Myeloproliferative Neoplasms in 2014: Morphology, Molecular Genetics and Test Utilization

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DISCLOSURES:

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None

Off Label Usage
None
Today’s Objectives

• Briefly review recommendations for cytogenetic and molecular monitoring in CML

• Understand the role of blood and bone marrow morphology in the subclassification of the MPNs

• Be familiar with the role of various molecular and genetic studies in the MPNs, including:
  • Chromosome analysis
  • Molecular testings of JAK2, CALR, MPL, CSF3R, other prognostic mutations

• Review an algorithmic approach to evaluating a possible MPN

• Understand how to overcome common utilization issues in the evaluation of the MPNs
Myeloproliferative Neoplasms (MPNs)

• Malignancy of hematopoietic stem cells

• Effective hematopoietic proliferation
  • Hypercellular bone marrow with elevated peripheral blood counts and/or organomegaly

• Fibrosis is common

• No (minimal) dysplasia

• Classified on the basis of dominant cell line involved
Imatinib responsiveness in CML is one of the original genetic success stories of cancer treatment!

CML is a genetic evaluation
Molecular Tests in CML

- Chromosome banding analysis
- **FISH:** $BCR-ABL1$ fusion detection
- **Qualitative PCR:** multiplex, identifies $BCR-ABL1$ fusion form, p210, p190, p230, etc.
- **Quantitative real time-PCR:** monitor specific $BCR-ABL1$ fusion transcript level in response to therapy
- **ABL kinase domain mutation (KDM) analysis:** detects mutations in the ABL kinase domain that confers acquired drug resistance; guide 2$^{rd}$/3$^{rd}$ line therapy choice
# Definition of Response to First-Line TKI Therapy

<table>
<thead>
<tr>
<th>Time</th>
<th>Optimal</th>
<th>Warning</th>
<th>Failure</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Baseline</strong></td>
<td></td>
<td>High risk CCA/Ph+</td>
<td></td>
</tr>
<tr>
<td><strong>3 months</strong></td>
<td>$BCR-ABL1 \leq 10%$ and/or Ph+ $\leq 35%$</td>
<td>$BCR-ABL1 \geq 10%$ and/or Ph+ $36-95%$</td>
<td>Non-CHR and/or Ph+ $&gt;95%$</td>
</tr>
<tr>
<td><strong>6 months</strong></td>
<td>$BCR-ABL1 \leq 1%$ and/or Ph+ $0$</td>
<td>$BCR-ABL1$ $1-10%$ and/or Ph+ $1-35%$</td>
<td>$BCR-ABL1 \geq 10%$ and/or Ph+ $&gt;35%$</td>
</tr>
<tr>
<td><strong>12 months</strong></td>
<td>$BCR-ABL1 \leq 0.1%$ (MMR)</td>
<td>$BCR-ABL1 &gt; 0.1-1%$</td>
<td>$BCR-ABL1 &gt; 1%$ and/or Ph+ $&gt;0$</td>
</tr>
<tr>
<td><strong>Then and any time</strong></td>
<td>$BCR-ABL1 \leq 0.1%$ (MMR)</td>
<td>CCA/Ph- (-7 or 7q-)</td>
<td>Loss of CHR Loss of CCyR Confirmed loss of MMR Mutation CCA/Ph+</td>
</tr>
</tbody>
</table>

## Recommendations for cytogenetic and molecular monitoring in CML

### At diagnosis
- Chromosome banding analysis (CBA)
- FISH (in case of Ph negativity to identify variant, cryptic translocations)
- Qualitative PCR (identification of transcript type)

### During treatment
- Quantitative real-time PCR (RQ-PCR) (international scale); every 3 months until an MMR (BCR-ABL ≤0.1%); then every 3 to 6 months
- CBA at 3, 6, and 12 months until a CCyR has been achieved, then every 12 months.
- If adequate molecular monitoring can be ensured, cytogenetics can be spared.

### Failure, progression
- RQ-PCR
- ABL kinase domain mutational analysis
- CBA

### Warning
- Molecular and cytogenetic tests to be performed more frequently.
- CBA in case of myelodysplasia or CCA/Ph– with chromosome 7 involvement.
- ABL kinase domain mutational analysis

Whenever possible, both cytogenetic and molecular tests are recommended until a CCyR and an MMR are achieved. Then RQ-PCR alone may be sufficient.

