Hematology Fellows Conference: The Changing Genetic Landscape of Myeloproliferative Neoplasms

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64 year-old M presented with 3 months of fatigue and dyspnea, found to have a platelet count of 3.2 million
Healthy until 3 months ago when he started developing extreme fatigue, dyspnea on exertion

Presented to PCP 2 weeks prior to admission and found to have plts of 1.7 million, HCT 20, iron sat 2%, total iron 9, TIBC 510

- Guaic positive, got 4 units prbcs and PPI, no scope
- He was seen by hematology, thought to have reactive thrombocytosis due to iron-deficiency anemia, and started on iron supplementation and aspirin

He continued to feel poorly so presented to HMC
History

- ROS: increased satiety, mild nausea, constipation, poor appetite, and pruritus. No fevers, chills, sweats, weight loss, increased abdominal girth or pain, swollen lymph nodes
- PMH: pre-diabetes
- Meds: all recent- Aspirin, iron, omeprazole
- FH: no blood disorders or cancers
- SH: Native American, used to work as a logger, now a landscaper. No etoh/tobacco/drugs
Admission Labs

- WBC: 37K, HCT 25, Plts 3.2 million
  - MCV 77
  - Smear notable for nucleated rbc
  - N77%, L8%, M3%, E2%, Baso 8%, Blasts 1%
- Fibrinogen 437, INR 1.2, PTT 29
- LDH 489, Uric acid 8.5
- K 6.7, Cr .94, phosphate 4.9, calcium 8.5
- Ferritin 6
- Guaic positive, no frank melena/BRBPR
- Normal CBC obtained from 2011
Initial Management

- Started on Hydrea 1 G BID, allopurinol
- Peripheral blood sent for flow cytometry, JAK2, BCR-ABL and bone marrow biopsy done
- HCT kept trending down without clear GIB, prbcs were transfused and iron continued
- Hydrea increased to 1.5 G BID after plts spiked, and then started trending down
- Aspirin resumed
Platelet Trend
Pathology

- Bone marrow: 90% cellular, moderate reticulin fibrosis, no increased blasts.
- Flow: basophilia (1.1% wbc) and CD34-positive myeloid blasts represent .41% of the wbc and showed mild immunophenotypic abnormalities including CD5 (subset), CD7 (subset) and CD34 (bright) with normal CD13, 33, 38, 45, 71, 117, 123, HLA-DR, no CD4, 14, 15, 16, 19, 56, or 64.
- FISH and RT-PCR negative for BCR-ABL rearrangement
- JAK2 V617F mutation negative
- Cytogenetics: 46, XY, del(13) (q13q14) in 2/20 cells
Diagnosis: Primary Myelofibrosis

- Per marrow interpretation, the marked thrombocytosis and leukocytosis in combination with the hypercellular marrow with moderately increased reticulin fibrosis are diagnostic of a myeloproliferative disorder. The differential includes ET and PMF, with the leuko-erythroblastic peripheral blood picture favoring PMF
- Flow interpretation: suggested chronic phase myeloproliferative disorder
Why is this patient bleeding with so many platelets?
Acquired Von Willebrand Syndrome (AVWS)

- **Patient Labs:**
  - F8 187, VW factor antigen 152, VW collagen binding 82 (ratio .54), multimers: decreased high molecular weight multimers
  - Consistent with acquired VWD type 2b

- **Can occur secondary to autoimmune, lymphoproliferative or myeloproliferative disorders (along with cardiovascular or multiple other disorders*)

- **Mechanisms:**
  - antibody-mediated increased clearance of VWF from the plasma
  - functional interference from autoantibodies to platelet or collagen binding with VWF
  - increased shear stress and subsequent proteolysis of VWF (Budde et al 1984)
AVWS: Diagnosis and Treatment

