

# T-cell lymphoblastic lymphoma and leukemia: different diseases from a common premalignant progenitor?

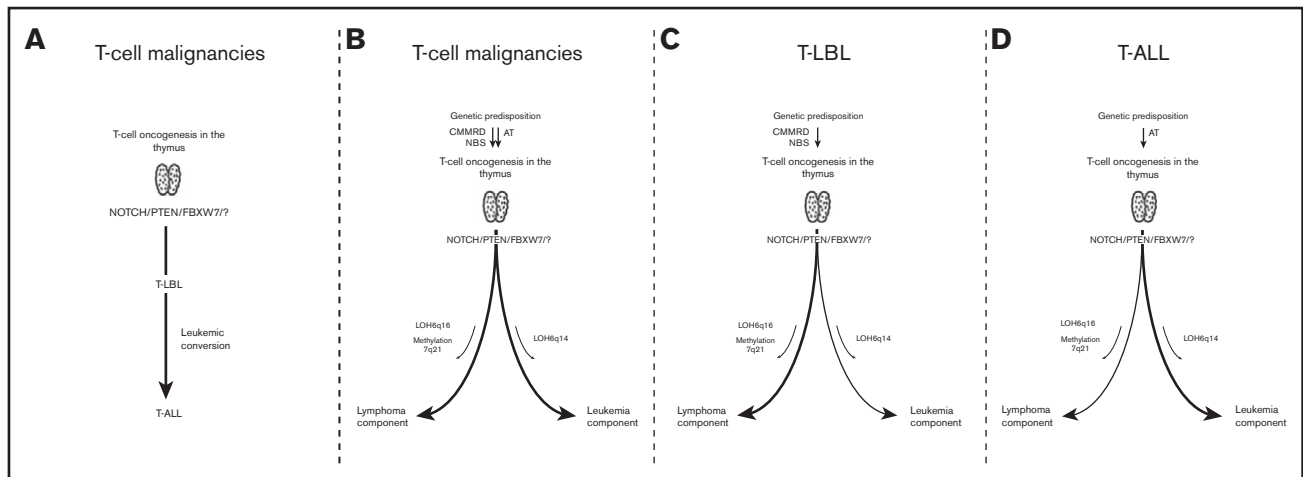
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T-cell lymphoblastic lymphoma (T-LBL) and lymphoblastic leukemia (T-ALL) represent malignancies that arise from the transformation of immature precursor T cells. Similarities in T-LBL and T-ALL have raised the question whether these entities represent 1 disease or reflect 2 different diseases. The genetic profiles of T-ALL have been thoroughly investigated over the last 2 decades, whereas fairly little is known about genetic driver mutations in T-LBL. Nevertheless, the comparison of clinical, immunophenotypic, and molecular observations from independent T-LBL and T-ALL studies lent strength to the theory that T-LBL and T-ALL reflect different presentations of the same disease. Alternatively, T-LBL and T-ALL may simultaneously evolve from a common malignant precursor cell, each having their own specific pathogenic requirements or cellular dependencies that differ among stroma-embedded blasts in lymphoid tissues compared with solitary leukemia cells. This review aims to cluster recent findings with regard to clinical presentation, genetic predisposition, and the acquisition of additional mutations that may give rise to differences in gene expression signatures among T-LBL and T-ALL patients. Improved insight in T-LBL in relation to T-ALL may further help to apply confirmed T-ALL therapies to T-LBL patients.

