80 Myeloproliferative Neoplasms: New Approaches to Diagnosis and Disease Monitoring

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This session will summarize the salient characteristics of the eight types of myeloproliferative neoplasms, with emphasis on recent developments in molecular pathophysiology and therapeutic monitoring of these disorders.

- Apply the current World Health Organization criteria to assess newly diagnosed cases of chronic myeloproliferative neoplasms.
- Describe advances in the therapeutic monitoring of patients with chronic myeloproliferative neoplasms.
- Use cytogenetic and molecular genetic data to aid clinical findings and histomorphology in the diagnosis of chronic myeloproliferative neoplasms.

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Myeloproliferative Neoplasms: New Approaches to Diagnosis and Disease Monitoring

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Myeloproliferative Neoplasms
2008 WHO Classification

• Chronic myelogenous leukemia, \( BCR-ABL1 \) positive
• Chronic neutrophilic leukemia
• Chronic eosinophilic leukemia, NOS
• Polycythemia vera
• Primary myelofibrosis
• Essential thrombocythemia
• Mastocytosis
• Chronic myeloproliferative disease, unclassifiable
Philadelphia Chromosome

- The Philadelphia chromosome (Ph) is commonly found in hematologic malignancies
  - CML: 90-95%
  - Adult ALL: 20%
  - Pediatric ALL: 5%
  - AML: 2%

- BCR-ABL functions
  - Aberrant tyrosine kinase activity
  - Activation of Ras
  - Secondary lesions that promote blast crisis
    - Differentiation arrest
    - Impaired genomic surveillance
    - DNA-repair defects

BCR-ABL

- Multiple potential gene products of the t(9;22) translocation based on site of breakpoint in BCR gene
Chronic Myelogenous Leukemia (CML)

- Clonal myeloproliferative disorder
- Occurs at any age; most patients 30-60 yrs old
- Symptoms - fatigue, weight loss, abdominal discomfort – 20-40% asymptomatic at diagnosis
- Physical exam - splenomegaly

Chronic Myelogenous Leukemia (CML)

- Chronic phase – inc. myelopoeisis, basophilia, eosinophilia
  - Median survival 7 years with interferon therapy
- Accelerated phase
  - 6-18 months
- Blast phase
  - Myeloid: median survival 3-4 months
  - Lymphoid: median survival 9-12 months

Chronic Myelogenous Leukemia
Accelerated Phase (WHO)

- Blasts 10-19% of WBCs in PB and/or of nucleated bone marrow cells
- Peripheral blood basophils ≥20%
- Persistent thrombocytopenia (<100x10^9/L) unrelated to therapy, or persistent thrombocytosis (>1000x10^9/L) unresponsive to therapy
- Increasing spleen size and increasing WBC count unresponsive to therapy
- Clonal evolution
Chronic Myelogenous Leukemia

Accelerated Phase

- Blasts >20% of PB WBCs/nucleated bone marrow cells
- Extramedullary blast proliferation
- Large foci/clusters of blasts in bone marrow biopsy

Chronic Myelogenous Leukemia Blast Phase (WHO)

Blast Phase of CML
Blast Phase of CML

Imatinib Mesylate
STI-571, Gleevec®, Glivec®

- Developed by Druker et al in collaboration with Ciba-Geigy (now Novartis)
- Inhibitor of Bcr-Abl tyrosine kinase activity
- Minimal crossover
  - Stem-cell factor receptor, c-kit (CD117)
  - Platelet derived growth factor receptor, PDGFR
- Blocks Abl tyrosine kinase activity by binding and inactivating the ATP-binding pocket of Abl

Actuarial probability of disease progression according to the level of cytogenetic and molecular response after 12 months of imatinib ($P < .001$; log-rank test).

