Received: 16 June 2024 | Accepted: 13 February 2025

DOI: 10.1002/hem3.70115

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TP53 deletion as an MRD-dependent risk factor in childhood B-ALL: A post hoc analysis from a prospective cohort

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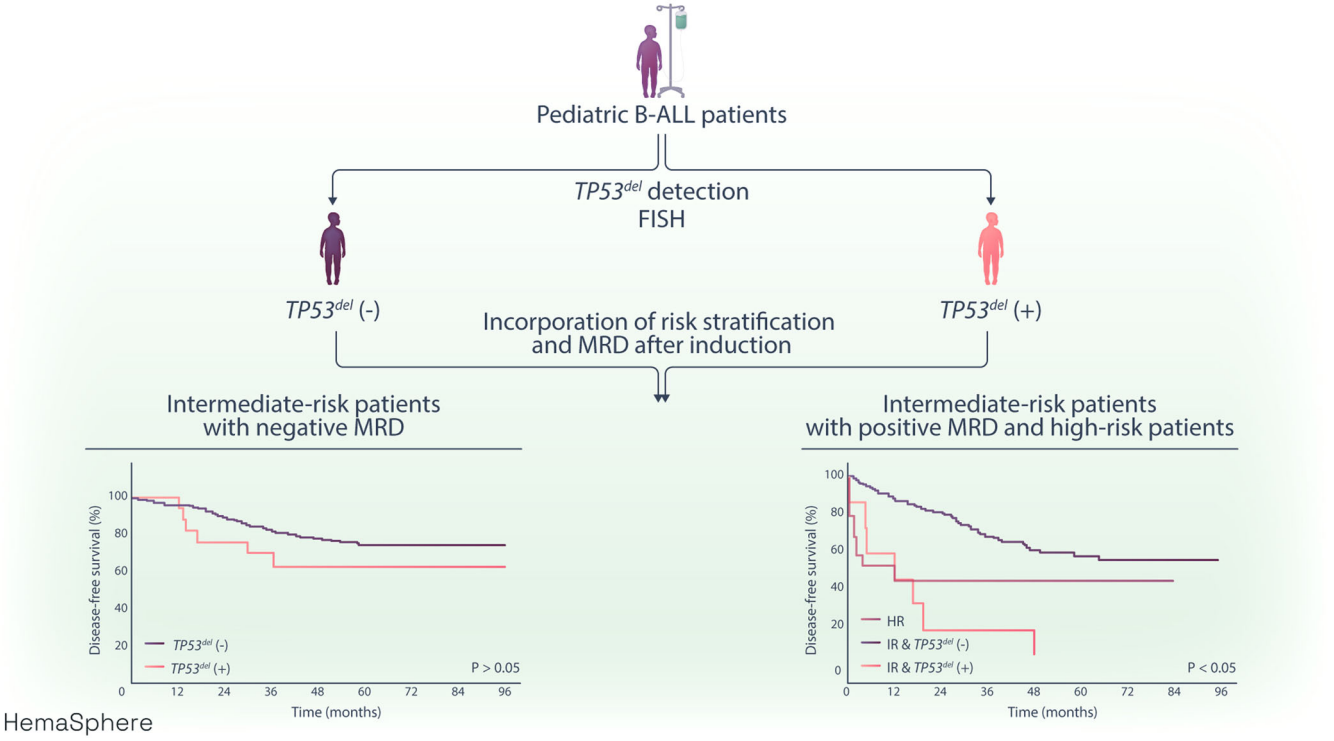
Graphical Abstract



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TP53 deletion as an MRD-dependent risk factor in childhood B-ALL: A post hoc analysis from a prospective cohort

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Abstract

The effect of *TP53* alterations on childhood B-cell acute lymphoblastic leukemia (B-ALL) remains unclear. To investigate the prognostic value of *TP53* deletion (*TP53*^{del}) and *TP53* mutation (*TP53*^{mut}), this post hoc study used fluorescence in situ hybridization test to detect *TP53*^{del} in 907 newly diagnosed B-ALL patients from a prospective cohort of Chinese Children's Cancer Group ALL-2015 trial. Targeted gene sequencing was used to identify *TP53*^{mut} in 342 out of the 907 patients. *TP53*^{del} was detected in 4.4% of patients. The frequency of hypodiploidy was higher in *TP53*^{del} subgroup (7.5% vs. 0.5%, $p = 0.002$), but patients with *TP53*^{del} were less likely to have other recurrent genetic abnormalities, including *BCR::ABL1*, *ETV6::RUNX1*, *TCF3::PBX1 and KMT2A* rearrangements. Univariable and multivariable analyses indicated that *TP53*^{del} was an independent risk factor for overall survival (OS) and disease-free survival (DFS). Furthermore, stratification analysis revealed that *TP53*^{del} was associated with lower 5-year DFS in patients with positive minimal residual disease (MRD) after induction in the intermediate-risk group (0.0% vs. 58.0% [95% confidence interval [CI] 49.2%–68.3%], $p < 0.001$), suggesting an MRD-dependent pattern. However, somatic *TP53*^{mut} was not associated with poor survival (81.8% [95% CI 61.9%–100.0%] vs. 84.9% [95% CI 81.1%-89.0%], $p = 0.971$). In summary, *TP53*^{del} may serve as a predictor for poor prognosis in pediatric B-ALL. In particular, children in the intermediate-risk group with positive MRD and *TP53*^{del} may require more aggressive treatment.

