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TP53 alterations in relapsed childhood acute lymphoblastic leukemia

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Abstract

TP53 alterations are frequent relapse-acquired mutations in childhood acute lymphoblastic leukemia (ALL). The present study evaluated the clinical significance of relapsed childhood ALL in Taiwan. Diagnostic and/or relapsed bone marrow or peripheral blood was obtained from 111 children with relapsed ALL who were initially treated by using Taiwan Pediatric Oncology Group (TPOG) ALL protocols from January 1997 to May 2018. Mutations were detected by PCR and sequencing, as well as by multiplex ligation-dependent probe amplification to detect copy number alterations. Copy number and/or sequence alterations of TP53 were detected in 29% (28 of 98) and in 46% (6 of 13) of patients with relapsed B-cell and T-cell ALL, respectively. This incidence was much higher than that in several similar studies conducted in Caucasian populations. Seventy percent of all TP53 alterations were gained at relapse in 67 matched samples by back-tracking matched diagnostic samples. TP53 alterations were associated with lower 5-year event-free survival (EFS) and overall survival (OS) rates ($P = .013$ and $P = .0002$, respectively). Multivariate analysis confirmed the prognostic significance of TP53 alterations. Forty-five patients received hematopoietic stem-cell transplantations

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post-relapse. Patients with *TP53* alterations (14/45) had inferior 5-year EFS and OS than patients without *TP53* alterations after transplantation (*P* = .002 and *P* = .001, respectively). The significance of these *TP53* alterations for patients who received transplantations was confirmed by multivariate analysis. In conclusion, *TP53* alterations were enriched and useful as prognostic markers in relapsed childhood ALL.

KEYWORDS

MLPA, relapse-acquired mutations, relapsed childhood ALL, Taiwan, *TP53* alterations

1 | INTRODUCTION

Acute lymphoblastic leukemia (ALL) is the most common childhood malignancy, accounting for approximately 25% of childhood malignancies.[1] Overall 5-year event-free survival (EFS) rate for this disease now exceeds 80% in developed countries;[2-4] however, 10%-20% of patients succumb to recurrences. The prognosis for relapsed ALL is dismal, even with aggressive salvage strategies.[5] Thus, relapsed ALL remains a leading cause of cancer-related deaths in children. Current comprehensive genome sequencing of triplicate diagnostic, remission, and relapsed samples from childhood ALL suggests that most relapsed patients have new, secondary genetic alterations or acquired lesions that likely occurred in a minor clone after the original diagnosis of ALL. One of the pathways enriched in recent relapse pair samples is *TP53* mutations.[6]

TP53 mutations are infrequent (2%-3%) at initial diagnosis and have not been found to be an important marker for prognosis or in the prediction of treatment response in most childhood ALL clinical trials.[7,8] However, *TP53* alterations are frequently found in low-hypodiploidy ALL, with a somatic mutation rate of up to 90%.[9] The incidence of *TP53* alterations is higher in relapsed childhood ALL samples than in diagnostic samples.[10,11] Back-tracking studies showed that most *TP53* genetic alterations were relapse-specific,[10] which was confirmed through whole exome sequencing.[6]

Numerous prognostic markers predict disease outcomes or relapse in newly diagnosed childhood ALL, especially B-cell ALL.[1,3,12,13] *TP53* alterations are important genetic events, but poor prognostic markers for relapsed childhood ALL in clinical trials.[10,11] After relapse, minimal residual disease measurements might be the only prognostic marker after reinduction of chemotherapy to predict survival after stem-cell transplantation.[14] *TP53* relapse-specific mutations were observed in approximately 10% of relapsed Caucasian patients.[10,11] However, there are currently few prognostic markers for relapsed patients that indicate a successful return to remission.[5]

The actual incidence of *TP53* alterations in childhood ALL in Taiwan remains unknown. Therefore, we profiled the frequency of genetic alterations and impact of *TP53* alterations on survival rate of relapsed childhood ALL patients treated with Taiwan Pediatric Oncology Group (TPOG) ALL protocols in a medical center in Taiwan.

2 | MATERIALS AND METHODS

2.1 | Patients and protocols

Bone marrow or peripheral blood samples taken at diagnosis, relapse, and/or clinical remission were available for 111 children with relapsed ALL; 98 with B-cell ALL, and 13 with T-cell ALL) from January 1997 to December 2015. These patients were enrolled in the TPOG ALL 93, ALL 97 very high risk (VHR), ALL 2002, or ALL 2013 protocols; details of these protocols have been published elsewhere.[15] The TPOG ALL 2013 was modified from ALL 2002 by incorporating minimal residual disease (MRD) measurements to stratify treatment. After disease relapse, physicians used different protocols, including the Berlin Frankfurt Münster (BFM) relapse protocol, upgrade to TPOG VHR protocols or FLAG.[5,16,17] This study was approved by the Institutional Review Board of National Taiwan University Hospital and all participants or their guardians provided written informed consent in accordance with the Declaration of Helsinki.

