Integrative clinicopathological and molecular analyses of angioimmunoblastic T-cell lymphoma and other nodal lymphomas of follicular helper T-cell origin

In addition to angioimmunoblastic T-cell lymphoma (AITL), the 2016 revised WHO classification of haematological malignancies recognizes two provisional lymphoma entities of follicular helper T-cell (TFH) derivation, namely follicular peripheral T-cell lymphoma (F-PTCL) and nodal PTCL with a TFH phenotype.[1] Here, we performed a comprehensive, integrative clinicopathological and molecular analysis of these three entities. We found that F-PTCL and other nodal PTCL with TFH phenotype share not only immunophenotypical features, but also similar clinical, genetic and molecular features with AITL. Our results support the view that these lymphomas belong to the spectrum of a common disease.

AITL and PTCL, not otherwise specified (PTCL-NOS), account for the majority of nodal PTCLs.[2,3] While PTCL-NOS is by definition heterogeneous and an exclusion diagnosis, AITL is characterized by a constellation of clinical, morphological, and immunophenotypical features, and defined by its cellular derivation from TFH cells.[1,4] The typical pathological features, i.e., clear cells, increased vascularization, follicular dendritic cell (FDC) proliferation, and the presence of eosinophils, inflammatory cells, and EBV-positive B-blasts, are variably developed.[5,6]

An overlap between AITL and PTCL-NOS was substantiated by the observation that a subset of cases diagnosed as PTCL-NOS, upon routine pathological evaluation, actually harbor imprints of the TFH signature[4] and/or express TFH-associated markers.[7,8] Furthermore, the rare "follicular" T-cell lymphoma (F-PTCL), initially classified as a PTCL-NOS variant, is characterized by a TFH immunophenotype and clinicopathological features overlapping with AITL.[9,10] Recently, recurrent AITL-associated *TET2, DNMT3A* and *RHOA* mutations were also found in a subset of PTCL-NOS, and tended to correlate with TFH features.[11,12] Based on these recent findings, the 2016 update of the WHO classification groups AITL and other nodal lymphomas of TFH origin under the same umbrella.[1] However, a thorough, systematic and multiparametric comparison of these entities is lacking.

Here, we performed a comprehensive integrative clinicopathological and molecular analysis comparing AITL and other nodal PTCLs of TFH origin. Twenty-one such cases, including five F-PTCL, were identified in the TENOMIC biobank of the LYSA, and compared to 94 AITL and 36 PTCL-NOS. All cases were reviewed by three hematopathologists according to the 2016 WHO classification criteria ([1] and *Online Supplementary Information*). The five F-PTCL by definition comprised FDCs associated to the follicles without extrafollicular FDC expansion, and were positive for all TFH markers tested (4 or 5) (Figure 1A-B). The 16 other nodal PTCL with TFH phenotype (referred to as "TFH-like PTCL") (Figure 1A, 1C, 1D) lacked typical morphological AITL

Table 1. Summary of clinical features, mutational status and copy number variations in AITL, other PTCL of TFH origin and PTCL-NOS.

