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ARTICLE

Oncogenetic landscape of T-cell lymphoblastic lymphomas compared to T-cell acute lymphoblastic leukemia

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In the latest 2016 World Health Organization classification of hematological malignancies, T-cell lymphoblastic lymphoma (T-LBL) and lymphoblastic leukemia (T-ALL) are grouped together into one entity called T-cell lymphoblastic leukemia/lymphoma (T-LBLL). However, the question of whether these entities represent one or two diseases remains. Multiple studies on driver alterations in T-ALL have led to a better understanding of the disease while, so far, little data on genetic profiles in T-LBL is available. We sought to define recurrent genetic alterations in T-LBL and provide a comprehensive comparison with T-ALL. Targeted whole-exome next-generation sequencing of 105 genes, multiplex ligation-dependent probe amplification, and quantitative PCR allowed comprehensive genotype assessment in 818, consecutive, unselected, newly diagnosed patients (342 T-LBL vs. 476 T-ALL). The median age at diagnosis was similar in T-LBL and T-ALL (17 vs. 15 years old, respectively; $p = 0.2$). Although we found commonly altered signaling pathways and co-occurring mutations, we identified recurrent dissimilarities in actionable gene alterations in T-LBL as compared to T-ALL. *HOX* abnormalities (*TLX1* and *TLX3* overexpression) were more frequent in T-ALL (5% of T-LBL vs 13% of T-ALL had *TLX1* overexpression; $p = 0.04$ and 6% of T-LBL vs 17% of T-ALL had *TLX3* overexpression; $p = 0.006$). The PI3K signaling pathway was significantly more frequently altered in T-LBL as compared to T-ALL (33% vs 19%; $p < 0.001$), especially through *PIK3CA* alterations (9% vs 2%; $p < 0.001$) with $PIK3CA^{H1047}$ as the most common hotspot. Similarly, T-LBL genotypes were significantly enriched in alterations in genes coding for the *EZH2* epigenetic regulator and in *TP53* mutations (respectively, 13% vs 8%; $p = 0.016$ and 7% vs 2%; $p < 0.001$). This genetic landscape of T-LBLL identifies differential involvement of recurrent alterations in T-LBL as compared to T-ALL, thus contributing to better understanding and management of this rare disease.

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INTRODUCTION

Precursor T-cell malignancies are rare clonal hematopoietic stem cell neoplasms of lymphoid precursors that are committed to the T-cell lineage and mainly affect pediatric patients. By convention, the designation of T-cell lymphoblastic lymphoma (T-LBL) is used when the neoplasm is confined to a tissue lesion without or with only minimal blood or bone marrow involvement while T-cell lymphoblastic leukemia (T-ALL) is used when there is extensive blood involvement and/or >25% bone marrow infiltration. The World Health Organization denominated both T-ALL and T-LBL as T-lymphoblastic leukemia/lymphoma (T-LBLL) in the 2016 Revised World Health Organization classification of hematological malignancies but without further specification[1]. Despite this, similarities and differences in T-LBL and T-ALL regarding clinical course, phenotypic and molecular features have raised the question of whether these entities represent one disease or reflect two different diseases[2]. Molecular aspects of T-ALL have been widely explored and, although T-ALL and T-LBL share several common aberrations, clinical and multiomic strategies suggested that the two entities may have independent pathogenic requirements and dependencies[3]. Evidence that leukemic conversion originating from the T-LBL cell in lymphoid tissue can occur has been reported[4,5]. About 40% of relapsed T-LBL patients have bone marrow (BM) involvement, whereas less than 20% of T-LBL patients have histological evidence of BM involvement at diagnosis[5]. Conversely, about 20% of ALL patients undergo isolated extramedullary relapse (mainly central nervous system or testis) that could be considered lymphoma[6]. Gene expression

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analysis of T-LBL and T-ALL patient samples also showed different signatures for T-LBL and T-ALL in both children and adults, implying specific requirements to invade lymphoid tissues (T-LBL) or involve systemic compartments (T-ALL)[7,8]. Genes involved in angiogenesis, chemotactic response, and nodal metastases were more highly expressed in T-LBL[7]. Genes coding for proteins involved in cell-cell adhesion such as *BCL2, S1P1,* and *ICAM1* have also been shown to be differentially expressed in T-LBL, leading to a blockade of tumor cell intravasation to blood[9]. This may explain why T-LBL and stromal cells are embedded in close proximity to lymphoid tissues. Phenotypically, it has been reported that T-LBL more frequently involves mature thymic cells than T-ALL, with a less frequent expression of myeloid antigens[10–12]. Finally, one DNA methylation study identified an epigenetic signature of differentially methylated CpG sites that segregates T-LBL from T-ALL. Those sites were associated with increased expression of membrane-associated protein domains[13].

Understanding the significance of gene mutations in the diagnostic or prognosis of T-LBLL has already helped risk stratification and the development of individualized treatment[14]. T-ALL and T-LBL, however, remain highly aggressive malignant tumors, notably in adults. Despite event-free survival (EFS) rates of up to 50–90%, the overall survival rate after relapse is only ~3–27%[15–20]. A precise description of the genetic landscape of T-LBLL and specifically T-LBL is therefore desirable, in order to identify potential therapeutic targets and improve survival rates while reducing acute and long-term toxicities.

In this study, we analyzed the genetic landscape of 818 T-LBLL and identified significant differences in the incidence of *PI3K/Akt, EZH2,* and *TP53* gene alterations between T-LBL and T-ALL. These alterations affect specific signaling pathways and may confer a susceptibility to recently developed targeted therapies.