- Lab workup similar to congenital VWD, distinguished by late onset bleeding and negative FH, especially in the presence of an associated underlying condition
  - If WWF:Ag reduced or WF:Rco/Ag ratio reduced or HMW multimers reduced/absent should consider AVWS if AVWS-associated condition present (if not, would consider VWD type 2 if HMW multimers low)
- Treatment options: the usual suspects
  - Treat underlying disorder
  - Desmopressin- response rate ranges 10-75% based on underlying disorder
  - VWF-containing concentrates, very short half-life if inhibitors present
  - Recombinant factor VIIa- particularly in those with alloantibodies against VWF- 96% effective (Tiede et al, Blood 2011)
  - Antifibrinolytics
  - IVIG
  - Plasma-exchange
AVWS and MPNs

- Results from adsorption of HMW multimers of VWF to transformed blood cells, in particular plts
- In a prospective study by Mohri et al (Blood 1998), AVWS was diagnosed in 14/124 (11%) consecutive patients with MPN
- Plts >1.5 million is a significant risk factor for bleeding
- Thrombocytosis has a concomitant risk for thrombosis*
- Cytoreductive therapy can address both bleeding and thrombotic risk but is not immediately effective
- Treatment: desmopressin preferred as newly released VWF from endothelial cells contain very large HMW multimers, larger than those in plasma-based concentrates (Federici et all Thomb Haemost 2000)
Primary Myelofibrosis

- The MPNs are a class of chronic myeloid cancers that are characterized by overproduction of mature blood cells, that may evolve into acute myeloid leukemia.
- Bone marrow fibrosis can accompany a number of hematologic and non-hematologic conditions: myeloid/lymphoid neoplasms, metastatic cancer, autoimmune disease, hyperparathyroidism, vitamin D deficiency, pulm HTN, infections (AIDs, leishmaniasis) or exposure to toxins.
- Primary myelofibrosis, in contrast, is classified by the WHO in the MPN category that includes PV, ET, CML, and MPN unclassifiable.
Diagnosis- PMF vs Secondary to ET or PV

Clinical: MF can present as a primary disease or can evolve from polycythemia vera or essential thrombocythemia, and is characterized by marrow fibrosis, progressive anemia, and extramedullary hematopoiesis, manifested primarily as splenomegaly. Severe constitutional symptoms such as night sweats and weight loss, pruritus, fatigue, and sequelae of splenomegaly common

- WHO Diagnostic Criteria:
  Major:
  - Megakaryocyte abnormalities
  - No evidence CML (bcr-abl neg)
  - No prior ET/PV
  - Evidence of a clonal marker such as JAK2 mutation
  - No systemic cause of marrow fibrosis identified

Minor: leuko-erythroblastosis, increased LDH, anemia, splenomegaly

International Working Group for Myeloproliferative Neoplasms Research and Treatment criteria for post-polycythemia vera/essential thrombocytemia (post-PV/ET) myelofibrosis requires meeting both major criteria and 2 minor criteria

- Major criteria
  - Megakaryocyte proliferation, including small-to-large megakaryocytes, with aberrant nuclear/ cytoplasmic ratio and hyperchromatic and irregularly folded nuclei and dense clustering accompanied by either reticulin and/or collagen fibrosis, or in the absence of reticulin fibrosis (ie, prefibrotic PMF), the megakaryocyte changes must be accompanied by increased marrow cellularity, granulocytic proliferation, and often decreased erythropoiesis (Figure 1).
  - Not meeting WHO criteria for chronic myelogenous leukemia, polycythemia vera, myelodysplastic syndromes, or other myeloid neoplasm
  - Demonstration of JAK2V617F or other clonal marker or no evidence of reactive marrow fibrosis

- Minor criteria
  - Leuko-erythroblastosis
  - Increased serum lactate dehydrogenase
  - Anemia
  - Palpable splenomegaly
Pathophysiology

- MPNs are monoclonal hemopathies without a clear known disease-initiating event, although a variety of mutations have been found in the diseases.
- Clonal myeloproliferation in MF is accompanied by a secondary inflammatory state characterized by bone marrow stromal changes and abnormal cytokine expression.
- Myeloid cell-derived transforming growth factor-B, platelet-derived growth factor, fibroblast growth factor B, and VEGF have been implicated as mediators of bone marrow fibrosis, osteosclerosis, and angiogenesis in MF.
- Elevated plasma levels of pro-inflammatory cytokines in MF might be responsible for disease-associated constitutional symptoms and cachexia.
PMF: Risk Stratification