	Nodal lymphomas of TFH cell origin (TFH-PTCL)			PTCL-NOS	*P* (Fisher test) across four entities
	AITL	Other TFH-PTCL			
		TFH-like PTCL	F-PTCL*		
Clinical variables					
Median age at diagnosis	67.8	65	67	62.5	
Sex (M)	53/94 (56%)	9/16 (60%)	2/5 (40%)	23/34 (68%)	0.54
Stage III-IV	84/85 (99%)	14/15 (93%)	4/4 (100%)	29/34 (85%)	0.02
ECOG ≥ 2	67/83 (53%)	3/12 (25%)	1/4 (25%)	11/33 (33%)	$< 1.0 \times 10^{-7}$
IPI ≥ 3	63/79 (80%)	11/15 (73%)	2/4 (50%)	20/31 (65%)	0.21
Coombs (+)	25/56 (45%)	1/3 (33%)	1/2 (50%)	0/6 (0%)	0.14[a]
Anemia	47/71 (66%)	5/10 (50%)	2/4 (50%)	10/27 (37%)	0.17[a]
Hypergammaglobulinemia (≥16 g/dl)	23/48 (48%)	1/8 (12.5)	1/3 (33%)	4/19 (21%)	0.08
Mutations (%)					
TET2	31/64 (48%)	7/11 (64%)	3/4 (75%)	4/24 (17%)	$< 1.0 \times 10^{-2}$
DNMT3A	19/64 (30%)	1/10 (10%)	1/4 (25%)	1/24 (4%)	0.02
IDH2	22/66 (33%)	1/11 (10%)	0/5 (0%)	0/23 (0%)	$< 1.0 \times 10^{-3}$
RHOA (G17V)	42/72 (58%)	8/14 (57%)	3/5 (60%)	0/23 (0%)	$< 1.0 \times 10^{-6}$
Copy number variations					
Events per patient (average)	3.17	3.94	2.36	10.8	
Patients with events	23/60 (38%)	4/12 (33%)	1/3 (33%)	17/27 (63%)	0.78
Heavily-rearranged (>10 events) patients	6/60 (8%)	3/12 (25%)	0/3 (0%)	11/27 (41%)	$< 1.0 \times 10^{-2}$
Patients with homozygous deletions or amplifications	3/60 (5%)	3/12 (25%)	0/3 (0%)	12/27 (44%)	$< 1.0 \times 10^{-4}$

*Two out of 5 F-PTCL (40%) had *ITK-SYK* translocation by FISH, 1/5 (20%) was negative and 2/5 (40%) had non- interpretable results. None of the other PTCL with interpretable signals (9 AITL, 5 TFH-like PTCL, and 4 PTCL-NOS) disclosed *ITK-SYK* rearrangement. [a]*P*-value < 0.05 when TFH-like PTCL and F-PTCL are calculated as a single entity (Other TFH-PTCL).

