



Frequent *TCR* rearrangements in pediatric B-cell lymphoblastic acute leukemia: genomic and phenotypic features

Jiajia Zheng¹ · Yiting Wang^{2,3} · Zhiheng Li⁴ · Peifang Xiao¹ · Jun Lu¹ · Hu Liu⁵ · Yixin Hu^{1,5} · Shuiyan Wu⁶ · Bohan Li¹ · Li Gao¹ · Qi Ji¹ · Hailong He¹ · Yi Wang¹ · Jie Li¹ · Nan Chen^{2,3} · Lijun Meng^{2,3} · Yuanyuan Tian⁴ · Yizhen Li⁵ · Sheng Xiao⁷ · Shaoyan Hu^{1,5,8}

Received: 19 June 2025 / Accepted: 5 October 2025 / Published online: 20 October 2025
© The Author(s) 2025

Abstract

T-cell receptor (*TCR*) loci undergo complex *V(D)J* rearrangements during T-cell maturation. However, errors in this process can cause *TCR* promoters and enhancers to aberrantly fuse with oncogenes, leading to their overexpression and the development of T-cell acute lymphoblastic leukemia (T-ALL). Unexpectedly, in a retrospective study of 97 pediatric B-ALL cases, all diagnosed according to WHO criteria at a single institution, we identified a subgroup of B-ALL harboring *TCR* fusion. These fusions involved diverse partner genes, including *LINC01656*, *TBC1D10B*, *TCL1A/TCL1B*, *CDKN2A*, *CEBPB/G*, and *AH11*. Patients with *TCR* fusions showed a gene expression profile enriched in hematopoietic stem cell (HSC) signatures and frequently carried other adverse genomic markers, such as *CDKN2A/B* alterations and *RAS* pathway activation. Clinically, *TCR*-rearranged B-ALL patients were associated with increased risk ($P=0.005$) and had higher minimal residual disease (MRD) at day 19 ($P=0.034$), and may benefit from MRD-based risk-directed therapy. Our study highlights frequent *TCR* fusions in B-ALL, suggesting a stem cell-derived leukemia with potential differentiation into both B-cell and T-cell lineages.

Keywords Pediatric · B-cell acute lymphoblastic leukemia · *TCR* fusion

Introduction

Acute lymphoblastic leukemia (ALL) is the most common childhood cancer, with 85% of cases classified as B-cell ALL (B-ALL). Advances in risk classification, incorporating

clinical factors such as white blood cell count (WBC) and age at diagnosis, genomic subtyping, and minimal residual disease (MRD) assessment after initial treatment, along with stratified therapies and new drug developments, have significantly improved survival rates for pediatric B-ALL.

Jiajia Zheng, Yiting Wang, Zhiheng Li, Peifang Xiao and Jun Lu contributed equally.

✉ Sheng Xiao
sxiao@partners.org

✉ Shaoyan Hu
hushaoyan@suda.edu.cn

¹ Department of Hematology & Oncology, Children's Hospital of Soochow University, Suzhou 215000, China

² Advanced Molecular Pathology, Institute of Soochow University and SANO, Suzhou 215123, China

³ Suzhou Sano Precision Medicine Ltd, Suzhou 215123, China

⁴ Pediatric Clinical Research Institute, Children's Hospital of Soochow University, Suzhou 215000, China

⁵ Jiangsu Pediatric Hematology & Oncology Center, Suzhou 215000, China

⁶ Department of Intensive Care Unit, Children's Hospital of Soochow University, Suzhou 215000, China

⁷ Department of Pathology, Brigham and Women's Hospital, Harvard Medical School, Boston, MA 02115, USA

⁸ Department of Hematology and Oncology, Jiangsu Pediatric Hematology & Oncology Center, The Children's Hospital of Soochow University, Suzhou Industrial Park, No.92 Zhongnan Street, Suzhou 215025, China

These advancements make B-ALL one of the significant milestones in the success of precision medicine. Over the past decade, developed countries have reported event-free survival (EFS) rates of 74–87% and overall survival (OS) rates of 85–90% [1].

Gene fusions involving the immunoglobulin (IG) and T-cell receptor (TCR) loci are well-established markers of B-ALL and T-ALL, respectively [2]. In B-ALL, *IG* rearrangements juxtaposition their potent enhancers to oncogenes such as *CRLF2*, *EPOR*, *IL3*, *BCL2*, *BCL6*, *MYC*, *DUX4*, and *CEBPs*, resulting in overexpression [3–6]. These *IG* rearrangements, depending on their fusion partners, have distinct prognostic and therapeutic implications. For example, *IGH::CRLF2* and *IGH::EPOR* rearrangements, which are associated with the Philadelphia-like (Ph-like) B-ALL subtype, may be sensitive to *JAK2* signaling inhibitors [7]. *IGH::DUX4* occurs in approximately 5% of pediatric B-ALL cases and is associated with a favorable prognosis [8]. Conversely, *IGH::IL3* B-ALL is linked to poor prognosis and often presents with eosinophilia due to *IL3* overexpression [6, 9].

TCR, with a gene structure and somatic recombination mechanism similar to *IG*, is rearranged in approximately one-third of T-ALL cases. Common fusion partners for *TCR* include *TAL1/2*, *LCK*, *TLX1/3*, *TCL1A*, *MYC*, *LYL1*, *LMO1/2*, *NKX2*, *BCL11B*, *NOTCH1*, and *IRS4* [10–16]. While the oncogenic mechanisms of these *TCR* rearrangements are mostly defined and serve as effective diagnostic and classification markers, their prognostic and therapeutic values are less well-established compared to *IG* rearrangements in B-ALL.

Although *IG* and *TCR* rearrangements are generally considered specific to B- and T-cell tumors, early studies showed unexpected *IGH* fusions in myeloid tumors. These *IGH* rearrangements in myeloid neoplasms lead to the translocation of the *IGH* enhancers and promoters to oncogenes, such as *MECOM* and *CCNG1*, leading to their

overexpression [17]. There have been occasional case reports of *TCR* rearrangements in B-ALL, with 7 cases documented to date, involving *TRA/D* (T-cell receptor alpha and delta) at chromosome 14q11 (Table S1). The fusion partners in these cases include *IGH*, *CDKN2A*, and *MYC* [4, 18–23]. In this study, we identified *TRA/D* rearrangements in 6 of 97 pediatric B-ALL using a targeted DNA next-generation sequencing (NGS) panel that covered both *IG* and *TCR* genes. Our findings reveal that *TRA/D* rearrangements are more common than previously reported in pediatric B-ALL. These tumors expressed hematopoietic stem cell (HSC) markers and were frequently associated with adverse genomic features, such as *CDKN2A/B* alterations and RAS

signaling mutations. Patients with these alterations may benefit from additional early intensification therapy.

Materials and methods

Patients

This study contained 97 continuous pediatric patients diagnosed with B-ALL at the Children's Hospital of Soochow University from June 2023 to January 2024. All patients provided informed consent in accordance with institutional guidelines. The details of the diagnosis, risk assessment, and treatment are described in the Online Supplementary Information.

