Genomics improves risk stratification of adults with T-cell acute lymphoblastic leukemia enrolled in measurable residual disease-oriented trials

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Abstract

Genetic information has been crucial to understand the pathogenesis of T-cell acute lymphoblastic leukemia (T-ALL) at diagnosis and at relapse, but still nowadays has a limited value in a clinical context. Few genetic markers are associated with the outcome of T-ALL patients, independently of measurable residual disease (MRD) status after therapy. In addition, the prognostic relevance of genetic features may be modulated by the specific treatment used. We analyzed the genetic profile of 145 T-ALL patients by targeted deep sequencing. Genomic information was integrated with the clinical-biological and survival data of a subset of 116 adult patients enrolled in two consecutive MRD-oriented trials of the

Spanish PETHEMA (Programa Español de Tratamientos en Hematología) group. Genetic analysis revealed a mutational profile defined by *DNMT3A/ N/KRAS/ MSH2/ U2AF1* gene mutations that identified refractory/resistant patients. Mutations in the *DMNT3A* gene were also found in the non-leukemic cell fraction of patients with T-ALL, revealing a possible mutational-driven clonal hematopoiesis event to prime T-ALL in elderly. The prognostic impact of this adverse genetic profile was independent of MRD status on day +35 of induction therapy. The combined worse-outcome genetic signature and MRD on day +35 allowed risk stratification of T-ALL into standard or high-risk groups with significantly different 5-year overall survival (OS) of 52% (95% confidence interval: 37-67) and 17% (95% confidence interval: 1-33), respectively. These results confirm the relevance of the tumor genetic profile in predicting patient outcome in adult T-ALL and highlight the need for novel gene-targeted chemotherapeutic schedules to improve the OS of poor-prognosis T-ALL patients.

Introduction

Acute lymphoblastic leukemia (ALL) is an infrequent aggressive cancer being more common in children.[1] ALL includes B- and T-cell lineage subtypes. T-cell acute lymphoblastic leukemia (T-ALL) accounts for 10–15% of pediatric and 20–25% of adult cases.[2,3] B-cell precursor ALL and T-ALL share some genetic features[4] and are considered together for treatment in many trials. The classical prognostic factors such as age, white blood cell (WBC) count, measurable residual disease (MRD) and some genetic features have been used to predict outcome and stratify the therapy in both types of ALL.[2,5-7] Although treatment response via MRD monitoring represents a milestone in virtually all ALL trials, MRD is not available at diagnosis and might not finely predict relapse in adult ALL patients,[6-8] emphasizing the need for additional prognostic markers that may be readily available for these patients at diagnosis. Studies by the cooperative Group for Research in Adult Acute Lymphoblastic Leukemia (GRAALL) demonstrated the prognostic relevance of some genetic markers in adult T-ALL.[7,9] The combination of mutations in the *NOTCH1/FBXW7* signaling pathway, in the absence of *N/KRAS* gene mutations and alterations in *PTEN*, together with adequate MRD clearance, identified a group of adult T-ALL patients with a good prognosis, who might not benefit from further intensification treatment by allogeneic stem cell transplantation (allo-SCT).[7] MRD and the oncogenetic pattern were independent prognostic factors used for patient stratification in the current protocols of the GRAALL Group.

It is well established that genetics plays a key role in the development and progression of T-ALL. The disease is sustained by genetic abnormalities that often determine the stage of maturation arrest (e.g., *MLL-r, CALM::AFA10, HOXA-r, TLX1-r, TLX3-r, SIL::TAL*), and/or the proliferation and/or survival rate of leukemic cells (e.g., *CDKN2A/B, K/NRAS, NOTCH1/FBXW7, NUP214::ABL1, JAK/STAT*).[10,11] However, the prognostic relevance of a genetic profile identified in a group of T-ALL patients may be influenced by the treatment protocol used, since several gene mutations generate resistance to treatment.[12–17] Therefore, assessment of the genetic profiles associated with patient outcome within specific clinical trials is essential for improved risk stratification and to move towards more personalized medicine. In the study reported here, we screened DNA from 145 T-ALL patients, based on a custom-built T-ALL-oriented next-generation sequencing panel (NGSp). Of these, 116 were adult patients treated as part of two consecutive high-risk MRD-oriented trials by the Spanish PETHEMA (Programa Español para el Tratamiento de Hemopatías Malignas) group. Our goal was to identify gene-mutational profiles (point mutations, small insertions and deletions and indels) at diagnosis that could help to predict response to therapy and outcome.

Methods

Targeted deep sequencing

DNA samples or cryopreserved leukemic cells from T-ALL patients (n=145) were collected from different national biobanks (see the *Online Supplementary Appendix* for information). The mutational profile was obtained employing a custom gene panel (SureSelectXT HS Target Enrichment System for Illumina Multiplexed Sequencing Platforms, Agilent Technologies, Santa Clara, CA, USA) and sequencing in a MiSeq instrument (Illumina, San Diego, CA, USA). Mutations were retrieved applying a home-made standard gold pipeline analysis (see detailed methods section in the *Online Supplementary Appendix*). Final selected variants were classified as pathogenic, benign or of uncertain significance if the majority (≥6/10), final version of the *in silico* predictors identified the variant as being in one of the categories. Information about predictors was extracted from dbNSFP[18] via ANNOVAR annotation. Benign variants were excluded from further analyses. Information of genes and regions included in the panel has been previously described[19] and it is shown in an Excel file in the *Online Supplementary Appendix*.

Patients and treatment protocols

For clinical and outcome correlations, 29 patients from our initial cohort were excluded (see the *Online Supplementary*

Appendix). Thus, a representative cohort of 116 adult T-ALL patients were studied. The diagnosis of T-ALL was made according to the World Health Organization criteria[20] and the cytogenetic classification was based on the Genesca *et al.*[19] study, instead of the classical cut-off of five genetic alteration[21] (see the *Online Supplementary Appendix*). Patients were treated with two consecutive MRD-oriented high-risk adult ALL protocols. Detailed information of both trials is shown in the *Online Supplementary Appendix* and the *Online Supplementary Figure S1*. In order to homogenize patient allocation according MRD levels at the end of induction treatment, we established a MRD cut-off of 0.1 to define a patient as good responder (≤ 0.1%) in the ALL-AR [Ph-]-03 trial (*clinicaltrials gov. Identifier: NCT00853008*), similarly as it was established for the ALL-HR [Ph-]-11 trial (*clinicaltrials gov. Identifier: NCT01540812*). Informed consent was obtained from all patients. Samples and clinical data were stored in accordance with the declaration of Helsinki. The study was approved by the Institutional Review Board of the Hospital Germans Trias i Pujol.

Statistical analyses

Genes mutated in at least five patients were included in the initial screening for impact on OS and cumulative incidence of relapse (CIR), as individual genes, except for *N/KRAs* and *JAK1/JAK3* mutations that were assessed together since are isoforms, respectively. OS curves were plotted using the Kaplan–Meier method and compared by the log-rank test. CIR was estimated by competing risks analysis using cumulative incidence functions. Gray's test was used to compare CIR curves. A Cox proportional hazard regression model was used to identify predictive factors for OS. Statistical significance was concluded for two-sided values of $P<0.05$. All statistical analyses were performed using SPSS version 24 (IBM Corp. Armonk, NY, USA), GraphPad Prism® version 8 (GraphPad Software Inc., La Jolla, CA, USA) and R version 4.1.0.

Combinations of mutations (affecting ≥12 patients) and biological traits at the time of diagnosis were pairwise assessed using Fisher's exact test. Multiple associations were corrected using a Benjamini-Hochberg q test, with significance concluded for co-existence for values of $q<0.05$.