2.2 | Genomic DNA extraction

Lymphoblasts were purified from bone marrow or peripheral blood specimens by the Ficoll-Paque centrifugation method according to the manufacturer's instructions (GE Healthcare). Genomic DNA was extracted from leukemic cells using standard phenol/chloroform-based methods. Briefly, 1 million cells were lysed in 10 mmol/L Tris-HCl, 10 mmol/L NaCl, 10 mmol/L EDTA, 20 µg proteinase K, and 0.5% SDS by incubating at 37°C for 16 hours. Total RNA was further removed by adding 500 µg PureLink RNase A (Invitrogen) and incubating for 10 minutes at 37°C. An equal volume of phenol-chloroform-isopropanol (25:24:1) was added to lysates and mixed by shaking vigorously, followed by centrifugation at 16 100 g at 4°C for 5 minutes. The upper aqueous phase was transferred to a fresh tube; genomic DNA was then precipitated by adding 2× volume −80°C 100% ethanol. The DNA pellet was washed with 75% ethanol and rehydrated with Tris-EDTA buffer. Concentration of DNA was determined using a NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific).

TABLE 1 TP53 alterations in patients with first relapse of childhood acute lymphoblastic leukemia

Patient ID	Type	MLPA results TP53 locus	TP53 mutation by Sanger	Exon	Coding DNA position	Base change	Codon number	AA change	Origin of alteration	BMT	Outcome
196	B	ND	Missense	5	523	G→A	175	R→H	Diagnosis	Yes	Deceased
331	B	ND	Missense	8	800	G→C	267	R→P	Relapse	No	Deceased
488	T	ND	Missense	7	733	G→A	245	G→S	Relapse	No	Deceased
549	B	ND	Missense	7	725	G→A	242	C→Y	Relapse	Yes	Deceased
656	B	ND	Missense	7	743	G→A	248	R→Q	Relapse	Yes	Deceased
306	B	NA	Missense	7	743	G→A	248	R→Q	Somatic	No	Deceased
328	B	NA	Missense	8	817	C→T	273	R→C	Other	No	Deceased
438	B	NA	Insertion	8	844-845	6nt insertion	281-282	LV insertion	Relapse	Yes	Deceased
463	B	NA	Missense	5	509	C→T	170	T→M	Relapse	No	Deceased
508	B	NA	Missense	6	583	A→T	195	I→F	Germline	No	Deceased
9001	B	NA	Missense	8	844	C→T	282	R→W	Other	No	Deceased
85	B	ND	Missense; Missense	5; 8	527; 818	G→A; G→A	176; 273	C→Y; R→H	Relapse; Relapse	Yes	Deceased
612	B	ND	Missense; Missense	7; 8	743; 799	G→A; C→G	248; 267	R→Q; R→G	Relapse; Relapse	No	Deceased
682	B	ND	Missense; Missense	7; 8	743; 817	G→A; C→T	248; 273	R→Q; R→C	Relapse; Relapse	Yes	Deceased
453	T	NA	Missense	7	743	G→C	248	R→P	Relapse	Yes	Deceased
522	B	NA	Missense; Missense	5; 7	396; 743	G→T; G→A	132; 248	K→N; R→Q	Relapse; Relapse	No	Deceased
9002	B	NA	Missense	7	743	G→A	248	R→Q	Other	No	Deceased
915	T	NA	Missense; Missense; Missense	5; 5; 7	404; 475; 731	G→A; G→C; G→C	135; 159; 244	C→Y; A→P; G→A	Somatic; Somatic; Somatic	Yes	Deceased
748	B	ND	Missense; Missense; Missense	5; 8; 8	473; 791; 811	G→A; T→G; G→C	158; 264; 271	R→H; L→R; E→Q	Relapse; Relapse; Relapse	No	Deceased
353	T	11	ND						Relapse	No	Deceased
180	B	2-11	ND						Other	Yes	Deceased
657	B	2-11	ND						Somatic	No	Deceased
753	B	2-11	ND						Other	No	Deceased
863	B	2-11	ND						Other	No	Deceased
368	B	2-11	ND						Other	Yes	Deceased

(Continues)

2.3 | Mutation analysis of *TP53*

For *TP53* (NG_017013) sequence analysis, coding regions were amplified using Phusion Hot Start II High-Fidelity PCR Master Mix (Thermo Fisher Scientific). PCR was carried out as follows: 98°C for 30 seconds, then 38 cycles of 98°C for 30 seconds, annealing for 30 seconds and 72°C for 30 seconds, followed by a final extension at 72°C for 5 minutes. PCR products were confirmed using 2% agarose gel electrophoresis. We cleaned up the remaining PCR products by incubating with a reaction mixture of FastAP Thermosensitive Alkaline Phosphatase and exonuclease I (both Thermo Fisher Scientific) for 15 minutes at 37°C followed by 85°C for 15 minutes to inactivate the reaction. Sanger sequencing was done using an ABI 3730XL DNA analyzer (Applied Biosystems). The sequencing results aligned to the NCBI GenBank entry NM_000546 using SnapGene 4.1.3 software. Details of PCR, sequencing primers, and annealing temperatures are provided in Table S1.

