Molecular Profiling and Prognosis in T-Cell Lymphomas

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Background

In 1994, the Revised European–American Lymphoma (REAL) Classification introduced new standards in the lymphoma field [1]. In particular, it stated for the first time that a classification of lymphoid tumors should consist in a list of "real" entities, each defined by the amalgamation of cell morphology, phenotype, molecular genetics, clinical data, and identification of a normal counterpart, if possible [1]. After a validation trial [2], the REAL Classification was adopted by the World Health Organization (WHO) as guideline for lymphoma diagnosis and therapy [3]. On such occasion, its methodology was extended to all tumors of the haematopoietic system [3]. According to patients' survival without any treatment, non-Hodgkin lymphomas (NHLs) are classified as indolent (survival measurable in years) and aggressive (survival measurable in months).

Peripheral T-cell lymphomas (PTCLs) belong to the aggressive lymphoma group [4]. They represent approximately 12% of all lymphoid neoplasms [4, 5]. Their incidence varies in different countries and races, being higher in HTLV-1 endemic areas (Asia, Caribbean basin, and some parts of the United States) [4, 5]. PTCLs are a heterogeneous group of tumors that can be roughly subdivided into specified and not otherwise specified (NOS) forms [5, 6]. In particular, the latter—corresponding to about 35% of T-cell lymphomas—cannot be further classified on the basis of morphology, phenotype, and conventional molecular studies [4]. Usually, they occur in the fifth–sixth decade of life, without sex predilection [7–10]. Although PTCLs/NOS can present as isolated disease, they more often have a widespread dissemination (stage III–IV) with nodal, skin, liver, spleen, bone-marrow, and peripheral blood involvement [7–10]. B-symptoms are recorded in about 45% of cases at diagnosis. A haemophagocytic syndrome may also be encountered [7–10].

The tumor morphology is highly variable, comprising cells of different size and shape [4]. PTCLs/NOS may contain prominent reactive components, including small lymphocytes, eosinophils, plasma cells, histiocytes, and epithelioid elements [4].

Immunohistochemistry does generally show T-cell associated molecule expression, although the phenotypic profile is aberrant in about 80% of cases [11].

Clonal rearrangements of T-cell receptor encoding genes are generally detected [12]. The karyotype is aberrant in more than 80% of cases and often characterized by complex abnormalities. However, specific alterations have not been identified [13]. Recently, some recurrent lesions have been documented by comparative genomic hybridization and SNPs analysis [14, 15].

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On clinical grounds, PTCLs/NOS are among the most aggressive NHLs. In the majority of cases, the response to conventional chemotherapy is indeed frustrating, with relapse free and overall survival (OS) rates at 5 years below 30% [5].

Besides the PTCL/NOS category, histological classification remains anyway a basic prognostic indicator in the PTCL setting [5, 6, 8, 16]. First, nodal and extranodal entities are clinically well distinct, as extranodal tumors, and specially the cutaneous forms, often display a relatively good outcome [5]. In addition, among nodal PTCLs, the distinction between anaplastic large cell lymphoma (ALCL) and other entities as well as the distinction of ALK^+ and ALK^- cases among ALCLs retain a significant prognostic impact [5, 6, 8, 16]. In fact, ALK^+ ALCL, particularly when occurring in children and young adults, have a significantly better clinical outcome if compared with all other forms [5, 6]. Importantly, it was recently suggested to include ALK^- ALCL within the PTCLs/NOS basing on the lack of evidences of clear biological differences between them. However, new clinical and molecular findings demonstrated that ALK^- ALCL and PTCL/NOS are distinct entities, also presenting with different clinical outcome [6, 17, 18].

In addition to the basic distinction of the different entities, in that last years several attempts have been made in order to further characterize the molecular pathology of PTCLs and identify reliable prognostic indicators to be offered to clinicians. Indeed, novel insights have been provided by gene expression profile (GEP) studies, specially as far as tumor histogenesis, molecular pathogenesis, and possibly new targeted therapies are concerned. On the other hand, novel and more refined prognostic scores have been proposed, which will be also discussed in this chapter.

