CASE REPORT

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Challenging the prognostic expectations: a rare case of *ZNF618*::*NUTM1*-positive B-cell lymphoblastic leukemia with poor outcome

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1. INTRODUCTION

NUT midline carcinoma family member 1 (*NUTM1*) fusions are primarily known for their association with poorly differentiated and aggressive carcinomas, predominantly affecting midline structures in children and young adults. Recently, *NUTM1* fusions have been identified various malignancies, including hematologic malignancies.[1] Among these, *NUTM1*-rearranged B-cell lymphoblastic leukemia (B-ALL) has emerged as a distinct subtype that typically presents in children and is associated with positive clinical outcomes. This recognition is reflected in 2 major guidelines published in 2022 to refine the diagnosis and classification of B-ALL: the 5th edition of the World Health Organization Classification of Lymphoid Neoplasms and International Consensus Classification, both of which classify B-ALL with *NUTM1* rearrangements as an independent subtype.[2,3] *NUTM1* fusions are characterized by the overexpression of the normally silent *NUTM1* gene and upregulation of the proto-oncogene *BMI1*.[4,5] Although this subtype is very rare, with an incidence of 0.28% to 0.86% among pediatric B-ALL cases, it has garnered attention owing to its typically good prognosis.[4,5] To date, 11 fusion partners of *NUTM1* have been reported in B-ALL: *ACIN1*, *BRD9*, *CUX1*, *ZNF618*, *AFF1*, *ATAD5*, *CHD4*, *RUNX1*, *SLC12A6*, *IKZF1*, and *KAT6A*.[4,6] *ZNF618*::*NUTM1* was observed in <20 patients, all of whom had successful outcomes.[4,6–11]

This case presents a unique case of *ZNF618*::*NUTM1*-positive B-ALL that exhibited a poor prognosis, challenging the current understanding that this subtype generally predicts favorable outcomes.

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The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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2. CASE PRESENTATION

A 3-year-old boy was initially admitted to a local hospital due to an unexplained fever. Blood tests showed a hemoglobin level of 111 g/L, platelet count of 33 × 10^9/L, and white blood cell count of 7.17 × 10^9/L, and bone marrow (BM) smears revealed 70% lymphoblasts. Flow cytometry (FCM) showed that 29.78% of blast cells were positive for HLA-DR, TdT, CD10, CD19, CD22, CD38, CD24, and cCD79a. Additionally, multiplex-nested reverse transcription polymerase chain reaction (RT-PCR) screening for 41 common fusion genes in leukemia yielded negative results. Fluorescence in situ hybridization analysis for *ABL1*, *ABL2*, *CSF1R*, *EPOR*, *CRLF2*, *P2RY8*, *PDGFRB*, and *JAK2* gene rearrangements was also negative, and chromosomal karyotyping showed normal results.

He was diagnosed with B-ALL and immediately started on an induction chemotherapy regimen comprising vincristine, daunorubicin, L-asparaginase, and prednisone. The patient achieved complete remission (CR) after 1 course and received 4 additional consolidation courses. However, 11 months after the initial diagnosis, the patient experienced relapse and was transferred to our hospital for further treatment.

At relapse, the BM smear showed 98.5% lymphoblasts, and FCM revealed 56.49% blast cells expressing B-lymphoid-related markers. Chromosomal karyotyping revealed 46, XY, del(6)(q13q23) in 20 analyzed metaphase cells; this deletion occurs in hematological malignancies and may involve the loss of tumor suppressor genes.[12–14] Rescreening of the 41 fusion genes yielded negative results. To further investigate additional genetic abnormalities, a targeted next-generation sequencing analysis was performed on 300 frequently mutated genes in hematological malignancies. No pathogenic mutations were identified, suggesting that the aggressive clinical course was likely driven by the *ZNF618*::*NUTM1* fusion, in conjunction with possible uncharacterized genetic or epigenetic changes.