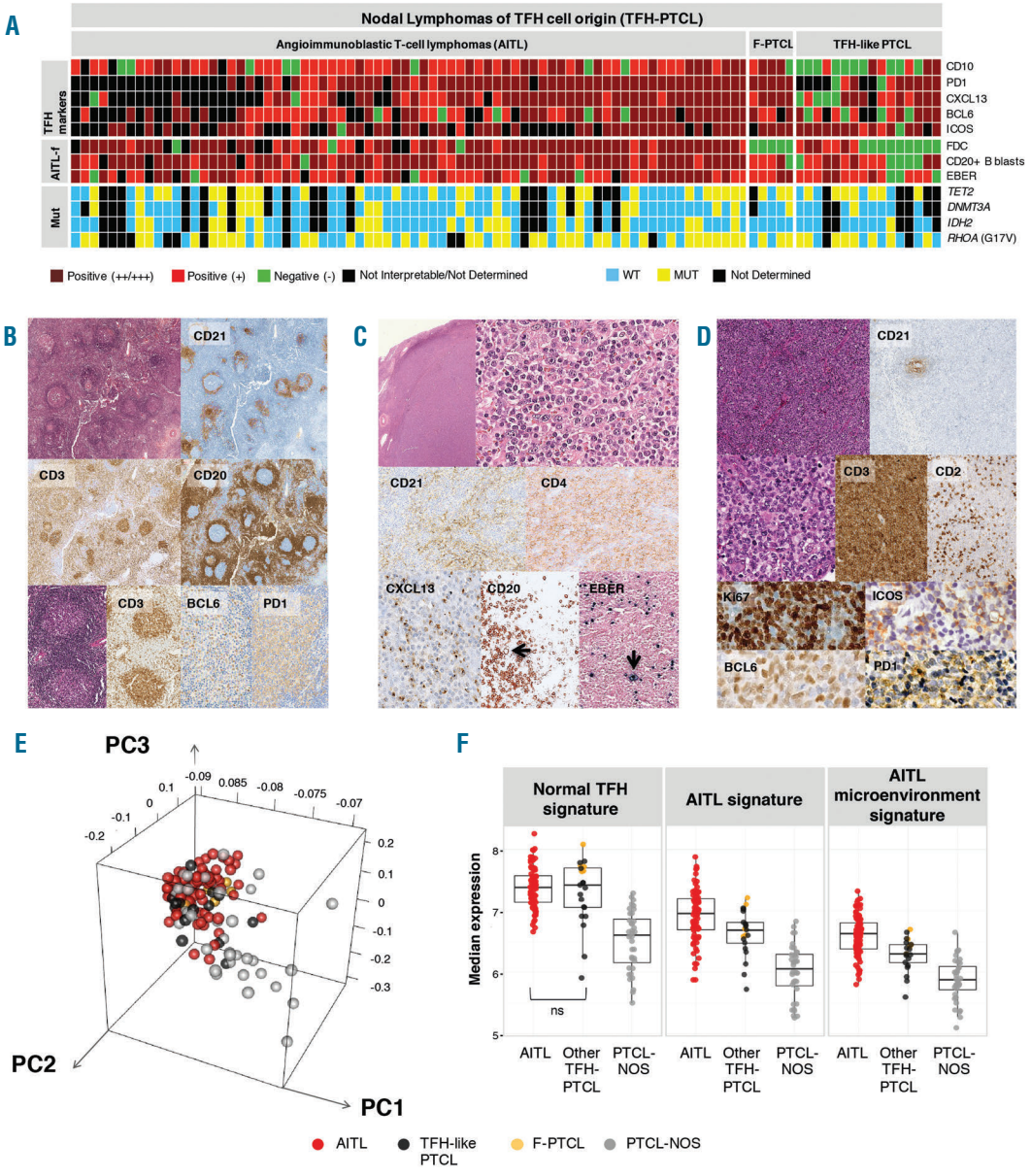

Figure 1. Morphological, immunophenotypical and molecular features of AITL and other PTCL of TFH origin. (A) Heatmap representation of TFH marker expression, AITL-like features and mutation status for epigenetic modifiers and *RHOA* in 74 AITL and 21 other nodal lymphomas of TFH cell origin including five F-PTCL. Of note, among the 16 cases with a diffuse pattern (TFH-like PTCL), 7 comprised some FDC expansion. Cases are arranged as a function of increasing column sums of the IHC scores; details on IHC scoring are included in *Online Supplementary Information*. Detailed score information is in *Online Supplementary Table S1*. TFH marker protein and mRNA expression correlations are in *Online Supplementary Table S2*. The mutational status for *RHOA, TET2, DNMT3A* and *IDH2* indicated for each case. (B) Representative example of a follicular PTCL (F-PTCL). Low-power view of the lymph node shows multiple nodules with pale centers surrounded by a rim of small, darker lymphocytes; CD21 staining underlines FDC meshworks in association with the nodules, but no extrafollicular FDC expansion; CD3 stains the cells in the center of the follicles, while CD20 is negative in the nodules and stains the cells forming the mantles. High-power view of the nodules shows pale lymphoid cells, expressing CD3, and the TFH markers BCL6 and PD1. (C-D) Representative examples of two TFH-like PTCL cases with (C) or without (D) FDC expansion. (C) At low-power, the lymph node shows diffuse architectural effacement, and extranodal infiltration without preservation of the peripheral sinus; high-power view of the lymphoproliferation shows a predominance of large transformed lymphoid cells featuring mitotic figures, and a minor reactive component of histiocytes and plasmacytoid cells. CD21 focally highlights the presence of a follicular dendritic cell meshwork in association with the neoplastic infiltrate; the large lymphoid cells are CD4^{+} and immunostaining confirms the abundance of the neoplastic component; many cells are CXCL13-positive; CD20 shows the presence of aggregates of small B cells and occasional larger B-cell blasts (arrow). *In situ* hybridization with EBER probes demonstrates Epstein-Barr virus in a small number of small lymphoid cells and occasional blastic cells. This case was also strongly positive for both PD1 and ICOS but negative for CD10 and BCL6. (D) Low-power view of the lymph node showing a diffuse lymphoma infiltrate; CD21 highlights small residual foci of follicular dendritic cells but no follicular dendritic cell expansion. High-power view of the lymphoproliferation composed of diffuse sheets of large lymphoid cells which are positive for CD3, negative for CD2, and feature a high proliferation index (Ki67). The lymphoma cells express several TFH markers including CXCL13 in scattered cells, BCL6 in most nuclei, and PD1. (E) Projection of samples on the first three component axes from principal component analysis (PCA) performed on 6000 genes with the most variable expression. Notably, with Consensus Clustering, which allows us to evaluate cluster stability based on ~1000 random subsamplings of 80% of the data, F-PTCL cases consistently clustered with AITLs. (F) Median levels of expression of the normal TFH cell signature, global AITL tumor signature and AITL microenvironment signature in AITL, other nodal PTCL of TFH origin, and PTCL-NOS. All but one (ns = not significant) of pairwise comparisons yielded statistically significant differences (P<0.01) between classes.