Results

Somatic mutational landscape of T-cell acute lymphoblastic leukemia

The presence of point mutations and insertions and deletions (indels) was investigated in 145 T-ALL patients by TDS using a customized NGSp. Overall, genetic variants were detected in 136 of 145 patients (94%) for a median of four genetic variants and three mutated genes per patient. No variants were observed in nine (6%) patients (Figure 1A). Of patients with variants, 92% (125/136) carried missense mutations and 32% (44/136) had nonsense mutations. Genes with predominate missense variants were *JAK3* (96.3%), *FBXW7* (92.3%), *FAT1* (100%), *JAK1* (100%) and *NRAS* (100%). In turn, insertions, deletions, and indels of up to 50 nucleotides, were identified in 67% (91/136) of the cases. Of these, 81% (74/91) presented short insertions (52 frameshift and 38 non-frameshift); 42% (38/91) had short deletions (28 frameshift and 11 non-frameshift); and 12% (11/91) had frameshift indels (small insertions or deletions). *PTEN* (71·4%) and *IL7R* (80%) were the two genes with the highest percentage of indels. Globally, combinations of point mutations and indels were observed in 60% (81/136) of patients, whereas isolated indels mutations were detected in 4% (6/136) of cases. According to the functional impact assigned to each variant, 89% (121/136) of patients presented pathogenic variants and 83% (113/136) had variants of uncertain significance, and only 15 of them exclusively presented variants of uncertain significance. Almost all pathogenic variants (98%) were missense mutations, whereas most variants of uncertain significance were nonsense variants (16%) or indels (53%) (*Online Supplementary Figure S2*).

Recurrently mutated genes found in at least five patients are listed in Figure 1B and all had been previously reported in T-ALL. Mutations were found in: i) transcription factor tumor suppressor genes (*PTEN, BCL11B, RUNX1, GATA3, ETV6*)[23–27]; ii) epigenetic regulators (*PHF6, DNMT3A, EP300, KMT2C*)[27–29]; iii) DNA mismatch repair genes (*MSH2*)[30]; iv) genes expressing ribosomal protein (*RPL5*)[31]; v) genes involved in RNA splicing (*U2AF1*)[32]; and vi) signaling pathways that regulate T-cell development, such as the RAS/MAPK signaling (*NRAS*)[33] WNT (*FAT1, FAT3*),[34,35] IL7R-JAK-STAT (*JAK3, JAK1, IL7R, DNM2*)[36-39] and the *NOTCH1/FBXW7* signalling.[40,41] The latter was found in 71% (97/136) of patients (*NOTCH1* and *FBXW7*), confirming the importance of this signaling pathway in the development of T-ALL.

Genetic-biological associations

Major biological features at diagnosis were available for 143/145 patients and their data were used to classify them by: i) age group: <18, 18-35, 36-60 and >60 years; ii) immunophenotype: ETP-ALL, pre-T, cortical, mature T-ALL and not determined immunophenotype (NDI); and iii) cytogenetic groups: 0-2 abnormalities, a complex karyotype with at least three abnormalities (CK ≥3) and non-evaluable (NE) cases.[19] Previously reported correlations between genetic variants occurring in genes mutated in ≥12 patients and diagnostic traits included a direct association between the presence of variants in *DNMT3A* gene and both ETP-ALL (OR=8.56; q= 0.02)[42,43] and advanced age.[43] In our analysis, however, *DNMT3A* mutations were restricted to the oldest patients (>60 years [y]) (OR=9.6; q= 0.08) and

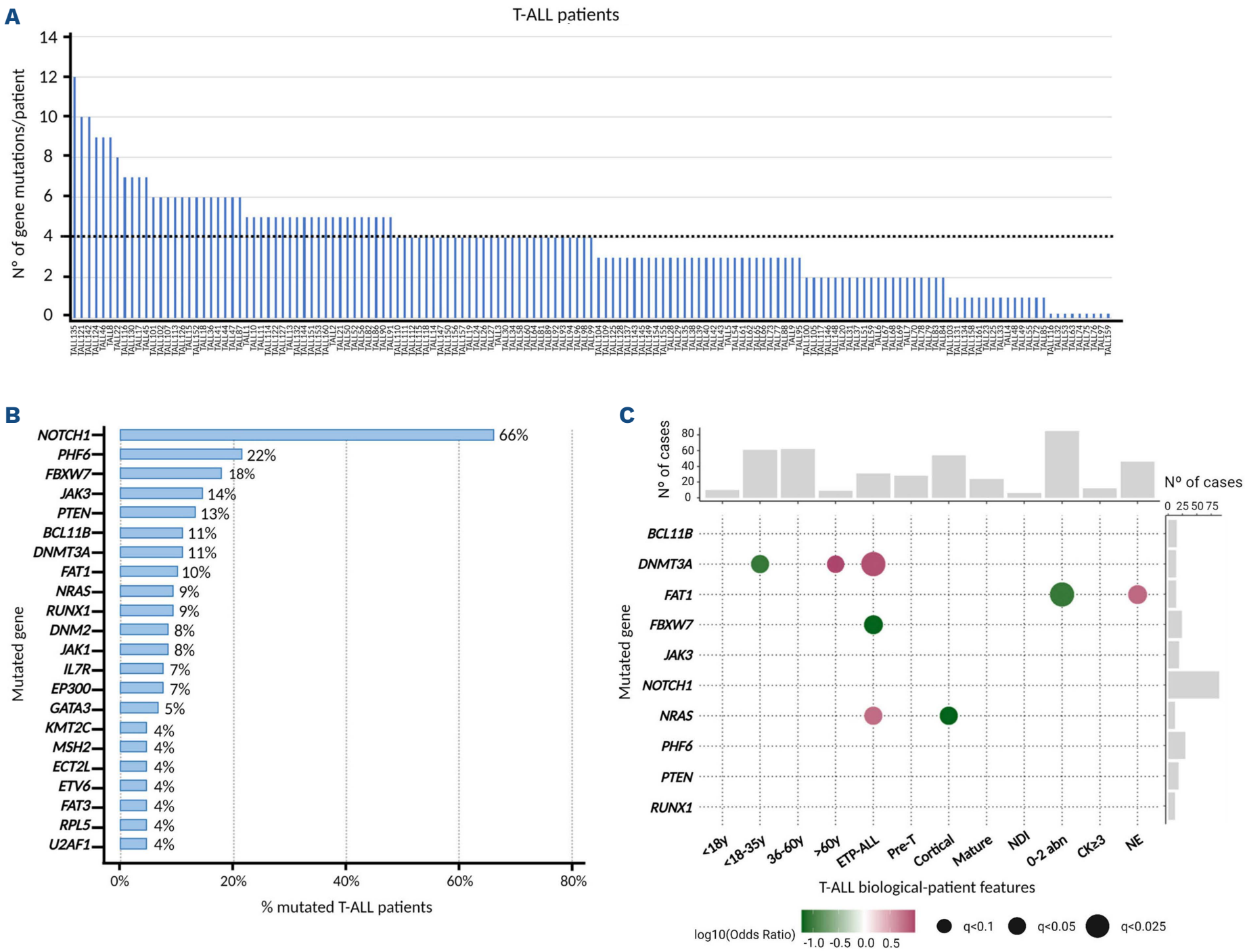


Figure 1. Genetic profile of T-cell acute lymphoblastic patients at diagnosis. (A) Frequency of variants per patient in the cohort (n=145). (B) Frequency of patients showing recurrently mutated genes (cut-off of ≥5 mutations/gene). (C) Pairwise associations observed between the recurrently mutated genes and the biological characteristics of the disease at presentation. Positive and negative correlations are depicted as magenta and green circles, respectively. Circle diameters indicate the degree of significance. Y: years; ETP-ALL: early T-cell precursor acute lymphoblastic leukemia; NDI: not determined immunophenotype; abn: abnormalities; CK: complex karyotype; NE: non-evaluable karyotype.

were not associated to adolescents and young adults (AYA, 18-35 y) (OR=0.09; q=0.07). Other new significant associations included a lower incidence of *FBXW7* variants in ETP-ALL (OR=0; q=0.07), higher frequency of *FAT1* gene variants in patients with an NE karyotype (OR=6.36; q=0.07) at the expense of a negative association between this gene and patients with 0-2 abnormalities (OR=0.09; q=0.02), and association of *NRAS* mutations with and ETP-ALL immunophenotype (OR=6.13; q=0.07) that contrasts with the negative association of these mutations with the cortical subtype (OR=0; q=0.07) (Figure 1C).