INTRODUCTION

Acute lymphoblastic leukemia (ALL) is caused by the malignant proliferation of B or T lymphocytes and is characterized by high biological and clinical heterogeneity.[1] It is the most common malignancy among children and accounts for approximately 25% of newly diagnosed pediatric cancers annually.[2] Nearly 85% of pediatric ALL is B-ALL.[1,3] The 5-year overall survival rate of pediatric ALL has significantly improved, exceeding 90% in recent decades, with advances in risk stratification and therapeutic regimens.[4] However, relapse occurs in 10%–20% of patients, leading to poor outcomes, and ALL remains the leading cause of cancer-related death in children.[3,5] Therefore, novel prognostic markers for the relapse risk of ALL are needed for adjustment of primary risk stratification-directed therapy to improve prognosis.[6]

TP53 is a tumor suppressor gene that is located on the short arm of chromosome 17 (17p13) and encodes the p53 protein.[1,7] The transcription factor p53 is an essential regulator of various signaling pathways, including cell cycle arrest, apoptosis, DNA repair, and genomic stability, and is known as a "genome guardian."[1,8–10] *TP53* aberrations, including mutations or deletions of the *TP53* gene, can cause p53 inactivation and are found in approximately 50% of human cancers.[1,9,11] In hematological malignancies, the frequency of *TP53* aberrations varies, with approximately 6% in pediatric ALL, but the frequency of *TP53* aberrations tends to increase with age.[12] As *TP53* gene mutation or deletion is rare in newly diagnosed childhood ALL, the relevant studies are few and heterogeneous, so its prognostic significance is still controversial.[9,13,14]

Due to large heterogeneity across studies, the prognostic significance of *TP53* aberrations in pediatric ALL remains to be explored. In this study, the frequency and clinical relevance of *TP53* aberrations, including mutations and deletions, were examined in a prospective cohort of pediatric B-ALL.

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METHODS

Patients and protocols

This study is a post hoc analysis of the prospective Chinese Children's Cancer Group ALL-2015 (CCCG-ALL-2015) cohort. A total of 907 patients aged 1–18 years with newly diagnosed B-ALL at the Institute of Hematology and Blood Diseases Hospital, Chinese Academy of Medical Sciences, and Peking Union Medical College (CAMS & PUMC) from May 2015 to October 2020 were enrolled in this study. All patients were treated with the CCCG-ALL-2015 protocol.[15] In this study, the peripheral blood blast count $>1.0 \times 10^9$/L on Day 5 of remission induction was defined as a poor dexamethasone response. Bone marrow minimal residual disease (MRD) was tested on Days 19 and 46 of induction therapy to assess the early treatment response. Bone marrow morphology on Day 46 with less than 5% blasts was considered to indicate complete remission (CR). The risk stratification criteria of the protocol have been described in detail in previous articles published by our Center.[15] According to the protocol, patients in the low-risk group would be adjusted to intermediate-risk if they had MRD of 1% or more on Day 19 of induction or the MRD on Day 19 was less than 1% but more than or equal to 0.1% and the MRD on Day 46 was more than 0.01%. All patients who had an MRD of 1% or more on Day 46 after induction therapy were assigned to the high-risk group.[15] Therefore, MRD on Day 19 equal to or greater than 1% and MRD on Day 46 equal to or greater than 0.01% were defined as positive in our study.

This study was approved by the institutional review boards of CAMS & PUMC. Informed consent was obtained from the guardians or patients before treatment for all patients.

Minimal residual disease testing

To assess early treatment response, we collected bone marrow for MRD analysis on Day 19 during remission induction and at the end of induction (Day 46). MRD was measured through eight-color multiparameter flow cytometry using a FACSCanto II flow cytometer (Becton Dickinson). And the data were analyzed using Kaluza software. We purchased all antibodies from Becton-Dickinson, Beckman-Coulter, BioLegend, and DAKO. The panel used for B-ALL MRD monitoring included the following antibodies: CD38-FITC, CD10-PE, CD34-PerCP-Cy5.5, CD19-PE-Cy7, CD81-APC, CD20-APC-H7, CD22-V450, and CD45-V500. The MRD detection sensitivity reached 10^{-4}, as clonal malignant cells <50 in 500,000 nucleated cells was defined as undetectable MRD. We performed MRD monitoring according to the identification of leukemia-associated immunophenotypes and abnormal differentiation markers.[16]

Cytogenetics and fusion genes

Karyotype analysis was performed on metaphase cells from unstimulated bone marrow after 24 h culture by traditional R-banding analysis for all patients at diagnosis. Cytogenetic alterations were analyzed and reported according to the International System for Human Cytogenetic Nomenclature (2020).[17] Furthermore, fusion genes such as *BCR::ABL1*, *ETV6::RUNX1*, and *TCF3::PBX1* and *KMT2A* rearrangement were tested with a polymerase chain reaction panel including 56 common fusion genes (Supporting Information S1: Table S1) in ALL.

TP53^{del} analysis

In all 907 patients, *TP53^{del}* was measured by interphase fluorescence in situ hybridization (FISH). Approximately 2 mL of bone marrow aspirates were obtained for *TP53^{del}* analysis before treatment. The LSI *TP53* (17p13.1) SpectrumRed/*CEP 17* (17p11.1-q11.1) SpectrumGreen Probes were obtained from Vysis laboratories (Abbott Laboratories). The *TP53* probe spanned approximately 172 kb and included *TP53*, *STA2*, *EFNB3*, *D17S655*, and *D17S812E* locus in the 17p13.1 chromosome region. *TP53* (17p13.1) gene was marked as red (R), and *CEP17* (17p11.1-q11.1) gene was marked as green (G). The normal signal feature was 2R2G, the positive signal feature was 2G1R, and -17 was 1R1G. At least 500 interphase cells were observed in each sample under fluorescence microscopy. Threshold rates <2.89% were negative, otherwise it was deletion.

