Bipartite *NUP98::RARA*-E412* fusion with a *cis*-aligned ligand binding domain truncation mutation in atypical acute promyelocytic leukemia

Acute promyelocytic leukemia (APL) is a distinct subtype of acute myeloid leukemia, typically characterized by the *PML::RARA* fusion gene. This fusion serves as the definitive therapeutic target for all-*trans* retinoic acid (ATRA) and arsenic trioxide (ATO), underpinning the excellent clinical outcomes achieved in classical APL.[1] However, a small subset of cases – referred to as atypical APL (aAPL) – harbors alternative retinoic acid receptor (*RAR*) fusions involving *RARA*, *RARB*, or *RARG*.[2-13] These rare fusion genes exhibit substantial molecular heterogeneity and markedly divergent ATRA responsiveness, ranging from high sensitivity to complete resistance.

In 2024, our group reported that all *RARG* fusions and certain *RARA* fusions are, in fact, previously unrecognized tripartite fusions.[2] Their ATRA resistance is driven by *RAR* 3′ splicing-mediated truncation of helix 11_12 (H11_12) within the ligand-binding domain (LBD), a finding subsequently confirmed by independent study.[3] We also demonstrated that the structural configuration of *RARA* fusions is associated with their 5′ partner gene: fusions with *STAT3*, *STAT5B*, and *HNRNPC* consistently form tripartite structures with LBD truncation, whereas *PML::RARA* and *TTMV::RARA* fusions do not.[2-11] Notably, these foundational studies encompassed 12 different *RARA* 5′ partners but did not include *NUP98*.

To date, only two cases of aAPL with *NUP98-RARA* rearrangements have been reported.[12,13] Both were conventionally annotated as bipartite *NUP98::RARA* fusions with presumably intact LBD. However, the structural integrity of their LBD was never experimentally verified. Furthermore, because these patients either discontinued ATRA early or died shortly after admission, their clinical ATRA responsiveness remained undetermined.

Here, we report the third known case of *NUP98-RARA*-rearranged aAPL, providing the first analysis of *RARA* 3′ region abnormalities. We identified a bipartite *NUP98::RARA*-E412* fusion harboring a *cis*-aligned LBD-H12 truncation mutation, which confers primary ATRA resistance via a novel molecular mechanism. This study was approved by the Institutional Ethics Committee of Tai'an City Central Hospital (2025-05-280).

A 14-year-old male was admitted with a 4-day history of fever, abdominal discomfort, dizziness, and fatigue. Initial evaluation revealed leukocytosis (white blood cell count 16.49×10^9/L), anemia (hemoglobin 84 g/L), and thrombocytopenia (platelet count 28×10^9/L). Peripheral blood smears revealed 60% abnormal promyelocytes. Coagulation studies demonstrated marked abnormalities, including prolonged prothrombin time (17.20 s, reference 10.00-14.00 s), prolonged fibrinogen clotting time (11.80 s, reference 5-10.2 s), and significantly elevated D-dimer (25.90 mg/L, reference 0-0.5 mg/L), alongside hypofibrinogenemia (1.99 g/L, reference 2.00-4.00g/L). Serum ferritin (1,203 ng/mL, reference 30-400 ng/mL), lactate dehydrogenase (367 U/L, reference 135-225 U/L), and C-reactive protein (53.30 mg/L, reference 0-10 mg/L) were also elevated. In accordance with current APL guidelines, treatment with ATRA was initiated promptly. However, after 3 days, the failure to achieve cytoreduction or a decrease in the promyelocyte proportion suggested a poor response to ATRA.

Bone marrow aspiration revealed hypercellularity with 94% abnormal promyelocytes (Figure 1A, B). Flow cytometry identified 95.8% abnormal myeloid blasts expressing CD9, CD13, CD33, CD117, and MPO, with partial expression of CD34, CD38, and HLA-DR. While the karyotype was normal, fluorescence *in situ* hybridization using break-apart probes revealed additional *RARA* 3′ signals (*Online Supplementary Figure S1A*). Furthermore, reverse transcription polymerase chain reaction (PCR) was negative for the canonical *PML::RARA* fusion gene.

