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Case Report

Homoharringtonine Combined with Pegylated Liposomal Doxorubicin Bridging to Haploidentical Hematopoietic Stem Cell Transplantation for Rare Acute Promyelocytic Leukemia with CPSF6-RARG Variants: A Case Report

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Received: 6 March 2024; Accepted: 20 May 2024; Available online: 30 June 2024

ABSTRACT: Acute promyelocytic leukemia (APL) with CPSF6-RARG fusion gene is a rare leukemia. Patients with this fusion are resistant to all-trans retinoic acid (ATRA) and arsenic trioxide (ATO), as well as conventional chemotherapy with anthracycline plus cytosine arabinoside. Therefore, it is necessary to explore new protocols for these patients to improve remission and survival. In this case, a 21-year-old female with APL of CPSF6-RARG fusion gene was resistant to daunorubicin combined with cytarabine, ATRA, and ATO. Then the patient underwent a combination of homoharringtonine and pegylated liposomal doxorubicin chemotherapy (homoharringtonine 2 mg/day from day 1–7, Ara-C 15 mg/m^2 every 12 hours from day 1–7, Peg-Dox 40 mg/m^2 divided over 3 days from day 1–3 and rhG-CSF 300 μg/day from day 0–8). Following one month of treatment, the patient reached CR. And after another two cycles of the HADG protocol with medium-dose Ara-C treatment, the CPSF6-RARG turned negative. Then the patient successfully received stem cell transplantation. Currently, the patient has survived disease-free for more than 2 years. This study indicates that a combination of homoharringtonine and pegylated liposomal doxorubicin chemotherapy is a successful treatment plan for AML resembling APL with the CPSF6-RARG fusion gene. However, further validation in larger sample sizes is required.

Keywords: Induction therapy, Acute promyelocytic leukemia, CPSF6-RARG, Novel protocol

1. Introduction

Acute promyelocytic leukemia (APL) with the promyelocytic leukemia–retinoic acid receptor alpha (PML-RARA) fusion gene caused by t (15; 17) translocation is uniquely sensitive to all-trans retinoic acid (ATRA) and arsenic trioxide (ATO) [1]. Some cases with acute myeloid leukemia (AML) resembling APL have retinoic acid receptor beta (RARB) or retinoic acid receptor gamma (RARG) rearrangement [2–4]. However, APL without the PML-RARA fusion gene is resistant to ATRA and ATO [5].

CPSF6-RARG, a very rare fusion gene of inv (12q13;12q15), is difficult to diagnose and treat effectively due to its resistance to ATRA and ATO [6–8]. Therefore, new protocols should be explored for these patients to improve remission and survival.

Homoharringtonine (Omacetaxine mepesuccinate, HHT), a cytotoxic alkaloid extracted from a Chinese natural plant, was proved to have an anti-proliferation effect, and was used in the treatment for leukemia as early as 1970s [9–11]. It was clarified that homoharringtonine reduces the expression of MCL1, blocks short-half-life oncoproteins, and induces myeloid leukemia cells apoptosis [12].