Bone Marrow Cellularity

Month 0  Month 3  Month 6

Bone Marrow Cellularity

Month 9  Month 12  Month 15  Month 18

Patient who became t(9;22) negative

Patient who remained t(9;22) positive


Progression to blast phase of CML


Gleevec Resistance

- Amplification and sequencing of the ATP-binding pocket of ABL
- T315I in 6 patients

Gleevec Resistance

- Primary resistance – 5% of patients in CP – BCR-ABL independent mechanisms
- Secondary resistance – 10-15% in CP –
  - Initial response, followed by increase in BCR-ABL transcripts
  - Point mutations in ATP binding pocket
  - 85% of cases: M224V, G250E, Y253F/H, E255K/V, T315I, M351T, F359V

BCR-ABL Wildtype  
BCR-ABL T315I Mutant

Kaplan-Meier survival curves for patients with mutations

**BCR-ABL MRD Testing**

- Conventional RT-PCR
  - Sensitive, specific
  - Specimen contamination, Suboptimal turnaround
- RT-PCR using closed tube techniques and fluorescence-based detection
  - ABI PRISM™, LightCycler™, TaqMan™, capillary electrophoresis, melting curve analysis
- Results normalized against housekeeping genes PBGD, ABL, G6PD, β-actin, RARα
- Lack of universally accepted standards for interlaboratory agreement

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**Presumptive CML**

- Conventional Cytogenetics
  - **t(9;22)+ (~95%)**
  - **t(9;22)-**
  - **Molecular**
    - **BCR-ABL1+** (~2.5%, likely not CML)
  - **Molecular**
    - **BCR-ABL1-**

CBC: Every 2 weeks, Every 3 months
Cytogenetics: Every 6 months, Yearly
FISH (PB): Every 2-3 months
Molecular (PB or BM): Every 3 months

**CBC**
**Cytogenetics**
**FISH (PB)**
**Molecular (PB or BM)**

* = qualitative RT-PCR
** = quantitative RT-PCR
*** = mutational testing

In cases of treatment failure, suboptimal response, or increasing transcript levels


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**Failure to Respond**

No CHR

<CHR, No CR

<PaCR

<CCR

Loss of CHR, Loss of CR

<CHR

<PaCr

<CCR

<MMR

ACA in Ph+ cells, Loss of MMR

Suboptimal Response


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**Gleevec Resistance Alternative Therapies**

### Philadelphia-Chromosome–Negative Classic MPNs
- Polycythemia vera (PV)
- Essential thrombocythemia (ET)
- Primary myelofibrosis (PMF)
- Clonal expansion of 1 or more bone marrow lineages

![W. Dameshek 1900-1969](image)

### Polycythemia Vera
- Absolute increase in erythrocyte cell mass
- Increased hematocrit
- Increased blood volume
- Increased blood viscosity

### Polycythemia Vera
**Clinical Features**
- Skin: Rubor, pruritus
- Vascular: Thromboses
- Gastrointestinal: Peptic ulcers, hemorrhage
- Splenomegaly
- Dyspnea
- CNS: Headache, vertigo, syncope, visual disturbance, tinnitus, stroke
Polycythemia Vera
Laboratory Features

- Increased absolute red blood cell mass
- Erythrocytosis (7-10,000,000/mm³)
- Increased hemoglobin (18-24 g/dL)
- Reticulocyte count not increased
- Leukocytosis (25-30,000/mm³)
- Thrombocytosis (500,000-1,000,000/mm³)
- Increased total blood volume
- Increased blood viscosity
- Increased leukocyte alkaline phosphatase (LAP)
- Increased serum vitamin B12 (increased transcobalamin I)

Polycythemia Vera
Morphologic Features

- Hypercellular bone marrow
- Multilineage hyperplasia
- Megakaryocyte clustering
- Minimal fibrosis


Polycythemia Vera
Natural History

10-15% mimic “ET”
Pre-polycythemic stage
- Jak2 +/-
- Jak2+/
- Epo ± a EECS

Definite increase in RBC mass
10-15 years
Splenomegaly

Fibrosis
Post-polycythemic myeloid Metaplasia “20%”

Post-PV/MF with blastic transformation “10%”

Terminal phase
Adapted from Swerdlow et al (2008)
Polycythemia Vera

Primary Myelofibrosis

- AKA: Agnogenic myeloid metaplasia, Myelofibrosis with myeloid metaplasia
- Neoplastic disorder of pluripotential hematopoietic stem cell
- Massive extramedullary hematopoiesis
- Cellular phase; progresses to fibrotic phase

Primary Myelofibrosis
Clinical Features

- Fatigue (anemia)
- Bleeding (thrombocytopenia)
- Infection (granulocytopenia)
- Abdominal mass (splenomegaly, due to extramedullary hematopoiesis)
### Primary Myelofibrosis