2.4 | Multiplex ligation-dependent probe amplification

To detect structural alterations of *TP53*, multiplex ligation-dependent probe amplification (MLPA) reactions were carried out according to the manufacturer's instructions. The MRC-Holland SALSA MLPA probe mix P056-C1 kit (MRC Holland) was used. PCR fragments were separated by capillary electrophoresis on a Life Technologies 3500 genetic analyzer (Thermo Fisher Scientific). MLPA data were analyzed using Coffalyser.Net v.140721.1958 (MRC Holland). Relative copy numbers were determined after normalization of peaks against controls. Values between 0.75 and 1.3 were considered within the normal range. Values below 0.75 or above 1.3 implied a deletion or gain, respectively. Values below 0.25 indicated biallelic deletion.

2.5 | Statistical analyses

Equality between categoric and continuous parameters was tested with the chi-squared or Fisher's exact tests. The Kaplan-Meier method was used to estimate survival curves associated with *TP53* alterations for relapsed ALL. The log-rank test compared different survival curves between *TP53* alterations and wild-type ALL-relapsed patients. EFS after first relapse was defined as the time from first relapse to any adverse events. OS was defined as time from diagnosis to death. Patients who did not suffer any adverse events within the follow-up period were censored. EFS of patients with no response to chemotherapy (refractory), death, and second relapse in induction was set to 0. Statistical software SAS 9.4 (SAS Institute) was used for all data analysis. Univariate and multivariate Cox regression analyses were carried out to evaluate hazard ratios (HR) and 95% confidence intervals (CI) of risk factors. We inspected the associations between the presence of alterations and the value of clinical parameters. MRD was not included in the parameters,

TABLE 1 (Continued)

Patient ID	Type	MLPA results TP53 locus	TP53 mutation by Sanger	Exon	Coding DNA position	Base change	Codon number	AA change	Origin of alteration	BMT	Outcome
649	B	2-11;10-11	ND						Other	Yes	Deceased
50	B	2-11	Missense	7	742	C→T	248	R→W	Diagnosis	No	Living
342	T	2-11	Missense	7	742	C→T	248	R→W	Relapse	No	Deceased
630	B	2-11	Missense	5	524	G→A	175	R→H	Relapse	Yes	Deceased
774	B	2-11	Frameshift	10	1022	1nt deletion	341	Frameshift	Diagnosis	No	Living
845	B	2-11	Nonsense	5	158	G→A	53	W→*	Diagnosis	No	Deceased
641	B	2-11	Missense	5	482	C→A	161	A→D	Relapse	Yes	Deceased
162	B	2-11	Indels; Indels	8; 8	818-843; 821-863	25nt deletion with 1nt insertion; 41nt deletion with 6nt insertion	274-281; 274-288	VCACPGRD deletion; Frameshift	Somatic; Somatic	Yes	Deceased
478	T	2-11	Missense; Nonsense	3; 4	91; 310	G→A; C→T	31; 104	V→I; Q→*	Relapse; Germline	No	Deceased

Abbreviations: AA, amino acid; BMT, bone marrow transplantation; Indels, insertion with deletion; MLPA, multiplex ligation-dependent probe amplification; NA, data not available; ND, not detected; nt, nucleotide.

attributable to inadequate data. All tests were two-sided, and values of P < .05 were considered significant.

3 | RESULTS

3.1 | Frequency and type of *TP53* alterations in relapsed childhood ALL

Detailed clinical characteristics of the studied cohort are described in Table 1. To obtain the associations of *TP53* alterations with different clinical, cytogenetic, and treatment parameters present at the time of relapse, these variables between patients with or without *TP53* alterations and between *TP53* alteration subgroups were compared statistically (Table 2). Hypodiploidy was enriched in *TP53* alterations. However, there were more patients with *KMT2A* rearrangements

and T-cell ALL in relapsed cases with *TP53* alterations than patients with *ETV6-RUNX1*, hypodiploidy, and *BCR-ABL1*.

TP53 alterations were identified in 34 patients (31%). Within the B-cell ALL relapse group, 28 patients (29%) had *TP53* gene alterations: six (21%) had deletions in the absence of a mutation (referred to as an "exclusive deletion"), 16 (57%) had mutation(s) in the absence of a deletion (referred to as an "exclusive mutation"), and six (21%) had mutation(s) and deletion(s). In T-cell ALL patients, *TP53* alterations were identified in six patients (46%): three had an exclusive mutation, one had an exclusive deletion, and two had mutation(s) and a deletion (Figure 1A). *TP53* P72R, a known polymorphism, exon 1 alterations, and intronic deletions were not evaluated in this study.