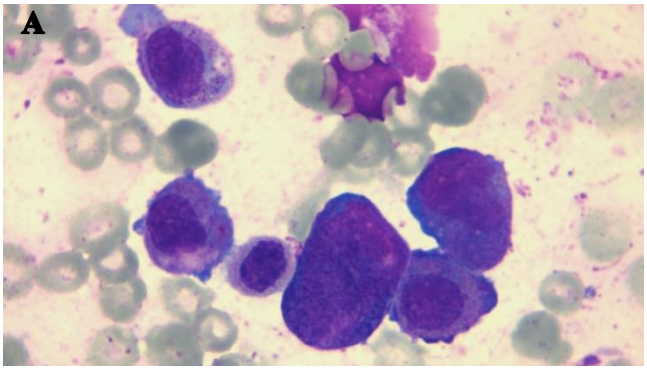
https://doi.org/10.70322/BG20240110008

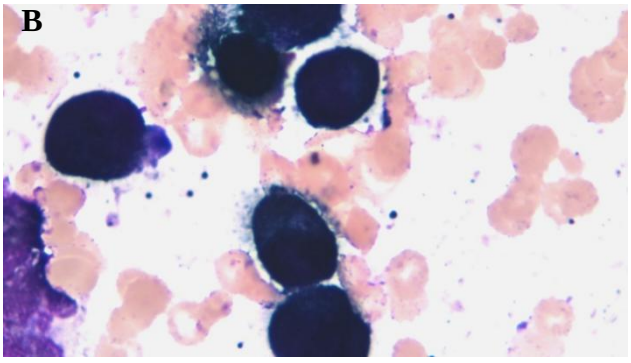
In this work, we describe a case of AML with a rare CPSF6-RARG fusion that morphologically and immunophenotypically resembles APL. Low-dose chemotherapy with homoharringtonine and pegylated liposomal doxorubicin produced a complete remission. After undergoing a successful haploidentical hematopoietic stem cell transplant (HSCT), the patient maintained remission for more than 28 months.

The study was reviewed and approved by the Institutional Review Board of Xinqiao Hospital, Army Medical University. Written informed consent was obtained from the patients. This work was in compliance with the Declaration of Helsinki.

2. Case Report

A 21-year-old, previously healthy female, was admitted due to excessive menstruation, dizziness, and fatigue. Blood tests showed the white blood cell count was 6.81 × 10^9/L (normal range, 4–10 × 10^9/L), the absolute neutrophil count was 0.61 × 10^9/L (normal range, 4–10 × 10^9/L), the hemoglobin level was 61 g/L (normal range, 110–150 g/L), and the platelet count was 23 × 10^9/L (normal range, 100–300 × 10^9/L). The fibrinogen was 1.67 g/L (normal range, 2.00–4.00 g/L). The D-dimer level was 2.605 μg/mL (normal range, 0.00–0.55 μg/mL). The activated partial thromboplastin time was 26.3 seconds (normal range, 23–35 seconds). The prothrombin time was 12.2 seconds (normal range, 10.5–13.0 seconds). The 82.5% blasts consisting of predominantly abnormal hypergranular promyelocytes with occasional Auer rods were observed in the bone marrow smear (Figure 1A). Moreover, myeloperoxidase was strongly positive in blasts, including promyelocytes (Figure 1B). Flow cytometric immunophenotypic studies demonstrated that the blasts were positive for CD13, CD33, CD117, and CD56 (Figure 1C), but negative for CD34, HLA-DR, CD38, CD14, CD15, CD2, CD3, CD4, CD7, CD8, CD19, CD20, and CD10. However, dual-color dual-fusion fluorescence in situ hybridization with a specific probe for PML and RARA failed to detect the PML-RARA fusion transcript in the bone marrow sample (Figure 1D). A chromosomal analysis using GTG banding indicated a normal karyotype of 46, XX (Figure 1E). Myeloid-related fusion genes (BCR-ABL, SIL-TAL1, E2A-HLF, TEL-AML1, MLL-AF4, E2A-PBX1, AML1-ETO, MLL-AF9, PML-RARA, PLZF-RARA, STAT5b-RARA, MLL-ELL, MLL-AF6, MLL-AF10, MLL-ENL, NPM-MLF1, TEL-PDGFFRB, FIP1L1-PDGFRA, AML1-MDS1/EVI1, AML1-MTG16, CBFβ-MYH1, DEK-CAN, TEL-ABL, ETV6-PDGFRA, NUP98-HoxA13, NUP98-HoxC11, NUP98-HoxD13, NUP98-HoxA9, NUP98-HoxA11, NUP98-PMX1, TEL-JAK2, MLL-AF17, MLL-AF1q, MLL-AF1p, MLL-AFX, MLL-SEPT6, NPM-RARA, PRKAR1A-RARA, FIP1L1-RARA, NUMA1-RARA, NPM-ALK, SET-CAN, and TLS-ERG) were negative by reverse transcription-polymerase chain reaction (RT-PCR).