Suspecting aAPL, the patient received ATRA combined with MAE (mitoxantrone, cytarabine, and etoposide) chemotherapy (Figure 1C). During therapy, the patient's condition deteriorated due to severe polymicrobial infections and persistent agranulocytosis (*Online Supplementary Figure S1B-F*). By day 11, the bone marrow still contained 81.5% abnormal promyelocytes. Due to this clinical worsening, the regimen was switched to a lower-intensity one, HAG (homoharringtonine, cytarabine, and granulocyte colony-stimulating factor). After 3 days of HAG, progressive multiorgan failure and hemorrhagic shock ensued. The patient died on day 23 from disseminated intravascular coagulation and overwhelming infection.

Whole-transcriptome sequencing was performed and analyzed on the diagnostic bone marrow sample according to our previously published methods.[2,14] The gene-expression profile of this case clustered with canonical *PML::RARA*-APL cases in an acute myeloid leukemia cohort[14] we previously reported (Figure 1D). Fusion analysis identified an in-frame *NUP98* (exon 11)::*RARA* (exon 3) fusion (Figure 2A-C), whereas no *RARA* 3' breakpoints or aberrant splicing events were detected. We further identified a c.1234G>T/p.E412* nonsense mutation in *RARA* exon 9 (Figure 2D). Subsequent reverse transcription PCR and Sanger sequencing verified that this mutation was *cis*-aligned within the *NUP98::RARA*

fusion transcript (Figure 2E). Notably, the Sanger sequencing chromatogram indicated that all *NUP98::RARA* fusion transcripts carried the c.1234G>T mutation, while the non-fused *RARA* transcripts did not harbor the mutation.

The E412* mutation is located within H12 of the RARA-LBD, a critical region for ligand-induced transcriptional activation.[2,15] This mutation is predicted to truncate H12 and disrupt coactivator binding, thereby conferring primary