TP53 alterations are summarized in Table 1. There were 25 missense mutations, four small indels, two exonic deletions, and deletion of the entire *TP53* gene among them. No mutations were detected in the remaining coding region in patients with an

TABLE 2 Clinical characteristics, genetics, and outcome of acute lymphoblastic leukemia relapsed patients with *TP53* alterations

Characteristics	Analysis of patients with altered vs wild-type *TP53*					Analysis of patients with mutated vs deleted vs mutated and deleted vs wild-type *TP53*									
	Wild-type		Alteration-type			Wild-type		Exclusive mutation		Exclusive deletion		Mutation and deletion			
	N	%	N	%	P-value	N	%	N	%	N	%	N	%	P-value
All patients	77	100	34	100		77	100	19	100	7	100	8	100	
Gender														
Male	46	59.7	22	64.7	.677	46	59.7	13	68.4	3	42.9	6	75.0	.597
Female	31	40.3	12	35.3		31	40.3	6	31.6	4	57.1	2	25.0	
Age at relapse, years														
<5	12	15.58	9	26.47	.388	12	15.6	4	21.1	3	42.9	2	25	.511
≥5 and <10	22	28.57	9	26.47		22	28.6	7	36.8	1	14.2	1	12.5	
≥10	43	55.84	16	47.06		43	55.8	8	42.1	3	42.9	5	62.5	
Time to relapse														
Very early	34	44.2	19	55.9	.022	34	44.2	11	57.9	4	57.1	4	50.0	.105
Early	8	10.4	8	23.5		8	10.4	5	26.3	2	28.6	1	12.5	
Late	35	45.5	7	20.6		35	45.5	3	15.8	1	14.3	3	37.5	
ALL subtypes														
T-ALL	7	9.09	6	17.6	.005	7	9.09	3	15.8	1	14.3	2	25.0	.089
TCF3-PBX1	1	1.3	1	2.9		1	1.3	1	5.3	0	0.0	0	0.0	
11q23	6	7.8	7	20.6		6	7.8	4	21.1	1	14.3	2	25.0	
Hyperdiploidy	5	6.5	1	2.9		5	6.5	1	5.3	0	0.0	0	0.0	
BCR-ABL1	5	6.5	0	0.0		5	6.5	0	0.0	0	0.0	0	0.0	
ETV6-RUNX1	6	7.8	2	5.9		6	7.8	2	10.5	0	0.0	0	0.0	
Hypodiploidy	0	0.0	4	11.8		0	0.0	1	5.3	1	14.3	2	25.0	
Other	47	61.0	13	38.2		47	61.0	7	36.8	4	57.1	2	25.0	
BMT														
None	46	59.7	20	58.8	1.000	46	59.7	11	57.9	4	57.1	5	62.5	1.000
BMT	31	40.3	14	41.2		31	40.3	8	42.1	3	42.9	3	37.5	

Abbreviations: ALL, acute lymphoblastic leukemia; BMT, bone marrow transplantation.

aTime to relapse: very early, within 18 months from diagnosis to first relapse of ALL; early, between 18 months and 30 months from diagnosis to first relapse of ALL; late, more than 30 months from diagnosis to first relapse of ALL.
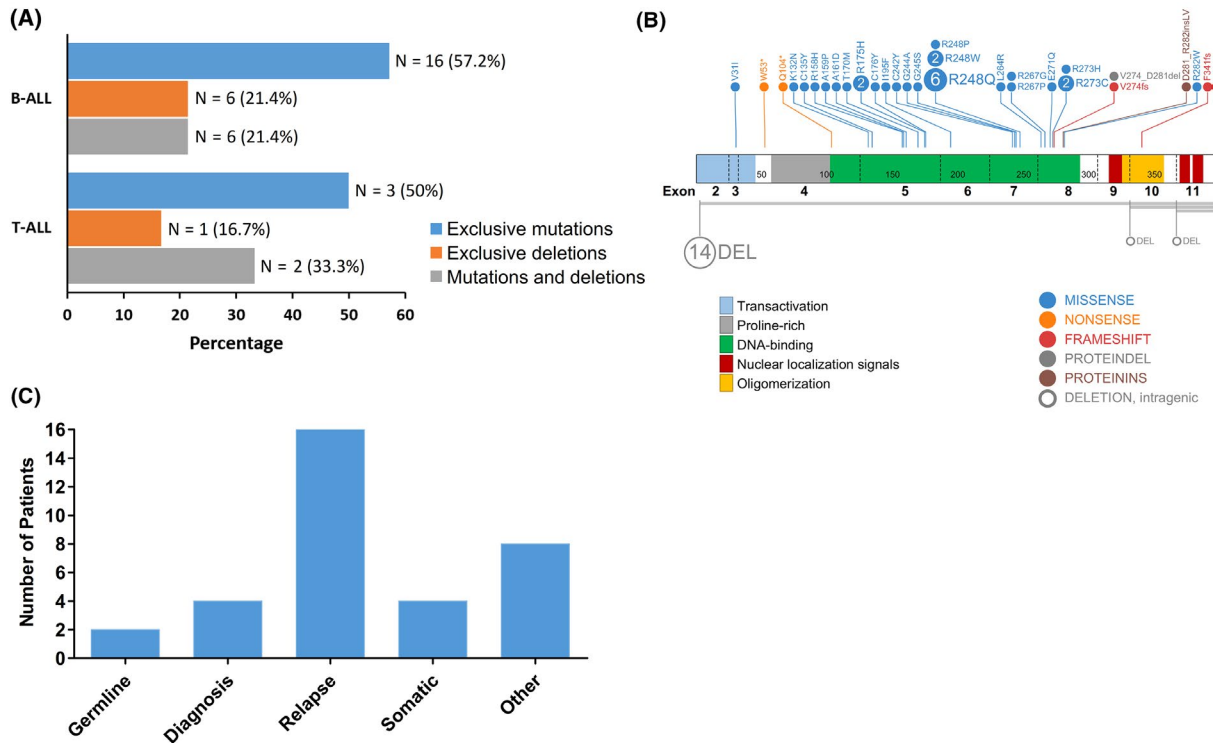
FIGURE 1 *TP53* alterations in first relapse of childhood acute lymphoblastic leukemia (ALL). A, Frequency of *TP53* alterations in patients with relapsed B-cell and T-cell ALL. B, Distribution of *TP53* alterations detected by sequencing in relapsed B-cell ALL and T-cell ALL over *TP53* coding regions. DNA-binding domains involved in most *TP53* sequence alterations. C, Retrospective copy number and sequence analysis of matched diagnostic and recurrent ALL samples from 67 patients with *TP53* alterations at relapse. Bar chart shows the frequency of mutations at both stages of the disease