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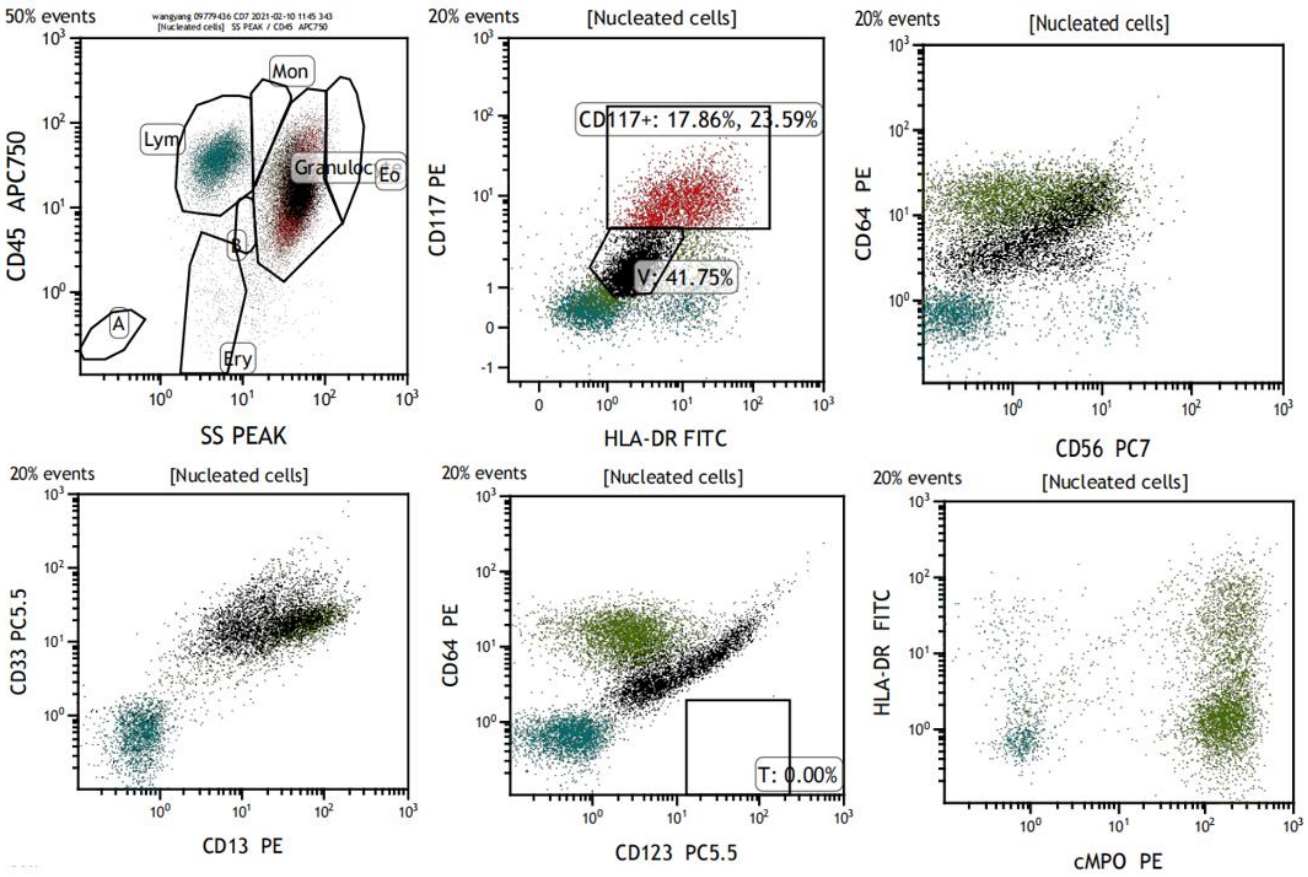
50% events
CD45 APC750
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HLA-DR FITC