## Introduction

Progenitor cells that give rise to myeloid and lymphoid cells reside in the bone marrow (BM). After entry in the thymus, lymphoid precursor cells proliferate and differentiate into the T-cell lineage and are denoted thymocytes. T-cell lymphoblastic lymphoma (T-LBL) and T-cell lymphoblastic leukemia (T-ALL) represent the malignant counterparts of these thymocytes and are characterized by massive infiltration of immature T cells mainly in the mediastinum and other lymphoid organs without or with involvement of peripheral blood (PB), BM, and cerebral spinal fluid compartments. T-ALL accounts for 15% of the ALL cases, whereas T-LBL represents approximately 20% of the non-Hodgkin lymphomas (NHLs) in children. The World Health Organization and the International Lymphoma Study Group denominated both T-ALL and T-LBL as T-lymphoblastic leukemia/lymphoma in the updated Revised European-American Classification of Lymphoid Neoplasms and World Health Organization classification but without further specification.<sup>1,2</sup> T-LBL and T-ALL represent malignancies that affect similar early thymocyte subsets that acquire genetic and epigenetic aberrations.<sup>3,4</sup> The molecular abnormalities in T-ALL are mostly known, and although aberrations in T-ALL and T-LBL seem comparable thus far, additional mutational differences are to be expected.<sup>5</sup> For example, the acquisition of signaling mutations in T-ALL may facilitate ligand or cytokine-independent cell proliferation and survival, which could drive disease dissemination toward systemic cytokine-low compartments including the PB and BM compartments.<sup>6,7</sup> It is presently not known whether similar oncogenic rearrangements and mutations drive the pathogenesis of T-LBL. This review will therefore discuss overlap and differences in clinical parameters, genetic predisposition, and somatic aberrations for pediatric T-LBL in comparison with T-ALL.



**Figure 1. Hypothesis on the pathogenic origin and acquisition of mutations that result in T-LBL or T-ALL.** (A) Hypothesis in which T-ALL emerges from a preexisting T-LBL clone. (B-D) Alternative hypothesis in which T-LBL and T-ALL share a common pathogenic origin but require additional and unique mutations.

## Clinical presentation of disease

Primary treatment of T-LBL and T-ALL is often aimed at reducing life-threatening respiratory distress. For T-LBL patients, this is followed by stage-specific treatment regimens based on the Murphy staging that is determined by disease localization and dissemination.<sup>8,9</sup> The T-LBL treatment protocols that resemble historic standard-risk T-ALL treatment protocol and variable outcomes for different disease stages have been reported among different studies. This illustrates the need for an improvement in stratification based on other disease markers.<sup>8,10-12</sup> Contemporary T-ALL treatment is based on minimal residual disease risk-adapted treatment. The current survival rates of both T-LBL and T-ALL patients are around 80%. Similar to T-ALL, survival rates of relapsed T-LBL patients are dismal because lymphoma cells at relapse are highly refractory to further treatment because of acquired therapy resistance. Burkhardt et al<sup>13</sup> found that approximately 40% of relapsed T-LBL patients have evidence of BM involvement, whereas less than 20% of the T-LBL patients present with BM involvement at diagnosis.<sup>14</sup> A quarter of these relapsed patients lack disease involvement of other tissues. This may provide some substantiation for the hypothesis that a leukemic conversion originating from the T-LBL can occur. Conversely, 15% to 20% of the relapses in ALL patients occur in so-called apparent isolated extramedullary (AIEM) compartments, mostly in the central nervous system (CNS) or the testis with no or low blast counts (<5%) in BM biopsies. Therefore, AIEM relapses could clinically be regarded as lymphomas, or alternatively, niches that are intrinsically resistant to chemotherapy.<sup>15</sup> In line, 11% of the relapse patients with AIEM also lack detectable minimal residual disease levels in the BM that are clonally related to the leukemia cells at diagnosis.<sup>15</sup> Whether these examples should illustrate evident lymphoma-to-leukemia transitions or vice versa remains questionable (Figure 1A). Thus far, no clear genetic evidence for such transitions has been provided. Alternatively, parallel and simultaneous development of both lymphoma and leukemia clones that evolve from the same common pathogenic precursor within a patient needs further genetic exploration (Figure 1B-D). This may provide an alternative explanation for the emergence of isolated BM relapses in a minority of T-LBL patients and apparent isolated extramedullary CNS or

testicular relapses without evidence of minimal residual disease in a quarter of relapsed T-ALL patients.

## Pathogenic requirements that distinguish T-LBL from T-ALL

In addition to overlap and differences in clinical presentation of T-LBL and T-ALL, there are also indications for (epi)genetic, immunophenotypic, and gene expression levels that suggest that the 2 disease entities may have independent pathogenic requirements and dependencies.