The patient underwent reinduction chemotherapy with vincristine, L-asparaginase, and prednisone. However, the BM smear and FCM revealed 60.5% and 25.33% of lymphoblasts, respectively. Then, the patient received lymphodepleting chemotherapy with fludarabine and cyclophosphamide, followed by a single-dose infusion of 1 × 10^6/kg autologous CD19 chimeric antigen receptor T (CAR-T) cells. However, 15 days post-infusion, BM examination indicated that CAR-T cell therapy was ineffective, with 89.5% blast cells in the BM smear and 16.07% in FCM.

Subsequently, the patient underwent additional chemotherapy and autologous CD19/CD22 dual-targeted CAR-T therapy but still did not achieve CR. The patient was then treated

with blinatumomab combined with cytarabine chemotherapy and allogeneic CD19/22-CAR T-cell infusion to achieve CR. Fifty days later, he underwent allogeneic hematopoietic stem cell transplantation (allo-HSCT) with his father as the 5/10 matched donor, however, the patient relapsed 7 months after the allo-HSCT.

After this, the patient was treated with chemotherapy (mitoxantrone, cytarabine, and etoposide) and donor-specific infusion. Methotrexate was administered for graft-versus-host disease (GVHD) prophylaxis. The patient achieved CR again; however, he developed severe grade IV GVHD, characterized by widespread skin lesions and critical gastrointestinal symptoms. Quantitative PCR chimerism analysis was conducted with markers targeting chromosome 6p, revealing a loss of recipient-specific HLA genes post-allo-HSCT, rendering leukemic cells resistant to donor graft-versus-leukemia effects.[15] Ultimately, the treatment was discontinued.

To explore potential fusion genes in this patient, we performed whole transcriptome sequencing (WTS) on the relapsed BM sample and identified the in-frame *ZNF618* (exon 10)::*NUTM1* (exon 2) fusion (**Fig. 1A**). We obtained a cDNA sample from the diagnostic BM and confirmed the presence of the same fusion transcript using RT-PCR followed by Sanger sequencing. The predicted structure of the fusion protein contains 1456 amino acids and preserves 2 intact NUT domains of NUTM1 protein (**Fig. 1B**).

Herein, we also analyzed the expression levels of *NUTM1* and *BMI1*, along with another 1013 B-ALL cases and 100 healthy individuals using WTS data. None of the control cases harbored *NUTM1*-related fusion genes. Notably, *ZNF618*::*NUTM1* exhibited significantly elevated *NUTM1* expression, whereas the other 2 groups showed minimal-to-no expression (**Fig. 1C**). To determine whether the upregulated *NUTM1* expression originated from the *ZNF618*::*NUTM1* fusion transcript or wild-type *NUTM1*, we examined the WTS read alignment. As shown in Figure 1A, most sequencing reads were mapped to the fusion junction and downstream region of *NUTM1*, whereas the 5' region of wild-type *NUTM1* exhibited minimal coverage. This suggests that the increased *NUTM1* expression was primarily derived from the fusion transcript rather than from the wild-type allele. The *BMI1* gene was also overexpressed in this patient; however, this finding was not unique to this case (**Fig. 1D**).

3. DISCUSSION

This case of *ZNF618*::*NUTM1*-positive B-ALL represents a rare instance that contradicts the generally favorable prognosis associated with *NUTM1*-rearranged B-ALL. Given the generally positive prognosis, the detection of *NUTM1* fusion has led to considerations for less intensive chemotherapy regimens.[4] Only 2 previously reported patients with B-ALL with *NUTM1* rearrangements demonstrated poor outcomes: one case involved concurrent *P2RY8*::*CRLF2* fusion and the other is the sole adult case documented to date.[16]This pediatric case did not present with any additional adverse molecular genetic abnormalities, however, it demonstrated a refractory and relapsed course despite aggressive treatment modalities, including CAR-T therapy and allo-HSCT. This highlights the complexity and variability of the clinical behavior of *NUTM1*-rearranged B-ALL, indicating that treatment strategies should be carefully considered and warrant further discussion.

