Peripheral T-cell lymphomas

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Peripheral T-cell lymphomas (PTCLs) account for about 12% of all lymphoid neoplasms in Western Countries. They show an endemic prevalence in some geographic areas, including southern Japan, Caribbean Basin, and regions of Mexico and USA, where they are related to HTVL1 infection.[1] PTCLs can be roughly subdivided into specified and not otherwise specified (NOS) forms (Table 1). While the former correspond to rare but distinct entities, the latter represent about 50% of these tumours. PTCL/NOS is a basket category characterised by great morphologic and phenotypic variability. Due to this, its distinction from angioimmunoblastic T-cell lymphoma (AITL) and anaplastic large cell lymphoma (ALCL) may be difficult based on conventional criteria. Such differential diagnosis is not irrelevant because of potential therapeutic and prognostic implications. In general, the behaviour of PTCLs is aggressive, with the exception of mycosis fungoides (in its early and plaque phases) and primary cutaneous small/medium CD4 positive T-cell lymphoma. Irrespectively of the fact that they have a leukaemic, nodal or extranodal presentation, they show a poor response to current therapies and dismal prognosis, the 5-year overall survival being about 20%.[2] Also the usage of supra-maximal approaches has not provided the expected results. This situation probably reflects the limited knowledge on their pathobiology, the interest of most researchers having been focused on the more common B-cell tumours for decades. Only recently, molecular biology studies have opened new scenarios and led to the proposal of novel targeted therapies.

In the following, the commonest varieties of T-cell lymphoma (i.e. PTCL/NOS, AITL and ALCL, both ALK-positive and ALK-negative) will be discussed according to the criteria given in the 4th edition of the WHO Classification of the tumours of lymphoid and haematopoietic tissues.[1] In particular, the new Classification will be used as main reference with appropriate selected integrations.

PTCL/NOS more often occurs in elderly with no sex predominance. Most patients present with lymphadenopathy, but any site may be affected. Advanced disease with B-symptoms is quite common with infiltrates in the bone marrow, liver, spleen and extra-nodal tissues. Peripheral blood involvement can occur, but leukaemic presentation is rare as is haemophagocytic syndrome.

In the lymph node, PTCL/NOS growths in the paracortex or diffusely producing partial or complete effacement of the normal

Table 1. Mature T-cell and NK-cell neoplasms.

T-cell prolymphocytic leukemia
T-cell large granular lymphocytic leukaemia
Aggressive NK cell leukemia
Systemic EBV+ T-cell lymphoproliferative disease of childhood (associated with chronic active EBV infection)
Hydroa vaccineforme-like lymphoma
Adult T-cell leukemia/lymphoma
Extranodal NK/T cell lymphoma, nasal type
Enteropathy-associated T-cell lymphoma
Hepatosplenic T-cell lymphoma
Subcutaneous panniculitis-like T-cell lymphoma
Mycosis Fungoides
Sezary Syndrome
Primary cutaneous anaplastic large-cell lymphoma
Primary cutaneous aggressive epidermotropic CD8 positive cytotoxic T-cell lymphoma^
Primary cutaneous gamma-delta T-cell lymphoma
Primary cutaneous small/medium CD4 positive T-cell lymphoma^
Peripheral T-cell lymphoma, not otherwise specified
Angioimmunoblastic T-cell lymphoma
Anaplastic large cell lymphoma (ALCL), ALK positive
Anaplastic large cell lymphoma (ALCL), ALK negative^

^Provisional entity.

structure. The cytological spectrum is extremely broad. Most cases consist of numerous medium-sized and/or large cells with irregular, polymorphic, hyper-chromatic or vesicular nuclei, prominent nucleoli and many mitotic figures. Clear cells and Reed-Sternberg (RS)-like elements can also be seen. An inflammatory background is often present, including small lymphocytes, eosinophils, plasma cells, large B-cells (that may be clonal irrespectively of EBV infection) and epithelioid histiocytes. The differential diagnosis with AITL may require extensive immunophenotyping.

