Prognostic value and oncogenic landscape of TP53 alterations in adult and pediatric T-ALL

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Prognostic value and oncogenic landscape of *TP53* alterations in adult and pediatric T-ALL

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Among hematological malignancies, T-cell acute lymphoblastic leukemia (T-ALL) represents a class of aggressive tumors with dismal clinical outcome in case of relapse or refractory diseases. While the intensification of multi-agent chemotherapy protocols has dramatically improved prognosis, refractory and relapsed cases are clinically challenging due to limited therapeutic options.[1,2] p53 is a transcription factor and a master tumor suppressor gene frequently altered in cancer.[3] In contrast to carcinomas and other hematological malignancies, *TP53* alterations (*TP53*^{Alt}), encompassing mutations (*TP53*^{Mut}) and/or pan-exon deletions (*TP53*^{Del}), are remarkably rare at diagnosis in T-ALL, and their clinical implication remains elusive.[4–6] Critically, *TP53*^{Alt} have been reported to be acquired in up to 20% of the relapsed T-ALL cases, where they convey a deleterious prognosis.[7–9] Here, we produce the first comprehensive analysis of *TP53*^{Alt} and the associated oncogenetic landscape in an extensive cohort of 476 patients newly diagnosed with T-ALL.

We investigated the clinical characteristics linked to *TP53*^{Alt} in 476 patients, including 215 adults, and 261 children. Adult patients were enrolled in the GRAALL-2003-2005 trials (GRAALL-2003, NCT00222027; GRAALL-2005, NCT00327678) and pediatric patients were enrolled in the FRALLE 2000 trial (Supplemental Figure 1). Based on DNA availability for molecular analysis, 215 adult patients out of 337 and 261 pediatric patients out of 427 were included in this study. No difference in clinical outcomes was observed between the included patients and the entire cohort (Supplemental Figure 2-3, Supplemental Table 1). Diagnostic peripheral blood or bone marrow samples were collected after informed consent was obtained, according to the Declaration of Helsinki. All samples contained ≥80% blasts, immunophenotypic of T-ALL samples, minimal residual disease (MRD) assessment and Multiplex ligation-dependent probe amplification analysis (MLPA) (P383 T-ALL from MRC Holland) were performed as previously described.[10–12]

Genomic analysis was performed by pan-exon targeted next-generation sequencing (NGS) of DNA extracted from diagnostic samples; DNA libraries were prepared using Nextera XT kit (Illumina) and sequenced on an Illumina MiSeq. The NGS panel included 63 genes known to be mutated in T-ALL (Supplemental Table 2.). Genetic lesion co-occurrences and mutual exclusions were computed using the DISCOVER R package. We performed a computational approach previously described for the detection of copy number variants (CNVs) from next-generation sequence data,[13,14] including systematic analysis of the depth of *TP53* gene coverage. This is based on variations in depth of coverage of aligned sequence reads, using a locally developed algorithm. The CNVs detected were confirmed by high-resolution CGH and/or MLPA analysis (kit P037-CLL-1 and P038-CLL-2 from MRC-Holland). Diagnostic DNA was hybridized on Affymetrix (Santa Clara, CA) Cytogenetics whole Genome 2.7M Arrays (CGH-array), according to the manufacturer's recommendations. Data analysis was performed with the Chromosome Analysis Suite (ChAS) software (Affymetrix®). Gene copy number aberrations were compared with the Database of Genomic variants (DGV) http://projects.tcag.ca/variation to study only non-variant GCV aberrations.

Comparisons for categorical and continuous variables between subgroups were performed with Fisher's exact test and Mann-Whitney test respectively. Overall survival (OS) was calculated from the date of diagnosis to the last follow-up date censoring patients alive. The cumulative incidence of relapse (CIR) was calculated from the complete remission (CR) date to the date of relapse censoring patients alive without relapse at the last follow-up date. Relapse and death in CR were considered competitive events. Univariate and multivariate analyses assessing the impact of categorical and continuous variables were performed with a Cox model. Proportional-hazards assumption was checked before conducting multivariate analyses. In univariate and multivariate analyses, age and log10(WBC) were considered continuous variables. All analyses were stratified on the trial. Variables with a p-value less

than 0.1 in univariate analysis were included in the multivariable models. Statistical analyses were performed with STATA software (STATA 12.0 Corporation, College Station, TX). All p-values were two-sided, with $p<0.05$ denoting statistical significance. Additional details are included in the Supplemental Methods.