The first indication comes from patients with a cancer predisposition syndrome that preferentially develops either T-LBL or T-ALL. Around 8% to 10% of childhood cancers result from genetic predisposition that predispose for specific malignancies, including ALL and NHL.<sup>16</sup> One of these syndromes is ataxia telangiectasia (AT), which is caused by the presence of biallelic pathogenic variants of *ATM*. *ATM* is a phosphatidylinositol 3-kinase-related kinase that is activated on DNA double-strand breaks (DSBs), resulting in defective DSB repair.<sup>17</sup> AT predisposes to T-ALL and mature B-cell NHL but almost never to T-LBL.<sup>18,19</sup> Patients with Nijmegen breakage syndrome (NBS) are diagnosed with a biallelic germline mutation in the *NBN* gene, encoding the nibrin protein. Nibrin is part of the MRE11, RAD50, and NBS1 (MRN) complex that localizes to sites of DSBs. The MRN complex is activated by *ATM*. In contrast to AT patients, NBS patients are predisposed to develop NHL, including mature B-NHL and T-LBL but less frequently T-ALL.<sup>17,20</sup> Constitutional mismatch repair deficiency (CMMRD) is associated with hematologic malignancies as well and is caused by a biallelic mutation in the mismatch repair genes *MLH1*, *MSH2*, *MSH6*, or *PMS2*. CMMRD also predisposes to the development of T-LBL but seldomly T-ALL.<sup>17,19</sup> This is inverted compared with sporadic cancer patients in which T-LBL is less frequent.

A second indication comes from gene expression analysis of T-LBL and T-ALL patient biopsies. Different gene expression signatures for T-LBL and T-ALL were demonstrated in both children and adult patients, implying specific requirements to sustain and colonize lymphoid tissues (T-LBL) or to manifest in systemic compartments (T-ALL) (Figure 1).<sup>21,22</sup> Differentially expressed genes were involved

in angiogenesis, chemotactic response, and nodal metastases, with a higher expression in T-LBL patients.<sup>21</sup> Another study showed high expression of BCL2 in T-LBL blasts, but also S1P1 and ICAM1, which are both involved in cell-cell adhesion.<sup>23</sup> This may explain why T-LBL cells remain embedded in lymphoid tissues in close proximity of stromal cells that may further raise chemoresistance and disease relapse.

A third indication comes from immunophenotypic analyses. Although, in general, the immunophenotype of T-LBL is fairly similar to T-ALL, it has been described that T-LBL more frequently affects a mature thymic entity than T-ALL with less frequent expression of myeloid antigens.<sup>4,24,25</sup>

A fourth indication comes from DNA methylation studies. For pediatric T-ALL, it was demonstrated that patients with low cytosine guanine dinucleotide (CpG) island methylation have a significantly worse outcome compared with T-ALL patients with high CpG island methylation.<sup>26</sup> It is currently not known whether this influences outcome of pediatric T-LBL patients. However, in 1 DNA methylation profiling study, an epigenetic signature of differentially methylated CpG sites was identified that clusters T-LBL and T-ALL separately.<sup>27</sup> Differentially methylated CpG islands point to differences in the expression of membrane-associated proteins. For example, sarcoglycan-ε (SGCE) and paternally expressed gene 10 (PEG10) are highly expressed in T-LBL. SGCE is involved in linking the actin cytoskeleton to the extracellular matrix and may therefore play an important role in embedding lymphoma cells in stromal niches, whereas PEG10 is associated with malignant transformation, affecting cell proliferation and apoptosis.<sup>27,28</sup> These indications demonstrate that T-LBL and T-ALL may reflect 2 different disease entities.

