

Taylor & Francis
Taylor & Francis Group

Scandinavian Journal of Clinical and Laboratory Investigation

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/iclb20

Impact of *TP53* gene variants on prognosis and survival of childhood acute lymphoblastic leukemia

Sinem Firtina, Yucel Erbilgin, Ozden Hatirnaz Ng, Serap Karaman, Zeynep Karakas, Tulin Tiraje Celkan, Sema Aylan Gelen, Yildiz Yildirmak, Ugur Ozbek & Muge Sayitoglu

To cite this article: Sinem Firtina, Yucel Erbilgin, Ozden Hatirnaz Ng, Serap Karaman, Zeynep Karakas, Tulin Tiraje Celkan, Sema Aylan Gelen, Yildiz Yildirmak, Ugur Ozbek & Muge Sayitoglu (2023) Impact of *TP53* gene variants on prognosis and survival of childhood acute lymphoblastic leukemia, Scandinavian Journal of Clinical and Laboratory Investigation, 83:3, 187-193, DOI: 10.1080/00365513.2023.2195682

To link to this article: https://doi.org/10.1080/00365513.2023.2195682

View supplementary material

Published online: 08 Apr 2023.

Submit your article to this journal

Article views: 140

View related articles

View Crossmark data

Full Terms & Conditions of access and use can be found at
https://www.tandfonline.com/action/journalInformation?journalCode=iclb20

SCANDINAVIAN JOURNAL OF CLINICAL AND LABORATORY INVESTIGATION
2023, VOL. 83, NO. 3, 187–193
https://doi.org/10.1080/00365513.2023.2195682

Taylor & Francis
Taylor & Francis Group

Check for updates

Impact of *TP53* gene variants on prognosis and survival of childhood acute lymphoblastic leukemia

Sinem Firtina[a,b], Yucel Erbilgin[a], Ozden Hatirnaz Ng[a,c], Serap Karaman[d], Zeynep Karakas[d], Tulin Tiraje Celkan[e], Sema Aylan Gelen[f], Yildiz Yildirmak[g], Ugur Ozbek[a,h] and Muge Sayitoglu[a]

[a]Department of Genetics, Aziz Sancar Institute of Experimental Medicine, Istanbul University, Istanbul, Turkey; [b]Department of Medical Genetics, Istanbul University-Cerrahpasa, Cerrahpasa School of Medicine, Istanbul, Turkey; [c]Department of Medical Biology, Acibadem University, Acibadem School of Medicine, Istanbul, Turkey; [d]Pediatric Hematology Oncology Department, Istanbul University, Istanbul Faculty of Medicine, Istanbul, Turkey; [e]Cerrahpasa Faculty of Medicine, Department of Pediatric Hematology Oncology, Istanbul University-Cerrahpasa, Istanbul, Turkey; [f]Department of Pediatric Hematology, Kocaeli University Hospital, Kocaeli, Turkey; [g]Pediatric Hematology Division, Ministry of Health Sisli Etfal Education and Research Hospital, Istanbul, Turkey; [h]Department of Medical Genetics, Acibadem University, Acibadem School of Medicine, Istanbul, Turkey

ABSTRACT

The *tumor suppressor protein 53* (*TP53*) gene is one of the most studied genes in cancer. Although *TP53* variants are rare events in acute leukemia, recent observations showed that relapse samples might harbor *TP53* variants. Here, we aimed to determine *TP53* variants (hotspot region, exon 4–11) in childhood acute lymphoblastic leukemia (B and T-ALL) patients ($n = 94$) including diagnostic-relapse pairs ($n = 15$) by amplicon sequencing and evaluate the clinical impact of these variants. In total, nine different (E298*, R283C, R273H, L252F, C229F, I195T, E286G, c.955_956insC, and c.920-1G > C) variants were identified in 17 (18%) childhood ALL patients. c.(920-1G>C) splice site variant and c.(955_956insC) insertion were reported for the first time. In diagnose-relapse pair samples, we identified acquired and/or loss of TP53 variants in the samples at the time of relapse. *TP53* variants were found to be more common in T-ALL (37%) than in B-ALL patients (9%). Pathogenic *TP53* variants were associated with a shorter overall survival time ($p = 0.001$).*TP53* variants were found to be associated with inferior outcomes in our cohort and can be an independent risk factor for poor prognosis in childhood acute leukemia patients. Identification of low-frequent variants with next-generation sequencing approaches enables better knowledge of the clonal dynamics of ALL.

Abbreviations: TP53: Tumor suppressor protein 53; ALL: Acute Lymphoblastic Leukemia; OS: overall survival; RFS: relapse-free survival; BFM: Berlin–Frankfurt–Munster protocol;; BM: bone marrow; CNS: central nervous system; MID: molecular identifier; IARC: International Agency for Research on Cancer; STD: standard risk group; MRG: medium risk group; HRG: High risk group; ; AML: Acute myeloid leukemia; MDS: myelodysplastic syndrome; CLL: chronic lymphoblastic leukemia

ARTICLE HISTORY
Received 6 October 2022
Revised 20 March 2023
Accepted 22 March 2023

KEYWORDS
TP53;
Next Generation Amplicon Sequencing;
B-ALL;
T-ALL;
prognosis

Introduction

Acute lymphoblastic leukemia (ALL) is one of the most common cancers in childhood characterized by uncontrolled cell proliferation, leading to a distinct block in an early developmental stage of lymphocytes [1]. Although the genetic background of B cell ALL (B-ALL) and T cell ALL (T-ALL) is substantially different, with the help of conventional and/or advanced personalized treatment options, the outcome of the patients is similar. Genetic alterations have a clinical impact on disease outcome, risk classification or treatment options and are used in clinical practice as biomarkers [2–4].