#### Cellular Phase: Laboratory Features

- **Peripheral blood**
  - Leukocytosis
  - Thrombocytosis
  - Basophilia
  - Eosinophilia
- **Bone marrow**
  - Hypercellularity
  - Granulocytic, megakaryocytic, erythroid hyperplasia
  - Minimal fibrosis
  - Megakaryocyte clustering

#### Fibrotic Phase: Laboratory Features

- **Peripheral blood**
  - Dacrocytes (teardrop RBCs)
  - Nucleated RBCs
  - Immature granulocytes
  - Anemia/leukopenia/thrombocytopenia
  - Increased LAP
- **Bone marrow**
  - Obliterative fibrosis
  - Osteosclerosis

### Natural History

- Progressive bone marrow failure
- Death due to infection or hemorrhage
- Conversion to acute leukemia in <10% of patients
Primary Myelofibrosis

Essential Thrombocytemia

- Clonal neoplasm derived from pluripotential hematopoietic stem cell
- Marked hyperplasia of bone marrow megakaryocytes
- Peripheral thrombocytosis

Essential Thrombocytemia

Clinical Manifestations

- Thrombocytemia/ bleeding due to platelet dysfunction
- Splenomegaly due to extramedullary hematopoiesis
### Essential Thrombocythemia

#### Laboratory Features, Peripheral Blood
- Thrombocytosis >1,000,000/mm³
- Abnormal platelet morphology
- Abnormal platelet function
- Leukocytosis 15-40,000/mm³
- Eosinophilia
- Basophilia

#### Laboratory Features, Bone Marrow
- Hypercellular bone marrow
- Marked megakaryocytic hyperplasia
- Variable hypercellularity of granulocytic and erythroid lineages
- Minimal bone marrow fibrosis

#### Natural History
- Episodic bleeding and/or thrombosis
- <1% progress to acute leukemia
Essential Thrombocythemia

Classic Myeloproliferative Neoplasms

Chronic Myeloid Leukemia → BCR-ABL1
(Philadelphia Chromosome)

Polycythemia vera (PV)

Essential thrombocythemia (ET)

Primary myelofibrosis (PMF)

Acquired mutation of the tyrosine kinase JAK2 in human myeloproliferative disorders
Janus Kinase 2
• Member of Janus family (Jak1, Jak2, Jak3 and Tyk2) of non-receptor tyrosine kinases that associate with cytokine/chemokine receptors
  - Shared structure consisting of adjacent kinase (JH1) and pseudokinase domain (JH2)
• Jak2 V617F: G to T somatic mutation in exon 14 (JH2) domain
  - Disrupts the interaction between JH2 and JH1, resulting in constitutive activity
  - Likely not disease initiating in humans but studies in mice do mimic components of the human disease

JAK2 V617F mutations

<table>
<thead>
<tr>
<th>Disease</th>
<th>JAK2 V617F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polycythemic Vera</td>
<td>95-100%</td>
</tr>
<tr>
<td>Primary Myelofibrosis</td>
<td>65%</td>
</tr>
<tr>
<td>Essential Thrombocytopenia</td>
<td>55%</td>
</tr>
</tbody>
</table>

- JAK2 V617F is not specific for MPNs
  - CMML: 5-10%  
  - MDS: 5-5%  
  - AML: <5%
  - Not associated with solid tumors or NHL
- JAK2 translocations have been described in hematologic malignancies
  - TEL-JAK2: ALL
  - P235-JAK2: acute myeloid leukemia, T cell lymphoma
  - BCR-JAK2: eosinophil MPD
- Jak family mutations are found in other hematologic malignancies
  - Jak1: AML
  - Jak3: MT-AML (megakaryoblastic leukemia)
  - SOCS mutations: Hodgkin lymphoma, primary mediastinal B-cell lymphoma (PMBL)

Jak2-negative ET/PMF/PV
• Polycythemia Vera
  - Jak2 exon 12 mutations
    - Clinically distinct: predominantly erythrocytosis without leukocytosis or thrombocytosis
• ET and PMF
  - MPL W515 (L/K)
    - Gain of function mutation
    - Found in ~5% of Jak2V617F negative PMF and ET
Mutational potpourri