## Mutational mechanisms in T-LBL compared with T-ALL

### Driving oncogenic rearrangements

Gene expression profiling studies distinguished the existence of at least 4 T-ALL subtypes denoted as immature, TLX, proliferative, and TALLMO.<sup>29</sup> These subtypes are characterized by unique driving aberrations and rearrangements including MEF2C-activating rearrangements or *HOXA* gene activation gene fusions in immature/ETP-ALL patients, *TLX3* or *HOXA*-activating gene fusion in TLX patients, *NKX2-1/2-2* or *TLX1* rearrangements in proliferative patients, and *TAL1/2*, *LYL1*, or *LMO1/2/3* rearrangements in TALLMO patients (Table 1).<sup>22,30-34</sup> Fairly little is known about the oncogenic drivers in T-LBL patients. Thus far, chromosomal rearrangements that have been described in pediatric T-LBL patients point to similar rearrangements as previously observed in T-ALL patients, except for 9q34 rearrangements that remarkably occur in approximately 10% of T-LBL patients compared with 1% to 3% of T-ALL patients and that frequently involves the t(9;17)(q34;q22-23) translocation.<sup>35</sup> This locus at 9q34 includes various oncogenes, such as *SET*, *ABL1*, *NUP214* and *NOTCH1*.<sup>5,36</sup> Different than in T-ALL, various T-LBL patients have been reported who carry the t(7;14)(p15;q32) translocation in which the T-cell receptor γ chain locus is coupled to the *TCL1A* oncogene.<sup>37</sup>

### NOTCH1 pathway mutations

Ligand induced activation of the NOTCH1 receptor depend on various proteolytic cleavages that result in the release of intracellular

NOTCH1 (ICN1), which acts as a transcription factor. Ubiquitination of the PEST domain of ICN1 by the E3-ubiquitin ligase FBXW7 leads to degradation of ICN1.<sup>38,39</sup> Mutations in *NOTCH1* or *FBXW7* result in active NOTCH1 signaling. These *NOTCH1* mutations have been identified in more than 70% of the T-ALL patients.<sup>40</sup> NOTCH pathway mutations in T-ALL have been associated with a favorable outcome and improved steroid responses.<sup>41,42</sup>

Similar *NOTCH1* and *FBXW7* mutations were found in 55% and 13% of pediatric T-LBL patients, respectively. These mutations were also associated with a favorable prognostic effect: the 5-year probability of event-free survival was shown to be 84% ± 5% for *NOTCH1* mutant patients vs 66% ± 7% for patients lacking *NOTCH1* mutations.<sup>43</sup> These findings were confirmed in a recent and independent study.<sup>34</sup> The 5-year cumulative incidence of relapse was 15% ± 5% for patients with a *NOTCH1* mutation vs 27% ± 7% for patients without *NOTCH1* mutations.<sup>43</sup> These results were comparable on inclusion of *FBXW7* mutations, albeit *FBXW7* mutations themselves did not contribute to a favorable outcome.<sup>43</sup>

In addition to mutations in *NOTCH1* or *FBXW7*, the TAL1 oncoprotein may directly repress *FBXW7* through upregulation of *miR-223* in T-ALL patients, resulting in activated *NOTCH1* signaling.<sup>44</sup> Expression of *miR-223* has also been reported in T-LBL, but seems unrelated to the NOTCH1 activity status, in contrast to T-ALL.<sup>45</sup> *NOTCH1* mutant T-LBL patients who have increased *miR-223* levels have an inferior probability of event-free survival compared with patients with *NOTCH1* mutations but low *miR-223* levels, suggesting that *miR-223* provides an unfavorable prognostic effect that outweighs the favorable prognostic value of NOTCH1 mutations in T-LBL.<sup>45</sup>

### Loss of heterozygosity at chromosome 6q (LOH6q)

In both hematologic malignancies and solid tumors, deletions of the long arm of chromosome 6 (also denoted as loss of heterozygosity [LOH]) have been detected that are possibly associated with the loss of important tumor suppressor genes.<sup>46,47</sup> LOH6q can be detected in approximately 13% of T-ALL patients and in 19% of T-LBL patients. The involved chromosomal bands differ between T-ALL and T-LBL, affecting chromosomal bands 6q14-15 and 6q16, respectively.<sup>43,48,49</sup> The commonly deleted area in T-ALL patients include the *SYNCRIP* and *SNH5G* genes, and 6q deletions are almost exclusively found in patients of the TALLMO subtype.<sup>50</sup> *SYNCRIP* and *SNH5G* are involved in the regulation of RNA maturation and translation. Downregulation of both genes accelerates leukemogenesis in a TAL1/LMO1/NOTCH1-driven T-ALL mouse model.<sup>50</sup> No association with disease outcome or risk of relapse has been identified for 6q deletions in pediatric T-ALL patients.<sup>51</sup>

