Outcome of PML-RARα Short Isoform and FLT3-ITD in a Patient with Several Adverse Prognostic Markers: a Case Report

Mohammed Bafail[1], Rahaf Altahan[1], Manar Samman[1], Suha Tashkandi[1], Ibraheem Motabi[1], and Abdul Ali Peer Zada[1]

[1]King Fahad Medical City

April 16, 2023

Τιτλε: Ουτςομε οφ ΠΜΛ-ΡΑΡα Σηορτ Ισοφορμ ανδ ΦΛΤ3-ΙΤΔ ιν α Πατιεντ ωιτη Σεεραλ Αδερσε Προγνοστις Μαρκερς: α ἅσε Ρεπορτ

Mohammed A Bafail[1], Rahaf AlTahan[1]Manar A Samman[1], Suha A Tashkandi[1], Ibraheem H Motabi[2] , Abdul Ali Peer-Zada[1*]

[1]*Department of Pathology and Clinical Laboratory Medicine Administration, Hematology, Molecular Pathology and Cytogenetics Sections King Fahad Medical City, Riyadh, Saudi Arabia*

[2]*Department of Adult Hematology and Bone Marrow Transplantation, King Fahad Medical City, Riyadh, Saudi Arabia*

Short Title: FLT3ITD with short isoform of PML-RARalpha

***Corresponding authors:**

Dr. Abdul A Peer Zada and Dr. Mohammed A Bafail

Consultant Clinical Lab Scientist (Molecular Genetics)

Department of Pathology and Clinical Laboratory Medicine,

King Fahad Medical City, 11525, Riyadh, Kingdom of Saudi Arabia

Tel: +966 11 288 9999 Ext. 17259; Mobile: +966 539417357

Email: azada@kfmc.med.sa; mbafail@kfmc.med.sa

Number of Tables: 0

Number of Figures: 2

Word Count:

Abstract: 125

Main Text: 1400

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

Abstract:

FLT3-ITD mutations are the most common activating mutations in FLT3 gene that occur in about 12 to 38% of acute promyelocytic leukemia cases, and are mainly associated with high white blood cell counts

and poor clinical outcome. We present here a case of short isoform [bcr3] of PML-RARα and FLT3-ITD who presented with adverse prognostic features characteristic of variant APL in the form of leukocytosis, hypogranular morphology, and unique immunophenotype. The patient received ATRA and ATO plus IDA instead of standard treatment protocol, and achieved a complete morphological, cytogenetic and molecular response. However, she experienced differentiation syndrome and coagulopathy that was subsequently resolved by appropriate management. The use of FLT3 inhibitor in APL induction management could prevent differentiation syndrome and coagulopathy in patients with FLT3-ITD.

Keywords: Acute promyelocytic leukemia, PML-RARα isoforms, Quantitative polymerase chain reaction ATRA-ATO plus IDA, FLT3-ITD, APL variant morphology and immunophenotype

Key Messages

Complete molecular remission in 'variant APL' patient with short isoform of PML-RARα and FLT3-ITD in response to ATRA and ATO plus IDA. The use of FLT3 inhibitor in APL induction management could prevent differentiation syndrome and coagulopathy experienced in the patient.

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 retinoic acid receptor alpha (RARα) gene at chromosome 17 that leads to the termination of maturation at the promyelocyte stage [1,2]. Prior to the introduction of all-trans retinoic acid (ATRA) and arsenic trioxide (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 [?]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 in cases with APL ranging from 12 to 38% [4]. The role of FLT3-ITDs 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-ITDs have been associated to 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 case of short isoform of PML-RARα and FLT3-ITD with characteristics of variant APL in terms of elevated WBC count, morphology and immunophenotype.

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 $\times$ 10^9/L, hemoglobin (Hb) 6.6 g/dL, platelets (PLT) 32 $\times$ 10^9/L and WBC differential revealed 68% blasts. The coagulation profile was abnormal; prothrombin time PT and activated partial thromboplastin time APTT were prolonged [PT 18.9 reference range 11,9-15,9 seconds); APTT 42,49 (reference range 28,7-39,7 seconds)], 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 biopsy revealed a hypercellular marrow with 94% blasts and abnormal promyelocytes characterized by a bilobed or butterfly nucleus and abundant cytoplasm with azurophilic granules and rare Auer rods (shown in Fig.1a-1c).

Immunophenotyping of bone marrow aspirate by multi-parameter flow cytometry identified the blast cells

that were positive for CD45 with an intermediate to high side scatter. The blasts showed positivity for CD34+ (partial), CD117+, CD33+, CD13+, MPO, CD64, CD38, CD58, CD11c (partial), CD11b+, CD56+ (dim), CD2+, and CD7+. They were negative for CD14, HLA-DR, and all other T/B lineage antigens (shown in Fig. 1d-1e].

