The Revised WHO Classification of Myeloproliferative Neoplasms

Robert P Hasserjian, MD
Associate Professor
Massachusetts General Hospital and Harvard Medical School
Myeloproliferative neoplasms

• Clonal hematopoietic stem cell disorders
• “Overexuberant” production of one or more hematopoietic cell types
• Erythroid and granulocytic elements generally appear normal, without dysplasia
• Treated differently from other myeloid neoplasms
### Myeloproliferative neoplasms

(WHO 2016)

<table>
<thead>
<tr>
<th>Entity</th>
<th>Genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chronic myeloid leukemia, Ph+</td>
<td><strong>BCR-ABL</strong></td>
</tr>
<tr>
<td>Polycythemia vera</td>
<td><strong>JAK2</strong></td>
</tr>
<tr>
<td>Essential thrombocytopenia</td>
<td><strong>MPL</strong></td>
</tr>
<tr>
<td>Primary myelofibrosis</td>
<td><strong>CALR</strong></td>
</tr>
<tr>
<td><strong>Rare entities</strong></td>
<td></td>
</tr>
<tr>
<td>– Chronic neutrophilic leukemia</td>
<td><strong>CSF3R</strong></td>
</tr>
<tr>
<td>– Chronic eosinophilic leukemia/hypereosinophilic syndrome</td>
<td></td>
</tr>
<tr>
<td>– Myeloproliferative neoplasm, unclassifiable</td>
<td></td>
</tr>
</tbody>
</table>

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**Genetically defined eosinophilic neoplasms**

- **PDGFRA**
- **PDGFRB**
- **FGFR1**
- **PCM1-JAK2**
Diagnostic issues with MPN

• Distinguishing MPN from reactive conditions that can produce elevated counts
• Separating MPN from other myeloid neoplasms (MDS and MDS/MPN)
• Providing a specific diagnosis
  – Requires integration of clinical and molecular genetic data with morphology
  – Important in predicting prognosis and dictating therapy
• Recognizing signs of progression
CML

• Hematopoietic stem cell neoplasm associated with \textit{BCR-ABL1} fusion gene

• Patients present with neutrophilic leukocytosis with morphologically normal and maturing granulocytic elements

• Natural history is that of genetic instability, with progressive accumulation of blasts culminating in acute leukemia
The Philadelphia chromosome

BCR-ABL fusion proteins

- p190
- p210
- p230
CML peripheral blood
CML bone marrow aspirate
CML bone marrow biopsy
Natural course of CML

• Patients may survive many years with relatively few symptoms
• Inexorably progress to an acute leukemia with loss of differentiation
  – Termed ‘Blast crisis’ or ‘Blast phase’
  – Blast crisis phenotype
    • 70% myeloid (≥20% BM/PB myeloblasts)
    • 30% B-lymphoid (any BM/PB lymphoblasts raises strong suspicion)
CML in blast crisis
Tyrosine kinase inhibitors

CML in the 21st century

- Treated very effectively with tyrosine kinase inhibitors (TKI)
  - Imatinib, nilotinib, dasatinib, bosutinib, ponatinib
- Disease progression no longer inevitable
- Patterns of disease evolution are closely linked to responsiveness versus resistance to TKI therapy
Assessing response to TKI

<table>
<thead>
<tr>
<th>Time on therapy</th>
<th>Optimal response</th>
<th>“Warning”</th>
<th>Failure</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 months</td>
<td>Hematologic remission ≤35% Ph+ metaphases ≤10% BCR-ABL1</td>
<td>36-95% Ph+ metaphases or &gt;10% BCR-ABL1</td>
<td>No hematologic remission or &gt;95% Ph+ metaphases</td>
</tr>
<tr>
<td>6 months</td>
<td>No Ph+ metaphases* &lt;1% BCR-ABL1</td>
<td>1-35% Ph+ metaphases or 1-10% BCR-ABL1</td>
<td>&gt;35% Ph+ metaphases or &gt;10% BCR-ABL1</td>
</tr>
<tr>
<td>1 year</td>
<td>≤0.1% BCR-ABL1**</td>
<td>&gt;0.1-1% BCR-ABL1</td>
<td>Any Ph+ metaphases or &gt;1% BCR-ABL1</td>
</tr>
<tr>
<td>Later</td>
<td>≤0.1% BCR-ABL1**</td>
<td>Clonal chromosomal abnormalities in Ph- cells</td>
<td>Loss of hematologic remission New Ph+ metaphases Loss of major molecular response on 2 consecutive tests</td>
</tr>
</tbody>
</table>