Prognostic significance of gene mutations and mutational profiles

The analysis of the clinical impact of the genetic variants and mutational profiles identified by TDS on patient outcome was restricted to 116 patients homogeneously treated in two high-risk consecutive MRD-oriented PETHEMA trials whose clinical, biological and outcome data were complete. Comparison of the outcome of the sequenced (n=116) *versus* non-sequenced patients (n=116) did not reach significant differences in terms of OS. Thus, our cohort was representative of the patients included in both trials. Like other adult T-ALL cohorts, this was mainly composed of males of median age (37 y). Overall, CR rate (Induction-1 + Induction-2) for the whole series was 88%, being 83% for cases who achieved MRD levels <0.1% at the end of Induction-1. Most patients (72%) were treated according to the chemotherapy schedules proposed in the trials. The remaining patients were assigned to allo-SCT.

Table 1. Prognostic impact of genes found to be recurrently mutated in the PETHEMA T-cell acute lymphoblastic leukemia

Gene	Number of patients (N=116)		OS 5 years, % (95% CI)			CIR 5 years, % (95% CI)	
	Patients with mutations	Patients without mutations	Patients with mutations	Patients without mutations	*P*	Patients with mutations	Patients without mutations
FBXW7	20	96	62 (36-88)	32 (21-43)	0.032	-	-
DNMT3A	10	106	13 (0-37)	38 (27-49)	0.001	-	-
N/KRAS	13	103	21 (0-45)	39 (28-50)	0.023	85 (37-97)	50 (39-61)
MSH2	5	111	20 (0-55)	37 (26-48)	0.036	-	-
U2AF1[*]	5	111	20 (0-55)	50 (40-60)	0.003	-	-

[*]results expressed as 2-year overall survival (OS) probability. CI: confidence interval; CIR: cumulative incidence of relapse.

The 5-year CIR and OS probabilities for the whole series was 54% (95% confidence interval [CI]: 43%-64%) and 36% (95% CI: 26-46), respectively.

In order to identify mutations in genes affecting patient outcome, we first assessed the impact on OS and CIR of each individual gene mutated in at least five patients. Strikingly, we could not confirm the previously reported association between *NOTCH1* mutations and prolonged OS (*Online Supplementary Figure S3A*), or the lower CIR rate (data not shown).[9,44] A more in-depth analysis of the distinct *NOTCH1* gene variants identified with respect to their functional impact (pathogenic *vs*. uncertain significance) (*Online Supplementary Figure S3B*) and according to the variant allele frequency (VAF) with a cut-off of 25% to define a variant as clonal (*Online Supplementary Figure S3C*), confirmed the lack of prognostic impact of *NOTCH1* variants on OS in our T-ALL patients. In contrast, patients carrying *FBXW7* mutations, were associated with a better OS (Table 1). Sixty-five percent of patients carrying mutations in *FBXW7* (13/20) also presented a mutation in the *NOTCH1* gene, with no differences in OS between both groups (*FBXW7* only *vs*. *FBXW7* and *NOTCH1*) (data not shown), suggesting that the good outcome observed in patients carrying *FBXW7* mutations could be attributed to a specific dysfunction of the FBXW7 protein (*Online Supplementary Figure S3D*). In addition, patients showing *DNMT3A*, *N/KRAS*, *MSH2* and/or *U2AF1* mutations had a lower OS than patients with no mutations in these genes (Table 1). It is of note that only patients with mutations in *N/KRAS* genes showed a high probability of CIR (Table 1).

Based on these results, we grouped mutations in genes associated with a worse outcome according to the same homeostatic processes affected and basal biologic characteristics. Thus, patients with mutations in *DNMT3A* and *U2AF1* were older (median age 54 y, *P*<0.001) and more frequently showed an ETP-ALL immunophenotype (*P*<0.001). Mutations in these two genes were consistent with clonal hematopoiesis of indeterminate potential (CHIP).[45–47] We grouped mutations in these two genes into the aging-genes cluster. In turn, *N/KRAS*, or *MSH2* gene mutations have previously been described as being involved in generating resistance to ALL treatment.[13,14,16] We named this group of gene mutations as treatment-resistance-gene cluster. Both clusters identified patients with a similarly poor response to treatment (*Online Supplementary Table S1*). Overall, 25% (29/116) of patients harbored mutations in genes conferring a worse outcome with more frequent slow response to initial treatment, high toxicity and early death (Figure 2). Of note, OS of patients with co-occurrence of more than one worse prognosis mutation was similar to that of patients carrying on only one worse outcome mutation (*P*=0.750). Therefore, we subsequently assessed the global prognostic impact of all adverse gene mutations collectively as a variable named worse-outcome genetics (WOG) by univariable and multivariable analyses. Similarly, patients carrying mutations in *FBXW7* were also included as a variable named good-outcome genetics (GOG). Basal characteristics at the time of diagnosis and treatment response of patients belonging to the WOG^- and WOG^+ groups are shown in Table 2 (*Online Supplementary Table S2* for patients GOG^+ and GOG^-). Importantly, only one patient presented mutations in *FBXW7* (GOG) and U2AF1 (WOG), whose death was associated to transplant-related mortality. This patient was included in the WOG group, to maintain both variables independent. Age, WBC count, central nervous system (CNS) infiltration, ETP-ALL phenotype, CK ≥3 alterations, MRD level at the 0.1% cut-off, WOG and GOG were included in the univariable analysis (Table 3). The two treatment protocols were also included in the analysis to exclude any possible protocol-dependent bias. By multivariable analysis, MRD level ≥0.1% after induction-1 (day +35) and the WOG cluster were independently associated with shorter OS rates with hazard ratios (HR) of 2.187 (range, 1.087-4.400) and 3.040 (range,