MATERIALS AND METHODS

Patients

Diagnostic peripheral blood, bone marrow, or lymphoid tissue samples from 818 unselected adults and children with T-LBLL (476 patients with T-ALL and 342 with T-LBL), newly diagnosed between 1999 and 2020, were analyzed centrally in Necker-Enfants Malades Hospital (AP-HP, Paris France) after informed consent was obtained at diagnosis according to the Declaration of Helsinki. According to WHO criteria, if a patient presents with a mass lesion and lymphoblasts in the marrow, a value of >25% marrow blasts is used to define leukemia versus lymphoma[1].

Gene mutation screening

A custom capture Nextera XT gene panel (Illumina, San Diego, CA) targeting all coding exons and their adjacent splice junctions of 105 genes was designed, based on available evidence in hematological neoplasms (Supplementary Table 2). DNA Libraries were prepared using Nextera Rapid Capture Enrichment protocol and underwent 2×150 bp paired-end sequencing on Illumina MiSeq sequencing system with MiSeq Reagent Kit v2 (Illumina). Briefly, sequence reads were filtered and mapped to the human genome (GRCh37/hg19) using in-house software (Polyweb, Institut Imagine, Paris). Annotated variants were selected after filtering out calls according to the following criteria: (1) coverage < 30×, <10 alternative reads or variant allelic fraction (VAF) < 7%; (2) polymorphisms described in dbSNP, 1000Genomes, EVS, Gnomad and EXAC with a calculated mean population frequency > 0.1%; (3) mutations with a frequency <2% in both T-LBL and T-ALL groups. Non-filtered variants were annotated using the somatic database COSMIC (version 78) and ProteinPaint (St Jude Children's Research Hospital—Pediatric Cancer data portal). Lollipop plots were generated with ProteinPaint (https://pecan.stjude.org/#/ proteinpaint) and splice mutations were not depicted.

Molecular characterization of oncogenic drivers in T-LBLL samples

Peripheral blood, bone marrow T-ALL samples, and lymphoid tissue T-LBL samples when available were analyzed for fusion transcripts (SIL-TAL1, CALM-AF10/PICALM-MLLT10), oncogenic transcripts (HOXA9, TLX1, and TLX3) and *NOTCH1/FBXW7/RAS/PTEN* mutations, as previously described[21,22].

Multiplex ligation-dependent probe amplification (MLPA) analysis

MLPA analysis was performed using the MRC Holland (Amsterdam, The Netherlands) SALSA MLPA probe mix P383-A1 TALL according to the manufacturer's recommendations. Polymerase chain reaction products were separated by capillary electrophoresis on an ABI-3130 device. Coffalyser software, available at http://www.mlpa.com, was used for the analysis.

Statistics

Comparisons for categorical variables between T-ALL and T-LBL subgroups were performed with Fisher's exact test or Wilcoxon rank-sum test. Statistical analyses were performed with STATA software (STATA 12.0 Corporation, College Station, TX, USA) and StatAid R package[23]. All *p*-values were two-sided, with $p < 0.05$ denoting statistical significance. Circos plots and oncoplots were generated using R software. Strong correlations are indicated by large ellipses, whereas weak correlations are indicated by small ellipses. Co-occurrences and mutual exclusions in T-LBL and T-ALL patients were computed with the DISCOVER algorithm (version 0.9.3).

RESULTS

Clinico-genomic comparison between T-ALL and T-LBL shows common features

Eight hundred and eighteen adults and children with T-LBLL (342 with T-LBL and 476 patients with T-ALL) were included in the study. Among the 476 T-ALL analyzed, 215 were adult patients (≥19 years old) and 261 were pediatric patients (<19 years old). Among the 342 T-LBL analyzed, 156 were adult patients (≥19 years old) and 186 were pediatric patients (<19 years old) (Supplementary Table 1). The median age at diagnosis was similar in T-LBL and T-ALL (17[1–72] vs 15[1–59] years old, respectively, ($p = 0.2$). 74% (249/335) of T-LBL vs 75% (357/476) of T-ALL were male ($p > 0.9$). CNS involvement occurred in 6% of T-LBL vs 11% of T-ALL ($p = 0.11$). Regarding oncogenetic classification, *CALM-AF10 (PICALM-MLLT10)* rearrangements were found in 4% of T-LBL vs 3% of T-ALL ($p = 0.8$). *SIL-TAL1* rearrangements were also comparable in both groups (13% of T-LBL vs 14% of T-ALL; $p > 0.9$). Clinico-biological features of the cohort are summarized in Table 1.

Of the 818 T-LBLL samples, 804 harbored at least one pathogenic mutation or MLPA alteration (330/96.5% T-LBL and 474/99.6% T-ALL). The global representation of the mutation landscape in T-LBL and T-ALL is displayed as an oncoplot (Fig. 1A). Circos plots depicting co-occurring mutations in T-LBL and T-ALL are shown in Fig. 1B.

Most alterations in T-LBLL affected *NOTCH1/FBXW7* pathway genes (68% of cases), which was the most frequently involved pathway in both T-LBL and T-ALL. The second most frequently altered pathway was the cell cycle, with *CDKN2A* being deleted in 50% of T-LBL vs. 70% of T-ALL. Epigenetic regulating factors were commonly altered in both categories (53% overall). Other signaling pathways, including PI3K, JAK/STAT, and RAS (including *KRAS, NRAS, NF1,* and *PTPN11*), were also commonly altered in T-LBL and T-ALL. Genes coding for transcription factors were similarly mutated in T-LBL (40% of cases) and in T-ALL (44% of cases); $p = 0.277$ (Fig. 1A and supplementary Table 3).