PTCL/NOS is usually characterised by an aberrant T-cell phenotype with frequent loss of CD5 and CD7. A $CD4^+/CD8^-$ phenotype predominates in nodal cases. CD4/CD8 double-positivity or double-negativity may also be seen, as is CD8, CD56 and cytotoxic granule expression. T-cell receptor (TCR) β-chain is expressed in most if not all cases, allowing the differentiation from γ/δ PTCLs and NK-cell lymphomas. CD52 is absent in about 60% of cases. CD30 can be expressed, exceptionally with CD15, but the global phenotypic profile and morphology allow the distinction from ALCL and classical Hodgkin lymphoma (CHL). Aberrant expression of CD20 and/or CD79a is occasionally encountered. Proliferation is usually high and Ki-67 rates ≥75% are associated with a worse prognosis.

TCR genes are clonally rearranged in most cases. PTCLs/NOS are usually neoplasms with complex karyotype. Recurrent chromosomal gains have been observed in chromosomes 7q, 8q, 17q and 22q, and recurrent losses in chromosomes 4q, 5q, 6q, 9p, 10q, 12q and 13q. Deletions in chromosomes 5q,10q and12q seem to be associated with a better prognosis. The genetic imbalances observed in PTCL/NOS differ from those of AITL and ALCL. Gene expression profiling studies have shown that the PTCL/NOS signature differs from those of AITL and ALCL. In comparison to normal T-lymphocytes, it is characterized by the recurrent deregulation of genes involved in relevant cell functions, e.g. matrix deposition, cytoskeleton organization, cell adhesion, apoptosis, proliferation, transcription, and signal transduction. The products of these genes (such as PDGFRα, GADD45A and GADD45B) might have therapeutic relevance.[3]

PTCLs/NOS are highly aggressive tumours, with poor response to therapy, frequent relapses and low 5-year overall survival and failure-free survival (20-30%). The only factors consistently associated with prognosis are stage and IPI. New scoring systems have recently been developed. Gene signature, high proliferation, EBV positivity, NF-κB pathway deregulation and cytotoxic granule expression have been found to correlate with a poor prognosis.

Lympho-epithelioid variant (Lennert lymphoma): it shows diffuse or, more rarely, interfollicular growth. Cytologically, it consists mostly of small cells with slightly irregular nuclei, confluent clusters of epithelioid histio-

cytes, and some larger, more atypical, proliferating blasts (at times with RS-like morphology and EBV-positivity). High-endothelial venules (HEV) are not prominent. In most cases, the neoplastic cells are CD8 positive.

Follicular variant: it usually consists of atypical clear cells forming intra-follicular aggregates (mimicking follicular lymphoma), small nodular aggregates in a background of progressively-transformed germinal centres (mimicking nodular lymphocyte-predominant HL), or enlarged peri-follicular zones/nodular aggregates surrounding hyperplastic follicles (mimicking nodal marginal-zone B-cell lymphoma). Despite a follicular TH phenotype, the early stage disease, partial lymph node involvement, lack of enlarged follicular dendritic cell (FDC) mesh-works, and lack of prominent HEV distinguish it from typical AITL Recently, a t(5;9) translocation has been reported.

AITL occurs in the middle aged and elderly, with no sex predominance. It accounts for 15-20% of all PTCLs. AITL typically presents with advanced stage disease, generalised lymphadenopathy, hepatosplenomegaly, frequent bone-marrow and skin involvement, systemic symptoms, and polyclonal hypergammaglobulinemia. Skin rash, often with pruritus, oedema, pleural effusion, arthritis, and ascites are frequently seen. Laboratory findings include circulating immune complexes, cold agglutinins with haemolytic anaemia, positive rheumatoid factor, and anti-smooth muscle antibodies. Patients exhibit immunodeficiency secondary to the neoplastic process, that probably sustains expansion of EBV^+ B-cells in 75% of cases.