The loss of function variants of *tumor suppressor protein 53* (*TP53*) gene is one the most frequent somatic pathogenic changes, which have been described in 38–50% of cancers [5]. p53 is induced by a stress response like DNA damage and transactivates its target genes that a role in the cell cycle or apoptosis. This p53-mediated cell cycle arrest is important for genomic stability because it allows DNA repair to correct the genome or eliminate the damaged cells through apoptosis [6,7]. Inactivation of p53 pathway gives a survival advantage in damaged cells and contributes to cancer development [8]. *TP53* variants are hallmarks of poor prognosis, metastasis, or poor outcome in various cancer types [9]. More than 20.000 somatic and 1500 germline variants have been identified in *TP53* so far (IARC database, R20 release). However, the role of *T53* variants in childhood acute leukemia is poorly understood. *TP53* variants were detected in up to 16% of ALL patients and

CONTACT Yucel Erbilgin yucel.erbilgin@istanbul.edu.tr Department of Genetics, Aziz Sancar Institute of Experimental Medicine, Istanbul University, Vakıf Gureba Cad. 34093, Istanbul, Turkey

Supplemental data for this article is available online at https://doi.org/10.1080/00365513.2023.2195682.

© 2023 Medisinsk Fysiologisk Forenings Forlag (MFFF)

found more frequently in low-hypodiploid ALL (specifically loss of chromosome 17) and relapsed ALL patients [3,10,11]. |The overall (OS) and relapse-free survival (RFS) rates were found to decrease in mutated ALL patients indicating that *TP53* variants were associated with poor outcomes [12,13].

High throughput sequencing techniques enable us to detect molecular targets for clonal changes in cancers such as leukemia, which is crucial to identify biomarkers for high-risk subgroups or individualized treatment options. Besides defining cancer-related biomarkers, subclonal (<20%) allele changes are also important for characterizing clonal dynamics in ALL [14]. Monitoring the genomic alterations in diagnosis and relapse patients by next-generation sequencing techniques and finding the relapse-related clonal changes might help to detect early relapse disease or chemotherapy-resistant clones [15,16].

Methods

Study cohort

Ninety-four samples of childhood ALL (67 males and 27 females) patients were enrolled in this study. The study group consisted of diagnosed time bone marrow (BM) samples of B-ALL ($n = 44$) and T-ALL ($n = 35$) cases and additional relapse samples of fifteen patients (B-ALL, $n = 9$ and T-ALL, $n = 6$). The patients were diagnosed and treated according to the BFM (Berlin–Frankfurt–Munster)-ALL 2000 protocol. The patients were treated uniformly according to the ALL-REZ BFM 2002 relapse protocol after the first BM relapses. The median age was 8.25 years (min:5 months-max:17 years). The median WBC count at diagnosis was 81.7 (min:13- max:603 10^9/L) and median hemoglobin was 10.7 (min:1.2 -max:13.5 10^9/L). Patients with tumor load of more than 90% at diagnosis were included in this study. Fusion genes were identified in 25% of our cohort; *BCR::ABL* ($n = 3$), *KMT2A::AFF1* ($n = 3$), *TEL::AML1* ($n = 3$), *E2A::PBX* ($n = 3$) and *SIL::TAL1* ($n = 6$). The median follow-up time was 48 months (range 1–234 months). Median RFS time was 21 months (range 0–140 months). Splenomegaly, hepatomegaly, and central nervous system (CNS) and other organ involvements (lymph node, testis, kidney, mediastinum, and lung) were also reported. Written informed consent was obtained from all patients and/or their parents and the study was approved by the Local Ethical Board of Istanbul University. The clinical characteristics of the patients are summarized in Supplementary Table 1.

Amplicon sequencing and data analysis

Genomic DNA was isolated by spin column Qiamp Genomic DNA isolation kit as described by the manufacturer (Qiagen, Germany). Eight exons (exon 4-11) of *TP53* gene (NM_000546.6) were amplified by eight primer pairs including a 10-base molecular identifier barcode sequence (MID) which were designed and validated by IRON II (**I**nterlaboratory **RO**bustness of **N**ext generation sequencing) Consortium [17]. Each ninety-six well plate were designed with primer pairs of eight exons of *TP53* gene with 11 different MID which leads us to screen 11 different samples (Roche Applied Science, Germany). Amplicons were amplified with FastStart High Fidelity PCR System (Roche Applied Science, Penzberg, Germany) and were purified by Ampure XP beads (Beckman Coulter, Krefeld, Germany). Libraries were quantified by Quant-iT PicoGreen dsDNA kit (Invitrogen, Carlsbad, CA, USA). *TP53* next-generation sequencing panel was applied on a Roche GS Junior (454-Life Sciences, Branford, CT) according to the manufacturer's instructions [18]. A data quality assessment was performed by GS Junior Sequencer Instrument 2.3 (Roche Applied Science, Germany). The amplicons were aligned to the reference *TP53* sequence and variants were analyzed by using GS Amplicon Variant Analyzer software version 2.5.3 (Roche Applied Science, Germany). The variants with a read depth of at least 500X were included. Variants with an allelic fraction of 15% or more were verified by bidirectional Sanger sequencing and variants which covered less than 15% were validated by a second NGS run. Only the verified variants were listed.