*Lack of Ph+ metaphases is considered “complete cytogenetic response”

**≤0.1% BCR-ABL1 is considered “major molecular response”

CML accelerated phase (WHO 2016)

- Accelerated phase definition
  - BM or PB blasts 10-19%
  - Basophils ≥20%
  - Platelets >1000 x 10^9/L or <100 x 10^9/L
  - WBC >10 x 10^9/L
  - Persistent or increasing splenomegaly
  - Clonal evolution
  - Second Ph, +8, i(17q), +19, complex karyotype, or 3q26.2 abnormalities at diagnosis

- Provisional TKI-response criteria
  - Hematologic resistance to first TKI therapy
  - Hematologic, cytogenetic, or molecular evidence of resistance to 2 sequential TKI therapies
  - 2 or more ABL1 mutations developing during TKI therapy

~10% of CML patients present in accelerated or blast phase
Role of pathology in the current era of CML management

• At the time of initial diagnosis of CML
  – Get the diagnosis right!
  – Provide prognostic information
    • Accurate blast count in blood and marrow aspirate, reticulin fibrosis grade

• At later timepoints, assess for progression and evaluate for other pathologic processes while on therapy
Caveats with CML diagnosis

- Increased erythroid proliferation in bone marrow, especially in patients with hemoglobinopathies
- Prominent thrombocytosis mimicking ET
  - Associated with p230 BCR-ABL1
- Minimal or no myeloid left-shift in blood
- Monocytosis mimicking CMML
  - Associated with p190 BCR-ABL1
- Blast crisis mimicking AML or ALL
Erythroid-rich CML
# Differential diagnosis of neutrophilia

<table>
<thead>
<tr>
<th>Disease</th>
<th>Peripheral counts</th>
<th>Neutrophil morphology</th>
<th>Genetics</th>
</tr>
</thead>
<tbody>
<tr>
<td>CML, <em>BCR-ABL1</em>+</td>
<td>↑Granulocytes with left-shift</td>
<td>Normal</td>
<td>t(9;22); <em>BCR-ABL1</em></td>
</tr>
<tr>
<td></td>
<td>Eosinophilia</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Basophilia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Atypical CML, <em>BCR-ABL1</em>-</td>
<td>↑Granulocytes with left-shift</td>
<td>Dysplastic</td>
<td><em>SETBP1</em> or <em>ETNK1</em> mutation (30%)</td>
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<tr>
<td></td>
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</tr>
<tr>
<td>Chronic neutrophilic leukemia</td>
<td>↑Granulocytes without left-shift</td>
<td>Normal</td>
<td><em>CSF3R</em> mutation (90%)</td>
</tr>
<tr>
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</tr>
<tr>
<td>Primary myelofibrosis</td>
<td>Leukoerythroblastic</td>
<td>Normal</td>
<td><em>JAK2</em>, <em>MPL</em>, or <em>CALR</em> mutations (90%)</td>
</tr>
</tbody>
</table>

The pathologist has a critical role in evaluating smear and biopsy morphology and integrating these findings with clinical and genetic features to make the diagnosis!
Atypical CML, *BCR-ABL1*-  

- **Misnomer**  
  Atypical CML is a distinct MDS/MPN, not a variant of CML (which is a pure MPN)

- **Features mimic CML, except:**  
  - No *BCR-ABL* translocation by definition  
  - Prominent granulocytic dysplasia  
  - No or minimal basophilia

- **Poor prognosis, not helped by TKI**
Atypical CML, BCR-ABL1- bone marrow biopsy
Atypical CML, *BCR-ABL1*- peripheral smear
Atypical CML, BCR-ABL1-bone marrow aspirate
Chronic neutrophilic leukemia

• Rare MPN with leukocytosis (>25 x 10⁹/L)
  – No dysplasia (hypogranulation) of neutrophils
  – Splenomegaly
  – <10% immature myeloid cells in blood
  – No $BCR-ABL1$ rearrangement
  – No significant basophilia or eosinophilia

• 83-89% have $CSF3R$ mutation

Chronic neutrophilic leukemia: peripheral blood smear

WBC $36.7 \times 10^9$/L
HCT 40.0% (MCV 98 fL)
PLT $253 \times 10^9$/L

82% polys, 14% lymphs, 2% metas, 2% myelos
Chronic neutrophilic leukemia: peripheral blood smear
Chronic neutrophilic leukemia: bone marrow
Algorithm for workup of persistent neutrophilia

Possible reactive causes excluded?