exclusive *TP53* deletion. The most frequent *TP53* mutations were detected in the DNA-binding domain (exons 5-8) (Figure 1B). In morphological remission samples, we identified missense mutations in two patients (478 and 508). There were eight patients with more than one *TP53* alteration. There were six patients with multiple mutations, six with single mutations and single deletions, and two with single deletions and multiple mutations.

3.2 | *TP53* alterations in matched diagnosis and relapsed patients

Diagnostic and relapse samples were available for 69 patients. Matched-initial ALL samples were compared with those from relapsed patients with *TP53* alterations to trace whether *TP53* alterations were persistent or acquired between initial diagnosis and first relapse. Approximately 70% of all mutations and deletions (16 of 24) were relapse-specific (Figure 1C). One patient acquired an exclusive deletion, seven patients acquired an exclusive mutation, and three patients acquired both a mutation and a deletion at relapse. Five patients had relapse-specific mutations, but we lacked additional material to check for deletions of *TP53*. Seven patients had somatic mutations of the *TP53* gene at first diagnosis. A single patient had both a mutation and a deletion at initial ALL diagnosis and acquired an additional mutation during relapse.

3.3 | Relationships between *TP53* alterations and clinical characteristics and survival rate

Five-year EFS and OS were analyzed for patients with and without *TP53* alterations. Five-year EFS and OS were inferior in patients with *TP53* alterations ($P = .013$ and $P = .0002$, respectively) after relapse (Figure 2). Of the 69 patients with paired samples, 5-year EFS and OS were inferior in patients with *TP53* alterations ($P = .06$ and $P = .018$, respectively) (Figure 3); the significance of *TP53* alterations on the inferior survival of relapsed B-cell ALL was similar (Figure S1). In T-cell ALL, patients with *TP53* alterations had inferior 5-year EFS and OS, although the number of cases in this subgroup was small (Figure S2). Patients with *TP53* alterations tended toward reinduction chemotherapy failure (Fisher's exact test, *P*-values .09 for the ALL cohort and .07 for the paired samples). Forty-five patients received bone-marrow transplantation as a rescue procedure; for them, *TP53* alterations correlated with lower 5-year EFS and OS (Figure 4).

3.4 | Multivariate analysis

Cox univariate regression model identified *TP53* alterations (HR = 1.7; 95% CI: 1.12-2.58, $P = .0130$) and age at onset of <1 year (HR = 2.34; 95% CI: 1.19-4.58, $P = .014$) as the strongest predictive factors for an unfavorable 5-year-EFS. Patients who

received bone marrow transplantation (BMT) presented with better 5-year EFS (HR = 0.49; 95% CI: 0.32-0.73, *P* = .001) than those who did not (Table 3). Multivariate Cox regression analysis showed that *TP53* alterations (HR = 1.52; 95% CI: 0.99-2.31, *P* = .054) resulted in marginal statistical significance. However, age at onset of <1 year (HR = 2.74; 95% CI: 1.36-5.52, *P* = .005) and BMT (HR = 0.46; 95% CI: 0.30-0.70, *P* = .0003) were clinically significant (Table 3).

TP53 alterations (HR = 2.38; 95% CI: 1.48-3.84, *P* = .0004) and age at onset of <1 year (HR = 4.07; 95% CI: 2.02-8.22, *P* < .0001) were the strongest predictive factors for an unfavorable 5-year OS. Clinical significance was noted in patients with *TP53* alterations (HR = 2.13; 95% CI: 1.30-3.47, *P* = .003) and age at onset of <1 year (HR = 3.94; 95% CI: 1.90-8.15, *P* = .0002; Table 3). Patients who received stem-cell transplantation presented with

a better 5-year OS; however, this trend did not reach statistical significance.

In patients who received BMT, *TP53* alterations (HR = 2.93; 95% CI: 1.46-5.87, *P* = .003) were identified as the strongest predictive factor for an unfavorable 5-year EFS. *TP53* alterations (HR = 2.88; 95% CI: 1.37-6.07, *P* = .005) and age at onset of <1 year (HR = 3.05; 95% CI: 1.19-7.79, *P* = .02) were clinically significant (Table S2).