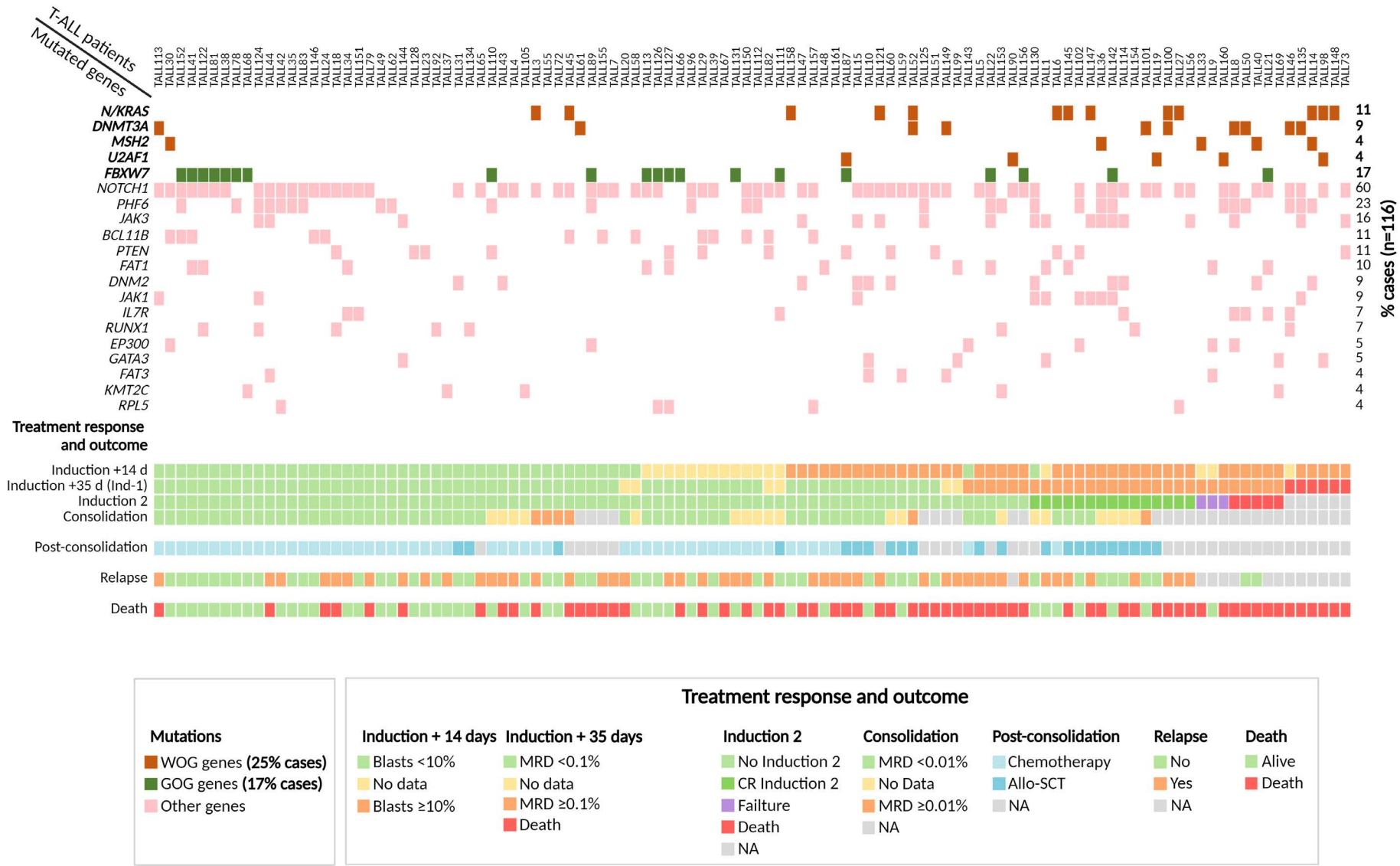


Figure 2. Scheme of the genetic profile of each T-cell acute lymphoblastic patient, its response and evolution during treatment. Only genes recurrently mutated in ≥5 patients are shown. Each mutation is indicated by a square: brown squares correspond to genes contained in the worse-outcome genetics (WOG) signature; dark green squares correspond to mutations in the *FBXW7* gene (good-outcome genetics [GOG] signature) and pink squares correspond to other mutated genes. Treatment response and patient evolution data are shown at the bottom. Induction + 14d indicates the percentage of blast cells in bone marrow 14 days after starting induction therapy; induction + 35d corresponds to measurable residual disease (MRD) values at the end of the first induction blocks (induction 1); induction-2 indicates patients that received or not an Induction-2 treatment block. Consolidation corresponds to MRD values at the end of consolidation chemo-block. Post-consolidation indicates treatment choice (allogeneic stem cell transplantation or chemotherapy) based on MRD values at the end of the consolidation treatment. On the right, the percentage of cases mutated in the different genes are indicated. NA: not available.

1.531-6.035) respectively (Table 3). The multivariable analysis for CIR, did not validate the predictive role of *N/KRAS* mutations observed in the univariable analysis (*Online Supplementary Table S3*). In fact, we could not identify any variable to predict CIR risk in our adult T-ALL cohort.

Based on these results, we re-stratified our patients according to their MRD level on day +35 after induction-1 and the presence or absence of WOG mutations. Two groups of patients were identified: standard-risk patients with MRD^{low} plus WOG^{-} (5-y OS of 52 %, 95% CI: 37-67, n=65); and high-risk patients that included MRD^{high} and WOG^{-} (n=11), MRD^{low} plus WOG^{+}(n=9) and MRD^{high} plus WOG^{+} (n=4) patients respectively, with a 5-y OS of 17% (95% CI: 1-33) (Figure 3).

Tracking aging cluster mutations in non-leukemic cells

In order to determine whether the *DNMT3A* and *U2AF1* genetic variants associated with the aging-genes cluster identified in our adult T-ALL patient cohort were related to clonal hematopoiesis of indeterminate potential (CHIP), we investigated those variants in the non-leukemic fraction of peripheral blood cells of the same patients. For this purpose, genomic DNA from purified polymorphonuclear cells of nine patients carrying mutations in these two genes was sequenced. However, no genetic variants of the *U2AF1* gene were identified in the polymorphonuclear cell fraction of the two patients with mutations in this gene in their leukemic cells. By contrast, identical *DNMT3A* mutations were found at lower or similar (VAF) in the polymorphonuclear fraction of seven of seven cases whose leukemic cells had mutations in this gene (Table 4), suggesting that these mutations were already present in a common lympho-myeloid early stem cell precursor and appear before the leukemia arises. Moreover, in two patients, copy number alterations and mutations in the *DNMT3* gene were apparent in the blast cells, highlighting the importance of this gene in the development of their leukemia. Further analysis of non-

hematopoietic cells would be needed to rule out a germinal basis for these alterations, although the *in silico* predictors indicated that those variants had an actual or potential impact on the function of the DNMT3A protein, which argues in favor of them being of non-germinal origin (Table 4).

Discussion

This study describes a gene mutational signature, named WOG, that identifies high-risk T-ALL patients with a slow response to initial chemotherapy treatment and low CR rates. These patients more often needed two induction cycles to achieve a good MRD response which could also account for the higher rate of early deaths, due to toxicity, observed at the end of induction treatment, and thereby their shorter OS. However, our WOG signature did not have an impact on the CIR, since did not identify patients at high *versus* low risk of relapse. This may be due to the very poor clinical outcome of the patients with a WOG mutational profile, yet observed at early stages of the treatment, that would eliminate the possibility of a higher rate of sub-

Table 2. Clinical-biological characteristics and response to treatment of T-cell acute lymphoblastic leukemia patients grouped according to their gene mutational profile.

	Genetic group		*P*	Total (N=116)
	WOG$^-$ (N=87)	WOG$^+$ (N=29)		
Patient-related features				
Median age, years (range)	34 (16-61)	44 (19-60)	0.068	37 (16-61)
Sex, M/F	71/16	15/14	0.001	86/30
Disease-related features				
Median WBC, x10^9/L (range)	66.3 (0.5-525.4)	23.4 (0.6-495)		
ECOG, N (%)				
0	31/83 (37)	12/29 (41)		43/112 (38)
1	42/83 (51)	11/29 (38)	0.546	53/112 (47)
2	9/83 (11)	5/29(17)		14/112 (13)
≥3	1/83 (1)	1/29 (4)		2/112 (2)
Adenopathies, N (%)	37/72 (51)	18/26 (69)	0.116	55/98 (56)
Splenomegaly, N (%)	30/83 (36)	9/28 (32)	0.701	39/111 (35)
Hepatomegaly, N (%)	20/82 (24)	5/28 (18)	0.476	25/110 (23)
Mediastinal mass, N (%)	41/84 (49)	8/29 (28)	0.047	49/113 (43)
CNS involvement, N (%)	10/83 (12)	4/27 (15)	0.743	14/110 (13)
Immunophenotype, N (%)				
ETP-ALL	8/81 (10)	14/29 (48)		22/110 (19)
Pre-T	15/81 (18)	4/29 (14)	<0.001	19/110 (16)
Cortical	42/81 (52)	5/29 (17)		47/110 (41)
Mature	16/81 (20)	6/29 (21)		22/110 (19
Cytogenetics, N (%)				
0-2 abn	49/87 (56)	17/29 (59)	0.926	66 (57)
CK ≥3	8/87 (9)	2/29 (7)		10 (9)
NE	30/87 (35)	10/29 (34)		40 (34)
Response-related features				
Slow response at day +14, N (%)	26/72 (36)	22/27 (82)	<0.001	48/99 (48)
Induction cycles to CR, N (%)				
1	77/87 (89)	16/29 (55)	<0.001	93 (80)
2	10/87 (11)	13/29 (45)		23 (20)
CR post Ind-1, N (%)	81/87 (93)	14/29 (48)	<0.001	95 (82)
CR (Ind-1 + Ind-2), N (%)	82/87 (94)	20/29 (69)	<0.001	102 (88)
MRD <0.1% at day +35, N (%)	65/76 (86)	9/13 (69)	0.221	74/89 (83)
Treatment				
Chemotherapy, N (%)	54/70 (77)	5/12 (42)	0.031	59/82 (72)
Allo-SCT, N (%)	16/70 (23)	7/12 (58)		23/82 (28)

