



# Transcriptome sequencing identifies novel *EVX* fusions involved in transcriptional activation of *HOX* family genes in pediatric immature T-cell acute lymphoblastic leukemia: two cases reports and a literature review

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Received: 17 January 2023 / Revised: 9 May 2023 / Accepted: 15 May 2023 / Published online: 27 May 2023  
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## Abstract

Driver genomic alterations in pediatric immature T-cell acute lymphoblastic leukemia are not fully known. We report two cases of novel *EVX* fusions involved in the transcriptional activation of *HOX* family genes, *ETV6::EVX2* and *MSI2::EVX1/HOXA13*, which activate *HOXD* and *HOXA* cluster genes transcription through enhancer hijacking. *HOXA* and *HOXD* were the only key transcription factors activated in these cases, which indicates their important roles in leukemogenesis. Our findings elucidate potential drivers for development of T-cell lymphoblastic leukemia, and are valuable for diagnosis and risk stratification of pediatric T-ALL in the era of precision medicine.

**Keywords** Pediatric immature T-ALL · Novel *EVX* fusions · *HOX* family genes · Transcriptome sequencing

## Introduction

T-cell Acute Lymphoblastic Leukemia (T-ALL) is an aggressive hematological malignancy, with high tumor heterogeneity and a poor prognosis: it accounts for approximately 10–15% of pediatric ALL cases and 25% of adult ALL cases. Early T-cell Precursor Acute Lymphoblastic Leukemia (ETP-ALL), which accounts for 15% of all T-ALL cases, has a poor prognosis with high rates of remission failure or relapse [1]. T-ALL is caused by the co-occurrence of multiple genetic abnormalities that are acquired over time and, once accumulated, lead to full-blown leukemia [2]. Although comprehensive cytogenetic and genomic landscape studies have revealed important molecular abnormalities [3], the drivers of genomic alterations in pediatric T-ALL cases remain unclear. In this study, we identified novel fusions in two pediatric T-ALLs cases diagnosed and treated at the Children's Hospital of Soochow University using transcriptome sequencing (RNA-seq). In both cases, fusion genes related to the *EVX* family were identified, forming *ETV6::EVX2* and *EVX1::MSI2*, which activated the *HOXD* and *HOXA* cluster genes. *HOXA* expression results in a subgroup of T-ALL with a wide spectrum of genomic abnormalities affecting *HOXA*, *KMT2A*, *MLLT10*,

