LYMPHOID NEOPLASIA

Prognostic relevance of integrated genetic profiling in adult T-cell acute lymphoblastic leukemia

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Key Points

- Integrated genomic profiling identifies high-risk adult T-ALL patients with poor response to intensified chemotherapy.
- Adult T-cell acute lymphoblastic leukemia (T-ALL) is an aggressive hematologic tumor associated with poor outcome. In this study, we analyzed the prognostic relevance of genetic alterations, immunophenotypic markers, and microarray gene expression signatures in a panel of 53 adult T-ALL patients treated in the Eastern Cooperative Oncology Group E2993 clinical trial. An early immature gene expression signature, the absence of bi-allelic TCRG deletion, CD13 surface expression, heterozygous deletions of the short arm of chromosome 17, and mutations in IDH1/IDH2 and DNMT3A genes are associated with poor prognosis in this series. In contrast, expression of CD8 or CD62L, homozygous deletion of CDKN2A/CDKN2B, NOTCH1 and/or FBXW7 mutations, and mutations or deletions in the BCL11B tumor suppressor gene were associated with improved overall survival. Importantly, the prognostic relevance of CD13 expression and homozygous CDKN2A/CDKN2B deletions was restricted to cortical and mature T-ALLs. Conversely, mutations in IDH1/IDH2 and DNMT3A were specifically associated with poor outcome in early immature adult T-ALLs. This trial was registered at www.clinicaltrials.gov as #NCT00802514. (Blood. 2013;122(1):74-82)

Introduction

T-cell acute lymphoblastic leukemias (T-ALLs) account for 25% of adult ALL cases.1 Clinically, T-ALL is characterized by 3 times more frequent in men than women and typically presents with hematopoietic failure resulting from bone marrow infiltration by an immature lymphoblast with a T-cell immunophenotype and high white blood cell counts. In addition, T-ALL patients frequently show mediastinal masses and leukemic infiltration of the central nervous system at diagnosis. T-ALL arises as a result of a multistep oncogenic process in which different genetic alterations induce aberrant cell proliferation, increased cell survival, and blocked differentiation of immature T-cell progenitors. In this context, deletion of the CDKN2A locus encompassing the p16/INK4A and p14/ARF suppressor genes in chromosome band 9p21 is the most frequent genetic alteration in T-ALL, present in >70% of all T-ALL cases.2,2 In addition, constitutive activation of NOTCH1 signaling as a result of activating mutations in the NOTCH1 gene and mutations in the FBXW7 tumor suppressor are found in 50% to 60% of T-ALLs.3 Moreover, and most uniquely, T-ALLs also show aberrant expression of oncogenic transcription factors as a result of chromosomal translocations involving strong regulatory elements associated with the TCR genes. T-ALL transcription factor oncogenes include basic helix-loop-helix factors such as TAL1; LIM-only domain (LMO) genes such as LMO1 and LMO2; the TLX1/HOX11, TLX3/HOX11L2, and HOXA homeobox genes; MYC; and MYB and TAL1, a truncated and constitutively activated form of the NOTCH1 receptor.4 Furthermore, recent studies have uncovered a growing number of mutations and deletions disrupting the function of key transcriptional regulators such as WT1, LEFT1, BCL11B, GATA3, RUNX1, and ETV6 and epigenetic factors including EP300, PHF6, SETD2, EZH2, EED, and SUZ12.4 The mutation landscape of T-ALL also includes chromosomal rearrangements and mutations driving increased proliferation, including translocations involving CCND2 and NUP214-ABL1 and mutations in IL7R, FLT3, PTEN, and NRAS.5 Finally, the genetic landscape of T-ALL is completed with mutations in genes such as the ribosomal protein RPL106 and the dymamin DNMT2 gene,6 whose functional role in T-cell transformation is yet to be fully understood.

