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Case Report
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Case Report of Three Co-Expression Gene Mutations in Pediatric AML Treated with Chemotherapy Combined with Haploid Conjugated Hematopoietic Stem Cell Transplantation

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Abstract

Background: In pediatric Acute Myeloid Leukemia (AML), the co-expression of NUP98/NSD1, FLT3/ITD, and WT1 constitutes a rare and complex genetic mutation profile, typically showing low responsiveness to chemotherapy alone and difficulty in achieving remission with the addition of targeted therapies. This case report introduces a combined transplantation treatment approach, exploring therapeutic options for this challenging AML subtype.

Methods: We reported on a pediatric patient with AML exhibiting co-expression of NUP98/NSD1, FLT3/ITD, and WT1 who failed to achieve remission after multiple courses of chemotherapy and targeted therapy. The patient subsequently underwent an innovative treatment consisting of Haploidentical allogeneic Hematopoietic Stem Cell Transplantation (Haplo-HSCT) supported by umbilical cord blood.

Results: Post-Haplo-HSCT, the patient achieved a complete molecular remission for 12 months, a remarkably rare occurrence in such cases. Bone marrow pathology and Minimal Residual Disease (MRD) analysis corroborated this significant and sustained therapeutic effect.

Conclusion: This case demonstrates that for pediatric AML patients with a specific combination of genetic mutations, a novel curative approach combining chemotherapy with innovative Haplo-HSCT treatment can be established. The findings provide valuable clinical insights for future treatment protocols of this AML subtype and could potentially influence therapeutic strategies for similar cases.

Keywords: Pediatric; AML; FLT3/ITD; NUP98/NSD1; WT1; Haplo-HSCT

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Introduction

This case report focuses on a rare but highly complex case of pediatric Acute Myeloid Leukemia (AML) that co-expresses three gene mutations: NUP98/NSD1, FLT3/ITD, and WT1. These genetic mutations are generally associated with the malignancy and poor prognosis of the disease [1]. Despite undergoing multiple rounds of treatment, including standard chemotherapy, salvage chemotherapy, and targeted therapy, the patient failed to achieve durable remission and continued to test positive for Minimal Residual Disease (MRD). Therefore, a haploidentical hematopoietic stem cell transplant from the patient's father was chosen as the treatment plan. Following the transplant, the patient received immunosuppressive therapy, along with medications such as azacitidine and venetoclax.

Case Presentation

In pediatric AML patients, we report a case with an extremely complex genetic background. Peripheral blood cell analysis revealed a WBC count of 343.45×10^9/L, hemoglobin levels at 68 g/L, and platelets at 119×10^{12}/L, with blasts comprising 75%. Further bone marrow morphology tests suggested Acute Monocytic Leukemia (AML-M5), while G-band chromosome analysis revealed