- **Reactive neutrophilia**: Usually self-limited

- **BCR-ABL1 +**
  - Signiﬁcant granulocytic left-shift and dysplasia
  - **CML**
    - Treat with TKI immediately to prevent progression

- **BCR-ABL1 -**
  - JAK2 mutation and typical bone marrow ﬁndings
  - **Primary myelofibrosis**
    - Several treatment options
  - CSF3R mutation and no left-shift or dysplasia
    - **Chronic neutrophilic leukemia**
      - May respond to ruxolitinib

Pathologist has critical role in evaluating smear and biopsy morphology and to integrate these findings with clinical and genetic findings

Karyotype, FISH, and/or RT-PCR

Atypical CML, BCR-ABL1-

Poor prognosis, difﬁcult to treat
Eosinophilia

• Reactive
  – Allergy, drug, parasitic or other infections

• Paraneoplastic (non-neoplastic eosinophils stimulated by tumor cytokines)
  – Lymphomas, mastocytosis, rare solid tumors

• Eosinophils part of a myeloid neoplasm
  – CML
  – AML with inv(16)
  – Genetically-defined eosinophilias
  – Chronic eosinophilic leukemia, NOS
Myeloid/lymphoid neoplasms with eosinophilia and abnormalities of *PDGFRA, PDGFRB, FGFR1*, or *PCM1-JAK2*

- Share similar molecular and biologic features
  - Appear to involve pluripotent stem cell with both lymphoid and myeloid differentiation capacity
  - Translocations activate genes encoding tyrosine kinases
  - Eosinophilia is characteristic

- Most entities respond to targeted tyrosine kinase inhibitors
<table>
<thead>
<tr>
<th>Disease</th>
<th>Presentation</th>
<th>Genetics</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PDGFRA</strong></td>
<td>Eosinophilia</td>
<td>Cryptic deletion at 4q12 FIP1L1-PDGFRB or other partners</td>
<td>TKI</td>
</tr>
<tr>
<td></td>
<td>↑Serum tryptase</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>↑Mast cells</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>PDGFRB</strong></td>
<td>Eosinophilia Monocytosis</td>
<td>t(5;12)(q32;p12) ETV6-PDGFRB or other partners</td>
<td>TKI</td>
</tr>
<tr>
<td><strong>FGFR1</strong></td>
<td>Eosinophilia Often presents as T-ALL/LBL or AML</td>
<td>Translocations of 8p11 FGFR1 with various partners</td>
<td></td>
</tr>
<tr>
<td><strong>PCM1-JAK2</strong></td>
<td>Eosinophilia Left-shifted erythroïds</td>
<td>t(8;9)(p22;p24.1) PCM1-JAK2</td>
<td>JAK2 inhibitor</td>
</tr>
</tbody>
</table>
45 year old woman with leukocytosis. WBC 100 x 10^9/L  34% polys, 26% bands, 6% lymphs, 9% eos, 3% metas, 9% myelos.
Bone marrow biopsy
Karyotype of both bone marrow and lymph node:
46, XX, t(8;13)(p12;q12)(ZNF198-FGFR1)

Diagnosis:
Myeloid and lymphoid neoplasm with FGFR1 rearrangement
Myeloid neoplasms with t(8;9)(p22;q24); PCM1-JAK2

- Eosinophilia, erythroid predominance with left-shift, prominent lymphoid aggregates
- Fibrosis often present, mimicking PMF
- Can rarely present as T- or B-ALL
- Respond to JAK2 inhibitor ruxolitinib
- Added as a *provisional entity* to the group of genetically defined eosinophilic leukemias

Chronic eosinophilic leukemia (CEL), not otherwise specified

- Persistent blood eosinophilia >1,500/mm³ with increased bone marrow eosinophils and end-organ damage by eosinophils
- Exclusion of all reactive, paraneoplastic, and specific cytogenetic causes of eosinophilia
- Evidence of clonality
  - Clonal cytogenetic abnormality present
  - Increased bone marrow (>5%) or peripheral blood (>2%) blasts (but <20% blasts)
- Classified as hypereosinophilic syndrome if clonality cannot be proven
Chronic eosinophilic leukemia, NOS
Chronic eosinophilic leukemia, NOS
Algorithm for workup of persistent eosinophilia >1.5 x 10⁹/L