In contrast to T-ALL, the 6q deletion in T-LBL patients affects the glutamate ionotropic receptor kainate type subunit 2 gene (*GRIK2*), the caspase 8 associated protein 2 gene (*CASP8AP2*) that can bind to the FAS-FADD-CASPASE8 death receptor complex, and the tyrosine kinase receptor gene ephrin type-A receptor 7 (*EPHA7*). Their exact roles in the pathogenesis of T-LBL remains unclear.<sup>52-54</sup> LOH6q in T-LBL patients has found to be associated with poor outcome and an increased risk of relapse, which is different for 6q deletions in T-ALL patients. This results in a 5-year cumulative

**Table 1. Subtypes, mutations, and prognostic effects in T-LBL and T-ALL**

Subtype	T-ALL		T-LBL	
	Aberrations/rearrangements	Outcome	Aberrations/rearrangements	Outcome
Immature	<i>MEF2C</i> , <i>HOXA</i> -activating fusions	Poor for <i>HOXA</i> <sup>+</sup> -ETP-ALL	<i>HOXA</i> -activating fusions, <i>TAL1</i> , <i>LMO2</i> , <i>MYC</i> , <i>NOTCH1</i> , <i>TCL1A</i>	Unknown
TLX	<i>TLX3</i> , <i>HOXA</i> -activating fusions	Unknown		
Proliferative	<i>TLX1</i> , <i>NKX2-1</i> , <i>NKX2-2</i>	Favorable		
TALLMO	<i>TAL1</i> , <i>TAL2</i> , <i>LYL1</i> , <i>LMO1</i> , <i>LMO2</i> , <i>LMO3</i> , <i>MYC</i>	Unknown		
Aberration	Incidence	Prognostic effect	Incidence	Prognostic effect
<i>NOTCH1</i> <sup>mut</sup>	>50% <sup>40</sup>	Strong trend toward favorable	55% <sup>43</sup>	Favorable
<i>FBXW7</i> <sup>mut</sup>	11%-31% <sup>40</sup>	No effect	13% <sup>43</sup>	No effect
<i>NOTCH1</i> <sup>mut</sup> <i>FBXW7</i> <sup>mut</sup>	Unknown	Trend toward favorable <sup>41</sup>	15% <sup>43</sup>	Favorable
<i>LOH6q</i> <sup>pos</sup>	13% <sup>49</sup>	No effect	19% <sup>48</sup>	Unfavorable
<i>PTEN</i> <sup>del/mut</sup>	13% <sup>67,72</sup>	Unfavorable	15% <sup>69,72</sup>	Unfavorable
<i>PTEN</i> <sup>del/mut</sup> <i>NOTCH1</i> <sup>mut</sup>	—	Favorable <sup>78</sup>	—	Favorable <sup>69</sup>
<i>PTEN</i> <sup>del/mut</sup> <i>LOH6q</i> <sup>pos</sup>	—	Unknown	—	Unfavorable <sup>69</sup>
<b><i>PHF6</i><sup>mut</sup></b>		No effect <sup>57</sup>		Favorable
Pediatric	16% <sup>55</sup>		—	
Adult	39% <sup>55</sup>		25% <sup>34</sup>	
<b><i>IL7R</i><sup>mut</sup></b>				
Pediatric	35% <sup>64</sup>	Unfavorable <sup>65,66</sup>		
Adult			26% <sup>34</sup>	No effect
<b><i>NRAS/KRAS</i><sup>mut</sup></b>				
Pediatric			10% <sup>69</sup>	No effect
Adult	10% <sup>67</sup>	Unfavorable		

incidence of relapse of 9% ± 3% for T-LBL patients without del6q compared with 63% ± 12% for T-LBL patients with del6q.<sup>49</sup>