features, seven of them disclosed some FDC expansion, and all expressed a TFH phenotype defined by expression of at least two TFH markers among ICOS (14/15, 93%), PD1 (8/10, 80%), CXCL13 (11/15, 73%), BCL6 (9/13, 69%) and CD10 (6/16, 37.5%). Overall, TFH-like PTCL cases tended towards a more incomplete TFH profile compared to AITL (Figure 1A, *Online Supplementary Table S1*); PTCL-NOS cases included cytotoxic PTCLs, non-cytotoxic PTCLs strongly positive for CD30, and others with a non-TFH immunophenotype (*Online Supplementary Figure S1*). All five F-PTCL and 13/16 TFH-like PTCL comprised scattered $CD20^+$ and/or EBV-positive blasts (Figure 1A).

Clinically, TFH-like and F-PTCL patients had a high incidence of disseminated disease (93% and 100%, respectively), closer to the incidence in AITL (99%) than PTCL-NOS cases (85%, $P<0.02$). Within the limits of available data, other AITL-related features, such as a positive Coombs test and anemia, also occurred more frequently in TFH-PTCL than in PTCL-NOS (Table 1). Overall survival compared across entities using the Kaplan-Meier and log-rank methods did not significantly differ (*Online Supplementary Figure S2*), consistent with previous reports of poor outcomes (30-35% 5-year OS) for AITL and PTCL-NOS.[2,3]

Targeted sequencing performed in 114 cases, including 85 previously reported,[11,13] revealed similar mutation frequencies in *TET2, DNMT3A* and *RHOA* in AITL and other nodal lymphomas of TFH origin, while *IDH2* mutations, with a single exception, were restricted to AITL, consistent with recent findings (Figure 1A, Table 1). The *RHOA* G17V mutation rate in TFH-like (57%) and F-PTCL (60%) was comparable to the 58% frequency found in AITL.[12] *TET2* mutations were even more frequent in TFH-like (64%) and F-PTCL (75%) than in AITL (48%). The mutational portrait of F-PTCL has not yet been reported. Here, we found that 3/5 F-PTCLs carried *RHOA* and *TET2* mutations, plus a *DNMT3A* mutation in one case (Figure 1A). *ITK-SYK* translocations occurred in 2/5 F-PTCLs, one of which was *TET2*-mutated, and the other wild-type for the four genes tested. The frequency of mutations in PTCL-NOS was significantly lower (*TET2*, 17%; *RHOA* G17V, 0%; *DNMT3A*, 4%; *IDH2*, 0%). These results extend previous findings, demonstrating overlapping genetic characteristics in AITL and other PTCL of TFH origin,[13] including F-PTCL.

To investigate the clustering of F-PTCL and TFH-like PTCL compared to AITL and PTCL-NOS, we performed principal component analysis (PCA) of the top 6000 most variably expressed genes based on gene expression profiles of 144 cases.[4] We calculated pairwise distances between cluster centroids ($\Delta C_{centroid}$) and cluster dispersion (D), measured by the distance of each sample from its cluster centroid, in the PCA space. Differences in D were evaluated using a one-tailed t-test. By PCA of GEP (Figure 1E, *Online Supplementary Figure S3A*), AITL was more homogenous (D=0.07) than PTCL-NOS (D=0.13). Other lymphomas of TFH origin clustered closer to AITLs ($\Delta C_{centroid}$=0.04) than to PTCL-NOS ($\Delta C_{centroid}$=0.10). AITL and other lymphomas of TFH origin (D=0.1) were also significantly less dispersed (P-value=0.03) than PTCL-NOS across the 3D principal component space. Interestingly, F-PTCLs were completely contained within AITLs, and were similarly dispersed (Figure 1E, *Online Supplementary Figure S3A*).