The incidence of $TP53^{Alt}$ at diagnosis in these cohorts was 4% (21/476). $TP53^{Mut}$ were detected in 9 patients (6 adults and 3 pediatric cases, Figure 1A, Supplemental Tables 3 and 4) and $TP53^{Del}$ were identified in 15 patients (7 adult and 8 pediatric cases), 3 patients harbored both $TP53^{Mut}$ and $TP53^{Del}$. Patients with $TP53^{Alt}$ did not significantly differ from *TP53 wild-type* ($TP53^{Wt}$) patients regarding sex, age and central nervous system (CNS) involvement (Table 1), but were associated with an immature phenotype (combining IM0 T-ALL; TCRd and TCRg germline, IMd T-ALL; TCRd rearranged but not TCRg and IMg T-ALL; both TCRd and TCRg rearranged)[12] (8/20, 40% vs 81/399, 20%, p = 0.048). The oncogenetic landscape of $TP53^{Alt}$ was comparable to $TP53^{Wt}$ T-ALLs (Figure 1B and 1C, Supplemental Figures 4, 5 and 6 and Supplemental Table 5). To investigate the prognostic value of $TP53^{Alt}$, survival analyses were performed on the series of 476 patients. $TP53^{Alt}$ did not confer increased poor prednisone response, defined by a peripheral blood blast count > 1.0 x 10^9/L at the end of the induction phase) (38% vs 45%, p = 0.7) (Table 1). Although $TP53^{Alt}$ did not significantly influence the morphological complete response rate at the end of the induction course (81% vs 93%, p = 0.07), patients harboring $TP53^{Alt}$ were associated with delayed early medullary blast clearance, as confirmed by end of induction MRD1 assessment, with more positivity ($\geq 10^{-4}$) in $TP53^{Alt}$ as compared with $TP53^{Wt}$ cases (75% vs 35%, p = 0.01). Patients (both adult and pediatric) with $TP53^{Alt}$ had an inferior outcome compared to $TP53^{Wt}$ (Table 1, Figures 1D-E, Supplemental Figure 7) with an increased CIR (5y-CIR: 65% vs. 27%; specific hazard ratio (SHR) 3.1, 95%CI (1.67 - 5.78); p <0.001) and a shorter OS (5y-OS: 48% vs.

72%; hazard ratio: 2.34, 95%CI (1.30 - 4.24); p = 0.005). In multivariate analysis *TP53*^{Alt} predicted a statistically lower OS (HR: 2.87, 95%CI (1.56 - 5.26); p = 0.001) and higher CIR (SHR, 2.90, 95%CI 1.55 - 5.44), p = 0.001) even after adjustment on the 4-genes *NOTCH1/FBXW7/RAS/PTEN (NFRP)* classifier, which identified poor prognosis patients in both GRAALL and FRALLE trials.[10,15]

The limited number of MRD1 assessments available among the *TP53*^{Alt} T-ALL patients (12/21, 57%), did not allow us to include this endpoint in the multivariate analysis, justifying further studies to establish whether this alteration remains an independent prognostic factor when combined with MRD1 status in T-ALL.

This study provides the largest comprehensive analysis of *TP53*^{Alt} in T-ALL, describing for the first time both their clinical profile and, most importantly, the extremely poor prognosis impact associated with *TP53*^{Alt} at diagnosis in T-ALL, urging the need to develop innovative targeted therapies for patients harboring *TP53*^{Alt}.

About 25% pediatric and 50% adult T-ALL patients relapse, with a 5-year survival inferior to 20% in both age groups.[1,2] The sole therapeutic approaches with a curative potential for T-ALL relapsed cases are limited to conventional chemotherapy or hematopoietic stem cell transplantation (HSCT). Molecular genetic analyses and sequencing studies have recently led to the identification of recurrent T-ALL alterations associated with the prognostic, allowing to refine the stratification of the relapse risk.[10,14,16,17] Yet, a significant proportion of T-ALL relapses remain unpredicted, underlining the urge for new predictive markers.