*Blood. 2013 Aug 8;122(6):872-84.
Baccarani M et al.*
Diagnostic Approach to the Ph- Classic MPNs

• Clinical findings

• Laboratory studies
  • CBC and diff, serum erythropoietin, LDH, iron studies, etc.

• Morphologic assessment of PB and BM

• Genetic studies
  • Chromosome analysis
  • JAK2, CALR and MPL
  • Other prognostic mutational analysis
Morphologic Assessment

• Goals:
  • Establish the diagnosis of a MPN
  • Subclassify the MPNs when possible
  • Determine the degree of fibrosis
  • Assess % blasts / transformation
Subclassifying MPNs by Morphology: A Practical Approach

• Acknowledge that you cannot accurately subclassify every MPN with absolute confidence

• Step 1: PV is the big “masquerader”
  • Diagnose or exclude PV on the basis of CBC, iron studies, serum EPO, JAK2, etc.

• Step 2:
  • Establish the diagnosis of those cases that are either straightforward ET or PMF

• Step 3:
  • Everything else is MPN, NOS
Possible MPN?

Morphology confirms MPN

Diagnose or exclude PV

“>15 to 20” year survivals

MPN, NOS. Clinical criteria needed

“3 to 7” year survivals
WHO Criteria for Diagnosis of PV

**Major criteria**
- Hemoglobin > 18.5 g/dL in men, > 16.5 g/dL in women, or evidence of increased red cell volume
- Presence of JAK2 (V617F) or other functionally similar mutation (eg, JAK2 exon 12 mutation)

**Minor criteria**
- BM biopsy showing hypercellularity for age with trilineage myeloproliferation
- Serum erythropoietin level below the normal reference range
- Endogenous erythroid colony formation in vitro

Requires either both major criteria and 1 minor criterion or the first major criterion and 2 minor criteria.
Diagnostic Criteria for PMF: Practical Approach

- WHO criteria
  - Morphologic features of a MPN
  - Exclude PV, ET, CML, MDS, CMML
  - JAK2V617F or other clonal markers; if none, exclusion of secondary causes of myelofibrosis or other changes
  - LEBR; increase in LDH; anemia; splenomegaly

- Present with neutrophilia, thrombocytosis or cytopenias
- Hypercellular bone marrow - with or without fibrosis
- Granulocytic hyperplasia
- Prominent megakaryocyte hyperplasia and marked megakaryocytic atypia
Diagnostic Criteria for ET: Practical Approach

- WHO criteria
  - Sustained platelet count ≥ 450 x 10⁹/L
  - Morphologic features of a MPN
  - Exclude PV, PMF, CML, MDS, CMML
  - JAK2V617F or other clonal marker; if none, exclusion of reactive thrombocytosis

- Normal to slightly hypercellular bone marrow
- Neutrophils <12 x 10⁹/L
- No granulocytic hyperplasia in bone marrow
- No reticulin fibrosis
- No marked megakaryocytic hyperplasia or striking atypia
Mayo Clinic Series of 605 ET Patients

Adverse risk factors

- Age $\geq$ 60 years
- Lower than normal hemoglobin
- WBC $\geq$ 15 x $10^9$/L

Gangat et al. Leukemia 2006
CSF3R Mutations in Chronic Neutrophilic Leukemia (CNL)

- GCSF receptor

- Two types of mutations:
  - Missense mutations affecting the extracellular domain:
    CNL (frequent, CSF3R T618I); hereditary neutrophilia
      - Strongly activates the JAK/signal transducer and activator of transcription pathway
      - Thus, are sensitive to JAK kinase inhibitors such as ruxolitinib
  - Truncation mutations of the cytoplasmic tail:
    CNL; severe congenital neutropenia patients developing AML following long term GCSF therapy
    - Downstream signaling operates predominantly through SRC kinases
    - Exhibits drug sensitivity to SRC kinase inhibitors such as dasatinib

- CSF3R mutations not seen in aCML or MGUS-associated neutrophilia

- CSF3R T618I absent among 170 patients with PMF or CMML

Pardanani, et al, Leukemia. 2013
MPNs: Distinctive Genetic Abnormalities

- 2005
  - MPL mutation in JAK2-neg ET and PMF
  - JAK2 exon 12 mutation in JAK2-neg PV