- Recent studies have established a lengthy list of mutations found at low frequencies in MPNs.
- These mutations are neither sensitive nor specific for MPNs and there are currently no implications for clinical testing.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Frequency in MPN (PV, PMF, ET)</th>
<th>Other myeloid disorders</th>
</tr>
</thead>
<tbody>
<tr>
<td>TET2</td>
<td>~10%</td>
<td>AML, BP-MPN, AML, CMML</td>
</tr>
<tr>
<td>EZH2</td>
<td>&gt;5%</td>
<td>AML, BP-MPN, AML, CMML</td>
</tr>
<tr>
<td>DNMT3a</td>
<td>5-10%</td>
<td>AML, BP-MPN, AML, CMML</td>
</tr>
<tr>
<td>EZH2</td>
<td>&gt;5%</td>
<td>AML, BP-MPN, AML, CMML</td>
</tr>
<tr>
<td>DNMT3A</td>
<td>&gt;5%</td>
<td>BP-MPN</td>
</tr>
<tr>
<td>ASXL1</td>
<td>&lt;5%</td>
<td>AML, BP-MPN, AML, CMML</td>
</tr>
</tbody>
</table>

Adapted from Tefferi, Leukemia (2011)

Polycythemia Vera

*Diagnosis requires both major and one minor OR the first major and two minor*

**Major criteria**
1. Hemoglobin >18.5 g/dL (men), 16.5 g/dL (women) or other evidence of increased RBC mass
2. Jak2V617F or Jak2 exon 12 mutations

**Minor criteria**
A. Bone marrow biopsy-hypercellular with pancytosis
B. Low serum Erythropoietin
C. Endogenous erythroid colony formation in vitro
Primary Myelofibrosis

Requires all three major criteria and two minor criteria

Major Criteria
1. Megakaryocyte proliferation and atypia with reticulin and/or collagen fibrosis (fibrotic) OR in the absence of reticulin fibrosis, megakaryocytic changes with increased marrow cellularity and granulocytic proliferation (pre-fibrotic)
2. Not meeting criteria for PV, CML, MDS
3. Jak2V617F OR other clonal markers OR no evidence of reactive marrow fibrosis

Minor Criteria
1. Leukoerythroblastosis
2. Increased serum LDH
3. Anemia
4. Palpable splenomegaly

Essential Thrombocythemia

Requires all four criteria

1. Sustained platelet count > 450 K/cumm
2. Bone marrow biopsy-megakaryocyte proliferation with increased numbers of enlarged, mature forms
   – No significant increase/left-shift in neutrophils or erythroblasts
   – No significant fibrosis
3. Not meeting criteria for PV, PMF, CML, MDS
4. Jak2V617F or other clonal markers OR no evidence of reactive thrombocytosis without a clonal marker

JAK2 V617F Detection

• Numerous methods (RFLP, allele-specific amplification) are currently available
• Washington University
   – Ipsogen JAK2 MutaScreen (qualitative assay)
     • 10ng gDNA as starting material, either from blood or bone marrow
     • Positive cutoff of 2%
JAK2 V617F Detection

• Issues to be resolved
  – Is there a role for reporting allele frequency?
    • Mouse models and clinical data suggest that allele burden helps shape the disease phenotype
      – ET has lowest allele burden
    • Increasing allele burden has been associated with increased fibrosis, splenomegaly and leukocyte count
  – Standardized JAK2 V617F monitoring has not been established
    • Currently unclear if JAK2V617F can be used for disease monitoring similar to BCR-ABL1 in CML

WHO 2008

• Myeloproliferative Neoplasms (MPN)
  – Chronic myelogenous leukemia
  – Polycythemia vera
  – Essential thrombocythemia
  – Primary myelofibrosis
  – Chronic neutrophilic leukemia
  – Chronic eosinophilic leukemia, not otherwise categorized
  – Hypereosinophilic syndrome
  – Mast cell disease
  – MPNs, unclassified

Mast Cell Disease

• Clinical heterogenous group of diseases due to clonal proliferation of mast cells
• Multiple WHO categories
  – Cutaneous mastocytosis
  – Indolent systemic mastocytosis
  – Systemic mastocytosis with associated clonal hematological non-mast cell lineage disease (SM-AHNMD)
  – Aggressive systemic mastocytosis
  – Mast cell leukemia
  – Mast cell sarcoma
  – Extracutaneous mastocytoma
Mast Cell Disease