Co-occurring gene alterations were also comparable between T-LBL and T-ALL (Fig. 1B). *NOTCH1* mutations frequently co-occurred with *CDKN2A* deletions, *FBXW7, PHF6,* and *BCL11B* mutations and are significantly less associated with *PTEN* alterations.

A thorough, comprehensive genetic landscape analysis of T-LBL, however, identified recurrent dissimilarities in oncogene alterations as compared to T-ALL

Molecular dissimilarities in T-LBL vs T-ALL

Several oncogenes were significantly differentially distributed and are described below. Gene and pathway alterations in T-LBL and T-LBL are depicted and detailed in Fig. 2 and supplementary Table 3. Regarding oncogenic drivers, *NKL* homeotic abnormalities

Table 1. Characteristics of the cohort.

Variable	Overall[a] ($n = 818$)	LBLT[a] ($n = 342$)	TALL[a] ($n = 476$)	p-value[b]
Age (y)				0.2
Median [range]	16 (1–72)	17 (1–72)	15 (1–59)	
Adult	371 (45)	156 (46)	215 (45)	
Ped	447 (55)	186 (54)	261 (55)	
Sex				>0.9
Female	208 (25)	89 (26)	119 (25)	
Male	610 (75)	253 (74)	357 (75)	
CNS involvement				0.11
No	525/582 (90)	102/108 (94)	423/474 (89)	
Yes	57/582 (10)	6/108 (6)	51/474 (11)	
Oncogenic drivers				
CALM-AF10 (PICALM-MLLT10)	17/529 (3)	4/106 (4)	13/415 (3)	0.8
TLX1	60/529 (11)	6/106 (6)	54/415 (13)	*0.04*
TLX3	79/529 (15)	7/106 (7)	72/415 (17)	*0.006*
SIL-TAL1	73/529 (14)	16/106 (15)	57/415 (14)	>0.9

P-values in italic are <0.05.
[a]n / N (%).
[b]Fisher's exact test; Wilcoxon rank-sum test.

(*TLX1* and *TLX3*) were more frequent in T-ALL as compared to T-LBL. *TLX1* overexpression was found in 6% of T-LBL vs. 13% of T-ALL ($p = 0.04$) and *TLX3* overexpression in 7% of T-LBL vs. 17% of T-ALL ($p = 0.006$).

NOTCH1/FBXW7 pathway. *NOTCH1* alterations were identified in 52% (170/330) of T-LBL patients versus 72% (342/474) of T-ALL ($p < 0.001$). Mutations mainly clustered in the *NOTCH1* HD domain (56% of *NOTCH1* mutations in T-LBL vs 42% of T-ALL; $p = 0.086$) (Supplementary Table 4). *FBXW7* mutations were found in 24% of T-LBL vs 20% of T-ALL ($p = 0.226$). However, the co-occurrence mutational profile of *FBXW7* differs between T-LBL and T-ALL. *FBXW7* mutations were significantly more commonly associated with *PTEN* and *STAT5B* alterations in T-LBL while *FBXW7* mutations were more commonly associated with *JAK1* and *RUNX1* mutations in T-ALL (Fig. 3).

Cell cycle. We observed significantly fewer *CDKN2A* alterations in T-LBL (50% of T-LBL vs 70% of T-ALL; $p < 0.001$). Conversely, T-LBL were enriched in *TP53* mutations (7% of T-LBL vs 2% of T-ALL; $p < 0.001$). *TP53* mutations had no significant co-occurrence with other gene alterations in T-ALL whereas they were less significantly associated with *CDKN2A* deletions in T-ALL (Fig. 3). *TP53* mutations mainly affected the p53 DNA-binding domain of the protein (exon 5–8) in both T-LBL and T-ALL (Fig. 4A). No differential incidence of mutations between adult and pediatric T-LBL regarding *TP53* was found (Supplementary Table 5).

Epigenetic deregulation. Epigenetic regulating factors were more frequently altered in T-ALL (52% of T-LBL *vs* 60% of T-ALL cases; $p = 0.02$). *PHF6* was the most frequently altered gene in this category, with mutations detected in 29% (232/804) of patients (71/330, 22% of T-LBL vs 161/474, 34% of T-ALL; $p < 0.001$). T-ALL patients were significantly enriched in *CTCF* and *EED* mutations as compared to T-LBL (respectively, 1% of T-LBL vs 5% of T-ALL; $p = 0.001$ and 1% of T-LBL vs 4% of T-ALL; $p = 0.002$). In addition, T-LBL patients were enriched in *EZH2* alterations (43/330, 13% of T-LBL vs 36/474, 8% of T-ALL; $p = 0.016$).

The main alterations were deletions (28% of *EZH2* alteration in T-LBL vs 31% in T-ALL) and missense mutations (42% in T-LBL vs 25% in T-ALL) (Supplementary Table 4). Of note, *EZH2* alterations were significantly less commonly associated with *NOTCH1* and *FBXW7* mutations in T-LBL (Fig. 3A). Mutations were mainly located in exons 16 to 20 in T-ALL and in T-LBL, affecting the SET domain of the protein (Fig. 4B).

Finally, *KMT2D* was mutated in 14% of T-LBL *vs* 8% of T-ALL ($p = 0.001$). The distribution of *KMT2D* variants in adult and pediatric patients is pictured in Supplementary Fig. 1. No differential incidence of alterations between adult and pediatric T-LBL regarding *EZH2* or *KMT2D* was found (Supplementary Table 5).