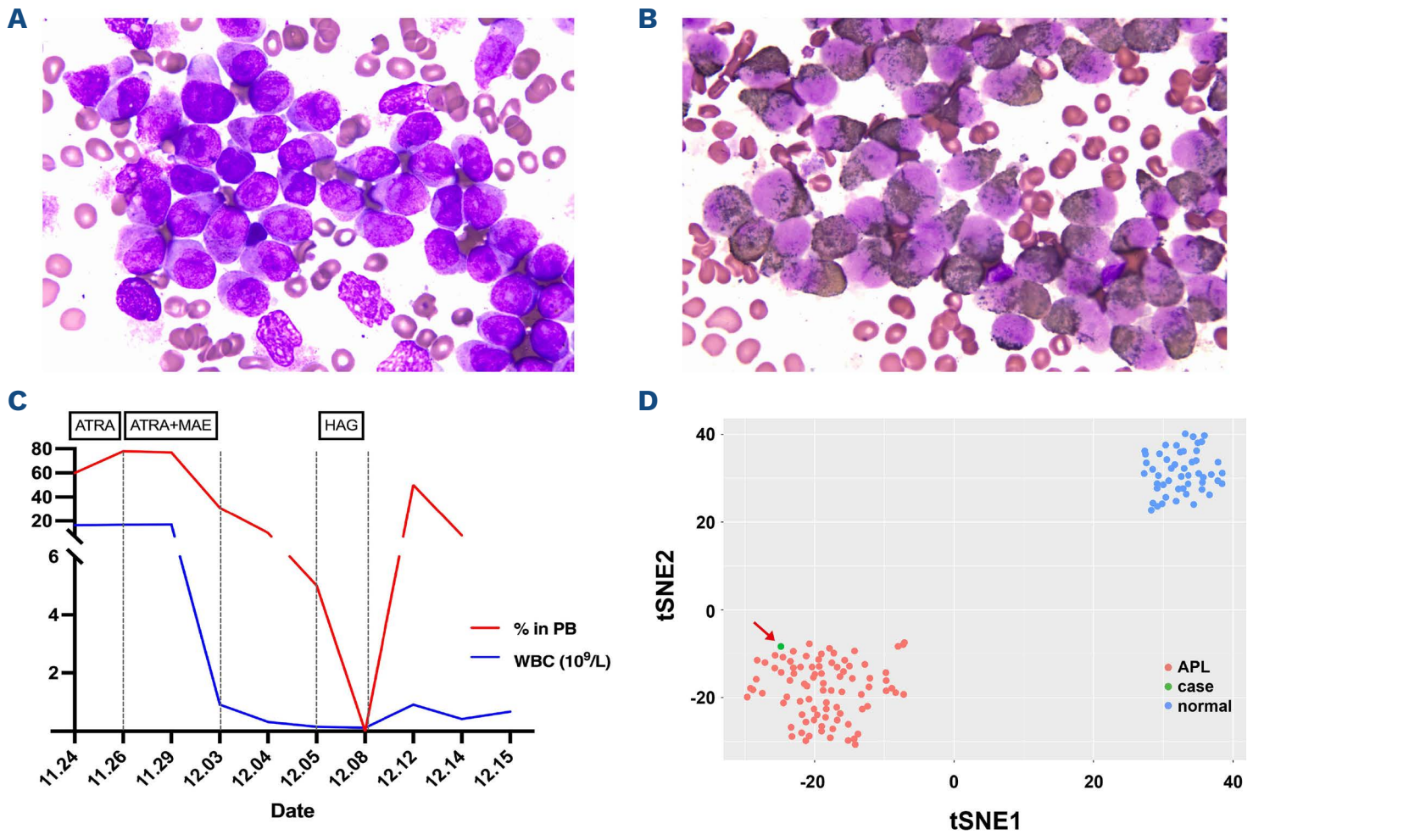
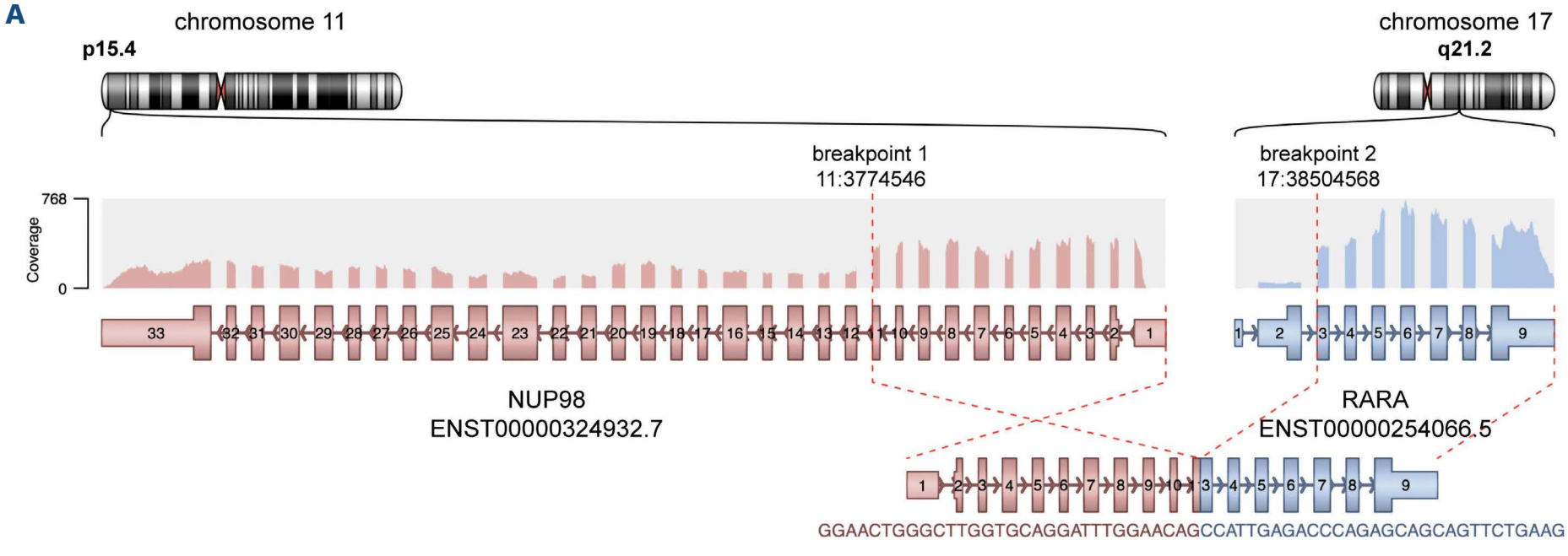


Figure 1. Laboratory findings and clinical course of the patient. (A) Wright staining of the bone marrow aspiration specimen at diagnosis showing aberrant promyelocytes with coarse azurophilic granules (×1,000). (B) Myeloperoxidase staining of the bone marrow aspirate demonstrating strong positivity in abnormal promyelocytes (×1,000). (C) Timeline of the clinical course of the patient. The graph shows the percentage of abnormal promyelocytes in peripheral blood (red line) and white blood cell count (light blue line) indicating treatment-related cytopenia. (D) t-distributed stochastic neighbor embedding analysis of the whole-transcriptome sequencing data showed that the gene expression profile of this case (green dot, indicated by red arrow) clustered closely with *PML::RARA*-APL cases (red dots), and was distinct from normal controls (blue dots). APL: acute promyelocytic leukemia; ATRA: all-*trans* retinoic acid; HAG: homoharringtonine, cytarabine, and granulocyte colony-stimulating factor; MAE: mitoxantrone, cytarabine, and etoposide; PB: peripheral blood; tSNE: t-distributed stochastic neighbor embedding; WBC: white blood cells.