Targeted DNA NGS covering TRA/D loci

DNA from bone marrow samples was isolated using QIAamp DNA Blood Mini Kits (Qiagen, Germany). Sequencing libraries were constructed with the Rapid Plus DNA Lib Prep Kit (ABclonal, China) and xGen® Hybridization and Wash Kit (IDT, USA). Briefly, DNA was fragmented, end polished, dA-tailed, and adapter-ligated. Library amplification was performed with initial denaturation at 98°C, followed by 7 cycles of denaturation, annealing, and extension, concluding with a final extension step. Target enrichment utilized biotin-labeled bait oligos targeting 187 leukemia-related genes, including both *IG* and *TCR*. Sequencing was performed on a NovaSeq 6000 sequencer (Illumina, US) with 2 × 150-bp bidirectional sequencing. Data analysis for SNVs, indels, CNVs, *IG/TCR* gene rearrangement, and variable (V), diversity (D) and joining (J) clonality was performed with custom pipelines developed at Sano Medical Laboratories (China). Variant nomenclature was interpreted following HGVS guidelines (GRCh38/hg38). The capture probe set comprised 5,396 probes, covering 464 kb of exon regions, 136 kb of *IG* regions, and 48 kb of *TCR* regions. Reporting criteria included mutations with a variant allele frequency (VAF) of ≥ 3% and at least 3 mutation variant reads. Sensitivity thresholds were established at a VAF of 3% and a sequencing depth of 325x.

Transcriptome and gene set enrichment analysis

RNA was purified from bone marrow specimens using the RNeasy Plus Mini Kit (Qiagen). Sequence libraries were constructed with the NEB Next Ultra II RNA Library Prep Kit (New England Biolabs), which included mRNA

enrichment, cDNA synthesis, fragmentation, adapter ligation, and amplification. Next-generation sequencing was performed using the NovaSeq 6000 sequencer. Reads count-based differential gene expression analysis was performed using DESeq2 R package [24]. Gene set enrichment analysis (GSEA) was performed according to the Developer's instructions and previous reports [25]. Differential expression was assessed with DESeq2 (v1.42.1), and GSEA was carried out using clusterProfiler (v4.10.1) against gene sets from MSigDB obtained via msigdb (v7.5.1) and two custom gene sets derived from GSE24759 analyzed with limma (v3.58.1). The four gene sets used were: (1) JAATINEN_HEMATOPOIETIC_STEM_CELL_UP (genes upregulated in CD133 + hematopoietic stem cells versus CD133- cells), (2) EPPERT_HSC_R (genes upregulated in human hematopoietic stem cells versus progenitors/mature cells), (3) genes upregulated (>2-fold, $p < 0.05$) in umbilical cord blood HSC compared with mature cells, and (4) genes upregulated (>2-fold, $p < 0.05$) in HSC compared with progenitors. Weighted enrichment statistics were calculated on the log2 fold-change ranked gene list; enrichment was considered significant at FDR < 0.25 and nominal $p < 0.05$.

Western blot analysis

Bone marrow aspirate was lysed by RIPA lysis buffer. Whole cell lysate was loaded onto polyacrylamide gels and probed by the following primary antibodies: SOCS2 (1:1000, A9190, Abclonal), ITGA6 (1: 2500, A20807, Abclonal), GATA3 (1:1000, 5852, cell signaling technology), and GAPDH (1:5000, AP0063, Bioworld Technology). The secondary antibodies were purchased from Jackson ImmunoResearch Laboratories. Blots were visualized by the LAS 4010 imaging system.

Data analysis

The results are presented as mean ± standard deviation, median, count, or percentage. Patient follow-up was continued until May 7, 2025. Fisher's exact test or χ^2 test was performed for mutual univariable analysis using SPSS version 2.0 software. A p -value < 0.05 is considered statistically significant.

Results

TRA/D rearrangement in pediatric B-ALL

A targeted DNA NGS panel, with probes covering all *IG* and *TCR* loci, detected *TRA/D* rearrangements in 6 of 97

pediatric B-ALL patients (6.2%) (Table 1; Fig. 1). The *TRA/D* breakpoints were clustered at *TRAJ* segments, with specific breakpoints observed at *J32* (patient 1), *J29* (patient 2), *J54* and *J28* (patient 3), *J12* (patient 4), *J29* (patient 5), and *TRDD3*, *J49*, *J9*, and *J6* (patient 6). These breakpoints are consistent with those seen in classic *TRA/D* fusions in T-ALL, preserving the enhancer region downstream of the *J* gene cluster (Fig. 2).

The fusion partners genes included long intergenic non-protein coding RNA 1656 (*LINC01656*), TBC1 domain family member 10B (*TBC1D10B*), T-cell leukemia/lymphoma 1 A/B (*TCL1A/B*), CCAAT enhancer-binding protein B and G (*CEBPB* and *CEBPG*), cyclin-dependent kinase inhibitor 2 A (*CDKN2A*) and Abelson helper integration site 1 (*AH1*). Among them, *CDKN2A*, *AH1*, and *TBC1D10B* contained intragenic breakpoints and gene truncation, likely leading to loss of function; in contrast, *TCL1A/B*, *CEBPG*, and *CEBPB* had breakpoints outside of genes, preserving the coding regions and likely resulting in a gain of function by overexpression (Figs. 1 and 2; Table 1).

Karyotype analysis revealed that three of these cases had rearrangements involving the *TRA/D* locus at 14q11, with the following abnormalities: a t(9;14) (p21; p11) resulting in *TRA::CDKN2A*, a t(6;14)(q23; q21) resulting in *TRA::AH1*, and a t(14;20) (q11; q13) resulting in *TRA::CEBPB* and *TRD::CEBPB* (Fig. 3A). FISH analysis confirmed *TRA/D* rearrangement in 4 of the 6 cases, including all 3 with karyotype abnormalities at 14q11 and one case without apparent 14q11 involvement (Fig. 3B). The remaining 2 cases, which were negative by both karyotyping and FISH, were further analyzed using PCR amplification with sequences obtained from the targeted DNA NGS. Sanger sequencing of the PCR products confirmed *TRA/D* rearrangement in these cases, identifying *TRD::LINC01656* and *TRA::TBC1D10B*, respectively (Fig. 3C). FISH-negative *TRA/D* rearrangements are likely the result of genomic insertions, which can lead to false negative FISH due to the involvement of small DNA fragments. For example, approximately 80% of *IGH::EPOR* are FISH-negative due to genomic insertion [26].

Genomic profiles of pediatric B-ALL with TRA/D rearrangement

A detailed genomic profile was obtained by concurrent transcriptome analysis and the targeted DNA NGS. In addition to the *TRA/D* rearrangement, these 6 cases also contained known dominant driver fusion transcripts including *TAF15::ZNF384*,

Table 1 Immunophenotype and genomic alterations of six patients with *TRA/D* rearrangement