### PHF6 mutations

*PHF6* is an epigenetic modifier that recognizes histone methylation marks. As identified in 16% of pediatric and 36% of adult T-ALL patients, inactivating mutations in the *PHF6* gene were identified in nearly 25% of adult T-LBL patients.<sup>34</sup> In T-ALL, these mutations were identified in patients having *TLX1* or *TLX3* rearrangements.<sup>55</sup> Despite the association of *PHF6* mutations with decreased prednisolone response in T-ALL cell lines, no negative impact on disease-free survival or overall survival was reported for *PHF6*-mutant adult T-ALL patients.<sup>56,57</sup> In contrast, *PHF6* mutations have been associated with a favorable outcome in adult T-LBL patients.<sup>34</sup>

### Interleukin-7 signaling mutations

Interleukin-7 (IL7) signaling is indispensable for both the development and survival of thymocytes.<sup>58-60</sup> In the presence of IL7, a receptor complex is formed that activates downstream Janus kinase (JAK)–signal transducer and activator of transcription and phosphatidylinositol 3-kinase (PI3K)–AKT signaling pathways.<sup>61-63</sup> Mutations in IL7R signaling molecules, as identified in nearly 35% of pediatric T-ALL patients,<sup>64,65</sup> result in high activation of IL7R signaling. These mutations, as well as IL7-induced signaling, raise steroid resistance in T-ALL.<sup>66</sup> Consequently, IL7R signaling mutations predict for poor outcome in pediatric T-ALL patients at diagnosis and relapse.<sup>65,67,68</sup> Similar activating mutations in *IL7R*, *JAK1*, and *JAK3* are known mutational hotspots and were identified in about 26% of the adult T-LBL patients.<sup>34</sup> It is presently unknown

whether these mutations are related to steroid resistance in T-LBL patients.

Mutations in the downstream IL7R signaling components *NRAS* or *KRAS* have been found in about 10% of T-ALL patients. RAS mutations were associated with poor outcome for adult T-ALL patients in the French Group for Research on Adult Lymphoblastic Leukemia (GRAALL)-2003/2005 study.<sup>67</sup> *NRAS* or *KRAS* mutations have been found in nearly 10% of T-LBL patients as well.<sup>69,70</sup> In contrast to T-ALL, these mutations do not have a negative impact on the prognosis of T-LBL patients.<sup>69</sup>

### PI3K-AKT pathway mutations

Mutations in the PI3K-AKT signaling pathway including *PTEN*-inactivating and *AKT*-activating mutations are found in approximately 18% of the pediatric T-ALL patients.<sup>71,72</sup> *PTEN* mutations/deletions are associated with decreased outcome, except for T-ALL patients in the MRC UKALL 2003 cohort.<sup>67,71,73-78</sup> When found in combination with activating *NOTCH1* mutations, the poor prognostic effect of *PTEN* mutations seems outweighed by the favorable effect of *NOTCH1* mutations.<sup>78</sup> *PTEN* mutations in T-ALL are most abundant in *TAL1*- or *LMO2*-rearranged patients and most frequently occur in the absence of *NOTCH*-activating mutations.<sup>67,71,79</sup>

In pediatric T-LBL patients, mutations in *PIK3R1*, *PIK3CA*, and *PTEN* have been found in, respectively, 4%, 6%, and 15% of the patients.<sup>69</sup> *PTEN* was identified as the most clinically relevant mutation because it defines a poor prognostic marker for pediatric T-LBL patients.<sup>69</sup> Although biology indicates that *PIK3R1*- and