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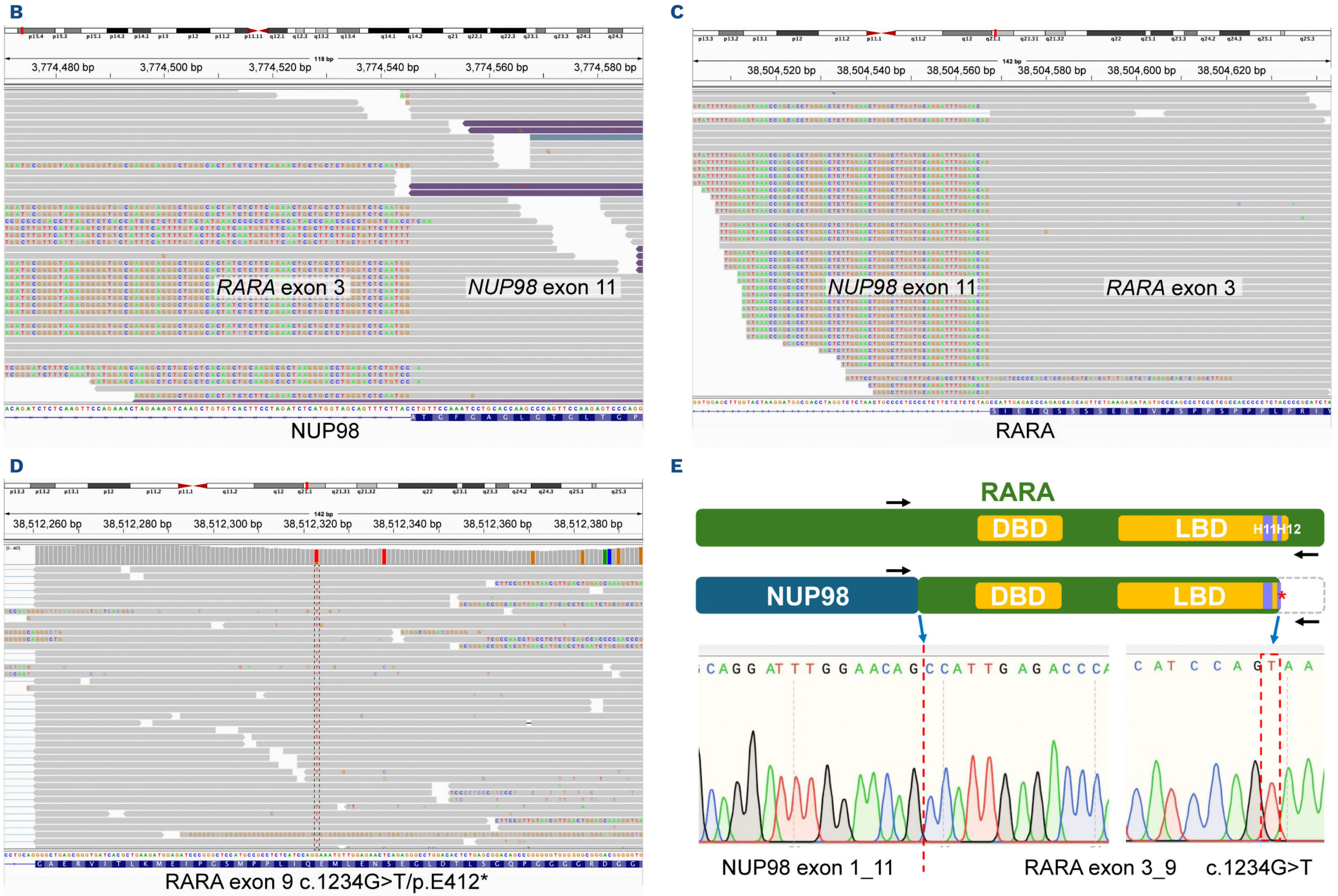