International Prognostic Scoring System (IPSS) evolved into Dynamic IPSS (DIPSS) and now to DIPSS-plus with 8 risk factors

- Age >65, Hgb <10, wbc >25, circulating blasts >1%, constitutional symptoms, red cell transfusion need, platelets <100, and unfavorable karyotype (complex, or 2+ abnormalities that include +8, -7, 7q-, i(17q), inv(3), -5, 5q-, 12p-, or 11q23)
- Specific mutations (such as jak2) have not yet been incorporated
- Low risk: 0 risk factors
- Intermediate 1: 1 risk factor
- Intermediate 2: 2-3 risk factors
- High risk: >3 risk factors
- Median survival 15.4, 6.5, 2.9 and 1.3 years respectively

Our patient: 4 risk factors (Hgb, wbc, transfusion need, constitutional?) \( \rightarrow \) high risk
But what about his non-mutated JAK2?

The genetic basic of MPNs (ET and PMF) is heterogeneous

Multiple mutations have been characterized for MPNs, the most common of which are JAK2 V16F and MPL exon 10 mutations

- 50-60% patients with ET/PMF have JAK2 gene mutation: gain of function mutation that constitutively activates JAK2 resulting in increased cytokine responsiveness of myeloid cells
- 5-10% have activating mutations in thrombopoietin receptor gene (MPL)
- Prior to 2013, there was no specific molecular marker described in the remaining 35-40%
- Somatic mutations such as TET2, CBL, EZH2, DNMT3A and ASKL1 are present in a proportion of cases of MPNs, but can co-occur with JAK2 and MPL and are found in multiple types of myeloid cancers, and thus may not have a primary role in the pathogenesis*
The sendout lab finally returns!!
(actually one month later)

- **New result**: Positive for CALR mutation in exon 9 (52 bp deletion), approximately 60% of the CALR DNA in this blood contains a deletion of 52 nucleotides between nucleotides 1092 and 1143.
Somatic CALR Mutations in Myeloproliferative Neoplasms with Nonmutated JAK2


Abstract

Somatic mutations in the Janus kinase 2 gene (JAK2) occur in many myeloproliferative neoplasms, but the molecular pathogenesis of myeloproliferative neoplasms with nonmutated JAK2 is obscure, and the diagnosis of these neoplasms remains a challenge.
Calreticulin: quality control protein

- Calreticulin is a multifunctional protein encoded by the CALR gene, whose job it is to bind to mis-folded proteins and prevents them from being exported from the endoplasmic reticulum (ER) to the golgi apparatus. Outside the ER it is found both intra- and extracellularly on the cell surface and has been implicated in diverse processes including proliferation, apoptosis, phagocytosis and immunogenic cell death.
Klampfl T et al (NEJM 2013) first performed whole genome sequencing in 6 patients who had PMF without mutations in JAK2 or MPL, then in a larger cohorts of myeloid neoplasms - 1215 pts with ET/PMF

They detected somatic insertions or deletions in exon 9 of CALR all patients who underwent whole genome sequencing

- No CALR mutations were found in PV, and few in other myeloid neoplasms
- CALR and JAK2/MPL mutations were mutually exclusive
- Among patients with ET/PMF negative for JAK2 and MPL, CALR was identified in 67% with ET and 88% with PMF
- 36 types of insertions or deletions were identified that all caused a frameshift to generate a novel C-terminal peptide in the mutant Calreticulin
- Overexpression of the most frequent CALR deletion caused cytokine-independent growth (and cytokine hypersensitivity) of a normally IL-3 dependent murine cell line Ba/F3 cells in vivo
- This cytokine-independent growth was still mediated through the JAK-STAT signaling pathway
Nangalia et al (NEJM 2013)