Focusing on molecular signatures characteristic of AITL,[4] the median level of TFH signature expression in PTCLs of TFH origin (7.33) was comparable to that in AITL (7.41) (P=0.47) (Figure 1F), further supporting that the expression of at least two TFH-associated molecules captures a TFH signature. In contrast, the AITL signature and AITL microenvironment signatures were both expressed at lower levels in PTCL of TFH origin than in AITL on average, but higher than in PTCL-NOS ($P<0.01$ for all pairwise comparisons), consistent with the lack of the characteristic AITL microenvironment in other PTCLs of TFH origin. Notably, the expression range of the AITL signature in PTCL of TFH origin was distributed within the observed range for AITL, except in one sample. We also performed pairwise gene set enrichment analyses using key biological signatures. Our results reinforce similarities between AITL and PTCL of TFH origin in terms of the expression of TFH lineage pathways, key signaling pathways such as JAK-STAT and IL2/IL17/IL15-mediated cytokine signaling as well as several transcription targets, including NFKB- and STAT3-induced genes (*Online Supplementary Figure S3B-C, Online Supplementary Table S3*). Altogether, the striking overlap of AITL, F-PTCL and TFH-like PTCLs in both global and specific gene expression patterns, suggests that these lymphomas, despite morphological differences, belong to the same spectrum. Interestingly, it was proposed that TFH-like PTCL with expanded FDC meshwork could represent a "tumor cell-rich" variant of AITL, as in seven of our patients.[5]

After correction for tumor content differences (*Online Supplementary information*), array CGH data showed a similar frequency of samples with events in AITL (23/60, 38%) and other PTCL of TFH origin (5/15, 33%), whereas a much higher proportion of PTCL-NOS (17/27, 63%) had abnormal profiles. There was, however, an increasing frequency of cases with heavy rearrangements and/or homozygous deletions or amplifications from AITL to other TFH-PTCL to PTCL-NOS (Table 1). In addition, AITL-linked copy number gains[14] in chromosomes 5 (9/60 AITL, 15%) and 7 (5/60 AITL, 8 %) occurred with similar frequency in other PTCL of TFH origin (1/15, 7%; and 2/15, 13%, respectively) (*Online Supplementary Figure 4*). Gene losses likely linked to secondary oncogenic events that potentially correlate with gene expression are given in *Online Supplementary Table S4*.

In conclusion, our results show that F-PTCL and nodal PTCL with TFH phenotype, as defined in the updated WHO classification,[1] share with AITL not only phenotypic features, but also similar clinical features, genetic events and molecular signatures. Our results therefore support the dissociation of F-PTCL and PTCL with TFH-phenotype out of the PTCL-NOS entity, and the grouping of these new provisional entities together with AITL, based on their TFH cell derivation as the unifying feature.[1] It is recommended that these lymphomas of TFH origin be identified by routinely investigating any PTCL-NOS for TFH-associated marker expression and AITL-like features and, when possible, key AITL-associated genetic lesions. While the distinction between PTCL of TFH origin *versus* PTCL-NOS has currently no impact on clinical management, the situation could change in the near future with the introduction of new therapeutic approaches and development of targeted interventions, more or less likely to be operant in one or the other category, such as hypomethylating agents.[15] Our results also support that AITL, F-PTCL and PTCL with TFH phenotype truly represent variations along the spectrum of a single disease entity. Whether the distinction between the prototypic TFH neoplasm (AITL) and the two less prevalent provisional entities needs to be maintained in the future remains an open question which should be addressed by prospective observational studies.

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