AITL is characterised by partial effacement of the lymph node structure, often with peri-nodal infiltration but sparing of the peripheral sinuses. There is marked proliferation of arborizing HEV. The infiltrate is predominantly located in the paracortex and is composed of small to medium-sized lymphocytes, with clear to pale cytoplasm and distinct cell membranes and minimal cytological atypia. The neoplastic cells often form small clusters around the follicles and HEV and are admixed with variable numbers of small reactive lymphocytes, eosinophils, plasma cells and histiocytes. Such polymorphic infiltrate is frequently associated with increased FDC mesh-works. Early cases may contain hyperplastic follicles with ill-defined borders, surrounded by clusters of clear neoplastic cells. An expansion of B-immunoblasts (usually EBV^+) is nearly always present. It may progress, either composite with AILT, or at relapse to EBV^+ diffuse large B-cell lymphoma (DLBCL).

The neoplastic T-cells express most T-cell antigens such as CD3, CD2 and CD5 and, in vast majority of the cases, CD4. Characteristically, they show phenotype of normal follicular T-helper cells (FTHL) expressing CD10, CXCL13 and PD-1 in 60-100% of the cases (*see below*). This phenotype is helpful in distinguishing AITL from atypical paracortical hyperplasia and PTCLs as well as diagnosing extra-nodal dissemination. B-immunoblasts and plasma cells are polytypic, however secondary EBV^+ B-cell proliferations including DLBCL, CHL or plasmacytoma may be seen. FDC mesh-works ($CD21^+$, $CD23^+$, $CD35^+$) are expanded, usually surrounding HEV.

T-cell receptor genes show clonal rearrangements in 75-90% of cases. Clonal immunoglobulin gene rearrangements may also be found in 25-30% of cases and correlate with expanded EBV^+ B-cells. The most frequent cytogenetic abnormalities are trisomy 3, trisomy 5, and an additional X chromosome. CGH has shown gains of 22q, 19, and 11q13 and losses of 13q in subset of cases. Gene expression profiling studies have revealed that the neoplastic cells show features of $CD4^+$ FTHL. In addition, they display over-expression of VEGF both at the genomic and protein level: this can lead to way to the usage of anti-angiogenetic drugs.[4-6]

The clinical course is aggressive with a medi-

an survival of less than 3 years. Patients often succumb to infectious complications, which makes delivery of aggressive chemotherapy difficult. Supervening DLBCL (often but not invariably EBV^+) can occur.

ALK-positive ALCL [anaplastic lymphoma kinase (ALK) positive anaplastic large cell lymphoma (ALK^+ ALCL)] is a T-cell lymphoma consisting of lymphoid cells that are usually large with abundant cytoplasm and polymorphic, often horseshoe-shaped nuclei, with a translocation involving the ALK gene and expression of ALK protein, and expression of CD30. ALK^+ ALC must be distinguished from ALK^- ALCL (*see below*), primary cutaneous ALCL and other subtypes of T- or B-cell lymphoma with anaplastic features and/or CD30 expression. ALK^+ ALCL accounts for approximately 3% of adult non-Hodgkin lymphomas and 10-20% of childhood lymphomas. ALK+ ALCL is most frequent in the first three decades of life and shows a male predominance (M/F ratio = 1.5).

The majority of patients (70%) present in stage III-IVB. The tumour involves both lymph nodes and extra-nodal sites (mainly skin, bone, soft tissues, lung and liver). The incidence of bone-marrow infiltration is about 30% when immunohistochemical stains are used. The small cell type of ALCL may have a leukaemic presentation.

ALK^+ ALCL shows a broad morphologic spectrum. However, all cases contain a variable proportion of cells with eccentric, horseshoe- or kidney-shaped nuclei often with an eosinophilic region near the nucleus. These cells have been referred to as hallmark cells since they are observed in all morphologic variants. Depending upon the plane of section, some cells may appear to contain cytoplasmic inclusions that are actually invaginations of the nuclear membrane: they are indicated as doughnut cells. Schematically, five morphologic subtypes of ALK^+ ALCL can be recognized.