Patient	Diagnosis	Immunophenotype		Karyotype	Concurrent fusion	TCR locus	TRA/D FISH (%)	Partner gene	Breakpoint ^a
		Positive	Negative						
1	B-ALL	CD19, CD22, CD13, CD33, CD117, HLA-DR, CD34 CD45, cCD79a, MPO, TdT, CD15, CD38, CD123	CD2, CD3, CD7	45,XY,der(12)t(12;17)(p13;q12), -17,i(17)(q10)[6]/46,XY[4]	TAF15::ZNF384	TRD	Negative	LINC01656	chr14:22509672
2	B-ALL	CD10, CD19, CD22, HLA-DR, CD34, CD45 cCD79a, TdT, CD38, CD123	CD2, CD3, CD7, CD13, CD14 CD117, cCD3, MPO, CD15	71-74,<3n>XXY,+2,-3,-4,+5,-7, +8,-9,+10,t(12;17)(p13;q12)*2,-14, -15,+16,+18,+20,+21,-22[cp12]/ 45,XY,t(12;17)(p13;q12)[3]/46,XY[5] 46,XY,del(9)(q21q31),add(10)(p13), inv(11)(p15q23)[15]	TAF15::ZNF384	TRA	Negative	TBC1D10B	chr14:22513942 chr16:30369921
3	B-ALL	CD10, CD19, CD22, HLA-DR, CD34, CD45 cCD79a, CD38, CD123	CD2, CD3, CD13, CD33, CD14 CD117, cCD3, MPO, CD15	45,XY,add(3)(q26),der(9), t(9;14)(p21;q11),-16[5]/46,XY[10]	KMT2A::MLLT10	TRA	IR1G1F(48)	TCL1A/B	chr14:22482294 chr14:95533875
4	B-ALL	CD10, CD19, CD22, HLA-DR, CD34, cCD79a TdT, CD38, CD123	CD2, CD3, CD7, CD20, CD13, CD33 CD14, CD117, CD45, cCD3, MPO, CD15	45,XY,add(3)(q26),der(9), t(9;14)(p21;q11),-16[5]/46,XY[10]	PAX5::FBRS	TRA	IR1G1F(77)	CDKN2A	chr14:22515631 Chr19:33406014 chr14:22531943
5	B-ALL	CD10, CD19, CD22, CD13, CD33, HLA-DR CD34, CD45, cCD79a, TdT, CD38, CD123	CD2, CD3, CD7, CD20, CD14 CD117, cCD3, MPO, CD15	46,XX,t(1;9)(p33;p23), t(6;14)(q23;q11)[5]/46,XX[3]	SMARCA2::ZNF362	TRA	IR1G1F(92)	AHL1	chr14:22513950 chr6:135325898
6	B-ALL	CD10, CD19, CD22, CD20, HLA-DR, CD34 cCD79a, TdT, CD38, CD123	CD2, CD3, CD7, CD13, CD33, CD14 CD117, CD45, cCD3, MPO, CD15	46,XX,t(9;22)(q34;q11.2), t(14;20)(q11;q13)[18]/46,XY[2]	BCR::ABL1	TRA	IR1G1F(69)	CEBPB	chr14:22449116/ chr14:22489488/ chr14:22535562/ chr14:22539076 chr20:50346656

Abbreviations: R red signal, 5' TRA/D, G green signal, 3' TRA/D, F fusion signal

^a Breakpoint location using hg38

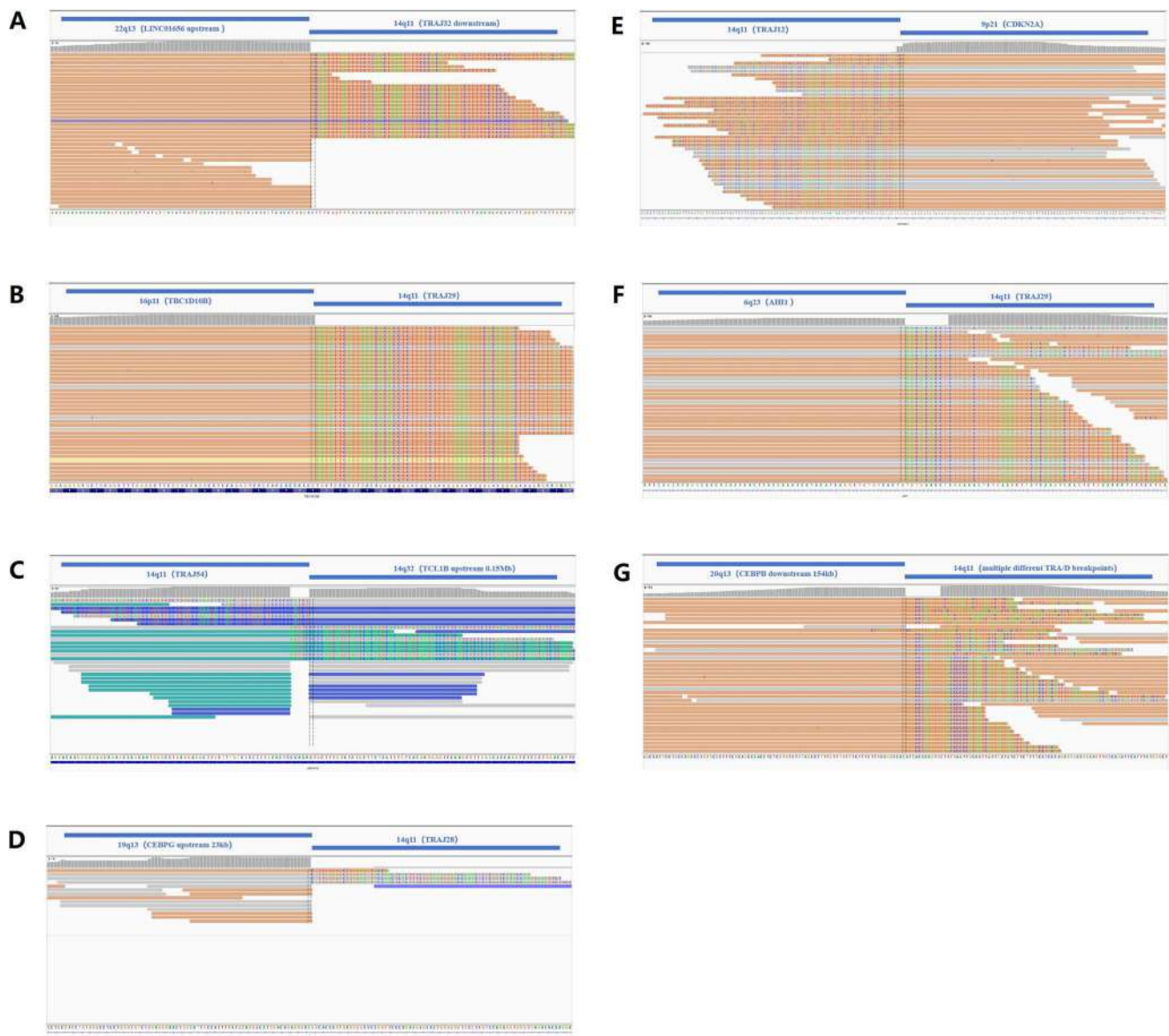


Fig. 1 Sequence of breakpoints from DNA NGS. IGV images show *TRAJ32::LINC01656* in patient 1 (A), *TRAJ29::TBC1D10B* in patient 2 (B), *TRAJ54::TCL1A/B* (C) and *TRAJ28::CEBPG* (D) in patient 3,

TRAJ12::CDKN2A in patient 4 (E), *TRAJ29::AH11* in patient 5 (F), and *TRDD3/TRAJ49/TRAJ9/TRAJ6::CEBPB* in patient 6 (G)

KMT2A::MLLT10, *PAX5::FBRS*, *SMARCA2::ZNF362*, and *BCR::ABL1* (Table 2; Fig. 3D). Notably, two of these cases contained the same *TAF15::ZNF384*, and one had *SMARCA2::ZNF362*, which belonged to the same B-ALL subgroup based on expression profiling [27]. *ZNF384* and *ZNF362* rearrangements are found in ~ 5% of pediatric B-ALL [28]. In our cohort of 97 cases, 4 had either *ZNF384* or *ZNF362* rearrangements, and 3 of these 4 also had *TRA/D*

rearrangements ($p < 0.001$). Other common genomic aberrations included *CDKN2A/B* deletions, detected in 3 of the 6 cases. Including the case with *TRA::CDKN2A*, which results in *CDKN2A* truncation, 4 of the 6 cases showed *CDKN2A/B* alterations, making them the most frequent genomic changes associated with *TRA/D* rearrangement in B-ALL. Additional common changes included RAS signaling gene mutations, found in 3 cases (Fig. 3D).