JAK/STAT signaling pathways. The JAK/STAT signaling pathway was significantly more affected in T-ALL vs T-LBL (207/474, 43% vs 110/330, 33%, respectively; $p = 0.004$), partly due to the higher incidence of *DNM2* mutations in T-ALL (70/474, 15% of T-ALL vs 23/330, 7% of T-LBL; $p < 0.001$). Regarding other major genes involved in JAK/STAT signaling, *IL7R*, *JAK1*, *JAK3,* and *STAT5B* were mutated in 10%, 6%, 14%, and 6% in T-LBLL, respectively, with no incidence difference between T-LBL and T-ALL (Fig. 1A and Supplementary Table 3).

PI3K signaling pathway. Evaluation of PI3K signaling pathway gene mutations in T-LBL highlights important dissimilarities with T-ALL. Overall, 5% (38/804) of patients had *PIK3CA* mutations (30/330, with 9% in T-LBL vs 8/474, 2% in T-ALL; $p < 0.001$), comprising 39 mutations (31 in T-LBL and 8 in T-ALL). Of these, 38 were missense mutations and one was a nonsense mutation. Interestingly, the most frequent mutation of *PIK3CA* in T-LBL was missense mutation at H1047 in exon 21 (kinase domain) (11/31 mutations, 35%) while T-ALL featured scattered mutations without preferential location. *PIK3CA* mutations are depicted in Fig. 4C and detailed in Supplementary Table 6. While no significant co-occurrence between *PIK3CA* and other gene alterations was reported in T-ALL, *PIK3CA* mutations were significantly less associated with *NOTCH1*, *PHF6*, *PTEN,* and *JAK3* alterations in T-LBL (Fig. 3).

PIK3R1 mutations were observed in 5% (42/804) of patients (24/330, 7% of T-LBL vs 18/474, 4% of T-ALL; $p = 0.036$) with 43 mutations reported (25 in T-LBL and 18 in T-ALL) featuring 25 missense, 11 indels, 3 frameshifts, and 4 splicing mutation. Most of the mutations observed were in the Inter-Src homology 2 (iSH2) helical domain of the protein, especially in exon 13. *PIK3R1* mutations were significantly less associated with *NOTCH1*, *PHF6,*

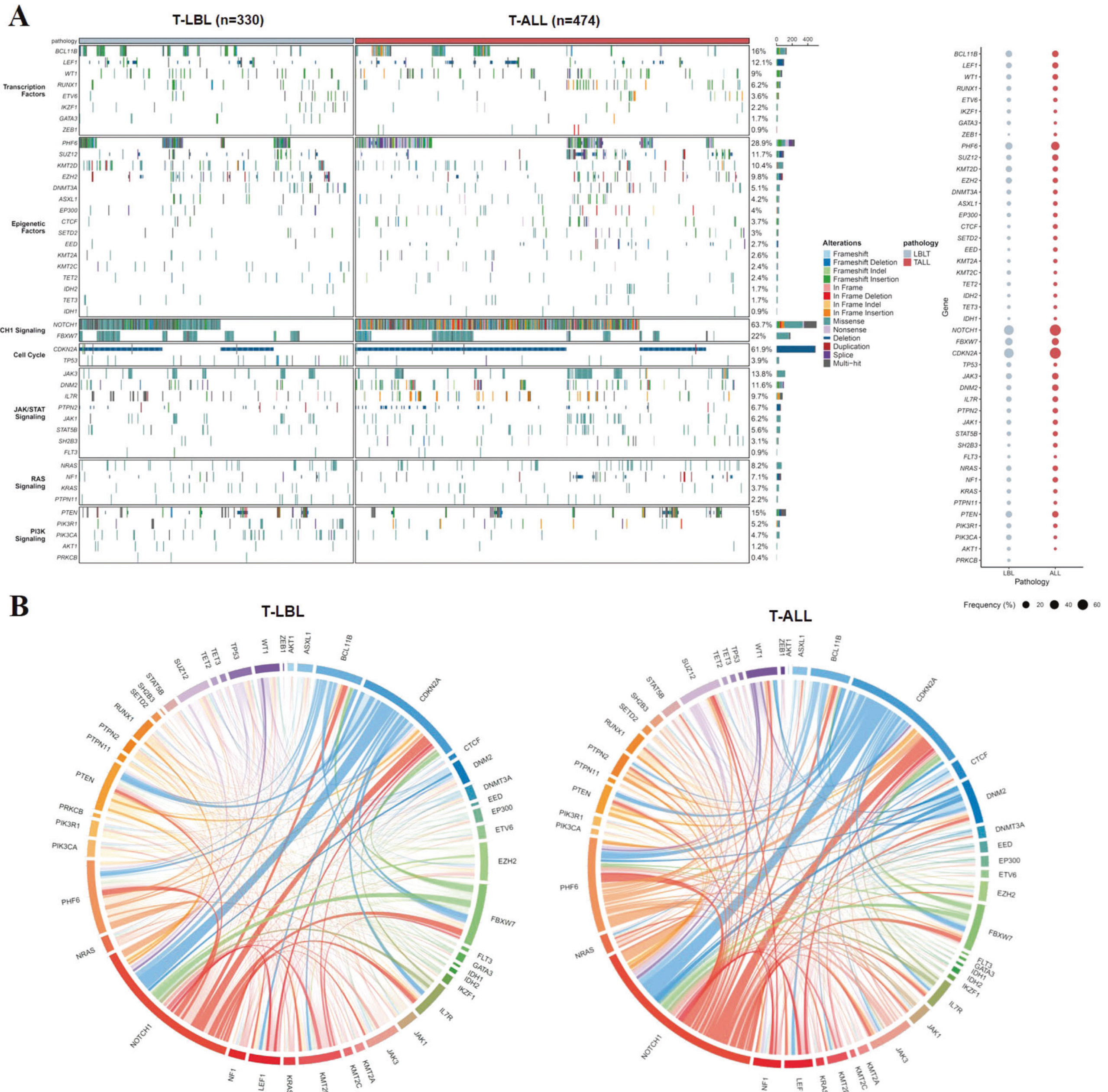

Fig. 1 Molecular comparison between T-LBL and T-ALL. A Oncoplot of T-ALL vs T-LBL. **B** Circos plots depicting co-occurring alterations in T-LBL (left) and T-ALL (right).

and *PIK3CA* alterations whilst no association or exclusion were observed in T-ALL. Details of *PIK3R1* mutations (splicing mutations excluded) are depicted in Fig. 4D.