The "common" type accounts for 60% of cases. The tumour cells have abundant cytoplasm that may appear clear, basophilic or eosinophilic. Multiple nuclei may occur in a wreath-like pattern and may give rise to cells resembling Reed-Sternberg cells. When the lymph node architecture is only partially effaced, the tumour characteristically grows within the sinuses and, thus, may resemble a metastatic process. The "lympho-histiocytic" type (10%) is characterised by tumour cells prevalently of small size, admixed with a large number of reactive histiocytes that may mask the malignant growth. The neoplastic cells often cluster around blood vessels and can be highlighted by immunostaining using antibodies to CD30 and/or ALK. The "small cell" type (5-10%) shows a predominant population of small to medium-sized neoplastic cells with irregular nuclei. Some cases consist of a predominant population of small cells with pale cytoplasm and centrally located nucleus, sometimes referred to as "fried egg cells". Hallmark cells are always present and are often concentrated around blood vessels. This morphologic variant of ALCL is often misdiagnosed as PTCL/NOS by conventional examination. When the blood is involved, atypical cells reminiscent of flower-like cells can be noted in smear preparations. The "Hodgkin's-like" type (3%) is characterized by morphological features mimicking nodular sclerosis CHL. More than one pattern may be seen in a single lymph node biopsy or may simultaneously occur at different anatomic sites (15%). Relapses may reveal morphologic features different from those seen at presentation.

At immunophenotyping, the tumour cells are positive for CD30 on the cell membrane and Golgi region, although smaller elements may be only weakly positive or even negative. ALK protein is invariably up-regulated with variable patterns of expression depending on the chromosomal translocation involving the correspon-

ding gene. In most cases carrying t(2;5)/NPM-ALK translocation, ALK staining of large cells is both cytoplasmic and nuclear. In the small cell type, ALK-positivity is usually restricted to the nucleus of tumour cells. In cases with variant translocations (*see below*), ALK staining may be membranous or cytoplasmic. Most ALK$^+$ ALCLs are positive for EMA but, in some cases, only a proportion of malignant cells is positive. The great majority of ALK$^+$ ALCLs express one or more T-cell antigens. However, due to loss of several pan T-cell antigens, some cases may have an apparent "null cell" phenotype, but show evidence for a T-cell lineage at the genetic level. Furthermore, most cases exhibit positivity for the cytotoxic associated antigens TIA-1, granzyme B, and/or perforin. CD43 is expressed in two thirds of the cases, but this antigen lacks lineage specificity. Tumour cells are variably positive for CD45 and CD45RO and strongly positive for CD25. CD15 expression is rarely observed. ALCLs are also consistently negative for EBV (i.e. EBER and LMP1). A number of other antigens are expressed in ALK$^+$ ALCL but are not of diagnostic value. These include clusterin, SHP1 phosphatase, BCL-6, C/EBPβ, serpinA1, and fascin.

Approximately 90% of ALK$^+$ ALCLs show clonal TCR rearrangement irrespective of the expression of T-cell antigens. ALK gene is involved in various chromosomal translocations that affect the subcellular distribution of chimeric proteins. The most frequent genetic alteration is a translocation, t(2;5)(p23;35), between the ALK gene on chromosome 2 and the nucleophosmin (NPM) gene on chromosome 5. Variant translocations involving ALK and other partner genes on chromosomes 1, 2, 3, 17, 19, 22 and X also occur. All these translocations result in up-regulation of ALK, but the subcellular distribution of the staining varies depending on the translocation.

The ALK gene encodes a tyrosine kinase receptor which is normally silent in lymphoid cells. In the t(2;5)(p23;35), the NPM gene fuses the ALK gene to produce a chimeric protein in which the N terminal portion of NPM is linked to the intracytoplasmic portion of ALK. The particular cytoplasmic, nuclear and nucleolar staining seen in cases associated with the t(2;5) id due to the formation of heterodimers between wild-type NPM (i.e. a shuttle-protein) and the fusion NPM-ALK protein. On the other hand, the formation of NPM-ALK homodimers using dimerisation sites at the N-terminus of NPM mimicks ligand binding and is responsible for the activation of the ALK catalytic domain and for the oncogenic properties of the ALK protein. In cases carrying different translocations, ALK staining is restricted to the cytoplasm of malignant cells.