CD117+: 17.86%, 23.59%
V: 41.75%
20% events
[Nucleated cells]
CD64 PE
CD56 PC7
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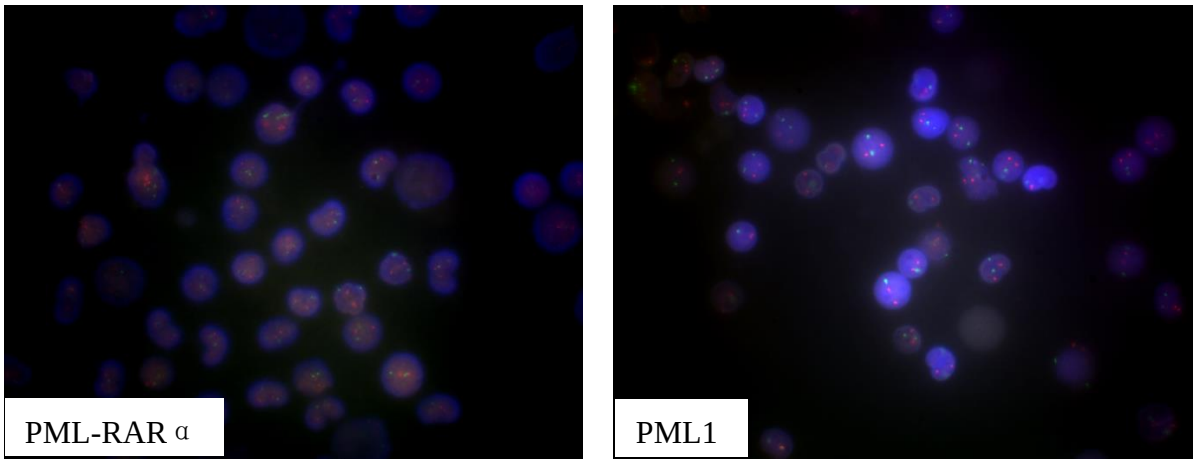