Figure 2. Identification and characterization of the *NUP98::RARA* fusion transcript. (A) Schematic diagram of the *NUP98* exon 11::*RARA* exon 3 reported by Arriba version 2.0.1 software with whole-transcriptome sequencing (WTS) data. (B, C) Integrative genomics viewer (IGV) snapshot showing WTS read alignment supporting the *NUP98* (exon 11)::*RARA* (exon 3) fusion junctions. (D) IGV view of the *RARA* c.1234G>T/p.E412* mutation detected in WTS. (E) Schematic diagram of the NUP98::RARA fusion protein showing the truncation of the RARA-LBD. Reverse transcription polymerase chain reaction and Sanger sequencing confirmed the *NUP98* (exon 11)::*RARA* (exon 3) junction and validated that the c.1234G>T/p.E412* mutation is *cis*-aligned within the fusion transcript. Black horizontal arrows indicate the respective positions of the polymerase chain reaction primers. DBD: DNA-binding domain; H11: helix 11; H12: helix 12; LBD: ligand-binding domain; NUP98: nucleoporin 98 and 96 precursor; RARA: retinoic acid receptor alpha.

resistance to ATRA. This molecular finding aligns with the patient's clinical failure to respond to ATRA-based induction therapy.

Cellular assays were performed according to our previously published methods.[2] Both NUP98::RARA and the E412* mutant exhibited exclusive nuclear localization (Figure 3A). Western blotting revealed that while NUP98::RARA showed partial degradation upon treatment, the E412* mutant was completely resistant to ATRA- or ATO-induced degradation (Figure 3B). In reporter assays, NUP98::RARA displayed attenuated but dose-dependent ATRA responsiveness, whereas the E412* mutant was entirely unresponsive (Figure 3C, *Online Supplementary Figure S2*).

Atypical APL driven by *RAR* fusions represents a clinically challenging subset of leukemia characterized by marked molecular heterogeneity.[2-13] Our recent work highlighted the importance of interrogating the *RAR* 3′ region, as previously overlooked abnormalities in this region can disrupt LBD integrity and dictate responsiveness to ATRA. These findings established the pivotal role of tripartite fusions and LBD-H11_12 truncation in the molecular pathogenesis of *RARG*-aAPL and certain *RARA*-aAPL cases. This discovery corrected earlier misannotations in which *X::RAR::X/Y* tripartite fusions were incompletely characterized as conventional bipartite *X::RAR* fusions. Given that earlier reports often overlooked *RAR* 3′ abnormalities, a systematic assessment of this region in both historical and novel *RAR* fusions is now essential.

By incorporating an analysis of the *RARA* 3′ region in this clinical ATRA-refractory *NUP98-RARA*-rearranged case, we found that although *RARA* did not undergo 3′ splicing to generate a tripartite fusion, a *cis*-aligned truncating mutation (E412*) produced a functionally equivalent effect. Functional assays corroborated these findings: bipartite NUP98::RARA retained dose-dependent ATRA responsiveness, consistent with prior *in vitro* reports,[12] whereas NUP98::RARA-E412*