CGH analysis shows that ALK$^+$ ALCLs carry frequent secondary chromosomal imbalance including losses of chromosome 4, 11q and 13q and gains of 7, 17p ans 17q. In addition, ALK$^+$ and ALK- ALCLs have a different representation of secondary genetic alterations, supporting the concept that they are different biological entities. Supervised analysis by class comparison between ALK$^+$ and ALK$^-$ ALCL tumours provided distinct molecular signatures. Among the 117 genes over-expressed in ALK$^+$ ALCL, BCL-6, PTPN12, serpinA1 and C/EBPβ were the four top genes.

The IPI appears to be of some value in predicting outcome in ALK$^+$ ALCL. The overall 5-year survival rate is close to 80%, in contrast to only 40% in ALK- ALCL. Relapses are not uncommon (30% of cases), but often remain sensitive to salvage therapies. Since it is now clearly demonstrated that ALK is essential for the proliferation and survival for ALK$^+$ ALCL cells, it can be expected that specific ALK inhibitors would be available in the near future for clinical trials.

ALK-negative ALCL (ALK$^-$ ALCL) is a CD30$^+$ PTCL that is not distinguishable on mor-

phological grounds from ALK+ ALCL, but lacks ALK protein. Importantly, it must be differentiated from primary cutaneous ALCL, other subtypes of CD30+ T- or B-cell lymphoma with anaplastic features, and CHL. In the third edition of the WHO classification, it was included together with ALK+ ALCL, in the broader entity of "anaplastic large cell lymphoma". However, it was also recognised that ALK- ALCL (mainly because of its older median age and more aggressive clinical course) may well prove to be a distinct entity from ALK+ ALCL. Some experienced pathologists have gone further by arguing that ALK- ALCL, in the light of its poor prognosis and partially overlapping phenotype, should simply be considered an "anaplastic variant" of PTCL/NOS. Although these questions are not fully resolved, in the light of available data (e.g. from gene expression profiling), it was felt appropriate to consider ALK-negative ALCL a provisional entity in the new WHL Classification, distinct from both ALK+ ALCL and PTCL/NOS.

The peak incidence of ALK- ALCL is in adults (40-65 years), unlike ALK+ ALCL. There is no clear sex preponderance.

Phenotypically, more than 50% of the cases express one or more T-cell markers, CD2 and CD3 being found more often than CD5, and CD43 being almost always expressed. CD4 is positive in a significant proportion of cases, whereas CD8 positivity is rare. Many cases express the cytotoxic-associated markers TIA-1, granzyme B, and/or perforin. A substantial minority of cases is EMA+. CD15 is occasionally expressed. ALK- ALCLs are consistently negative for EBV. The latter finding along with lack of PAX-5 staining does assist in differentiating ALK- ALCL with null phenotype from CHL.

The majority of cases show clonal rearrangement of the TCR genes, whether or not they express T-cell antigens. EBV sequences are absent. No recurrent primary cytogenetic abnormalities occur with any frequency. Some studies indicate a tendency of ALK- ALCL to differ (e.g. in terms of chromosome losses or gains) both from PTCL/NOS and ALK+ ALCL, although overlapping features can also be found. Similarly, published gene expression studies (in which the number of cases studied is relatively small) suggest that ALK- ALCL has a distinct profile but these results do not provide definitive evidence as to whether the disease is more closely related to ALK+ ALCL or PTCL/NOS.

The clinical outcome of ALK- ALCL with conventional therapy is clearly poorer than that of ALK+ ALCL. There is some suggestion that there is a plateau of long-term survivors not seen in PTCL/NOS).[7]

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