- Exome sequencing of 151 patients with MPNs as well as CALR mutation testing in 2862 other cancers
- CALR mutations present in 70% of patients with MPNs lacking the JAK2 or MPL mutations
  - 71% ET, 56% PMF
  - 86% of patients with progression from ET to PMF
- No CALR mutations were found in 511 MPNs that were JAK2 or MPL mutated
- CALR mutations were found in 8% MDS, 1 patient each with CMML and atypical CML, no mutations found in lymphoid cancers or solid tumors
- Used multiple lab and statistical approaches to determine if the mutation occurred as an early event
  - Genotyped 300 individual hematopoietic colonies in 5 patient with mutated CALR: in all patients CALR mutations arose in the earliest phylogenetic node *
A  Distribution of JAK2, MPL, and CALR Mutations in Philadelphia Chromosome–Negative Myeloproliferative Neoplasms

Polycythemia Vera (N=382)
- Nonmutated JAK2, MPL, and CALR
- JAK2 mutation

Essential Thrombocytemia (N=311)
- Nonmutated JAK2, MPL, and CALR
- CALR mutation
- JAK2 mutation
- MPL mutation

Primary Myelofibrosis (N=203)
- Nonmutated JAK2, MPL, and CALR
- CALR mutation
- JAK2 mutation
- MPL mutation

B  Frequency of CALR Mutations in Myeloid Cancers

<table>
<thead>
<tr>
<th>Condition</th>
<th>Patients with CALR Mutation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polycythemia Vera (N=382)</td>
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<tr>
<td>Essential Thrombocytemia (N=311)</td>
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<tr>
<td>Primary Myelofibrosis (N=203)</td>
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<td>AML (N=254)</td>
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<td>RARS-T (N=24)</td>
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<tr>
<td>Healthy Controls (N=524)</td>
<td>0</td>
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Clinical Significance of CALR

- Both key papers found a clinical phenotype associated with the CALR mutation.
- In ET, patients with CALR mutation had lower hemoglobin levels, lower wbc, higher platelet counts at dx than JAK2 mutants (p<.001 both studies)*
- In PMF, patients with CALR had lower wbc and higher platelets (P<.001) than mutated JAK2.
- For PMF, patients with CALR had longer OS than those with JAK2/MPL (p<.001), although only noted to adjust for age.
- In ET, patients with CALR had lower thrombosis risk than JAK2 (p=.003); this group too small to evaluate for PMF.

- The altered peptide sequence at the C-terminal domain of the mutated CALR offers opportunity for immunologic targeting because it represents a cancer-specific epitope.
A driver mutation is a genome abnormality that causes a selective advantage in a cell with capacity for self-renewal, leading to formation of a clone of mutated cells.

Driver mutation subtypes:
- Founding/initiating mutations which give rise to the initial clone of a malignancy
- Subclonal/cooperating mutations occur in a cell of an already established clone, propagate the initial clones, and commonly occur with disease progression

Then clonal evolution occurs in which the founding malignant clones generates subclones through the acquisition of additional mutations
- In MPNs, increased chromosomal lesions are associated with disease progression, poor survival and leukemic transformation (Klamft T et al, Blood 2011)
- Include ASXl1, SRSF2 and EZh2 in PMF
Driver mutations

- In MPNs, somatic mutations of JAK2, MPL and CALR are thought to behave as founding driver mutations for responsible for the MPN phenotype
  - These mutations may not be first somatic event
  - TET2, for example, can precede JAK2 in hematopoietic stem cells in MPNs (Lundberg et al, Busque et al)
  - But the acquisition of the JAK2 or CALR is thought to actually give rise to the phenotype
- 2 types of genetic predispositions to develop an MPN have been identified in the general population to support this theory (Cazzola et al)
  - A population level genetic predisposition, (haplotype 46/1 or GGCC) a mutation of the JAK2 gene itself predisposes to both JAK2 and MPL mutant MPNs
  - Familial cases of ET/PMF have been found to carry somatic mutations of CALR
A recent study of gene expression profiling indicates that activated JAK2 signaling is seen in all patients with MPNs regardless of founding driver mutation or clinical diagnosis (Rampal et al). This is consistent with observations that patients with PMF may respond to JAK inhibitors regardless of genotype (Cazzola et al).

