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CASE REPORT

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Favorable outcome of *PML-RARα* short isoform and *FLT3-ITD* mutation in a patient with several adverse prognostic markers: A case report

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Key Clinical Message

Complete molecular remission in a "variant APL" patient with short isoform of *PML-RARα* and *FLT3-ITD* mutation was achieved in response to ATRA and ATO plus IDA instead of standard treatment protocol. The use of *FLT3* inhibitor in APL induction management is implicated to prevent differentiation syndrome and coagulopathy experienced in in patients with *FLT3-ITD*.

Abstract

FLT3-ITD mutations are the most common activating mutations in *FLT3* gene, occurring in about 12 to 38% of acute promyelocytic leukemia cases, and are mainly associated with high white blood cell counts and poor clinical outcomes. Here, we present a case of APL variant with adverse prognostic features who showed short isoform [bcr3] of *PML-RARα* and *FLT3*-ITD mutation at diagnosis. The patient received all-trans retinoic acid (ATRA) and arsenic trioxide (ATO) plus idarubicin (IDA) instead of standard treatment protocol, and achieved a complete morphological, cytogenetic and molecular response. However, the patient experienced differentiation syndrome, and coagulopathy that was subsequently resolved by continuous oxygen therapy, dexamethasone, and enoxaparin. The use of *FLT3* inhibitor in APL induction management is implicated to prevent differentiation syndrome and coagulopathy in patients with *FLT3*-ITD mutation.

KEYWORDS

acute promyelocytic leukemia, APL variant, ATRA-ATO plus IDA, *FLT3*-ITD mutation, *PML-RARα* isoforms, qPCR

1 | INTRODUCTION

Acute promyelocytic leukemia (APL) is a subtype of acute myeloid leukemia (AML) that has a distinctive molecular pathophysiology and clinical manifestations. It is cytogenetically characterized by reciprocal translocation of promyelocytic leukemia (*PML*) gene at chromosome 15 and the retinoic acid receptor alpha (*RARα*) gene at chromosome 17 leading to the termination of maturation at the promyelocyte stage.[1,2] Prior to the introduction of ATRA

and ATO, APL was the most fatal subtype. Subsequently, therapy with ATRA and ATO has remarkably improved the outcome of APL patients.[1,2] The long-term survival rate is now greater than 95%, yet refractory/relapsed disease is still seen in around 5% of patients.[1]

FLT3 (FMS-like tyrosine kinase 3), located on human chromosome 13q12-q13 is a cell membrane-expressed proto-oncogene that belongs to the tyrosine kinase receptor family. The most common activating mutation in *FLT3* gene that occur in leukemia is internal tandem duplication (ITD) in exon 14 and 15 of the gene.[3] *FLT3*-ITD mutations have a significant incidence rate of about 12%–38% in APL.[4] The role of *FLT3-ITD* mutations in APL as a prognostic factor for long-term outcome has not yet been clarified, and the significance of these genetic alterations remains controversial. *FLT3*-ITD mutations have been associated with a variety of characteristics in APL including high while blood cell (WBC) count, short bcr-3, or microgranular morphology (M3v).[1]

Here, we present a rare APL variant in a patient presenting with an elevated white blood cell (WBC) count, hypogranular morphology, a unique immunophenotype, and expressing a short isoform of *PML-RARα* and *FLT3*-ITD mutation. Despite various adverse prognostic indicators and differentiation syndrome/coagulopathy, the patient had a favorable outcome with the use of ATRA and ATO plus IDA.

2 | CASE PRESENTATION

A 32-year-old female patient was presented to emergency department with hematuria, heavy menorrhagia, and mild epistaxis. Scattered petechiae and ecchymosis were observed during physical examination. Initial work up showed high WBC count and accordingly patient was admitted for further evaluation.

Complete blood cell count showed WBC 93.60×10^9/L (reference range $4.5–11.0\times10^9$/L), hemoglobin (Hb) 6.6 g/dL (reference range 12–16 g/dL), platelets (PLT) 32×10^9/L (reference range $150–450\times10^9$/L) and WBC differential revealed 68% blasts. The coagulation profile was requested due to the presence of bleeding signs and was found to be abnormal; prothrombin time (PT) and activated partial thromboplastin time (APTT) were prolonged (PT 18.9: reference range 11.9–15.9 sec); (APTT 42.49: reference range 28.7–39.7 sec), and D-dimer was elevated to 10.11 µg/mL (reference range is ≤0.5 µg/mL). Renal and liver profiles were unremarkable.

