Author(s): Dale Bixby, M.D., Ph.D., 2009

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Chronic Myeloid Leukemia and other Myeloproliferative Neoplasms (MPNs)

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Department of Internal Medicine
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Winter 2010
Definitions

Myeloproliferative Neoplasms (MPNs): are a group of clonal myeloid neoplasms in which a genetic alteration occurs in a hematopoietic progenitor cell leading to its proliferation resulting in an increase in the peripheral blood white blood cells (WBCs), red blood cells (RBCs), platelets, or a combination of these cells.
Hematopoietic Progenitors and MPNs

Genetic Mutation

Blood stem cell

Myeloid stem cell

Myeloblast

Platelets

Lymphoid stem cell

Lymphoblast

Red blood cells

White blood cells

National Cancer Institute
The type of disorder is often based on the predominant cell line that is affected, but because blood counts are often abnormal in more than one cell line, diagnoses based upon blood counts alone may be inaccurate.

<table>
<thead>
<tr>
<th>Four Main MPNs:</th>
<th>Additional MPNs:</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Chronic Myelogenous Leukemia (CML)</td>
<td>1. Systemic Mastocytosis</td>
</tr>
<tr>
<td>2. Polycythemia Vera (PV)</td>
<td>2. Hypereosinophilic Syndrome</td>
</tr>
<tr>
<td>3. Essential Thrombocytosis (ET)</td>
<td>3. Chronic Myelomonocytic Leukemia</td>
</tr>
<tr>
<td>4. Primary Myelofibrosis (PMF)</td>
<td>4. Chronic Neutrophilic Leukemia</td>
</tr>
<tr>
<td></td>
<td>5. Chronic Eosinophilic Leukemia</td>
</tr>
</tbody>
</table>
In CML, the predominant feature is a leukocytosis with a left shift. A mild anemia, normal to elevated platelet count, and a peripheral blood basophilia is often seen.

In PV, the predominant features are elevated red blood cell indices (RBC count, hemoglobin, and hematocrit). Patients often also have a mild leukocytosis and thrombocytosis.

In ET, the predominant feature is an elevated platelet count. Patients also often have a mild leukocytosis and polycythemia.

In PMF, the predominant feature is evidence of extramedullary hematopoiesis in the form of hepatomegaly, splenomegaly, and lymphadenopathy. Patients often have a mild anemia, but their WBC and platelet counts can be quite variable. Leukoerythroblastosis (tear drops, nucleated RBCs and early myeloid progenitors (including blasts) are often seen in the peripheral blood.
Clonal Genetic Abnormalities Define Many MPNs


Chronic Myeloid Leukemia (CML)
Epidemiology of CML

Approximately 5,050 cases in the U.S. in 2009 (11% of all leukemias) with an incidence that increases significantly with age (median age ~ 55)

Risk Factors include:
- prior high dose radiation exposure (WW II / Chernobyl / etc…)
- exposure to certain organic solvents (benzene)
- age
- gender (male > female)

A very small percentage (< 0.1%) of individuals can express Bcr-Abl but not develop CML (wrong cell of origin, multiple genetic mutations leading to non-viability, immune surveillance)
CML – Pathophysiology –
the Philadelphia Chromosome
The Philadelphia chromosome results when a piece of chromosome #9 switches places with a piece of chromosome #22. The translocation forms an extra-long chromosome #9 (called der 9) and an extra-short chromosome #22, which is the Philadelphia chromosome that contains the abnormal, fused BCR ABL gene.
Multiple Breakpoints in Bcr-Abl
Bcr-Abl expression **alone** is necessary and sufficient for the development of CML.
Chronic Myeloid Leukemia
Clinical Presentation

- Asymptomatic (~30%)

- Fatigue, weight loss, fever

- Abdominal fullness, pain and/or early satiety due to splenomegaly (~50-90%)

- Easy bruising and purpura

- Leukostasis
  - Pulmonary symptoms
  - Neurologic symptoms
Peripheral smear can only give a presumptive diagnosis of CML [you need to confirm the t(9;22)]:
1) leukocytosis with a ‘left shift’
2) normocytic anemia
3) thrombocytosis in 50% of pts
4) absolute eosinophilia with a normal % of Eos.
5) absolute and relative increase in basophils
6) LAP score is low (not frequently employed)
Diagnosing Chronic Myeloid Leukemia
Diagnostic Considerations in Chronic Myeloid Leukemia

Karyotyping in CML

1) Allows for the diagnosis of CML
2) Requires a bone marrow aspirate for optimal metaphases
3) Allows for evaluation of clonal evolution as well as additional chromosomal abnormalities in the non-Ph+ clones
4) Occasional cryptic and complex karyotypes can result in the missed identification of the t(9;22)