Megakaryocytes play a major role in pathophysiology of MPNs by mediating bone marrow fibrosis. Driver mutations appear to alter megakaryocyte differentiation, migration ability and pro-platelet formation, leading to increased platelet production.

Possible hypothesis: the founding driver mutation (JAK2, CALR or MPL) activates the JAK-STAT pathway in megakaryocytes, resulting in thrombocytosis initially and later in bone marrow fibrosis (Cazzola et al).
Treatment: Old School

- **Low risk: conservative management**
  - Asymptomatic: Watch and wait
  - Symptomatic: Erythropoiesis-stimulating agents (epo level <125, no splenomegaly)

- **Intermediate risk 2/high risk**
  - Steroids, Androgens, Danazol
  - Lenalidomide +/- Prednisone for 5q-
  - Hydroxyurea, especially if splenomegaly
  - Splenectomy for hydroxyurea-refractory splenomegaly with symptoms
  - Allogeneic transplant: only curative option
The future is now: JAK-inhibitors

- JAK inhibitors are a new targeted therapy for MF that reduce spleen size, improve constitutional symptoms, and have now been shown to improve survival (Harrison et al, Vertovsek et al)
  - Clinical trials show ruxolitinib (JAK1 and 2 inhibitor) was effective regardless of whether patients were JAK2 mutated or not (calr not measured in those trials)
- Studies have shown that JAK-SAT signaling is involved in cytokine-independent growth of mutant CALR-expressing Ba/F3 cells and that these cells are sensitive to JAK inhibitor fedratinib in vivo (Passamonti et al NEJM 2014)
- Passamonti et al reported in NEJM 2014 letter that they successfully treated 2 patients with PMF and CALR mutations with decreased spleen size and symptom improvement
  - Reported as proof of principle that these drugs can be used in CALR mutant patients
Patient Follow-Up

- Patient being seen locally in Aberdeen
- He remains on Hydroxyurea, and platelets now improved to 684,000
- Per outpatient oncologist, no current plan to add a JAK-inhibitor
Future Directions

- Should the classification of MPNs take genetic information into account?
- Should mutational status now be incorporated into prognostic scoring systems and guide clinical decision making?
- Can we target the CALR mutation therapeutically?
- All ongoing . . .
Take Home Points

• AVWS is an under-recognized bleeding disorder associated with multiple common clinical conditions, including myeloproliferative and cardiac disorders.

• In MPNs with significant thrombocytosis, patients are at risk for both bleeding and clotting, which cytoreductive therapies can address.

• Because CALR mutations are found in about 70-80% of MPN patients who do not have mutations in JAK2/MPL, they help fill a molecular diagnostic gap in diagnosing, and potentially treating, MPNs.

• JAK-inhibitors can be used in patients who are non-JAK2 mutated, and their role in treating MPNs as a whole is still evolving.
References

- Rampal R, Al-Shahrour F, Abdel-Wahab O et al. Inegrated genomic analysis illustrates the central role of JAK-STAT pathway activation in myeloproliferative neoplasma pathogenesis. Blood. 2014; April 16 (Epub ahead of print)
Thanks!

- Thanks to Dr. Richard for helping me with the talk and being my discussant
- Thanks to Dr. Martin for helping on the inpatient side
Our patient had a high risk for thrombosis given his platelet counts, based on studies in ET/PV.

A large randomized trial demonstrated a reduction of thrombotic events in PV in those taking ASA without increase risk of hemorrhage, thus we applied here given thrombocytosis (Landolfi, NEJM 2004).

Hydrea is the only cytoreductive agent proven to reduce thrombotic events in a randomized clinical trial for ET (Cortelazzo et al, NEJM 1995).