PML-RAR α
PML1

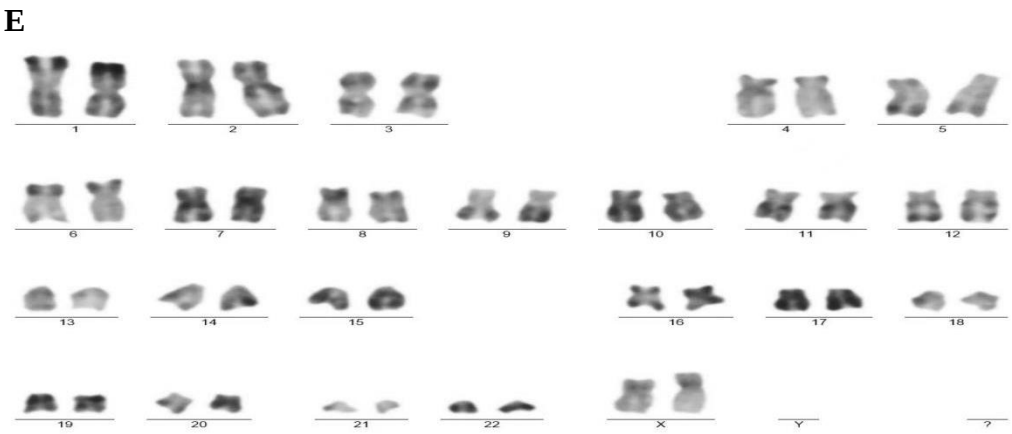
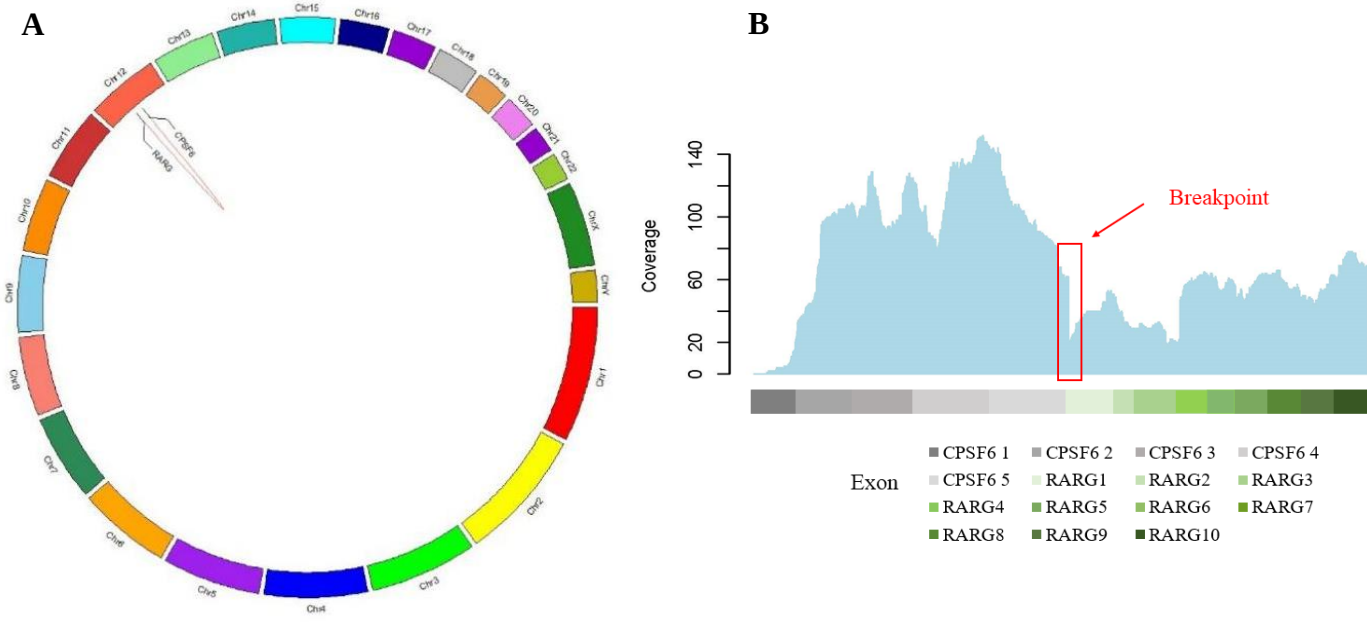


Figure 1. Results of bone marrow puncture. (**A**): Immature cells consisted of predominantly abnormal hypergranular promyelocytes with occasional Auer rods. (**B**): Myeloperoxidase was strongly positive in blasts, including promyelocytes. (**C**): Flow cytometric immunophenotypic study showed that the blasts were positive for CD13, CD33, CD117, and CD56. (**D**): DCDF-FISH failed to detect the PML-RARA fusion transcript. (**E**): A chromosomal analysis using GTG banding indicated a normal karyotype of 46, XX.

The patient was diagnosed with APL and underwent induction therapy with daunorubicin (60 mg on days 1 to 3) and Ara-C (150 mg on days 1 to 7). However, 68.5% of the cells found in the bone marrow were identified as promyelocytes. Subsequently, the patient was admitted to our hospital. We performed RNA sequencing of the peripheral blood samples with NovaSeq (Illumina, Inc., San Diego, CA, USA) for potential fusion genes and found 1 fusion gene using FuseCatcher software (Figure 2A,B). The fusion gene was the fusion of CPSF6 exon 5 with RARG exon 1, and the fusion protein consisted of 828 amino acids (Figure 2C). We also performed next-generation sequencing (NGS) to identify GATA2 and KMT2A mutations.