Open-source programs such as PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/), Variant Effect Predictor (VEP) (https://www.ensembl.org/info/docs/tools/vep/index.html) and Mutation Taster (https://www.mutationtaster.org/), dbSNP database https://www.ncbi.nlm.nih.gov/SNP/), The Human Gene Mutation Database (HGMD) (http://www.hgmd.cf.ac.uk/ac/index.php) and Varsome (https://varsome.com/) were used for frequency data and/or to predict the functional impact of the variants. The variant prediction was done according to TP53-specific ACMG criteria [19,20] and annotated according to the International Agency for Research on Cancer (IARC) *TP53* database [21]. After interpreting variants using the specific guidelines, we made the final interpretations publicly available through ClinVar [22].

Statistical analysis

Clinical differences between *TP53* mutated and non-mutated groups were statistically analyzed by the nonparametric Mann–Whitney *U* test. Categorical variables were compared by Pearson's χ^2 or Fisher's exact test and $p < 0.05$ was considered statistically significant. OS was defined by the interval from the diagnosis date to the death or last follow-up time. RFS was defined by the duration from the complete remission (CR) date to the relapse, or last follow-up. The end of the last follow-up period was December 2019. The Kaplan–Meier method and univariate and multivariate COX regression analysis was performed for the survival analysis. All statistical analyses were done by GraphPad Prism 8 (San Diego, CA, USA) and IBM SPSS statistics 20 (IBM Corp. Armonk, NY, USA).

Results

Here we identified 17 *TP53* variants in 79 diagnostic ALL samples and three variants among 15 relapse samples. Clonal

(VAF >15%) pathogenic and likely pathogenic variants' frequency was 12.6% in the cohort. The p.(E298*) was found in eight (two B-ALL and six T-ALL) patients. Beside p.(E298*), c.955_956insC ($n = 3$), p.(R283C) ($n = 2$), p.(R273H) ($n = 2$), p.(L252F) ($n = 1$), p.(C229F) ($n = 1$), p.(I195T) ($n = 1$), p.(E286G) ($n = 1$) and c.(920-1g > c) ($n = 1$) alterations were also detected (Table 1). The frameshift c.(955_956insC) and splice site c.(920-1G > C) *TP53* variants were reported for the first time in this study. All the missense variants were located in the DNA-binding domain of *TP53* protein (Figure 1). The presence of all clonal variants was confirmed by Sanger sequencing.

TP53 variants were found more frequently in T-ALL (13 in 35, 37%) than B-ALL (4 in 44, 9%) patients. Although most of our patients only carried one *TP53* variant, two T-ALL patients (#P11 and #P5) possessed concurrent monoallelic *TP53* variants. Patient #P11 harboured the p.(E298*) and the c.(955_956insC) novel variant with low variant allele fraction (52% and 2.69% respectively). Patient #P5 showed the p.(E298*) and the p.(L252F) variants. In addition to the pathogenic/likely pathogenic *TP53* variants, we also detected benign variants in the cohort; rs1625895 (91%), rs1042522 (82%), rs12951053 (7%), rs12947788 (7%), rs1800372 (5%), rs1800899 (4%), and rs1800370 (2%).

TP53 variants in the paired diagnose-relapse samples

Herein, 15 paired diagnostic-relapse (nine B-ALL and six T-ALL) samples were analyzed to identify the acquired or lost alterations during the treatment. In total, five ALL patients showed acquired or lost *TP53* variants (Figure 2). While the diagnosed sample #P28D did not show any *TP53* variant, the paired relapse sample #P28R acquired a splice site variant (Table 1). A new subclonal change with p.(C229R) missense variant was detected in the relapse sample of a B-ALL patient (#P47R) while this variant was absent at the diagnosis (#P47D). Similarly, diagnose sample of a B-ALL patient (#P07D) had an intronic *TP53* variant (rs1625895) and p.(E298*) variant, #P07D lost both variants at relapse (#P07R). B-ALL patient (#P03D) was detected as wild type at diagnosis and acquired a synonymous *TP53* variant (rs1800372) at relapse. Furthermore, a B-ALL patient (#P49D) had a low-level p.(E286G) (2.1%) variant but this subclone was lost in the relapse sample (#P49R) (Figure 2). In addition to acquired and/or loss of clonal changes, we identified the same pathogenic variants or polymorphisms at diagnosis and relapse samples with similar frequencies. We identified p.(R273H) variant with 92.89% read depth in the diagnostic sample of one T-ALL patient (#P29) and the same pathogenic variant was detected with 90% read depth at the relapse sample (#P29R). The other diagnose-relapse pair samples carried the same polymorphisms both at diagnosis and relapse time with similar frequencies (Supplemental Table 3).

Clinical impact of TP53 variants in childhood ALL patients

We evaluated the correlation between *TP53* variants and the clinical features of the patients. T-ALL patients (17%) had significantly higher mutation rates compared to B-ALL (4.5%) ($p = 0.01$, Supplementary Table 2). Gender (male vs female), Age (0–9 vs >10), WBC count (>50,000 vs <50,000), Platelet (Plt) count (>50,000 vs <50,000), Hb level (>10 vs

Table 1. Identified *TP53* variants in ALL patients.