Still, and despite this diversity of molecular and cytogenetic lesions, microarray gene expression studies have shown that T-ALLs can be classified in a limited number of transcriptional groups, which are related to the activation of specific transcription factor oncogenes and show distinct gene expression signatures related to their arrest at different stages of T-cell differentiation.1,7-9 Thus, early immature T-ALLs, which are arrested early at the double-negative stage of thymocyte development,1,10,11 show a transcriptional program related to hematopoietic stem cells and myeloid progenitors,6,11 higher levels of LYL1 and LMO2 expression,1 and mutations in acute myeloid leukemia genes,6,11 together with inactivation of important transcription factors such as RUNX1, GATA3, and ETV6.5,11 Notably, these
early immature tumors frequently show the absence of bi-allelic TCRG deletions\(^\text{12}\) and are associated with very poor prognosis.\(^\text{10,12}\) In contrast, CD1a\(^+\) T-ALLs show an early cortical gene expression signature, frequent activation of TLX1, and a favorable prognosis.\(^\text{1,13}\)

Finally, leukemias with aberrant expression of TAL1 together with LMO1 and LMO2 are characterized by a gene expression program related to those of late cortical thymocytes.\(^\text{1}\)

To gain insight in the clinical relevance of different genetic alterations and oncogenic pathways involved in T-cell transformation, we analyzed the prognostic significance of transcriptional, cytogenetic, immunophenotypic, and molecular features of T-ALLs treated in the Eastern Cooperative Oncology Group (ECOG) E2993 clinical trial.\(^\text{14}\)

Methods

Patient samples

Bone marrow lymphoblast samples from 53 T-ALL patients treated in the E2993 ECOG clinical trial\(^\text{14}\) were included in this study. All samples were collected with informed consent at trial entry according to the declaration of Helsinki and analyzed under the supervision of the Columbia University Medical Center Institutional Review Board.

Flow cytometry

Immunophenotypic analysis was performed at the ECOG leukemia reference laboratory as previously described\(^\text{14}\) (supplemental Table 1 on the website). Briefly, lymphoblasts were gated by 3-color flow cytometry based on antigen expression of the leukemic cells. In addition to cytoplasmic CD3 (cCD3), which established the diagnosis of T-ALL, T-lymphoid antigens included CD45, CD34, HLA-DR, and TdT. Uncommitted antigens included CD19 and CD10. Myeloid antigens were characterized by a gene expression program

Microarray gene expression profiling of primary adult T-ALL samples

RNA was isolated using the RNaseasy plus mini kit (Qiagen) according to the manufacturer’s protocol. Next, 500 ng of RNA was amplified and biotinylated using the TotalPrep RNA Amplification Kit (Ambion) and hybridized to the HumanHT-12 v4 Expression BeadChip (Illumina). Gene expression profiling data were normalized using log2 transformation and quantile normalization. Unsupervised consensus clustering was performed using the GenePattern application.\(^\text{16}\) Enrichment of the gene set associated with early T-cell precursor (ETP) T-ALL was analyzed by Gene Set Enrichment Analysis using the Student \(t\) test metric and 10,000 permutations of the genes. Microarray gene expression data are available in Gene Expression Omnibus (GEO accession code GSE42328).

Microarray-based array-CGH

Comparative genomic hybridization (array-CGH) analysis was performed using the SurePrint G3 Human 1 × 1M oligonucleotide array platform according to the manufacturer’s instructions (Agilent). Slides were scanned in a 2565AA DNA microarray scanner (Agilent). Microarray images were analyzed using feature extraction software (Agilent), and the data were subsequently imported into array-CGH analytics software (Agilent).

Mutation analysis

Exon sequences from NOTCH1, FBXW7, Pten, IL7R, Dnm2, Phf6, Bcl11b, WT1, EzH2, Etv6, Idh1, Idh2, Flt3, Nras, Dnm3a, Gata3, Tp53, and Runx1 were amplified from genomic DNA by polymerase chain reaction and analyzed by direct dyeoxynucleotide sequencing. Mutational hotspot regions were sequenced for NRAS, Pten, FLt3, DNM3A, IDH1, IDH2, NOTCH1, IL7R, and FBXW7. Primer sequences used for FLt3, DNM3A, IDH1, IDH2, NOTCH1, IL7R, FBXW7, DNM2, Phf6, Bcl11b, WT1, EzH2, Etv6, Idh1, Idh2, Flt3, Nras, Dnm3a, Gata3, Tp53, and Pten\(^\text{18}\) have been previously described.