Bone marrow aspirate and biopsy were indicated because of high WBC and presence of blasts. These results revealed a hypercellular marrow with 94% blasts and abnormal promyelocytes characterized by a bilobed or butterfly nucleus, abundant cytoplasm with azurophilic granules and rare Auer rods (shown in Figure 1A–C).

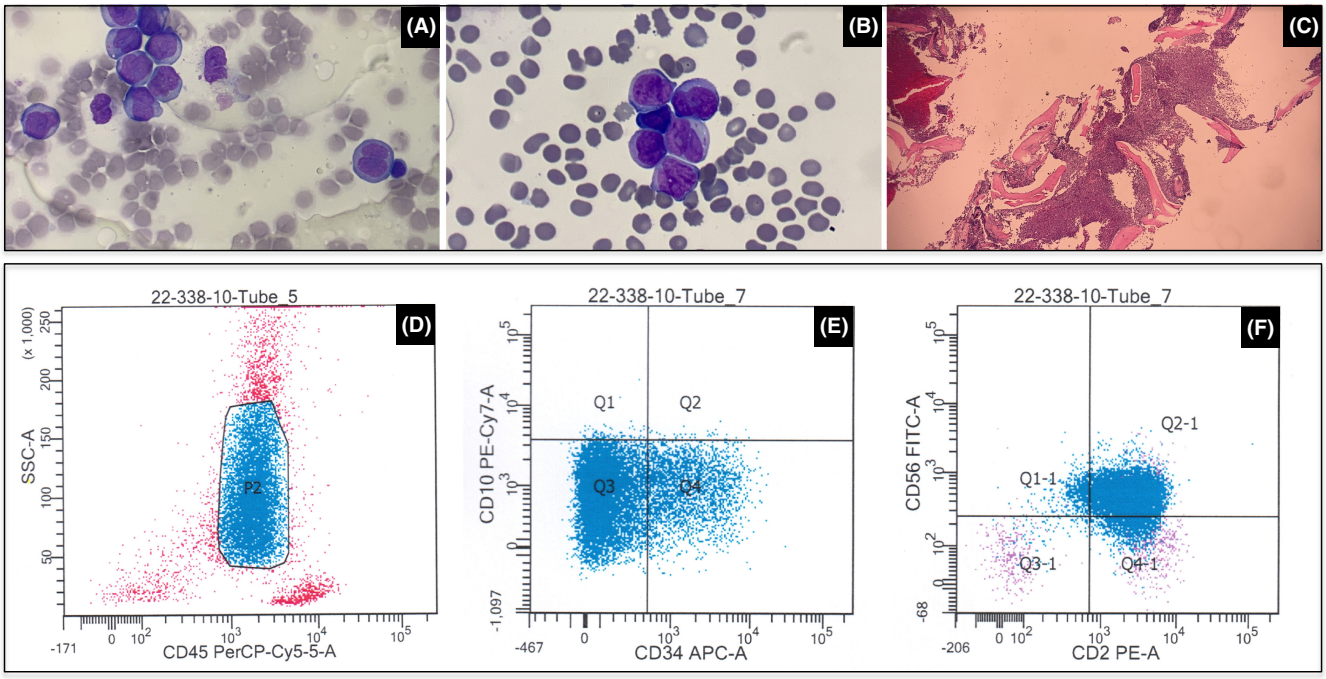

FIGURE 1 Bone marrow aspirate, biopsy and flow cytometry at diagnosis showing atypical APL morphology and immunophenotype. (A) morphologic review of bone marrow aspirate showing heavy infiltration by bilobed promyelocytes (wright-giemsa stain ×500, see arrow); (B) abnormal promyelocyte with Auer rods, (wright-giemsa stain ×500, see arrow); (C) bone marrow biopsy showing hypercellular marrow infiltrated by sheets of promyelocytes (H&E×40); (D) flow cytometry of bone marrow aspirate, illustrating a blast population positive for CD45 with an intermediate to high side scatter; (E) CD34+ (partial); (F) CD56+ (dim) and CD2 positive flow.