*PIK3CA*-activating mutations and *PTEN*-inactivating mutations result in activated PI3K-AKT signaling, the negative prognostic impact of mutant *PTEN* became weaker on inclusion of patients with PI3K mutations. This suggests that *PTEN* inactivation may affect additional signaling routes other than the PI3K-AKT pathway.<sup>69,80</sup> As for T-ALL, the unfavorable effect of *PTEN* mutations when coexisting with *NOTCH1* mutations is outweighed by the favorable prognostic effect of *NOTCH1*-activating mutations in pediatric T-LBL patients.<sup>69</sup> Also, patients that are wild type for both *PTEN* and *NOTCH1* have a significantly superior survival, as is described for T-ALL.<sup>67,69,71,81</sup>

For T-LBL, similar results were found for the combination of LOH6q and *PTEN* mutations; absence of LOH6q diminished the unfavorable prognostic effect of mutated *PTEN*, whereas patients with combined *PTEN* mutations and presence of LOH6q had a significantly worse outcome.<sup>69</sup> Co-occurrence of *PTEN* mutations and LOH6q has been shown in T-ALL as well, but the prognostic significance of this combination is presently unknown. An overview of the prognostic impact for these various mutations for T-ALL and T-LBL can be found in Table 1.

## Conclusion

Molecular cytogenetic observations in various T-LBL and T-ALL studies have fueled the hypothesis that these immature T-cell malignancies may reflect different representations of the same disease. However, the availability of immunophenotypic and genetic expression data for T-LBL patients is relatively limited in comparison with T-ALL patients, and our present view may be biased by analyses of T-ALL mutational hotspots in T-LBL patients in various studies. Unbiased whole-genome sequencing has not been performed yet. Additionally, paired analysis on the genetic makeup for tissue-embedded blasts vs circulating blasts has not been performed and is hampered by limited availability of tissue biopsies in T-ALL, impairing a direct comparison between blasts from different disease sites within a patient. As reviewed, there is ample evidence that T-LBL differs in various aspects from T-ALL in addition to differences in clinical presentation of disease. First, a strong predisposition toward development of T-LBL or T-ALL from various DNA repair deficiency syndromes including AT, NBS, and CMMRD is evident. Second, T-LBL blasts seem to be more frequently arrested at a mature thymic developmental stage than T-ALL blasts, and third, genetic differences are seen between T-LBL and T-ALL patients. The most remarkable genetic difference is the deletion in the 6q chromosomal arm, for which the common

deleted areas have been associated with different chromosomal bands, affecting different genes and being associated with different outcomes. In addition, differentially methylated CpG sites are seen, and the frequency of 9q34 rearrangements is higher in T-LBL patients compared with T-ALL patients. Also, the prognostic impact of RAS mutations is poor for relapsed T-ALL patients, whereas these mutations do not have a negative impact on the outcome of T-LBL patients. Therefore, despite a common pathogenic origin for T-LBL and T-ALL in the thymus, T-LBL blasts that remain in close contact with stromal cells may require different (genetic) dependencies than solitary T-ALL cells in the PB and BM compartments. This could possibly explain the differences in gene expression signatures between T-LBL and T-ALL. That indicates that T-ALL does not merely reflect the disseminated state from a stromal-bound ancestral T-LBL clone but supports the alternative hypothesis that stromal bound blasts and solitary blasts may evolve in parallel from a premalignant clone, requiring specific pathogenic aberrations (Figure 1). This could explain the presence of low blast counts in the BM in T-LBL patients, the emergence of solitary BM (leukemia) relapses in T-LBL patients, or local (lymphoma) relapses in the CNS or testis in T-ALL patients.

To further substantiate this hypothesis, genetic screening studies should be performed on T-LBL and T-ALL patient biopsies from paired tissue resources that compare the genetic makeup of tissue-embedded blasts to PB or BM blasts. This could elucidate whether T-LBL and T-ALL represent different states of a single disease that is derived from a common, premalignant ancestor or reflect independent diseases.

## Acknowledgments

E.K. was sponsored by the Princess Máxima Center for Pediatric Oncology. V.M.P. was sponsored by Stichting Kinderen Kankervrij (KiKa-335).

## Authorship

Contributions: E.K., V.M.P., J.L.C.L., and J.P.P.M. wrote the paper.

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

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