TP53^{mut} analysis

TP53^{mut} testing was done in 342 of the 907 patients who were diagnosed between September 2018 to October 2020 using targeted gene sequencing as previously described.[18] DNA was extracted from bone marrow or peripheral blood at diagnosis. And bone marrow during complete remission was obtained for germline mutation identification. Mutations in the hotspot regions of *TP53* (exons 2–10) gene were identified through next-generation amplicon deep sequencing via the Illumina high-throughput sequencing platform (MiSeq platform). The variants were annotated by 1000 Genomes, ESP6500, Inhouse, dbSNP database, Human Gene Mutation Database, PolyPhen, SIFT and COSMIC to determine pathogenicity.[19] Only pathogenic and likely pathogenic somatic mutations were included in the prognostic analysis.

Statistical analysis

The Mann–Whitney *U* test was used for continuous variables. Categorical parameters were compared by Fisher's exact tests. The primary endpoint of the study was disease-free survival (DFS), which was calculated from CR to death, relapse, second malignancy or the last follow-up. For patients receiving chimeric antigen receptor T-cell therapy (CART) or hematopoietic stem-cell transplantation (HSCT), DFS was censored at the day of receiving CART or HSCT. Details of the patients receiving CART or HSCT treatments were described in Supporting Information S1: Tables S3 and S4. The secondary endpoint overall survival (OS) was measured from diagnosis to death or the last follow-up. DFS and OS were compared using the log-rank test. Univariable and multivariable analyses of DFS and OS were performed using the Cox proportional hazard model. All reported *p* values are two-sided, and factors with $p < 0.05$ were regarded as significant. Variables with $p < 0.05$ in the univariable analysis were included in the multivariable analysis. All the statistical analyses were performed in SPSS version 26.0 and R software 4.0.4.

RESULTS

Patient characteristics

A total of 954 patients newly diagnosed with B-ALL in our center were enrolled in the cohort, among whom 47 patients were excluded from this study (Figure 1). The exclusion criteria included the absence of FISH results for *TP53* ($n = 5$), treatment abandonment ($n = 4$), treatment-related deaths during remission induction ($n = 7$), infantile acute leukemia ($n = 5$), and incomplete marrow aspiration or unsatisfactory karyotype results at initial diagnosis ($n = 26$). The male-to-female ratio in this cohort was 1.5:1, and the median age was 5 years (range 1–17 years). After remission induction, 704/907 (77.6%) and 740/907 (81.6%) patients achieved MRD negativity on

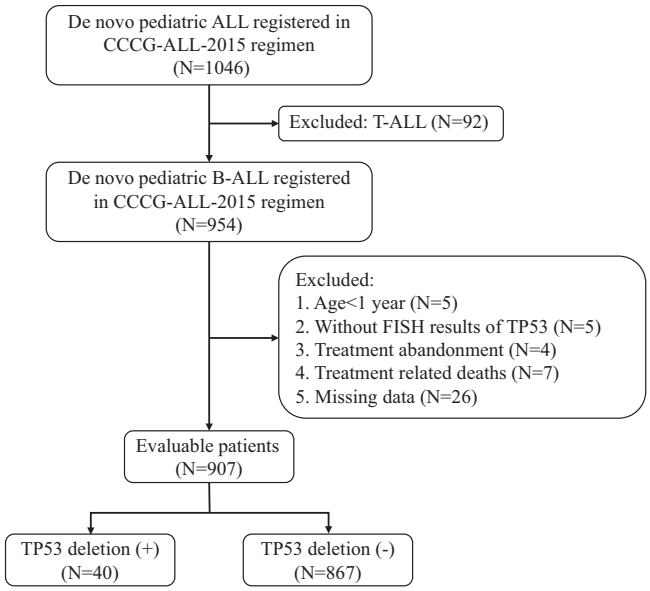

FIGURE 1 **Flow diagram summarizing the reasons for exclusion and the number of patients with or without *TP53* deletion.** ALL, acute lymphoblastic leukemia; CCCG-ALL-2015, Chinese Children's Cancer Group ALL-2015 protocol.

Days 19 and 46, respectively. Based on initial clinical features and MRD on Day 19 and Day 46 of remission induction, 460 (50.7%) patients were classified as low-risk, 429 (47.3%) as intermediate-risk, and 18 (2.0%) as high-risk. A total of 168 patients relapsed, and 64 patients died by the end of follow-up. The median follow-up of the whole cohort was 59.3 months (range 3.1–99.4 months). The 5-year OS was 92.3% (95% confidence interval [CI] 90.5–94.2%), and the DFS was 78.8% (95% CI 76.0%–81.8%) in the entire cohort (Supporting Information S1: Figure S1).

Clinical characteristics of patients with *TP53*^{del}

TP53^{del} was detected in 40 (4.4%) patients at initial diagnosis. Differences in baseline characteristics between patients with and without *TP53*^{del} are shown in Table 1. There was no significant difference between the two groups in terms of sex, age, white blood cell count, blast counts in the bone marrow, central nervous system (CNS) involvement or final risk subgroup. There were three patients with a hypodiploidy karyotype in the *TP53*^{del} group, and the positive rate was higher than that without *TP53*^{del} (7.5% vs. 0.5%, $p = 0.002$). However, there was a lower rate of *ETV6::RUNX1* positivity in patients with *TP53*^{del} (10.0% vs. 25.1%, $p = 0.029$). Table 1 shows that patients with *TP53*^{del} were less likely to harbor other recurrent genetic abnormalities; only two patients were positive for *BCR::ABL1*, one patient was positive for *TCF3::PBX1*, and all patients were negative for *KMT2A* rearrangement. Among the 40 patients with *TP53*^{del}, 15 (37.5%) patients relapsed by the end of follow-up, of whom 1 was hypodiploid, 2 had *ETV6::RUNX1*, and the rest had no other recurrent genetic abnormalities.