4 | DISCUSSION

Approximately 31% of relapsed childhood ALL cases harbor genetic alterations of *TP53*, and the *TP53*-acquired alteration rate was 35% in sixty-nine patients with diagnostic samples to backtrack mutations, which is higher than that observed in Caucasian populations.
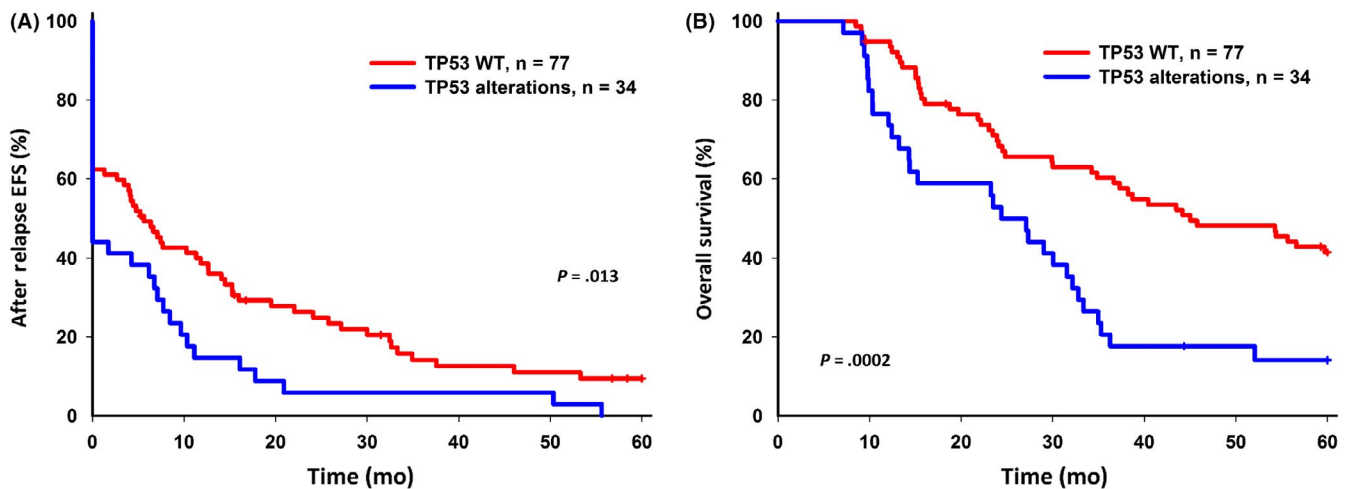

FIGURE 2 Five-year event-free survival (EFS) and overall survival (OS) of relapsed acute lymphoblastic leukemia (ALL) with *TP53* alterations. A, 5-year EFS for relapsed ALL with *TP53* wild-type (WT) and *TP53* alterations. B, 5-year OS for relapsed ALL with *TP53* WT and *TP53* alterations
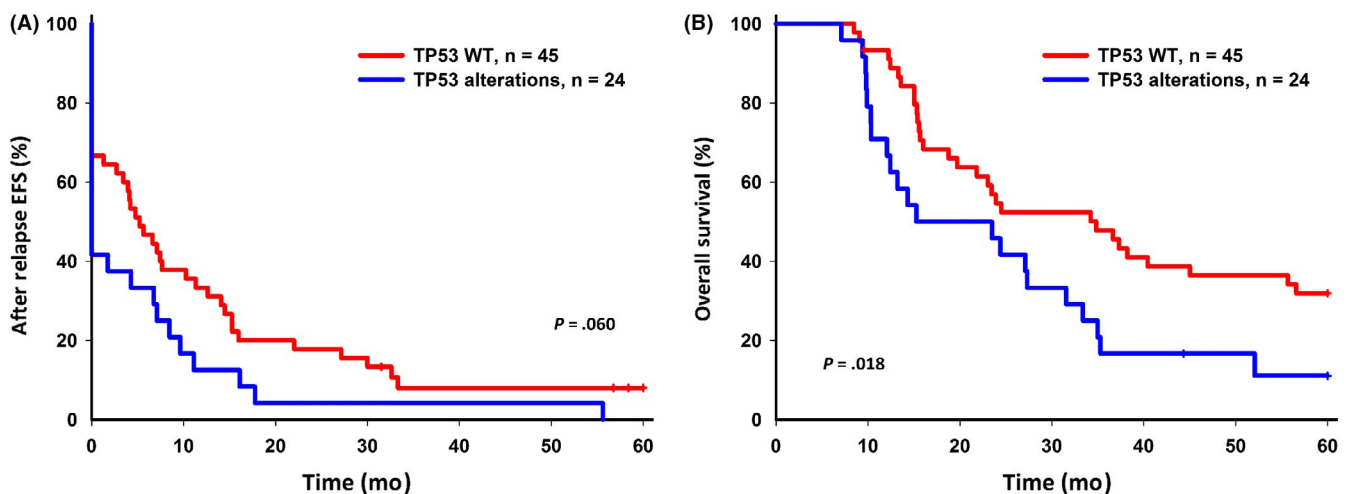

