdes-Montenegro JF, Tedoldi S, et al. Expression of two markers of germinal center T cells (SAP and PD-1) in angioimmunoblastic T-cell lymphoma. Haematologica. 2007;92:1059-1066.
21. Krenacs L, Schaerli P, Kis G, Bagdi E. Phenotype of neoplastic cells in angioimmunoblastic T-cell lymphoma is consistent with activated follicular B helper T cells. Blood. 2006;108:1110-1111.
22. Pizzolo G, Vinante F, Agostini C, et al. Immunologic abnormalities in angioimmunoblastic lymphadenopathy. Cancer. 1987;60:2412-2418.
23. Foss HD, Anagnostopoulos I, Herbst H, et al. Patterns of cytokine gene expression in peripheral T-cell lymphoma of angioimmunoblastic lymphadenopathy type. Blood. 1995;85:2862-2869.
24. Zhao WL, Mourah S, Mounier N, et al. Vascular endothelial growth factor-A is expressed both on lymphoma cells and endothelial cells in angioimmunoblastic T-cell lymphoma and related to lymphoma progression. Lab Invest. 2004;84:1512-1519.
25. Zhou Y, Attygalle AD, Chuang SS, et al. Angioimmunoblastic T-cell lymphoma: histological progression associates with EBV and HHV6B viral load. Br J Haematol. 2007;138:44-53.
26. Brauninger A, Spieker T, Willenbrock K, et al. Survival and clonal expansion of mutating „forbidden" (immunoglobulin receptor-deficient) Epstein-Barr virus-infected B cells in angioimmunoblastic T-cell lymphoma. J Exp Med. 2001;194:927-940.
27. Gesk S, Martin-Subero JI, Harder L, et al. Molecular cytogenetic detection of chromosomal breakpoints in T-cell receptor gene loci. Leukemia. 2003;17:738-745.
28. Leich E, Haralambieva E, Zettl A, et al. Tissue microarray-based screening for chromosomal breakpoints affecting the T-cell receptor gene loci in mature T-cell lymphomas. J

Pathol. 2007;213:99-105.
29. Nelson M, Horsman DE, Weisenburger DD, et al. Cytogenetic abnormalities and clinical correlations in peripheral T-cell lymphoma. Br J Haematol. 2008;141:461-469.
30. Thorns C, Bastian B, Pinkel D, et al. Chromosomal aberrations in angioimmunoblastic T-cell lymphoma and peripheral T-cell lymphoma unspecified: A matrix-based CGH approach. Genes Chromosomes Cancer. 2007;46:37-44.
31. Dogan A, Attygalle AD, Kyriakou C. Angioimmunoblastic T-cell lymphoma. Br J Haematol. 2003;121:681-691.
32. Kerl K, Vonlanthen R, Nagy M, et al. Alterations on the 5' noncoding region of the BCL-6 gene are not correlated with BCL-6 protein expression in T cell non-Hodgkin lymphomas. Lab Invest. 2001;81:1693-1702.
33. Murakami YI, Yatabe Y, Sakaguchi T, et al. c-Maf expression in angioimmunoblastic T-cell lymphoma. Am J Surg Pathol. 2007;31:1695-1702.
34. Bruggemann M, White H, Gaulard P, et al. Powerful strategy for polymerase chain reaction-based clonality assessment in T-cell malignancies Report of the BIOMED-2 Concerted Action BHM4 CT98-3936. Leukemia. 2007;21:215-221.
35. Zettl A, Lee SS, Rudiger T, et al. Epstein-Barr virus-associated B-cell lymphoproliferative disorders in angioimmunoblastic T-cell lymphoma and peripheral T-cell lymphoma, unspecified. Am J Clin Pathol. 2002;117:368-379.
36. Willenbrock K, Brauninger A, Hansmann ML. Frequent occurrence of B-cell lymphomas in angioimmunoblastic T-cell lymphoma and proliferation of Epstein-Barr virus-infected cells in early cases. Br J Haematol. 2007;138:733-739.
37. Stein H, Foss HD, Durkop H, et al. CD30(+) anaplastic large cell lymphoma: a review of its histopathologic, genetic, and clinical features. Blood. 2000;96:3681-3695.
38. Savage KJ, Harris NL, Vose JM, et al. ALK-negative anaplastic large-cell lymphoma (ALCL) is clinically and immunophenotypically different from both ALK-positive ALCL and peripheral T-cell lymphoma, not otherwise specified: report from the International Peripheral T-Cell Lymphoma Project. Blood. 2008;111:5496-5504.
39. Pulford K, Lamant L, Espinos E, et al. The emerging normal and disease-related roles of anaplastic lymphoma kinase. Cell Mol Life Sci. 2004;61:2939-2953.
40. Amin HM, Lai R. Pathobiology of ALK+ anaplastic large-cell lymphoma. Blood. 2007;110:2259-2267.
41. Tsuchiya T, Ohshima K, Karube K, et al. Th1, Th2, and activated T-cell marker and clinical prognosis in peripheral T-cell lymphoma, unspecified: comparison with AILD, ALCL, lymphoblastic lymphoma, and ATLL. Blood. 2004;103:236-241.