Gene Expression Profiling of Peripheral T-Cell Pymphoma

The pathobiology of PTCLs has been neglected for a long time. The main reasons for that probably relied on the relative rarity of the disease, as well as the extreme difficulty to culture these neoplastic cells ex vivo. However, in the last few years, a new interest on PTCLs did emerge. Specially, these lymphomas have been then the object of different studies based on the application of high throughput technologies and several reports dealt with the GEP of the different subtypes [17–32] (Table 3.1). In particular, on one hand, some authors focused on specific topics: that is, the GEP of mycosis fungoides, ALK^+ and ALK^- ALCLs, angioimmunoblastic lymphomas (AITL), γδ-T-cell lymphomas, adult T-cell lymphoma/leukemia (ATLL), and extranodal NK/T lymphoma nasal-type, respectively [19, 25, 26, 28–31]. On the other hand, others analyzed larger collections of PTCLs of the NOS, AITL, and ALCL types [17, 18, 20, 23, 24, 32]. However, some of these studies suffer of limitations that vary from the usage of chips with a restricted number of genes [20, 23, 24] to the lack of a reliable normal counterpart for comparison [20, 24]. Specifically, Martinez-Delgado et al. reported that PTCL/NOS corresponds to a heterogeneous group of tumors, whose GEP is difficult to interpret due to the significant amount of infiltrating reactive cells. According to these authors, the only clinically relevant information provided by GEP pertains the expression level of genes belonging to the NFκB pathway (see below) [20]. Ballester et al. [23] found that the GEP could discriminate among PTCLs of the NOS, AITL, and ALCL types, although the former did not share a single profile. Using a multi-class predictor, the authors separated their cases into three molecular subgroups called U1, U2, and U3. The U1 gene expression signature included genes known to be associated with poor outcome in other tumors, such as *CCND2*. The U2 subgroup was associated with over-expression of genes involved in T-cell activation, including *NFKB1* and *BCL2*. The third group was mainly defined by the over-expression of genes involved in the IFN/JAK/STAT pathway and comprised most histiocyte-rich tumors. This finding suggests that the signatures recorded by Ballester et al. might be at least in part influenced by reactive components. Nevertheless, at present, it is not defined yet whether the presence of specific reactive components may significantly affect the tumor behavior

Table 3.1 Summary of the main gene expression profile studies dealing with peripheral T-cell lymphomas

Reference	Disease(s) explored	Comments
Tracey et al. [19]	MF	The manuscript explored GEP of MF and showed concurrent deregulation of multiple genes involved in the TNF signaling pathway
Martinez-Delgado et al. [20]	PTCL/NOS	The authors found significant differences between the peripheral and lymphoblastic T-cell lymphomas, which include a deregulation of nuclear factor-κB signaling pathway
Martinez-Delgado et al. [21]	PTCL/NOS	The Authors found two different subgroups of PTCL based on the expression of NFκB-related genes. One-third of PTCL showed clearly reduced expression of NFκB genes, while the other group was characterized by high expression of these genes. Of interest, the expression profile associated to reduced expression of NFκB genes was significantly associated with shorter survival of patients
Ballester et al. [23]	PTCL/NOS, AILT, ALCL	According to this study, PTCL/NOS could be divided into three molecular subgroups called U1, U2, and U3. The U1 gene expression signature included genes known to be associated with poor outcome in other tumors, such as *CCND2*. The U2 subgroup was associated with over-expression of genes involved in T-cell activation and apoptosis, including NFKB1 and BCL2. The U3 subgroup was mainly defined by over-expression of genes involved in the IFN/JAK/STAT pathway. Notably, such distinction possibly reflected, at least in part, the presence of reactive components in the PTCL samples
De Leval et al. [26]	AILT	The molecular profile of AITL was characterized by a strong microenvironment and over-expression of several genes characteristic of normal follicular helper T (TFH) cells (*CXCL13, BCL6, PDCD1, CD40L, NFATC1*). Such finding was reinforced by gene set enrichment analysis, which demonstrated that the AITL molecular signature was significantly enriched in TFH-specific genes
Piccaluga et al. [17]	PTCL/NOS	The Authors showed that PTCLs/NOS are most closely related to activated peripheral T-lymphocytes, either CD4+ or CD8+, basing on the GEP. In addition, PTCLs/NOS displayed deregulation of relevant functional cell programs. In particular, among others, *PDGFRA*, a gene encoding for a tyrosine–kinase receptor, turned out to be aberrantly expressed by PTCL/NOS. Notably, both phosphorylation of PDGFRA and sensitivity of cultured PTCL cells to imatinib were demonstrated
Piccaluga et al. [81]	PTCL/NOS	In this study, the Authors found that CD52 is expressed in approximately 40% of PTCLs/NOS at the same level as in normal T-lymphocytes, being aberrantly down-regulated in the remaining cases. Notably, they concluded that the estimation of CD52 expression may provide a rationale for the selection of patients with a higher probability of response to the anti-CD52 antibody, alemtuzumab
Piccaluga et al. [28]	AILT	In this manuscript, the Authors reported that AILT and other PTCLs have rather similar GEP, possibly sharing common oncogenic pathways. In addition, they found that the molecular signature of follicular T-helper cells was significantly over-expressed in AILT. Finally, several genes deregulated in AILT, representing potential therapeutic targets such as *PDGFRA* and *VEGF*, were identified
Lamant et al. [25]	ALCL	This study focused, for the first time, on ALCLs. Unsupervised analysis classified ALCL in 2 clusters, corresponding essentially to morphologic subgroups and clinical variables. Supervised analysis showed that ALK + ALCL and ALK − ALCL have different GEPs, further confirming that they are different entities

(continued)

Table 3.1 (continued)

