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

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**Key Point**

Integrated genomic profiling identifies high-risk adult T-ALL patients with poor response to intensified chemotherapy.
Abstract

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 (ECOG) 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 were 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.

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Introduction

T-cell acute lymphoblastic leukemias (T-ALL) account for 25% of adult ALL cases\(^1\). Clinically, T-ALL is characteristically three times more frequent in males than females and typically presents with hematopoietic failure resulting from bone marrow infiltration by immature lymphoblast with a T-cell immunophenotype and high white cell counts. In addition T-ALL patients frequently show mediastinal masses and leukemic infiltration of the central nervous system at diagnosis. T-ALLs arise as result of a multistep oncogenic process in which different genetic alterations induce aberrant cell proliferation, increased cell survival and blocked differentiation immature T-cell progenitors. In this context deletions of the \textit{CDKN2A} locus encompassing the \textit{p16/INK4A} and \textit{p14/ARF} suppressor genes in chromosome band 9p21 is the most frequent genetic alteration in T-ALL, present in more than 70% of all T-ALL cases\(^1,2\). In addition, constitutive activation of \textit{NOTCH1} signaling as result of activating mutations in the \textit{NOTCH1} gene and mutations in the \textit{FBXW7} tumor suppressor are found in 50-60% of T-ALLs\(^3\). Moreover, and most uniquely, T-ALLs also show aberrant expression of oncogenic transcription factors as result of chromosomal translocations involving strong regulatory elements associated with the \textit{TCR} genes. T-ALL transcription factor oncogenes include basic helix-loop-helix (bHLH) factors such as \textit{TAL1}; LIM-only domain (LMO) genes such as \textit{LMO1} and \textit{LMO2}; the \textit{TLX1/HOX11}, \textit{TLX3/HOX11L2} and \textit{HOXA} homeobox genes; \textit{MYC}; \textit{MYB} and \textit{TAN1}, a truncated and constitutively activated form of the \textit{NOTCH1} receptor\(^4\). Furthermore, recent studies have uncovered a growing number of mutations and deletions disrupting the function of key transcriptional regulators such as \textit{WT1}, \textit{LEF1}, \textit{BCL11B}, \textit{GATA3}, \textit{RUNX1} and \textit{ETV6}; and epigenetic factors including \textit{EP300}, \textit{PHF6}, \textit{SETD2}, \textit{EZH2}, \textit{EED} and \textit{SUZ12}\(^4\). The mutation landscape of T-ALL also includes chromosomal rearrangements and mutations driving increased proliferation, including translocations involving \textit{CCND2} and \textit{NUP214-ABL1} and mutations in \textit{IL7R}, \textit{FLT3}, \textit{PTEN}, and \textit{NRAS}\(^4\). Finally, a the genetic landscape of T-ALL is
completed with mutations in genes such as the ribosomal protein RPL10\textsuperscript{5} and the dynamin DNM2 gene\textsuperscript{5} 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\textsuperscript{1,7-9}. Thus, early immature T-ALLs, which are arrested early at the double negative stage of thymocyte development\textsuperscript{1,10,11}, show a transcriptional program related to hematopoietic stem cells and myeloid progenitors\textsuperscript{6,11}, higher levels of LYL1 and LMO2 expression\textsuperscript{1} and mutations in acute myeloid leukemia genes\textsuperscript{6,11}, together with inactivation of important transcription factors such as RUNX1, GATA3 and ETV6\textsuperscript{6,11}. Notably, these early immature tumors frequently show absence of biallelic TCRG deletions\textsuperscript{12} and are associated with very poor prognosis\textsuperscript{10,12}. In contrast, CD1a positive T-ALLs show an early cortical gene expression signature, frequent activation of TLX1 and a favorable prognosis\textsuperscript{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\textsuperscript{1}.