Demonstrating the presence of the t(9;22) or its gene product is absolutely essential in diagnosing a patient with CML.
Diagnostic Considerations in Chronic Myeloid Leukemia

**Fluorescence in-situ hybridization (FISH) in CML:**

1. Allows for the diagnosis of CML
2. Does not require a bone marrow aspirate for optimal results
3. Allows for the identification of potential duplications of the Ph chromosome
4. Allows for the identification of the loss of the der (9) chromosome
5. Allows for the identification of cryptic translocations involving Bcr-Abl

![Fluorescence in-situ hybridization image](image-url)
FISH in CML

Red → Bcr probe
Green → Abl Probe
Yellow → fusion of Bcr and Abl
Diagnostic Considerations in Chronic Myeloid Leukemia

Quantitative RT-PCR for Bcr-Abl in CML

1) Allows for the diagnosis of CML
2) Does not require a bone marrow aspirate for optimal results
3) Can quantify the amount of disease
4) Allows for the identification of cryptic translocations involving Bcr-Abl
5) Many primers sets only detect the p190 and/or the p210 translocation and may miss the p230 or alternative translocations
Quantitative RT-PCR for Bcr-Abl in CML

Amount of Fluorescence

PCR Cycle Number

High Concentration
Moderate Concentration
Low Concentration
# Disease Diagnosis and Monitoring in CML

<table>
<thead>
<tr>
<th>Test</th>
<th>Target</th>
<th>Tissue</th>
<th>Sensitivity (%)*</th>
<th>Use</th>
</tr>
</thead>
</table>
| Cytogenetics | Ph chromosome          | BM     | 1-10             | ▪ Confirm diagnosis of CML  
▪ Evaluate karyotypic abnormalities other than Ph chromosome (ie, clonal evolution) |
| FISH   | Juxtaposition of \( \text{bcr} \) and \( \text{abl} \) | PB/BM  | 0.5-5            | ▪ Confirm diagnosis of CML  
▪ Routine monitoring of cytogenetic response in clinically stable patients  
▪ Routine measurement of MRD |
| RT-PCR | \( \text{bcr-abl} \) mRNA | PB/BM  | 0.0001-0.001    | ▪ Routine measurement of MRD  
▪ Determine the breakpoints of the fusion genes |

*Number of leukemic cells detectable per 100 cells.

BM = bone marrow; FISH = fluorescence in situ hybridization; PB = peripheral blood; MRD = minimal residual disease; RT-PCR = reverse transcriptase polymerase chain reaction.

**Chronic Myeloid Leukemia - Diagnostic Criteria for the 3 Phases of the Disease**

**CML, chronic phase (CP-CML)**
- A myeloproliferative disorder caused by the balanced translocation between the long arms of chromosome 9 and chromosome 22: (9:22)(q34;q11)
- Not meeting criteria for accelerated or blastic phase

**CML, accelerated phase (AP-CML)**
- Diagnose if one or more of the following is present:
  - Blasts 10% to 19% of peripheral blood white cells or bone marrow cells
  - Peripheral blood basophils at least 20%
  - Persistent thrombocytopenia (<100 X 10^9/L) unrelated to therapy, or persistent thrombocytosis (>1000 X 10^9/L) unresponsive to therapy
  - Increasing spleen size and increasing WBC count unresponsive to therapy
  - Cytogenetic evidence of clonal evolution (i.e., the appearance of an additional genetic abnormality that was not present in the initial specimen at the time of diagnosis of chronic phase CML)
  - Megakaryocytic proliferation in sizable sheets and clusters, associated with marked reticulin or collagen fibrosis, and/or severe granulocytic dysplasia, should be considered as suggestive of CML-AP. These findings have not yet been analyzed in large clinical studies, however, so it is not clear if they are independent criteria for accelerated phase.

**CML, blast phase (BP-CML)**
- Diagnose if one or more of the following is present:
  - Blasts 20% or more of peripheral blood white cells or bone marrow cells
  - Extramedullary blast proliferation
  - Large foci or clusters of blasts in bone marrow biopsy
Therapeutic Options in Chronic Myeloid Leukemia
History of CP-CML Therapies

- Interferon – α +/- AraC
- Early Interferon – α trials
- Intensive chemotherapy
- Hydrea, or radiation therapy or Busulphan

Imatinib (Gleevec, Novartis)  
a small molecule tyrosine kinase inhibitor
Frontline Therapy in Chronic Phase - Chronic Myeloid Leukemia