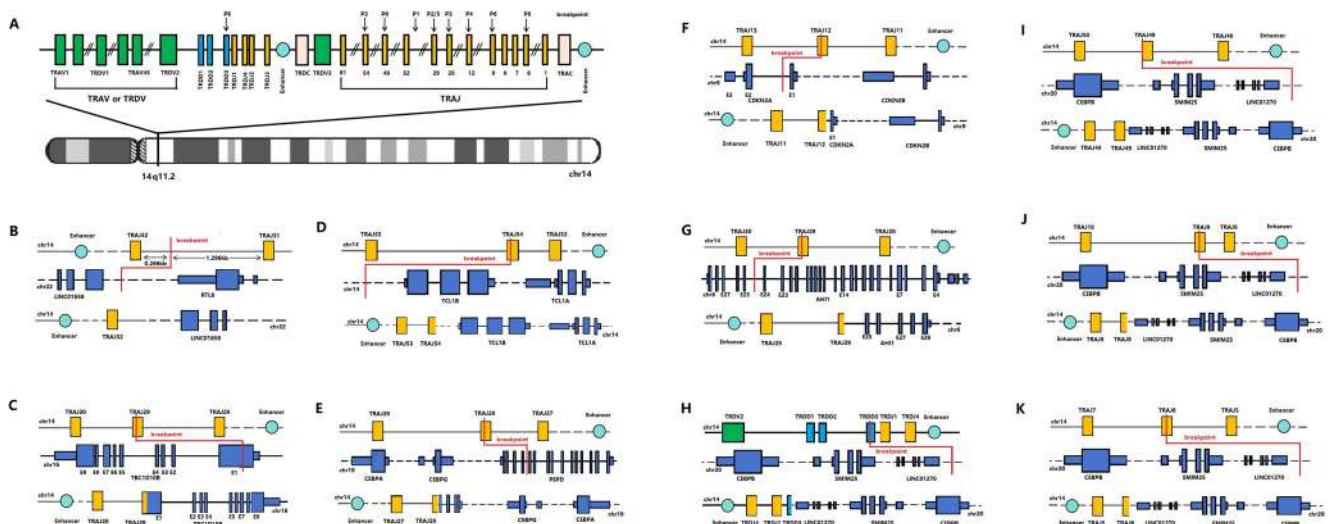


Fig. 2 The genomic structure and breakpoint locations of *TRA/D* rearrangements. *TRA/D* breakpoints (arrows) in all 6 B-ALL cases (P1-6) clustered in the *TRAJ* region (A). Individual fusion and their breakpoints are illustrated, including *TRD::LINC01656* in patient 1 (B),

TRA::TBC1D10B in patient 2 (C), *TRA::TCL1A/B* (D) and *TRA::CEBPG* (E) in patient 3, *TRA::CDKN2A* in patient 4 (F), *TRA::AH1* in patient 5 (G), and *TRA/D::CEBPB* (H) and *TRA::CEBPB* in patient 6 (I-K)

Expression signature in pediatric B-ALL with *TRA/D* rearrangement

The GSEA reported in the manuscript was performed on a carefully selected subset of seven cases to minimise confounding by other B-ALL subtypes with similar stem-cell-like signatures. To identify these cases, we integrated our RNA-seq data with the microarray dataset GSE79533 [28] using the GEDI R package (v1.1) for cross-platform normalization, followed by UMAP (v0.2.10.0) for dimensionality reduction. Seven cases that clustered with *TCF3::PBX1* reference samples were retained for subsequent analysis. The results indicate significant enrichment of HSC genes in B-ALL cases with *TRA/D* rearrangement, suggesting that these tumors may originate from primitive hematopoietic progenitors (Fig. 3E). Consistently, patients with *TRA/D* rearrangement showed significantly higher CD34 fluorescence intensity (MFI) compared to other B-ALL cases by flow cytometry ($p=0.002$) and expressed typical stem cell markers, including *ITGA6*, *SOCS2*, and *GATA3*, as confirmed by Western blot analysis (Figs. 3F-H).

Clinic features of pediatric B-ALL with *TRA/D* rearrangement

The clinical and laboratory features of the 6 patients with *TRA/D* rearrangements are summarized in Tables 2 and 3. The patients' ages range from 4 months to 14

years at presentation, with a median age of 5 years. The cohort included 5 males and 1 female. Initial WBC counts varied from 19.11 to $202.8 \times 10^9/L$, with a median of $30.6 \times 10^9/L$. Analysis of cerebrospinal fluid obtained via lumbar puncture revealed no CNS involvement, and none of the patients experienced extramedullary invasion. Bone marrow aspirate morphology showed blasts typical of B-ALL, with medium to large in size, oval or round-shaped, with fine chromatin, often indistinct nucleoli, and minimal cytoplasm. Immunophenotypically, 4 cases showed typical B-ALL immunophenotypes, including positive B-cell markers and early lymphoid progenitor cell marker TdT. Two cases also expressed myeloid markers CD13 and CD33, with one patient additionally expressing MPO (48.7%), satisfying the criteria for mixed phenotype acute leukemia (Table 2, Figure S1). Similar myeloid marker expression was observed in 8 of 91 cases without *TRA/D* rearrangement in our series ($p=0.055$). Although these data suggest that the *TRA/D*-rearranged patients are more likely to be male and to have additional myeloid markers, statistical significance was not achieved. Future studies with a larger population are needed. Prognostically, 4 of 6 patients (66.7%) were initially classified as intermediate risk (IR) based on various criteria: WBC counts $> 50 \times 10^9/L$ (patient 1), age > 10 years (patient 2), *KMT2A::MLLT10* gene fusion (patient 3), and *BCR::ABL1* gene fusion (patient 6). Patients 4 and 5 were re-classified as IR due to MRD $> 1\%$ at day 19. Four of the 6 patients

received an additional cycle of cyclophosphamide, cytarabine, 6-mercaptopurine, vincristine, and pegaspargase from days 50 to 57. Patient 6, with *BCR::ABL1*, was treated with Dasatinib and achieved MRD < 0.01% by day 19. All 6 patients achieved MRD < 0.01% by day 46, with 5 currently in complete remission. However, patient 3 experienced a CNS relapse.

Correlation analysis was performed between *TRA/D* rearrangements and various clinical parameters, including age at initial diagnosis, sex, peripheral blood WBC count, extramedullary infiltration, risk stratification, and MRD levels on days 19 and 46 of treatment. Patients with *TRA/D* rearrangements had higher MRD levels on day 19 ($P=0.034$). Initial risk classification did not differ significantly between the two groups ($P=0.081$). However, when integrating MRD response into the risk classification algorithm, patients with *TRA/D* rearrangements were more frequently classified as IR ALL ($P=0.005$). No significant differences were observed in other clinical parameters between the groups (Table 3).