- Typical morphologic/immunophenotypic features
  - Clusters (>15 cells) of spindled mast cells
  - Atypical expression of CD2 and CD25
- SM-AHNMD
  - Concurrent clonal hematologic malignancy (commonly CMML.)
- Activating mutations in c-kit
  - D816V is most common and occurs within kinase domain and thus is insensitive to Gleevec
  - Other mutations may be present depending on additional hematologic disorders (SM-AHNMD)

Myeloid neoplasms associated with eosinophilia

- Chronic eosinophilic leukemia, not otherwise categorized
- Hypereosinophilic syndrome
- Myeloid and lymphoid neoplasms with eosinophilia and abnormalities of PDGFRB
- Myeloid and lymphoid neoplasms with eosinophilia and abnormalities of PDGFR
- Myeloid and lymphoid neoplasms with eosinophilia and abnormalities of FGFR
Case presentation

• 42 year old man with mild leukocytosis (15.1 K/cumm)
  – Eosinophils: 12% (1.8 K/cumm; nl 0.0-0.5)
  – Neutrophils: 68% (8.26 K/cumm; nl 1.8-6.6)
  – Monocytes: 4% (0.48 K/cumm; nl 0.2-1.2)
• Presented with chief complaint of fatigue
• All other indices were within normal limits

Eosinophilia

• Classically defined as >0.6 K/cumm
  – Mild: 0.6-1.49
  – Moderate: 1.5-5.0
  – Severe:>5.0
• Primary (part of a clonal hematopoietic neoplasm)
• Secondary (reactive,non-neoplastic)-most common
  • Parasites, allergies, medications

Primary Eosinophilia

• Hypereosinophilic Syndrome (non-clonal)
  – Persistent eosinophilia (>6 mo) of >1.5 K/cumm
  – Rule out all reactive conditions
  – Rule out all other hematolymphoid neoplasms associated with eosinophilia
  – Presence of tissue damage due to eosinophilia
    • If absent-idiopathic hypereosinophilia
• Chronic eosinophilic leukemia
  – Rule out all other hematolymphoid neoplasms associated with eosinophilia
  – Cytogenetic abnormality or blasts >2% in PB or >5% in BM
Other Hematolymphoid malignancies associated with eosinophilia

- Chronic myelogenous leukemia
  - Evaluate for BCR-ABL1
- Mast cell disease
  - Evaluate for D816V C-Kit mutation
- B lymphoblastic leukemia/lymphoma with t(5;14)(q31;q32)
  - IL3-IgH
- Acute myeloid leukemia with inv(16)(p13.1q22) or t(16;16)(p13.1; q22)
  - CBFB-MYH11
  - Myelomonocytic leukemia with immature eosinophils with basophilic granules
- Myeloid and lymphoid neoplasms with associated abnormalities of PDGFRα, PDGFRβ and FGFR1
  - Eosinophilia is characteristic but not always present
- Other disorders: T cell lymphoma, Hodgkin lymphoma, LCH
46,XY,t(5;12)(q33;p13)[20]

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nuc insh(PDGFRBx2)(5’PDGFRB sep 3’PDGFRBx3)[181/200]

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### Myeloproliferative Neoplasm Molecular Alteration

<table>
<thead>
<tr>
<th>Alteration</th>
<th>Age/Gender</th>
<th>Clinical Presentation</th>
<th>Histologic Features</th>
<th>Molecular Confirmation</th>
<th>Molecular Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>PDGFRA</td>
<td>M&gt;F (20:1)</td>
<td>Chronic inflammatory</td>
<td>Tissue infiltrated by eosinophils</td>
<td>FISH/PCR reported</td>
<td>Yes</td>
</tr>
<tr>
<td>PDGFRB</td>
<td>M&gt;F (2:1)</td>
<td>Eosinophilic leukemia</td>
<td>Hypercellular BM with eosinophilia</td>
<td>Karyotype or PCR</td>
<td>Yes</td>
</tr>
<tr>
<td>FGFR1 (aka 8p11 syndrome)</td>
<td>M&gt;F</td>
<td>Maternal origin</td>
<td>Variable</td>
<td>Karyotype or PCR</td>
<td>No</td>
</tr>
</tbody>
</table>

### Conclusions

- Majority of MPNs now have a defined molecular event that can be used in their diagnosis

