Genetics of MPNs – insights from genomic and functional studies

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Scientific Questions/Advances

• Are mutations which activate JAK2 a hallmark of all MPN patients?

• What do mutations which occur in concert with JAK2/MPL mutations do?

• What have we learned about JAK2 inhibitors

• What novel therapies are of potential benefit for MPN patients?
JAK2V617F Mutations in MPN patients

No mutation in normal tissue  
Heterozygous mutation in MPN Cell

Valine→Phenylalanine Amino Acid Change

- 90% of PV
- 60% of ET/PMF
- <10% of CMML/AML

*James et al. Nature 2005
Levine et al. Cancer Cell 2005
Baxter et al. Lancet 2005
Kralovics et al. NEJM 2005
Conserved domains in JAK family members

Cytokine Receptor Binding

- JH7
- JH6
- JH5
- JH4
- JH3
- JH2
- JH1

“Pseudo kinase” domain
- autoinhibition of kinase

- STAT Binding

Expression of JAK2V617F in vivo results in PV phenotype*

*Wernig et al. Blood 2006
Lacout et al. Blood 2006

Constitutively active tyrosine kinase

IP JAK2

WB: p-tyr

WT V617F

WB: JAK2

V617F
JAK2V617F negative MPN

• JAK2V617F-negative PV
  - JAK2 exon 12 mutations
  - loss of function mutations in LNK, negative regulator of JAK2 (Oh et al Blood 2010)

• JAK2V617F-negative ET/PMF
  - MPL mutations in 10%
  - LNK mutations in <5%

• Somatic mutations have not been identified in 30-40% of MPN patients
  - Sequencing known genes in the JAK2 pathway has not provided the answer->how to proceed?
Whole Exome Sequencing to identify MPN Alleles*

• To date we have sequenced 40 exomes from MPN patients
  - 20 JAK2/MPL negative patients
  - 15 patients with myelofibrosis
  - 5 patients with MPN which transformed to leukemia

• We have sequenced members of 2 families with high penetrance MPN – try to find familial predisposition locus

• Complements efforts by Sanger/European group focusing on JAK2+ disease, PV/ET/PMF

*Jay Patel, Ann Mullally, Ben Ebert (MPN Foundation Grant)
Lessons from Exome Sequencing to Date

• Easy to generate data – much more difficult to accurately analyze it

• Recurrence/testing large number of samples will be key

• Functional studies will take months to years to find true “drivers” which cause MPN versus “passengers” along for the ride

• Many mutations may not be specific to MPN, but might be seen in MPN, MDS, AML

• We hope to find lesions with clinical significance
  - Novel therapeutic targets
  - Lesions which predict outcome to ensure we aggressively treat patients with poor prognosis and leave good prognosis patients alone
  - Lesions which occur at transformation to AML->prevent or treat leukemic transformation
Are there cooperating somatic mutations?

• If most MPN patients are JAK2 positive, why do some people develop PV, or ET, or PMF?

• Perhaps it is the presence of a second mutation, which occurs in concert with JAK2, which determines the specific MPN?
TET2 Deletions/Mutations in Myeloid Malignancies*

LOH/deletions involving a single gene → \textit{TET2}

Sequence analysis of \textit{TET2} in MDS/MPN samples identified somatic mutations in 10-20% of MPN and MDS patients

*Delhommeau \textit{et al} NEJM 2009
Langemeijer \textit{et al.} Nat Gen 2009
**TET2 Mutations in Myeloid Malignancies**

*Abdel-Wahab et al Blood 2009

- TET2 mutations are not specific to MPN – seen in all myeloid malignancies, and likely in other leukemias as well!

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### A) MPN 7.6% (27/354)

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Frequency</th>
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<td>1 2 3 4</td>
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### B) CMML 42% (29/69)

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### C) AML 10.1% (12/119)

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Somatic ASXL1 Mutations in MPN*

Like TET2 seen in all myeloid malignancies->not specific to MPN

*Omar Abdel-Wahab, Jay Patel
Leukemic Transformation of MPN

- Patients with PV, ET, and PMF are at high risk for transformation to AML-associated with a dismal prognosis
- Genetic/Epigenetic events which contribute to leukemic transformation are not known
- Approximately 50% of JAK2+ MPN patients transform to a JAK2-negative MPN*

*Campbell et al. Blood 2006
Theocarides et al. Blood 2007
TET2 Mutations, but not ASXL1 Mutations are Acquired at Leukemic Transformation