Fluorescence in situ hybridization (FISH) was positive for translocation t(15;17) and quantitative real-time polymerase chain reaction (qPCR) confirmed the presence of short isoform bcr-3 of PML/RARα (shown in Fig. 2a-2c). As a routine work-up for mutation testing in AML, FLT3-ITD was detected by PCR followed by gel electrophoresis (shown in Fig. 2d].

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 $\times 10^9$/L, as well as fresh frozen plasma to correct coagulopathy.

After eight days of ATO administration, the patient developed fever (39 °C), chest tightness and shortness of breath. Upon 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. Head Computerized Tomography was unremarkable. Electrocardiogram revealed 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 was not detected (shown in Fig. 2b). At the time of writing this report, patient was started on the consolidation chemotherapy.

Discussion

We report a rare case of short isoform of PML-RARα and FLT3-ITD with characteristics of variant APL in terms of elevated WBC, hypogranular morphology and unique immunophenotype. This is a very distinctive case that presented with several adverse prognostic factors and yet the patient achieved remission post induction phase. There are only two case reports in the literature that have shown PML-RARα and FLT3-ITD 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-RARa, 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 [8,9]. 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 [10]. 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 [11,12]. It is interesting to note that our patient is an adult of 32 years who presented with bcr3 and FLT3-ITD and a good outcome.

FLT3 mutations are often associated with an important adverse marker of APL, leukocytosis status (WBC

count > 10 × 109/L), low-fibrinogen concentration, hemoglobin levels and high lactate dehydrogenase (LDH) level [13]. 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 [14]. Some authors have suggested that FLT3 inhibitor treatment might potentially intercept differentiation syndrome or coagulopathy [15,16]. Our 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 may be beneficial.

Our patient presented with variant APL morphology. The morphology of malignant promyelocyte is classified into 4 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 our 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 [17,18].

Our patient expressed CD34, CD2 and CD56 but lacks HLA-DR. These markers are characteristic of the microgranular variant of APL with CD34 as 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 that are present in our patient are occasionally expressed and associated with adverse prognosis and increased risk of thrombosis [20,21,22].

In conclusion, APL patient having several adverse prognostic markers including PML-RARα short isoform and FLT3-ITD mutation shows a good response in achieving complete remission to ATRA and ATO plus IDA. The use of FLT3 inhibitors in the induction regimen of APL patients with FLT3-ITD may be beneficial to prevent differentiation syndrome and coagulopathy in such patients.

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Acknowledgement

We thank KFMC Research Center, Faculty of Medicine for their support. This study is approved by King Fahad Medical City Institutional Review Board (IRB Log No. 23-072).

Conflict of interest: The authors declare that there is no conflict of interest regarding the publication of this article.

Funding: None

List of abbreviations:

APL: Acute promyelocytic leukemia; MR: molecular remission; MRD: minimal residual disease; qPCR: Quantitative polymerase chain reaction; CNS: central nervous system; CT: computerized tomography; ATRA: All-trans-retinoic acid; ATO: Arsenic trioxide; IDA: idarubicin

Figure legends:

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.

Φιγυρε 2. Μολεςυλαρ γενετις αναλψσες βψ ΠCΡ σηοωινγ ΠΜΛ-ΡΑΡα ανδ ΦΛΤ3-ΙΤΔ δετεςτιον. qPCR data obtained from amplification of*ΠΜΛ-PAPα* 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 a high positive (***), low positive (*) and abl1 gene (c, lower panel) as internal control used in each qPCR run amplification curves; d) PCR followed by gel electrophoresis for the detection of FLT3-ITD using commercially available kit (invivoscribe, USA). Lanes are marked as: ladder, 100 bp marker; water control; PC positive control for FLT3-ITD; NC wild type FLT3; #1 and #2 (our case) patient specimen. The expected size range is marked between 300-400 bp.

Figure 1. Bone marrow aspirate, biopsy and flow cytometry at diagnosis showing atypical APL morphology and immunophenotype

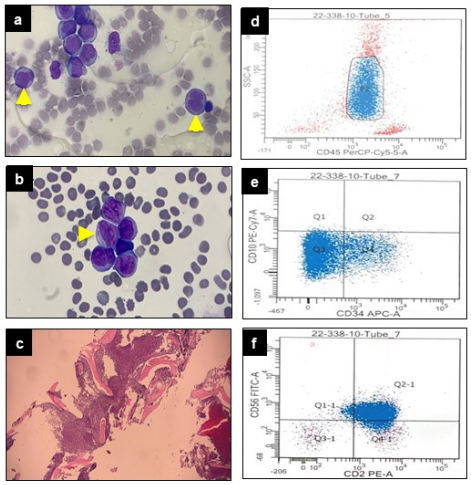

Figure 2. Molecular genetic analyses by PCR showing PML-RARα and FLT3-ITD detection