## Treatment Milestones for CML

### Definitions of Responses to Treatments

**Hematologic Response**
- Complete Hematologic response
  1) Normal PB counts (WBC < 10 and plt < 450)
  2) Normal WBC differential
  3) No Dz symptoms
  4) Normalization of the size of the liver and spleen

**Cytogenetic Responses: Ph\(^+\) Metaphases**
- 1) complete: 0%
- 2) partial: 1% - 35%
- 3) minor: 36% - 65%
- 4) minimal: 66% - 95%
- 5) none: 96% - 100%

**Molecular Responses: ratio of Bcr-Abl/Abl**
- Major Molecular Response
  3-log\(_{10}\) reduction from initial diagnosis sample (i.e. 25 \(\rightarrow\)0.025)
Imatinib has Revolutionized the Treatment of CML – IRIS Trial

1. Newly diagnosed CML patients were randomized to receive either Imatinib 400 mg daily or Interferon-α at approximately $5 \times 10^6$ U/day as well as Ara-C 20 mg/m² d1-10 q 8 days. Graph shows outcomes of 553 pts randomized to Imatinib.

# 2009 ELN Recommendations for Response Assessment for Treatment

<table>
<thead>
<tr>
<th>Evaluation Time, Months</th>
<th>Optimal</th>
<th>Suboptimal</th>
<th>Failure</th>
<th>Warnings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>High risk; CCA/Ph +</td>
</tr>
<tr>
<td>3</td>
<td>CHR and at least minor CgR (Ph+ ≥ 65%)</td>
<td>No CgR (Ph+ &gt; 95%)</td>
<td>Less than CHR</td>
<td>NA</td>
</tr>
<tr>
<td>8</td>
<td>At least PCgR (Ph+ ≤ 35%)</td>
<td>Less than PCgR (Ph+ &gt; 35%)</td>
<td>No CgR (Ph− &gt; 95%)</td>
<td>NA</td>
</tr>
<tr>
<td>12</td>
<td>CCgR</td>
<td>PCgR (Ph− 1% to 35%)</td>
<td>Less than CCA/Ph+</td>
<td>NA</td>
</tr>
<tr>
<td>18</td>
<td>MMdrR†</td>
<td>Less than MMdrR–</td>
<td>Less than CgR</td>
<td>NA</td>
</tr>
<tr>
<td>Any time during treatment</td>
<td>Stable or improving MMdrR†</td>
<td>Loss of MMdrR†; mutations†</td>
<td>Loss of CHR; loss of CCgR; mutations†; CCA/Ph+</td>
<td>Increase in transcript levels; CCA/Ph−</td>
</tr>
</tbody>
</table>

Mechanisms of Imatinib Resistance

**Primary resistance**
- failure to achieve preset hematologic and/or cytogenetic milestones
  - IRIS data indicates a rate of ~ 15% by failing to achieve a PCyR at 12 months and 24% by failing to achieve a CCyr by 18 months of therapy.
  - rates higher in accelerated and blast phase disease

**Secondary resistance**
- loss of a previously achieved hematologic or cytogenetic milestone
  - rates may be 10-15% on Imatinib, but become rarer as time on therapy progresses
  - rates higher in accelerated and blast phase disease

**Resistance Mechanisms**

1) **Bcr-Abl Kinase mutations**
   - > 50 known mutations within Abl sequence which inhibits Imatinib from binding
   - mutations identified in 30-80% of individuals with resistant disease

2) **Bcr-Abl duplication**
   - duplication of the *Bcr-Abl* sequence has been identified in cell lines with Im resistance

3) **Pgp over-expression**
   - export pump of many chemotherapeutics leading to lower intracellular Im concentration

4) **hOct-1 under-expression**
   - import pump for Im which may lead to lower intracellular levels of IM

5) **Src-Family kinase (SFK) expression**
   - activation may circumnavigate the Bcr-Abl ‘addiction’ of the transformed cell

<table>
<thead>
<tr>
<th></th>
<th>Parental</th>
<th>38.31</th>
<th>10.78</th>
<th>&gt; 50</th>
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<tr>
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<td>Q252H</td>
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<td>0.81</td>
<td>1.39</td>
<td>3.05</td>
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<td>Y253F</td>
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<td>0.96</td>
<td>3.58</td>
<td>1.58</td>
<td>3.23</td>
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<tr>
<td>E255K</td>
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<td>6.02</td>
<td>5.61</td>
<td>6.69</td>
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<td>D276G</td>
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<td>0.60</td>
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<td>1.64</td>
<td>2.05</td>
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<tr>
<td>V299L</td>
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<td>8.65</td>
<td>1.34</td>
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<tr>
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<td>75.03</td>
<td>39.41</td>
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<td>4.46</td>
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<td>0.93</td>
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<td>L384M</td>
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<td>1.07</td>
<td>2.41</td>
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<tr>
<td>H396R</td>
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<td>3.91</td>
<td>1.63</td>
<td>3.10</td>
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<td>G398R</td>
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<td>1.16</td>
<td>0.35</td>
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<td>0.49</td>
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<tr>
<td>F486S</td>
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<td>2.31</td>
<td>8.10</td>
<td>3.04</td>
<td>1.85</td>
</tr>
</tbody>
</table>

