1. Introduction

Essential thrombocythemia (ET) is a clonal disorder of the hematopoietic stem cell that is closely related to two other myeloproliferative neoplasms (MPNs) polycythemia vera (PV) and primary myelofibrosis (PMF). The Janus-Associated-Kinase 2 (JAK2) V617F mutation is present in the vast majority of PV patients and in 50% of patients with ET and PMF [1]. JAK2 V617F produces constitutive activation of the thrombopoietin and erythropoietin signaling pathways, driving ET and PV respectively. Over time, ET and PV can both progress to myelofibrosis [1]. Further, all three conditions can transform into acute myelogenous leukemia (AML), although this occurs most frequently in PMF [2]. Transformation carries a dismal prognosis [2] and is often associated with complex genetic changes. Transformation of ET, PV or PMF into acute promyelocytic leukemia (APL), a favorable risk leukemia characterized by the t(15;17) translocation, is exceedingly rare with only eight documented cases in the literature [3–9]. Of these, only two prior cases have been reported of ET transforming into APL with molecular documentation of the t(15;17) [3,4]. We report an 83-year-old woman with a history of ET and PMF who then transformed to APL. To our knowledge, this is the first case to demonstrate co-expression of PML-RARα and JAK2 V617F by leukemic blasts. Clinically she also developed severe differentiation syndrome during induction with ATRA plus arsenic trioxide (ATO), suggesting that JAK2 signaling may augment differentiation syndrome.

2. Case study

The patient was diagnosed with ET in 1995 and began treatment with hydroxyurea and aspirin. In 2011 she noted worsening fatigue and left lower quadrant abdominal pain. She underwent a bone marrow biopsy that revealed myelofibrosis with a JAK2 V617F allelic burden of 92% and was found to have splenomegaly. Treatment with the JAK1/2 inhibitor ruxolitinib was initiated, which resulted in a significant improvement in energy and appetite. She did well until May of 2013 when she was noted to be pancytopenic with a WBC of 2.0 × 10^3/mm³, hemoglobin of 8.5 mg/dL and platelets of 96 × 10^3/mm³. Concerned that her cytopenias were the result of ruxolitinib, it was discontinued; however, she remained persistently pancytopenic. A bone marrow biopsy revealed a hypercellular marrow with 70% blasts, megakaryocytic hyperplasia and grade 3/3 fibrosis. Fluorescence in-situ hybridization (FISH) confirmed the t(15;17) in 90% of cells and diagnosis of APL. A GeneTrails AML/MDS next-generation sequencing panel (Supplementary Table 1, Knight Diagnostics Lab, Portland, OR) was performed on marrow aspirate and was significant
for a JAK2 V617F allelic burden of ~90%, indicating that the APL cells also contained the JAK2 V617F mutation. A FLT3 D835 mutation was also found with this panel.

Given her advanced age she was started on ATRA+arsenic trioxide (ATO) induction [10]. After two days of ATRA her WBC count began to increase rapidly and despite escalating doses of hydroxyurea her WBC count continued to rise, reaching $6.74 \times 10^3$/mm$^3$ on day 6 of ATRA (Fig. 1A,B). During this time she was started on treatment-dose dexamethasone for differentiation syndrome. She developed severe abdominal pain and ultrasound demonstrated splenomegaly (15.8 x 6.8 x 13.7 cm). Despite dexamethasone, her symptoms were felt to be secondary to severe differentiation syndrome and she was given a single dose of idarubicin (12 mg/m$^2$) for rapid cytoreduction. Her WBC count peaked on day 7 at $95.9 \times 10^3$/mm$^3$. At this time she developed increasing oxygen requirements with a chest X-ray demonstrating evolving bilateral patchy opacities (Fig. 1C). Given her clinical deterioration, ATRA was held for two days but by day 9 her WBC had decreased to $27.5 \times 10^3$/mm$^3$. ATRA was restarted, hydroxyurea was tapered, and on day 10 ATO (0.15 mg/kg/day) was initiated. However, despite her declining WBC count, her oxygen requirement again increased to 10 L on day 12 and she was aggressively diuresed in addition to continued dexamethasone. By day 17 she showed dramatic clinical improvement and was successfully weaned from oxygen. A repeat ultrasound at this time demonstrated a greater than 50% reduction in splenic volume (11.2 x 10.8 x 5.5 cm), suggesting that extramedullary APL cells were abundant in the spleen and the bone marrow. A bone marrow biopsy performed on day 34 revealed a morphologic complete remission and she was discharged to a rehab facility to recover. On day 47 after initiation of therapy, her peripheral blood had counts recovered with a WBC count of $29.9 \times 10^3$/mm$^3$, hemoglobin of 11.0 mg/dL and platelets of $603 \times 10^3$/mm$^3$, consistent with her previous ET, so she was restarted on 10 mg twice daily ruxolitinib and 81 mg aspirin. She then received four cycles of consolidation therapy with ATRA plus ATO [10]. Ruxolitinib was well tolerated during consolidation and dose reduced only when her platelet count decreased with ATO. A bone marrow biopsy after completion of consolidation revealed complete molecular remission and a return to her underlying myeloproliferative disease with grade 2/3 myelofibrosis.