- 1960
## MPNs: Distinctive Genetic Abnormalities

<table>
<thead>
<tr>
<th>Disease</th>
<th>Cytogenetics</th>
<th>Molecular genetics</th>
</tr>
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<tbody>
<tr>
<td>CML</td>
<td>t(9;22)(q34;q11.2)</td>
<td><em>BCR-ABL1</em></td>
</tr>
<tr>
<td>PV</td>
<td>+8, +9, del(20q), del(13q), del(9p)</td>
<td><em>JAK2 V617F</em> (95-98%)&lt;br&gt;<em>JAK2 exon12</em> (2-5%)</td>
</tr>
<tr>
<td>ET</td>
<td>Rare abnormalities</td>
<td><em>JAK2 V617F</em> (50-60%)&lt;br&gt;<em>CALR</em> (20-30%)&lt;br&gt;<em>MPL</em> exon10 (3-5%)</td>
</tr>
<tr>
<td>PMF</td>
<td>+8, +9, +1q, del(20q), del(13q), der(6)t(1;6)</td>
<td><em>JAK2 V617F</em> (50-60%)&lt;br&gt;<em>CALR</em> (20-30%)&lt;br&gt;<em>MPL</em> exon10 (5-10%)</td>
</tr>
</tbody>
</table>
Molecular Landscaping of Ph- Classic MPNs

Three Major Driver Mutations (JAK2, CALR and MPL)

PV

ET

PMF
JAK2 V617F Mutation

(+)  
• PV  95%  
• PMF  ~ 50-60%  
• ET  ~ 50-60%  
• RARS-T  ~ 60%  
• MDS  0-5%  
• AML  0-5%  

(-)  
• Healthy volunteers  
• Secondary polycythemia  
• Reactive thrombocytosis  
• Reactive leukocytosis  
• Solid tumor  
• Lymphoid disorders  
• CML
JAK2 V617F Mutation Assay

- **Positive result:**
  - Confirms the presence of a myeloid disorder
  - Strongly favors MPN over MDS diagnosis
  - Cannot distinguish between MPN’s

- **Negative result**
  - In general, diagnostically not helpful
  - Argues against a diagnosis of PV or PPMF

- **Assay sensitivity**
  - Sanger sequencing: \( \sim 20\% \)
  - Quantitative AS-PCR: \(<0.1\%\)
Calreticulin (CALR) Mutations

- Multifunctional protein
- Somatic insertions and deletions of CALR exon 9
- Resulting in the same alternative reading frame in C-terminus of CALR
- Mutually exclusive of JAK2 and MPL mutations
  - ~50 to 80% of JAK2 and MPL-negative PMF and ET
  - 20-30% PMF and ET
- CALR mutations found in hematopoietic stem and progenitor cells, converges on JAK/STAT pathway

Klampfl, et al. NEJM 2013
Nangalia, et al. NEJM 2013
**CALR Exon 9 Indel Mutations**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDS</td>
<td>8%</td>
</tr>
<tr>
<td>RARS-T</td>
<td>12%</td>
</tr>
<tr>
<td>CMML</td>
<td>3%</td>
</tr>
<tr>
<td>aCML</td>
<td>3%</td>
</tr>
<tr>
<td>AML</td>
<td>0%</td>
</tr>
<tr>
<td>CML</td>
<td>0%</td>
</tr>
<tr>
<td>Lymphoid neoplasm</td>
<td>0%</td>
</tr>
<tr>
<td>Solid tumor</td>
<td>0%</td>
</tr>
<tr>
<td>Normal controls</td>
<td>0%</td>
</tr>
</tbody>
</table>

*Klampfl, et al. NEJM 2013*
*Nangalia, et al. NEJM 2013*
Clinical Features and Prognosis Impact of CALR mutation

ET
- Lower Hb
- Lower WBC
- Higher platelets
- Lower risk of thrombosis
- ? OS

PMF
- Less likely to have transfusion-dependent anemia or leukocytosis
- Higher platelet count
- Lower risk of thrombosis
- Better OS

Klampfl, et al. NEJM 2013
Nangalia, et al. NEJM 2013
Rumi, et al, Blood 2014
Tefferi, et al, Leukemia 2014
**MPL Exon 10 Mutations**

- Thrombopoietin receptor
- *MPL* mutations present in 5-10% PMF and 3-5% ET
- Mutations are essentially exclusive of *JAK2* and *CALR*
- Especially useful when there is a clinical suspicion of a MPN but the bone marrow morphology is equivocal
  - Fibrosis without megakaryocyte clusters
  - Thrombocytosis and/or neutrophilia without megakaryocyte clusters
- Sanger sequencing: 20% sensitivity
Prognosis Impact of Three Driver mutations in PMF
JAK2, MPL, and CALR

• **CALR** mutation showed a favorable impact on survival, over **JAK2** mutation and triple negative (**JAK2-CALR-MPL-**) cases, independent of IPSS/DIPSS+.