Patient No	Pheno type	Sample time	Position	Protein level	dbSNP ID	COSMIC ID	VAR (%)	READ DEPTH	ACMG CLASS
P11	T-ALL	diagnosis	NM_001126114.2:c.955_956insC	Lys319Thrfs*28	NOVEL		2.69	557	Likely pathogenic
P04	T-ALL	diagnosis	NM_001126114.2:c.955_956insC	Lys319Thrfs*28	NOVEL		3.07	391	Likely pathogenic
P63	T-ALL	diagnosis	NM_001126114.2:c.955_956insC	Lys319Thrfs*28	NOVEL		2.94	579	Likely pathogenic
P22	B-ALL	diagnosis	NM_001126114.1:c.892G > T	E298*	rs201744589	COSV52661551	41	318	Pathogenic
P55	B-ALL	diagnosis	NM_001126114.1:c.892G > T	E298*	rs201744589	COSV52661551	1.7	400	Pathogenic
P11	T-ALL	diagnosis	NM_001126114.1:c.892G > T	E298*	rs201744589	COSV52661551	52	557	Pathogenic
P13	T-ALL	diagnosis	NM_001126114.1:c.892G > T	E298*	rs201744589	COSV52661551	2.5	466	Pathogenic
P33	T-ALL	diagnosis	NM_001126114.1:c.892G > T	E298*	rs201744589	COSV52661551	44.6	468	Pathogenic
P44	T-ALL	diagnosis	NM_001126114.1:c.892G > T	E298*	rs201744589	COSV52661551	27	603	Pathogenic
P05	T-ALL	diagnosis	NM_001126114.1:c.892G > T	E298*	rs201744589	COSV52661551	17	177	Pathogenic
P07D	T-ALL	diagnosis	NM_001126114.1:c.892G > T	E298*	rs201744589	COSV52661551	16.5	242	Pathogenic
P73	B-ALL	diagnosis	NM_001126114.2:c.847C > A	R283C	rs149633775	COSV52676681	8.7	472	Likely pathogenic
P40	T-ALL	diagnosis	NM_001126114.2:c.847C > A	R283C	rs149633775	COSV52676681	63	1530	Likely pathogenic
P29D	T-ALL	diagnosis	NM_001126114.2:c.818G > A	R273H	rs28934576	COSV52660980	92.89	731	Pathogenic
P29R	T-ALL	relapse of P29	NM_001126114.2:c.818G > A	R273H	rs28934576	COSV52660980	90	1005	Pathogenic
P05	T-ALL	diagnosis	NM_001126114.2:c.754C > T	L252F	NA	COSV52730003	48	31	Pathogenic
P47R	B-ALL	relapse of P47	NM_001126114.1:c.685T > C	C229R	rs1064794312	COSV52741302	1.81	380	VUS
P41	T-ALL	diagnosis	NM_001126114.2:c.584T > C	I195T	rs760043106	COSV52664264	29	333	Pathogenic
P28R	T-ALL	relapse of P28	NM_001126114.2:c.920-1G > C	splice site region	NOVEL		32	637	VUS
P49D	B-ALL	diagnosis	NM_001126114.2:c.857A > G	E286G	NA	COSV52661405	2.1	286	Pathogenic

The letter "D" at the end of the patient codes defines the "diagnosis" time sample of the patient and the letter "R" defines the "relapse" time sample of the patient. VAR: Variant allele frequency; NA: Not Avaliable; VUS: Variant of unknown significance.

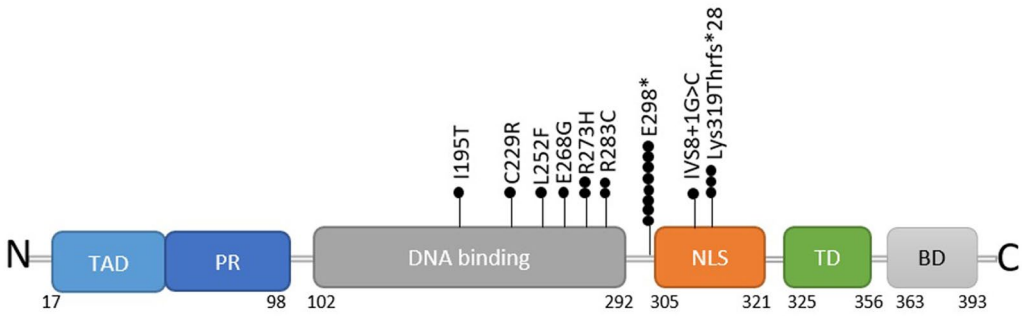


Figure 1. Gene mutation mapper plot of *TP53* variants. Each dot represents a single variant. TAD: Transcriptional activation domain; PR: Proline-rich domain; NLS: Nuclear localisation signal; TD: tetramerization domain; BD: C-terminal basic domain.