FIGURE 3 Five-year event-free survival (EFS) and overall survival (OS) of relapsed acute lymphoblastic leukemia (ALL) with *TP53* alterations in *69* patients with paired samples. A, 5-year EFS for relapsed ALL patients with *TP53* wild-type (WT) and *TP53* alterations. B, 5-year OS for relapsed ALL patients with *TP53* WT and *TP53* alterations

FIGURE 4 Comparison of bone marrow transplantation (BMT) therapy for 5-year event-free survival (EFS) and overall survival (OS) in relapsed acute lymphoblastic leukemia (ALL) patients with *TP53* alterations. A, 5-year EFS for relapsed ALL patients with *TP53* wild-type (WT) and *TP53* alterations. B, 5-year OS for relapsed ALL patients with *TP53* WT and *TP53* alterations
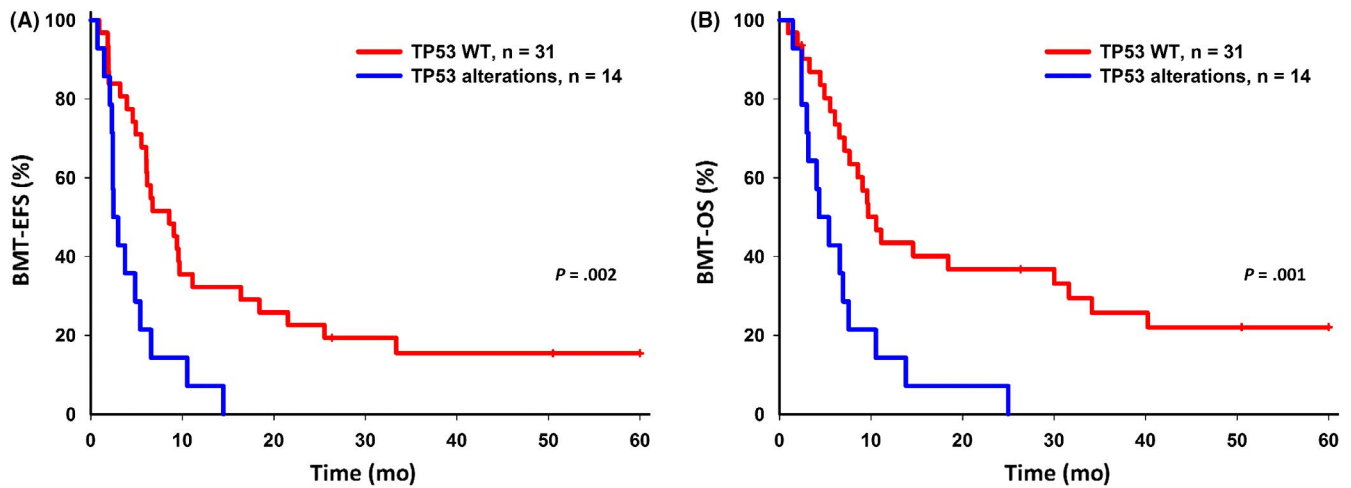
TABLE 3 Five-year EFS and OS using univariate and multivariate survival analysis in relapsed pediatric ALL patients

	Univariate			Multivariate		
	HR	95% CI	*P*-value	HR	95% CI	*P*-value
5-year EFS						
TP53 alterations	1.70	1.12-2.58	.013	1.52	0.99-2.31	.054
Gender	1.44	0.95-2.17	.088	1.40	0.91-2.14	.127
Age (<1 y)	2.34	1.19-4.58	.014	2.74	1.36-5.52	.005
ALL type	1.15	0.63-2.10	.658	1.29	0.68-2.44	.430
BMT	0.49	0.32-0.73	.001	0.46	0.30-0.70	.0003
5-year OS						
TP53 alterations	2.38	1.48-3.84	.0004	2.13	1.30-3.47	.003
Gender	1.41	0.87-2.29	.162	1.22	0.74-2.00	.438
Age (<1 y)	4.07	2.02-8.22	<.0001	3.94	1.90-8.15	.0002
ALL type	1.83	0.96-3.48	.065	2.24	1.13-4.46	.021
BMT	0.81	0.51-1.30	.389	0.71	0.44-1.16	.173

Abbreviations: ALL, acute lymphoblastic leukemia; BMT, bone marrow transplantation; CI, confidence interval; EFS, event-free survival; HR, hazard ratio; OS, overall survival. Mutation type (reference = *TP53* wild-type); Gender (reference = female); Age (reference = ≥1 y); ALL type (reference = T-ALL).

These alterations were associated with a higher rate of reinduction failure; most were relapse-specific.