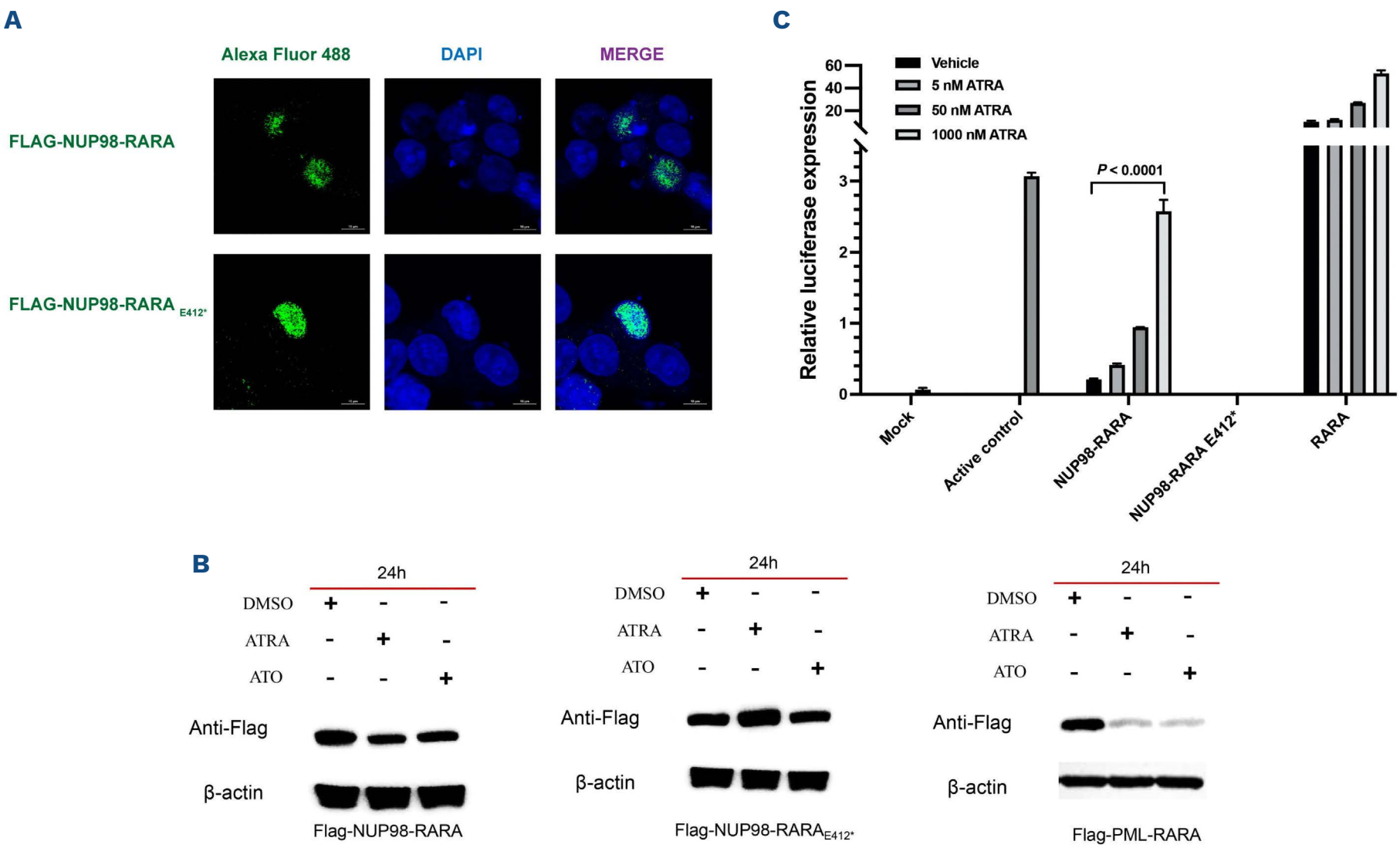


Figure 3. Functional characterization of the fusion protein: subcellular localization and responses to all-*trans* retinoic acid and arsenic trioxide. (A) Immunofluorescence analysis of NUP98::RARA and NUP98::RARA-E412* expressed in 293T cells showing exclusive nuclear localization of both fusion proteins. Scale bars: 10 μm. (B) Western blot analysis demonstrates differential degradation of RARA fusion proteins upon treatment. NUP98::RARA shows limited partial degradation, whereas the NUP98::RARA-E412* mutant is completely resistant to degradation after 24 hours of treatment with 1 μM all-*trans* retinoic acid (ATRA) or arsenic trioxide; PML::RARA, included as a control, displays efficient degradation. (C) Assessment of ATRA responsiveness was performed using a modified GAL4-UAS luciferase reporter assay in 293T cells. Constructs encoding wild-type RARA, NUP98::RARA, and NUP98::RARA-E412* were cloned into the pBIND vector containing Renilla luciferase. NUP98::RARA exhibited dose-dependent responsiveness to ATRA, whereas NUP98::RARA-E412* showed no response. ATO: arsenic trioxide; DAPI: 4',6-diamidino-2-phenylindole; DMSO: dimethylsulfoxide.

was entirely ATRA-unresponsive, in full agreement with the patient's clinical phenotype.

Despite the sequential administration of multiple chemotherapy regimens, the leukemic burden remained persistently uncontrolled, and the patient rapidly succumbed to disease progression and overwhelming infections. This fatal outcome underscores the formidable therapeutic challenges posed by aAPL. The coexistence of a bipartite fusion and a *cis*-aligned mutation suggests a "two-hit" model underlying the loss of ATRA sensitivity. In this scenario, the *NUP98::RARA* fusion exhibits attenuated ATRA responsiveness and likely provides an initial growth advantage, although it may be insufficient on its own to drive the full APL phenotype. The subsequent E412* mutation confers absolute transcriptional resistance to the fusion protein, ultimately precipitating the malignant APL phenotype.