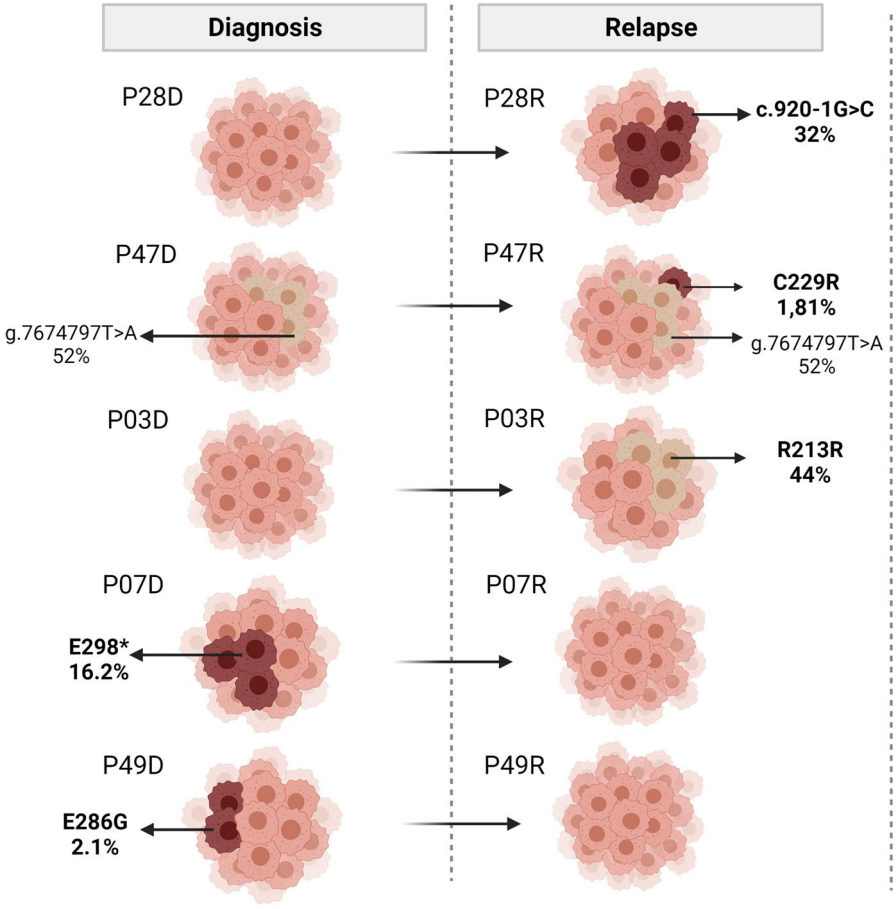


Figure 2. Clonal dynamics in paired diagnosis-relapse pediatric T-ALL and B-ALL samples. This figure is created with BioRender.com.

<10), central nervous system (CNS) or other involvements (yes vs no), translocation (positive vs negative) and risk groups (STD-MRG vs HRG) did not show any statistical differences between mutated and unmutated patients. Then we analyzed the patients who had both p.(E298*) variant and any other *TP53* variants and found no significant difference in any clinical findings. In addition, the association of clinical features and ALL subgroups were also analyzed. Within the limited cohort size of T-ALL subgroup, we found no T-ALL patients with *TP53* variants who had WBC $>100\times10^9$/L at the time of diagnosis ($p=0.03$). However, no other clinical correlation was found between ALL subtypes and clinical findings.

Cox univariate regression model showed the presence of translocation ($p=0.005$), lack of induction treatment response at day 33 ($p<0.001$) and *TP53* mutation status ($p=0.004$) with shorter OS. Lack of induction treatment response at day 33 ($p=0.001$) was also found associated with inferior RFS (Table 2). On the other hand, multivariate analysis showed that induction treatment response at day 33 ($p=0.001$) is associated with shorter RFS, CNS involvement ($p=0.002$) and shorter OS ($p<0.001$).

In the Kaplan-Meier survival analysis, OS and RFS were inferior in patients with *TP53* variants (OS $p=0.01$ and RFS $p=0.02$ respectively; Figure 3(A,B)). When we focused on OS and RFS rates on subtype levels, there is no significant differences in mutated or unmutated patients among T-ALL and B-ALL patients. Furthermore, we performed Kaplan Meier analysis by defining nonsense variant p.(E298*) and other missense variants separately for OS

Table 2. Univariate and multivariate COX regression models of *TP53* variants and clinical associations.

	Relapse-free survival (RFS)				Overall survival (OS)			
	Univariate analysis		Multivariate analysis		Univariate analysis		Multivariate analysis	
Variable	*p*–value	HR(95%)	*p*–value	HR (95% CI)	*p*–value	HR (95% CI)	*p*–value	HR (95% CI)
Age (<1 year or >10 years vs. 1–10 years)	0.842	1.084 (0.490–2.398)	0.909	1.052 (437–2.534)	0.149	1.789 (0.812–3.943)	0.063	2.427 (0.953–6.184)
Gender (female vs. male)	0.814	0.906 (0.399–2.059)	0.437	1.504 (0.537–4.211)	0.98	0.405 (0.139–1.180)	0.406	0.538 (0.166–2.070)
WBC count (>50.000 vs. ≤50.000)	0.063	2.028 (0.962–4.275)	**0.026**	2.996 (1.138–7.887)	0.36	2.353 (1.055–5.245)	0.407	1.517 (0.566–4.062)
Translocation (presence vs. absence)	0.384	1.423 (0.643–3.148)	0.563	1.322 (0.513–3.404)	**0.005**	3.097 (1.408–6.813)	0.459	1.455 (0.538–3.933)
Treatment Response Day 33 (presence vs. absence)	**0.001**	64.383 (5.167–802.287)	**0.001**	78.138 (5.331–1145.210)	**<0.001**	10.524 (3.95–28.43)	**<0.001**	10.344 (2.717–39.378)
Subtype	0.693	0.856 (0.394–1.857)	0.093	0.426 (0.158–1.153)	0.61	2.154 (0.966–4.803)	0.450	0.650 (0.212–1.990)
CNS	0.64	0.75 (0.22–2.50)	0.69	0.74 (0.17–3.16)	0.75	1.18 (0.40–3.46)	**0.02**	0.21 (0.05–0.82)
Organ involvement	0.965	0.984 (0.64–2.083)	0.66	0.849 (0.371–1.940)	0.079	0.438 (0.175–1.099)	0.092	0.438 (0.168–1.146)
TP53	0.609	1.288 (0.489–3.394)	0.35	1.668 (0.566–4.877)	**0.004**	2.439 (1.01–5.863)	0.274	1.824 (0.621–5.355)
Overall model P value			**<0.001**				**<0.001**	

WBC: White Blood Cell count at diagnosis; CNS: Central nervous system involvement. Statistically significant results were indicated at bold format.

and RFS estimations. Patients showed equality of OS and RFS distributions for variant subtypes (OS $p = 0.094$, RFS $p = 0.074$).