<table>
<thead>
<tr>
<th>Myeloproliferative Neoplasm</th>
<th>Molecular Alteration</th>
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<tbody>
<tr>
<td>Chronic Myelogenous Leukemia</td>
<td>BCR-ABL1</td>
</tr>
<tr>
<td>Polycythemia Vera</td>
<td>JAK2 V617F</td>
</tr>
<tr>
<td>Essential Thrombocythaemia/Primary Myelofibrosis</td>
<td>JAK2 V617F, MPL W515</td>
</tr>
<tr>
<td>Mast Cell Disease</td>
<td>ITD 816F</td>
</tr>
<tr>
<td>Myeloid diseases associated with clonal eosinophilia</td>
<td>PDGFR/FGFR/FGFR1 translocations</td>
</tr>
</tbody>
</table>

### Future Clinical Directions

- Detection of these events in MRD testing has yet to be universally accepted and validated
- Role of detecting less common mutations (i.e. DNMT3a, TET2) in the diagnosis of myeloproliferative neoplasms is unclear
Molecular Pathology of Myeloproliferative Neoplasms

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Key Words: Molecular pathology; Myeloproliferative neoplasms; Chronic myelogenous leukemia; Chronic neutrophilic leukemia; Polycythemia vera; Primary myelofibrosis; Essential thrombocytopenia; Chronic eosinophilic leukemia; Mastocytosis; Myeloproliferative neoplasm, unclassifiable

Abstract

Myeloproliferative neoplasms (MPNs; formerly chronic myeloproliferative disorders) are a class of myeloid hematologic malignancies that represent a stem cell–derived expansion of 1 or more hematopoietic cell lineages. The current 2008 World Health Organization system recognizes 8 types of MPN: chronic myelogenous leukemia, chronic neutrophilic leukemia, polycythemia vera, primary myelofibrosis, essential thrombocytopenia, chronic eosinophilic leukemia, mastocytosis, and myeloproliferative neoplasm, unclassifiable. This review summarizes the salient characteristics of the MPNs, with emphasis on recent developments in the molecular pathophysiology and therapeutic monitoring of these disorders.

Chronic Myelogenous Leukemia

Myeloproliferative neoplasms (MPNs), formerly referred to as chronic myeloproliferative disorders, are a class of stem cell–derived myeloid hematologic malignancies, primarily occurring in adults, characterized by an expansion of 1 or more hematopoietic cell lineages with resulting bone marrow hypercellularity. Mature and immature marrow elements readily traffic into the peripheral blood, as evidenced by increases in the WBC count, hemoglobin and/or hematocrit values, and/or platelet count.

The World Health Organization (WHO) classification of tumors from 20011 recognized 7 distinct categories within this family of diseases. Since 2001, the molecular pathogenesis of a number of these entities has been elucidated, necessitating a revised classification system. Furthermore, the former designation of chronic myeloproliferative disorders has been replaced by MPNs, emphasizing the stem cell–derived heritage of these diseases. The new classification2 includes 8 subcategories, now including mastocytosis. In addition, the distinction between chronic eosinophilic leukemia and the hypereosinophilic syndrome subcategory has been refined,3 and a new category of myeloid and lymphoid neoplasms with eosinophilia, based on recurrent genetic abnormalities involving receptor tyrosine kinases, has been added. This review highlights recent advances in the understanding of the molecular pathophysiology of MPNs and how the insights have altered diagnostic algorithms and led to advances in therapy and disease monitoring.
granulocytic lineage, although all hematopoietic lineages may be affected. Patients usually seek care in the chronic (or stable) phase of disease with vague symptoms, including fatigue, weight loss, and abdominal discomfort (due to hepatosplenomegaly), although 20% to 40% of patients are asymptomatic at diagnosis, and disease is incidentally detected following CBC and peripheral blood review.6,7 In addition to increased myelopoiesis, bone marrow and peripheral blood basophilia and eosinophilia and bone marrow micromegakaryocytes may also be present.8 With time, the disease progresses to a more aggressive phase characterized by increased blasts in the peripheral blood and bone marrow9 that have a myeloid phenotype in the majority of cases, but about 20% to 30% of patients can have a lymphoblastic or, rarely, an ambiguous phenotype.10 If untreated, disease inevitably progresses to an acute leukemia with a short median survival.