Negative outcomes observed in *TP53*^{Alt} T-ALL are likely to be related to p53-induced therapeutic resistance previously described for other malignancies combining loss of p53 tumor suppressor activity and acquisition of novel functions that disrupt the DNA-damage response pathway.[18,19] APR-246 (Eprenetapopt), a small molecule interacting with mutated p53 protein to restore its WT conformation[20,21], has recently shown promising results in

154 myeloid malignancies and in B-ALL [22–25] justifying further studies to explore the potential
155 efficacy of this new drug in T-ALL *TP53*^{Mut}.
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Authorship Contributions

V.A, O.H, M.S and G.P.A conceived and designed the research and oversaw the project; M.B, C.G, N.G, J-M.C, F.H, Y.C, Y.B, E.M, V.G, A.P, P.R, A.B, D.B, O.H, N.B, V.A provided study materials or patients; M.S, G.P.A and V.A performed molecular analyses; M.S and G.P.A collected and assembled data; N.B and M.S performed statistical analysis; M.S, G.P.A, R.B, O.H V.A, N.B analyzed and interpreted data; M.S, G.P.A, N.B and V.A wrote the manuscript. M.S and G.P.A contributed equally to this work.

Disclosure of Conflicts of Interest

The authors declare no competing financial interests

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Table Legends

Table 1: Clinico-biological and outcome characteristics of adult and pediatric T-ALL (GRAALL and FRALLE protocols) according to *TP53* status.

Abbreviations: T-ALL: T-cell acute lymphoblastic leukemia; WBC, white blood count; CNS, central nervous system; ETP, early thymic precursor; *High Risk* classifier, *NOTCH1/FBXW7-RAS/PTEN* classifier as previously described[10,15]; CR, complete remission; MRD, minimal residual disease; Allo-HSCT, allogenic hematopoietic stem cell transplantation; CIR, cumulative incidence of relapse; OS, overall survival; HR: hazard ratio, SHR: specific hazard ratio, CI: confidence interval.

MRD1 correspond to MRD evaluation after induction and was performed by allele-specific oligonucleotides polymerase chain reaction. T-cell receptor status and oncogenic were performed as described in supplemental methods.

* CNS involvement; CNS3 in FRALLE2000 trial, CNS2 and/or CNS3 in GRAALL2003 and GRAALL2005 trial
** T-ALL are divided into three subclasses as following **(i)** immature (no detectable TCRb VDJ): IM0 (TCRd and TCRg germline), IMd (TCRd rearranged but not TCRg) and IMg (both TCRd and TCRg rearranged); **(ii)** T-ALL with TCRab lineage (including both early-cortical IMb/Pre-ab and mature sTCRab+) and **(iii)** mature sTCRgd.[12]
*** Low Risk Classifier: *NOTCH1* and/or *FBXW7 (N/F)* mutation without *N/K-RAS* and *PTEN (R/P)* alteration. High-Risk Classifier: *N/F* mutation with *R/P* alteration, *N/F* Wild-type with or without *R/P* alteration.[10,15]

[1]Statistics presented: Median (Minimum-Maximum)

[2]Statistical tests performed: Fisher's exact test; Wilcoxon rank-sum test.

[3]Univariate and multivariate Cox analyses stratified on protocol

p-values < 0.05 are indicated in bold

Figure Legends

Figure 1: ***TP53* Alterations in the GRAALL03/05 and FRALLE2000 studies**

A. Lollipop plots indicating the observed *TP53* mutations in 476 T-ALL patients. More details reporting the pathogenicity of the *TP53* mutations are provided in Supplemental Table 4.

B. Circos plots illustrating pairwise relationships across the relatively common mutated genes in $TP53^{Alt}$ T-ALL. The width of the ribbon corresponds to the number of cases who have simultaneous presence of a first and a second gene mutation.

C. Frequency of alterations per gene among the $TP53^{Alt}$ vs $TP53^{WT}$ T-ALL. The width of the circles is proportional to the frequency of alterations observed in the two T-ALL subgroups ($TP53^{Alt}$ in red, vs $TP53^{WT}$ T-ALL in blue).

D, E Clinical impact of *TP53* Alterations in the GRAALL0305 and FRALLE2000 studies. Overall survival (**D**) and cumulative incidence of relapse (**E**). The red curve represents the $TP53^{Alt}$ T-ALL, and the black curve the $TP53^{WT}$ patients

288 Table

Table 1.