Sensitive: ≤ 2
Imatinib Poorly Control Advanced Phase Disease

Treatment Options for Resistant Disease

1) Dose Escalation of imatinib
2) Second Generation TKIs
3) Bone Marrow Transplant
4) Clinical Trial Participation
Dose Escalation of imatinib
START-R Trial

Patients resistant to 400mg-600 mg of imatinib were treated with either 70 mg BID of dasatinib or 800 mg of imatinib

▪ primary endpoint of the trial was the rate of MCyR at 12 weeks and this was equal (D=36%; IM=29%; p=.40)

▪ At a minimum follow-up of 2 years, dasatinib demonstrated higher rates of:
  ▪ complete hematologic response (93% vs 82%; \( P = .034 \))
  ▪ major cytogenetic response (MCyR) (53% vs 33%; \( P = .017 \))
  ▪ complete cytogenetic response (44% vs 18%; \( P = .0025 \))

The depth of the previous response to imatinib may be associated with the proportion of patients responding to dose escalation. Patients having achieved a prior major cytogenetic response (MCyR) with imatinib reported a greater than 50% chance of re-achieving that response with high-dose imatinib, yet only 7% of patients who did not achieve any cytogenetic response on standard dose imatinib were able to achieve a MCyR.

Second Generation
Tyrosine Kinase Inhibitors (TKIs)

The FDA has approved 2 additional oral TKIs for the treatment of imatinib relapsed/refractory or imatinib intolerant CML

<table>
<thead>
<tr>
<th>dasatinib (Sprycel – BMS)</th>
<th>nilotinib (Tasigna – Novartis)</th>
</tr>
</thead>
<tbody>
<tr>
<td>oral multi-kinase inhibitor</td>
<td>oral multi-kinase inhibitor</td>
</tr>
<tr>
<td>~ 325 times more potent than IM</td>
<td>~ 30 times more potent than IM</td>
</tr>
<tr>
<td>active against the ‘open’ and ‘closed’ confirmation of Bcr-Abl</td>
<td>active against only the closed confirmation of Bcr-Abl</td>
</tr>
<tr>
<td>active against many of the identified kinase domain (KD) mutations</td>
<td>active against many of the KD mutations</td>
</tr>
<tr>
<td>active against the SFKs</td>
<td>not active against the SKFs</td>
</tr>
<tr>
<td>may not be a substraight for Pgp or hOct-1</td>
<td>may not be a substraight for hOct-1</td>
</tr>
</tbody>
</table>
Bone Marrow Transplantation

Allogeneic bone marrow transplant remains the only known curative option in CML

Associated with an increased morbidity and mortality (TRM -10%-30%)

Therefore, not typically applied for upfront therapy for CML
  - considered only in cases of matched-related Txp for extremely young pts (pediatrics)

However, often considered in those with relapsed/refractory disease to TKI based therapies
  - efficacy of the transplant dependent upon the phase of the disease at the time of the transplant: CP>AP>BP
Clinical Trial Options in CML

<table>
<thead>
<tr>
<th>Clinical Trial</th>
<th>Sponsor</th>
<th>Indications</th>
<th>Targets</th>
</tr>
</thead>
<tbody>
<tr>
<td>NS-107</td>
<td>CytRx</td>
<td>CML, Ph- ALL</td>
<td>ABL, KIT, LYN, PDGFR</td>
</tr>
<tr>
<td>AP215349</td>
<td>Arad</td>
<td>CML advanced hematologic malignancies</td>
<td>ABL, FGFR1, FLT3, KIT, VEGFR</td>
</tr>
<tr>
<td>XL225</td>
<td>Exelixis</td>
<td>CML, Ph+ ALL, lymphoma, myeloma, solid tumors</td>
<td>ABL, Aurora A, FGFR1, SRC</td>
</tr>
<tr>
<td>AT3283</td>
<td>Astex Therapeutics</td>
<td>CML, AML, ALL, MDS, myelofibrosis, NHL, solid cancers</td>
<td>ABL, Aurora A&amp;B, FLT3, JAK2, JAK3</td>
</tr>
<tr>
<td>PHA986358*</td>
<td>Nerviano Medical Sciences</td>
<td>CML, myeloma, prostate</td>
<td>ABL, Aurora A&amp;B, FGFR1, RET, THK</td>
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<tr>
<td>KW-2449*</td>
<td>Kyowa Hakko Kirin Pharma</td>
<td>CML, AML</td>
<td>ABL, Aurora A, FGFR1, FLT3</td>
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<tr>
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<td>Merck</td>
<td>CML, ALL, MDS</td>
<td>ABL, Aurora A&amp;B, FLT3, JAK2</td>
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<tr>
<td>Horcharringtonine (HHT)*</td>
<td>ChemGenex</td>
<td>CML</td>
<td>cytochrome C, MCL-1</td>
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<tr>
<td>DCC2036*</td>
<td>Dociphora</td>
<td>CML, Ph+ ALL</td>
<td>ABL, FLT3, KDR, SFK, TIE2</td>
</tr>
</tbody>
</table>