Prognostic significance of *TP53*^{del}

We explored the effect of *TP53*^{del} on early treatment response and ultimately found no significant difference in the poor dexamethasone response rate between patients with or without *TP53*^{del} (27.5% vs. 22.3%, $p = 0.439$). Moreover, *TP53*^{del} was not associated with MRD positivity on either Day 19 (35.0% vs. 21.8%, $p = 0.078$) or Day 46 (20.0% vs. 18.3%, $p = 0.791$) of remission induction, although the rates were slightly higher in patients with *TP53*^{del} (Table 1).

Survival analyses revealed that the 5-year DFS and OS in patients with *TP53*^{del} were significantly lower than those without *TP53*^{del}, with 5-year DFS of 57.0% (95% CI 42.8%–76.1%) and 79.8% (95% CI 77.0%–82.8%) ($p < 0.001$, Figure 2A), respectively, and 5-year OS of 81.7% (95% CI 70.2%–95.0%) and 92.8% (95% CI 90.9%–94.7%) ($p = 0.006$, Figure 2B) in patients with and without *TP53*^{del}, respectively. Univariate Cox regression analysis demonstrated that age ≥10 years, white blood cell count (WBC) > 50×10^9/L, CNS involvement, *TP53*^{del}, *BCR::ABL1* fusion gene, *KMT2A* rearrangement, and positive MRD on Day 19 and Day 46 had negative impacts on OS and DFS (Table 2). Multivariable analysis indicated that *TP53*^{del} was an independent risk factor for both OS (hazard ratio [HR] 2.75, 95% CI

TABLE 1 Clinical characteristics of B-ALL children with or without *TP53*^{del}.

Variable	no *TP53*^{del} ($n = 867$)	*TP53*^{del} ($n = 40$)	*p* value
Age, years (%)			0.053
1–9	729 (84.1)	29 (72.5)	
≥10	138 (15.9)	11 (27.5)	
Gender (%)			0.967
Female	344 (39.7)	16 (40.0)	
Male	523 (60.3)	24 (60.0)	
Leukocyte, $\times 10^9$/L, median (range)	9.2 (0.7-846.5)	9.3 (1.2–307.8)	0.775
Hemoglobin, g/l, median (range)	78 (21–155)	85 (51–136)	0.212
Platelets, $\times 10^9$/L, median (range)	60.0 (3–919)	38.5 (7–341)	0.062
Bone marrow blasts, %, median (range)	91.0 (20.0–100.0)	90.0 (21.0–99.5)	0.406
Karyotype (%)			
Hyperdiploid	85 (9.8)	2 (5.0)	0.463
Hypodiploid	4 (0.5)	3 (7.5)	0.002
Others	778 (89.7)	35 (87.5)	0.851
Genetic fusion subtype (%)			
ETV6::RUNX1	218 (25.1)	4 (10.0)	0.029
BCR::ABL1	57 (6.6)	2 (5.0)	0.947
TCF3::PBX1	44 (5.1)	1 (2.5)	0.718
KMT2Ar	13 (1.5)	0 (0.0)	1.000
CNS involvement[a] (%)	72 (8.3)	3 (7.5)	1.000
Risk stratification (%)			0.159
Low risk	445 (51.3)	15 (37.5)	
Intermediate risk	405 (46.7)	24 (60.0)	
High risk	17 (2.0)	1 (2.5)	
D19 MRD positive (%)	189 (21.8)	14 (35.0)	0.078
D46 MRD positive (%)	159 (18.3)	8 (20.0)	0.791

Abbreviations: *KMT2Ar*, *KMT2A* rearrangement; MRD, minimal residual disease; *TP53*^{del}, *TP53* deletion.

[a]CNS involvement, central nervous system involvement, including CNS3, CNS2, or traumatic lumbar puncture.