Discussion

Genomic profiling of ALL patients is important for diagnosis, subgroup identification, risk assessment and/or targeted treatment options [23]. *TP53* gene is one of the most known tumor suppressor genes which was shown to be inactivated in 2–16% of pediatric ALL [24–27]. In this study, we identified nine different *TP53* variants with low/high levels in 17 (18%) pediatric ALL patients. Diagnostic samples of pediatric T-ALL patients showed significantly higher mutation frequency (37%) than B-ALL patients (9%). *TP53* plays an important role in all hematological malignancies [28], and further studies showed that *TP53* gene variants may also have an impact on disease progression and treatment resistance, and can be used as a biomarker to identify high-risk T-ALL [29].

Recent studies demonstrated the importance of heterozygous *TP53* variants and the tp53 haploinsufficiency. The heterozygous forms of rare *TP53* variants were previously reported in acute and chronic leukemia patients [30–33]. Monoallelic missense *TP53* variants that were identified in this study, were located in the DNA-binding domain which was associated with decreased p53 activity [34]. The most common variant was p.(E298*) in our cohort, found as clonal and subclonal in eight (six T-ALL, two B-ALL) ALL patients. The p.(E298*) variant was classified as pathogenic and predicted to lead to a loss of TP53 protein function. The p.(E298*) was observed in Li-Fraumeni syndrome, several types of carcinomas and cancer tissues. Even though, there is no functional evidence for p.(E298*) in ClinVar, ovarian cancer tissues with this variant showed an intermediate tp53 expression level [35,36].

Two novel *TP53* variants were also detected (c.955_956insC and c.920-1G>C) in T-ALL patients. Two of the three patients who harbored c.(955_956insC) were high-risk patients with CNS involvement and positive for *SIL::TAL* translocation. One of these patients (#P11D) who was deceased, had clonal p.(E298*) variant and an additional low-level subclonal insertion c.(955_956insC) (2.6%) in *TP53* gene. #P28R was also a high-risk patient, had higher WBC counts (>100.000) at relapse and acquired c.(920-1G>C) clonal *TP53* variant with 32% frequency at relapse time.

Association of *TP53* variants with poor outcomes in pediatric ALL [37] and the effect of *TP53* variants on survival rate in B-cell malignancies, AML, ALL, MDS, MM and CLL patients are known [28,38,39]. Here, our results showed that *TP53* variants were significantly associated with resistance to induction therapy at day 33, high WBC count at diagnosis and shorter RFS time. Independently, the patients with *TP53* mutations also showed inferior OS and RFS rates compared to nonmutated patients. The somatic *TP53* mutations were commonly present in the low hypodiploidy ALL patients [13]. Two T-ALL patients were hypodiploid and one of them carried p.(E298*) variant. Unfortunately, we were unable to analyze the correlation between hypodiploidy status and *TP53* mutations in our cohort due to the lack of cytogenetics data for the other patients.

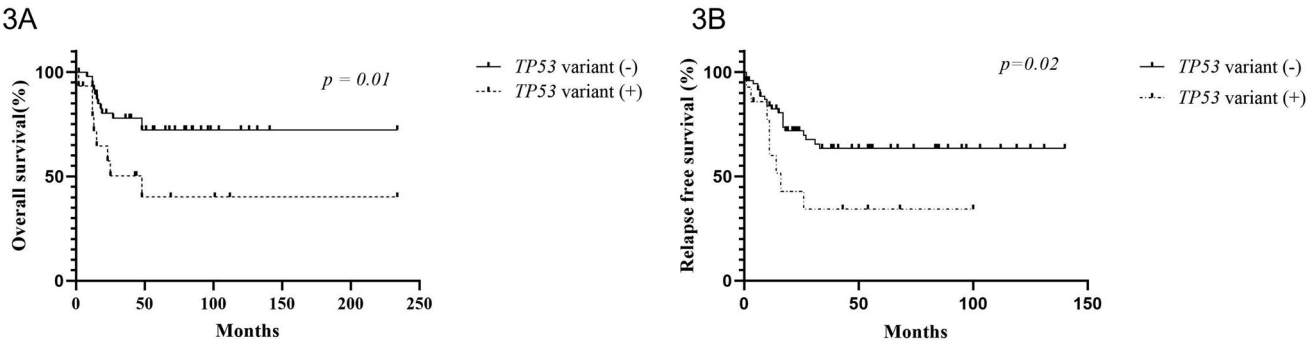


Figure 3. (A) Overall survival (OS) rates (B) Relapse-free survival (RFS) rates. The Kaplan–Meier graph shows overall and relapse-free survival rates between cases with *TP53* variant or not.

The subclonal *TP53* variants have an impact on low remission rates, and poor overall and event-free survival and can be used for risk stratification [40–42]. Zhang et al. pointed out that screening the subclonal variants in diagnosis and relapse time points can improve the treatment success [25]. Fifteen diagnose-relapse paired ALL samples were also analyzed to show the clonal dynamic changes in this study. A novel splice site variant that was located within the splice site of intron 8 and might cause alternative splicing, was detected in a T-ALL (#P28R) patient at the time of relapse, but not at diagnosis time. On the other hand, #P07D lost p.(E298*) pathogenic variant at the time of relapse. A subclonal missense variant p.(C229R) was detected in relapse samples of #P47R which has a dominant negative effect [43]. p.(R273H) variant was detected in diagnosis and relapse samples of a T-ALL (#P29D and #P29R) patient. This variant can cause a less stable p53 protein and leads to multi-drug resistance and metastasis [44–46]. Although more evidence is needed; clonal evidence in this study showed us that relapse samples acquire novel *TP53* variants and might contribute to subclonal progression or treatment failure.