Reference	Disease(s) explored	Comments
Cuadros et al. [27]	PTCL/NOS	In this study, the Authors identified five clusters of genes, the expression of which varied significantly among the samples. Genes in these clusters were functionally related to different cellular processes such as proliferation, inflammatory response, and T-cell or B-cell lineages. Notably, over-expression of genes in the proliferation signature was associated significantly with shorter survival of patients
Miyazaki [29]	γδ-TCL	This study focused on the GEP of 7 γδ-TCL cases Notably, hepatosplenic γδ-TCLs were clustered together, while the other γδ-TCLs were scattered within the αβ-TCLs In addition, a classifier based on GEP was developed, able to distinguish γδTCL
Pise-Masison [30]	ATLL	This study focused on ATLL and provided evidence that BIRC5 plays an important role in ATL cell viability as well as other important insights into ATLL pathogenesis and potential targeted therapies
Huang et al. [31]	NKTCL	This study highlighted emerging oncogenic pathways in NKTCL and identified novel diagnostic and therapeutic targets. In particular, deregulation of the AKT, JAKSTAT, and NFκB pathways was documented. In addition, aberrant expression of *PDGFRA,* and sensitivity to imatinib were demonstrated
Iqbal et al. [32]	PTCL/NOS, AILT, ALCL, ATLL	This study collected a large panel of PTCLs within the ITCLP and provided important information. First, confirmed the existence of at least 2 PTCL/NOS subtypes, based on the cellular derivation, the helper and cytotoxic ones. Second, the author suggested that the latter are provided with a worse prognosis. Finally, a molecular classificatory was built for AITL, ATLL, and ALK^+ ALCL
Piva et al. [18]	PTCL/NOS, AILT, ALCL	This study specially focused on ALCLs. It was shown that the molecular signature of ALK^+ cases largely relies on STAT3 signaling. In addition, it was shown that ALCLs are distinct from other PTCLs and selected genes can discriminate ALK^+ vs. ALK^- or ALK^- vs. PTCL/NOS

MF mycosis fungoides, *PTCL/*NOS peripheral T-cell lymphoma, not otherwise specified, *AILT* peripheral T-cell lymphoma, angioimmunoblastic type, *ALCL* anaplastic large cell lymphoma, *ATLL* Adult T-cell leukemia/lymphoma, *γδ-TCL* γδ T-cell lymphoma, *NKTCL* NK/T-cell lymphoma, nasal-type, *ITCLP* International T-cell lymphoma project, *GEP* gene expression profile

in the field of PTCL/NOS, as it appeared in the case of some B-cell derived lymphomas (namely, follicular and Hodgkin lymphomas) [33, 34], and possibly AITL (see below) [32].

Subsequently, Piccaluga et al. [17] have published a GEP study based on the analysis of 28 PTCLs/NOS, all corresponding to lymph node biopsies and containing an amount of neoplastic cells that exceeded the 70% value of the whole examined population. The mRNA extracted from these cases was hybridized on the HG U133 2.0 *Plus* gene chip. The obtained results were compared with those of six AITLs, six ALCLs (two ALK^+ and four ALK^-), and 20 samples of normal T-lymphocytes, purified from the peripheral blood and tonsil and corresponding to the main T-cell subsets ($CD4^+$, $CD8^+$, resting, and activated). Thus, the study of Piccaluga et al. significantly differs from most previous reports [20, 23, 24] in terms of methodology and selection criteria. In addition, it provides for the first time the rationale for possible targeted therapies in PTCL/NOS by offering clear evidence of their effectiveness ex vivo. In particular, the GEP detected by Piccaluga et al. [17] indicates that PTCLs/NOS are distinct from the other lymphoid malignancies and normal T-lymphocytes, establishing a clear relationship between PTCL/NOS and normal cellular counterparts and providing the basis for a better understanding of their pathogenesis.

More recently, Iqbal and Colleagues analyzed a large series of PTCLs, collected within the International T-cell lymphoma Project (ITCLP) [32]. Importantly, they could build a

robust molecular classifier for ATLL, AITL and ALK$^+$ ALCL. On the other hand, PTCLs/NOS were confirmed to have a more heterogeneous profile possibly related to those of the normal counterparts. In addition, importantly, this study provided novel evidences on AITL and PTCL/NOS prognostication (see below). Additional, important, information have been then offered by Piva et al. [18]. In their study, the authors mainly focused on the molecular pathogenesis of ALCLs but also established the relationship between ALCL and PTCL/NOS. Importantly, they showed that ALCLs are molecularly distinct from PTCL/NOS, thus flattening the diffuse, though not biologically based, proposal of including ALK$^-$ ALCL within the group of PTCL/NOS. Consistently, in the course of their analysis, Piccaluga et al. [17] already found that all ALCLs tended to cluster together, irrespectively of their ALK positivity or negativity, though in a more limited number of cases. In all, this suggests that—besides the occurrence or not of a translocation involving the ALK gene at 2p23—these tumors share a set of deregulated pathways. On the other hand, it is possible to clearly differentiate ALK$^+$ and ALK$^-$ cases basing on GEP, as shown by different authors [18, 25, 32]. To this regard, in particular, it was demonstrated the strong biological relevance of the ALK/STAT3 signaling in characterizing the global molecular profile of ALK+ALCL [18].