AKT1 were mutated in 1% (10/804) of patients overall, including 2% (8/330) of T-LBL vs 2/474, 0.4% of T-ALL; $p = 0.019$). 12 missense mutations were reported (8 mutations in T-LBL and 4 mutations in T-ALL). Eighty three percent (10/12) of mutations were E17K in exon 3 (kinase domain). Seven *AKT1* E17K mutations were identified in T-LBL and 2 in T-ALL (Fig. 4E).

The incidence of *PTEN* alterations was comparable (52/330, 16% of T-LBL vs 69/474, 15% of T-ALL; $p = 0.689$) with *PTEN* deletions identified in 27% of *PTEN* altered T-LBL cases and in 22% of T-ALL cases (Supplementary Table 4).

No difference was seen in the incidence of mutations between adult and pediatric T-LBL regarding *PIK3CA, PIK3R1,* and *AKT1*. (Supplementary Table 5).

DISCUSSION

This study provides a comprehensive genetic study of T-LBLL and demonstrates that the T-LBL oncogenetic landscape differs from T-ALL.

The incidence of specific driver oncogenic rearrangement was significantly different in T-LBL and T-ALL regarding *HOX* abnormalities, since T-ALL were relatively enriched in *TLX1* and *TLX3* overexpression. TLX1 overexpression, found in a "proliferative" molecular cluster, is associated with a better outcome in T-ALL[24] while the prognostic impact of *TLX3* overexpression, found in a "TLX" molecular cluster, remains contested[25,26]. So far, little is known about oncogenic drivers in T-LBL patients. Few T-LBL patients benefit from RT-qPCR or rearrangement screening, as is systematically performed for T-ALL.

T-LBL also demonstrated differences in recurrent gene alterations affecting actionable signaling pathways as compared to T-ALL.

