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Recurrent *TET2* mutations in Peripheral T-Cell Lymphomas correlate with T_{FH}-like features and adverse clinical parameters.

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Abstract

Inactivating mutations of the *Ten-Eleven translocation (TET)2* gene were first identified in myeloid malignancies and more recently in peripheral T-cell lymphomas (PTCL). We investigated the presence of *TET2* coding sequence mutations and their clinical relevance in a large cohort of 190 PTCL patients. *TET2* mutations were identified in 40/86 (47%) angioimmunoblastic T-cell lymphomas (AITL), 22/58 (38%) peripheral T-cell lymphomas, not otherwise specified (PTCL-NOS), but were absent in all other PTCL entities except 2/10 enteropathy-associated T-cell lymphoma.

Among PTCL-NOS, a heterogeneous group of lymphoma comprising cases likely to derive from T helper follicular (T_{FH}) cells similarly to AITL, *TET2* mutations were more frequent when PTCL-NOS expressed T_{FH} markers and/or had features reminiscent of AITL (58% vs 24%, $p=0.01$). In AITL and PTCL-NOS subgroups, *TET2* mutations were associated with an advanced stage disease, thrombocytopenia, high International Prognostic Index scores, and a shorter progression free survival.

Introduction

Inactivating mutations of the *Ten-Eleven translocation (TET)2* gene were first identified in myeloid malignancies^{1,2}. *TET2* encodes a 2-oxoglutarate/Fe²⁺ dependent oxygenase that catalyses the oxidation of 5-methylcytosine to 5-hydroxymethylcytosine³⁻⁵, and its inactivation in mouse results in expansion of the hematopoietic progenitor cells and in pleiotropic abnormalities affecting both myeloid and lymphoid lineages⁶⁻⁸. Recently, *TET2* mutations have been reported in human lymphomas with a higher frequency in T-cell than in B-cell-derived neoplasms (12% versus 2%)^{7,9}. Among peripheral T-cell lymphomas (PTCL), the mutations identified were almost exclusively in angioimmunoblastic T-cell lymphoma (AITL) and PTCL, not otherwise specified (PTCL-NOS) subtypes, the two most common PTCL entities in western countries¹⁰. These mutations were demonstrated in tumor cells, with evidence that they may be acquired either in early CD34+ progenitors or at later steps of lymphoid development⁷.

Here, we extend the analysis of *TET2* mutations to a larger independent series of PTCL samples, with an attempt to correlate the mutational status to pathological features and clinical outcome.

Materials and methods

Patients and tumour samples

A series of 190 PTCL patients with frozen lymphoma samples available at diagnosis were selected within the framework of a multicentric T-cell lymphoma consortium (Tenomic). The study was approved by the local ethic committee (CPP Ile de France IX= 08-009). Clinical data were retrospectively collected. All cases were reviewed and a consensus diagnosis was made at multihead microscope by 3 hematopathologists, according to the criteria of the 2008 WHO classification¹¹.

Within PTCL-NOS, a group without typical morphology of AITL but expressing T_{FH} markers (PD1, BCL6 and/or CXCL13) and/or having some other features reminiscent of AITL [i.e. presence of 2 of the following criteria: CD20-positive large B-cells, EBV-encoded small RNAs(EBER)-positive cells , CD21 and/or CD23 follicular dendritic cell expansion, CD10 expression] was designated "TFH-like" PTCL-NOS.

***TET2* genotyping**

DNA was extracted from several frozen tissue sections with morphological control using QIAamp DNA mini Kit (Quiagen, LesUlis, France) and an aliquot amplified by linear amplification. Analysis of the coding sequences of *TET2* was performed by direct sequencing of the PCR fragments and candidate mutations were confirmed by sequencing an independent PCR product amplified from native genomic DNA, as previously described⁷. Frameshift, nonsense mutations, mutations in splice site, and missense mutations (only those affecting the evolutionary conserved regions of the protein) were considered. Single nucleotide polymorphisms (SNP) either previously published or recorded in the National Center for Biotechnology Information SNP database were excluded.

Statistical analysis

Continuous and dichotomic variables were compared with the Wilcoxon sum rank test or Cochran Armitage and Khi-2 or Fisher's exact tests. Overall survival (OS) and progression-free survival (PFS) were estimated using the Kaplan-Meier method and compared with the log rank test. All tests were two-sided and P values <0.05 were considered significant.

