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Early T-cell Precursor Acute Lymphoblastic Leukemia (ETP T-ALL)

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Abstract

Purpose of review—Early T-cell Precursor (ETP) leukemias have been recently recognized as a form of T-cell acute lymphoblastic leukemia (T-ALL) with poor prognosis. The purpose of this review is to outline the most recent advances in the biology, genetics and prognostic significance of this aggressive disease.

Recent findings—Detailed immunophenotypic analyses have defined ETP T-ALLs as a distinct group of T-cell acute lymphoblastic leukemia (T-ALL) with poor prognosis. Transcriptionally, ETP T-ALLs and early immature T-ALLs, a broader group of tumors characterized by very early arrest in T-cell differentiation, are most related to hematopoietic stem cells and myeloid progenitors. Consistently these leukemias show lower frequencies of prototypical T-ALL lesions such as *CDKN2A/B* deletions and activating mutations in *NOTCH1* and show a higher prevalence of mutations typically associated with the pathogenesis of acute myeloid leukemias (AML).

Summary—ETP and early immature T-ALLs are characterized by a very early differentiation arrest and show unique genetic and transcriptional features that overlap both with T-ALL and AML. Given the unique biology and poor prognosis associated with the ETP T-ALL group there is an urgent need of new tailored therapeutic strategies for the treatment of this disease.

Keywords

T-ALL; ETP T-ALL; gene expression profiling; genomics

Introduction

T cell acute lymphoblastic leukemia (T-ALL) is an aggressive hematologic tumor resulting from the malignant transformation of T cell progenitors [1]. As such, intrathymic T-cell development constitutes the pathophysiological framework underlying much of the basic mechanisms involved in the pathogenesis of T-ALL and a key conceptual background for understanding the biological heterogeneity of this disease. Early immunophenotype based classifications grouped T-ALLs based on the expression of surface markers differentially expressed during T-cell development. Moreover, the introduction of microarray gene expression profiling analyses showed a marked relationship between the expression of T-ALL oncogenes activated by chromosomal translocations, gene expression signatures related to differential arrest at different stages of T-cell development, immunophenotype and clinical outcome [2]. Recently, genetic studies have shed new light on the biology of ETP T-

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Conflicts of interests

There are no conflicts of interest

ALLs, a distinct disease entity associated with poor prognosis and defined by a characteristic immunophenotype and a gene expression signature indicative of a very early arrest in T-cell development.

Gene expression profiling studies define a group of early immature T-ALLs

Over the last decade a number of microarray gene expression profiling studies have recognized early immature T-ALLs as a transcriptionally distinct group of leukemias. Early on, supervised analysis of microarray data from pediatric T-ALLs based on the expression of T-ALL transcription factor oncogenes activated by chromosomal translocations identified a distinct group of T-ALLs with the highest levels of expression of the *LYL1* oncogene and coexpression of *LMO2* [2]. Notably, *LYL1* tumors not only showed a gene expression signature distinct from leukemias driven by *TLX1* and *TAL1* oncogenes, but also showed a gene expression signature reflecting an early arrest in T-cell differentiation. In contrast *TLX1* positive tumors were more related to early cortical thymocytes and expressed high levels of *CD1* genes, while *TAL1* positive leukemias showed transcriptional upregulation of *CD3* and *TCR* genes corresponding with a late cortical thymocyte stage of development arrest. Consistently, immunophenotype analysis showed that transcriptionally defined *LYL1* and *LYL1*-related T-ALLs universally expressed the early hematopoietic marker CD34, myeloid antigens (CD13 or CD33), and for the most part lacked expression of both CD4 and CD8. In addition, and supporting a unique genetic basis for these leukemias, transcriptionally defined *LYL1* and *LYL1*-related T-ALLs showed a uniquely high prevalence of 5q, 13q and 11q chromosomal deletions and in contrast lacked deletions involving the short arm of chromosome 9 which delete the *CDKN2A/B* tumor suppressor gene in over 70% of all T-ALLs.

These results were subsequently validated and extended in independent series. First, unsupervised analyses of adult and pediatric T-ALLs identified an immature cluster with frequent expression of myeloid CD13 or CD33 surface antigens [3]. As before, these immature leukemias strongly expressed *LYL1* and *CD34* genes and were highly related to the previously reported *LYL1* group [3]. In addition and most notably these immature leukemias transcriptionally clustered with the most immature ETP and Pro-T subpopulations when compared with normal T-cell progenitors [3]. More recently, similar results have been reported in one of the largest microarray series analyzed to date, which recognized an immature cluster of T-ALLs highly enriched for immature CD4 CD8 double-negative cases [4]. Consistently with previous studies, samples in this immature cluster expressed CD34, frequently co-expressed the myeloid markers CD13 and/or CD33; expressed high levels of *LYL1* and overlapped with the *LYL1*-positive cluster originally defined by of Ferrando *et al.* [4].