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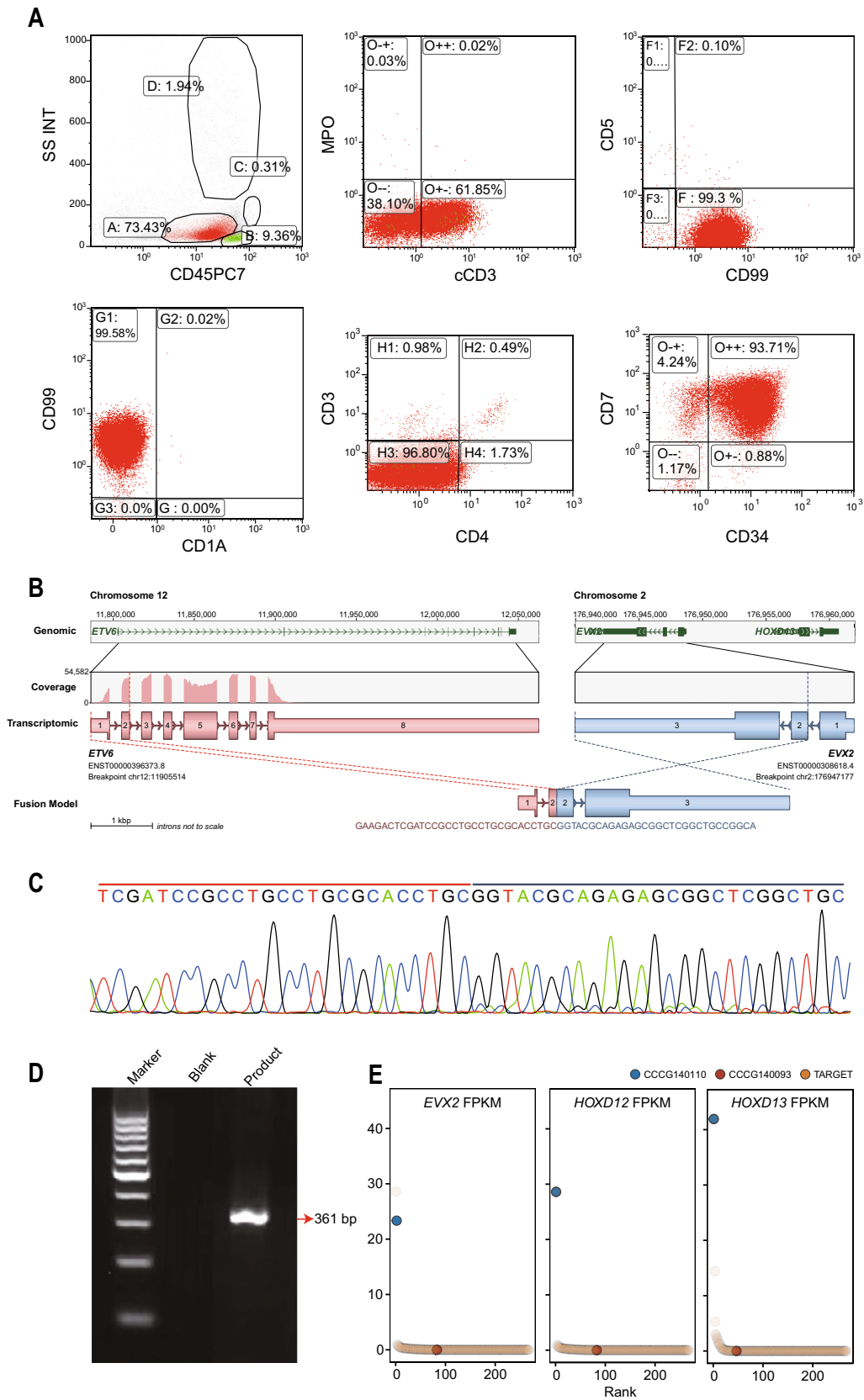
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**Fig. 1** The molecular data for CCCG140110 at diagnosis. **A** Immunophenotyping of bone marrow cells at diagnosis in CCCG140110. FCM analyses of leukemia cells revealed that the blast cells were positive for CD7, CD34, and cCD3 and negative for CD5, CD3, and CD1a. **B** RNA sequencing analysis revealed *ETV6::EVX2* fusion with breakpoints in exon 2 of *ETV6* and exon 2 of *EVX2*. **C** and **D** The *ETV6::EVX2* fusion was validated by Sanger sequencing and RT-PCR in CCCG140110. **E** Transcription of *EVX2*, *HOXD12*, and *HOXD13* in CCCG140110 and CCCG140093 together with 264 pediatric T-ALLs from the TARGET project. The Y-axis represents FPKM. The X-axis represents the rank of T-ALL cases

and *NUP214* [3, 4]. However, *HOXD* activation in T-ALL has not been previously described.