Statistical analyses

Survival time was measured from the date of randomization to date of death for patients who died and to the date of the last follow-up for those who were alive at the time of the analysis. The prognostic value of the different covariates was evaluated with the use of the Kaplan-Meier estimate of survival function. Differences between the survival functions were assessed with the log-rank test. The covariates included transcriptional gene signature status (early immature vs typical/mature), cytogenetic characteristics (gene mutations and genomic deletions/amplifications), and expression of immunophenotypic markers. Any antibody binding by a distinct subset of gated T-lymphoblasts was considered a positive finding. Weak intensity of antibody binding by the entire blast cell population above background staining was interpreted as positivity of all blasts for the respective antigen, albeit at low density.

Multivariate survival analyses were performed with the Cox proportional-hazards model. Covariates were selected using the least absolute shrinkage and selection operator (lasso)\(^\text{27}\) as this approach is particularly well suited when the number of explanatory variables is high with respect to the sample size. We checked the proportional-hazards assumption by testing for a nonzero slope in a regression of the scaled Schoenfeld residuals on functions of time. The Cox model was cross-validated using the leave-one-out cross-validation method proposed by Verweij and Van Houwelingen.\(^\text{30}\) The variables included in the final model appeared in >90% of the leave-one-out lasso–selected sets. We used the libraries survival and glmnet in R2.15.0. All analyses were performed with the use of Stata version 11.0 (www.stata.com) and R statistical software 2.15.0 (www.r-project.org).

Results

Prognostic value of transcriptionally defined early immature and cortical-mature adult T-ALL groups

To analyze the prognostic relevance of molecular groups defined by gene expression profiling in adult T-ALL, we analyzed a series of 53 primary leukemia samples using gene expression oligonucleotide microarrays. As previously described,\(^\text{15}\) unsupervised clustering analysis of microarray gene expression data in this series revealed the presence of 2 robust clusters of samples (Figure 1A). The first of these clusters encompassed 28 samples and corresponded to early immature leukemias (Figure 1A-B), a group characterized by a gene expression signature related to that of hematopoietic stem cells and myeloid progenitors.\(^\text{11}\) In contrast, the second of these clusters, referred here as cortical/mature leukemias, contained 28 samples (Figure 1A) whose gene expression signatures were related to those of cortical and mature thymocytes\(^\text{11}\) (Figure 1B). Notably, univariate analysis in our patient series showed that early immature adult T-ALLs are associated with poor prognosis and reduced overall survival compared with cortical/mature adult T-ALL (5-year survival: early immature 34% [95% confidence interval: 17%-52%] vs cortical/mature 62% [95% confidence interval: 40%-78%], \(P = .0112\); Figure 1C).