Variable	*TP53*^{Alt} n = 21 **(4%)**	*TP53*^{WT} n = 455 (**96**%)	Total n = 476	*p*-value[2]
Male	14 / 21 (67%)	343 / 455 (75%)	357 / 476 (75%)	0.4
Age (y)[1]	23.4 (4.0-51.8)	15.3 (1.1-59.1)	15.3 (1.1-59.1)	0.5
WBC (G/l)[1]	25 (5-674)	66 (0-980)	64 (0-980)	**0.01**
CNS Involvement*	3 / 21 (14%)	48 / 453 (11%)	51 / 474 (11%)	0.5
Immunophenotype				
ETP phenotype	6 / 16 (38%)	50 / 291 (17%)	56 / 307 (18%)	0.09
Immature (IM0/d/g)**	8 / 20 (40%)	81 / 399 (20%)	89 / 419 (21%)	**0.048**
ab lineage	6 / 20 (30%)	205 / 399 (51%)	211 / 419 (50%)	0.07
Mature TCRgd	3 / 20 (15%)	63 / 399 (16%)	66 / 419 (16%)	>0.9
Oncogenetic classification				
TLX1	1 / 18 (6%)	53 / 397 (13%)	54 / 415 (13%)	0.5
TLX3	2 / 18 (11%)	70 / 397 (18%)	72 / 415 (17%)	0.8
SIL-TAL1	1 / 18 (6%)	56 / 397 (14%)	57 / 415 (14%)	0.5
CALM-AF10	0 / 18 (0%)	13 / 397 (3%)	13 / 415 (3%)	>0.9
High-Risk Classifier***	13 / 21 (62%)	196 / 455 (43%)	209 / 476 (44%)	0.12
Treatment Response				
Prednisone response	13 / 21 (62%)	246 / 446 (55%)	259 / 467 (55%)	0.7
Chemosensitivity	12 / 21 (57%)	325 / 446 (73%)	337 / 467 (72%)	0.1
MRD1 > 10^{-4}	9 / 12 (75%)	114 / 328 (35%)	123 / 340 (36%)	**0.01**
Complete Remission	17 / 21 (81%)	423 / 455 (93%)	440 / 476 (92%)	0.07
Allo-HSCT	4/20 (20%)	97 / 436 (22%)	101 / 456 (22%)	0.7
Outcome				
5-year CIR [95% CI]	65% [11;43]	27% [23;32]	29% [25;33]	**<0.001**
5-year OS [95% CI]	48% [26;67]	72% [68;76]	71% [67;75]	**0.005**

Univariate and Multivariate Analysis[3]

	Univariate			Multivariate		
CIR	SHR	95%CI	p	SHR	95%CI	p
Age	1.01	(0.98 ; 1.03)	0.57	-	-	-
CNS	1.57	(0.85 ; 2.59)	0.08	1.28	(0.77 ; 2.13)	0.34
Log(WBC)	1.62	(1.2 ; 2.18)	**0.002**	1.62	(1.19 ; 2.19)	**0.002**
Prednisone response	0.67	(0.47 ; 0.95)	**0.03**	0.93	(0.64 ; 1.35)	0.70
High-risk Classifier**	2.78	(1.94 ; 3.99)	**< 0.001**	2.58	(1.78 ; 3.74)	**< 0.001**
TP53^{Alt}	3.11	(1.67 ; 5.78)	**< 0.001**	2.90	(1.55 ; 5.44)	**0.001**
OS	HR	95%CI	p	HR	95%CI	p
Age	1.03	(1.01 ; 1.05)	**0.001**	1.05	(1.03 ; 1.07)	**< 0.001**
CNS	2.00	(1.28 ; 3.14)	**0.002**	1.64	(1.02 ; 2.64)	**0.04**
Log(WBC)	1.99	(1.48 ; 2.67)	**< 0.001**	2.01	(1.51 ; 2.86)	**< 0.001**
Prednisone response	0.54	(0.38 ; 0.76)	**< 0.001**	0.83	(0.57 ; 1.20)	0.31
High-risk Classifier	2.93	(2.06 ; 4.17)	**< 0.001**	2.90	(2.01 ; 4.18)	**< 0.001**
TP53^{Alt}	2.34	(1.30 ; 4.24)	**0.005**	2.87	(1.56 ; 5.26)	**0.001**

Table 1.

Table 1.