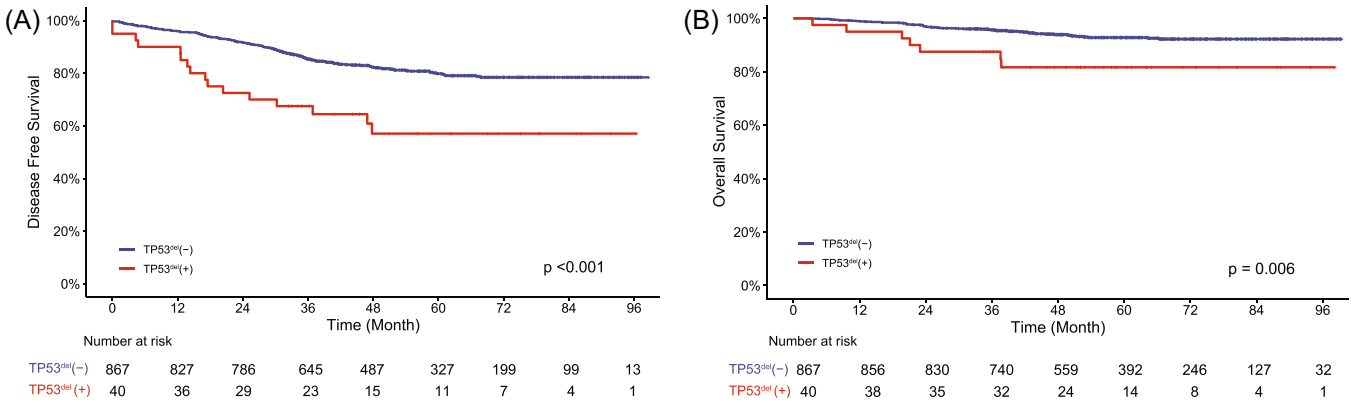

FIGURE 2 **(A) The 5-year disease-free survival of patients with or without *TP53* deletion. (B) The 5-year overall survival of patients with or without *TP53* deletion.** *TP53*^{del} (+), *TP53* deletion; *TP53*^{del} (−), without *TP53* deletion.

TABLE 2 Univariate analysis for DFS and OS.

	Univariate analysis					
	DFS			OS		
Parameter	HR	95% CI	*p* value	HR	95% CI	*p* value
Age (≥10 years)	1.89	1.34–2.65	<0.001	2.49	1.47–4.23	0.001
Male	1.18	0.87–1.60	0.285	1.48	0.87–2.51	0.145
WBC > 50 × 10^9/L	3.50	2.57–4.75	<0.001	4.66	2.84–7.62	<0.001
Bone marrow blasts	1.01	1.00–1.02	0.275	1.02	1.00–1.04	0.149
CNS involvement[a]	1.72	1.10–2.68	0.018	2.17	1.10–4.26	0.025
Hyperdiploid	0.93	0.56–1.56	0.783	0.80	0.32–1.99	0.631
Hypodiploid	0.74	0.10–5.31	0.768	–	–	–
ETV6::RUNX1	0.39	0.25–0.61	<0.001	0.35	0.16–0.77	0.009
BCR::ABL1	2.75	1.80–4.20	<0.001	2.19	1.04–4.59	0.038
TCF3::PBX1	0.89	0.44–1.81	0.753	1.28	0.46–3.51	0.637
KMT2Ar	7.94	4.05–15.59	<0.001	12.28	5.26–28.66	<0.001
TP53^{del}	2.60	1.55–4.34	<0.001	2.87	1.31–6.28	0.009
D19 MRD positive	1.89	1.38–2.59	<0.001	2.63	1.60–4.33	<0.001
D46 MRD positive	3.63	2.69–4.91	<0.001	4.12	2.52–6.74	<0.001

Abbreviations: CI, confidence interval; DFS, disease-free survival; HR, hazard ratio; *KMT2Ar*, *KMT2A* rearrangement; MRD, minimal residual disease; OS, overall survival; *TP53*^{del}, *TP53* deletion; WBC, white blood cell count.

[a]CNS involvement: central nervous system involvement, including CNS3, CNS2, or traumatic lumbar puncture.

1.23–6.16, $p = 0.014$) and DFS (HR 3.04, 95% CI 1.80–5.14, $p < 0.001$). WBC > 50 × 10^9/L, positive *BCR::ABL1* fusion gene, *KMT2A* rearrangement, and positive MRD on Day 46 were also independently correlated with poor DFS (Figure 3A). In addition to *TP53*^{del}, WBC > 50 × 10^9/L, *KMT2A* rearrangement, and positive MRD on Day 46 were also independent risk factors for OS (Figure 3B).

Our protocol showed that MRD, especially MRD on Day 46 of induction, is a main stratification criterion and prognostic factor. Patients were assigned to the low-, intermediate- or high-risk group according to their MRD status. Patients in the three risk groups accepted different treatments. Therefore, we further analyzed the prognostic impact of *TP53*^{del} stratified by the final risk stratifications and MRD level after induction. DFS was not significantly different between patients with or without *TP53*^{del} in the final low-risk group (83.0% [95% CI 63.5%–100.0%] vs. 89.6% [95% CI 86.6%–92.8%], $p = 0.646$; Figure 4A). Moreover, there was also no significant difference in DFS between patients with or without *TP53*^{del} in the intermediate-risk group with negative MRD on Day 46 (62.7% [95% CI 42.7%–92.1%] vs. 75.6% [95% CI 70.2%–81.4%], $p = 0.132$, Figure 4B). However, for intermediate-risk patients with positive MRD on Day 46, the 5-year DFS was significantly lower in those with *TP53*^{del} (0.0% vs. 58.0% [95% CI 49.2%–68.3%], $p < 0.001$; Figure 4C). The 5-year DFS of patients with *TP53*^{del} in the intermediate-risk group with positive MRD was even not better than the high-risk group (0.0% vs. 41.7% [95% CI 23.2%–74.7%], $p = 0.465$, Figure 4C). All seven patients in the intermediate-risk group with positive MRD on Day 46 and *TP53*^{del} relapsed. The high-risk group was not analyzed separately because only one patient with *TP53*^{del} was assigned to the high-risk group.