Discussion

Leukemias originating from hematopoietic cells can exhibit varying degrees of differentiation into downstream lineages [29]. This includes mixed phenotype acute leukemia (MPAL), where cells can show both myeloid and lymphoid characteristics [30], and chronic myeloid leukemia (CML), which can progress to either a myeloid blast crisis or a lymphoid blast crisis [31]. Our study identified *TCR* rearrangements in 6.2% of pediatric B-ALL. B-ALL most commonly arises from pre-B cells, with some originating from pro-B cells, an earlier stage in B-cell development [32]. Our analysis of *TCR*-rearranged B-ALL patients revealed an mRNA expression profile enriched in HSC markers, consistent with stem cell leukemia characteristics. Although *TCR* rearrangement suggests potential T-cell differentiation [33], the tumor cells did not express T-cell markers by flow cytometry, likely due to limited T-cell development. *TCR V(D)J* gene segment rearrangement occurs at the double-negative (DN) stage of T-cell development, when specific T-cell markers are not yet expressed.

The impact of *TCR* rearrangements on tumorigenesis in these B-ALL remains unclear. In T-ALL, potent enhancers from *TCR* genes can drive overexpression when fused with oncogenes [34]. A common fusion partner is *TCL1A/B*, which, when overexpressed, promotes the proliferation of early T-cell progenitors by activating the PI3K/AKT pathway [35]. One of our patients (patient 3) showed a *TRA::TCL1A/B* fusion, suggesting it may share similar mechanisms of tumorigenesis. Additionally, *TRA::CDKN2A*

was identified in 1 of the 6 patients studied. The *CDKN2A* breakpoint occurs within intron 1, leading to truncation of the p16^{ink4a} tumor suppressor protein. Interestingly, an identical *TRA/D::CDKN2A* fusion was found in another B-ALL case (Table 1), indicating a potentially recurrent event [36]. *CDKN2A* inactivation is a common genomic alteration in ALL, occurring in approximately 20% of B-ALL cases and 50% of T-ALL cases [37, 38], and is indicative of poor prognosis [39]. In our study, 4 of the 6 cases with *TCR* rearrangements contained *CDKN2A* alterations, likely contributing to the increased prognostic risk in this subset of B-ALL. Additionally, Patient 3, who had a *KMT2A::MLLT10* rearrangement, a known high-risk marker, had a CNS relapse. Notably, 2 of the 7 B-ALL cases with *TCR* rearrangements reported in the literature also had *KMT2A* rearrangements (Table 1), and both of them experienced relapse [20, 23].

Two cases in our series showed rearrangements between *TRA/D* and two *CEBP* family members, *CEBPB* and *CEBPG*. *CEBP* family members are known to be rearranged with *IGH* in B-ALL, leading to their overexpression [40]. The *CEBP* family of bZIP transcription factors plays a crucial role in regulating hematopoiesis, including stem cell function and the differentiation of myeloid and B lymphoid cells [41–43]. The *TRA/D::CEBPB/G* fusions likely contribute to leukemogenesis in a manner similar to *IGH::CEBP* by deregulating their expression [40]. The roles of the remaining partner genes, *TBC1D10B*, *AH11*, and *LINC01656*, are not well defined in B-ALL.

Since all 6 patients with *TCR* rearrangements harbor known dominant drivers, including *TAF15::ZNF384* (2 patients), *SMARCA2::ZNF362*, *BCR::ABL1*, *KMT2A::MLLT10*, and *PAX5::FBRS*, *TCR* rearrangement is likely a late event that contributes to tumor progression rather than an initiating event. Expression profiling and GSEA of these *TRA/D*-rearranged B-ALL cases revealed characteristics of HSC. Notably, 5 of the 6 fusions are associated with stem cell features and multi-lineage potential. For example, B-ALL with *ZNF384* rearrangement often expresses CD10 dim/−, CD33+, and CD13+ [44], and this rearrangement is found in 48% of MPAL cases [29]. *ZNF362* fusions have a similar expression profile to *ZNF384*-rearranged B-ALL and are included in the same subgroup [27]. *KMT2A* rearrangements are frequently associated with MPAL [45], and Ph + B-ALL often expresses myeloid markers such as CD13 and CD33 [46]. Recent research on Ph + B-ALL has shown that the differentiation stage of leukemia significantly impacts patient prognosis. Specifically, patients with Ph + B-ALL who are differentiated at early progenitor and inter-progenitor stages have poorer OS and EFS rates compared to those whose B-ALL is arrested at the late progenitor stage [47].

The reason for the limited number of previously reported cases of *TCR* rearrangement in B-ALL, despite an occurrence