Variable	*TP53*^{Alt} n = 21 **(4%)**	*TP53*^{WT} n = 455 (**96**%)	Total n = 476	*p*-value[2]
Male	14 / 21 (67%)	343 / 455 (75%)	357 / 476 (75%)	0.4
Age (y)[1]	23.4 (4.0-51.8)	15.3 (1.1-59.1)	15.3 (1.1-59.1)	0.5
WBC (G/l)[1]	25 (5-674)	66 (0-980)	64 (0-980)	**0.01**
CNS Involvement*	3 / 21 (14%)	48 / 453 (11%)	51 / 474 (11%)	0.5
Immunophenotype				
ETP phenotype	6 / 16 (38%)	50 / 291 (17%)	56 / 307 (18%)	0.09
Immature (IM0/d/g)**	8 / 20 (40%)	81 / 399 (20%)	89 / 419 (21%)	**0.048**
ab lineage	6 / 20 (30%)	205 / 399 (51%)	211 / 419 (50%)	0.07
Mature TCRgd	3 / 20 (15%)	63 / 399 (16%)	66 / 419 (16%)	>0.9
Oncogenetic classification				
TLX1	1 / 18 (6%)	53 / 397 (13%)	54 / 415 (13%)	0.5
TLX3	2 / 18 (11%)	70 / 397 (18%)	72 / 415 (17%)	0.8
SIL-TAL1	1 / 18 (6%)	56 / 397 (14%)	57 / 415 (14%)	0.5
CALM-AF10	0 / 18 (0%)	13 / 397 (3%)	13 / 415 (3%)	>0.9
High-Risk Classifier***	13 / 21 (62%)	196 / 455 (43%)	209 / 476 (44%)	0.12
Treatment Response				
Prednisone response	13 / 21 (62%)	246 / 446 (55%)	259 / 467 (55%)	0.7
Chemosensitivity	12 / 21 (57%)	325 / 446 (73%)	337 / 467 (72%)	0.1
MRD1 > 10^{-4}	9 / 12 (75%)	114 / 328 (35%)	123 / 340 (36%)	**0.01**
Complete Remission	17 / 21 (81%)	423 / 455 (93%)	440 / 476 (92%)	0.07
Allo-HSCT	4/20 (20%)	97 / 436 (22%)	101 / 456 (22%)	0.7
Outcome				
5-year CIR [95% CI]	65% [11;43]	27% [23;32]	29% [25;33]	**<0.001**
5-year OS [95% CI]	48% [26;67]	72% [68;76]	71% [67;75]	**0.005**

Univariate and Multivariate Analysis[3]

	Univariate			Multivariate		
CIR	SHR	95%CI	p	SHR	95%CI	p
Age	1.01	(0.98 ; 1.03)	0.57	-	-	-
CNS	1.57	(0.85 ; 2.59)	0.08	1.28	(0.77 ; 2.13)	0.34
Log(WBC)	1.62	(1.2 ; 2.18)	**0.002**	1.62	(1.19 ; 2.19)	**0.002**
Prednisone response	0.67	(0.47 ; 0.95)	**0.03**	0.93	(0.64 ; 1.35)	0.70
High-risk Classifier**	2.78	(1.94 ; 3.99)	**< 0.001**	2.58	(1.78 ; 3.74)	**< 0.001**
TP53^{Alt}	3.11	(1.67 ; 5.78)	**< 0.001**	2.90	(1.55 ; 5.44)	**0.001**
OS	HR	95%CI	p	HR	95%CI	p
Age	1.03	(1.01 ; 1.05)	**0.001**	1.05	(1.03 ; 1.07)	**< 0.001**
CNS	2.00	(1.28 ; 3.14)	**0.002**	1.64	(1.02 ; 2.64)	**0.04**
Log(WBC)	1.99	(1.48 ; 2.67)	**< 0.001**	2.01	(1.51 ; 2.86)	**< 0.001**
Prednisone response	0.54	(0.38 ; 0.76)	**< 0.001**	0.83	(0.57 ; 1.20)	0.31
High-risk Classifier	2.93	(2.06 ; 4.17)	**< 0.001**	2.90	(2.01 ; 4.18)	**< 0.001**
TP53^{Alt}	2.34	(1.30 ; 4.24)	**0.005**	2.87	(1.56 ; 5.26)	**0.001**

Figure 1.