Immunophenotyping of bone marrow aspirate by multi-parameter flow cytometry identified the blast cells that were positive for CD45 (leukocyte common antigen) with an intermediate to high side scatter. The blasts showed positivity for CD34+(hematopoietic progenitor cell antigen, partial), CD117+(stem cell factor receptor), CD33+(common myeloid antigen), CD13+(common myeloid antigen), MPO (Myeloperoxidase enzyme), CD64 (granulo-monocytic lineage marker), CD38 (hematopoietic stem cells marker in conjunction with CD34), CD58, CD11c (monocyte marker, partial), CD11b+(Common myeloid marker), CD56+(expression of this marker consider unfavorable, dim), CD2+(aberrant expression of early T cell marker), and CD7+(aberrant expression T cell surface protein). They were negative for CD14 (mature monocyte marker), HLA-DR (major histocompatibility complex), and all other T/B lineage antigens (shown in Figure 1D–F).

Fluorescence in situ hybridization (FISH) was positive for translocation t (15;17) and qPCR confirmed the presence of short isoform bcr-3 of *PML/RARα* (shown in Figure 2A–D). As a routine work up for mutation testing in AML, *FLT3-ITD* mutation was detected by PCR followed by gel electrophoresis (shown in Figure 2E).

The patient was diagnosed with APL and was started on ATRA and ATO plus IDA protocol as an induction regimen. Prednisone 100 mg daily was administered for ATRA syndrome prophylaxis and platelets were infused to maintain the patient's platelets above 50×10^9/L, as well as fresh frozen plasma to correct coagulopathy.

After 8 days of ATO administration, the patient developed fever (39°C), and shortness of breath. Upon