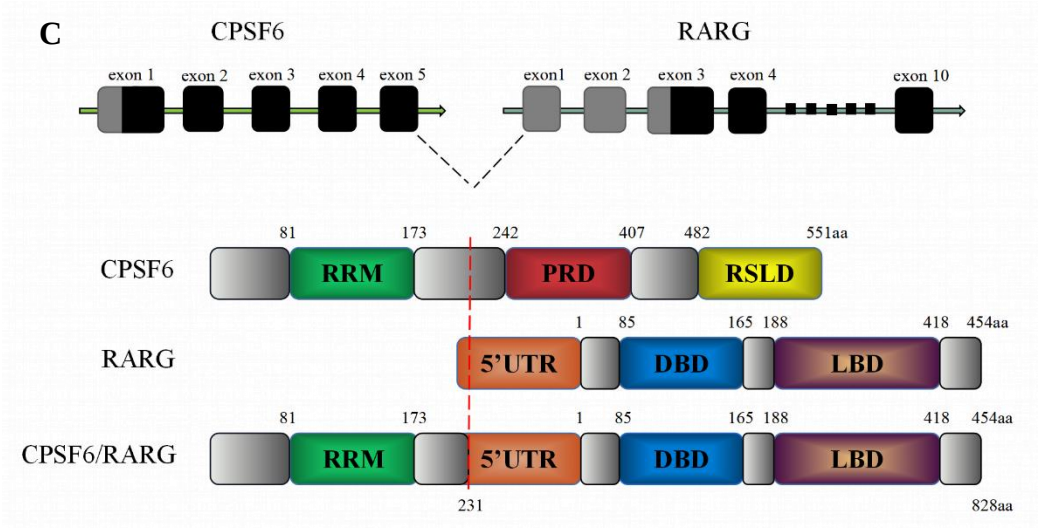


Figure 2. Molecular analysis of the CPSF6-RARG fusion. (**A**): RNA sequencing analysis indicated 1 fusion gene using FuseCatcher software. (**B**): RNA sequencing reads based on pseudoalignment to the RARG-CPSF6 predicted fusion transcript. (**C**): Schematic comparison of the CPSF6-RARG fusion transcript and the fusion protein of the patient, whose fusion site is consistent with another reported case [13].

Based on morphological and immunophenotypical results, we presumptively diagnosed APL. The patient was treated with a dose of 20 mg of ATRA and 10 mg of ATO per day. After around three weeks of treatment, promyelocytes made up 1% of the bone marrow cells. However, her peripheral blood cell did not recover in the next month. Even continuous administration of ATRA and ATO, 56% promyelocytes were found in the repeated bone marrow. The patient underwent a course of low-dose chemotherapy with homoharringtonine combined with pegylated liposomal doxorubicin (homoharringtonine 2 mg/day from day 1–7, Ara-C 15 mg/m2 every 12 hours from day 1–7, Peg-Dox 40 mg/m2 divided over 3 days from day 1–3, and rhG-CSF 300 μg/day from day 0–8). After one month of treatment, the patient reached CR. After another two cycles of the HADG protocol with medium-dose Ara-C treatment with negative CPSF6-RARG, GATA2, and KMT2A, the patient received haploidentical HSCT from her father. Following a Me-CCNU+BU+CY+Ara-C+ATG conditioning regimen (semustine 0.2 mg/kg qd×1 d, busulfan 0.8 mg/kg q6h×3 d, Ara-C 2 g/m2 q12h×2 d, cyclophosphamide 1.8 g/m2 qd×2 d, rabbit anti-human thymocyte immunoglobulin 2.5 mg/kg qd×4 d)，she transplanted with peripheral blood stem cells and bone marrow from the haploidentical donor on September 16 and 17, 2021, and achieved engraftment at +26 d after infusion of the stem cells. Currently, the patient has survived disease-free for more than 33 months.

3. Discussion

Because both the CPSF6 and RARG genes are located at 12q1, even with NGS, it is difficult to identify the CPSF6-RARG fusion gene using traditional technologies. RNA sequencing is used to identify the fusion gene from most patients [6–8,13,14], and also detected the CPSF6-RARG fusion gene for this patient in this study. Finally, the patient, with a CPSF6-RARG or RARG-CPSF6 fusion gene morphologically diagnosed with APL, was not sensitive to ATRA and ATO, which indicates that this is a novel subtype of AML.