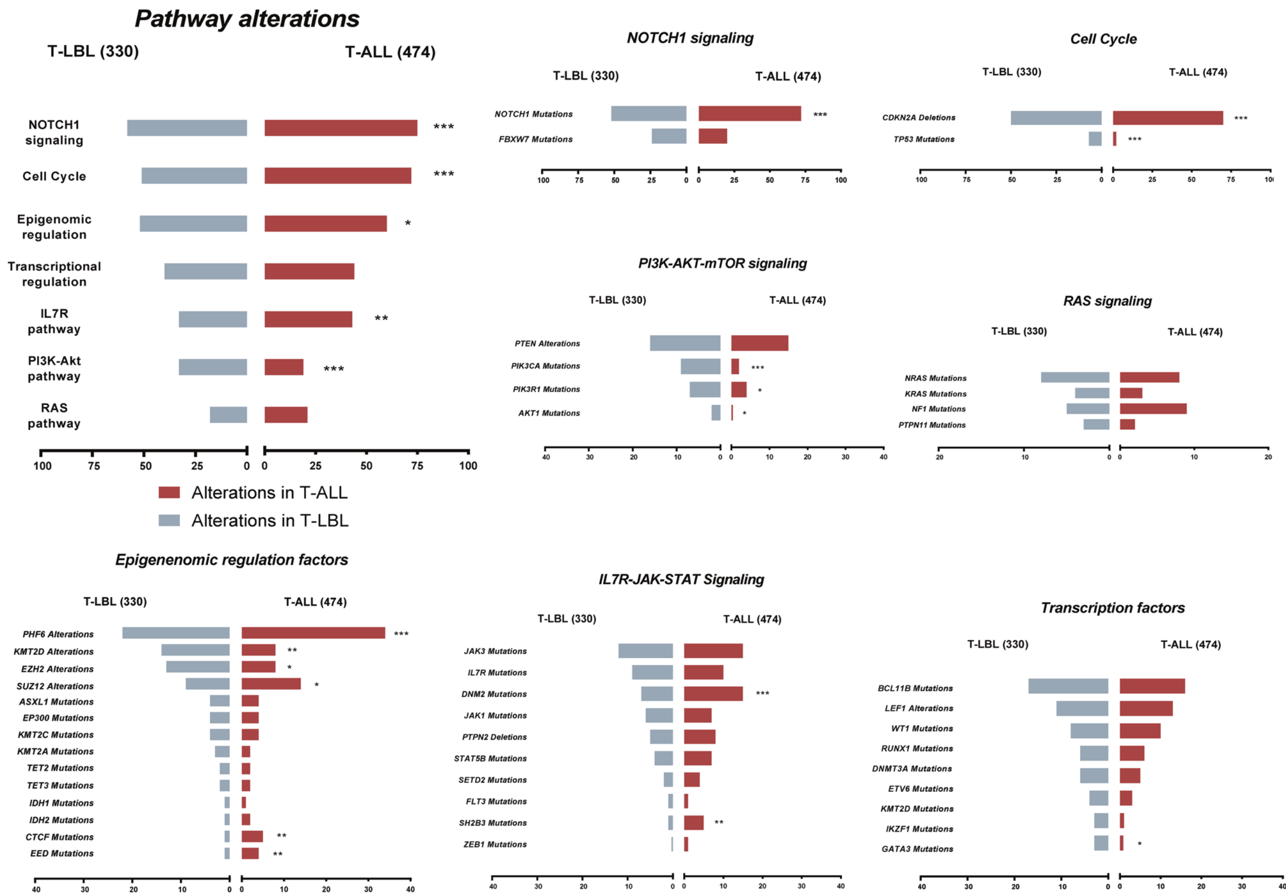

Fig. 2 Bar plots featuring the incidence of signaling pathway and gene alterations in T-ALL (red bars) versus T-LBL (gray bars). The significance level of co-occurrences or mutual exclusions are depicted as followed: * indicating a p-value < 0.05, ** a p-value < 0.01, and *** a p-value < 0.001.

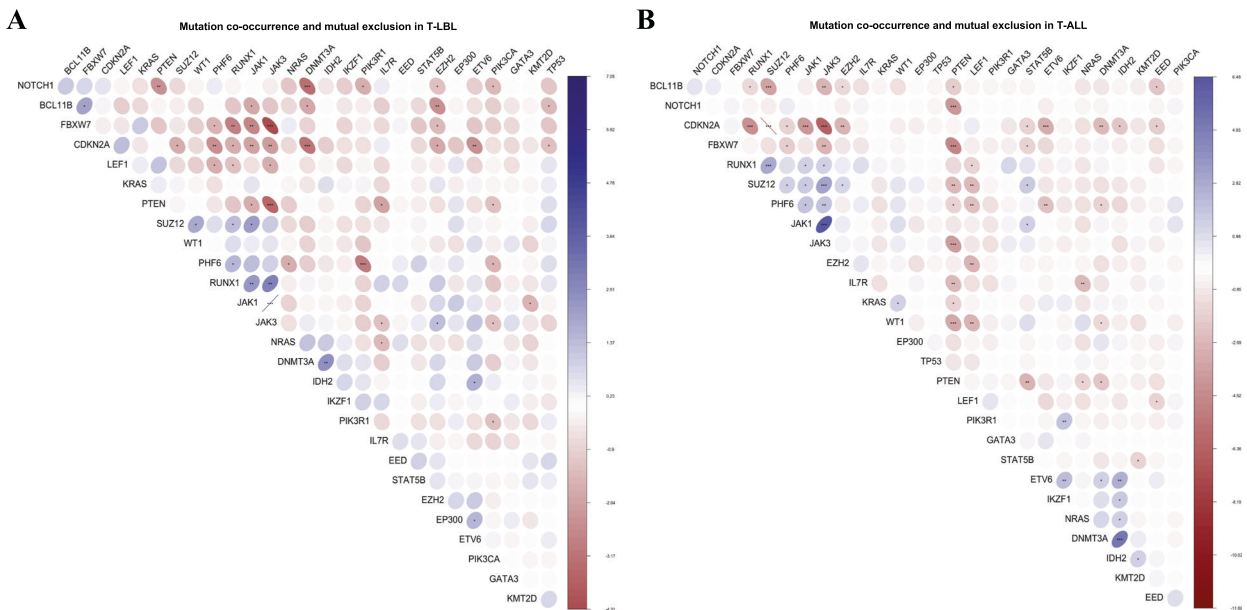

Fig. 3 Mutation co-occurrence and mutual exclusion in T-LBL and T-ALL. Corrplot showing the co-mutations in: **A** T-LBL patients and **B** T-ALL patients. The colors of the scale bar denote the nature of the correlation, with +1 indicating a perfectly positive correlation (blue) and −1 indicating a perfectly negative correlation (red) between two alterations. The significance level of co-occurrences or mutual exclusions are depicted as followed: * indicating a p-value < 0.05, ** a p-value < 0.01, and *** a p-value < 0.001.