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

**Methods**

**Patient samples**

Bone marrow lymphoblast samples from 53 T-ALL patients treated in the E2993 Eastern Cooperative Oncology Group clinical trial\textsuperscript{14} (ClinicalTrials.gov identifier: NCT00002514) 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\(^\text{14}\) (Supplementary Table 1). 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–affiliated antigens tested included CD1a, CD2, surface CD3, CD4, CD5, CD8, CD62L, CD57, and surface α/β and γ/δ. The intensity of CD5 staining was determined as mean fluorescent intensity (mfi) as previously described\(^\text{15}\). Myeloid antigens included myeloperoxidase, CD117, CD33, CD13, CD65(s), CD15(s), CD11b, and CD14, and B-lymphoid-affiliated antigens included CD19 and CD10. Finally, uncommitted antigens included CD45, CD34, HLA-DR, and TdT.

**Microarray gene expression profiling of primary adult T-ALL samples**

RNA was isolated using the RNeasy plus mini kit (Qiagen) according to manufacturer’s protocol. Next, 500 ng of RNA were 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 GSEA using the t-test metric and 10,000 permutations of the genes. Microarray gene expression data is available in Gene Expression Omnibus (GEO accession code GSE42328).
**Microarray-based comparative genomic hybridization (array-CGH)**

Array-CGH analysis was performed using the SurePrint G3 Human 1x1M oligonucleotide array platform (Agilent, Santa Clara, USA) according to manufacturer 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**, **DNMT3A**, **GATA3**, **TP53** and **RUNX1** were amplified from genomic DNA by PCR and analyzed by direct dideoxynucleotide sequencing. Mutational hotspot regions were sequenced for **NRAS**, **PTEN**, **FLT3**, **DNMT3A**, **IDH1**, **IDH2**, **NOTCH1**, **IL7R** and **FBXW7**. Primer sequences used for **FLT3**17, **DNMT3A**18, **IDH1**19, **IDH2**19, **NOTCH1**3, **IL7R**20, **FBXW7**21, **DNM2**6, **PHF6**22, **BCL11B**23, **WT1**24, **EZH2**25, **ETV6**11, **GATA3**6, **RUNX1**26, **TP53**27 and **PTEN**28 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), genetic 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) 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 leave-one-out cross-validation method proposed by Verweij
and van Houwelingen. The variables included in the final model appeared in more than 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 the 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, 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. 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\textsuperscript{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\% CI: 17\%-52\%] vs. cortical/mature 62\% [95\% CI: 40\%-78\%]; \( P = 0.0112 \); 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 (Supplementary Tables 2 and 3). Recurrent deletions of the short arm of chromosome 9 centered in 9p22 and encompassing the \textit{CDKN2A/CDKN2B} tumor suppressor genes were present in 22 samples (Figure 2A). Additional recurrent copy number aberrations included 1p33 deletions generating the \textit{SIL-TAL1} fusion transcript (\( n = 3 \)); 4q25 deletions targeting the \textit{LEF1} (\( n = 2 \)); 4q31.3 deletions targeting the \textit{FBXW7} tumor suppressor gene (\( n = 3 \)); duplications of the \textit{MYB} oncogene (\( n = 10 \)) at 6q23.3; 9q34 deletions generating the \textit{SET-NUP214} fusion oncogene (\( n = 2 \)); 11p13 deletions targeting the \textit{WT1} tumor suppressor (\( n = 3 \)); 12p13 deletions targeting the \textit{ETV6 and CDKN1B} (\( n = 5 \)); 13q deletions targeting \textit{RB1} (\( n = 9 \)); 14q32 deletions targeting the \textit{BCL11B} gene (\( n = 2 \)); \textit{TP53} deletions at 17p (\( n = 5 \)) and 17q11 deletions encompassing the \textit{NF1} and \textit{SUZ12} tumor suppressors (\( n = 3 \)) (Figure 2A, Supplementary Table 3). Finally, array CGH analysis also revealed the rearrangement status of the TCR loci evidenced as highly recurrent deletions in chromosome bands 7p14 (\textit{TCRG}), 7q34 (\textit{TCRB}) and 14q11 (\textit{TCRA/D}).