## Cases

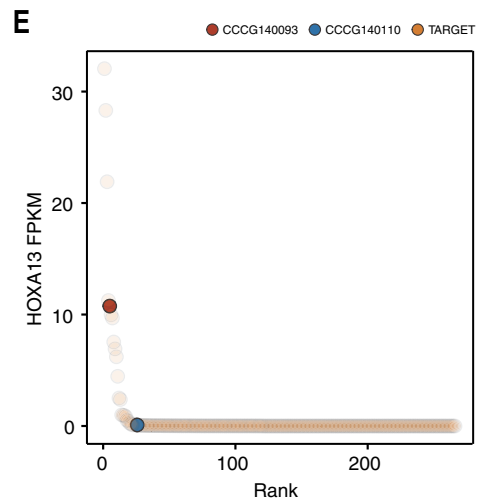
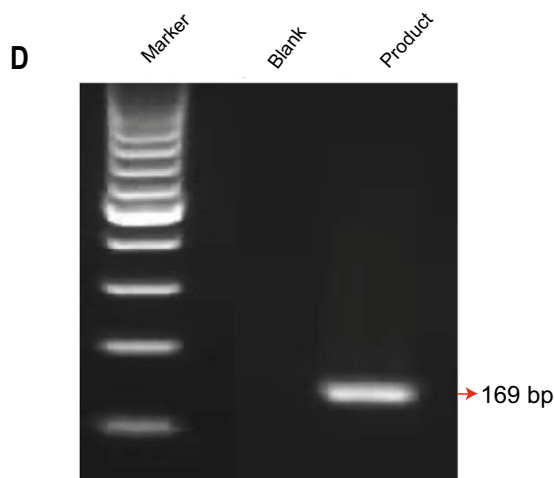
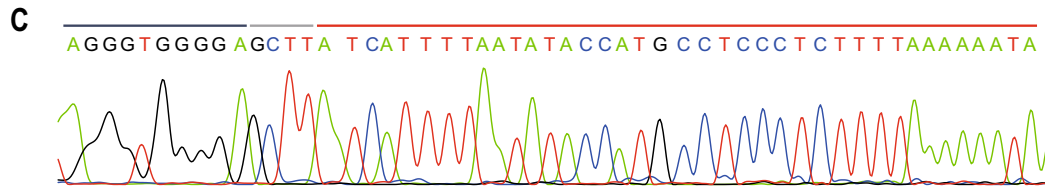
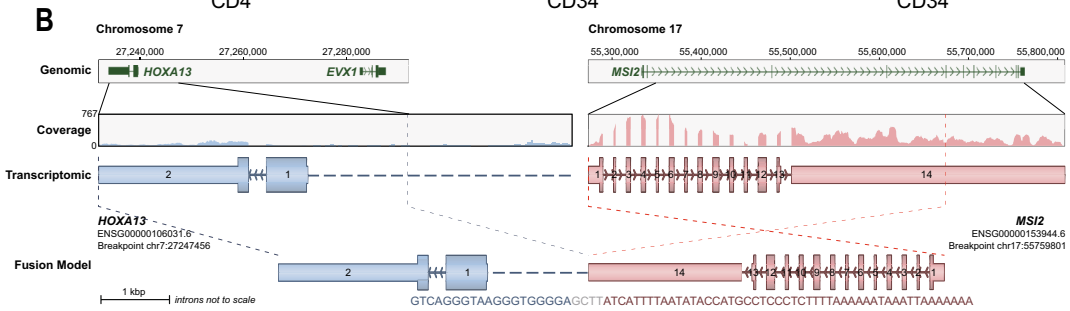
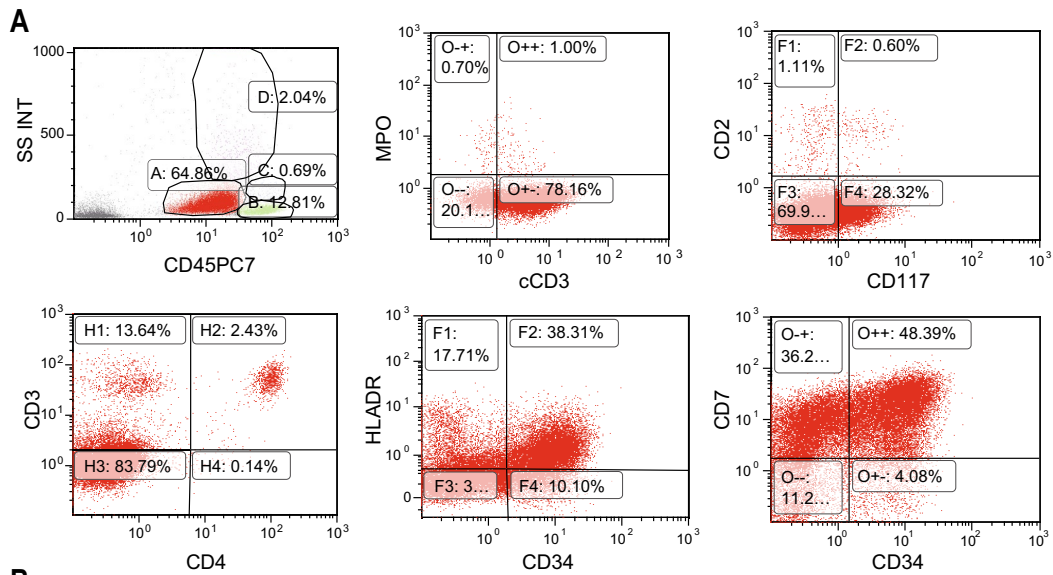
In the first patient (CCCG140110), a 7-year-old boy was hospitalized for pallor and general weakness for one week. A complete blood count showed a white blood cell (WBC) count of  $8.04 \times 10^9/L$ , hemoglobin (Hb) of 79 g/L, a platelet count of  $118 \times 10^9/L$ , and 32% blast cells. Bone marrow aspiration smears showed the presence of 79% lymphoblasts. Flow cytometry (FCM) revealed that 79.9% of blast cells expressed T-lymphoid-related markers. The blasts were positive for CD7, CD34, and cCD3 and negative for CD3, CD5, and CD1a (Fig. 1A), which are characteristic criteria for an early T-cell precursor ALL (ETP-ALL) diagnosis. Conventional cytogenetic analyses failed, because no mitotic cells were observed. RNA-seq identified an in-frame fusion from *ETV6* exon 2 on chromosome 12 to *EVX2* exon 2 on chromosome 2 (Fig. 1B), which was further validated using Sanger sequencing and RT-PCR (Fig. 1C and D, Supplementary Table 1). The fusion product retained the DNA-binding domain of *EVX2* but had no functional domain of *ETV6*, suggesting a potential transcription factor function of *ETV6::EVX2*. Meanwhile, the *EVX2* gene is located adjacent to the *HOXD* gene cluster on chromosome 2. We observed aberrantly high transcription of *EVX2* (FPKM = 23.3384), *HOXD12* (FPKM = 28.2090), and *HOXD13* (FPKM = 41.9178) in this case compared to other T-ALLs from the TARGET project, which do not carry this fusion [5] (Fig. 1E). We hypothesized that the activation of these transcription factors occurs through enhancer hijacking due to the disruption of the three-dimensional architecture of this genomic neighborhood by translocation. Other pathogenic or likely pathogenic variants detected in this case included *NRAS G12S* and *SETD2* at P1571\_Esplice and variants of uncertain significance (VUS) for *CTNBN1*, *U2AF2*, *PHF12*, *PDS5A*, *BCR* and *SREBF2* (Supplementary Table 2). The patient was placed in the intermediate-risk (IR) group for treatment according to the Chinese Children's Cancer Group (CCCG) ALL 2015 protocol [6]. Minimal