INNO 406
- Phase III
- NCT0352677 (c)

INNO 406 Phase I
- NCT00464113

INNO 406 Phase II
- NCT0022990

INNO 406 Phase III
- NCT00466868
- NCT00979480

AEL indicates Bcr-Abl (Abelson) kinase; SFK, Src family kinase; KIT, CD117; TEC, Tec protein kinase; STE20, serine/threonine 20 kinase; CAMK2G, calcium/calmodulin-dependent protein kinase (CaM kinase) II gamma kinase; PDGFR, platelet-derived growth factor receptor; FGFR1, fibroblast growth factor receptor 1; IGFIR, insulin like growth factor receptor; JAK, Janus kinase; FLT3, fms-like tyrosine kinase.
Polycythemia Vera (PV)
Polycythemia

A hematocrit greater than 48% (♀) or 52% (♂) constitutes polycythemia

Likewise, a hemoglobin of >16.5 g/dL (♀) or >18.5 g/dL (♂) raises the suspicion for polycythemia

**Absolute polycythemia** is characterized by an increase in red blood cell (RBC) mass
- Five common causes include: 1) primary polycythemia, 2) hypoxia, 3) carboxyhemoglobinemia, 4) cushing’s syndrome or corticosteroids, and 5) erythropoietin-secreting tumors

**Relative polycythemia** is characterized by a decrease in plasma volume.
Two common causes:
- Dehydration (e.g., from vomiting, diarrhea, excessive sweating, or diuretics) can deplete plasma volume, leading to a relative polycythemia.
- Stress erythrocytosis (Gaisböck’s polycythemia) actually results from contraction of the plasma volume and is therefore a misnomer. This benign disorder is seen most often in hypertensive, obese men.

Red Blood Cell Mass Assay:
- used to distinguish an absolute versus a relative polycythemia
- does not subclassify absolute polycythemias
Clinical Presentation of Primary PV

**Symptoms:**
- non-specific complaints: headache, weakness, dizziness, and excessive sweating
- pruritus, especially following a warm bath or shower
- erythromelalgia, or burning pain in the feet or hands accompanied by erythema, pallor, or cyanosis
- symptoms related to either an arterial or venous thrombosis (CVA, MI, DVT, Budd Chiari syndrome or other portal venous thrombosis)

**Signs:**
- facial plethora (ruddy cyanosis)
- splenomegaly
- hepatomegaly
- gouty arthritis and tophi
Diagnostic Criteria for Primary PV

**Polycythemia Vera Study Group (PVSG) Criteria for PV**

<table>
<thead>
<tr>
<th>Major Criteria</th>
<th>Minor Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elevated RBC mass</td>
<td>Plt count &gt; 400,000</td>
</tr>
<tr>
<td>&gt;36 cc/kg in men</td>
<td>WBC &gt; 12,000</td>
</tr>
<tr>
<td>&gt;32 cc/kg in women</td>
<td>Elevated LAP score (&gt;100)</td>
</tr>
<tr>
<td>Oxygen saturation &gt;92%</td>
<td>Serum vitamin B12 &gt;900 pg/mL or serum</td>
</tr>
<tr>
<td>Splenomegaly</td>
<td>unbound B12 binding capacity &gt;2,200 pg/mL</td>
</tr>
</tbody>
</table>