- Reactive eosinophilia
  - Abnormal T-cell clone
  - Other lymphoma with eosinophilia
  - Clonal eosinophilia due to CML or AML
  - Systemic mastocytosis

Screen for secondary causes of eosinophilia

- Evaluate peripheral blood & bone marrow
  - Cytogenetics
    - + Myeloid/lymphoid neoplasm with PDGFRA, PDGFRB, or FGFR1
      - CEL
      - - HES
    - - Other clonal abnormality or increased blasts

Courtesy of Tracy George, University of New Mexico
The JAK2-associated MPNs

• Essential thrombocythemia (ET)
  – Increased platelet count
  – Only rarely progress to a fibrotic phase or AML

• Polycythemia vera (PV)
  – Increased red cell production
  – May progress to a fibrotic phase or rarely AML
  – Can have thrombotic complications

• Primary myelofibrosis (PMF)
  – Often thrombocytosis at presentation
  – Progressive splenomegaly and marrow fibrosis
The spectrum of megakaryocyte morphology

MDS and CML

Myeloproliferative

Normal/Reactive
The non-CML MPN: Deregulation of the JAK/STAT pathway


[Diagram showing deregulation of JAK/STAT pathway with mentions of CSF3R, EPOR, TPOR, CALR, MPLW515L/K, JAK2V617F or JAK2 Exon 12, PI3K, Akt, mTOR, FoxO, Ras, Raf, MEK, ERK, Stat, SOCS1, SOCS3, Cytokines, Cyclin D1, FGFB, VEGF, and Mutant calreticulin.]
Distribution of mutation types in the non-CML MPN

Klampfl T et al. NEJM 2013;369:2379
Essential thrombocythemia (ET)
2016 WHO Criteria

• Platelet count \( \geq 450 \times 10^9/L \)
• Bone marrow biopsy showing typical morphology of ET and no or (rarely) minor increase in reticulin fibers.
• Does not meet WHO criteria for another myeloid neoplasm AND

  \[ \text{AND} \]

• Presence of \( JAK2 \), \( CALR \) or \( MPL \) mutation or other proof of clonality (cytogenetic abnormality or other mutation)*

*Must rigorously exclude reactive thrombocytosis if there is no proof of clonality
Other myeloid neoplasms that can present with thrombocytosis

- Other MPNs
  - Polycythemia vera
  - Primary myelofibrosis (early stages)
  - Chronic myeloid leukemia
- Myelodysplastic syndrome with isolated del(5q) or inv(3)/t(3;3)
- MDS/MPNs
  - Refractory anemia with ring sideroblasts and thrombocytosis (RARS-T)
Typical ET morphology

• Normocellular for age
• Increased megakaryocytes with minimal clustering
• Megakaryocytes are large forms
  – Predominantly ‘staghorn’ nuclei with complex lobation and abundant cytoplasm
• Reticulin is not increased
Essential thrombocytopenia
Essential thrombocythemia
Essential thrombocythemia
Polycythemia vera (PV) 
2016 WHO Criteria

• Evidence of increased red cell production

• Bone marrow showing typical PV histology
  – Hypercellular for age
  – Panmyelosis with increased erythroids and megakaryocytes
  – Spectrum of small, medium, and large megakaryocytes with bulbous and hyperlobated nuclei

  AND

• JAK2 mutation*

*Must have subnormal EPO level in the rare cases (<2%) lacking JAK2 mutation
Polycythemia vera
Polycythemia vera
“Masked” polycythemia vera

• Some patients have bone marrow findings typical of PV, but do not meet required 2008 WHO hemoglobin levels
  – Male ≥18.5 g/dL, Female ≥16.5 g/dL)

• These patients mimic ET in their clinical presentation (thrombocytosis), but have PV-like clinical behavior
  – Can progress to fibrotic phase of disease
  – Risk of thrombosis

Masked polycythemia vera

65 year-old woman
WBC 14.2 x 10^9/L
HGB 16 g/dL
PLT 744 x 10^9/L
Polycythemia vera (PV)
2016 WHO Criteria

• Male HGB > 16.5 g/dL or HCT > 49%, Female HGB > 16.0 g/dL or HCT > 48%

• Bone marrow showing typical PV histology
  – Hypercellular for age
  – Panmyelosis with increased erythroids and megakaryocytes
  – Spectrum of small, medium, and large megakaryocytes with bulbous and hyperlobated nuclei