• Triple negative: high-risk molecular signature

*Tefferi, et al, Leukemia, 2014
Rumi, et al, Blood, 2014*
Emerging Value of Other Mutations as Prognostic Markers

- ASXL1, SRSF2, IDH1/2, and EZH2 mutations identified as “prognostically detrimental” markers, associated with shortened survival in PMF
- Only ASXL1 mutations remain significant independent of IPSS/DIPSS+
- High risk molecular signature:
  - ≥2 mutations (ASXL1, SRSF2, EZH2, IDH1/2)
  - CALR-ASXL1+
  - triple negative (JAK2-CALR-MPL-)
- CALR mutations favorably impact survival, independent of number of mutations, ASXL1 mutation, and IPSS/DIPSS+

Tenedini, et al, Leukemia, 2014

- NRAS (G12V and G12D) mutation- association with highest DIPSS+ score in MPN

Lundbery, et al, Blood, 2014

- TP53 somatic mutation with loss of heterozygosity -association with leukemic transformation
“For now, presence of these alterations in the absence of other poor prognostic features is not sufficient to support the indication of intensive therapies such as allo-SCT. They can be considered as a warning, recommending the patient’s closer monitoring to detect early changes indicating the need for a different therapeutic strategy. In summary, this new information will require validation and consolidation before its routine incorporation into decision-making.”

JAK2 Exon 12 Mutations

- JAK2 Exon 12 (Sanger sequencing)
  - 2-5% PV
  - ~0% PMF and ET
  - Less sensitive than V617F PCR (~20% vs <<1%)
    - Not an appropriate JAK2 screening assay
  - Use for suspected PVs with negative V617F
    - Erythrocytosis, low/normal serum erythropoietin, normal LDH, and JAK2 V617F negative by PCR
Molecular Landscaping Guides Appropriate Test Utilization in MPN
Common Test Utilization Issues in MPNs

- **JAK2 V617F** studies in concurrent blood and marrow
- **JAK2** exon 12 sequencing studies without **JAK2 V617F** studies
- Both **JAK2** exon 12 sequencing and **JAK2 V617F** studies ordered at the same time
- **JAK2 V617F**, **JAK2** exon 12 sequencing and **MPL** exon 10 sequencing ordered at the same time
- Multiple **BCR-ABL** assays in obvious classic MPN
- Flow cytometry requests in MPNs
- Mast cell or eosinophil studies (**KIT**, **PDGFR-A** or **PDGFR-B**, etc.) in classic MPNs
**JAK2 V617F Testing on PB & BM**

- All JAK2 V617F tests performed on MCR patients from 2006 to 2009 were reviewed (n=1624).
- 267 patients with concurrent PB and BM studies were identified

<table>
<thead>
<tr>
<th>JAK2 V617F</th>
<th>PB+</th>
<th>PB-</th>
</tr>
</thead>
<tbody>
<tr>
<td>BM+</td>
<td>137</td>
<td>2*</td>
</tr>
<tr>
<td>BM-</td>
<td>2*</td>
<td>126</td>
</tr>
</tbody>
</table>

- *Mutation burden at lower limit of detection
- *BM diagnoses in discrepant cases
  - 1 MDS/CMPN, unclassified
  - 3 normal bone marrow
- *No change in dx, management, outcome
- **Action:** cancel duplicate tests
JAK2 Exon 12 Sequencing Data (n = 92)

Test covers exons 12-15

- Mutation present (n=20)
  - 16 positive V617F
  - 4 with non-V617F mutation (all had PV lab features)*

- Failed study (n=3)
  - none with PV lab features

- No mutation (n=69)
  - 58 negative V617F
    - 19: PV lab features*
    - 39: other MPN and non-PV CBC
  - 4 V617F + by PCR
  - 7 had no V617F done
    - none with PV lab features

*Appropriate testing 23 of 92 cases (25%)
**MPL Exon 10 Sequencing: (n = 60)**

- **Mutation present (n=7)**
  - 7 negative V617F
    - 4 classic MPN*
    - 3 PB studies only*