Histogenesis of PTCLs

In the REAL and subsequent WHO classifications of lymphomas, the recognition of the non-neoplastic cellular counterpart is regarded as a main factor contributing to the definition of the single disease entity. However, differently from the field of B-NHLs, the vast majority of PTCLs have not yet definitely associated to a normal counterpart, mainly due to the complexity of T-cell compartment, as well as the bizarre morphology and largely aberrant phenotype of the neoplastic elements. Nevertheless, the recent GEP studies provided evidences which may be the basis for a future classification of these tumors. First, robust data were generated supporting the concept that AITL cells correspond to follicular T-helper (F_{TH}) cells [26, 28]. Specifically, De Leval and Colleagues studied 18 AITL cases and by gene set enrichment analysis [35] found that AITL signature is significantly enriched in molecules characteristic of normal T_{FH}, including *CXCL13, BCL6, PDCD1, CD40L*, and *NFATC1* [26]. At the same time, Piccaluga et al., by using a different algorithm, also showed that the GEP of AITL is definitely related to that of T_{FH} lymphocytes [28]. Noteworthy, both the studies proved that such feature is restricted to AITL cases [26, 28], though a small fraction of PTCLs/NOS, more often characterized by clear cell cytology, presence of blastic EBV$^+$ B-cells, and sometimes, follicular architecture [4] also presents with T_{FH} molecular pattern [26]. Importantly, GEP results were validated by the immunohistochemical demonstration of F_{TH} markers, such as CD10, BCL6, CXCL13, PD1, CCR5, SAP, and ICOS, in AITL [26, 28, 36–40], and were largely in keeping with the observations previously made by Rüdiger et al. [41]. Subsequently, the expression of the same molecules was confirmed in follicular PTCL/NOS [42].

As far as PTCLs/NOS are concerned, interestingly, GEP results suggested that these tumors are more closely related to activated rather than resting T-cells [17]. As in normal mature T-lymphocytes, it was possible to identify two main subgroups of PTCL/NOS, with GEPs related to either CD4 or CD8 elements [17]. Notably, this characteristic did not correspond to the immunophenotype with regards to the expression of the single CD4 and CD8 molecules [17], reflecting the aberrancy in tumor phenotypes [11]. Importantly, the existence of these two molecularly distinct subgroups of PTCL/NOS was later confirmed by the ITCLP study [32]. Remarkably, the latter, also suggested that cases with cytotoxic molecular profile may be provided with a worse prognosis (see below). Finally, ALCLs, according to GEP, appeared to be related to either T_{H17} [32] (Piccaluga, unpublished) or T_{H1} lymphocytes (Piccaluga, unpublished).

Overall, the recognition of normal counterparts for different PTCLs has both biological and practical relevance. On one hand, in fact, it provides the basis for the recognition of cellular

abnormalities and comprehension of the interaction with the microenvironment (i.e., the relationship of TFH-derived neoplastic cells and follicular dendritic cells, mast cells etc. in AITL). On the other, it can be used in clinics for easier differential diagnosis, by applying new cell specific markers (i.e., a panel of F_{TH}-associated markers for the distinction of AITL and PTCL/NOS) [40].

Molecular Pathogenesis of PTCL

Besides histogenetic information, different GEP studies provided relevant insights into the functional alterations of PTCLs. First, a careful comparison of PTCL/NOS with the closest normal cellular counterparts revealed, in fact, the extensive deregulation of genes, which control functions that are typically damaged in malignant cells, such as matrix remodeling, cell adhesion, transcription regulation, proliferation, and apoptosis. In particular, the analysis of Piccaluga et al. [17] might explain the dissemination pattern of PTCL/NOS, with frequent extranodal and bone-marrow involvement and spread to peripheral blood [4], by showing the up-regulation of *FN1*, *LAMB1*, *COL1A2*, *COL3A1*, *COL4A1*, *COL4A2*, and *COL12A1*, that is, of genes which promote local invasion and metastasis in different types of human cancers [43–45]. In addition, it revealed the deregulation of genes involved in apoptosis (e.g., *MOAP1*, *ING3*, *GADD45A,* and *GADD45B*) [46–52] and chemo-resistance (such as *CYR61* and *NNMT*) [43–45, 53–64], which may be responsible for the poor response to conventional chemotherapy. Secondly, a couple of GEP studies suggested the possible deregulation of NFκB pathway in a certain number of PTCL/NOS cases [20, 21, 23]. Indeed, it was shown that around 30–40% of cases present with nuclear localization (i.e., activation) of NFκB elements and peculiar GEP [65]. Noteworthy, it was then demonstrated that a fraction of PTCL/NOS presents with REL locus abnormalities, including amplifications and translocations, finally leading to NFκB constitutive activation [15]. Interestingly, on the other hand, down-regulation of *BCL10* (an upstream activator of NFκB in human lymphocytes) was reported to occur in PTCL/NOS [17, 66] with consequent NFκB shut off. However, it is still debated whether (1) such cases NFκB negative cases have a worse clinical outcome and (2) whether anti-NFκB approaches can be eventually effective.