<table>
<thead>
<tr>
<th>Sample</th>
<th>MPD</th>
<th>Genotype during MPN</th>
<th>Genotype during AML</th>
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<tbody>
<tr>
<td></td>
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<td>JAK2</td>
<td>TET2</td>
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<tr>
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<td>MF</td>
<td>V617F</td>
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<td>MF</td>
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Frequency of mutation: TET2 78.6%, ASXL1 21.4%, ASXL1 50%, ASXL1 42.9%, ASXL1 28.6%
Cooperating Mutations in MPN Patients

- Recent studies have identified somatic disease alleles which occur in concert with JAK2/MPL mutations
  - TET2 loss of function mutations in 10% of MPN patients
  - ASXL1 mutations in 8-10% of MPN patients
  - IDH1/2 mutations in 3-5% of MPN patients
  - EZH2 mutations in 10-15% of patients

- Same mutations are seen in MDS and AML patients - they do not explain the PV/ET/MF conundrum

- In some cases (TET2, IDH1/2) these mutations occur most commonly at progression to AML

- Limited functional data suggest these mutations affect the epigenetic state of MPN cells - affect the way DNA is packaged and which parts of it are used in MPN cells
What about gene expression – can we measure gene expression and learn something about pathogenesis of MPN*

- Determine if there is a common genetic signature associated with MPN or with *JAK2V617F* mutations
- Identify genes which segregate with clinical phenotype
- Identify candidate genes in JAK2/MPL-negative MPN

*Ben Ebert/Todd Golub
Gene Expression Profiling in MPN

- Purified neutrophils from MPN patients
- All patients had JAK2 allele burden, MPL, TET2, ASXL1 mutations
- Only patients who had clonal disease were included in expression array analysis
  - Mutational allele burden > 51%
  - X inactivation DS > 0.25 in females
  - Clonal abnormality on SNP Array
- Compared to purified neutrophils from normal donors
- Integrated with Affy SNP Array, genotyping for > 500 known disease alleles using Oncomap (Broad Institute)

*Ben Ebert/Todd Golub
Gene Expression Profiling of MPN Samples clearly distinguishes MPN Patients from Normal Blood Cells
Gene expression profiling does not distinguish patients based on clinical diagnosis

**JAK2 V617F Homozygous**

comparison:

- PV vs MMM

**JAK2 WT comparison**:

- ET vs MMM

No genes significantly differentially expressed (FDR < 0.05)
Dominant Gene Expression Signature in MPN is Homozygous JAK2 Mutant Signature
Differential JAK2 Expression in MPN patients

- JAK2 expression levels differ according to mutational status and allele burden
  - Not explained by JAK2 haplotype

- In vitro data suggests JAK2 regulates its own expression

- Suggests JAK2 expression level, and not just mutational status, relevant to MPN pathogenesis – not clear this is recapitulated in murine models
Is there a JAK2 signature in heterozygous/WT MPN patients?

JAK2 shRNA in HEL cells to generate JAK2 signature

Similar data with JAK inhibitor
JAK2 shRNA signature in MPN and Normal samples

JAK2 V617F Homozygous (PV/MMM) vs normal

JAK2 V617F Heterozygous (ET/MMM) vs normal

JAK2 WT (ET/MMM) vs normal

D

E

F

Enrichment Score (ES)

Homozygous

Normal

FDR qvalue= 0.018

Heterozygous

Normal

FDR qvalue= 0.035

WT

Normal

FDR qvalue= 0.038

JAK2 V617F Homozygous (PV/MMM) vs normal

JAK2 V617F Heterozygous (ET/MMM) vs normal

JAK2 WT (ET/MMM) vs normal

Enrichment Score (ES)

Homozygous

Normal

FDR qvalue= 0.018

Heterozygous

Normal

FDR qvalue= 0.035

WT

Normal

FDR qvalue= 0.038
JAK2 shRNA signature in MPN and Normal samples

<table>
<thead>
<tr>
<th>HOMOZYGOUS</th>
<th>HETEROZYGOUS</th>
<th>NORMAL</th>
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<tbody>
<tr>
<td>PV</td>
<td>MMM</td>
<td>ET</td>
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Seen in all MPN patients, not in normals

Suggests JAK2 is activated in all MPN patients regardless of specific mutation
Model of MPN Pathogenesis

- Mutations which activate JAK2 are the most common lesion -> best therapeutic target
- Possible other mutations affect response to JAK inhibitors
Preclinical/Clinical Development of JAK inhibitors