Germline pathogenic variant of *TP53* is associated with Li-Fraumeni syndrome and is predisposed to leukemia and early cancer development [26]. On the other hand, a low variant allelic fraction of *TP53* may be detected on germline samples as a result of clonal hematopoiesis of indeterminate potential. Herein, we analyzed somatic variants of *TP53* and did not discriminate germline variants which is a limitation of the study. A small number of cohort sizes and screening only the hot-spot region of *TP53* gene are also the limitations of our study. Screening both copy number variations (CNVs) and mutations in *TP53* in a large-size cohort might increase the impact of our findings.

In conclusion, *TP53* variants were found to be associated with inferior outcomes in our study and can be an independent risk factor for poor prognosis in childhood acute leukemia patients. Identifying *TP53* variants with next-generation techniques may be impactful to detect low-level variants which is important for future risk classification and treatment strategies.

Acknowledgements

Concept: M.S., O.H.N., Y.E., U.O., S.F. Data collection or processing: S.K., Z.K., T.T.C., S.A.G., Y.Y., S.F., O.H.N. Analysis or interpretation: M.S., O.H.N., Y.E., U.O., S.F. Literature review: M.S., O.H.N., Y.E., U.O., S.F. Writing: S.F., M.S., O.H.N., Y.E. .

Authorship contributions

Concept: M.S., O.H.N., Y.E., U.O., S.F. Data collection or processing: S.K., Z.K., T.T.C., S.A.G., Y.Y., S.F., O.H.N. Analysis or interpretation: M.S., O.H.N., Y.E., U.O., S.F. Literature review: M.S., O.H.N., Y.E., U.O., S.F. Writing: S.F., M.S., O.H.N., Y.E.

Ethics committee approval

The study was approved by the local ethics committee and conducted in accordance with the Declaration of Helsinki.

Patient consent

The patient consent form was signed and obtained from all the participants of the study.

Disclosure statement

No conflict of interest was declared by the authors.

Funding

This study was funded by Scientific Research Project Coordination Unit of Istanbul University (Project No:34101 and TDP-2016-20520).

ORCID

Yucel Erbilgin http://orcid.org/0000-0001-6969-6649
Ozden Hatirnaz Ng http://orcid.org/0000-0001-7728-6527
Zeynep Karakas http://orcid.org/0000-0002-8835-3235
Ugur Ozbek http://orcid.org/0000-0001-5319-0547
Muge Sayitoglu http://orcid.org/0000-0002-8648-213X

References

[1] Inaba H, Greaves M, Mullighan CG. Acute lymphoblastic leukaemia. Lancet. 2013;381:1943–1955.
[2] Zhang HH, Wang HS, Qian XW, et al. Genetic variants and clinical significance of pediatric acute lymphoblastic leukemia. Ann Transl Med. 2019;7:296.
[3] Demir S, Boldrin E, Sun Q, et al. Therapeutic targeting of mutant p53 in pediatric acute lymphoblastic leukemia. Haematologica. 2020;105:170–181.