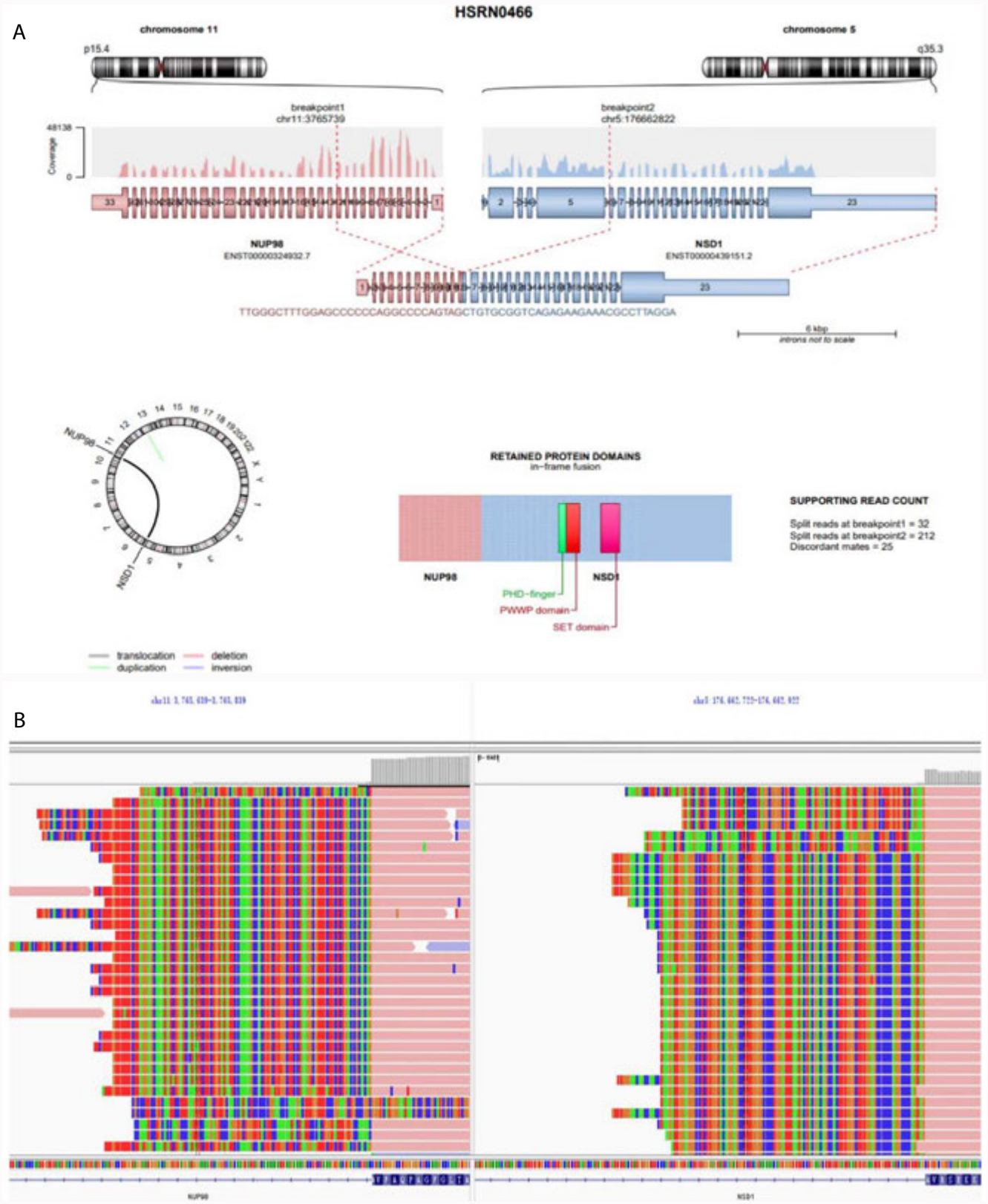

Figure 1: IGV visualization of NUP98-NSD1 fusion gene sequencing data. **A)** Zoomed-in view of the fusion junction region, indicating the precise breakpoint. **B)** Gene annotation track showing the positions of NUP98 and NSD1 genes.

a karyotype of 46, XY, inv(5)(q15q35). Flow cytometry phenotypic analysis showed a non-M3 AML immunophenotype, with CD34+ cells making up about 84.3% of the total nucleated cells.

Genetic testing further uncovered the complexity of the patient's pathological state. Somatic mutations included clinically relevant Class I mutations (FLT3-ITD, WT1(p.S386fs), WT1(p.A387fs)) and

potentially clinically relevant Class II mutations (WT1(p.R467P)). Additionally, multiple lineage mutations were detected, including AKT1S1(p.R51G), BLM(p.K812Q), DDX41(p.S154R), RB1(p.S82L), RYR1(p.E3689dup), TYK2(p.R221W), and UBR4(p.T2677I). At the chromosomal level, a heterozygous deletion of a normal copy number of chr13 was identified. Blood tumor fusion gene panel tests revealed positive fusion genes NUP98-NSD1 (Figure 1A, 1B) and ST8SIA4-NUP98.

Based on the patient's medical history and examination results, we employed the DAH (Daunorubicin, Cytarabine, and Homoharringtonine) chemotherapy regimen, combined with targeted drugs sorafenib. Flow cytometry detected approximately 2.3% residual AML tumor cells in the sample taken on day 15, indicating suboptimal treatment efficacy. Subsequent salvage chemotherapy with the C+HAG (Clofarabine, Homoharringtonine, Cytarabine, G-CSF) regimen and targeted therapy was administered, but bone marrow MRD revealed 8.11% residual cells, indicating ongoing chemotherapy resistance. Despite multiple rounds of standard chemotherapy, salvage chemotherapy, and targeted therapies, the patient's condition did not show significant improvement. Persistent MRD tests yielded positive results, suggesting the continuous presence of residual leukemia cells. Therefore, we decided to adopt a more aggressive treatment approach. The study opted for non-remission transplantation therapy assisted by cord blood peripheral blood hematopoietic stem cells. Typing results: Cord blood HLA typing: GVH 6/10, HVG 7/10; the patient's father's HLA +Cw+DRB1+DQB1+DPB1 typing: 6/12; DSA negative; KIR receptor-ligand, ligand-ligand were both mismatched. Pre-treatment regimen: BU+FLU+IDA+ CTX +ATG, Busulfex 0.95 mg/kg for 3 days from -d10 to -d8, fludarabine 30 mg/m^2 from -d7 to -d5 and idarubicin hydrochloride 10 mg/m^2 for 3 days from -d7 to -d5, ATG 2.5 mg/kg for 4 days from -d6 to -d3, and cyclophosphamide 40 mg/kg for 2 days from -d4 to -d3. On d-1, 4.11 × 10^6/L CD34+ cord blood stem cells were infused, and on d0, 12.6 × 10^6/kg of CD34+ stem cells were harvested and reinfused (Figure 2).