residual disease (MRD) monitoring showed that the child responded well to treatment. The MRD was  $< 0.025\%$  and  $0.01\%$  on days 19 and 46, respectively. The patient had been treated for more than 19 months, and MRD remained negative as detected by FCM and RT-PCR.

The second patient (CCCG140093) was a 13-year-old boy with enlarged lymph nodes in multiple body parts, including the neck, armpit, and groin, with no pain and good mobility. Complete blood counts showed a WBC of  $5.30 \times 10^9/L$ , a hemoglobin level of 152 g/L, a platelet count of  $209 \times 10^9/L$ , and peripheral blast cells at 16%. Morphological examination of BM smears revealed 55% lymphoblast infiltration. FCM identified 74.9% of the blastic cells expressing the human leukocyte antigen-DR isotype (HLA-DR), CD7, CD2, and cCD3, whereas CD3 expression was negative, indicating early T-ALL (Fig. 2A). Karyotype was 46, XY. Total RNA-seq identified an out-of-frame fusion between the *MSI2* locus on chromosome 17 and the *EVX1/HOXA13* locus on chromosome 7 (Fig. 2B), which was confirmed by Sanger sequencing and RT-PCR (Fig. 2C and D, Supplementary Table 1). The breakpoint on chr 7 was 7731 bp upstream of *HOXA13*. Aberrant activation of *HOXA13* was observed in this case (FPKM = 10.7565), comparable to other T-ALLs of the *HOXA* subtype from the TARGET project with a known mechanism activating *HOXA* genes [5] (Fig. 2E). Another likely pathogenic genomic variant detected in this case was the *KDM6A* H702fs mutation, whereas the VUS variants included *SUZ12*, *BCR*, and *CHD4* (Supplementary Table 2). The patient was treated according to the Chinese Children's Leukemia Group (CCLG) ALL 2008 protocol [7] and was classified into the high-risk (HR) group, because the MRD was 53.7% on day 15 and 8.1% on day 35. The patient underwent allogeneic hematopoietic stem cell transplantation for 14 months and MRD remained negative as detected by FCM and RT-PCR.

## Discussion

In pediatric T-ALL, we observed two fusions, *ETV6::EVX2* and *MSI2::EVX1/HOXA13*. Total RNA-seq was applied in this analysis, which contained the coding exons and non-transcribed regions of the genome like regulatory elements and led to the discovery of an out-of-frame rearrangement of *MSI2::EVX1/HOXA13* in the second case. *EVX1* and *EVX2* are located at the 5' end of *HOXA13* and *HOXD13*, respectively [8]. These rearrangements activate the transcription of *HOXD* and *HOXA* cluster genes through enhancer hijacking, like previously reported [9, 10]. T-ALL is characterized by the dysregulation of key transcription factors involved in T-cell differentiation, including *TALI2*, *LMO12*, *TLX13*, *NKX2-1/NKX2-5*, *LYL1* and *HOXA* cluster genes [5]. In the two cases reported in the current analysis, *HOXA13* and



**Fig. 2** The molecular data for CCCG140093 at diagnosis. **A** Immunophenotyping of bone marrow cells at diagnosis in CCCG140093. FCM analyses of leukemia cells revealed that the blast cells expressed HLA-DR, CD7, CD2, and cCD3, while CD3 expression was negative. **B** RNA sequencing analysis showed the *MSI2::EVX1/HOXA13* fusion between chr17 and chr7. **C** and **D** Validation of *EVX1::MSI2* fusion using Sanger sequencing and PCR in CCCG140093. **E** Transcription of *HOXA13* in CCCG140093 and CCCG140110 together with 264 pediatric T-ALLs from the TARGET project. The Y-axis represents FPKM. The X-axis represents the rank of T-ALL cases

*HOXD12/HOXD13* were the only key transcription factors activated, suggesting a likely pathogenic role for these rearrangements. Immature, ETP, and near-ETP phenotypes are involved in 40–45% of HOXA-positive T-ALL [11–13]. The molecular findings of the two cases in this study were consistent with the ETP/near-ETP phenotype observed in multiple lines. First, both patients harbored activated HOX family genes. The first patient with the *ETV6::EVX2* fusion gene had an ETP immunophenotype with high expression of *HOXD13* and *HOXD12* but not *HOXA*. The second patient with *MSI2::EVX1/HOXA13* fusion showed high transcription of *HOXA13* and had the characteristic immature immunophenotype of T-ALL (early T-ALL), which is between ETP and cortical T-ALL. As shown in the tSNE plot (Fig. 3), the two cases grouped with HOXA and LMO2/LYL1 T-ALLs from the TARGET project, representing T-ALL at an early T-cell differentiation stage [5]. Moreover, activating mutations in *RAS* signaling pathway genes and abnormalities in histone-modifying genes are common in ETP and early T-ALL [14]. Mutations in *NRAS* and *SETD2* were observed in patient CCCG140110 and *KDM6A* mutations were observed in patient CCCG140093. Notably, *EVX2* was activated in

CCCG140110 cells by *ETV6::EVX2* fusion. As a homeobox transcription factor, the role of *EVX2* in leukemia remains largely unknown. *EVX2* plays an important role in leukemogenesis in this case, together with *HOXD12* and *HOXD13*. To our knowledge, a translocation involving the *EVX1/2* locus has been reported in three T-ALLs, including *RUNX1::EVX1* rearrangements in two cases [15, 16] and the *ETV6::HOXD* locus in one case [17]. The two cases reported here suggest likely pathogenic role for these recurrent translocations and expand our understanding of T-ALL leukemogenesis.