ETP TALLs as an immunophenotypically defined group of T-ALLs associated with poor prognosis

The relevance of the transcriptionally defined early immature group of T-ALLs was highlighted by early indications that these leukemias could be associated with a less favorable outcome. Thus, the original *LYL1* positive group of pediatric T-ALLs showed a poor prognosis particularly when compared with the more favorable group of *TLX1* positive leukemias [2]. In addition, expression of *ERG*, an early immature gene was associated with inferior relapse-free survival in adult T-ALL [5]. However, the poor prognosis associated with early immature leukemias was best recognized in a group of immunophenotypically defined leukemias characterized by the absence (<5% positive cells) of CD1a and CD8 expression, weak CD5 (<75% positive cells), and expression (>25% positive cells) of at least one or more of the following myeloid or stem-cell markers: CD117, CD34, human

leucocyte antigen (HLA)-DR, CD13, CD33, CD11b, or CD65 [6]. In this study, leukemias matching this immunophenotype were named ETP T-ALLs as they were more closely related to early T-cell precursors than to more mature thymocyte populations when analyzed by gene expression profiling. ETP T-ALLs accounted for about 12% of pediatric T-ALLs in this series and were associated with a very poor prognosis [6]. Thus, for T-ALLs treated in Total Therapy Studies XIII, XIV, and XV at St. Jude Children's Research Hospital, ETP-ALL patients showed a significantly worse clinical response to the first phase of remission induction therapy as measured by minimal residual disease [6], which ultimately translated in a significantly worse outcome, with a 10-year overall survival of 19% for patients with ETP-ALL compared with 84% for all other T-ALLs [6]. In the same study, the strong association of ETP T-ALLs with poor outcome was confirmed in an independent series of 100 consecutive patients treated in the ALL-2000 protocol of the Associazione Italiana Ematologica Oncologica Pediatrica (AIEOP) protocol. In this second cohort, ETP T-ALLs showed reduced 2-year overall and event-free survival rates, as well as an increased cumulative incidence of remission failure or hematological relapse [6].

Several subsequent studies analyzing the prognostic significance of immunophenotypically defined ETP T-ALLs have now confirmed these results. In this regard, ETP T-ALLs accounted for 5.5% of pediatric T-ALL patients treated in the Tokyo Children's Cancer Study Group Study L99-15, showed an inferior response to early remission induction therapy and were associated with worse prognosis (4-year event-free survival 40% in ETP T-ALLs vs. 70% in non-ETP cases) [7]. As in the study of Coustan-Smith *et al.*, ETP-ALLs showed no significant differences in clinical features and the ETP immunophenotype was the strongest risk factor for relapse in univariate and multivariate analyses [7]. For patients treated in Shanghai Children's Medical Center studies XH-ALL-99 and SCMC-ALL-2005, patients with an ETP T-ALL immunophenotype accounted for 16% of all T-ALLs [8]. ETP T-ALL cases in these series had a significantly worse event-free survival (EFS 11.1% for ETP T-ALLs vs. 57.6% for non-ETP-ALL); higher rates of relapse and remission failure (58.3% for ETP T-ALLs vs. 24.2% for non-ETP T-ALLs); and a worse overall survival (13.3% for ETP T-ALL vs. 64.7% for non-ETP T-ALL cases) [8].

Early immature T-ALLs encompass ETP T-ALLs and related “close to ETP” leukemias

Cross examination of gene sets associated with the ETP T-ALL defined by Coustan Smith *et al.*, the *LYL1* leukemias originally identified by Ferrando *et al.* and the immature T-ALL clusters reported by Soulier *et al.* and Homminga *et al.* reveal that these are closely related groups. However, it seems that transcriptionally identified early immature T-ALLs encompass a broader group than the immunophenotypically defined ETP T-ALLs. Thus, the original report by Coustan-Smith *et al.* showed four T-ALLs that clustered together with ETP T-ALLs in an unsupervised analysis of the gene expression even though they did not fulfill all immunophenotypic criteria for their classification as ETP T-ALLs. Of these, three had expression of CD5 (with absence of myeloid or stem cell markers in one case) and a fourth case showed very high expression of surface CD3 and TCR gamma/delta [6]. Conversely, gene expression analysis of adult T-ALL patients identified a robust cluster of early immature leukemias encompassing about 50% of T-ALLs [9*]. As in previous gene expression studies this transcriptionally defined cluster of early immature leukemias was highly related to the original *LYL1* group identified by Ferrando *et al.* and showed a characteristic immunophenotype with expression of early hematopoietic markers such as CD34 and CD133 and myeloid-associated antigens such as CD13, CD33, and CD11b [9*]. However, based on CD5 expression only a fraction of these leukemias seem to fulfill the strict ETP immunophenotype criteria, as 34% of them showed complete absence of surface

CD5 expression, 55% showed low levels of surface CD5 expression, and 11% were strongly CD5 positive [9*].