Results expressed as number of cases/total cases (percentage). WOG: worse-outcome genetics; measurable residual disease (MRD) values were considered for those patients that reach complete remission (CR); M: male; F: female; WBC: white blood cells; ECOG: Eastern Cooperative Oncology Group; CNS: central nervous system; ETP-ALL: early T-cell precursor acute lymphoblastic leukemia; abn: abnormalities; CK: complex karyotype; day +14: 14 days after induction treatment; day +35: 35 days after induction treatment; allo-SCT: allogeneic stem cell transplantation. Ind: induction.

sequent relapses. Thus, the WOG mutational signature allows to identify at diagnosis those patients who will be resistant and refractory to conventional frontline treatment. The WOG signature was an independent risk factor for OS, together with the end-induction MRD levels (0.1% cut-off) indicating that the two parameters cooperate to confer a poor outcome to T-ALL patients. Thus, we demonstrated that the WOG signature contributes to the improved risk-stratification of adult T-ALL patients, confirming the relevance of genetic data in this clinical setting. The lack of co-occurrence observed in the genes included in the WOG signature (*Online Supplementary Figure S4*) highlights the importance of the contribution of each individual gene in the outcome significance of the WOG signature, although we could not demonstrate its individual contribution in the multivariable analysis except for patients with *DNMT3A* mutations (data not showed), probably due to the limited number of patients included in each individual gene-group. Here also, the impact of WOG variable was probably underestimated since the MRD threshold employed in the two retrospective trials to discriminate patients with good *versus* worse response was not sensitive enough. With a more sensitive MRD cut-off, the number of patients with MRD^{high} would be higher and therefore the WOG signature could contribute to discriminate an intermediate-risk group (those with MRD^{high}plus WOG^{-} and MRD^{low} plus WOG^{+}) within the high-risk group. In addition, other genes that did not reach statistical significance when analyzed individually for OS impact, due to the limited number of mutated patients for the specific gene assessed (i.e., patients with *JAK3* and *JAK1* mutations), could also impair the selection of a very low-risk group of patients. The assessment of the WOG genetics in the current ALL-PETHEMA trial (ALL19), in which a more sensitive MRD cut-off is employed, will help to corroborate the utility of these genetic marker to stratify adult T-ALL patients within PETHEMA trials.

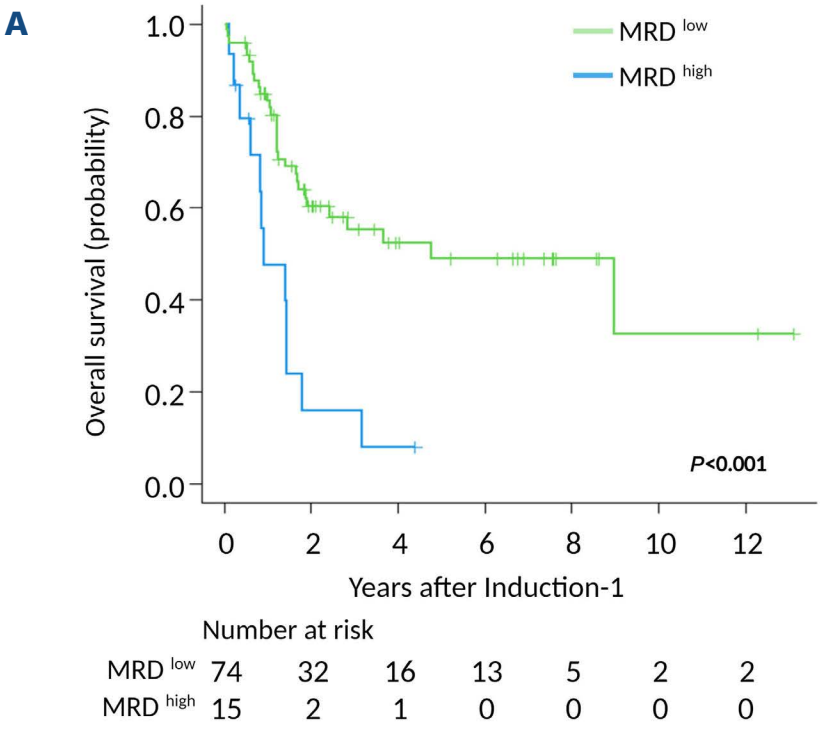
The WOG signature identified here is defined by the mutational status of genes known to be involved in ALL treatment resistance.[13-16] These included activating mutations in the *N/KRAS* genes (n=13). The two G-proteins (N/KRAS) are involved in the RAF/MEK/ERK signaling pathway[48] and have been identified as being responsible for steroid resistance.[12,13] Importantly, these mutations are associated with poor prognosis in both pediatric and adult T-ALL.[9,49] The WOG signature described here also included *MSH2* gene mutations (n=5) that affected the interaction with MSH3 and MSH6 proteins, respectively, and the formation of MSH3-MSH2 and MSH6-MSH2 heterodimers, two components of the post-replicative DNA mismatch repair sys-

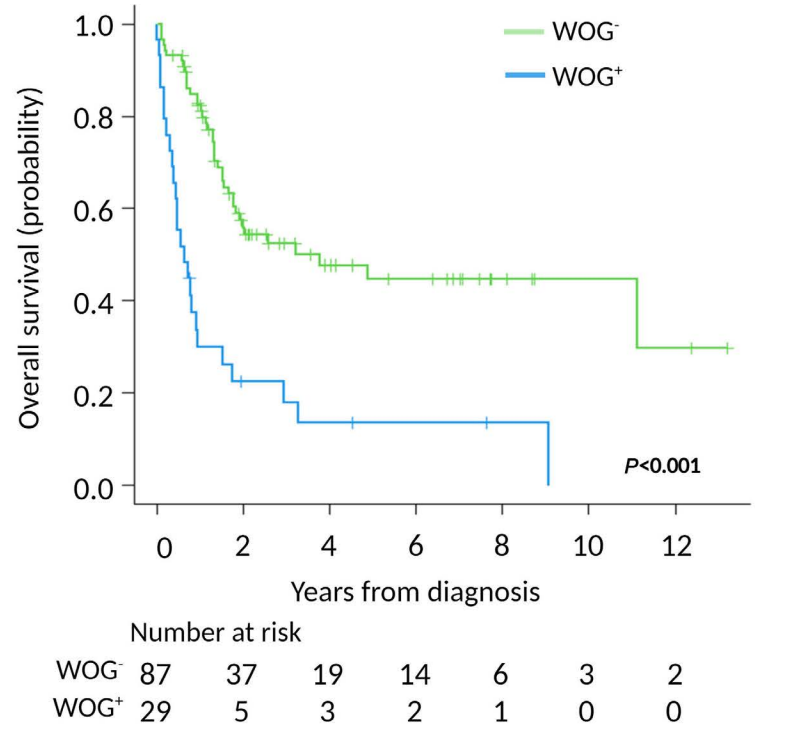
Table 3. Prognostic factors for overall survival identified in the univariable and multivariable analyses in the PETHEMA adult T-cell acute lymphoblastic cohort.