- **No mutation (n=53)**
  - 10 positive V617F
  - 40 negative V617F
    - 16 normal BM or other disease processes
    - 5 classic MPN*
    - 12 borderline BMs*
    - 7 PB studies only*
  - 3 with no V617F done

*Appropriate testing 31 of 60 cases (52%)
Myeloproliferative Neoplasm: A Diagnostic Approach to Bone Marrow Evaluation

Clinical suspicion of myeloproliferative neoplasm

Bone marrow testing begins with:
- 70610 / Hematopathology Consultation, Wet Tissue
- BM / Cytogenetic Analysis, Hematologic Disorders, Bone Marrow
- JAK2 / JAK2 V617F Mutation Detection, Bone marrow
- BADX / BCR/ABL, mRNA Detection, Reverse Transcription-PCR (RT-PCR), Qualitative, Diagnostic Assay
- OR MBCR / BCR/ABL, Translocation t(9;22), FISH (D-FISH)

Negative for BCR/ABL

Bone marrow morphology: MPN?

NO

Erythrocytosis?

YES

REVP / Erythrocytosis Evaluation

YES

PV possible

JAKXM / JAK2 Exon 12 and Other Non-V617F Mutation Detection, Bone Marrow

POSITIVE

NEGATIVE

PV

NEGATIVE

Not supportive of PV

CALR / CALR Mutation Analysis, Myeloproliferative Neoplasm (MPN)

POSITIVE

NEGATIVE

MPLM / MPL Exon 10 Mutation Detection, Bone Marrow

POSITIVE

NEGATIVE

ET

PMF

MPN, not otherwise specified (ET or PMF)

P

E

F

M

POSITIVE

NEGATIVE

No further testing

Legend:
Pv: Polycythemia vera
E: Essential thrombocythemia
P: Myelofibrosis
M: Myeloproliferative neoplasm

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Initial Tests For Patients With Suspected MPNs

- Bone marrow aspirate and biopsy
- Chromosome analysis
- *BCR/ABL* Diagnostic PCR to quickly confirm or exclude CML (FISH can also be used)
- *JAK2 V617F* (either PB or BM; not both)*
- Then follow the algorithm
MPN Diagnostic Algorithm

Clinical Suspicion for MPN

BCR-ABL (PCR/FISH)

- **positive**
  - Diagnosis: Chronic Myelogenous Leukemia

- **negative**
  - Bone marrow morphology MPN?
    - **no**
      - Erythrocytosis?
        - **no**
          - STOP
        - **yes**
          - Follow erythrocytosis algorithm
    - **yes**
      - Continue in MPN algorithm
    - **equivocal**
      - Follow erythrocytosis algorithm
MPN Diagnostic Algorithm

- **Bone marrow morphology MPN+**
  - **Negative**
    - **JAK2 V617F**
      - **Positive**
        - Clinical CBC, LDH, EPO
      - **Negative**
        - **CBC, Serum Epo**
          - **PV possible**
            - **JAK2 exon 12 sequencing**
              - **Positive**
                - Diagnosis: PV
              - **Negative**
                - **Not PV**
                  - **CALR mutation analysis**
                    - **Positive**
                      - Diagnosis: PV
                    - **Negative**
                      - **MPL mutation analysis**
                        - **Positive**
                          - Diagnosis: PMF; ET; MPN, unclassified
                        - **Negative**
                          - Diagnosis: PV ET PMF MPN, unclassified
Bone marrow morphology **equivocal** for MPN

JAK2 V617F

- **Negative**
  - CALR mutation analysis
    - positive
    - Diagnosis: PV
    - ET
    - PMF
    - MPN, unclassifiable
    - STOP

- **Positive**
  - Clinical CBC
    - LDH
    - EPO
  - Diagnosis: PV
  - ET
  - PMF
  - MPN, unclassifiable
MPN Reflex Testing - Available in January 2015
Next Generation Sequencing - 35 Gene Panel

- Kit
- TP53
- RUNX1
- PHF6
- U2AF1
- TET2
- GATA2
- ETV6
- GATA1
- IDH2
- MYD88
- NPM1
- NRAS
- PTPN11
- SRSF2
- FLT3
- BRAF
- CEBPA
- EZH2
- JAK2
- ZRSR2
- CBL
- MPL
- IDH1
- SF3B1
- ASXL1
- SETBP1
- NOTCH1
- DNMT3A
- WT1
- KRAS
- CSF3R
- TERT
- CALR
- BCOR
Link to Algorithms

www.mayomedicallaboratories.com/articles/resources/index.html
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