Pediatric T-ALL patients with absence of bi-allelic \textit{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\textsuperscript{12}. Array CGH analysis of \textit{TCRG} copy number in our series showed ABD of the \textit{TCRG} locus in 27 out of 53 (51\%) adult T-ALL samples. In addition,
22 out 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% CI: 18%-53%] vs. bi-allelic TCRG deletion 59% [95% CI: 38%-76%]; P = 0.0217; Figure 2B). In contrast, homozygous deletion of the CDKN2A/CDKN2B (n=15) was associated with favorable outcome (5 year survival: no bi-allelic CDKN2A/CDKN2B deletion 38% [95% CI: 23%-53%] vs. bi-allelic CDKN2A/CDKN2B deletion 71% [95% CI: 41%-88%]; P = 0.0119; Figure 2C), while heterozygous deletions of the short arm of chromosome 17 covering the TP53 tumor suppressor gene (n=15) predicted for worse clinical outcome (2 year survival: TP53 deletion 20% [95% CI: 9%-58%] vs. no TP53 deletion 66% [95% CI: 50%-77%]; P = 0.0005; Figure 2D). Within these TP53 deleted leukemia samples, 4 out of 5 (80%) patients were ABD positive, and 3 out 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 and Supplementary 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 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 PTEN) were found in 36% (19/53) of T-ALL patients, whereas mutations targeting the endocytosis and membrane trafficking factor gene DNM2, were present in 8 out 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% CI: 7%-46%] vs. mutant 59% [95% CI: 41%-74%]; P = 0.0214; Figure 4A); and in cases with heterozygous inactivating mutations or deletions in the BCL11B tumor suppressor gene (5 year survival: wild type 43% [95% CI: 28%-56%] vs. mutation/deletion 100%; P = 0.0415; Figure 4B). In contrast, somatic mutations in genes targeting the epigenetic regulators DNMT3A (1 year survival: mutant 50% [95% CI: 6%-84%] vs. wild type 77% [95% CI: 63%-87%]; P = 0.0026; Figure 4C) and IDH1/2 (2 year survival: mutant 20% [95% CI: 8%-58%] vs. wild type 66% [95% CI: 50%-77%]; P = 0.0113; Figure 4D) were associated with worse prognosis. Consistent with previous reports11, 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 out 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 out of 53 (28%) leukemia samples (Figure 3, Supplementary Table 1).

Prognosis analysis of these immunophenotypic markers revealed that expression of CD13 was associated with poor survival (5 year survival: CD13 positive 34% [95% CI: 18%-50%] vs. CD13 negative 69% [95% CI: 44%-85%], P = 0.0023, Figure 5A), as previously described14. In addition, expression of CD8 (5 year survival: CD8 negative 33% [95% CI: 17%-51%] vs. CD8 positive 63% [95% CI: 41%-79%], P = 0.0035, Figure 5B) and CD62L (5 year survival: CD62L negative 37% [95% CI: 17%-57%] vs. CD62L positive 54% [95% CI: 35%-70%], P = 0.0337, 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 (Supplementary 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 two groups. This analysis revealed that the poor prognosis CD13 expression was restricted to the otherwise favorable clinical group of mature/cortical leukemias (5 year survival: CD13 positive 36% [95% CI: 11%-63%] vs. CD13 negative 85% [95% CI: 51%-96%], P = 0.0031, Figure 6A) and was not observed in T-ALLs with an early immature gene expression signature (5 year survival: CD13 positive 33% [95% CI: 15%-53%] vs. CD13 negative 38% [95% CI: 6%-72%], P = 0.77, 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% CI: 15%-67%] vs. bi-allelic CDKN2A/CDKN2B deletion 83% [95% CI: 48%-96%]; P = 0.0104; 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% CI: 9%-67%] vs. wild type 62% [95% CI: 40%-78%]; P = 0.0407; Figure 6D). This is in contrast with IDH1/IDH2 mutations, which are also restricted to early immature adult T-ALLs, but only showed a trend towards worse clinical outcome (2 year survival: mutant 20% [95% CI: 8%-58%] vs. wild type 56% [95% CI: 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. These leukemias are transcriptionally related to early T-cell progenitors, a subtype of thymocytes that retain multi-lineage 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, the ALL-2000 protocol of the Associazione Italiana Ematologia Oncologia Pediatrica (AIEOP) and the L99-15 study of the Tokyo Children's Cancer Study Group. 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. Of note, the German Multicenter Study Group for Adult ALL (GMALL) 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 (alloSCT).