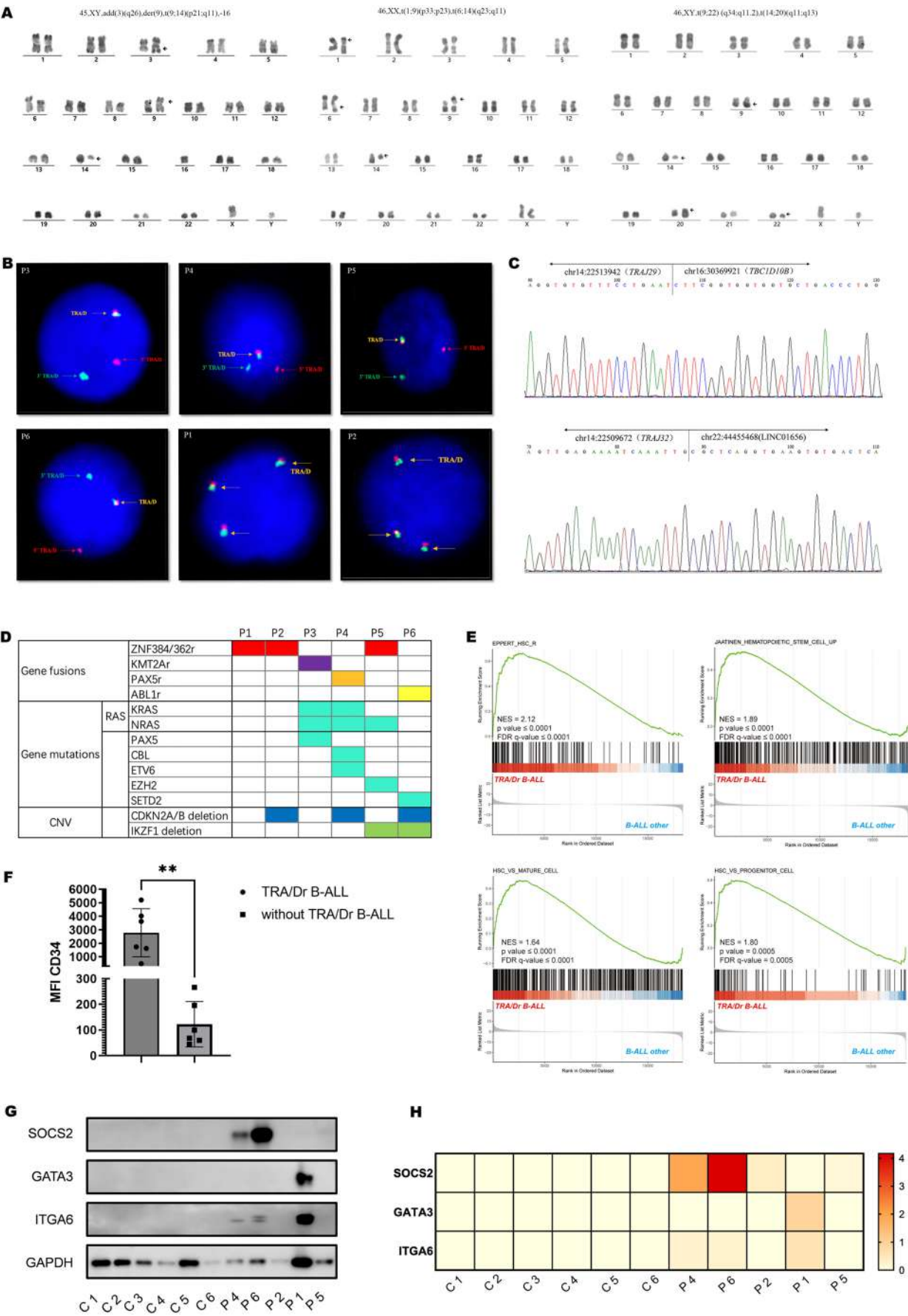


Fig. 3 Genomic and transcriptomic characteristics of *TRA/D*-rearranged B-ALL. **(A)** Karyotyping of bone marrow using R-banding showed chromosome rearrangements involving the *TRA/D* locus at 14q11.2, including t(9;14)(p21;p11) in patient 4, t(6;14)(q23;q21) in patient 5, and t(14;20)(q11;q13) in patient 6. **(B)** FISH with a split-apart probe set showed separation of 5' *TRA/D* (red) and 3' *TRA/D* (green) signals in patients 3, 4, 5, and 6, but not in patients 1 and 2. **(C)** Sanger sequencing of PCR products from patients 1 and 2 confirmed *TRAJ29::LINC01656* and *TRAJ32::TBC1D10B*, respectively. **(D)** Additional genomic alterations in the 6 cases of *TRA/D*-rearranged B-ALL were detected, with enrichment in *ZNF384/ZNF362* rearrangements, *NRAS* mutations, and *CDKN2A/B* deletions. **(E)** Gene set enrichment analysis (GSEA) using four different gene sets showed significant enrichment of hematopoietic stem cell genes in *TRA/D*-rearranged B-ALL compared to non-*TRA/D*-rearranged B-ALL. Bold lines indicate significant enrichments {false discovery rate (FDR) q -value < 0.25 and/or nominal (NOM) P -value < 0.05 }. NES: normalized enrichment score. **(F)** Patients with *TRA/D* rearrangement showed significantly higher CD34 fluorescence intensity (MFI) compared to other B-ALL cases. Nonparametric Mann-Whitney test; p -value of 0.002. **(G)** Western blot analysis confirmed the expression of stem cell markers ITGA6, SOCS2, and GATA3 in *TRA/D*-rearranged B-ALL samples (P1-5; specimen unavailable for P6). These markers were absent in randomly selected B-ALL samples without *TRA/D* rearrangement (C1-6). **(H)** Expression levels were normalized to GAPDH and presented as a heatmap

rate of 6.2% in our series, remains unclear. Two factors may contribute to this discrepancy. First, our study utilized a DNA NGS assay covering all *IG* and *TCR* regions, which can detect small genomic rearrangements, including small insertions that might escape detection by karyotyping and FISH. Indeed, 2 of the 6 cases in our series were negative by both karyotyping and FISH. Second, only a portion of B-ALL cases can proliferate in vitro, while others may not divide and only show a normal karyotype from non-neoplastic hematopoietic cells, missing the opportunity to detect chromosome rearrangements, including 14q11 (*TRA/D*) rearrangements.

In summary, we identified a subgroup of pediatric B-ALL cases with *TCR* rearrangements. These tumors

exhibit expression of HSC genes and frequently harbor other adverse genomic markers, including *CDKN2A/B* alterations and *RAS* mutations. In our series of 97 cases, 59 were classified as low risk and 38 as IR, with no cases classified as high risk. Notably, all 6 patients with *TCR* rearrangement were classified as IR finally, and the proportion of patients with an MRD level higher than 0.1% at day 19 was significantly higher in patients with *TRA/D* rearrangements than in a comparator group of 11 patients with primary drivers such as *BCR::ABL1*, *KMT2A*, *PAX5r* or *ZNF384* fusions but without *TRA/D* rearrangements (4/5 vs. 2/11; $p = 0.0179$). These findings suggest that *TCR* rearrangement may be associated with a poor initial therapeutic response, and these patients could potentially benefit from MRD-based risk-directed therapy. However, further studies are required to validate its role as a prognostic marker for IR. The overall prognostic significance of *TCR* rearrangement remains unclear due to the short follow-up period. It is noteworthy that 5 of 6 B-ALL cases with *TRA/D* rearrangement were male; however, the p -value suggests a lack of statistical significance, likely due to the limited sample size. Additionally, 6 of the 7 previously published B-ALL cases with *TCR* rearrangements were pediatric patients, with the remaining case involving a 20-year-old (Table S1). Whether *TCR*-rearranged B-ALL is enriched to pediatric cases remains to be determined. Based on these findings, we suggest that *TRA/D* rearrangement screening could be considered as part of risk assessment algorithms for pediatric B-ALL patients. However, it is important to note that models have yet to be developed to demonstrate its functional role in the poor prognosis of ALL patients. Further research is necessary to explore the biological role of *TRA/D* rearrangements and better understand their molecular contribution to B-ALL development.

Table 2 Clinical and laboratory characteristics of the six B-ALL with *TRA/D* rearrangement

Patient	Age(Years)	Sex	WBC count ($\times 10^9/L$)	Blast in BM	Blast in PB	Initial risk classification	Final risk classification	MRD on day 19	MRD on day 46	Current status
1	6.8/M	M	52.36	93%	91%	IR	IR	1.24×10^{-2}	$< 1 \times 10^{-4}$	First CR for 21 months
2	13.9/M	M	23	74.50%	50%	IR	IR	1.58×10^{-3}	$< 1 \times 10^{-4}$	First CR for 20 months
3	0.3/M	M	202.8	96%	96%	IR	IR	NA	$< 1 \times 10^{-4}$	CNS relapse at 5th month
4	2.4/M	M	19.11	90%	50%	LR	IR	2.17×10^{-2}	$< 1 \times 10^{-4}$	First CR for 18 months
5	3/F	F	30.6	90%	78%	LR	IR	$< 1 \times 10^{-2}$	$< 1 \times 10^{-4}$	First CR for 23 months
6	10/M	M	31.85	92%	85%	IR	IR	$< 1 \times 10^{-4}$	$< 1 \times 10^{-4}$	First CR for 17 months

IR intermediate-risk, LR low-risk

Table 3 Statistical evaluation of clinical parameters between patients with and without TRA/D rearrangement

Variable	TRA/D rearrangement				<i>P</i>
	Yes		No		
	No	%	No	%	
Age at diagnosis(years)					
1-10	4	5.33	71	94.70	0.52
>10	2	9.10	20	90.90	
Sex					
Male	5	9.80	46	90.20	0.119
Female	1	2.17	45	97.83	
WBC count(× 109/L)					
<50	4	5.13	74	94.87	0.381
≥50	2	10.53	17	89.47	
Extramedullary infiltration					
Yes	0	0.0	2	100.00	0.714
No	6	6.25	89	93.75	
ETV6::RUNX1					
Absent	6	7.14	78	92.86	0.32
Present	0	0.00	13	100.00	
BCR::ABL1					
Absent	5	5.49	86	94.51	0.271
Present	1	16.67	5	83.33	
TCF3::PBX1					
Absent	6	6.67	84	93.33	0.48
Present	0	0.00	7	100.00	
KMT2Ar					
Absent	5	5.38	88	94.62	0.111
Present	1	25.00	3	75.00	
Hyperdiploidy					
Absent	6	8.45	65	91.55	0.126
Present	0	0.00	26	100.00	
ZNF384r/ZNF362r					
Absent	3	3.23	90	96.77	<0.001
Present	3	75.00	1	25.00	
MEF2Dr					
Absent	6	6.32	89	93.68	0.714
Present	0	0.00	2	100.00	
Ph-like					
Absent	6	6.59	85	93.40	0.516
Present	0	0.00	6	100.00	
DUX4r					
Absent	6	6.52	86	93.48	0.555
Present	0	0.00	5	100.00	
PAX5r					
Absent	6	5.38	88	94.62	0.111
Present	0	25.00	3	75.00	
Initial risk classification					
Low	2	3.13	62	96.87	0.081
Immediate	4	12.12	29	87.88	
High	0	0.00	0	0.00	
Final risk classification ^a					
Low	0	0.85	53	98.15	0.005
Immediate	6	11.63	38	88.37	
High	0	0.00	0	0.00	
MRD on day 19 ^b					
<0.1%	1	16.39	60	98.36	0.034
≥0.1%	4	11.76	30	88.24	