Finally, different studies characterized an aberrant tyrosine–kinase (TK) signaling in PTCLs [17, 18, 22, 31]. In particular, Piccaluga et al. showed that PTCLs/NOS constantly express the *PDGRA* gene and present with consistent phosphorylation (i.e., activation) of the encoded protein [17, 22]. Intriguingly, the same authors also found PDGF ligands to be produced by PTCL/NOS cells, proposing the intriguing hypothesis of an autocrine/paracrine loop activating this signaling [67]. Noteworthy, it was later on showed that also other T/NK-derived tumors present with such phenomenon with relevant therapeutic implications (see below) [31]. Moreover, Piva et al. demonstrated that STAT3 activation induced by ALK is a major contributor to ALK$^+$ ALCL molecular signature [18].

Importantly, immunohistochemistry was largely adopted in order to provide in situ validation of the genomic data by showing correspondence between mRNA and protein expression, as seen, for example, with *PDGFRA* [17, 22] and *BCL10 [*17, 66]. In addition, by comparison with normal tissues, immunohistochemistry allowed the identification of staining patterns corresponding to the synthesis of ectopic or paraphysiologic products by neoplastic cells. On the other hand, the phenotypic test highlighted the possibility that some of the results obtained by gene expression profiling may depend on non-neoplastic cellular components present in the analyzed sample, as seen for caldesmon [17].

Identifying Novel Targeted Therapies for PTCL

Tyrosine–Kinase Inhibitors

Basing on the evidence of TK deregulation in different subtypes of PTCLs, the application of TK inhibitors (TKI) has been tested ex vivo [17, 31]. In particular, Piccaluga et al. [17] designed experiments aiming to test the sensitivity of PTCL/NOS cells to different TKI, including imatinib mesylate. The results obtained were of

interest, with about 50% cytotoxic effect seen at 48 h with a 1 μmol concentration. Notably, imatinib exerted a limited effect on the viability of normal lymphocytes. On the other hand, Huang et al. treated with imatinib T/NK-tumor-derived cell lines, and obtained analog results [31]. Finally, as far as ALCL are concerned, consistently with GEP data, Chiarle et al. clearly showed that targeting ALK$^+$ and its downstream STAT3 is an effective strategy in ALK$^+$ ALCL [68, 69].

Histone–Deacetylase Inhibitors

Interestingly, GEP analyses provided evidence for the silencing of genes, possibly regulated by epigenetic mechanisms such as acetylation (e.g., *GADD45A* and *GADD45B*), and suggested to test histone–deacetylase inhibitors (HDACi) against PTCL/NOS primary cells and cell lines [17]. Notably, these compounds induced a dramatic reduction in cell viability, with G0–G1 cell cycle arrest and apoptosis at therapeutic concentrations, suggesting a possible role for this class of drugs in PTCL/NOS therapy. Noteworthy, this idea was also supported by some clinical preliminary observations [70]. Interestingly, the association of HDACi and daunorubicin apparently had a slight additive effect, as already observed in other settings [71]. Notably, the triple combination of TKI, HDACi, and anthracyclines produced a remarkable effect on cell viability: it might represent a promising option for future therapeutic applications.

Anti-angiogenetic Therapy

Increased angiogenesis is a major characteristic of AITL. However, its molecular basis has been unknown for a long time. Recently, a couple of studies documented the up-regulation of the *VEGF* gene in this tumor [26, 28]. Importantly, immunohistochemistry, extensively applied to a large series of cases on tissue microarrays, demonstrated that VEGF is mainly expressed by the neoplastic elements [28] and not only by the abundant vascular component, as initially proposed [26]. Remarkably, it was further shown that AITL cells do also express a VEGF-receptor±±±, VEGFR2/KDR [28], suggesting the hypothesis of an autocrine/paracrine stimulation also in this setting. In addition, it suggested the possible AITL sensitivity to anti-angiogenetic drugs, such as thalidomide and bevacizumab. Indeed, several reports have then documented their positive activity in AITL cases [72–77]

Monoclonal Antibodies

During the last few years, therapeutic monoclonal antibodies (MoAb) have become a major component of anti-lymphoma approaches. However, as far as PTCLs are concerned, a significant limitation emerged, differently from what seen in B-NHL. In particular, PTCLs were demonstrated to extensively present with the aberrant expression of T-cell-associated molecules [11, 17]. This phenomenon is indeed relevant in the clinical practice. In fact, some antigens against which, MoAb have been designed, such as CD4 [78] and, specially, CD52 [79, 80], are frequently down-regulated in T-cell tumors [11, 17, 81–83]. Based on these findings, different authors agreed that the estimation of CD52 expression may provide a rationale for the selection of patients with higher probability of responding to alemtuzumab, by avoiding the risk of unwanted toxicity [81]. Similar consideration will have to be applied to other antigens/MoAbs available in the future.