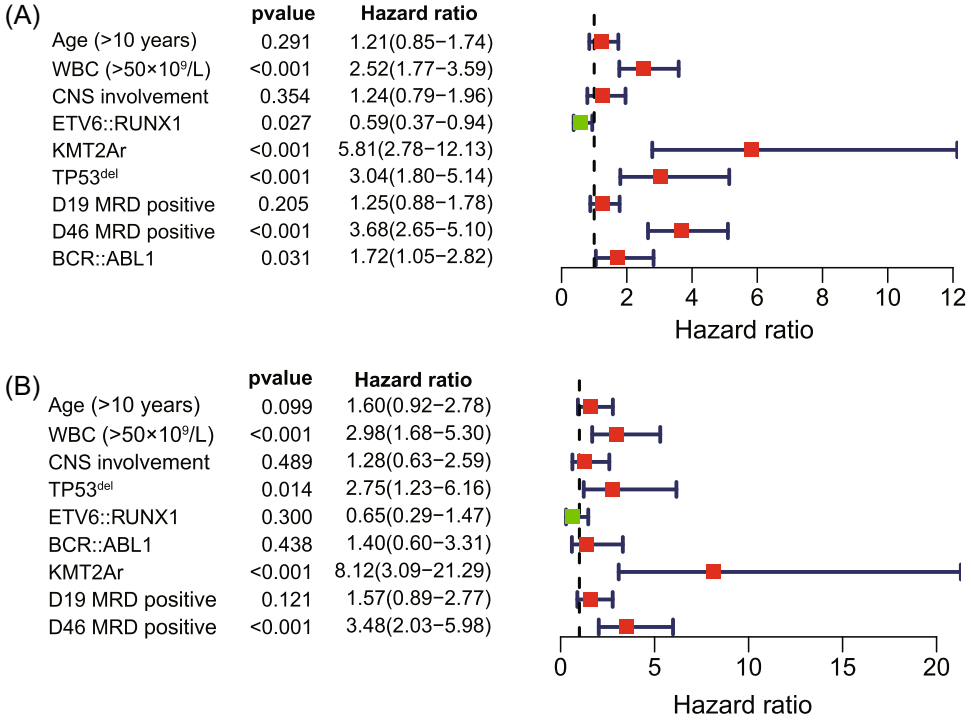

FIGURE 3 **(A) Forest plot based on the results of multivariable analysis of the factors affecting disease-free survival (DFS). (B) Forest plot based on the results of multivariable analysis of the factors affecting overall survival (OS).** CNS involvement: central nervous system involvement, including CNS3, CNS2, or traumatic lumbar puncture; *KMT2Ar*, KMT2A rearrangement; MRD, minimal residual disease; $TP53^{del}$, *TP53* deletion; WBC, white blood cell.

Prognostic significance of $TP53^{mut}$

With the application of next-generation sequencing, we were able to obtain the $TP53^{mut}$ data of 342 patients who were enrolled after September 2018. Somatic $TP53^{mut}$ was detected in 11 (3.2%) patients. The positions and mutation types are depicted in Supporting Information S1: Figure S2A and Table S2. Prognostic analysis revealed no significant difference in 3-year DFS for patients with or without $TP53^{mut}$ (81.8% [95% CI 61.9%–100.0%] vs. 84.9% [95% CI 81.1%–89.0%], $p = 0.971$; Supporting Information S1: Figure S2B). $TP53^{mut}$ was detected in 3 of the 18 patients with $TP53^{del}$ (16.7%), indicating a tendency to co-occur (16.7% vs. 2.5%, $p = 0.016$). The cohort was divided into four groups: patients with $TP53^{mut+del}$, $TP53^{del}$ only, $TP53^{mut}$ only and the wild type ($TP53^{wt}$.) The 3-year DFS of patients with $TP53^{mut+del}$, $TP53^{del}$ only, $TP53^{mut}$ only and $TP53^{wt}$ were 33.3% (95% CI 6.7%–100.0%), 66.7% (95% CI 46.6%–95.3%), 100.0% and 85.8% (95% CI 81.9%–89.8%), respectively ($p < 0.001$). Comparisons of DFS among the four groups revealed that patients with $TP53^{del}$ had lower 3-year DFS, independent of the status of $TP53^{mut}$ (Supporting Information S1: Figure S2C). After adjustment to the previously identified risk factors by multivariable analysis, TP53 mutation remained insignificant (Supporting Information S1: Figure S3).

Next generation sequencing analysis of bone marrow samples in complete remission revealed that 4 (1.2%) patients carried *TP53* germline mutations, and 2 of which were hypodiploidy karyotype with $TP53^{del}$. There was a higher rate of *TP53* germline mutation in patients with hypodiploidy karyotype (50.0% vs. 0.6%, $p = 0.001$). And the two patients with *TP53* germline mutations, $TP53^{del}$ and hypodiploidy karyotype eventually suffered bone marrow relapse. Due to the small number of cases, no prognostic analysis was performed.

DISCUSSION

Previous studies have investigated the clinical features and prognostic significance of *TP53* alterations, mostly $TP53^{mut}$ *alterations, in ALL patients*.[1,8–11,13,20–22] However, the clinical characteristics and prognostic impact of $TP53^{del}$ in pediatric ALL at diagnosis have not been well characterized due to limited research and large heterogeneity across studies. In this study, we found that $TP53^{del}$ served as a prognostic factor for pediatric B-ALL, especially those in the intermediate-risk group who were MRD- and $TP53^{del}$ positive, had the worst outcome and may benefit from more aggressive treatment.