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. More recently, the prognostic relevance of ABD was confirmed in pediatric T-ALL patients treated according to the Taiwan Pediatric Oncology Group (TPOG) protocols and pediatric T-cell lymphoblastic lymphoma patients treated following a Berlin-Frankfurt-Munich (BFM)-ALL-type strategy. In our patient series, ABD was identified in about half of adult T-ALL samples and found to confer poor overall survival. In addition, 22 out 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 RB1 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 20-30% 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 CDKN2A/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 ALL-BFM-95 study protocol41.

Activation of NOTCH signaling through NOTCH1 and/or FBXW7 mutations has been associated with a favorable prognosis in several, but not all, pediatric study protocols42-45. Similarly, controversial results on the prognostic effect of NOTCH activation have been obtained in adult T-ALL46-49. 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 BFM-treated children with T-ALL50. In our study, both univariate and multivariate analyses revealed that patients whose leukemias showed mutations in NOTCH1 and/or FBXW7 had favorable outcome.

Recently, we documented a high prevalence of epigenetic and signaling mutations targeting FLT3, NRAS, DNMT3A, IDH1 and IDH2, typically found in myeloid leukemias, in adult early immature T-ALLs11. Notably, activating internal tandem duplication mutations in the FLT3 tyrosine kinase gene correlate with poor overall survival and an increased risk of relapse in AML51. Similarly, mutations in DNMT3A occur in about 20% of AML patients and seem to confer poor overall survival in this disease18,52. In contrast, mutations in IDH1 and IDH2 are associated with a favorable clinical outcome in AML, provided they co-occur together with NPM1 mutations53. 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 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 protocols54.

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.

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Authorship

P.V.V. performed experiments and wrote the manuscript. A.A. 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.R., M.T. and J.M.R provided samples and correlative clinical and immunophenotypic data from ECOG. A.F. designed the studies, directed research and wrote the manuscript.

The authors have no conflicts of interest to declare.
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Figure Legends

Figure 1. Prognostic value of immature adult T-ALL.

(A) Top 50 differentially expressed genes between early immature and cortical/mature adult T-ALLs. Genes in the heat map are shown in rows and each individual sample is shown in one column. The scale bar shows color coded differential expression from the mean in standard deviation units with red indicating higher levels and blue lower levels of expression. (B) GSEA analysis of genes associated with pediatric ETP leukemias in early immature vs. cortical/mature adult T-ALLs. (C) Kaplan-Meier survival curves in adult T-ALL patients treated in Eastern Cooperative Oncology Group clinical trial ECOG2993 with early immature vs. cortical/mature gene expression signatures.

Figure 2. Prognostic value of copy number defects in adult T-ALL.

(A) Human chromosomal ideograms showing the areas of genetic gain and loss identified by aCGH in adult T-ALL. Red bars represent areas of gain. Light blue bars represent areas of heterozygous copy number loss and dark blue bars indicate homozygous deletions. (B-D) Kaplan-Meier survival curves in adult T-ALL patients with or without the absence of biallelic deletion of the TCRG locus (ABD) (B); with or without homozygous CDKN2A/CDKN2B deletion (C) and with or without heterozygous TP53 (17q) deletion (D), treated in the ECOG2993 clinical trial.