Prognostication of Peripheral T-Cell Lymphomas

Several prognostic indicators (summarised in Table 3.2) have been proposed that will be detailedly discussed in the following.

International Prognostic Index

The international prognostic index (IPI) was first introduced in 1993 with the intent of identifying patients with aggressive NHL and different

risk of treatment failure, relapse, and death [84]. It is based on clinical parameters, such as tumor stage, presence of extranodal localizations, age, lactate dehydrogenase (LDH) level, and performance status (PS). Notably, it was built up on B-NHL rather than T-NHL cases, and, specially, diffuses large B-cell lymphomas (DLBCL). However, its ability to stratify PTCL patients was reported in the following years. In particular, the International Lymphoma Study Group showed that overall and relapse free survival were significantly different in patients with low (0/1) vs. high (4/5) IPI, in patients with all PTCL type but ALCL (5-year OS, 36 vs. 15%; 5-year failure free survival, FFS, 27 vs. 10%) [2]. On the other hand, subsequent studies showed that IPI was particularly effective for prognostication of both ALK$^+$ and ALK$^-$ ALCL [6, 85]. In particular, in ALK$^+$ ALCL, the 5-year OS was 94 ± 5% for the low/low intermediate risk group vs. 41 ± 12% for the high/high intermediate group ($p < 0.0001$) [85]. Noteworthy, the IPI was more relevant than ALK expression in stratifying patients with ALCL (relative risk, 3.50 vs. 0.29, respectively), though both were significant prognostic factors [85]. In addition, in a large study within the ITCLP, the IPI effectively identified risk groups with different prognoses within both ALK$^+$ and ALK$^-$ ALCL, although those with an IPI score of 3 or more fell into the poor-risk category regardless of ALK status [6].

Furthermore, Suzumiya et al. recently reported on a large series of patients with aggressive adult T-cell leukemia/lymphoma (ATLL), providing evidence that IPI, platelet count, and B-symptoms were significant prognostic factors [86]. Interestingly, multivariate analysis indicated that only the IPI was an independent predictor of OS in this series, though the IPI significantly predicted for OS only in the lymphoma type of ATLL ($p = 0.04$), but not in the acute one ($p = 0.24$) [86].

Overall, IPI identifies patients at higher risk, being the expression of disease extension on one hand, and patient's frailty on the other. Indeed, it does offer neither specific biological hints nor potential target for overcoming drug resistance. In addition, it was soon clear that IPI was not as effective in PTCLs/NOS and AITLs (the two commonest PTCL types) as in the original series of DLBCLs, probably reflecting, at least in part, the fact that PTCL therapy is basically derived from B-NHLs and specific trials have been lacking for a long time. In particular, the extensive use of anthracyclines did not appear to provide significant benefits in PTCL patients [5]. Thus, novel scores have been investigated in this setting in the last few years (see below).

Prognostic Index for PTCL/NOS

In 2004, an Italian group (*Intergruppo Italiano Linfomi*, *IIL*) proposed a novel prognostic model based on a retrospective multi-centric clinical analysis of 385 patients [87]. Specifically, the new model, named *Prognostic Index for PTCL-U* (PIT), included bone-marrow involvement, age, PS, and LDH. When these four variables were combined in four groups, the PIT could identify patient subgroups with different outcomes. Noteworthy, the PIT turned out to be slightly more effective than IPI in stratifying PTCL patients (log-rank 66.79 vs. 55.94) and was then proposed as reference tool. In addition a simplified, two-classes PIT appeared to be superior to a simplified two-classes IPI (log-rank 49.36 vs. 30.23) [87]. However, PIT was based on a series lacking systematic histological review and though its value was confirmed within the ITCLP, it did not resulted superior to IPI. In particular, the PIT was also applied to ALK$^+$ and ALK$^-$ ALCL and was similarly predictive of FFS and OS in both groups [6]. However, given that the distribution of patients across the risk groups was very similar with the two prognostic models and that bone-marrow involvement is rarely observed in ALCL, the PIT actually seems to mirror the IPI in this setting [6].

In addition, as it does not include tumor-specific biologic factors, cannot be intended for the future application of targeted therapies.