Prognostic value of copy number alterations

Array-CGH analysis in our adult T-ALL series revealed on average 6 copy number alterations (5 deletions and 1 amplification) per sample (supplemental Tables 2 and 3). Recurrent deletions of the short arm of chromosome 9 centered in 9p22 and encompassing the CDKN2A/CDKN2B tumor suppressor genes were present in 22 samples (Figure 2A). Additional recurrent copy number aberrations
included 1p33 deletions generating the *SIL-TAL1* fusion transcript (n = 3); 4q25 deletions targeting *LEF1* (n = 2); 4q31.3 deletions targeting the *FBXW7* tumor suppressor gene (n = 3); duplications of the *MYB* oncogene (n = 10) at 6q23.3; 9q34 deletions generating the *SET-NUP214* fusion oncogene (n = 2); 11p13 deletions targeting the *WT1* tumor suppressor (n = 3); 12p13 deletions targeting the *ETV6* and *CDKN1B* (n = 5); 13q deletions targeting *RB1* (n = 9); 14q32 deletions targeting the *BCL11B* gene (n = 2); *TP53* deletions at 17p (n = 5); and 17q11 deletions encompassing the *NF1* and *SUZ12* tumor suppressors (n = 3; Figure 2A; supplemental Table 3). Finally, array-CGH analysis also revealed the rearrangement status of the T-cell receptor (TCR) loci evidenced as highly recurrent deletions in chromosome bands 7p14 (*TCRG*), 7q34 (*TCRB*), and 14q11 (*TCRA/D*).
Pediatric T-ALL patients with the absence of the bi-allelic TCRG deletion (ABD), a feature linked with developmental arrest at the earliest stages of thymocyte development, exhibit early induction failure and inferior overall survival rates. Array-CGH analysis of TCRG copy number in our series showed ABD of the TCRG locus in 27 of 53 (51%) adult T-ALL samples. In addition, 22 of 27 (81%) ABD adult T-ALL samples showed an early immature gene expression signature, suggesting biological overlap between the ABD and the early immature subtype in adult T-ALL. Notably, as in the case of pediatric T-ALLs, ABD adult T-ALL patients showed a worse overall survival (ABD 35% [95% confidence interval: 18%-53%] vs bi-allelic TCRG deletion 59% [95% confidence interval: 38%-76%]; P = 0.0217; Figure 2B). In contrast, homozygous deletion of CDKN2A/CDKN2B (n = 15) was associated with favorable outcome (5-year survival: no bi-allelic CDKN2A/CDKN2B deletion 38% [95% confidence interval: 23%-53%] vs
bi-allelic CDKN2A/CDKN2B deletion 71% [95% confidence interval: 41%-88%]; P = .0119; Figure 2C), whereas heterozygous deletions of the short arm of chromosome 17 covering the TP53 tumor suppressor gene (n = 15) predicted worse clinical outcome (2-year survival: TP53 deletion 20% [95% confidence interval: 9%-58%] vs no TP53 deletion 66% [95% confidence interval: 50%-77%]; P = .0005; Figure 2D). Within these TP53-deleted leukemia samples, 4 of 5 (80%) patients were ABD positive and 3 of 5 (60%) showed an early immature gene expression signature (Figure 3).

**Prognostic value of somatic gene mutations**

Mutation analysis of T-ALL oncogenes and tumor suppressor genes (Figure 3; supplemental Table 4) identified NOTCH1 and FBXW7 mutations, resulting in activation of NOTCH signaling, in 62% (33/53) of adult T-ALL patients. In addition, 49% (27/53) of adult T-ALL cases showed mutations targeting epigenetic and/or chromatin remodeling factors including DNMT3A, IDH1, IDH2, EZH2, and PHF6. Moreover, mutations targeting transcription factors including

![Figure 3. Genetic and immunophenotypic characteristics of adult T-ALL. Schematic comparison of copy number lesions, genetic mutations, and surface marker expression between early immature and mature/cortical adult T-ALL. Solid circles represent positive leukemia samples. Data were not available for leukemia patients represented by gray filled circles. T-ALL oncogenic subtypes are based on aCGH alterations and microarray expression of T-ALL transcription factor oncogenes.](image)

![Figure 4. Prognostic value of somatic gene mutations in adult T-ALL.](image)
GATA3, RUNX1, WT1, BCL11B, and ETV6 were identified in 38% (20/53) of adult T-ALL patients. Finally, mutations in signaling pathways (FLT3, NRAS, IL7R, and PTK2) were found in 36% (19/53) of T-ALL patients, whereas mutations targeting the endocytosis and membrane trafficking factor gene DNMT2 were present in 8 of 53 T-cell leukemias. No mutations were identified in TP53.