In our cohort, the frequency of $TP53^{del}$ was 4.4% (40/907), which is in line with the frequency of $TP53^{del}$ reported in previous large cohort studies[12,23] but lower than that reported in adults.[9] Stengel et al reported that the frequency of $TP53^{del}$ in ALL increased with age,[1] and the data from our cohort showed the same trend. The greater frequency of $TP53^{del}$ in older patients may be one of the reasons for the worse prognosis in adults with ALL. Yu et al reported that in relapsed pediatric ALL, there were more patients with hypodiploidy and fewer patients with *ETV6::RUNX1* among those with *TP53* alterations.[24] Our data showed that newly diagnosed childhood ALL with $TP53^{del}$ were also associated with a higher frequency of hypodiploid karyotype and a lower frequency of *ETV6::RUNX1* fusion gene. Previous studies have shown that ALL patients with hypodiploid have a greater possibility of treatment failure,[1,20,25–28] and it can be postulated that poor outcomes are partly associated with the higher positive rate of $TP53^{del}$, as *TP53* alterations represent a preleukemic event that can lead to aneuploidy in B-ALL patients.[29]

International collaborative groups have included $TP53^{del}$ in the risk stratification of myeloid tumors. However, the impact of $TP53^{del}$

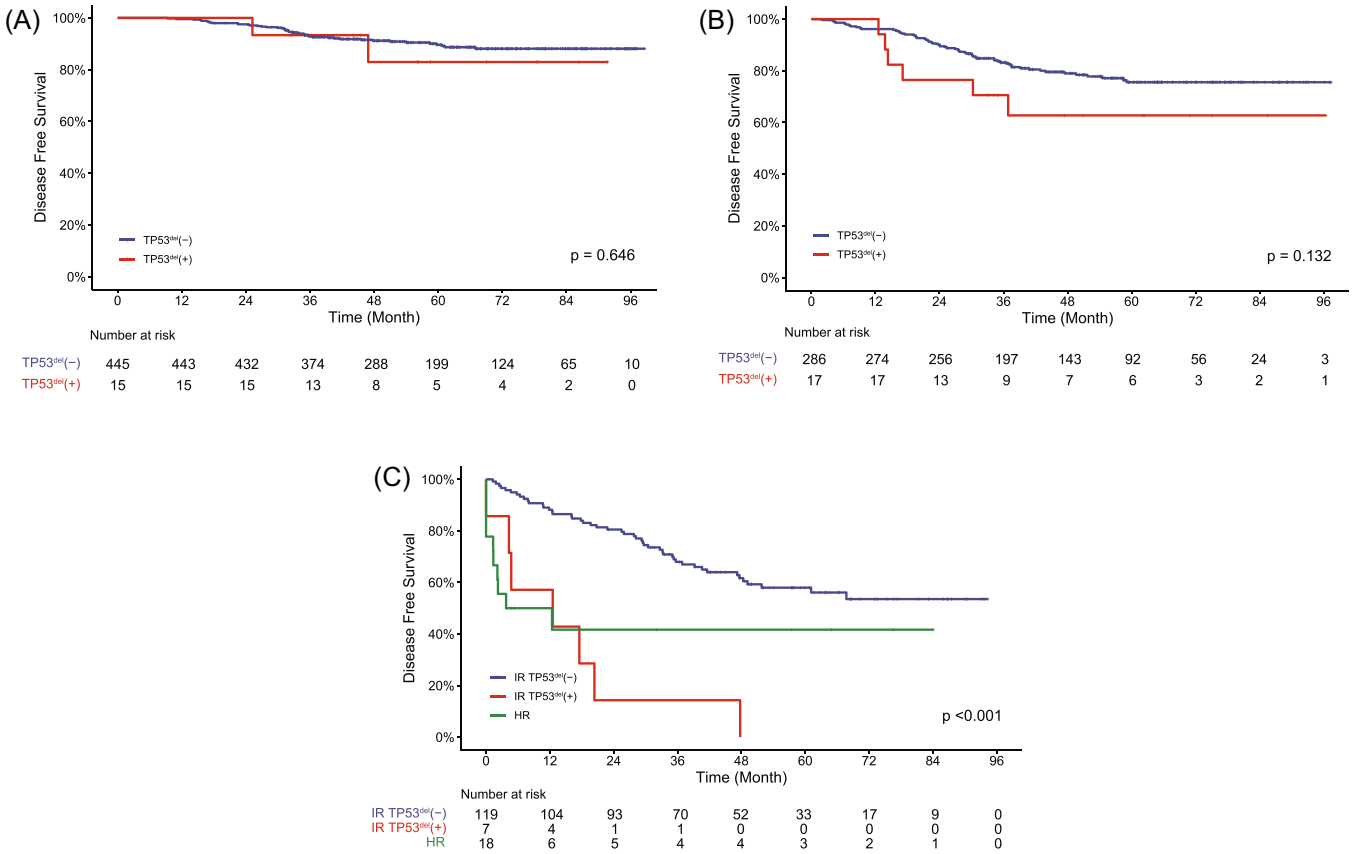

FIGURE 4 **The 5-year disease-free survival (DFS) of patients with or without *TP53* deletion in different risk groups and minimal residual disease (MRD) levels.** **(A)** DFS in the low-risk group. **(B)** DFS of intermediate-risk patients with negative MRD. **(C)** DFS of patients with positive MRD in the intermediate-risk group with or without *TP53* deletion and the DFS of the whole high-risk group. HR, high-risk group; IR, intermediate-risk group; $TP53^{del}$ (+), *TP53* deletion; $TP53^{del}$ (−), without *TP53* deletion.

on acute lymphoblastic leukemia is still unclear. Several domestic and foreign studies have shown that $TP53^{del}$ is associated with poor prognosis.[7,11–13,30] However, some studies have come to the opposite conclusion. For example, $TP53^{del}$ was found to have no adverse effect on survival for ALL in a study by Stengel et al.[9] Therefore, more studies are needed to further clarify the significance of $TP53^{del}$. By analyzing the effect of $TP53^{del}$ on prognosis based on different risk groups and MRD levels, we found that $TP53^{del}$ was an MRD-dependent prognostic factor. As all patients in the intermediate-risk group with positive MRD after induction and $TP53^{del}$ relapsed, it is reasonable to assume that patients with these characteristics are at high risk of recurrence and that $TP53^{del}$ is an MRD-dependent risk factor.