• Agents in Current Clinical Development:
  • INCB18424: Potent dual JAK1/JAK2 inhibitor (approved)
  • TG101348/SAR302503: Most JAK2 selective, has FLT3 inhibitory activity (phase I completed)
  • CYT387: JAK1/JAK2 inhibitor (phase I completed)
  • SB1518: fairly JAK2 selective (phase I completed)
  • AZD1480: JAK2/JAK1 inhibitory activity (phase I)
  • LY2784544: phase I trial
  • Others in earlier phase development

• XL019: discontinued due to neurotoxicity->not clear if this is a JAK dependent effect or due to off-target effects

• Differences between these drugs may have a lot to do with pharmacokinetics and half-life, not just targets
INCB18424 treatment improves outcome in MPLW515L-mutant PMF mice*

- Improved splenomegaly, thrombocytosis, leukocytosis, and myelofibrosis
- No reduction in mutant population in stem/progenitor or in differentiated cells

*Sachie Marubayashi, Priya Koppikar
JAK Inhibitor Treatment Decreases Circulating Cytokine Levels and Improves Body Weight
Clinical Trials to Date with JAK2 Inhibitors

- JAK2 inhibitors improve spleen size, elevated blood counts, clinical symptoms - Is this due to effects on the malignant clone?

- Main side effect of JAK2 inhibitors is anemia/thrombocytopenia - likely due to “on-target” effects of inhibiting JAK2 in normal cells

- To date we have seen minimal effects on mutant allele burden - incomplete dependence on JAK2 - inherent or acquired resistance/persistence

In murine model if we stop rx -> all mice succumb to disease within 21 days
Can we improve our ability to target JAK2*

- It is presumed the hematopoietic toxicities are due to inhibition of JAK2 in normal cells—has this been clearly delineated in vivo?

- Can we develop better therapies which improve the therapeutic window and target the malignant cell?
  - additional therapies
  - alternate dosing strategies for JAK2 inhibitors

- Collaborated with Gabriela Chiosis and Jay Bradner to test ability of additional compounds to inhibit JAK2 dependent proliferation

PU-H71

Sachie Marubyashi, Priya Koppikar
PU-H71 Inhibits Growth and Signaling of MPN cells

**Vf neo with PU-H71**

IC50 = 35 nM

**W515L neo with PU-H71**

IC50 = 190 nM

**NOMO1 with PU-H71**

IC50 = 10.3 nM

**SET-2 with PU-H71**

Growth inhibition associated with degradation of JAK2
PU-H71 demonstrates efficacy in vivo in JAK2V617F and MPLW515L transplant models

![Survival](image)

- **Survival**
  - Graph showing survival over treatment days.
  - Comparison between Vehicle and PU-H71.
  - p < 0.0004

![Spleen Weight](image)

- **Spleen Weight**
  - Bar graph comparing mJAK2 V617F Vehicle (n=5), mJAK2 V617F PU-H71 (n=4), hMPL W515L Vehicle (n=3), hMPL W515L PU-H71 (n=4).
  - *p < 0.01
PU-H71 Depletes JAK2 in leukemic, but not normal hematopoietic cells

PK/PD studies show PU-H71 is selectively taken up and maintained in tumor, but not normal cells – basis for therapeutic index
PU-H71 Degrades JAK2/Inhibits JAK-STAT signaling in 1° MPN Samples

Clinical studies of HSP90 inhibitors and preclinical studies of combination JAK2/HSP90 inhibitor therapies are underway.
Summary

• Mutations which activate JAK-STAT Signaling are seen in almost all MPN patients but there are additional genetic lesions seen in MPN patients which contribute to MPN/MDS/AML stem cell survival.

• Additional novel therapeutic approaches targeted at JAK2 and at other oncogenic signaling pathways might offer benefit alone or in conjunction with JAK2 inhibitors.

• Genetic studies of myeloid malignancies will likely identify novel mutations with pathogenetic and therapeutic relevance.
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  - Levine et al. Cancer Cell 2005
  - Levine et al. Blood 2005
  - Levine et al. Blood 2006
  - Pikman et al. Plos Medicine 2006
  - Scott et al. NEJM 2007
  - Kawamata et al. Experimental Hematol 2007
  - Kilpivaara et al. Nature Genetics 2009
  - Abdel-Wahab et al. Blood 2009
  - Abdel-Wahab, Verstovsek et al. Canc Res 2010
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