→ All 3 major criteria OR the first 2 major and any 2 minor criteria ←

**2008 WHO Diagnostic Criteria for Primary Polycythemia Vera**

<table>
<thead>
<tr>
<th>Major Criteria</th>
<th>Minor Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) Hgb &gt; 18.5 g/dl (♂) or 16.5 g/dl (♀) or Hgb or Hct &gt; 99%</td>
<td>1) Bone marrow trilineage expansion</td>
</tr>
<tr>
<td>or Hgb or Hct &gt; 99%</td>
<td>2) Subnormal EPO level</td>
</tr>
<tr>
<td>or Hgb &gt; 17 g/dl (♂) or 15 g/dl (♀) and a documented increase of 2 g/dl</td>
<td>3) Endogenous erythroid colony growth</td>
</tr>
<tr>
<td>or RBC mass &gt; 25% of mean normal</td>
<td></td>
</tr>
<tr>
<td>2) Presence of a JAK2 V617F or similar mutation</td>
<td></td>
</tr>
</tbody>
</table>

→ two major or first major and two minor criteria ←

Tefferi et al. Leukemia (2008) 22, 14–22
JAK2 Mutations Seen in Three Different MPNs

See online at: http://img.medscape.com/fullsize/migrated/563/885/nrc563885.fig1.gif

JAK2 Mutations and MPNs

- Receptor Tyrosine Kinase - maps to chromosome 9p

- Valine to phenylalanine substitution at amino acid 617 (V617F) in pseudokinase domain of JAK2 allows for the constitutive activation of the receptor

- Somatic acquired mutation

- High incidence in PCV (~95%)
  - Not present in every patient with PCV

- Lower incidence in ET (~50%) and PMF (~50%)
Outcomes and Treatment of PV

Survival outcomes in PV are affected by:
1) hyperviscosity and associated ischemic sequela
2) thromboses independent of hyperviscosity
3) transformation to myelofibrosis or acute myeloid leukemia (~3%-10%)

Therapeutic Options in PV:
1) Low Risk: phlebotomy (to an Hct of <45 in ♂ and <42 in ♀) + low dose aspirin (81 mg daily) – decreases risk of thrombosis
2) High Risk: phlebotomy + ASA + hydroxyurea

High Risk for Thrombosis:
- age over 70
- prior thrombosis
- platelet count >1,500,000/µl
- presence of cardiovascular risk factors
A number of inhibitors of the JAK2 kinase have been developed and inhibit the proliferation and survival of JAK2 V617F transformed cell lines in-vitro.

Clinical studies (Phase I and Phase II) have been initiated and demonstrate some symptomatic improvement as well as improvement in splenomegaly in a number of patients, but unlike CML, the percentage of JAK2⁺ progenitor cells have not been significantly altered. However, a large number of trials continue at this time.

Leads to speculation that JAK2 may not be sufficient for the development of MPNs and there may be an earlier genetic mutation that is driving the phenotype.
Essential Thrombocythemia (ET)
Thrombocytosis

**Definition:** thrombocytosis is defined as a platelet count > 450,000 cells/µL

**Etiology of Thrombocytosis**

- **Primary** - if the thrombocytosis is caused by a myeloproliferative neoplasm, the platelets are frequently abnormal and the patient may be prone to both bleeding and clotting events.

- **Secondary** - if thrombocytosis is secondary to another disorder (reactive), even patients with extremely high platelet counts (e.g., > 1,000,000 cells/µl) are usually asymptomatic.

**Differential Diagnosis of secondary thrombocytosis:**

1. Malignancies
2. Infections and inflammatory disorders (e.g., Crohn’s disease)
3. Post surgical status
4. Connective tissue disorders
5. Iron deficiency anemia
6. Splenectomy
7. Recovery of the bone marrow from a stress (chemotherapy or alcohol)
8. Essential Thrombocythemia
Clinical Presentation of Essential Thrombocythemia (ET)

Asymptomatic (~ 30-50%)

Vasomotor symptoms including headache, syncope, atypical chest pain, acral paresthesia, livedo reticularis, and erythromelalgia

Thrombosis and hemorrhage occur to various degrees in 5%-25% of patients

Early satiety and abdominal bloating due to splenomegaly

JAK 2⁺ (V617F) in approximately 50% of patients
Diagnostic Criteria for ET

2008 WHO Diagnostic Criteria for Essential Thrombocytosis

1. Platelet count > 450,000
2. Megakaryocytic proliferation with large, mature morphology and with little granulocytic or erythroid expansion
3. Not meeting WHO criteria for CML, PV, PMF, MDS or other myeloid neoplasm
4. Demonstration of the JAK2V617F or other clonal marker or lack of evidence of a secondary (reactive thrombocytosis)

Diagnosis of essential thrombocytemia requires meeting all four major criteria

Teferri et al. Leukemia (2008) 22, 14–22
Outcomes in ET

Most patients with ET enjoy a normal life expectancy.