Patients with this new type of leukemia are resistant to ATRA and ATO, as well as the conventional chemotherapy with anthracycline plus cytosine arabinoside [6–8,13,14]. Only one patient underwent chemotherapy with daunorubicin and cytosine arabinoside (DA), and one patient underwent treatment using homoharringtonine and cytosine arabinoside (HA) [6–8]. However, other patients who received the same treatment with DA or HA, did not show any responses (Table 1). Therefore, it is important to explore new protocols for these patients to improve remission and survival.

Table 1. The outcome for patients with CPSF6-RARG or RARG-CPSF6 fusion gene.

No.	Cycle 1			Cycle 2		
	Fusion gene	**Drugs**	**Response**	**Drugs**	**Response**	**Ref.**
Pt.1	CPSF6-RARG	ATRA+ATO+IDA	No	IAG+ATRA+ATO	No	6
Pt.2	CPSF6-RARG	ATRA+DNR	No	DA	Yes	6
Pt.3	CPSF6-RARG	ATRA+ATO	No	MA	No	7
Pt.4	CPSF6-RARG	ATRA+HA	No	/	/	8
Pt.5	RARG-CPSF6	ATRA+IA	N/A	/	/	14
Pt.6	CPSF6-RARG	ATRA+ATO	No	HA	Yes	13

Pt: patient; DNR: daunorubicin; IDA: idarubicin; ATRA: all-trans retinoic acid; ATO: arsenic trioxide; IAG: idarubicin, cytosine arabinoside and granulocyte colony-stimulating factor; DA: daunorubicin and cytosine arabinoside; HA: homoharringtonine and cytosine arabinoside; MA: mitoxantrone and cytosine arabinoside; IA: idarubicin and cytarabine; N/A: not available. Ref.: references.

Peg-Dox was shown to slighter myelosuppression, lower infection rates, and fewer cardiac events when used to treat hematological malignancies [15]. We have previously explored the role of Peg-Dox in myeloid neoplasms, despite the fact that there were no indications or reports regarding the use of Peg-Dox for these conditions. According to our research, treating myeloid neoplasms with Peg-Dox and a Peg-Dox-based protocol may be a potential strategy [16]. Next, we had positive results when treated refractory/relapsed myeloid neoplasms with Peg-Dox-based protocol (HADG and DAC: Peg-Dox, cytarabine, cladribine). Additionally, we successfully treated refractory blastic plasmacytoid dendritic cell neoplasms used a Peg-Dox-based protocol [17].In China, homoharringtonine (HHT) has been widely used for the treatment of myeloid neoplasms and acute leukemia [18]. Therefore, in this study, we treated the patient using the combination of homoharringtonine chemotherapy and pegylated liposomal doxorubicin, resulting in full remission with no detectable residual disease and a negative CPSF6-RARG fusion. After two additional cycles of chemotherapy, the patient successfully underwent haploidentical hematopoietic stem cell transplantation (HSCT) and survived disease-free for more than two (2) years.

This case indicates that the combination of homoharringtonine chemotherapy and pegylated liposomal doxorubicin, followed by haploidentical hematopoietic stem cell transplantation, is an effective treatment protocol for APL with CPSF6-RARG fusion. Further research involving a larger number of patients is required to validate the efficacy of this protocol.

Author Contributions

Resources, G.C., H.Y.; Investigation, Y.M., X.T., and M.W.; Writing—Original Draft Preparation, G.C., C.Z.; Writing—Review & Editing, Q.W., P.K., C.Z., and X.Z.; Funding Acquisition, C.Z.

Ethics Statement

The studies were reviewed and approved by the Institutional Review Board of Xinqiao Hospital, Army Medical Universtiy. Written informed consent was obtained from the patients.

Informed Consent Statement

Informed consent was obtained from all subjects involved in the study.

Funding

This work was supported by grants from the National Natural Science Foundation of China (No. 82170212 and No. 2023A037).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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