Table 3 (continued)

Variable	TRA/D rearrangement				<i>P</i>
	Yes		No		
	No	%	No	%	
MRD on day 46 ^c					
<0.01%	6	6.59	85	93.41	0.553
≥0.01%	0	0.00	5	100.00	

^a Based on MRD levels measured on days 19 and 46 of remission induction

^b Undetected MRD in two patients

^c Undetected MRD in one patient

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s00277-025-06687-2>.

Author contributions J.J.Z. performed clinical studies, results interpretation, and wrote the manuscript. Y.T.W. performed laboratory work and result analysis. Z.H.L. performed result interpretation and data analysis. P.F.X., J.L., H.L. performed critical clinical studies. Y.X.H., S.Y.W., B.H.L. supported experimental execution and data verification. L.G., Q.J. assisted with statistical analysis and manuscript preparation. H.L.H., Y.W., J.L. contributed to data analysis and interpretation. L.J.M. assisted in experimental design and manuscript review. Y.Y.T. provided support with experimental setup and data analysis. Y.Z.L. contributed to data analysis and manuscript revision. N.C. performed expression analysis. S.X., S.Y.H. designed the study and supervised the research. All authors reviewed and approved the manuscript.

Funding The National Key Research and Development Program of China (no. 2022YFC2502700), Jiangsu Key project, BE2021654 to Shaoyan Hu, the National Natural Science Foundation of China (NSFC 82100229 to Yuanyuan Tian, 82200177 to Li Gao, 82170218, and 81970163 to Shaoyan Hu, and Suzhou Projects (DZXYJ202305, GSWS2023048, 2020ZKPB02), and the Suzhou Municipal Key Laboratory (SZS201615, SKY2022012, SZS2023014) to Shaoyan Hu. Soochow University of Medical School, ML13101223 to Shaoyan Hu.

Data availability No datasets were generated or analysed during the current study.

Declarations

Ethics approval The study protocol was approved by the ethics committee of the Children's Hospital of Soochow University and conducted according to the Declaration of Helsinki. Written informed consent was obtained from the patients' parents or legal guardians.

Consent to publish Not applicable.

Competing interests The authors declare no competing interests.

Open Access This article is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License, which permits any non-commercial use, sharing, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if you modified the licensed material. You do not have permission under this licence to share

adapted material derived from this article or parts of it. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by-nc-nd/4.0/>.

References

1. Inaba H, Mullighan CG (2020) Pediatric acute lymphoblastic leukemia. *Haematologica* 105(11):2524–2539
2. Shahkarami S, Mehrasa R, Younesian S, Yaghmaie M, Chahardouli B, Vaezi M et al (2018) Minimal residual disease (MRD) detection using rearrangement of immunoglobulin/T cell receptor genes in adult patients with acute lymphoblastic leukemia (ALL). *Ann Hematol* 97(4):585–595
3. Dyer MJ, Akasaka T, Capasso M, Dusanj P, Lee YF, Karran EL et al (2010) Immunoglobulin heavy chain locus chromosomal translocations in B-cell precursor acute lymphoblastic leukemia: rare clinical curios or potent genetic drivers? *Blood* 115(8):1490–1499
4. Chapiro E, Radford-Weiss I, Cung HA, Dastugue N, Nadal N, Taviaux S et al (2013) Chromosomal translocations involving the IGH@ locus in B-cell precursor acute lymphoblastic leukemia: 29 new cases and a review of the literature. *Cancer Genet* 206(5):162–173
5. Yasuda T, Tsuzuki S, Kawazu M, Hayakawa F, Kojima S, Ueno T et al (2016) Recurrent DUX4 fusions in B cell acute lymphoblastic leukemia of adolescents and young adults. *Nat Genet* 48(5):569–574
6. Davis K, Sheikh T, Aggarwal N (2023) Emerging molecular subtypes and therapies in acute lymphoblastic leukemia. *Semin Diagn Pathol* 40(3):202–215
7. Roberts KG, Li Y, Payne-Turner D, Harvey RC, Yang YL, Pei D et al (2014) Targetable kinase-activating lesions in Ph-like acute lymphoblastic leukemia. *N Engl J Med* 371(11):1005–1015
8. Jeha S, Choi J, Roberts KG, Pei D, Coustan-Smith E, Inaba H et al (2021) Clinical significance of novel subtypes of acute lymphoblastic leukemia in the context of minimal residual disease-directed therapy. *Blood Cancer Discov* 2(4):326–337
9. Guenzel AJ, Smadbeck JB, Golden CL, Williamson CM, Benvides Demasi JC, Vasmatzis G et al (2021) Clinical utility of next generation sequencing to detect IGH/IL3 rearrangements [t(5;14) (q31.1; q32.1)] in B-lymphoblastic leukemia/lymphoma. *Ann Diagn Pathol* 53:151761