AND

• JAK2 mutation*

*Must have subnormal EPO level in the rare cases (<2%) lacking JAK2 mutation
Primary myelofibrosis (PMF)
2016 WHO Criteria

• Bone marrow showing typical PMF histology
  – Hypercellular for age
  – Increased M:E ratio, atypical megakaryocytes

• Presence of \textit{JAK2}, \textit{CALR} or \textit{MPL} mutation or other proof of clonality (cytogenetic abnormality or other mutation)*

• Anemia, WBC $\geq 11 \times 10^9$/L, splenomegaly, increased LDH, or leukoerythroblasticosis

*Must exclude possible reactive causes of marrow fibrosis if there is no clonal marker
Stages of Primary Myelofibrosis

Evolution → Manifestation → Transformation

Initial stage

Reticulin → Collagen fibrosis → Osteosclerosis

PMF blast phase
BM - insufficiency

Spleen size (cm)
Erythro-/myeloblasts (%)
Haemoglobin (g/dL)
LDH (U/L)
Thrombocytes (x10^9/L)

Grade of myelofibrosis

MF-0 MF-1 MF-2 MF-3
Prefibrotic-early PMF Advanced PMF (MMM)

WHO Classification of Tumors of Hematopoietic and Lymphoid Tissues; 4th edition
Early/prefibrotic primary myelofibrosis
<table>
<thead>
<tr>
<th><strong>ET</strong></th>
<th><strong>PMF (early-prefibrotic stage)</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>- no or only slight increase in age-matched cellularity</td>
<td>- marked increase in age-matched cellularity</td>
</tr>
<tr>
<td>- no significant increase in granulo- and erythropoiesis</td>
<td>- pronounced proliferation of granulopoiesis and reduction of erythroid precursors</td>
</tr>
<tr>
<td>- prominent large to giant mature megakaryocytes with hyperlobulated or deeply folded nuclei, dispersed or loosely clustered in the marrow space</td>
<td>- dense or loose clustering and frequent endosteal translocation of medium sized to giant megakaryocytes showing hyperchromatic, hypolobulated, bulbous, or irregularly folded nuclei and an aberrant nuclear/cytoplasmic ratio</td>
</tr>
<tr>
<td>- no or very rarely minor increase in reticulin fibers</td>
<td>- no or no significant increase in reticulin fibers</td>
</tr>
</tbody>
</table>

![Diagram showing megakaryopoiesis, granulopoiesis, erythropoiesis, and reticulin fibers.](image_url)

Courtesy of Hans-Michael Kvasnicka, University Hospital, Frankfurt
Primary myelofibrosis, fibrotic phase
Primary myelofibrosis, fibrotic phase
Advanced fibrosis on trichrome stain
Survival in the non-CML MPN (n=826)

Tefferi et al. Blood 2014;124:2507-2513

Expected survival

ET vs Expected p < 0.001

Median survival = 19.8 yrs

PV vs PMF p < 0.001

Median survival = 5.9 yrs

ET vs PV vs PMF p < 0.001

Median survival = 13.7 yrs

Number at Risk

292

267

207

153

85

34

11

7

37

1
Importance of accurate diagnosis of MPN to inform prognosis and guide therapy

<table>
<thead>
<tr>
<th></th>
<th>PV</th>
<th>ET</th>
<th>PMF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukemic transformation</td>
<td>3% at 10 years</td>
<td>1% at 10 years</td>
<td>12-30% at 10 years</td>
</tr>
<tr>
<td>Fibrosis progression</td>
<td>15-25%</td>
<td>Rare</td>
<td>100%</td>
</tr>
<tr>
<td>Thrombosis, per 100 patients/year</td>
<td>5.5</td>
<td>1-3</td>
<td>2</td>
</tr>
<tr>
<td>Initial treatment</td>
<td>Phlebotomy +/- HU</td>
<td>None, aspirin +/- HU</td>
<td>Allo-SCT, JAK inhibitors, chemotherapy</td>
</tr>
</tbody>
</table>

Courtesy of Olga Pozdnyakova, BWH
Summary

• Myeloproliferative neoplasms have distinctive morphologies and distinctive genetic aberrations
  – Important to correctly diagnose the various MPN diseases, which have different patterns of progression and are treated differently
  – Pathologists must utilize a combination of information from morphology, clinical features, and cytogenetics/molecular genetics to
• Eosinophilic myeloid disorders are characterized by recurrent genetic abnormalities and some are amenable to targeted therapies with TKIs