Disease/patient feature	Univariable analyses			Multivariable analyses		
	N	HR (95% CI)	*P*	N	HR (95% CI)	*P*
Age*	95	1.007 (0.984–1.031)	0.540	-	-	-
WBC count*	93	1.001 (0.999–1.003)	0.440	-	-	-
CNS involvement No Yes	 82 8	 Reference 0.598 (0.185–1.937)	0.391	-	-	-
ETP-ALL No ETP-ALL ETP-ALL	 77 11	 Reference 1.687 (0.803–3.544)	0.168	-	-	-
Karyotype 0-2 abn. CK ≥3	 59 6	 Reference 2.623 (0.993–6.928)	0.052	-	-	-
PETHEMA treatment protocol ALL-HR-11 ALL-AR-03	 69 26	 Reference 1.162 (0.628–2.148)	0.633	-	-	-
GOG Non-mutated Mutated	 77 18	 Reference 0.343 (0.120–0.981)	0.046	-	-	-
WOG Non-mutated Mutated	 81 14	 Reference 2.636 (1.363 – 5.095)	0.004	 76 13	 Reference 2.187 (1.087 –4.400)	0.028
MRD at day +35 <0.1% ≥0.1%	 74 15	 Reference 3.339 (1.691 – 6.592)	0.001	 74 15	 Reference 3.040 (1.531 –6.035)	0.001

*Age and white blood cell (WBC) count were considered as continuous variables. N: number of cases; HR: hazard ratio; CI: confidence interval; OS: overall survival; ETP-ALL: early T-cell precursor acute lymphoblastic leukemia; abn: abnormalities; CK: complex karyotype; CNS: central nervous system; GOG: good-outcome genetics; WOG: worse-outcome genetics; MRD: measurable residual disease.

tem (MMR).[50] Under defective MMR function conditions, blasts may lack the capacity to recognize mismatched DNA pairs, leading to the generation of chemoresistance.[14,15] Despite the pathogenic role of mutations in this gene, limited information is available about its clinical relevance, probably due to the low frequency of these mutations in adult T-ALL. Nevertheless, leukemic cells from relapsed pediatric cases are enriched in *MSH2* gene mutations, suggesting that relapse in these patients may be due to a drug resistance mechanism driven by *MSH2* mutations.[17,51] The two other genetic components of WOG are the *DNMT3A* (n=10) and *U2AF1* (n=5) genes. *U2AF1* gene mutations have been previously identified in adult and childhood T-ALL[32,52] and predict poorer prognosis in de *novo* acute myeloid leukemia (AML) patients.[53] However, as far as we know, no data are available concerning the consequences of this mutation in T-ALL. *DMNT3A* mutations have previously been identified in older patients and in immature leukemic subtypes[42,54-56] and like other epigenetic regulators, they are thought to be an early event in leukemogenesis, conferring self-renewal properties on uncommitted hematopoietic progenitors, facilitating the subsequent acquisition of secondary mutations.[57,58] Importantly, most *DNMT3A* mutations observed in the blast cells were also present in the non-leukemic cell fraction of polymorphonucleated cells of the same patients at lower or similar VAF. It is of particular interest that the genetic signature that defines CHIP includes mutations in these two genes at very low VAF.[45,46] These genes have also been described in AML at higher VAF.[59,60] Together, these observations favor an explanation of a *DNMT3A* mutation-driven clonal hematopoiesis event in a common lymphoid-myeloid early progenitor that, together with other alterations, could determine the transformation into AML or T-ALL. In contrast, we did not find *U2AF1* mutations in non-leukemic cells, although we were able to investigate only a few T-ALL cases.





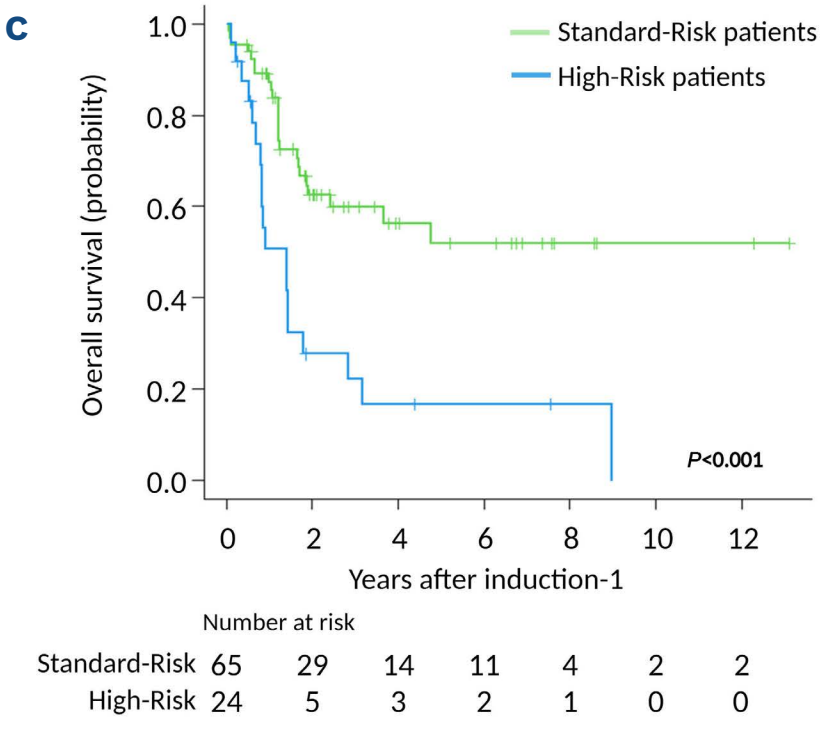


Figure 3. Prognostic stratification of adult T-cell acute lymphoblastic patients according to overall survival defined by the presence of worse-outcome genetic mutations and measurable residual disease status 35 days after starting therapy. (A) Overall survival (OS) according to measurable residual disease (MRD) levels at 4 years (y) showed rates of (95% confidence interval [CI]: 35-63) in patients with MRD^{low} (<0.1%) and 8% (95% CI: 0-23) for those with MRD^{high} (≥0.1%). (B) OS according to worse-outcome genetics (WOG) mutational status at 5 y showed rates of 13% (95% CI: 0-26) in the WOG-mutated patients (WOG^{+}) and 45% (95% CI: 32-58) in non-mutated patients (WOG^{-}). (C) OS according to WOG mutational status and MRD values (d+35) at 5 y was 52% (95% CI: 37-67) for patients with MRD^{low} (<0.1%) and WOG- (standard-risk-patients), compared with 17% (95% CI: 1-33) for high-risk patients including MRD^{high} plus WOG^{-}, MRD^{low} plus WOG^{+} and MRD^{high} plus WOG^{+}.

Table 4. *DNMT3A* genetic variants identified in patients associated with the aging cluster in the non-leukemic and leukemic cell.

Patient ID	Age, years	Immunophenotype	DNMT3A aa change	VAF leukemic cells	VAF non-leukemic cells	CNA status	Functional impact of *DNMT3A* mutations	
							In silico predictors	COSMIC
TALL91	64	ETP-ALL	p.F827fs	28%	34%	2	Uncertain significance	..
			p.R771X	59%	3.6%	3	Uncertain significance	Pathogenic
TALL95	61	ETP-ALL	p.F732V	88%	38%	1	Pathogenic	..
TALL93	78	ETP-ALL	p.R882H	43%	3.5%	..	Pathogenic	Pathogenic
TALL50	60	ETP-ALL	p.R882H	48%	46%	..	Pathogenic	Pathogenic
TALL101	48	Cortical	p.R882C	50%	21%	..	Pathogenic	Pathogenic
TALL46	57	ETP-ALL	p.R882H	51%	40%	..	Pathogenic	Pathogenic
TALL8	51	ETP-ALL	p.D876E	41%	30%	2	Pathogenic	..
			p.I715fs	45%	31%	2	Uncertain significance	..

ID: identifier; aa: amino acid; VAF: variant allelic frequency; CNA: copy number alteration; ETP-ALL: early T-cell precursor acute lymphoblastic leukaemia. RefSeq ID *DNMT3A*: NM_022552.

The incidence of *NOTCH1* and *FBXW7* mutations in our patients was similar to that reported in other adult and pediatric cohorts,[40,44,61-63] but we found no association between mutations in *NOTCH1* gene and patient outcome. In fact, the clinical impact of *NOTCH1/FBXW7* mutations in adult T-ALL is still a matter of debate. Here we have shown that patients with mutations in *FBXW7*, including or not mutations in *NOTCH1*, have a better outcome comparing with patients carrying on only mutations in *NOTCH1* gene, but this association do not reach statistical significance in the multivariable analysis. The GRAALL group showed that mutations in *NOTCH1*, together with *FBXW7*, identified patients with better event-free survival and OS, with an independent predictive value.[44,61] They also showed that the combination of low-risk genetics (defined by the presence of mutations in the *NOTCH1* signaling pathway [*NOTCH1* and *FBXW7*] and wild-type *N/KRAS* or *PTEN*) and MRD negativity, allowed identification of a fraction of adult T-ALL patients with a very good outcome[7]. However, the same cooperative group also showed that the favorable prognostic impact of *NOTCH1/FBXW7* mutations was influenced by the treatment protocol used.[61] Other studies in adult T-ALL cohorts have not been able to confirm this benefit,[62,64] whereas the large differences in the prevalence of *NOTCH1/FBXW7* mutations reported in other series may compromise the clinical impact of these mutations in the studied cohort.[65] Compared with the GRAALL trials,[7,9] patients from the PETHEMA trials[6,22] included in this study are older, and were treated slightly different (i.e., they did not receive cyclophosphamide during the induction or late intensification stages of the treatment).