Clinical-Pathologic Prognostic Score (Bologna Score)

Immunohistochemical markers have been largely proposed for prognostication of malignant

Table 3.2 Summary of prognostic markers and scores in peripheral T-cell lymphomas

Prognostic indicator	PTCL subtype	Reference
Histotype	All	The Non-Hodgkin's Lymphoma Classification Project [2]
		Vose et al. [5]
		Savage et al. [6]
		Lopez-Guillermo et al. [8]
		Ascani et al. [16]
IPI	All	The Non-Hodgkin's Lymphoma Classification Project [2]
	ALCL	Savage et al. [6]
		Falini et al. [85]
	ATLL	Suzumiya et al. [86]
	NK/TCL	Au et al. [108]
PIT	PTCL/NOS	Gallamini et al. [87]
		Savage et al. [6]
Bologna score	PTCL/NOS	Went et al. [11]
	AITL	Briones et al. [66]
Korean prgnostic index	NKTCL	Lee et al. [105]
NK prognostic index	NKTCL	Suzuki et al. [118]
EBV integration	PTCL/NOS	Went et al. [11]
	AITL	Kluin et al. [89]
		Dupuis et al. [90]
	NKTCL	Au et al. [108]Cheung et al. [60]
		Chim et al. [115]
		Huang et al. [116]
		Lee et al. [117]
		Ng et al. [114]
Proliferation (evaluated by)	PTCL/NOS	Went et al. [11]
Ki-67		Cuadros et al. [27]
Molecular signature		
Cellular derivation	PTCL/NOS	Went et al. [11]
		Bekkenk et al. [91]
		Kojima et al. [92]
		Iqbal et al. [32]
NFκB activation	PTCL/NOS	Martinez-Delgado et al. [21]
		Ballester et al. [23]
		Briones et al. [66]
CYP3A	PTCL/NOS	Rodriguez-Antona et al. [100]

PTCL/NOS peripheral T-cell lymphoma, not otherwise specified, *AILT* peripheral T-cell lymphoma, angioimmunoblastic type, ALCL anaplastic large cell lymphoma, *ATLL* Adult T-cell leukemia/lymphoma, *NKTCL* NK/T-cell lymphoma, nasal-type

lymphomas. As far as PTCLs are concerned, in a large collection of Italian cases, Went et al. recently found that high Ki-67 expression, Epstein–Barr virus (EBV) status, and CD15 staining were associated with the worst outcome in PTCL/NOS [11]. Interestingly, EBV has repeatedly been proposed as a negative prognosticator in PTCLs [88], both among Asian and European patients [89, 90]. Specifically, Went et al. found EBV-positivity in 5 and 3% of PTCLs, NOS, and AITL respectively: this value is definitely lower than the one recorded by Dupuis et al. in a

French cohort. Such discrepancies might reflect geographic or racial differences. No other immunohistochemical marker alone or in combination was associated with a poor outcome, although patients with tumors expressing CD57 or $CD4^+/CD8^-$ phenotype showed a tendency for a better outcome, the possible prognostic relevance of the latter having also been proposed by others [91, 92].

Furthermore, based on their collective largely provided with follow-up data and previous experience in the literature [87, 93–98], Went et al. developed a new score integrating both patient- and tumor-specific characteristics (age >60 years, PS, LDH, and Ki-67 marking ≥80%) and identifying three clear-cut groups of patients with different responses to therapy and life-expectancy. Such score seemed to show an improved ability to predict patient-outcome compared to previous indices, including IPI ($p<0.001$ vs. 0.1) and PIT ($p<0.001$ vs. 0.0043) [11]. In particular, according to the Bologna score, patients were clustered into three groups, which showed significantly different clinical outcome (median OS 37 vs. 23 vs. 6 months, respectively; $p<0.001$) [11].

Interestingly, the ability of the Bologna score was recently validated by a Spanish group [66].

Remarkably, all the factors contributing to the scoring system proposed by Went et al. [11] are part of the routine workup, making their integration simple and cost-effective, and incorporate both patient- and tumor-specific characteristics.

Gene Expression Profiling

In the last few years, several studies dealt with the gene expression profiling (GEP) of nodal PTCLs [17, 18, 20–23, 25–32, 81, 99], possibly providing novel insight into PTCL prognostication. First, a few reports suggested that PTCLs/NOS may present with up- or down-regulation of NFκB molecules [20, 21, 23], with possible prognostic relevance [21, 23]. In particular, cases with higher levels of NFκB-related molecules or other evidence of NFκB activation showed a better median OS (25 months, range 0–124 months, vs. 12 months, range 0–19 months; $p=0.032$) [21, 23]. This observation was then confirmed by another Spanish group, the 5-year OS being 45% vs. 0%, in $NF\kappa B^+$ and $NF\kappa B^-$ cases, respectively ($p=0.04$) [66]. However, all these studies included a relatively limited number of cases, by mixing different histotypes [21, 66], or cases with prominent non-neoplastic components [23], which might have influenced, at least in part the results.