After the successful transplantation on day 60, the patient started three courses of azacitidine combined with venetoclax chemotherapy to prevent relapse. Two months post-transplant, bone marrow pathology and MRD results indicated remission, and to date, the patient has reached complete molecular remission for 12 months. Our case illustrates that pediatric AML patients with NUP98-NSD1, FLT3-ITD, and WT1 mutations are difficult to bring into remission through chemotherapy drugs alone and may require rescue transplantation for better therapeutic outcomes.

Discussion

Pediatric Acute Myeloid Leukemia (AML) accounts for 15% to 20% of all childhood leukemias. Despite significant advances in treatment methods and diagnostic techniques in recent years, the overall survival rate for this disease remains less than 82%, with a 5-year event-free survival rate ranging from approximately 46% to 69% [2]. These figures reflect the immense challenges still facing the treatment of pediatric AML.

In the field of pediatric AML treatment, the co-expression of NUP98-NSD1, FLT3-ITD, and WT1 is relatively rare in clinical settings [3], adding to the complexity and challenges of treatment. This particular gene combination is not only associated with a high degree of malignancy but also with poor responsiveness to conventional and salvage chemotherapy [1,3,4]. In recent years, many studies have focused on the role of gene mutations in pediatric AML to better understand the disease mechanisms and to provide new targets for clinical treatment. Various molecular targeted therapies have been developed to study these genetic changes and are under investigation in multiple clinical trials. In the treatment of adult AML, innovative treatment methods have been introduced and are making significant progress. These methods are also gradually changing traditional treatment approaches for pediatric AML. Although the clinical trial results of some new drugs are encouraging, further research on a larger pediatric patient population is still needed to improve the prognosis of children with AML [5].

In pediatric AML, NUP98 gene rearrangements are relatively rare, accounting for about 3.8%. This is a fusion gene caused by the chromosomal translocation t(5;11)(q35;p15.5), involving Nuclear Pore Complex Protein 98 (NUP98) and Nuclear SET Domain-containing protein 1 (NSD1). It is associated with high malignancy and poor treatment response in pediatric AML [3]. NUP98-NSD1 may regulate gene expression by affecting chromatin structure and histone modifications, thereby enhancing the proliferation and survival of leukemia cells. The NUP98-NSD1 fusion gene, associated with poor prognosis, is relatively common in pediatric AML cases with normal cytogenetic characteristics, making it one of the more frequent genetic alterations in this population [6].

Persistent activation of the FLT3 tyrosine kinase receptor is caused by FLT3-ITD (Internal Tandem Duplication) mutations in the FLT3 gene, promoting cell proliferation and inhibiting apoptosis. FLT3 gene mutations are a relatively frequent genetic alteration and are closely associated with poor prognosis in AML patients [7]. In pediatric AML cases, the presence of FLT3-ITD mutations is closely

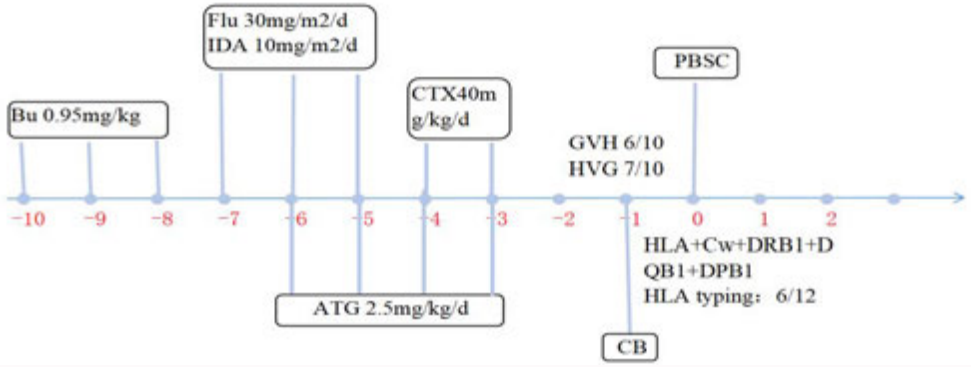

Figure 2: Pre-treatment regimen for peripheral blood hematopoietic stem cell transplantation assisted by cord blood.

related to high-risk disease and increased rates of treatment failure and relapse. Some progress has been made in targeting FLT3-ITD, but further optimization is needed to improve patient outcomes [8].