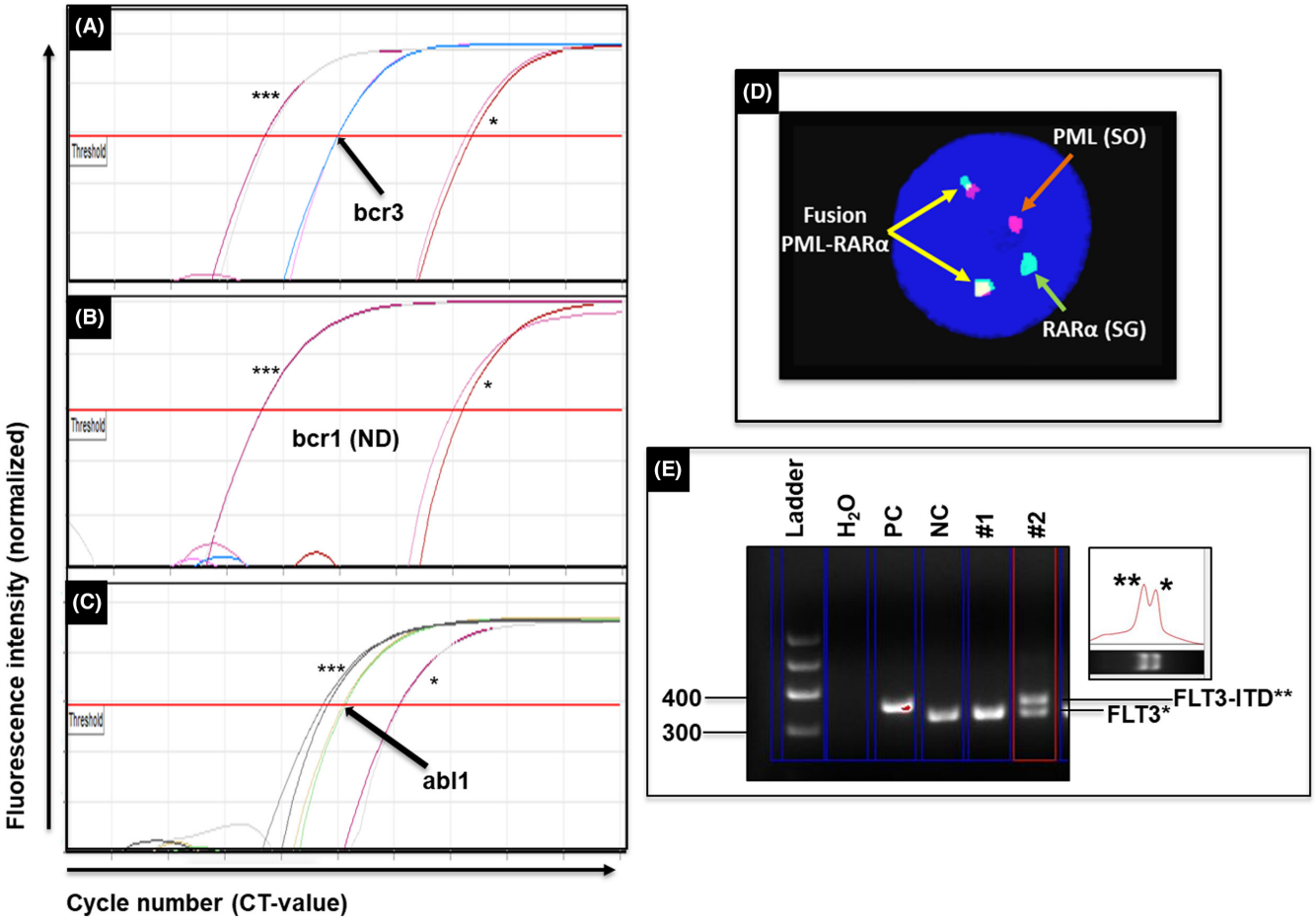

FIGURE 2 Molecular genetic analyses by PCR showing *PML-RARα* and *FLT3-ITD* detection. qPCR data obtained from amplification of *PML-RARα* isoforms bcr3 (A, upper panel) and bcr1 (B middle panel) at diagnosis on RotorGene instrument using commercially available kits (Ipsogen Qiagen, Germany). The graph shows amplification curves for a high positive (***), low positive (*) and abl1 gene (C, lower panel) as internal control used in each qPCR run; D) FISH image showing the presence of *PML-RARα* fusion. Dual color dual fusion probe (Abbott Molecular) of *PML-RARα* was used in FISH procedure with signal orange (SO) for *PML* gene and signal green (SG) for *RARα* gene (see arrows); E) PCR followed by gel electrophoresis for the detection of *FLT3-ITD* mutation using commercially available kit (invivoscribe). Lanes are marked as: ladder, 100 bp marker; water control; PC positive control for *FLT3-ITD*; NC wild type *FLT3*; #1 and #2 (this case) patient specimen. The expected size range is marked between 300 to 400 bp. The graphical insert represents area under the curve of each band for semiquantitative determination of allelic ratio (mutant/wild-type).

examination, right arm was swollen and mildly tender. Chest X-ray showed mild bilateral infiltrate mainly right side. Doppler ultrasound of right upper limb confirmed acute deep vein thrombosis (DVT) involving right median and distal cephalic vein. Computed tomography pulmonary angiogram showed no evidence of pulmonary embolism. Computerized Tomography (CT) scan of the brain to rule out the presence of blood clots was unremarkable. To rule out common cardiac complications of ATRA, an electrocardiogram (ECG) was requested that showed sinus tachycardia. Considering these as promyelocyte differentiation syndrome, chemotherapy was put on hold and patient was placed under continuous oxygen therapy, with 10 mg BID of dexamethasone. Enoxaparin 80 mg was administered daily for upper DVT. Three days after chemotherapy was on hold, symptoms improved, and chemotherapy was continued.

The patient then entered a bone marrow suppression period and developed a second episode of high grade fever. Blood culture was positive for methicillin-susceptible Staphylococcus aureus. Patient was started on cefazolin, and ciprofloxacin. Caspofungin was also given considering the possibility of fungal infection. During that time chemotherapy was put on hold and then resumed after the infection was resolved.

Repeated bone marrow was done on the 51st day of induction chemotherapy, which has been interrupted several times, and it showed complete remission. *PML/RARα by qPCR* was below detection limit and *FLT3*-ITD mutation was not detected (shown in Figure 2B). At the time of writing this report, the patient had completed the consolidation phase with undetectable *PML-RARA* by molecular qPCR and maintenance phase has been initiated with continual monitoring.