[4] Li B, Brady SW, Ma X, et al. Therapy-induced mutations drive the genomic landscape of relapsed acute lymphoblastic leukemia. Blood. 2020;135:41–55.
[5] Olivier M, Hollstein M, Hainaut P. TP53 mutations in human cancers: origins, consequences, and clinical use. Cold Spring Harb Perspect Biol. 2010;2:a001008.
[6] Ozaki T, Nakagawara A. Role of p53 in cell death and human cancers. Cancers . 2011;3:994–1013.
[7] Mantovani F, Collavin L, Sal D. G. Mutant p53 as a guardian of the cancer cell. Cell Death Differ. 2019;26:199–212.
[8] Hernandez Borrero LJ, El-Deiry WS. Tumor suppressor p53: biology, signaling pathways, and therapeutic targeting. Biochim Biophys Acta Rev Cancer. 2021;1876(1):188556.
[9] Petitjean A, Achatz MI, Borresen-Dale AL, et al. TP53 mutations in human cancers: functional selection and impact on cancer prognosis and outcomes. Oncogene. 2007;26:2157–2165.
[10] Hof J, Krentz S, van Schewick C, et al. Mutations and deletions of the TP53 gene predict nonresponse to treatment and poor outcome in first relapse of childhood acute lymphoblastic leukemia. J Clin Oncol. 2011;29:3185–3193.
[11] Irving JA, Enshaei A, Parker CA, et al. Integration of genetic and clinical risk factors improves prognostication in relapsed childhood B-cell precursor acute lymphoblastic leukemia. Blood. 2016;128:911–922.
[12] Weng W, Zhang P, Ruan J, et al. Prognostic significance of the tumor suppressor protein p53 gene in childhood acute lymphoblastic leukemia. Oncol Lett. 2020;19:549–556.
[13] Holmfeldt L, Wei L, Diaz-Flores E, et al. The genomic landscape of hypodiploid acute lymphoblastic leukemia. Nat Genet. 2013;45:242–252.
[14] Tran TH, Hunger SP. The genomic landscape of pediatric acute lymphoblastic leukemia and precision medicine opportunities. Semin Cancer Biol. 2020;84:144–152.
[15] Flach J, Shumilov E, Wiedemann G, et al. Clinical potential of introducing next-generation sequencing in patients at relapse of acute myeloid leukemia. Hematol Oncol. 2020;38:425–431.
[16] Ishida H, Iguchi A, Aoe M, et al. Panel-based next-generation sequencing identifies prognostic and actionable genes in childhood acute lymphoblastic leukemia and is suitable for clinical sequencing. Ann Hematol. 2019;98:657–668.
[17] Kohlmann A, Klein HU, Weissmann S, et al. The ınterlaboratory RObustness of next-generation sequencing (IRON) study: a deep sequencing investigation of TET2, CBL and KRAS mutations by an international consortium involving 10 laboratories. Leukemia. 2011;25:1840–1848.
[18] Metzker ML. Sequencing technologies – the next generation. Nat Rev Genet. 2010;11:31–46.
[19] Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American college of medical genetics and genomics and the association for molecular pathology. Genet Med. 2015;17:405–424.
[20] Fortuno C, Lee K, Olivier M, et al. Specifications of the ACMG/AMP variant interpretation guidelines for germline TP53 variants. Hum Mutat. 2021;42:223–236.
[21] Bouaoun L, Sonkin D, Ardin M, et al. TP53 variations in human cancers: new lessons from the IARC TP53 database and genomics data. Hum Mutat. 2016;37:865–876.
[22] Landrum MJ, Chitipiralla S, Brown GR, et al. ClinVar: improvements to accessing data. Nucleic Acids Res. 2020;48: D835–D844.
[23] Pui CH. Precision medicine in acute lymphoblastic leukemia. Front Med. 2020;14:689–700.
[24] Zhang J, Mullighan CG, Harvey RC, et al. Key pathways are frequently mutated in high-risk childhood acute lymphoblastic leukemia: a report from the children's oncology group. Blood. 2011;118:3080–3087.
[25] Ma X, Edmonson M, Yergeau D, et al. Rise and fall of subclones from diagnosis to relapse in pediatric B-acute lymphoblastic leukaemia. Nat Commun. 2015;6:6604.
[26] Qian M, Cao X, Devidas M, et al. TP53 germline variations ınfluence the predisposition and prognosis of B-Cell acute lymphoblastic leukemia in children. J Clin Oncol. 2018;36:591–599.
[27] Stengel A, Schnittger S, Weissmann S, et al. TP53 mutations occur in 15.7% of ALL and are associated with MYC-rearrangement, low hypodiploidy, and a poor prognosis. Blood. 2014;124:251–258.
[28] Stengel A, Kern W, Haferlach T, et al. The impact of TP53 mutations and TP53 deletions on survival varies between AML, ALL, MDS and CLL: an analysis of 3307 cases. Leukemia. 2017;31:705–711.
[29] Richter-Pechanska P, Kunz JB, Hof J, et al. Identification of a genetically defined ultra-high-risk group in relapsed pediatric T-lymphoblastic leukemia. Blood Cancer J. 2017;7:e523.
[30] Harutyunyan A, Klampfl T, Cazzola M, et al. p53 lesions in leukemic transformation. N Engl J Med. 2011;364(5):488–490.
[31] Jethwa A, Hullein J, Stolz T, et al. Targeted resequencing for analysis of clonal composition of recurrent gene mutations in chronic lymphocytic leukaemia. Br J Haematol. 2013;163:496–500.
[32] Chang MT, Asthana S, Gao SP, et al. Identifying recurrent mutations in cancer reveals widespread lineage diversity and mutational specificity. Nat Biotechnol. 2016;34:155–163.
[33] Kurniawan AN, Hongyo T, Hardjolukito ES, et al. Gene mutation analysis of sinonasal lymphomas in Indonesia. Oncol Rep. 2006;15:1257–1263.
[34] Natan E, Baloglu C, Pagel K, et al. Interaction of the p53 DNA-binding domain with its n-terminal extension modulates the stability of the p53 tetramer. J Mol Biol. 2011;409(3):358–368.
[35] Murnyak B, Hortobagyi T. Immunohistochemical correlates of TP53 somatic mutations in cancer. Oncotarget. 2016;7:64910–64920.
[36] Cole AJ, Dwight T, Gill AJ, et al. Assessing mutant p53 in primary high-grade serous ovarian cancer using immunohistochemistry and massively parallel sequencing. Sci Rep. 2016;6:26191.
[37] Yu CH, Chang WT, Jou ST, et al. TP53 alterations in relapsed childhood acute lymphoblastic leukemia. Cancer Sci. 2020;111:229–238.
[38] Tessoulin B, Eveillard M, Lok A, et al. p53 dysregulation in B-cell malignancies: more than a single gene in the pathway to hell. Blood Rev. 2017;31:251–259.
[39] Ye F, Wang T, Liu A, et al. Clinical significance of TP53 abnormalities in newly diagnosed multiple myeloma. Turk J Haematol. 2021;38:246–253.
[40] Ueno H, Yoshida K, Shiozawa Y, et al. Landscape of driver mutations and their clinical impacts in pediatric B-cell precursor acute lymphoblastic leukemia. Blood Adv. 2020;4:5165–5173.
[41] Prochazka KT, Pregartner G, Rucker FG, et al. Clinical implications of subclonal TP53 mutations in acute myeloid leukemia. Haematologica. 2019;104:516–523.
[42] Malcikova J, Stano-Kozubik K, Tichy B, et al. Detailed analysis of therapy-driven clonal evolution of TP53 mutations in chronic lymphocytic leukemia. Leukemia. 2015;29:877–885.
[43] Zerdoumi Y, Lanos R, Raad S, et al. Germline TP53 mutations result into a constitutive defect of p53 DNA binding and transcriptional response to DNA damage. Hum Mol Genet. 2017;26:2812.
[44] Wong RP, Tsang WP, Chau PY, et al. p53-R273H gains new function in induction of drug resistance through down-regulation of procaspase-3. Mol Cancer Ther. 2007;6:1054-1061.
[45] Uddin MB, Roy KR, Hosain SB, et al. An N(6)-methyladenosine at the transited codon 273 of p53 pre-mRNA promotes the expression of R273H mutant protein and drug resistance of cancer cells. Biochem Pharmacol. 2019;160:134–145.
[46] Olive KP, Tuveson DA, Ruhe ZC, et al. Mutant p53 gain of function in two mouse models of Li-Fraumeni syndrome. Cell. 2004;119:847–860.