CML is molecularly defined by the Philadelphia chromosome, t(9;22)(q34;q11.2),11 which encodes the BCR-ABL1 fusion protein that has dysregulated tyrosine kinase function.12 Rare cases have cryptic abnormalities involving BCR and ABL1 that require higher resolution molecular techniques for detection or involve complex translocations. Other cytogenetic abnormalities are identified in CML, particularly in accelerated and blast phases, and include additional Philadelphia chromosomes, an additional copy of chromosome 8, and isochromosome 17q.13 In addition, a variety of cellular processes are dysregulated in CML, such as monitoring genomic instability through ATM14 and DNA repair.15,16 Last, the tumor suppressor PP2A is inhibited by BCR-ABL1, a key feature of disease progression.17

Because BCR-ABL1 is identified in all cases of CML, it has been the subject of considerable study by the pharmacogenomics community, and CML became the first human disease with a specific targeted therapy directed at the causative molecular lesion. Imatinib mesylate (Gleevec, formerly STI571), developed by Druker et al in collaboration with Ciba-Geigy (now Novartis), is a small-molecule tyrosine kinase inhibitor that works by binding and inactivating the adenosine triphosphate binding pocket of the BCR-ABL1 protein.18-21 It is a phenylaminopyrimidine with a molecular weight of 589.7 kDa that has minimal crossover with other tyrosine kinases but has been proven to partially inhibit the stem cell factor receptor c-KIT (CD117) and platelet-derived growth factor receptor (PDGFR).22

The International Randomized Study of Interferon and STI571 (IRIS) study has established the superiority of imatinib to interferon and cytarabine in the treatment of CML, particularly in patients with chronic phase disease.23,24 Because
imatinib is now the mainstay of CML therapy, its impact on the natural history of the disease has been well studied and is important to consider when developing a therapeutic monitoring assay. Clinical assessment is performed at 4 levels, listed in order of increasing sensitivity for detection of disease: hematologic (eg, CBC), conventional cytogenetic, fluorescence in situ hybridization (FISH), and molecular. At initial diagnosis, most patients now have an initial genetic interrogation, including conventional cytogenetic analysis and quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) analysis for BCR-ABL1, which confirms the hematologic and clinical suspicion of CML. Conventional cytogenetic analysis is useful for identification of cytogenetic abnormalities in addition to the Philadelphia chromosome, which may impact prognosis. FISH for BCR-ABL1 fusion is sometimes performed in addition to conventional cytogenetic analysis, which has the ability to detect a potential BCR-ABL1 fusion in the context of a complex karyotype and to identify the presence of multiple BCR-ABL1 fusions in a single cell. RT-PCR analysis provides an important baseline BCR-ABL1 level to which future test results are compared. In general, initial RT-PCR analysis is performed using a quantitative rather than a qualitative assay because quantitative analysis of BCR-ABL1 is now considered standard of care for detection of minimal residual disease.

A number of studies have established that the quantitative analysis of BCR-ABL1 is technically feasible, reproducible with excellent intralaboratory agreement, and useful in assessing response to therapy. Current recommendations are for serial assessment of BCR-ABL1 levels at 3-month intervals in patients treated with imatinib. Data thus far have shown that 4 transcript level patterns follow imatinib treatment: continual decline, undetectable, stable/plateau, or rising. The therapeutic goal, as determined by the IRIS study, is a 3-log reduction or more in BCR-ABL1 transcript compared with the standard-of-care level of <10%, which is referred to as the major molecular response (MMR). The attainment of MMR is of demonstrated clinical significance in imatinib-treated patients. Patients with complete cytogenetic response (lack of detection of the Philadelphia chromosome by conventional cytogenetic analysis) and MMR at 12 months have a 100% rate of progression-free survival.

Minimal residual disease testing for BCR-ABL1 has developed in tandem with advances in RT-PCR technology. Early efforts used conventional RT-PCR that, while sensitive and specific, had potential problems with sample contamination and suboptimal turnaround time. These drawbacks were significantly improved with the development of closed-tube techniques and fluorescence-based detection of RT-PCR end products. Subsequently, assays were reported using ABI PRISM (Perkin Elmer, Applied Biosystems, Foster City, CA), LightCycler (Roche, Indianapolis, IN), TaqMan (Applied Biosystems), capillary electrophoresis, and melting curve techniques. Most clinical laboratories report the results normalized against housekeeping genes including the following: 30,31,35,40 32,33,35,36 37,38,40 39, and 40. Although intralaboratory reproducibility and sensitivity of RT-PCR assays are excellent, a major limitation in minimal residual disease testing for BCR-ABL1 is a lack of universally accepted standards for interlaboratory agreement.