3 | DISCUSSION

We report a rare case of APL variant presenting with elevated WBC, hypogranular morphology, and unique immunophenotype including short isoform of *PML-RARα* and *FLT3*-ITD mutation. This is a very distinctive case that presented with several adverse prognostic factors and yet the patient achieved remission post induction phase, albeit for a longer duration (51 days' vs. 28). There are only two case reports in the literature that have shown short isoform of *PML-RARα* and *FLT3*-ITD mutations in patients with poor outcome. Both the reported cases carried *WT1* gene mutation and died during induction phase.[5,6]

In the diagnostic setting, *PML-RARα* is detected by qPCR as three different isoforms: the long bcr-1, the variant bcr-2, and the short bcr-3.[2] Approximately, 70% of APL patients express the long/variant type *PML-RARα*, whereas the S type isoform is seen in ~30% of APL patients.[7] Patients with bcr-3 subtype of APL are less sensitive to ATRA treatment, take longer time to achieve complete remission, and are at a higher risk of relapse compared to patients with other isoforms.[8–10] Additionally, in an in vitro study, bcr-3 cells showed unique anti-apoptotic properties that were not seen in bcr-1, which may explain why patients with bcr-3 APL have stronger drug resistance to ATRA.[11] Several studies have mentioned that there is a high-degree of correlation between bcr-3 subtype and *FLT3* mutations with high incidence in pediatric and yet better outcome compared to adults.[12,13] It is interesting to note that this patient is an adult of 32 years who presented with bcr3 and *FLT3-ITD* mutation and a good outcome.

FLT3 mutations are often associated with an important adverse marker of APL, leukocytosis status (WBC count $>10\times109$/L), low-fibrinogen concentration, hemoglobin levels, and high lactate dehydrogenase (LDH) level.[14] In a meta-analysis, Picharski et al conclude that APL patients with *FLT3*-ITD mutations have significantly higher WBC counts at diagnosis and higher risk of induction deaths.[15] Some authors have suggested that *FLT3* inhibitor treatment might potentially intercept differentiation syndrome or coagulopathy.[16,17] This patient did not receive any *FLT3* inhibitors and developed both differentiation syndrome and DVT. This suggests the use of *FLT3* inhibitors in the induction regimen of APL patients with *FLT3*-ITD mutations may be beneficial.

The current patient presented with APL variant. The morphology of malignant promyelocyte is classified into four types: first, classical or hypergranular type, which is morphologically diagnostic for APL, has heavy granular cytoplasm and numerous fused Auer rods, faggot cells; second, microgranular variant or hypogranular, as this case, has folded nuclei, fine granules and Auer rods are rarely seen; third, high nucleocytoplasmic ratio with irregular nuclear borders, with rare granules, and lack Auer rods; fourth, round regular nuclei that lack granules and subsequently lack Auer rods.[18,19]

This patient expressed CD34, CD2, and CD56 but lacks HLA-DR. These markers are characteristic of the of APL variant, with CD34 being the most expressed marker more frequently seen in bcr-3 subtype females followed by HLA-DR. On the other hand, the immunophenotype of classical APL is positive for CD13, CD33, CD64, and CD117 but lacks HLA-DR and CD34.[19] CD2 and CD56, which are present in the patient, are occasionally expressed and associated with adverse prognosis and increased risk of thrombosis.[20–23]

In conclusion, an APL patient with several adverse prognostic markers, including *PML-RARα* short isoform and *FLT3-ITD* mutation, showed a good response in achieving complete remission to ATRA and ATO plus IDA, despite regimen-related complications. The use of

FLT3 inhibitors in the induction regimen of APL patients with *FLT3-ITD* mutations may be beneficial to prevent differentiation syndrome and coagulopathy in such patients.

AUTHOR CONTRIBUTIONS

Mohammed A Bafail: Data curation; writing – original draft. **Rahaf Altahan:** Writing – review and editing. **Manar A Samman:** Data curation; supervision. **Suha A Tashkandi:** Data curation; supervision. **Ibraheem H Motabi:** Supervision; writing – review and editing. **Abdul Ali Peer Zada:** Conceptualization; writing – review and editing.

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None.

CONFLICT OF INTEREST STATEMENT

The authors declare that there is no conflict of interest regarding the publication of this article. PZ AA and MB are the Principal Investigators of the project, performed data analyses, involved in conceptual design, writing of the manuscript. RT is a Consultant Hematopathologist (MD physician) who analyzed and interpreted morphology. MS is a co-supervisor involved in editing the manuscript. ST is a cytogeneticist at KFMC who analyzed and reported karyotype and FISH aanalyses results. IM is a Consultant Hematologist (MD physician) who was involved in the clinical management of the patient.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

CONSENT STATEMENT

Written informed consent was obtained from the patient to publish this report in accordance with the journal's patient consent policy. Institutional Review Board (IRB Log No. 23–072) was obtained after patient's consent.

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