In addition, basing on GEP obtained from 35 nodal PTCL cases (23 PTCLs/NOS and 12 AITLs), it was suggested that over-expression of genes involved in a so-called "proliferation signature" was associated significantly with shorter survival of patients [27]. This proliferation signature included genes commonly associated with the cell cycle, such as *CCNA*, *CCNB*, *TOP2A*, and *PCNA* [27]. Notably, this evidence of high proliferation as a possible adverse prognostic factor was definitely in line with what reported by Went et al. [11] and what observed within the ITCLP (unpublished), highlighting the importance of such parameter.

Finally, our Group, basing on GEP analyses, indicated that PTCLs/NOS can be subclassified according to their histogenesis. In particular, at least two subgroups were described, derived from activated helper and cytotoxic elements, respectively [17]. Importantly, such finding was recently confirmed by Iqbal et al. [32]. Intriguingly, in this report, it was also suggested that the cytotoxic profile may be associated with unfavorable outcome, though this evidence was based on a limited series and warrants further validation. On the other hand, a possible more favorable outcome for PTCL cases with helper phenotype had been also previously suggested by others [11, 91, 92].

Overall, GEP studies provided evidences that molecular features may be useful in defining the prognosis of PTCL patients. However, no complete explanation has been offered as far as the molecular bases of drug resistance are concerned. Notably, our group described for the first time the expression of molecules associated to drug resistance in solid tumors such as *CYR61* and *NNMT* in PTCL/NOS [17]. Furthermore, Rodríguez-Antona et al. recently found that a high expression of cytochrome P450 3A (CYP3A), an enzyme

involved in the inactivation of chemotherapy drugs, was associated to poor response to the standard PTCL chemotherapy, suggesting that CYP3A could be useful as a predictor of response [100]. Indeed, the molecular classification of PTCLs and the identification of key events in their molecular pathology will be probably the basis for future prognostication and targeted treatment in this field as in the case of DLBCL [101, 102].

Prognostication of NK/T-Cell Lymphoma, Nasal-Type

Extranodal natural killer/T-cell lymphoma, nasal-type is a distinct entity in the WHO classification of lymphoid tumors, more frequent in Asia and Central–South America than in Western countries [103–108]. Morphologically, tissue invasion, vascular destruction, and necrosis are the most prominent features; EBV is always integrated in the genome of neoplastic cells [107]. Most of the cases derive from natural killer (NK) cells and are characterized by a typical NK phenotype and T-cell receptor genes in germ-line configuration; however, in some instances a cytotoxic T lymphocyte origin was recognized [107]. The nasal cavity and the upper aerodigestive tract (nasal NK/T-cell lymphoma) are the most commonly involved sites, but skin, gastrointestinal tract, lung, testis, and soft-tissues (extra-nasal NK/T-cell lymphoma) can be also affected [103, 104, 107, 109].

The prognosis of extranodal natural killer/T-cell lymphoma is poor, being the worst among the PTCL categories [108]: survival rate is 30–40%, anyway some differences exist between nasal and non-nasal disease as the latter is more aggressive [107, 108, 110], therefore the inclusion of radiotherapy in treatment protocols improved outcome of nasal natural killer/T-cell lymphoma in stage I or II [106–108, 111, 112]. Among nasal forms, adverse prognostic factors are unfavorable IPI, advanced stage disease (stage III or IV), high circulating EBV DNA levels, and detection of EBV in bone-marrow cells by in situ hybridization [107, 108, 111, 113–117]. Some studies suggest that high proportion of large/transformed cells in tumoral population have a negative impact on survival: anyway the significance of cytological features as prognostic indicator is still uncertain [106–109]. The primary extra-nasal cases are highly aggressive with poor response to therapy even in patients with localized disease [107, 108].

Importantly, a new prognostic index, proposed by a Korean group, specifically developed for NK/T-cell tumors and based on four parameters, ("B" symptoms, LDH levels, stage and regional lymph node involvement) demonstrated a better prognostic stratification of NK/T-cell lymphomas as compared with IPI [105].

In addition, recently, another study showed that four factors (non-nasal-type, stage, performance status and numbers of extranodal involvement) were significant prognostic factors in NK/T-cell lymphomas [118]. Using these four variables, a NK prognostic index was successfully constructed, the 4-year OS of patients with zero, one, two and three or four adverse factors being 55, 33, 15, and 6%, respectively [118].

Conclusion

PTCLs have represented for a long time an orphan pathology. This can be explained by their relatively low incidence (that is anyway higher that of a "common" tumor, such as Hodgkin's lymphoma), the difficulties encountered in their analysis, and their dismal prognosis. During the last few years, however, a great deal of interest has developed shedding new light on the pathobiology of these tumors and leading to the proposal of more effective prognosticators. In particular, though IPI is somehow effective for PTCL prognostication, novel more refined and possibly disease specific scores have been explored, and several models including clinical–pathological and molecular features have been proposed, their validation process being now ongoing. In addition, innovative therapeutic schedules have been recently proposed, based on the application of the newly developed micro-array techniques. The morning of a new era seems quite close that will actually dissipate the shadows which have wrapped PTCLs for several decades.

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