The incidence of *TP53* alterations has increased in relapsed childhood ALL over the incidence in diagnostic samples, which has been documented in several large clinical trials. Hof et al[10] sequenced *TP53* hot-spot exons 5-8 (DNA-binding domain) and deletion status in 265 first-relapsed childhood ALL patients, whereas Irving et al[11] analyzed a larger cohort of relapsed childhood B-cell ALL patients. These large international trials reported similar frequencies of *TP53* alterations for relapsed B-cell ALL, 11%-12%. Richter-Pechańska et al[18] conducted another cohort study of relapsed T-cell ALL patients, reporting that 13% of samples from relapsed T-cell ALL patients harbored *TP53*

alterations; only 1.4% of the initial diagnostic samples had *TP53* alterations. Our case number is approximately half of those of these recent studies; however, the results are similar to those of previous studies using small case numbers, irrespective of B-cell or T-cell ALL.[19-21] The reason for the higher incidence of *TP53* alterations in relapsed samples remains unknown, although we did not use next-generation sequencing to target the sequences. *TP53* alterations might be identified in other pediatric cancers, such as medulloblastoma, osteosarcoma, and rhabdomyosarcoma. However, this information is not available from Taiwanese patients; there are no reports of the incidence of somatic, acquired *TP53* alterations or germline variants in childhood ALL or other pediatric malignancies. This is the first report of acquired somatic

mutations in relapsed ALL patients in Taiwan. Given the much higher incidence of acquired *TP53* alterations in childhood ALL, a larger clinical trial to detail the true somatic and germline variants of *TP53* in other pediatric malignancies, particularly in relapsed samples, is worthy of investigation in Taiwan.

The prognostic values of *TP53* alterations have been documented in several large-scale international clinical trials for relapsed childhood ALL. *TP53* alterations are associated with nonresponse to chemotherapeutic treatment and poor EFS and OS.[10] Most groups used time to relapse, relapse site, and cytogenetic alterations to define risk groups after recurrence.[10] Patients with a *TP53* alteration tended to have a very early relapse and should be classified and treated as high-risk patients.[11] Irrespective of the initial chemotherapy response, patients with *TP53* alterations tend to have refractory disease, second relapses, and dismal outcomes. For relapsed T-cell ALL, *TP53* mutations were the best predictor of a second event in the cohort of Richter-Pechańska.[18] Almost all patients with *TP53* alterations died of disease very soon after relapse, whereas patients without *TP53* alterations survived. In this cohort, 47 patients received BMT; infant ALL patients apparently did not benefit from stem-cell transplantation. Other treatment strategies might be indicated for this aggressive ALL.[22-24] Our analyses suggest that somatic alteration of *TP53* at relapse is a good predictor of transplantation outcomes.

We identified two patients with *TP53* mutations from remission samples, suggesting that these mutations were not somatic, but of germline origin. *TP53* germline mutations are enriched in low-hypodiploidy patients and are common in Li-Fraumeni syndrome.[9] *TP53* germline mutations result in a poorer outcome than other common subtypes of childhood ALL, such as *ETV6-RUNX1* and hyperdiploidy. A recent study showed *TP53* germline variants (not common mutations) also contribute to significant clinical outcomes.[25] Excluding the low hypodiploidy subtype, which occurred in close relationship with *TP53* germline mutations, children with *TP53* pathogenic variants still experienced significantly worse outcomes than those without.

There have also been studies investigating the significance of *TP53* alterations in diagnostic ALL samples. Such alterations were profiled in B-cell ALL by Forero-Castro et al[26] They reported that patients with *TP53* alterations had lower 5-year OS and EFS and higher relapse rates after multivariate analysis. In a study of 625 ALL patients by Stengel et al,[27] overall *TP53* mutation incidence was 16%, increasing with age. In addition to the low hypodiploidy subtype, *TP53* mutations were also enriched in ALL with *MYC* rearrangements, recently classified as a subtype of B-cell ALL.[28] Stengel et al used next-generation sequencing to profile the *TP53* mutations. In addition to low hypodiploidy, high *TP53* mutation loads also correlated with high hyperdiploidy and complex karyotypes. B-cell ALL had a higher mutation load than T-cell ALL. Median OS was significantly shorter for patients with *TP53* mutations than for patients with wild-type TP53.[27] Although the clinical significance of diagnostic *TP53* alterations requires validation in other childhood ALL clinical trials, *TP53* alterations, including diagnostic, germline, and relapse alterations, might have clinical significance in childhood ALL and, therefore, warrant identification in future ALL clinical trials.

Although *TP53* alterations have clinical significance, it remains difficult to ablate poor clinical outcomes. Just as in other cancers, in childhood ALL, the most common *TP53* missense mutations are detected in the DNA-binding domain. This might result in accumulation of mutant p53 and poor therapy response, with inferior outcomes. Restoration of the wild-type p53 protein or ablation of the mutant p53 protein might be a fruitful approach for targeting these *TP53* alterations. In a preclinical patient-derived *TP53*-mutant ALL xenograft model by Demir et al, a small molecule, APR-246, had a therapeutic effect and resulted in excellent leukemia-free survival. Rebuilding tumor-suppressive functions by targeting mutant p53 proteins seems to be a reasonable therapeutic strategy and is likely worth future clinical trials.[29,30]

In conclusion, our findings indicate that *TP53* alterations at relapse may be used as a molecular marker for therapeutic failure in relapsed pediatric ALL patients. The frequency of *TP53* alterations at relapse in the present study was much higher than that reported in Caucasians. This finding shows the need for a larger prospective clinical trial in Taiwan. *TP53* alterations may aid in future risk classification of children with relapsed ALL. Targeting of mutant p53 proteins might warrant further clinical trials as a treatment strategy.

CONFLICTS OF INTEREST

Authors declare no conflicts of interest for this article.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.