Like PV, the major risks are secondary to thrombosis and disease transformation:
- 15-year cumulative risks:
  - thrombosis - 17% risk
  - clonal evolution into either myelofibrosis (4%) or AML (2%)

High risk for thrombosis:
- age ≥ 60
- prior thrombosis
- long-term exposure to a plt count of > 1,000,000
Treatment of ET

**Low Risk:**
- Age <60 years
- No previous history of thrombosis
- Platelet count <1 million/µl
  → aspirin (81 mg daily) if vasomotor Sx or other medical need for ASA
  → if otherwise low risk and plt >1.5 X 10^6, screen for an acquired von Willebrand disease before instituting ASA

**High Risk:**
- Age ≥60 years
- A previous history of thrombosis
  → hydroxyurea + aspirin (81 mg daily)
  → if plt >1.5 X 10^6, screen for an acquired von Willebrand disease before instituting ASA
  → anagrelide is an option, but when c/w hydroxyurea, it was assn with an increased risk of arterial thrombosis, venous thrombosis, serious hemorrhage, or death from vascular causes
Primary Myelofibrosis (PMF)
Primary Myelofibrosis
(Chronic Idiopathic Myelofibrosis)

**Signs and Symptoms:**
- asymptomatic (15% - 30%)
- severe fatigue
- splenomegaly
- hepatomegaly
- fever and night sweats
- signs or symptoms of anemia or thrombocytopenia
- foci of extramedullary hematopoiesis may occur in almost any organ
- bone or joint involvement

**CBC Findings:**
- anemia (hgb<10 in 50% of pts); anisocytosis, poikilocytosis, teardrop-shaped red blood cells (dacrocytes), and nucleated red blood cells
- leukoerythroblastosis (increased presence of immature myeloid cells and nucleated erythrocytes in the circulating blood.
- WBC and Plt counts are variable (ranging from low to high) with increased circulating CD34+ precursor cells
- BM Biopsy shows increased fibrosis (reticulin fibers or mature collagen)
- JAK2+ (V617F) in approximately 50% of cases
Diagnostic Criteria for PMF

2008 WHO Diagnostic Criteria for Primary Myelofibrosis

Major:
1. Megakaryocytic proliferation and atypia with either reticulin or collagen fibrosis
   or
   If no fibrosis, mekakaryocytic expansion must be assn. w/ increased BM cellularity
2. Does not meet WHO criteria for CML, PV, MDS, or other myeloid neoplasm
3. Demonstration of the JAK2 V617F mutation or other clone marker
   or
   no other evidence of a reactive marrow fibrosis

Minor:
1. Leukoerythroblastosis (immature RBCs and WBCs in the PB)
2. Increased LDH
3. Anemia
4. splenomegaly

Diagnosis of primary myelofibrosis (PMF) requires meeting all three major criteria and two minor criteria

Teferri et al. Leukemia (2008) 22, 14–22
## DDx of Myelofibrosis

### Myeloid Neoplasms
- PMF
- CML
- ET
- PV
- MDS
- Acute myelofibrosis (potentially assn. w/ FAB M7 AML)
- AML
- Mast Cell Disease

### Lymphoid Neoplasms
- lymphoma
- Hairy Cell Leukemia
- Multiple Myeloma

### Non-Hematologic Disorders
- Metastatic cancer
- Connective tissue diseases
- Rickets
- Infections
- Renal Osteodystrophy

Source: Undetermined
Outcomes in PMF

As fibrosis progresses, cytopenias worsen leading to a transfusion dependency
  ▪ symptoms related to extramedullary hematopoiesis increase (worsening splenomegaly and ‘B’ symptoms) also are frequently identified

Rarely do patients transform to Acute Leukemia (~ 4%)
  ▪ clonal evolution was common in these patients
  ▪ some evidence that in all MPNs, cases of JAK2 (-) Acute Leukemia arise out of a JAK+ MPN, causing speculation that there are additional genetic changes that either initiate and/or propagate these diseases

Despite the lack of transformation to leukemia, three-year survival rate is approximately 52%
### Risk Assessment in PMF

#### Mayo Scoring System (pts age < 60)

<table>
<thead>
<tr>
<th>Score</th>
<th>Median Survival</th>
</tr>
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<tbody>
<tr>
<td>0</td>
<td>173 mo</td>
</tr>
<tr>
<td>1</td>
<td>61 mo</td>
</tr>
<tr>
<td>≥ 2</td>
<td>26 mo</td>
</tr>
</tbody>
</table>

#### Transplant Scoring System (pts age < 55)

<table>
<thead>
<tr>
<th>Score</th>
<th>Median Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 or 1</td>
<td>15 yrs</td>
</tr>
<tr>
<td>≥ 2</td>
<td>3 yrs</td>
</tr>
</tbody>
</table>