Wilms' Tumor-1 protein (WT1) is a transcription factor that plays a crucial role in regulating cellular growth, differentiation, and apoptosis by selectively activating or inhibiting genes. Mutations in the WT1 gene may promote the development of leukemia cells by affecting pathways of apoptosis and proliferation. Various interacting partners play a critical role in regulating the cellular functions of WT1. Specifically, the WT1 protein carries out functions related to cell growth, maturation, and apoptosis (cell death) to promote differentiation. To achieve this, WT1 regulates the activity of other genes by binding to specific regions of DNA. The occurrence rate of WT1 mutations in pediatric AML patients is about 8.3%. The Overall Survival rate (OS) for the WT1 wild-type group is higher than for the WT1 mutant group (54% *vs.* 41%) [9].

The different combinations of WT1 gene mutations, FLT3-ITD alterations, and NUP98-NSD1 fusion genes characterize subgroups of pediatric AML patients with poor prognosis. Therefore, more aggressive and personalized treatment strategies are needed for these high-risk patients. Targeted therapy against these specific gene mutations could be an effective approach. Specific inhibitors targeting FLT3-ITD have been shown to reduce malignancy and enhance treatment response. Guidelines now recommend timely molecular testing for FLT3 mutations at the time of diagnosis and early implementation of targeted therapy to achieve deeper remission and facilitate timely consideration of allogeneic hematopoietic stem cell transplantation [10].

Several clinical trials have been conducted to explore the efficacy of FLT3 inhibitors in preventing and treating AML recurrence after allogeneic hematopoietic stem cell transplantation, following the successful use of sorafenib for treating FLT3-ITD mutated AML. However, many questions about the mechanism of action of these inhibitors remain unanswered [11]. In this case, we promptly combined sorafenib with chemotherapy (DAH) during the early stages of treatment. However, no significant improvements were observed in the patient's inflammatory markers, liver function, kidney function, or hematological parameters. Therefore, even with the timely addition of sorafenib, the clinical indicators of some patients co-expressing NUP98-NSD1, FLT3-ITD, and WT1 may still not show significant improvement. This may be due to a combination of factors such as individual patient variability, disease severity, treatment dosage, and duration. The use of FLT3 inhibitors should be further observed in more clinical cases in the future. Currently, there are limited clinical reports on the successful treatment of patients exhibiting simultaneous mutations in NUP98-NSD1, FLT3-ITD, and WT1. Our case study emphasizes the potential difficulties in achieving complete remission for pediatric AML patients with NUP98-NSD1, FLT3-ITD, and WT1 mutations using conventional chemotherapy, salvage chemotherapy, and targeted therapies. Haplo-HSCT may be necessary to achieve complete molecular remission. We have explored the possibility of using allogeneic hematopoietic stem cell transplantation as a viable treatment strategy for high-risk pediatric AML cases with these mutations. However, further studies of additional cases are needed to more broadly assess the role of this approach in treating pediatric AML patients with mutations in NUP98-NSD1, FLT3-ITD, and WT1.

In summary, for pediatric AML patients carrying mutations in NUP98-NSD1, FLT3-ITD, and WT1, a comprehensive treatment plan involving intensified genetic testing, personalized treatment plans, targeted therapies, and allogeneic hematopoietic stem cell transplantation should be employed to achieve biological remission and improve prognosis.

Conclusion

This case report reveals that pediatric AML patients carrying triple mutations in NUP98-NSD1, FLT3-ITD, and WT1 may encounter significant challenges when undergoing conventional chemotherapy, salvage chemotherapy, and targeted therapies. Although these patients have extremely complex genetic and molecular backgrounds, Haplo-HSCT still shows potential as a possibly effective treatment method. However, given the uniqueness and complexity of each case, more case studies and long-term follow-ups are needed to further assess the broad applicability and long-term efficacy of this treatment approach.

Through this case, we strongly recommend comprehensive molecular and genetic testing at the initial stage of diagnosis to more accurately assess disease risk and formulate personalized treatment plans. Additionally, this case provides valuable clinical experience for managing pediatric AML patients with similar complex genetic backgrounds.

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