42. Chiarle R, Voena C, Ambrogio C, Piva R, Inghirami G. The anaplastic lymphoma kinase in the pathogenesis of cancer. Nat Rev Cancer. 2008;8:11-23.
43. Salaverria I, Bea S, Lopez-Guillermo A, et al. Genomic profiling reveals different genetic aberrations in systemic ALK-positive and ALK-negative anaplastic large cell lymphomas. Br J Haematol. 2008;140:516-526.
44. Thompson MA, Stumph J, Henrickson SE, et al. Differential gene expression in anaplastic lymphoma kinase-positive and anaplastic lymphoma kinase-negative anaplastic large cell lymphomas. Hum Pathol. 2005;36:494-504.
45. Lamant L, de Reynies A, Duplantier MM, et al. Gene-expression profiling of systemic anaplastic large-cell lymphoma reveals differences based on ALK status and two distinct morphologic ALK+ subtypes. Blood. 2007;109:2156-2164.
46. Quintanilla-Martinez L, Pittaluga S, Miething C, et al. NPM-ALK-dependent expression of the transcription factor CCAAT/enhancer binding protein beta in ALK-positive anaplastic large cell lymphoma. Blood. 2006;108:2029-2036.
47. Piva R, Pellegrino E, Mattioli M, et al. Functional validation of the anaplastic lymphoma kinase signature identifies CEBPB and BCL2A1 as critical target genes. J Clin Invest. 2006;116:3171-3182.
48. Duplantier MM, Lamant L, Sabourdy F, de Reynies A, Delsol G, Espinos E. Serpin A1 is overexpressed in ALK+ anaplastic large cell lymphoma and its expression correlates with extranodal dissemination. Leukemia. 2006;20:1848-1854.
49. Gisselbrecht C, Gaulard P, Lepage E, et al. Prognostic significance of T-cell phenotype in aggressive non-Hodgkin's lymphomas. Groupe d'Etudes des Lymphomes de l'Adulte (GELA). Blood. 1998;92:76-82.
50. Geissinger E, Odenwald T, Lee SS, et al. Nodal peripheral T-cell lymphomas and, in particular, their lymphoepithelioid (Lennert's) variant are often derived from CD8(+) cytotoxic T-cells. Virchows Arch. 2004;445:334-343.
51. de Leval L, Savilo E, Longtine J, Ferry JA, Harris NL. Peripheral T-cell lymphoma with follicular involvement and a CD4+/bcl-6+ phenotype. Am J Surg Pathol. 2001;25:395-400.
52. Rudiger T, Ichinohasama R, Ott MM, et al. Peripheral T-cell lymphoma with distinct perifollicular growth pattern: a distinct subtype of T-cell lymphoma? Am J Surg Pathol. 2000;24:117-122.
53. Ikonomou IM, Tierens A, Troen G, et al. Peripheral T-cell lymphoma with involvement of the expanded mantle zone. Virchows Arch. 2006;449:78-87.
54. Streubel B, Vinatzer U, Willheim M, Raderer M, Chott A. Novel t(5;9)(q33;q22) fuses ITK to SYK in unspecified peripheral T-cell lymphoma. Leukemia. 2006;20:313-318.
55. Feldman AL, Sun DX, Law ME, et al. Overexpression of Syk tyrosine kinase in peripheral T-cell lymphomas. Leukemia. 2008;22:1139-1143.
56. Rudiger T, Geissinger E, Muller-Hermelink HK. 'Normal counterparts' of nodal peripheral T-cell lymphoma. Hematol Oncol. 2006;24:175-180.
57. Went P, Agostinelli C, Gallamini A, et al. Marker expression in peripheral T-cell lymphoma: a proposed clinical-pathologic prognostic score. J Clin Oncol. 2006;24:2472-2479.
58. Zettl A, Rudiger T, Konrad MA, et al. Genomic profiling of peripheral T-cell lymphoma, unspecified, and anaplastic large T-cell lymphoma delineates novel recurrent chromosomal alterations. Am J Pathol. 2004;164:1837-1848.
59. Nagel S, Leich E, Quentmeier H, et al. Amplification at 7q22 targets cyclin-dependent kinase 6 in T-cell lymphoma. Leukemia. 2008;22:387-392.
60. Piccaluga PP, Agostinelli C, Zinzani PL, Baccarani M, Dalla Favera R, Pileri SA. Expression of platelet-derived growth factor receptor alpha in peripheral T-cell lymphoma not otherwise specified. Lancet Oncol. 2005;6:440.
61. de Leval L, Rickman D, Thielen C, et al. The gene expression profile of nodal T-cell lymphomas identifies a molecular link between angioimmunoblastic T-cell lymphoma (AITL) and Follicular Helper T Cells (TFH), and between CD30+ Peripheral T-cell lymphoma and ALK-negative anaplastic large cell lymphoma (ALCL) [abstract]. Blood. 2006;108. Abstract #289.