This clinical course can be explained using several mechanisms. First, the presence of del(6)(q13q23) may contribute to leukemogenesis by affecting tumor suppressor genes, as 6q deletions are recurrent in hematological malignancies and have been implicated in therapy resistance.[12–14] Second, immune evasion mechanisms may contribute to this course, particularly in CAR-T cell therapy failure, where antigen escape or immune checkpoint upregulation causes relapse. FCM analysis of the relapsed BM revealed that malignant B lymphoblasts partially expressed PD-1 (CD279)—an uncommon finding in B-ALL. Although the functional significance of PD-1 expression in leukemic blasts remains unclear, recent studies have suggested that PD-1 expression in B-ALL may contribute to immune evasion and resistance to CAR-T therapy.[17,18] However, PD-L1 (CD274) was not detected in the malignant blasts, indicating that PD-L1-mediated immune suppression might not have played a major role in this case. These findings highlight the complexity of immune interactions in *NUTM1*-rearranged B-ALL and suggest that additional immune profiling could provide deeper insights into treatment resistance mechanisms. Finally, epigenetic alterations may influence leukemic cell plasticity, immune regulation, and resistance to therapy. DNA methylation and histone modifications shape the transcriptional landscape of leukemia cells, potentially affecting key pathways involved in treatment response.[19] For instance, epigenetic dysregulation is linked to the altered expression of immune checkpoint molecules and leukemic stem cell properties, which may contribute to resistance against CAR-T therapy and chemotherapy. Further studies, such as single-cell RNA sequencing and assays for transposase-accessible chromatin with high-throughput sequencing, are necessary to investigate the interplay between epigenetic mechanisms and immune escape in *NUTM1*-rearranged B-ALL.

Currently, no direct experimental evidence exists that *ZNF618*::*NUTM1* acts as an oncogenic driver in pediatric patients with B-ALL. However, previous studies have shown that *NUTM1* fusion-positive B-ALL represents a distinct subtype with unique transcriptional activation and high *BMI1* expression, which are features associated with leukemogenesis.[4,5] Considering that other *NUTM1* fusion partners (eg, *ACIN1*, *BRD9*, *CUX1*, *AFF1*, *RUNX1*) have well-characterized oncogenic functions,[6] *ZNF618*::*NUTM1* may drive leukemogenesis through similar mechanisms. Further functional studies are required to elucidate their precise roles. Our case underscores the importance of continued vigilance in the clinical monitoring of *ZNF618*::*NUTM1*-positive B-ALL and suggests that the prognostic expectations for this fusion may require reassessment.

In conclusion, our findings provide valuable insights into the complexities of *NUTM1*-rearranged B-ALL and underscore the need for further research to better understand the molecular mechanisms underlying variability in its clinical outcomes.

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ETHICAL APPROVAL

Samples were obtained in accordance with the principles of the Declaration of Helsinki and the Chinese legislation for the protection of personal data and research on human samples. The study was approved by the Institutional Review Board and Ethical Committee of the Hebei Yanda Lu Daopei Hospital. Informed consent was obtained from the guardian of the patient.

AUTHOR CONTRIBUTIONS

H.L. designed the research; X.C. designed molecular studies and wrote the paper; L.Y., X.M., J.W., F.W., Y.Z., and J.C. supervised clinical and experimental findings; P.C. performed bioinformatics analysis; J.Y. and R.S. were involved in the management of the patient and provided clinical data. X.Z. performed molecular studies. All authors reviewed the manuscript and contributed to the final draft.