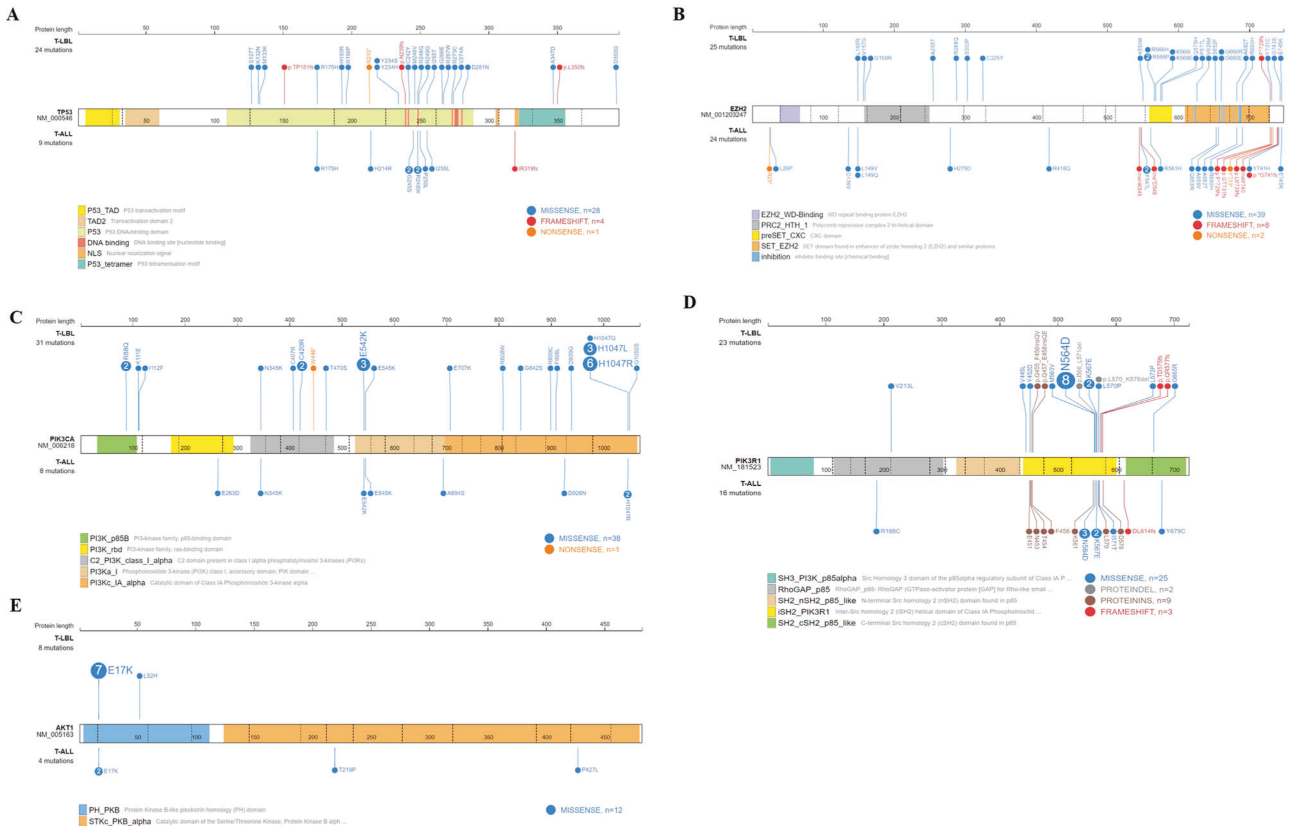

Fig. 4 Representation and distribution of mutations observed in T-LBL confront to T-ALL for selected genes. Lollipop plots indicating the observed mutations and their consequences for: **A** TP53. **B** EZH2. **C** PIK3CA. **D** PIK3R1. **E** AKT1.

Most importantly, NGS-based molecular screening reveals recurrent PI3K signaling pathway alterations in T-LBL as compared to T-ALL. Nine percent of T-LBL patients had *PIK3CA* mutations, mainly involving exons 10 and 21. This is slightly higher than previously reported in pediatric and adult T-LBL cohorts (7% and 6%, respectively),[27,28]. *PIK3R1* mutations in our cohort were also higher compared to previous observations (7% vs 4%)[27]. Regarding T-ALL, there were few *PIK3CA* (2%), *PIK3R1* (4%), and *AKT1* (0.4%) mutations, which is consistent with the literature, although slightly lower than previously reported, albeit limited, cohorts[29,30]. A recent NGS-based study of 87 adult T-LBL did not identify significant *PIK3CA*, *PIK3R1*, or *AKT1* mutations[31]. *PIK3CA* mutations were significantly less associated with *NOTCH1*, *PHF6*, and *PTEN* alteration in T-LBL, suggesting a specific oncogenic role. The PI3K/Akt signaling pathway plays an important role in the early stages of thymocyte development, specifically for the transition of double-negative to double-positive thymocytes. Whereas a loss of regular PI3K/Akt signaling leads to defective thymocyte development, its aberrant activation triggers uncontrolled cell survival, growth, and proliferation that may lead to the formation of T-cell lymphomas[32]. The PI3K/Akt pathway also has a crucial role in cell-to-cell adhesion and is implicated in tumor-induced extracellular matrix reorganization and tumor dissemination[33–36]. We hypothesize that activating mutations in this pathway might affect thymocyte-stroma interaction to promote tumor formation and dissemination preferentially in T-LBL. Clinically, specific inhibition of PIK3CA in *PIK3CA*-related overgrowth CLOVES syndrome patients induces spectacular disease regression[37]. Interestingly, the 19 T-LBL patients in this cohort showed a spectrum of mutations very similar to those reported in this study, with $PIK3CA^{H1047}$ as the most frequent alteration (Fig. 4C). PI3K/Akt pathway alterations and specifically *PIK3CA* alterations are frequent targetable lesions in solid tumors. Promising clinical trials have led to FDA approval for alpelisib in metastatic breast cancer[38]. Our results show that mutations in the PI3K/Akt signaling pathway are recurrent features and potentially actionable targets in T-LBL.

Additional genomic differences between T-LBL and T-ALL affect *NOTCH1*, *CDKN2A* genes, and epigenetic regulating factors. *NOTCH1* mutations affect the HD or TAD/PEST domain of the protein, resulting in increased *NOTCH1* signaling. These mutations have been widely described in T-ALL, when they are found in up to 70% of diagnostic samples[39,40]. NOTCH pathway mutations in T-ALL have been associated with a favorable outcome and improved steroid responses[41,42]. *NOTCH1* mutations have been reported in 55–60% of pediatric T-LBL[2,28,43] and 36–52% of adult T-LBL[31,44], while we now report *NOTCH1* mutations in 52% of T-LBL patients. These mutations were also associated with a favorable prognostic effect[31,43]. Interestingly, T-ALL were significantly enriched in *NOTCH1* mutations as compared to T-LBL, particularly those leading to single in-frame mutations, which were absent in T-LBL, compared to 17% of T-LBL.