In conclusion, we describe a WOG mutational signature, which identifies older, refractory/resistant high-risk T-ALL patients with a poorer OS due to the suboptimal response to induction therapy. Patients in this group emerge as candidates for novel, personalized frontline therapeutic schedules.

Disclosures

No conflicts of interest to disclose.

Contributions

CG-G performed the experiments and analyzed the data, produced the figures and contributed to the writing process. MM did the statistical analyses. TL performed library preparations; FF-T analyzed the sequencing data. JG-C developed the initial NGSp design. PM, AT, MD-B, RC, JR, LH, SM, JG-C, LZ, TA, FV-L, MT, CG-C, PB, AN, TB, PLdU, M-PQ, PM-S, AG, TG, AC, JC, RF, MAA, MJV and ÁB provided clinical data. N-LB and AB provided support for the project through the AECC T-ALL consortium. RZ and JM created Figure 1C. AO and JMR contributed to the study conceptualization, data analysis and reviewed the manuscript. EG designed the study, reviewed the data, and wrote and reviewed the manuscript. All authors have read and approved the manuscript.

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Data-sharing statement

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

References

1. Yi M, Zhou L, Li A, Luo S, Wu K. Global burden and trend of acute lymphoblastic leukemia from 1990 to 2017. Aging. 2020;12(22):22869-22891.
2. Hunger SP, Mullighan CG. Acute lymphoblastic leukemia in children. N Engl J Med. 2015;373(16):1541-1552.
3. Teachey DT, Pui C-H. Comparative features and outcomes between paediatric T-cell and B-cell acute lymphoblastic leukaemia. Lancet Oncol. 2019;20(3):e142-e154.
4. Kimura S, Mullighan CG. Molecular markers in ALL: clinical implications. Best Pract Res Clin Haematol. 2020;33(3):101193.
5. Thomas X, Le Q-H. Prognostic factors in adult acute lymphoblastic leukemia. Hematol Amst Neth. 2003;8(4):233-242.
6. Ribera J-M, Morgades M, Ciudad J, et al. Chemotherapy or allogeneic transplantation in high-risk Philadelphia chromosome-negative adult lymphoblastic leukemia. Blood. 2021;137(14):1879-1894.
7. Beldjord K, Chevret S, Asnafi V, et al. Oncogenetics and minimal residual disease are independent outcome predictors in adult patients with acute lymphoblastic leukemia. Blood. 2014;123(24):3739-3749.
8. Lussana F, Intermesoli T, Gianni F, et al. Achieving molecular remission before allogeneic stem cell transplantation in adult patients with Philadelphia chromosome–positive acute lymphoblastic leukemia: impact on relapse and long-term outcome. Biol Blood Marrow Transplant. 2016;22(11):1983-1987.
9. Trinquand A, Tanguy-Schmidt A, Ben Abdelali R, et al. Toward a NOTCH1/FBXW7/RAS/PTEN–based oncogenetic risk classification of adult T-cell acute lymphoblastic leukemia: a group for research in adult acute lymphoblastic leukemia study. J Clin Oncol. 2013;31(34):4333-4342.
10. Belver L, Ferrando A. The genetics and mechanisms of T cell acute lymphoblastic leukaemia. Nat Rev Cancer. 2016;16(8):494-507.
11. Girardi T, Vicente C, Cools J, De Keersmaecker K. The genetics and molecular biology of T-ALL. Blood. 2017;129(9):1113-1123.
12. Li Y, Buijs-Gladdines JGCAM, Canté-Barrett K, et al. IL-7 receptor mutations and steroid resistance in pediatric T cell acute lymphoblastic leukemia: a genome sequencing study. PLoS Med. 2016;13(12):e1002200.
13. Ariës IM, van den Dungen RE, Koudijs MJ, et al. Towards personalized therapy in pediatric acute lymphoblastic leukemia: RAS mutations and prednisolone resistance. Haematologica 2015;100(4):e132–e136.
14. Fordham SE, Matheson EC, Scott K, Irving JAE, Allan JM. DNA mismatch repair status affects cellular response to Ara-C and other anti-leukemic nucleoside analogs. Leukemia. 2011;25(6):1046-1049.
15. Diouf B, Cheng Q, Krynetskaia NF, et al. Somatic deletions of genes regulating MSH2 protein stability cause DNA mismatch repair deficiency and drug resistance in human leukemia cells. Nat Med. 2011;17(10):1298-1303.
16. Fedier A, Schwarz VA, Walt H, Carpini RD, Haller U, Fink D. Resistance to topoisomerase poisons due to loss of DNA mismatch repair. Int J Cancer. 2001;93(4):571-576.
17. Li B, Brady SW, Ma X, et al. Therapy-induced mutations drive the genomic landscape of relapsed acute lymphoblastic leukemia. Blood. 2020;135(1):41-55.
18. Liu X, Wu C, Li C, Boerwinkle E. dbNSFP v3.0: a one-stop database of functional predictions and annotations for human non-synonymous and splice site SNVs. Hum Mutat. 2016;37(3):235-241.
19. Genescà E, Morgades M, González-Gil C, et al. Adverse prognostic impact of complex karyotype (≥3 cytogenetic alterations) in adult T-cell acute lymphoblastic leukemia (T-ALL). Leuk Res. 2021;109:106612.
20. Arber DA, Orazi A, Hasserjian R, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood. 2016;127(20):2391-2405.
21. Moorman AV, Harrison CJ, Buck GAN, et al. Karyotype is an independent prognostic factor in adult acute lymphoblastic leukemia (ALL): analysis of cytogenetic data from patients treated on the Medical Research Council (MRC) UKALLXII/Eastern Cooperative Oncology Group (ECOG) 2993 trial. Blood. 2007;109(8):3189-3197.
22. Ribera J-M, Oriol A, Morgades M, et al. Treatment of high-risk Philadelphia chromosome-negative acute lymphoblastic leukemia in adolescents and adults according to early cytologic response and minimal residual disease after consolidation assessed by flow cytometry: final results of the PETHEMA ALL-AR-03 trial. J Clin Oncol. 2014;32(15):1595-1604.
23. Palomero T, Sulis ML, Cortina M, et al. Mutational loss of PTEN induces resistance to NOTCH1 inhibition in T-cell leukemia. Nat Med. 2007;13(10):1203-1210.
24. Gutierrez A, Kentsis A, Sanda T, et al. The BCL11B tumor suppressor is mutated across the major molecular subtypes of T-cell acute lymphoblastic leukemia. Blood. 2011;118(15):4169-4173.
25. Della Gatta G, Palomero T, Perez-Garcia A, et al. Reverse engineering of TLX oncogenic transcriptional networks identifies RUNX1 as tumor suppressor in T-ALL. Nat Med. 2012;18(3):436-440.
26. Ting CN, Olson MC, Barton KP, Leiden JM. Transcription factor GATA-3 is required for development of the T-cell lineage. Nature. 1996;384(6608):474-478.
27. Van Vlierberghe P, Ambesi-Impiombato A, Perez-Garcia A, et al. ETV6 mutations in early immature human T cell leukemias. J Exp Med. 2011;208(13):2571-2579.