**Risk Factors:**
- Hemoglobin <10 g/dL
- White blood cell count <4000/µL or >30,000/ µL
- Absolute monocyte count >1000 µL
- Platelet count <100,000/ µL
- ‘B’ symptoms present (eg, fever, NS, weight loss)
- Circulating blasts >1 percent

---


Treatment of PMF

Risk stratification is critical in deciding on therapeutic options (see previous scoring systems)

‘Low Risk’ without symptoms – expectant management

‘Low Risk’ with symptoms – hydroxyurea
  androgenic and corticosteroids
  splenectomy if adequate BM hematopoiesis
  splenic irradiation
  thalidomide or lenalidomide

‘High Risk’ and age < 55(?) – consider a reduced intensity allogeneic BMT
One Genetic Abnormality and Three Diseases Possible
Role of Allele Burden

Figure 1. Box-plots showing the JAK2 V617F allele percentage in essential thrombocythemia (ET), polycythemia vera (PV), and primary myelofibrosis (PMF) for: upper left - the total JAK2 V617F positive population analysed with qPCR (n = 124). The difference in JAK2 V617F allele burden was highly significant between the disease entities (ET vs. PV, P = 0.001 and PV vs. PMF, P < 0.00001); upper right - newly diagnosed patients (n = 61) (ET vs. PV, P = 0.02 and PV vs. PMF, P = 0.0008); lower left - non-newly diagnosed patients (n = 63).
Review Question # 1

42 yo woman with no past medical Hx presented to her PCP for an annual health maintenance examination. Physical exam was normal. A CBC was drawn and revealed a WBC of 14.2 (normal differential), Hbg of 13.5 and a plt count of 752,000.

Her diagnosis is:
A) Polycythemia Vera (PV)
B) Essential Thrombocythemia (ET)
C) Chronic Myeloid Leukemia (CML)
D) Reactive Thrombocytosis
E) Not sure – need more data
Iron studies are normal and there was no evidence of inflammation on history or examination. There was no history of recurrent infections or connective tissue diseases. Further blood testing demonstrated no evidence of the JAK2 V617F mutation by gene sequencing.

Her diagnosis is:
A) Polycythemia Vera (PV)
B) Essential Thrombocythemia (ET)
C) Chronic Myeloid Leukemia (CML)
D) Reactive Thrombocytosis
E) Not sure – need more data
Additional testing of her peripheral blood demonstrated a negative RT-PCR for the $Bcr-Abl$ p210 and p190 gene products but the peripheral blood FISH for the $Bcr-Abl$ translocation was positive in 72% of cells. Repeat testing confirmed both of these findings.

Her diagnosis is:
A) Polycythemia Vera (PV)
B) Essential Thrombocythemia (ET)
C) Chronic Myeloid Leukemia (CML)
D) Reactive Thrombocytosis
E) Not sure – need more data
Review Question #1 (cont)
A 34 yo woman presents for her annual HME and a CBC reveals a WBC count of 11.2, hgb of 17.1 and a platelet count of 390,000. Peripheral blood was sent to evaluate for the JAK2 mutation and was negative. What is the most appropriate next step in the evaluation of the patient?

A) Bone marrow biopsy to evaluate for a myeloproliferative neoplasm
B) Repeat CBC in 3 months
C) Repeat JAK2 testing to ensure laboratory accuracy
D) Red cell mass assay to determine a primary versus a secondary erythrocytosis
E) Referral to hematology
The patient underwent a red cell mass assay that demonstrated a true erythrocytosis (increased red cell mass). Upon further questioning, she states that she was previously treated with phlebotomy for the elevated Hgb and felt horrible for 3-4 weeks. She also indicates that her brother has a similar condition as did her mother and her mothers sister, but no one has been able to find a cause. What is the most appropriate next step in the management of this patient.

A) Repeat phlebotomy, but take only 250 cc/session
B) Initiate treatment with low dose aspirin (81 mg/day) and hydroxyurea
C) Repeat phlebotomy, but take only 250 cc/session and also treat with low dose aspirin (81 mg/day)
D) Evaluate for an inherited cause of polycythemia
Hemoglobin (Hb) Ypsilanti is a rare, high-oxygen-affinity hemoglobin first described in 1967 and named for the Michigan city in which the index family resided.\textsuperscript{1-3}

Like other high-oxygen-affinity hemoglobins, of which there are now substantially more than 100 described, Hb Ypsilanti manifests as a true erythrocytosis.

Phlebotomy in individuals with an appropriate erythrocytosis (high affinity Hgb, CO poisoning, living at altitude, sleep apnea) will increase symptoms because the erythrocytosis is an appropriate correction for the primary disorder.

Additional Source Information

for more information see: http://open.umich.edu/wiki/CitationPolicy

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