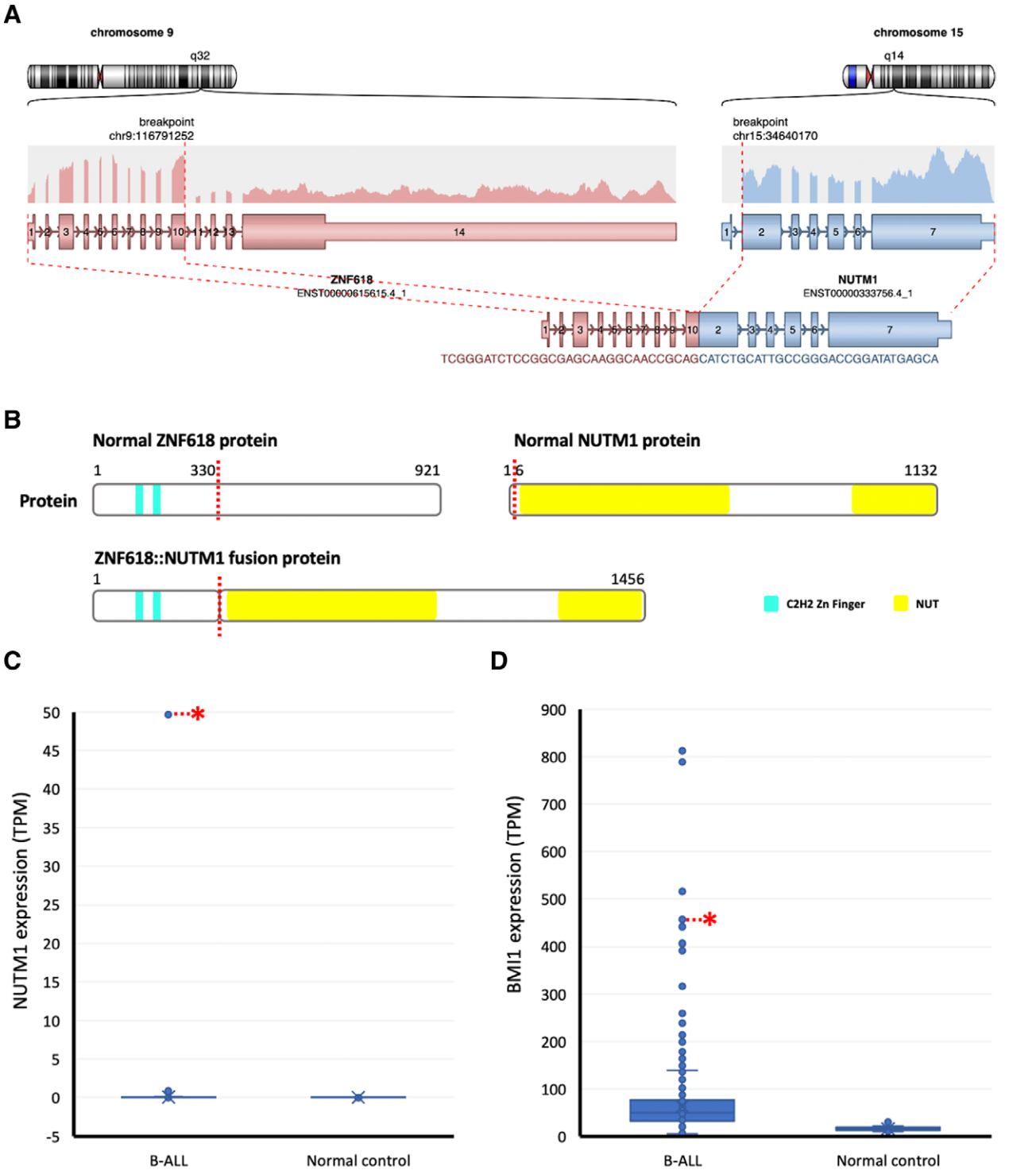

Figure 1. Transcriptomic sequencing results of the *ZNF618*::*NUTM1*-positive B-ALL patient. (A) Schematic representation of the *ZNF618*::*NUTM1* fusion, where exon 10 of *ZNF618* is fused with exon 2 of *NUTM1*. (B) Schematic diagrams of ZNF618, NUTM1, and the predicted ZNF618::NUTM1 fusion protein. Cleavage sites on the 2 proteins are indicated by red dashed lines. (C) Gene expression levels of *NUTM1* as determined by transcriptomic sequencing. (D) Gene expression levels of *BMI1* as determined by transcriptomic sequencing. Red asterisks indicate the expression levels of *NUTM1* and *BMI1* in the present case with the *ZNF618*::*NUTM1* fusion. B-ALL = B-cell lymphoblastic leukemia.

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