The E412* mutation has not been previously reported as a secondary resistance mutation following ATRA therapy in APL cases. Instead, it was identified in this treatment-naïve patient, representing a primary molecular event. The precise pathophysiological mechanisms governing this synergistic two-hit process remain to be elucidated in future studies. This finding also suggests that the two previously reported cases,[12,13] which were annotated solely as bipartite *NUP98::RARA* fusions, were likely incompletely characterized. Consequently, a systematic reassessment of the *RARA* 3′ region, particularly LBD-H11_12 integrity, is warranted in such cases.

Our previous work demonstrated that the requirement for LBD-H11_12 truncation is tightly linked to the identity of the specific *RARA* 5′ fusion partner.[2] This suggests a partner-dependent mechanism that dictates whether structural disruption of the LBD is necessary to drive leukemogenesis and ATRA resistance. The identification of mutation-mediated LBD-H12 truncation in the current case establishes a distinct molecular mechanism that achieves functional equivalence with tripartite fusions. Whether such *cis*-aligned truncating mutations represent a recurrent phenomenon in *NUP98-RARA*-rearranged aAPL, or arise in other *RAR* fusions, warrants further investigation in larger cohorts. Nevertheless, these findings highlight the expanding molecular complexity of *RAR* fusions in aAPL and reinforce the critical requirement of an intact LBD architecture for ATRA responsiveness.

The underlying defect of RARA-LBD in this scenario is a structural loss of function rather than a reduction in ligand-binding affinity. Consequently, this resistance is unlikely to be overcome by higher doses or high-affinity agonists. Because the truncated LBD is structurally incapable of adopting an "active" conformation, neither standard nor synthetic retinoids are likely to be effective. However, emerging molecular glue technologies offer a promising strategy to rescue coactivator interactions and warrant further exploration.

In summary, we report a case of primary refractory pediatric aAPL harboring a *NUP98::RARA* fusion with a *cis*-aligned E412* mutation. This defines a novel molecular mechanism involving mutation-mediated LBD truncation. While this mechanism is distinct from tripartite RAR fusions, it achieves functional convergence by mediating ATRA resistance. Our findings reinforce the critical role of LBD-H11_12 allosteric dysfunction across a subset of aAPL. These results highlight the urgent need for rapid and comprehensive molecular characterization in suspected cases. Specifically, assessing *RAR* 3′ region integrity is essential, particularly when ATRA-based therapy is initiated empirically.

Authors

Qinqin Liu,[1*] Jiaqi Chen,[2,3*] Xiaosu Zhou,[2*] Qingliang Teng,[1] Xue Chen,[3] Panxiang Cao,[3] Jiancheng Fang,[3] Xudong Wang,[4] Chunmei Zhang,[1] Xiaoli Ma,[3] Zheng Wang,[3] Fang Wang,[3] Yang Zhang,[3] Li Xin[5] and Hongxing Liu[2,3,6]

[1]Department of Hematology, the Affiliated Tai'an City Central Hospital of Qingdao University, Tai'an; [2]Precision Medicine Center, Beijing Lu Daopei Institute of Hematology, Beijing; [3]Division of Pathology & Laboratory Medicine, Hebei Yanda Lu Daopei Hospital, Langfang; [4]Department of Pediatric Hematology, the Affiliated Tai'an City Central Hospital of Qingdao University, Tai'an; [5]Department of Cardiology, the Affiliated Tai'an City Central Hospital of Qingdao University, Tai'an and [6]Division of Pathology & Laboratory Medicine, Beijing Lu Daopei Hospital, Beijing, China

**QL, JC, and XZ contributed equally as first authors.*

Correspondence:
H. LIU - starliu@pku.edu.cn
L. XIN - liuqinxinli@163.com

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Disclosures

No conflicts of interest to disclose.

Contributions

HL, JC, and XZ designed the experiments, supervised the study and analysis, and wrote the manuscript. XC, XM, ZW, FW, and YZ performed and analyzed the experiments. PC and JF analyzed the bioinformation data. QL, QT, XW, CZ, and LX collected clinical data. All authors discussed the results and implications of the manuscript.

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Data-sharing statement

For original data, please contact starliu@pku.edu.cn.

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