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 grey filled circles. T-ALL oncogenic subtypes are based on aCGH alterations and microarray expression of T-ALL transcription factor oncogenes.
Figure 4. Prognostic value of somatic gene mutations in adult T-ALL.

(A) Kaplan-Meier survival curve in adult T-ALLs treated in the ECOG2993 clinical trial with or without NOTCH1/FBXW7 mutations. (B) Kaplan-Meier survival curve in adult T-ALLs treated in the ECOG2993 clinical trial with or without BCL11B mutations/deletions. (C) Kaplan-Meier survival curve in adult T-ALLs treated in the ECOG2993 clinical trial with or without DNMT3A mutations. (D) Kaplan-Meier survival curves in adult T-ALLs treated in the ECOG2993 clinical trial with or without IDH1/IDH2 mutations, treated in the ECOG2993 clinical trial.

Figure 5. Prognostic value of cell surface markers in adult T-ALL.

(A) Kaplan-Meier survival curves in adult T-ALLs treated in the ECOG2993 clinical trial according to CD13 expression. (B) Kaplan-Meier survival curves in adult T-ALLs treated in the ECOG2993 clinical trial according to CD8 expression. (C) Kaplan-Meier survival curves in adult T-ALLs treated in the ECOG2993 clinical trial according to CD62L antigen expression.

Figure 6. Risk stratification in early immature and cortical/mature adult T-ALL.

(A) Kaplan-Meier survival curve of early immature adult T-ALLs treated in the ECOG2993 clinical trial according to CD13 expression. (B) Kaplan-Meier survival curve of cortical/mature adult T-ALLs treated in the ECOG2993 clinical trial according to CD13 expression. (C) Kaplan-Meier survival curve of cortical/mature adult T-ALLs treated in the ECOG2993 clinical trial according to the presence or absence of homozygous CDKN2A/CDKN2B deletion. (D) Kaplan-Meier survival curve of early immature adult T-ALLs treated in the ECOG2993 clinical trial according to the presence or absence of DNMT3A mutations. (E) Kaplan-Meier survival curve of early immature adult T-ALLs treated in the ECOG2993 clinical trial according to the presence or absence of IDH1/IDH2 mutations.
Figure 1

A

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<th>Early Immature T-ALL</th>
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B

**Enrichment Score** vs **t-score**

- Early Immature T-ALL
- Cortical/mature T-ALL

C

**Overall survival (%)**

- early immature: p = 0.0112
- cortical / mature
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Figure 5

A: Overall survival (%) for CD13 positive and CD13 negative. The p-value is 0.0023.

B: Overall survival (%) for CD8 positive and CD8 negative. The p-value is 0.0035.

C: Overall survival (%) for CD62L positive and CD62L negative. The p-value is 0.0337.
Figure 6

A) Overall survival for Cortical / mature adult T-ALL, comparing CD13 positive and CD13 negative groups. The log-rank test yields a p-value of 0.0031.

B) Overall survival for Early immature adult T-ALL, comparing CD13 positive and CD13 negative groups. The log-rank test yields a p-value of 0.77.

C) Overall survival for Cortical / mature adult T-ALL, comparing No homo del CDKN2A/2B and Homo del CDKN2A/2B groups. The log-rank test yields a p-value of 0.0104.

D) Overall survival for Early immature adult T-ALL, comparing DNMT3A wildtype and DNMT3A mutation groups. The log-rank test yields a p-value of 0.0407.

E) Overall survival for Early immature adult T-ALL, comparing IDH1/IDH2 wildtype and IDH1/IDH2 mutation groups. The log-rank test yields a p-value of 0.12.
Prognostic relevance of integrated genetic profiling in adult T-cell acute lymphoblastic leukemia

Pieter Van Vlierberghe, Alberto Ambesi-Impiombato, Kim De Keersmaecker, Michael Hadler, Elisabeth Paietta, Martin S. Tallman, Jacob M. Rowe, Carles Forne, Montserrat Rue and Adolfo A. Ferrando