Proliferating genetics in the MPNs

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Case

• 74 yo man with persistent, isolated thrombocytosis (~800k) observed by PCP over the course of several months
• Otherwise healthy: No underlying cause for reactive thrombocytosis (e.g. anemia, cancer, infection)
  – BCR/ABL mutation negative
• Seen in consultation in Nov 2013:
  – Suspected ET
  – Recommended testing for JAK2 mutation
Outline

• Overview – MPNs
• Genetics through November 2013
• Calreticulin
  – Discovery
  – Prognosis
  – Function
  – Treatment
Overview of the MPNs: Dameshek 1951 - WHO 2008

Myeloid Malignancies

- AML
- MDS
- MPN
- MDS/MPN
- PDGFR – Eos (1)

- CML
- PV
- ET
- PMF

- CNL(2)
- CEL(3)
- SM(4)
- MPN-U(5)

1 Platelet-derived growth factor-rearranged neoplasms associated with eosinophilia
2 Chronic neutrophilic leukemia
3 Chronic eosinophilic leukemia
4 Systemic mastocytosis
5 MPN unclassifiable
Ph-negative ‘classic’ MPNs: presentation and complications

J Kiladjian, Hematology 2012;2012:561-566
Ph-neg MPNs genetics: Nov ‘13

MPL, JAK2-exon 12, LNK, TET2, DNMT1, IDH*, ASXL1*, EZH2*

J Kiladjian, Hematology 2012;2012:561-566
Epo, Tpo, GM-CSF, IL 3, IL 5, GH, IFNγ, IL 6, IL 10, IL 11, IL 19, IL 20, IL 22

JAK-STAT: *MPL* (thrombopoietin receptor)

- Mutations to W515 of MPL present in 1% ET and 5% PMF
- Also causes over-activation of JAK-STAT

CALR mutation: discovery

- Whole-exome sequencing performed on 6 subjects with JAK2/MPL WT PMF.
- Genomic DNA obtained from PB granulocytes (tumor) and compared with T-lymphocytes (control).
- ROIs were then re-sequenced after PCR amplification.
- 2-12 mutations were identified per patient.
- CALR = only gene with recurrent mutations (all in exon 9): 2/6 = deletions, 4/6 = insertions.
- CALR not previously recognized as an oncogene.

Klampfl et al. NEJM Dec 2013
CALR mutation: discovery

- CALR mutations mutually exclusive of JAK2 and MPL mutations
- Mutational frequency in WT JAK2/MPL = 67% ET (195 / 289) and 88% PMF (105 / 120)

Klampfl et al. NEJM Dec 2013
CALR mutuation: discovery (2)

• 151 samples with Ph-neg MNPs (48 PV, 62 ET, 39 MF): exomes sequenced
• 0–32 mutations per sample, including in JAK2, MPL, and epigenetic regulators (ASXL1, IDH1)
• 26/31 patients WT for JAK2/MPL with ET or PMF had CALR mutations; 0/70 with JAK2 or MPL mutations had CALR mutated
• In the 26 patients, all CALR mutations = indels in exon 9
• Not identified in AML (n = 48), lymphoid malignancies (287), solid tumors (502), controls (550)

Nangalia et al. (UK), NEJM Dec 2013
CALR in ET: clinical impact

- OS at 10 yrs: 96.9% (CI 91.7-98.8) in CALR group vs 91.1% (CI 87.1 – 93.9) JAK2 group (p = 0.04)
- Cumulative incidence at 10 yrs of thrombosis is 11.0% (CI 6.3 – 17.1) in CALR group versus 21% (CI 16.6 – 25.7) in JAK2 group (p = 0.003)

Klampfl et al, NEJM Dec 2013
**CALR in ET: clinical impact**

- 576 patients with ET: U Florence
- *CALR* mutations present in 49% of *JAK2*/*MPL* WT patients with ET: probably reflects stricter patient selection – others may have included early PMF
- Lower thrombosis risk (*p* = 0.01)
- No impact on survival or transformation to PET MF

CALR in PMF: clinical impact

Median OS with CALR mutation 21.4 yrs (CI 17.1 – 22.9) vs 11.0 yrs (CI 7.8 – 14.4) with JAK2 mutation and 8.2 (CI 2.0 – NR) with MPL mutation

Klampfl et al, NEJM Dec 2013
CALR in PMF: clinical impact

• Lower prbc transfusion dependency (not shown) and superior survival (not accounted for by DIPSS+ in multivariate analysis)

Calreticulin

Multiple reported functions:

**Within ER**
1. Quality control for glycoprotein folding in ER
2. Calcium homeostasis

**Outside of ER**
1. Proliferation
2. Apoptosis

http://www.nature.com/scitable/topicpage
CALR mutation: exon 9

- Type 1 = 52 bp deletion
- Type 2 = 5 bp insertion

Klampfl et al. NEJM Dec 2013
Calreticulin

- Pattern of mutations is remarkably stereotypical: +1 frameshift
- Result is a less acidic domain without an ER retention motif
- Dose-effect -- Nearly all negatively charged AA’s in C-terminal domain are lost in type 1 mutation, which is more frequently associated with PMF (p < 0.001), compared with loss of about ½ these AA’s in the type 2 mutation
- Theorized to allow more protein chaperone function at the expense of impaired calcium binding

http://michalaklab.com/
**CALR analysis in the clinic**

- SCCA/FHCRC pathology currently planning to offer mutational testing – validation of assay underway
- At present may send specimen to OHSU
Case: management of ET

- Goal = prevent thrombosis
- Risk stratify with age (> 60) or thrombosis history
- Low risk: ASA for those with cardiovascular RFs
- High risk: ASA and cytoreduction with hydrea
- Will this change with CALR screening?
Conclusions

• Somatic CALR mutations are present in a large proportion of JAK2/MPL-WT ET and PMF
• Mutations in CALR in these cases confer a relatively favorable survival profile
• Expect incorporation into prognostic models
• Modification of treatment recommendations?
• Awaiting deeper understanding of functional consequences and targetability of CALR mutations
Thank you

• Dr. Vivian Oehler
• Dr. David Wu
Calreticulin: localization

No obvious alteration in subcellular localization (arguably the localization is not as strict in the GFP-tagged mutant form)

Klampfl et al. NEJM Dec 2013
Nangalia et al. NEJM Dec 2013
Calreticulin: function

- In an IL-3 dependent pro-B cell line, mutant CALR enhances viability and proliferation

Klampfl et al, NEJM Dec 2013