Favorable outcome was observed in adult T-ALL patient samples with NOTCH1 and/or FBXW7 mutations (5-year survival: wild type 24% [95% confidence interval: 7%-46%] vs mutant 59% [95% confidence interval: 41%-74%]; P = .0214; Figure 4A); and in cases with heterozygous inactivating mutations or deletions in the BCL11B tumor suppressor gene (5-year survival: wild type 43% [95% confidence interval: 28%-56%] vs mutation/deletion 100%; P = .0415; Figure 4B). In contrast, somatic mutations in genes targeting the epigenetic regulators DNMT3A (1-year survival: mutant 50% [95% confidence interval: 6%-84%] vs wild type 77% [95% confidence interval: 63%-87%]; P = .0026; Figure 4C) and IDH1/2 (2-year survival: mutant 20% [95% confidence interval: 8%-58%] vs wild type 66% [95% confidence interval: 50%-77%]; P = .0113; Figure 4D) were associated with worse prognosis. Consistent with previous reports, alterations in DNMT3A and IDH1/IDH2 were uniquely present in the early immature adult T-ALL group.

Prognostic value of cell surface markers

Immunophenotype analysis showed that our 53 adult T-ALL series encompasses 25 immature (CD4+ CD8+ CD1a-), 13 early cortical (CD1a+), 6 late cortical/mature (CD8+ CD3+) leukemias, and 9 cases with overlapping patterns of antigen expression. Notably, leukemic blasts from 39 of 53 adult T-ALL patients (73%) showed expression of the myeloid antigens CD13 and/or CD33 (Figure 3). In addition, expression of the stem cell marker c-KIT (CD117) was identified in 15 of 53 (28%) leukemia samples (Figure 3; supplemental Table 1).

Prognosis analysis of these immunophenotypic markers revealed that expression of CD13 was associated with poor survival (5-year survival: CD13+ 34% [95% confidence interval: 18%-50%] vs CD13- 69% [95% confidence interval: 44%-85%]; P = .0023; Figure 5A), as previously described. In addition, expression of CD8 (5-year survival: CD8+ 33% [95% confidence interval: 17%-51%] vs CD8- 63% [95% confidence interval: 41%-79%]; P = .0035; Figure 5B) and CD62L (5-year survival: CD62L- 37% [95% confidence interval: 17%-57%] vs CD62L+ 54% [95% confidence interval: 35%-70%]; P = .0337; Figure 5C) conferred better prognosis.

Multivariate analysis

Next, multivariate Cox regression analysis revealed that CD13 and CD62L antigen expression, heterozygous 17p deletions, and mutations in NOTCH1/FBXW7 and DNMT3A are independent prognostic markers in adult T-ALL treated on the E2993 protocol (supplemental Table 5).

Risk stratification in early immature and cortical/mature adult T-cell leukemias

Given the broad gene expression, immunophenotypic, and genetic differences between early immature and cortical/mature adult T-cell leukemias, we tested the significance of different prognostic markers in these 2 groups. This analysis revealed that the poor prognostic effect of CD13 expression was restricted to the otherwise favorable clinical group of immature T-cell leukemias (5-year survival: CD13+ 36% [95% confidence interval: 11%-63%] vs CD13- 85% [95% confidence interval: 51%-96%]; P = .0031; Figure 6A) and was not observed in T-ALLs with an early immature gene expression signature (5-year survival: CD13+ 33% [95% confidence interval: 15%-53%] vs CD13- 38% [95% confidence interval: 6%-72%]; P = .77; Figure 6B). In addition, the overall favorable prognostic effect of homozygous CDKN2A/CDKN2B deletions was confirmed within the mature/cortical gene expression profile group (5-year survival: no bi-allelic CDKN2A/CDKN2B deletion 42% [95% confidence interval: 15%-67%] vs bi-allelic CDKN2A/CDKN2B deletion 83% [95% confidence interval: 48%-96%]; P = .0104; Figure 6C), and the overall poor prognosis of DNMT3A mutations was confirmed within the high-risk group of T-ALLs with an early immature gene expression signature (1-year survival: mutant 25% [95% confidence interval: 9%-67%] vs wild type 62% [95% confidence interval: 40%-78%]; P = .0407; Figure 6D). This is in contrast with IDH1/2 mutations, which are also restricted to early immature adult T-ALLs, but only showed a trend toward
worse clinical outcome (2-year survival: mutant 20% [95% confidence interval: 8%-58%] vs wild type 56% [95% confidence interval: 34%-74%]; \(P = 0.12\); Figure 6E) in this group.