MRD monitoring has significantly improved the prediction of relapse and directed subsequent treatment adjustments for balancing high survival rates and quality of life in pediatric ALL.[31,32] However, although patients who are classified into the high-risk group according to MRD truly have poor outcomes, a large proportion of recurrences still occur in the intermediate-risk group.[33] Therefore, it is urgent to find new markers to further enhance the existing risk stratification and to identify relapse-prone patients and provide timely appropriate treatment. Previous studies have shown that combining MRD data with genetic data could further improve risk stratification in pediatric B-cell precursor ALL.[33] In this study, we found that patients with $TP53^{del}$ had lower 5-year DFS and OS, 37.5% (15/40) of the patients with $TP53^{del}$ relapsed, and most relapses occurred in the intermediate-risk group. Furthermore, all patients with both $TP53^{del}$ and positive MRD on Day 46 in the intermediate-risk group relapsed, indicating that the prognostic effect of $TP53^{del}$ in pediatric B-ALL was MRD dependent. Intensive treatment could be beneficial for intermediate-risk patients who are positive for both $TP53^{del}$ and MRD on Day 46, but this needs further verification in the future.

With the application of targeted gene sequencing, our study revealed that $TP53^{mut}$ frequency is higher in $TP53^{del}$ group. In this study, $TP53^{mut}$ occurred in 16.7% of patients with $TP53^{del}$. Stengel's study found that possessing both $TP53^{mut}$ and $TP53^{del}$ ($TP53^{mut+del}$) had a significant negative impact on the prognosis of ALL, but $TP53^{mut}$ only or $TP53^{del}$ only had no influence on survival.[9] However, Fang's research showed that the 3-year OS and DFS rates were not significantly different among patients with $TP53^{del}$ only, $TP53^{mut}$ only, and $TP53^{mut+del11}$. Research on the effect of *TP53* gene alterations on prognosis has not reached a unanimous conclusion. In our cohort, $TP53^{mut}$ had no significant impact on 3-year DFS. Subsequent analysis showed that the prognostic impact of $TP53^{del}$ on DFS was not affected by the presence or absence of $TP53^{mut}$. Our research yielded different findings, possibly because previous studies mostly focused on adult ALL or included both adult and pediatric population, and the treatment schedules were quite different between children and adults.[34] However, the intensity of treatment can affect the prognosis of patients[35]; for instance, Shamanna et al. concluded that $TP53^{mut}$ did not indicate a poor outcome in adults with ALL who received intensified treatment regimen.[36] In addition, the majority of previous studies analyzed T-ALL and B-ALL together, and even included

Burkitt lymphoma. However, these disease subgroups are heterogeneous in terms of genetic background and treatment intensity. Our cohort specifically comprised children who underwent treatment according to the CCCG-ALL-2015 protocol, thus eliminating the impact of the aforementioned factors on our findings.

Despite the new findings, there are still some limitations in this study. Although a total of 907 children were included in this study and finished the $TP53^{del}$ test, only 342 children completed $TP53^{mut}$ detection due to the limited application of targeted gene sequencing. Therefore, the effect of $TP53^{mut}$ on the prognosis of pediatric B-ALL requires further study with large datasets. In addition, our study was a single-center retrospective study. Multicenter researches are needed to further validate our conclusions and explore the underlying mechanisms involved.

In conclusion, this study reported the incidence and prognostic impact of $TP53^{del}$ in pediatric B-ALL patients. For patients in the intermediate-risk group, $TP53^{del}$ at diagnosis combined with positive MRD on Day 46 could be a promising indicator for risk stratification adjustment and more aggressive treatment to improve DFS.

ACKNOWLEDGMENTS

The authors thank all participants for their contributions and time investment in the study.

AUTHOR CONTRIBUTIONS

Study design: Yangyang Gao, Jun Li, and Ning Wang. *Data collection*: Yangyang Gao and Junxia Wang. *Data analysis*: Yangyang Gao and Jun Li. *Writing of the manuscript*: Yangyang Gao, Jun Li, and Ning Wang. *Manuscript revision*: Xiaojuan Chen and Xiaofan Zhu. *Data analysis and manuscript writing guidance*: Wenbin An, Zixi Yin, Xia Chen, Yumei Chen, Ye Guo, Wenyu Yang, Li Zhang, Yao Zou, Xiaojuan Chen, and Xiaofan Zhu.

CLINICAL TRIAL REGISTRATION

This trial is registered with the Chinese Clinical Trial Registry, ChiCTR-IPR-14005706.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

All data for this study, including basic clinical and genetic characteristics of patients, are available by contacting the corresponding authors.

ETHICS STATEMENT

This study was approved by the institutional review boards of CAMS & PUMC. Informed consent was obtained from the guardians or patients before treatment for all patients.

FUNDING

This work was supported by the Ministry of Science and Technology of China (2021YFA1101603, 2021YFE0106900), the CAMS Innovation Fund for Medical Sciences (2022-I2M-1-022, 2021-1-I2M-1-040, 2022-I2M-C&T-B-088), and the National Natural Science Foundation of China (82270144, 82100198, 81870131, 81270651).

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SUPPORTING INFORMATION

Additional supporting information can be found in the online version of this article.

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