10. Cauwelier B, Dastugue N, Cools J, Poppe B, Herens C, De Paepe A et al (2006) Molecular cytogenetic study of 126 unselected T-ALL cases reveals high incidence of TCR beta locus rearrangements and putative new T-cell oncogenes. *Leukemia* 20(7):1238–1244
11. Przybylski GK, Dik WA, Wanzeck J, Grabarczyk P, Majunke S, Martin-Subero JI et al (2005) Disruption of the BCL11B gene through Inv (14) (q11.2q32.31) results in the expression of BCL11B-TRDC fusion transcripts and is associated with the absence of wild-type BCL11B transcripts in T-ALL. *Leukemia* 19(2):201–208
12. Przybylski GK, Dik WA, Grabarczyk P, Wanzeck J, Chudobska P, Jankowski K et al (2006) The effect of a novel recombination between the homeobox gene NKX2-5 and the TRD locus in T-cell acute lymphoblastic leukemia on activation of the NKX2-5 gene. *Haematologica* 91(3):317–321
13. Chiaretti S, Foà R (2009) T-cell acute lymphoblastic leukemia. *Haematologica* 94(2):160–162
14. Karman K, Forestier E, Heyman M, Andersen MK, Autio K, Blennow E et al (2009) Clinical and cytogenetic features of a population-based consecutive series of 285 pediatric T-cell acute lymphoblastic leukemias: rare T-cell receptor gene rearrangements are associated with poor outcome. *Genes Chromosomes Cancer* 48(9):795–805
15. Van Vlierberghe P, Pieters R, Beverloo HB, Meijerink JP (2008) Molecular-genetic insights in paediatric T-cell acute lymphoblastic leukaemia. *Br J Haematol* 143(2):153–168
16. Kang DH, Kim SH, Jun JW, Lee YW, Shin HB, Ahn JY et al (2012) Simultaneous translocation of both TCR loci (14q11) with rare partner loci (Xq22 and 12p13) in a case of T-lymphoblastic leukemia. *Ann Lab Med* 32(3):220–224
17. Xiao AW, Jia Y, Baughn LB, Pearce KE, Pitel BA, Aster JC (2020) IGH rearrangement in myeloid neoplasms. *Haematologica* 105(6):e315–e317
18. Denny CT, Hollis GF, Hecht F, Morgan R, Link MP, Smith SD et al (1986) Common mechanism of chromosome inversion in B- and T-cell tumors: relevance to lymphoid development. *Science* 234(4773):197–200
19. Duro D, Bernard O, Della Valle V, Berger R, Larsen CJ (1995) A new type of p16INK4/MTS1 gene transcript expressed in B-cell malignancies. *Oncogene* 11(1):21–29
20. Chervinsky DS, Grossi M, Kakati S, Block AW, Aplan PD (1995) Concurrent presence of Inv (14) (q11q32) and t (4;11) (q21; q23) in pre-B acute lymphoblastic leukemia. *Genes Chromosomes Cancer* 12(3):229–236
21. Berger R, Busson M, Daniel MT (2001) B-cell acute lymphoblastic leukemia with tandem t (14;14) (q11; q32). *Cancer Genet Cytogenet* 130(1):84–86
22. da Silva WF, Cordeiro MG, Kishimoto RK, Velloso EDRP (2022) TCRAD rearrangement in B-cell precursor leukemia: an unexpected finding. *Hematol Transfus Cell Ther* 44(4):595–597
23. Wang T, Ni J, Zhang H, Hui S, Liu T, Zhang X et al (2022) Genetic characteristics and CD7-CAR-T therapy of TRAD::MYC translocation positive acute lymphoblastic leukemia patients. *EHA Libr* P377
24. Love MI, Huber W, Anders S (2014) Moderated Estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol* 15(12):550
25. Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA et al (2005) Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. *Proc Natl Acad Sci USA* 102(43):15545–15550
26. Iacobucci I, Li Y, Roberts KG, Dobson SM, Kim JC, Payne-Turner D et al (2016) Truncating erythropoietin receptor rearrangements in acute lymphoblastic leukemia. *Cancer Cell* 29(2):186–200
27. Li JF, Dai YT, Lilljebjörn H, Shen SH, Cui BW, Bai L et al (2018) Transcriptional landscape of B cell precursor acute lymphoblastic leukemia based on an international study of 1,223 cases. *Proc Natl Acad Sci U S A* 115(50):E11711–E11720
28. Hirabayashi S, Ohki K, Nakabayashi K, Ichikawa H, Momozawa Y, Okamura K et al (2017) ZNF384-related fusion genes define a subgroup of childhood B-cell precursor acute lymphoblastic leukemia with a characteristic immunotype. *Haematologica* 102(1):118–129
29. Brown G (2022) Oncogenes and the origins of leukemias. *Int J Mol Sci* 23(4):2293
30. Alexander TB, Gu Z, Iacobucci I, Dickerson K, Choi JK, Xu B et al (2018) The genetic basis and cell of origin of mixed phenotype acute leukaemia. *Nature* 562(7727):373–379
31. Naresh KN, Medeiros LJ, WHO Fifth Edition Classification Project (2023) Introduction to the fifth edition of the world health organization classification of tumors of hematopoietic and lymphoid tissues. *Mod Pathol* 36(12):100330
32. Jackson TR, Ling RE, Roy A (2021) The origin of B-cells: human fetal B cell development and implications for the pathogenesis of childhood acute lymphoblastic leukemia. *Front Immunol* 12:637975
33. Greaves MF, Chan LC, Furley AJ, Watt SM, Molgaard HV (1986) Lineage promiscuity in hemopoietic differentiation and leukemia. *Blood* 67(1):1–11
34. Dik WA, Nadel B, Przybylski GK, Asnafi V, Grabarczyk P, Navarro JM et al (2007) Different chromosomal breakpoints impact the level of LMO2 expression in T-ALL. *Blood* 110(1):388–392
35. Narducci MG, Stoppacciaro A, Imada K, Uchiyama T, Virgilio L, Lazzeri C et al (1997) TCL1 is overexpressed in patients affected by adult T-cell leukemias. *Cancer Res* 57(24):5452–5456
36. Duro D, Bernard O, Della Valle V, Leblanc T, Berger R, Larsen CJ (1996) Inactivation of the P16INK4/MTS1 gene by a chromosome translocation t (9;14) (p21-22; q11) in an acute lymphoblastic leukemia of B-cell type. *Cancer Res* 56(4):848–854
37. Sulong S, Moorman AV, Irving JA, Strefford JC, Konn ZJ, Case MC et al (2009) A comprehensive analysis of the CDKN2A gene in childhood acute lymphoblastic leukemia reveals genomic deletion, copy number neutral loss of heterozygosity, and association with specific cytogenetic subgroups. *Blood* 113(1):100–107
38. Vijayakrishnan J, Henrion M, Moorman AV, Fiege B, Kumar R, da Silva Filho MI et al (2015) The 9p21.3 risk of childhood acute lymphoblastic leukaemia is explained by a rare high-impact variant in CDKN2A. *Sci Rep* 5:15065
39. Feng J, Guo Y, Yang W, Zou Y, Zhang L, Chen Y et al (2022) Childhood acute B-Lineage lymphoblastic leukemia with CDKN2A/B deletion is a distinct entity with adverse genetic features and poor clinical outcomes. *Front Oncol* 12:878098
40. Akasaka T, Balasas T, Russell LJ, Sugimoto KJ, Majid A, Walewska R et al (2007) Five members of the CEBP transcription factor family are targeted by recurrent IGH translocations in B-cell precursor acute lymphoblastic leukemia (BCP-ALL). *Blood* 109(8):3451–3461
41. Lekstrom-Himes J, Xanthopoulos KG (1998) Biological role of the CCAAT/enhancer-binding protein family of transcription factors. *J Biol Chem* 273(44):28545–28548
42. Nerlov C (2004) C/EBPalpha mutations in acute myeloid leukaemias. *Nat Rev Cancer* 4(5):394–400
43. Laiosa CV, Stadtfeld M, Graf T (2006) Determinants of lymphoid-myeloid lineage diversification. *Annu Rev Immunol* 24:705–738
44. Zaliouva M, Winkowska L, Stuchly J, Fiser K, Triska P, Zwyrtekova M (2021) A novel class of ZNF384 aberrations in acute leukemia. *Blood Adv* 5(21):4393–4397
45. Parkin JL, Arthur DC, Abramson CS, McKenna RW, Kersey JH, Heideman RL et al (1982) Acute leukemia associated with the t

- (4;11) chromosome rearrangement: ultrastructural and immunologic characteristics. *Blood* 60(6):1321–1331
46. Corrente F, Bellesi S, Metafuni E, Puggioni PL, Marietti S, Ciminello AM et al (2018) Role of flow-cytometric immunophenotyping in prediction of BCR/ABL1 gene rearrangement in adult B-cell acute lymphoblastic leukemia. *Cytometry B Clin Cytom* 94(3):468–476
47. Kim JC, Chan-Seng-Yue M, Ge S, Zeng AGX, Ng K, Gan OI et al (2023) Transcriptomic classes of BCR-ABL1 lymphoblastic leukemia. *Nat Genet* 55(7):1186–1197

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.