Absence of biallelic deletion of *TCRG* (ABD) as prognostic marker in T-ALL

To date, it remains to be established if the poor prognosis associated with immunophenotypically defined ETP T-ALLs would also apply to the broader group of early immature T-ALLs identified by gene expression profiling. However, the recent identification of absence of biallelic *TCRG* deletion (ABD), a marker of developmental arrest at the earliest stages of thymocyte development, as a poor prognostic marker strongly associated with a unfavorable response to chemotherapy and induction failure in pediatric T-ALL suggests that the adverse prognosis of ETP T-ALLs might extend to other early immature T-ALL cases [10]. Thus, even though *TCR* loci deletions are significantly less frequent in ETP T-ALL [6], and the majority of ABD patients possessed the ETP T-ALL gene expression signature [10], only 1 out of 8 ABD T-ALL patients in this study met the ETP T-ALL immunophenotype criteria [10]. These discrepant results may very well reflect, at least in part, the challenge to accurately assess the ETP T-ALL immunophenotype across different laboratories. As of today, the ETP T-ALL immunophenotype is the most validated prognostic marker for the identification of high risk early immature T-ALLs. However, its implementation as a diagnostic tool is not trivial. Further studies will determine if T-ALLs with ABD are broadly overlapping with immunophenotypically defined ETP-TALLs and the comparative sensitivity and specificity of these two markers for the identification of high risk T-ALLs.

ETP and early immature T-ALLs are transcriptionally related to hematopoietic stem cells and myeloid progenitors

ETP T-ALLs were originally defined as an immunophenotypically homogeneous group of leukemias with gene expression signatures related to those of early immature T-cell precursors and distinct from those of more mature T-cell progenitors [6]. However, recent reanalysis of the gene expression signatures associated with immunophenotypically defined T-ALLs has shown that the gene expression programs of these leukemias are indeed most closely related to those of human hematopoietic stem cells and myeloid progenitors [11**]. Similarly, and more broadly, the early immature cluster of adult T-ALLs identified by Van Vlierberghe *et al.* showed a marked enrichment in hematopoietic stem cell and granulocyte monocyte/macrophage precursor gene sets [9*].

The genetics of ETP and early immature T-ALLs

Several lines of evidence suggested a distinct genetic landscape for both transcriptionally defined early immature T-ALLs and the ETP T-ALLs. As mentioned before, the absence of chromosomal deletions encompassing the *CDKN2A/B* locus suggested early on that the original *LYL1* positive group of early immature T-ALLs could be genetically distinct from most other TALLs [2]. In addition, the initial group of immunophenotypically defined ETP ALLs seemed to have increased genomic instability as evidenced by an increased number and size of genomic lesions in their karyotypes [6]. The first indication of a specific genetic lesion possibly associated with the pathogenesis of early immature leukemias was the identification via 4C analysis of rare but recurrent rearrangements resulting in aberrantly high levels of expression of the *MEF2C* gene in this group [4]. *MEF2C* encodes an important transcriptional regulator of lymphoid development which is expressed at high levels in very early pre-DN1 and DN1 thymocytes and whose expression dramatically drops beyond the DN2 stage of thymocyte development. Notably, and supporting a potential role as a master regulator of the early immature T-ALL transcriptional signature, *MEF2C* can

directly induce the expression of *LYL1*, *LMO2*, *HHEX*, three oncogenic transcription factors expressed at high levels in early immature leukemias [4].

Given the lack of a unifying lesion for ETP T-ALL and to define the genetic landscape of this high risk leukemia group, Zhang *et al.* analyzed 12 ETP T-ALL cases using whole-genome sequencing and determined the recurrence and frequency of mutations in an extended cohort of 52 ETP T-ALL and 42 non-ETP T-ALL cases [11**]. This seminal work identified a high frequency of activating mutations in factors mediating cytokine receptor and RAS signaling including *NRAS*, *KRAS*, *FLT3*, *IL7R*, *JAK3*, *JAK1*, *SH2B3*, and *BRAF* in ETP T-ALL [11**]. In addition ETP T-ALLs frequently showed inactivating mutations in genes encoding key transcription factors involved in hematopoietic development including *GATA3*, *ETV6*, *RUNX1*, *IKZF1*, and *EP300* and in genes encoding histone modifiers such as *EZH2*, *EED*, *SUZ12*, *SETD2* and *EP300* [11**]. Interestingly, several ETP T-ALL cases showed multiple genomic rearrangements suggestive of genomic instability, which seemed to be associated with lesions in genes involved in DNA mismatch repair (*MLH3* and *MSH5*) and non homologous end joining (*DCLRE1C*) [11**]. Finally, and in agreement with previous reports, biallelic deletion of *TCRG* and deletions in the short arm of chromosome 9 encompassing the *CDKN2A/B* tumor suppressor genes were less prevalent in ETP T-ALLs compared with non-ETP leukemias in this series [11**].