28. Van Vlierberghe P, Palomero T, Khiabanian H, et al. PHF6 mutations in T-cell acute lymphoblastic leukemia. Nat Genet. 2010;42(4):338-342.
29. Zhang J, Ding L, Holmfeldt L, et al. The genetic basis of early T-cell precursor acute lymphoblastic leukaemia. Nature. 2012;481(7380):157-163.
30. Matheson EC, Hall AG. Expression of DNA mismatch repair proteins in acute lymphoblastic leukaemia and normal bone marrow. Adv Exp Med Biol. 1999;457:579-583.
31. De Keersmaecker K, Atak ZK, Li N, et al. Exome sequencing identifies mutation in CNOT3 and ribosomal genes RPL5 and RPL10 in T-cell acute lymphoblastic leukemia. Nat Genet. 2013;45(2):186-190.
32. Spinella J-F, Cassart P, Richer C, et al. Genomic characterization of pediatric T-cell acute lymphoblastic leukemia reveals novel recurrent driver mutations. Oncotarget. 2016;7(40):65485-65503.
33. von Lintig FC, Huvar I, Law P, Diccianni MB, Yu AL, Boss GR. Ras activation in normal white blood cells and childhood acute lymphoblastic leukemia. Clin Cancer Res. 2000;6(5):1804-1810.
34. Neumann M, Seehawer M, Schlee C, et al. FAT1 expression and mutations in adult acute lymphoblastic leukemia. Blood Cancer J. 2014;4(6):e224.
35. Morris LGT, Kaufman AM, Gong Y, et al. Recurrent somatic mutation of FAT1 in multiple human cancers leads to aberrant Wnt activation. Nat Genet. 2013;45(3):253-261.
36. Shochat C, Tal N, Bandapalli OR, et al. Gain-of-function mutations in interleukin-7 receptor-α (IL7R) in childhood acute lymphoblastic leukemias. J Exp Med. 2011;208(5):901-908.
37. Zenatti PP, Ribeiro D, Li W, et al. Oncogenic IL7R gain-of-function mutations in childhood T-cell acute lymphoblastic leukemia. Nat Genet. 2011;43(10):932-939.
38. Maude SL, Dolai S, Delgado-Martin C, et al. Efficacy of JAK/STAT pathway inhibition in murine xenograft models of early T-cell precursor (ETP) acute lymphoblastic leukemia. Blood. 2015;125(11):1759-1767.
39. Tremblay CS, Brown FC, Collett M, et al. Loss-of-function mutations of Dynamin 2 promote T-ALL by enhancing IL-7 signalling. Leukemia. 2016;30(10):1993-2001.
40. Weng AP, Ferrando AA, Lee W, et al. Activating mutations of NOTCH1 in human T cell acute lymphoblastic leukemia. Science. 2004;306(5694):269-271.
41. Thompson BJ, Buonamici S, Sulis ML, et al. The SCFFBW7 ubiquitin ligase complex as a tumor suppressor in T cell leukemia. J Exp Med. 2007;204(8):1825-1835.
42. Neumann M, Heesch S, Schlee C, et al. Whole-exome sequencing in adult ETP-ALL reveals a high rate of DNMT3A mutations. Blood. 2013;121(23):4749-4752.
43. Bond J, Touzart A, Leprêtre S, et al. DNMT3A mutation is associated with increased age and adverse outcome in adult T-cell acute lymphoblastic leukemia. Haematologica. 2019;104(8):1617-1625.
44. Asnafi V, Buzyn A, Le Noir S, et al. NOTCH1/FBXW7 mutation identifies a large subgroup with favorable outcome in adult T-cell acute lymphoblastic leukemia (T-ALL): a Group for Research on Adult Acute Lymphoblastic Leukemia (GRAALL) study. Blood. 2009;113(17):3918-3924.
45. Kurosawa S, Iwama A. Aging and leukemic evolution of hematopoietic stem cells under various stress conditions. Inflamm Regen. 2020;40(1):1-10.
46. Buscarlet M, Provost S, Zada YF, et al. DNMT3A and TET2 dominate clonal hematopoiesis and demonstrate benign phenotypes and different genetic predispositions. Blood. 2017;130(6):753-762.
47. Desai P, Mencia-Trinchant N, Savenkov O, et al. Somatic mutations precede acute myeloid leukemia years before diagnosis. Nat Med. 2018;24(7):1015-1023.
48. Knight T, Irving JAE. Ras/Raf/MEK/ERK pathway activation in childhood acute lymphoblastic leukemia and its therapeutic targeting. Front Oncol. 2014;4:160.
49. Oshima K, Khiabanian H, Silva-Almeida AC da, et al. Mutational landscape, clonal evolution patterns, and role of RAS mutations in relapsed acute lymphoblastic leukemia. Proc Natl Acad Sci U S A. 2016;113(40):11306-11311.
50. Edelbrock MA, Kaliyaperumal S, Williams KJ. Structural, molecular and cellular functions of MSH2 and MSH6 during DNA mismatch repair, damage signaling and other noncanonical activities. Mutat Res. 2013;743–744:53-66.
51. Irving JAE. Towards an understanding of the biology and targeted treatment of paediatric relapsed acute lymphoblastic leukaemia. Br J Haematol. 2016;172(5):655-666.
52. Liu Y, Easton J, Shao Y, et al. The genomic landscape of pediatric and young adult T-lineage acute lymphoblastic leukemia. Nat Genet. 2017;49(8):1211-1218.
53. Hou H-A, Liu C-Y, Kuo Y-Y, et al. Splicing factor mutations predict poor prognosis in patients with de novo acute myeloid leukemia. Oncotarget. 2016;7(8):9084-9101.
54. Roller A, Grossmann V, Bacher U, et al. Landmark analysis of DNMT3A mutations in hematological malignancies. Leukemia. 2013;27(7):1573-1578.
55. Grossmann V, Haferlach C, Weissmann S, et al. The molecular profile of adult T-cell acute lymphoblastic leukemia: mutations in RUNX1 and DNMT3A are associated with poor prognosis in T-ALL. Genes Chromosomes Cancer. 2013;52(4):410-422.
56. Van Vlierberghe P, Ambesi-Impiombato A, De Keersmaecker K, et al. Prognostic relevance of integrated genetic profiling in adult T-cell acute lymphoblastic leukemia. Blood. 2013;122(1):74-82.
57. Feinberg AP, Koldobskiy MA, Göndör A. Epigenetic modulators, modifiers and mediators in cancer aetiology and progression. Nat Rev Genet. 2016;17(5):284-299.
58. Challen GA, Sun D, Jeong M, et al. Dnmt3a is essential for hematopoietic stem cell differentiation. Nat Genet. 2011;44(1):23-31.
59. Sasaki K, Kanagal-Shamanna R, Montalban-Bravo G, et al. Impact of the variant allele frequency of ASXL1, DNMT3A, JAK2, TET2, TP53, and NPM1 on the outcomes of patients with newly diagnosed acute myeloid leukemia. Cancer. 2020;126(4):765-774.
60. Saygin C, Hirsch C, Przychodzen B, et al. Mutations in DNMT3A, U2AF1, and EZH2 identify intermediate-risk acute myeloid leukemia patients with poor outcome after CR1. Blood Cancer J. 2018;8(1):1-12.
61. Ben Abdelali R, Asnafi V, Leguay T, et al. Pediatric-inspired intensified therapy of adult T-ALL reveals the favorable outcome of NOTCH1/FBXW7 mutations, but not of low ERG/BAALC expression: a GRAALL study. Blood. 2011;118(19):5099-5107.
62. Baldus CD, Thibaut J, Goekbuget N, et al. Prognostic implications of NOTCH1 and FBXW7 mutations in adult acute T-lymphoblastic leukemia. Haematologica. 2009;94(10):1383-1390.
63. Breit S, Stanulla M, Flohr T, et al. Activating NOTCH1 mutations predict favorable early treatment response and long-term outcome in childhood precursor T-cell lymphoblastic leukemia. Blood. 2006;108(4):1151-1157.
64. Mansour MR, Sulis ML, Duke V, et al. Prognostic Implications of NOTCH1 and FBXW7 Mutations in Adults With T-Cell Acute Lymphoblastic Leukemia Treated on the MRC UKALLXII/ECOG E2993 Protocol. J Clin Oncol. 2009;27(26):4352-4356.
65. Feng J, Li Y, Jia Y, et al. Spectrum of somatic mutations detected by targeted next-generation sequencing and their prognostic significance in adult patients with acute lymphoblastic leukemia. J Hematol Oncol. 2017;10(1):61.