3. Discussion

ATRA and arsenic are both known to induce differentiation syndrome but this case was notable for its severity. Previous studies of differentiation syndrome have demonstrated that APL cells treated with ATRA secrete the chemokines CCL2 and IL-8, which in combination with chemokine production from alveolar

![Fig. 1. Development of Severe Leukocytosis and Differentiation Syndrome.](image.png)
epithelial cells, are thought to initiate a hyperinflammatory cascade in the lung [11]. While dexamethasone does not directly affect the production of CCL2 or IL-8 from APL cells, it does reduce their production in the lung and thus attenuates the clinical development of differentiation syndrome [11]. Since inflammatory cytokine signaling plays a significant role both in the pathogenesis of JAK2 V617F MPNs as well as in the development of differentiation syndrome, we postulated that robust differentiation syndrome experienced by this patient was the result of excessive inflammatory response related to her underlying JAK2 V617F mutation.

To test whether JAK2 inhibition has any direct effects on the production of cytokines by APL cells, we used ATRA to differentiate the NB-4 APL cell line in the presence or absence of ruxolitinib. Cytokine release into the media was measured over three days of ATRA-induced differentiation. There was a substantial increase in both CCL2 and IL-8 with ATRA treatment, although this was not abrogated by JAK inhibition (Fig. 2). In fact, ruxolitinib had very little effect overall on cytokine production of NB-4 cells. Therefore, CCL2 and IL-8 production by APL cells is unaffected by JAK inhibition, similar to what has been shown for dexamethasone [11]. However, there is substantial evidence that signaling downstream of cytokines is through activation of JAK2 [1]. Indeed, it is possible that the presence of JAK2 V617F in APL and non-leukemic cells enhances CCR2 and IL-8 signaling during differentiation syndrome, accelerating leukocyte extravasation and inflammatory amplification within the lungs and other organs. This suggests that JAK inhibitors may have clinical utility in attenuating the inflammatory amplification step in differentiation syndrome in a manner similar to glucocorticoids. Further studies with in vivo models of differentiation syndrome are needed to evaluate this possibility.

In summary, this case details a previously undescribed pathologic entity, the co-expression of the PML/RARα and JAK2 V617F in APL. Further, the clinical presentation with severe differentiation syndrome may be the result of this rare molecular partnership, providing a framework for future studies exploring the role of JAK2 in differentiation syndrome. In so doing, it offers the opportunity for the development of novel non-steroidal therapies in the management of differentiation syndrome.

Conflict of interest

The authors declare no conflict of interest.

Author Contributions

TPB, JEM and ET were responsible for experimental design, literature review, data collection, statistical analysis and manuscript writing. AA performed the cytokine analysis. JD reviewed pathology and took pictures. SES and ET cared for patient. All authors contributed to writing manuscript and reviewed before submission.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.lrr.2014.12.003.

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