In parallel and following the close relationship between early hematopoietic and myeloid gene expression signatures and the transcriptional programs characteristic of early immature adult TALLs, Van Vlierberghe *et al.* tested the possibility that transcriptionally defined early immature T-ALLs could be genetically related to myeloid tumors [9*]. Consistent with this hypothesis, 50% of adult early immature T-ALLs showed mutations in myeloid-specific oncogenes and tumor suppressor genes including *IDH1*, *IDH2*, *DNMT3A*, *FLT3*, and *NRAS* and a lower prevalence of prototypical T-ALL genetic alterations such as activating mutations in the *IL7R* gene and mutations in *NOTCH1* and *FBXW7* activating the NOTCH-signaling pathway [9*]. These results highlighted the close relationship between early immature T-ALLs and acute myeloid leukemias. However, and most notably, even 25% of early immature T-ALLs showed concurrence of a myeloid-specific gene alteration and a prototypical T-ALL mutation. These results suggest that early immature adult T-ALLs comprise a heterogeneous leukemia category characterized by overlapping myeloid and T-lymphoid genetic alterations. In addition, this study also identified and functionally characterized mutations in the *ETV6* tumor suppressor gene resulting in expression of truncated forms of ETV6 with dominant negative activity in almost 25% of early immature T-ALLs [9*]. Interestingly, rare but recurrent fusions of the *ETV6* tumor suppressor gene with *NCOA2* were previously identified in rare leukemias harboring both T lymphoid and myeloid characteristics [12] and two cases harboring a *ETV6-INO80D* fusion oncogene were identified in the genomic studies of Zhang *et al.* [11**].

Conclusion

Early immature T-ALLs encompassing ETP T-ALLs and related “close to” ETP tumors constitute a genetically heterogeneous group of leukemias characterized by a gene expression signature most related to that of hematopoietic stem cells and myeloid progenitors. Prognostically, the ETP T-ALL immunophenotype and the absence of biallelic deletion of the *TCRG* locus are associated with very poor prognosis in children. Early immature T-ALLs seem to be more prevalent in adults, however there is limited information of the prognosis of these leukemias in the context of adult T-ALL [13,14]. Notably, and despite harboring a distinct transcriptional signature, early immature T-ALLs seem to lack a distinct unifying genetic alteration and are characterized by a lower prevalence of NOTCH1 activating mutations, mutations in myeloid oncogenes and tumor suppressor genes, and

genetic alterations disrupting key transcription factors involved in hematopoietic and lymphoid development. Two recent studies have reported a possible worse prognosis for immature adult T-ALL leukemias harboring mutations in *RUNX1* and *DNMT3A* [14,15]. Further dissection of this heterogeneous leukemia group is needed to address the prognostic implications of specific immunophenotypes, transcriptional signatures and genetic alterations. Therapeutically, new chemotherapy regimens, perhaps including drugs active in myeloid tumors, validated in relevant animal models are urgently needed for the treatment of high risk ETP T-ALLs. In addition, targeted therapies may be available in the near future for those leukemias harboring activating mutations in druggable factors and signaling pathways such as FLT3, JAK1, JAK3, IL7R and NOTCH1.

Acknowledgments

The authors confirm that this manuscript has not been published in its current or substantially similar form elsewhere.

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Key Points

ETP T-ALLs are an immunophenotypically distinct group of T-ALLs with poor prognosis.

Early immature T-ALLs defined on the basis of gene expression signatures and ETP T-ALLs are transcriptionally related to hematopoietic stem cells and myeloid progenitors.

Genetically, ETP T-ALLs show mutations driving activation of growth factor receptors and RAS signaling and genetic lesions disrupting lymphoid and hematopoietic development and epigenetic regulation of gene expression.

Early immature T-ALLs show a high prevalence of mutations involving AML oncogenes and tumor suppressor genes together with a lower incidence of mutations in NOTCH1 signaling and deletions encompassing the *CDKN2A/B* tumor suppressor loci.