**Supplementary Information** The online version contains supplementary material available at <https://doi.org/10.1007/s12185-023-03619-6>.

**Acknowledgements** This work was supported by the following projects: National Natural Science Foundation of China (81970163 and 82170218 to SH), Jiangsu Project (BE2021654 to SH), Suzhou Project (GSWS2020039 to SH), the National Clinical Research Center for Hematological Disorders (2020ZKPB02 to SH), Shanghai Key Laboratory of Clinical Molecular Diagnostics for Pediatrics (20dz2260900 to YL) and Innovative research team of high-level local universities in Shanghai (to YL).

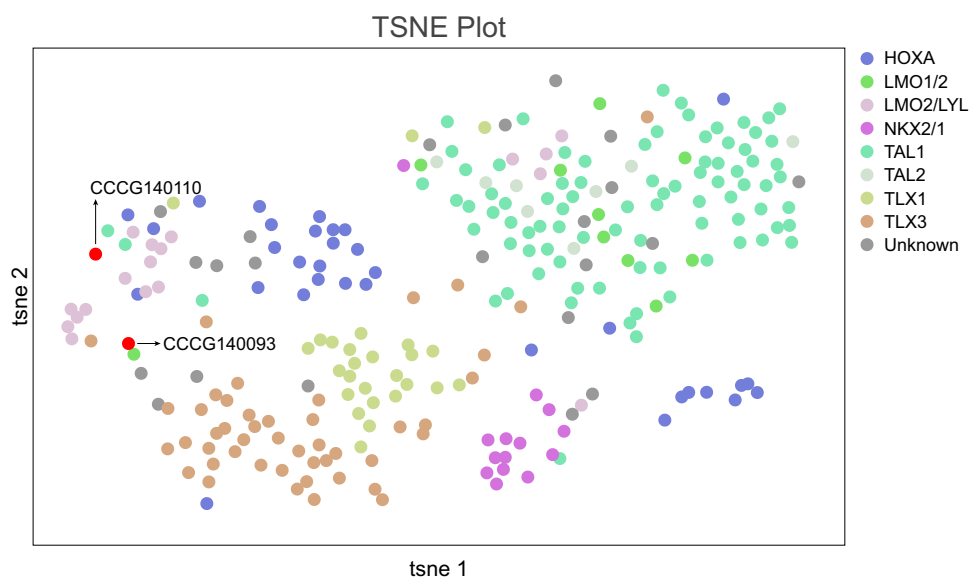
**Author contributions** XZ put all data together and drafted this manuscript. BC and YL analyzed the data. ZL interpreted the fusion genes' action and JZ collected the clinical data. PX and JL made the treatment decision. XC, ZW and JC did RT-PCR for monitoring the MRD. YL and SH designed the study, reviewed the cases, and revised the manuscript.

**Data availability** The data that support the findings of this study are openly available in [repository name] at [URL].

## Declarations

**Conflict of interest** The authors declare that they have no conflict of interest.

**Fig. 3** tSNE plot of CCCG140093, CCCG140110, and 264 pediatric T-ALLs from the TARGET project based on gene transcription. Each dot represents an individual T-ALL with molecular subgroups color coded as shown on the right. The two cases in this study, CCCG140093 and CCCG140110, are shown in red and labeled



**Ethical approval** The studies involving human participants were reviewed and approved by the Ethics Committee of the Children's Hospital of Soochow University. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin. Written informed consent was obtained from the individual(s), and minor(s)' legal guardian/next of kin, for the publication of any potentially identifiable images or data included in this article.

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