Results and Discussion

TET2 mutations were found in 64/190 samples at diagnosis (34%). The frequency of *TET2* mutations was extremely variable according to the diagnosis category. *TET2* mutations were

present in 40/86 (47%) AITL, in 22/58 (38%) PTCL-NOS and in 2/10 (20%) enteropathy-associated T-cell lymphoma. By contrast, no mutation was observed in any of the 18 anaplastic large cell lymphomas (12 ALK-positive, 6 ALK-negative), the 12 extranodal NK/T lymphomas, nasal-type and the 6 hepatosplenic T-cell lymphomas tested (Table 1).

Interestingly, and in accordance with the previous report⁷ the highest frequency of mutations was found in AITL, which is thought to derive from T_{FH} cells normally present in germinal centers¹²⁻¹⁴. Within the large group of PTCL-NOS, it is also now recognized that a subset has “T_{FH}-like” features in the form of a molecular profile bearing imprints of T_{FH} cell signature, the expression of T_{FH}-associated molecules¹⁵⁻¹⁷ and/or overlapping pathological features¹⁶. Thus, we questioned whether *TET2* mutations correlated with T_{FH}-like features in PTCL-NOS. Among the 58 PTCL-NOS in our series, 24 were classified as “T_{FH}-like” PTCL-NOS. *TET2* mutations were present in 14/24 (58%) of the T_{FH}-like PTCL subgroup compared to 8/34 (22%) in those lacking AITL features and T_{FH}-markers. Therefore, *TET2* mutations in PTCL appear to correlate with T_{FH} derivation, i.e occur at higher frequency in AITL and T_{FH}-like PTCL-NOS, than in non-T_{FH}-like PTCL-NOS and other PTCL entities (49% vs 12,5%, p<0.0001).

Among the 64 PTCL patients with *TET2* mutations, 37 had single mutations and 27 had 2 or more mutations. *TET2* mutations were mainly insertions/deletions generating frameshifts and nonsense mutations, similar to those found in myeloid malignancies (Table S1). Eighteen of 19 missense mutations were predicted as probably damaging by the polyphen software (<http://genetics.bwh.harvard.edu/pph2/>) and one as possibly damaging. For three mutated patients with available biopsy at relapse, an identical pattern of *TET2* mutations was found in paired samples (Figure S1), suggesting that *TET2* mutations are associated with the main driving clone.

When focusing on AITL and PTCL-NOS patients (n=144), 62 patients had *TET2* mutations and 82 were *TET2* wildtype. Clinical factors associated with *TET2* mutated status (Table 2 and Figure S2) were advanced stage disease (p=0.02), increased number of involved extranodal sites (p=0.017), presence of B symptoms (p=0.02), thrombocytopenia (p=0.04), high International Prognosis Index (IPI) (p=0.028) and Prognosis Model for PTCL-NOS (PIT)¹⁸ (p=0.025) scores, indicating that *TET2* mutations are associated with adverse clinical parameters at presentation. Despite incomplete clinical data and heterogeneous therapies in this multicentric retrospective cohort, anthracyclin-based chemotherapy was the most common regimen in both mutated and non-mutated groups (73% and 70% respectively). In this retrospective study, *TET2*-mutated patients with AITL or PTCL-NOS appeared to have a shorter PFS (p=0.04) than *TET2*-wildtype patients, but no significant difference in OS was observed (p=0.1) (Figure S2). The association between *TET2* mutations, poor prognosis clinical parameters and outcome was even stronger when focusing on the group of patients with AITL and T_{FH}-like PTCL-NOS. In this group, *TET2* mutations were associated with a shorter PFS (p=0.022) and a trend to a shorter overall survival (p=0.058) (Table S2 and Figure S3)

We recently reported recurrent *IDH2* mutations in AITL¹⁹. These mutations alter IDH enzymatic function and may result in the functional inactivation of TET protein activity²⁰. In acute myeloid leukemia, *IDH* and *TET2* mutations are reported mutually exclusive²⁰. Of the 31 AITL samples from the current study also analysed for *IDH2* mutations¹⁹, 6 cases disclosed both *IDH2* and *TET2* mutations, 2 cases were *IDH2* mutated/*TET2* wildtype and 10 cases were *TET2* mutated/*IDH2* wildtype, indicating that both mutations can accumulate in AITL. Ultimately, both *IDH2* and *TET2* mutations, recurrent in AITL and in T_{FH}-derived PTCL may deregulate the control of chromatin structure. This situation may be reminiscent of that observed in B-cell lymphomas, in which mutations of several genes involving epigenetic

changes and chromatin remodelling, such as CREBBP, EP300²¹, EZH2²², MLL2 or MEF2B²³ have recently been described.