**Discussion**

ETP leukemias are characterized by a cell surface antigen profile consisting of lack of CD1 and CD8, weak CD5, and the expression of at least one myeloid- or stem cell–related marker.10 These leukemias are transcriptionally related to early T-cell progenitors, a subtype of thymocytes that retain multilineage potential, and have been associated with poor prognosis in different clinical series including the Total Therapy Studies XIII, XIV, and XV at St. Jude Children’s Research Hospital,10 the ALL-2000 protocol of the Associazione Italiana Ematologia Oncologia Pediatrica,10 and the L99-15 study of the Tokyo Children’s Cancer Study Group.31 Our microarray gene expression signature analyses demonstrate a high prevalence of early immature leukemias closely related to pediatric ETP-ALLs associated with reduced overall survival in adult T-ALL. This observation is in line with previous immunophenotype-based correlative analyses showing that adult T-ALLs with immature immunophenotypic features are associated with poor outcome.14,32 Of note, the German Multicenter Study Group for Adult ALL study protocols currently applies an immunophenotype-based risk stratification in which early adult T-ALLs (sCD3\(^+\), CD1a\(^+\)) are selected for a more intensive treatment regimen including allogeneic stem cell transplantation.33

Absence of a bi-allelic TCRG deletion, a molecular marker linked with a very early block in T-cell differentiation, has been associated with poor response to induction therapy and dismal 5-year event-free and overall survival in pediatric T-ALL patients treated on COG P9404 or DFCI 00-01 protocols.12 More recently, the prognostic relevance of ABD was confirmed in pediatric T-ALL patients treated according to the Taiwan Pediatric Oncology Group protocols34 and pediatric T-cell lymphoblastic lymphoma patients treated following a Berlin-Frankfurt-Munich ALL-type strategy.35 In our patient series, ABD was identified in about half of adult T-ALL samples and found to confer with poor overall survival. In addition, 22 of 27 ABD adult T-ALL samples showed an early immature gene expression signature, suggesting biological overlap between the ABD and early immature subgroups.
in adult T-ALL. Altogether, these data show that early immature leukemias (defined by their transcriptional profile or TCRG status) are associated with poor prognosis in adult T-ALL. In addition, and as previously documented, CD13 expression was strongly associated with poor survival in our patient series, whereas other early immature or myeloid antigens including CD34 and CD33 showed no clinical impact. Importantly, our results suggest that CD13 expression could identify patients at increased risk of therapeutic failure in the otherwise good prognostic subtype of adult T-ALLs with a mature/cortical gene expression profile. However, given the small number of patients in the different subgroups, these results should be interpreted with caution and await validation in an independent series.

T-ALL is a cytogenetically stable disease with a limited number of chromosomal alterations per sample. Comparison of our aCGH analysis results with those of previously reported pediatric series showed major overlap and some differences. Among these, the frequency of MYB duplications (10/53; 19%) and RBL1 deletions (9/53; 17%) in our adult patient series was higher than previously documented in pediatric studies. In addition, we failed to identify genomic deletions at the 6q14-6q22 locus, which occur in 20% to 30% of pediatric T-ALLs. Finally, the presence of multiple amplifications at 13q and 17q, and recurrent genomic deletions at 1p36 and 5q, suggest that these chromosomal regions could harbor novel T-ALL oncogenes and tumor suppressors, respectively.