With respect to epigenetic abnormalities, the *EZH2* gene was more frequently mutated in T-LBL. In previous studies, *EZH2* was mutated in 7% of T-ALL adult cases while *EZH2* gain was observed in 13% of T-LBL pediatric cases with SNP array[28,31]. Loss-of-function *EZH2* mutations were found in T-ALL, along with deletions and mutations of other Polycomb Repressive Complex 2 (PRC2) subunits[45–47]. EZH2 is the functional enzymatic component of PRC2 and is responsible for its methylation activity. PRC2 loss-of-function alterations can profoundly reshape the genetic and epigenetic landscape of T-ALL, leading to the reactivation of stem cell programs that cooperate with Bromodomain and Extraterminal (BET) proteins to sustain T-ALL. We recently identified a targetable vulnerability to BET inhibition in PRC2-altered T-ALL patients[47]. The present data suggest that *EZH2*-altered T-LBL patients should also be considered for such targeted therapy. On the other hand, other mutations in

PRC2 subunits (i.e., SUZ12) and in epigenetic factor *PHF6* were enriched in T-ALL. Similar to what has been reported, *SUZ12* inactivation and *PHF6* alterations significantly co-occurred with *JAK3* mutations in our T-ALL patients as compared to T-LBL[48,49]. Based on our results, selective PRC2 subunits alterations seem to be preferentially associated with leukemic or tumoral involvement and should be further explored. In the same manner, *PHF6* gene does not seem to play a major role in lymphomagenesis as compared to what has been reported in T-ALL[50]. In contrast to T-ALL cell lines, *PHF6* mutations have been associated with a favorable outcome in adult T-LBL patients[31]. The role *PHF6* plays in leukemogenesis is still actively under investigation and further study is therefore required to assess its true role and significance in T-LBLL patients.

Intriguingly, we found a higher incidence of *KMT2D* mutation, another epigenetic modifier, in T-LBL vs T-ALL. *KMT2D* have been previously shown to be associated with poor prognosis in pediatric T-LBL[28]. We reported a similar *KMT2D* mutation incidence (14% vs 13%) in T-LBL, but we did not observe a differential incidence of *KMT2D* mutation in pediatric vs. adult patients. In addition, we found that mutations in T-LBL were rather localized in a region ranging from 2000 to 3000 amino acids, as previously described, and, specifically to our cohort, in exon 11. For T-ALL, we found 8% of patients with *KMT2D* mutation which is higher than data previously reported, albeit in limited cohorts of purely pediatric patients (2% and 3%)[51,52]. Although *KMT2D* gene has been very little studied in T-LBLL, it seems to have an important role in leukemogenesis specifically in T-LBL. Because of a heterogenous distribution of mutations, further studies are needed for better characterization.

T-LBL were also highly enriched in *TP53* missense mutations as compared to T-ALL. *TP53* is the most commonly mutated somatic gene in human cancer and is often associated with a poor outcome in hematological malignancies[53]. The *TP53* targeting drug APR 246 (eprenetapopt) has recently shown promising results in myeloid malignancies with mutant *TP53*[54,55]. APR 246 is a small molecule that restores wild-type p53 functions in *TP53*-mutant cells and could be part of the future of care in T-LBL/T-ALL harboring *TP53* Mutation.

The JAK/STAT signaling pathway was frequently altered in T-LBLL (39% of cases), particularly in T-ALL, with *JAK3* as the main mutated gene in both T-LBL (12%) and T-ALL (15%). The incidence of *IL7R* was frequent and similar in T-LBL and T-ALL (about 10%). *IL7R/JAK/STAT* pathway alterations are known to participate in leukemic development and are associated with reduced steroid sensitivity and poor clinical outcome[56], although this may improve with targeted use of JAK inhibitors (tofacitinib and ruxolitinib)[57–59].

In conclusion, we provide the largest comparative study exploring the oncogenic landscape of T-LBL vs T-ALL and reveal for the first time significant preferential alterations in T-LBL vs T-ALL (PI3K/Akt pathway, *TP53,* and *PRC2/EZH2* alterations) using a pan-exon NGS-based approach. We identified recurrent mutations in targetable oncogenic signaling pathways in T-LBL and suggest that certain somatic abnormalities may affect the degree of tissue dissemination to blood and or bone marrow. Nevertheless, further prospective studies in T-LBL and T-ALL patients homogeneously treated are needed to better characterize the impact of genomic alterations in these rare diseases.

DATA AVAILABILITY

Data will be made publicly available.

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AUTHOR CONTRIBUTIONS

C.B.: Data collection, methodology, analysis, validation, visualization, graphic representation, statistics, writing, original draft preparation; M.S.: Data collection, methodology, analysis, validation, visualization, graphic representation, statistics, reviewing and editing; N.G.: Reviewing and editing; L.L.: Analysis, reviewing and editing; A.T.: Reviewing and editing; G.A.: Reviewing and editing; J.B.: Reviewing and editing; E.L.: Reviewing and editing; A.P.: Reviewing and editing; N.B.: Resources, reviewing and editing; A.B.: Resources, reviewing and editing; Y.B.: Resources,

reviewing and editing T.J.M.: Resources, reviewing and editing; V.A.: Conceptualization, methodology, validation, resources, reviewing and editing, supervision, project administration.

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ETHICS APPROVAL/CONSENT TO PARTICIPATE
Diagnostic peripheral blood, bone marrow, or lymphoid tissue samples from 818 adults and children with T-LBLL (476 patients with T-ALL and 342 with T-LBL) were centrally analyzed in Necker-Enfants Malades Hospital (AP-HP, Paris France) after informed consent was obtained at diagnosis according to the Declaration of Helsinki.

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