In addition to similarities in their ontogeny, AITL and a subset of T_{FH}-like PTCL share common oncogenic alterations including *TET2* abnormalities, further supporting their close relationship. The strong association between *TET2* mutations, AITL and T_{FH}-like PTCL may provide molecular rationale to merge together AITL and PTCL-NOS expressing T_{FH} markers as previously suggested^{15,16} and to refine the spectrum of AITL. Overall, the identification of frequent *TET2* mutations in AITL and other T_{FH}-related PTCL extends the importance of epigenetic alterations in lymphomagenesis. These results suggest that, in these diseases in which conventional chemotherapies are ineffective²⁴ in most patients, the use of new agents, such as demethylating agents should be considered.

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Authorship

PG, CB and OAB designed the research. FL, LdL and PG wrote the paper. LC performed DNA sequencing. FL collected and interpreted clinical data. MP, LdL, PG performed histological review. JPJ performed statistical analysis.

Conflict-of-interest disclosure: the authors declare no competing financial interest.

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Table 1: *TET2* mutations in the different PTCL entities.

PTCL-NOS: PTCL, not otherwise specified, AITL: angioimmunoblastic T-cell lymphoma, ALCL: anaplastic large cell lymphoma, HSTL: hepatosplenic T-cell lymphoma, EATL: enteropathy-associated T-cell lymphoma, Extranodal NK/T: extranodal NK/T-cell lymphoma, nasal-type.

(°)*TET2* mutations were found in 2 intestinal tissue samples diagnosed as EATL and (*) in 3 other extranodal tissue samples (1 skin, 1 spleen, 1 liver) diagnosed as PTCL-NOS

PTCL entities	<i>TET2</i> mutation		
	N	N	%
AITL	86	40	47%
PTCL NOS *	58	22	38%
<i>T_{FH}-like</i>	24	14	58%
<i>Others</i>	34	8	24%
ALCL	18	0	0%
EATL °	10	2	20%
Extranodal NK/T	12	0	0%
HSTL	6	0	0%
Total	190	64	34%

Table 2: Clinical data according to *TET2* status in AITL and PTCL-NOS patients

¹ : Fisher exact test, ² : Wilcoxon sum rank test, ³ :Cochran Armitage test, ⁴ : log rank test

	<i>TET2</i> mutation		<i>TET2</i> WT		
N	62		82		
Sex/ gender					
Male	38	61%	43	52%	p=0.18 ¹
Female	24	39%	39	48%	
Age (median)	67		65		p=0.3 ²
Stage					
1	0	0%	4	5%	p=0.028 ³
2	0	0%	3	4%	
3	11	21%	17	23%	
4	42	79%	49	67%	
Number of extra nodal localizations (median)	2		1		p=0.056 ²
Lactate dehydrogenase					
Elevated	41	76%	46	65%	p=0,24 ¹
Normal	13	24%	25	35%	
Hemoglobin					
≥10g/dL	35	74%	33	60%	p=0.14 ¹
<10g/dL	12	26%	22	40%	
Platelet count					
≥150000/mm3	34	72%	48	89%	p=0.04 ¹
<150000/mm3	13	28%	6	11%	
Direct Coombs test					
Positive	14	52%	13	43%	p=0.6 ¹
Negative	13	48%	17	57%	
Hypergammaglobulinemia					
Yes	11	32%	12	33%	p=1 ¹
No	23	68%	24	67%	
B symptoms					
Yes	40	78%	38	58%	p=0.028 ¹
No	11	22%	27	42%	
Performance status					
0-1	22	44%	41	62%	p=0.06 ¹
2-4	28	56%	25	38%	
International Prognosis Index					
0	0	0%	4	6%	p=0.0062 ²
1	2	4%	4	6%	
2	8	16%	11	16%	
3	10	20%	22	33%	
4	19	37%	23	34%	
5	12	24%	3	4%	
Prognosis Model for PTCL¹⁸					
0	2	5%	4	9%	p=0.0045 ²
1	3	7%	11	24%	
2	9	21%	13	29%	
3	18	43%	13	29%	
4	10	24%	4	9%	
Treatment					
Anthracycline based regimen	37	69%	40	58%	P=0,5 ¹
Anthracycline based regimen and frontline transplantation	2	4%	8	12%	
Other combination of chemotherapy	7	13%	8	12%	

Single agent chemotherapy	5	9%	7	10%
Palliative treatment	3	6%	6	9%
5 years OS	28%		31%	p=0.11 ⁴
5 years PFS	10%		21%	p=0.047 ⁴