Importantly, TP53 mutations have been previously associated with poor survival in pediatric T-ALL patients treated on the Pediatric Oncology Group protocol POG8862. Moreover, TP53 deletions and mutations at relapse are associated with chemotherapy resistance, failure to achieve a second complete remission, and poor survival. In line with this notion, heterozygous deletions of the short arm of chromosome 17 encompassing the TP53 tumor suppressor gene predicted for worse clinical outcome in adult T-ALL. In contrast, homozygous deletions of the CDKN2AC/CDKN2B locus on the short arm of chromosome 9 conferred better clinical outcome in our series. Consistently, loss of heterozygosity at the short arm of chromosome 9 has been associated with a favorable initial treatment response in pediatric T-ALL patients treated in the Berlin-Frankfurt-Munster ALL-95 study protocol. Activation of NOTCH signaling through NOTCH1 and/or FBXW7 mutations have been associated with a favorable prognosis in several, but not all, pediatric study protocols. Similarly, controversial results on the prognostic effect of NOTCH activation have been obtained in adult T-ALL. These inconsistent results might at least in part be explained by the prognostic relevance of functional interplay between specific genetic lesions in T-ALL, as recently shown for NOTCH1 activation and inactivation of Pten in children with T-ALL treated according to Berlin-Frankfurt-Munster study protocols. In our study, both univariate and multivariate analyses revealed that patients whose leukemias showed mutations in NOTCH1 and/or FBXW7 had a favorable outcome.

Recently, we documented a high prevalence of epigenetic and signaling mutations targeting FLT3, NRRAS, DNMT3A, IDH1, and IDH2, typically found in myeloid leukemias, in adult early immature T-ALLs. Notably, activating internal tandem duplication mutations in the FLT3 tyrosine kinase gene correlated with poor overall survival and an increased risk of relapse in AML. Similarly, mutations in DNMT3A occur in ~20% of AML patients and seem to confer poor overall survival in this disease. In contrast, mutations in IDH1 and IDH2 are associated with a favorable clinical outcome in AML, provided they co-occur together with NPM1 mutations. In our series, mutations targeting the epigenetic regulators DNMT3A, IDH1, and IDH2 conferred worse prognosis in adult T-ALL, and multivariate analysis pointed to DNMT3A mutations as an independent prognostic factor. Importantly, these findings confirm the recent association of DNMT3A mutations with poor outcome in a panel of 90 adult T-ALL patients treated according to the German Multicenter Study Group for Adult ALL protocols.

Overall, the comprehensive analysis presented here shows that an early immature gene expression signature and the absence of bi-allelic TCRG deletion are associated with poor prognosis in adult T-ALL. In addition, we show that CD13 expression and homozygous CDKN2A/CDKN2B deletions might serve as prognostic markers to stratify low-risk cortical/mature adult T-ALLs, whereas DNMT3A mutations may be useful for risk stratification within high-risk early immature adult T-ALLs. Together, these analyses identified a subset high risk adult T-ALLs who may benefit from new emerging targeted therapies or alternative chemotherapy approaches.

Acknowledgments

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Authorship

P.V.V. performed experiments and wrote the manuscript; A.A.-I. performed bioinformatic analyses; K.D.K. provided samples and correlative clinical and immunophenotypic findings concerning the recent genetic lesion in T-ALL, as recently shown for NOTCH1 activation and inactivation of Pten in children with T-ALL treated according to Berlin-Frankfurt-Munster study protocols. In our study, both univariate and multivariate analyses revealed that patients whose leukemias showed mutations in NOTCH1 and/or FBXW7 had a favorable outcome.

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Over all, the comprehensive analysis presented here shows that an early immature gene expression signature and the absence of bi-allelic TCRG deletion are associated with poor prognosis in adult T-ALL. In addition, we show that CD13 expression and homozygous CDKN2A/CDKN2B deletions might serve as prognostic markers to stratify low-risk cortical/mature adult T-ALLs, whereas DNMT3A mutations may be useful for risk stratification within high-risk early immature adult T-ALLs. Together, these analyses identified a subset high risk adult T-ALLs who may benefit from new emerging targeted therapies or alternative chemotherapy approaches.

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Authorship

P.V.V. performed experiments and wrote the manuscript; A.A.-I. performed bioinformatic analyses; K.D.K. performed aCGH analysis; M.H. performed sequencing analysis; M.R. and C.F. performed statistical analysis; E.P., J.M.R., M.S.T., and J.M.R. provided samples and correlative clinical and immunophenotypic data from ECOG; and A.A.F. designed the studies, directed research, and wrote the manuscript.

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