Resistance

Approximately 5% of patients with chronic phase CML have initial treatment failure with imatinib owing to BCR-ABL1–independent mechanisms through a process known as primary resistance. Secondary resistance, identified in 10% to 15% of patients with chronic phase CML, manifests as an initial response to imatinib followed by an increase in BCR-ABL1 transcripts. It generally results from point mutations in the adenosine triphosphate binding pocket in ABL1, of which 7 distinct mutations account for approximately 85% of cases: M244V, E250V, Y253F/H, E255K/V, T315I, M351T, and F359V. Overall, secondary resistance develops in about 10% to 30% of patients with chronic phase, 60% of patients with accelerated phase, and 90% of patients with blast phase CML. Testing for these mutations is performed using a variety of modalities. Mutational analysis is indicated in patients with a suboptimal or failed response to imatinib, although specific guidelines have not been developed.

Philadelphia-Chromosome–Negative Classic MPNs

Along with CML, polycythemia vera (PV), essential thrombocytopenia (ET), and primary myelofibrosis (PMF) have been historically referred to as the classic MPNs. As with CML, these entities are characterized by a clonal expansion of 1 or more bone marrow lineages. PV, for example, is characterized by a proliferation of the erythroid lineage, resulting in increased erythroid cell mass, hemoglobin concentration, hematocrit value, and blood viscosity. Patients with PV have symptoms related to these findings and have an increased incidence of thromboses, hemorrhage, peptic ulcers, and stroke. Although not frequently performed on cases of PV at diagnosis, a bone marrow biopsy will show expansion of all 3 myeloid lineages (panmyelosis) and is frequently used to evaluate patients for other MPNs and to assess fibrosis, which may be significant late in the disease. In contrast with the other classic MPNs, megakaryocytes in PV are described as normal appearing to slightly enlarged, with a minimal amount of clustering.

In PMF Image 2, the dominant pathologic change is progressive marrow fibrosis. Early in the disease, however, the diagnosis may be problematic because most patients initially have a nonspecific, usually multilineage, expansion of hematopoiesis with minimal fibrosis and resulting neutrophilic
leukocytosis, thrombocytosis, basophilia, or eosinophilia. This constellation of findings makes the distinction from reactive conditions and from the other MPNs challenging.\(^{54,55}\) As marrow fibrosis increases, the peripheral counts frequently decline and patients become symptomatic with fatigue, bleeding, and/or infections due to cytopenias. Splenomegaly, as a result of extramedullary hematopoiesis, may be marked.\(^{50}\) Morphologic evaluation of the peripheral blood in late cases shows numerous dacrocytes (teardrop RBCs), nucleated RBCs, and circulating immature granulocyte precursors.\(^{53,54}\) Megakaryocytes are frequently clustered and consist of normal-appearing forms along with enlarged megakaryocytes with bizarre, hypolobated nuclei. Megakaryocytes may become abnormally localized within the lumina of marrow sinuses (intrasinusoidal hematopoiesis), a feature that is not unique to PMF and may be encountered in other diseases with marrow fibrosis.\(^{56}\)

Last, ET is characterized by dysregulated proliferation of megakaryocytes and platelets in the bone marrow and peripheral blood.\(^{57}\) Platelets are increased in number and often morphologically and functionally abnormal, and, thus, patients are at an increased risk of thrombosis and bleeding.\(^{58}\) The bone marrow is frequently hypercellular owing to a marked increase in megakaryocytes, which may be enlarged and hyperlobated, with minimal to no expansion of the granulocytic and erythroid lineages.\(^{59}\) In contrast with PMF, fibrosis is minimal.\(^{59}\)

Of the three Ph– classic MPNs, PMF has the worst outcome, and death is frequently related to bone marrow failure with resultant systemic infection or fatal hemorrhage in many cases.\(^{48,51,52}\) PV, in contrast, has a more indolent course, but there is considerable associated morbidity and mortality due to thromboses and/or hemorrhage, which can be treated with moderate success by therapeutic phlebotomy.\(^{60}\) The risk of transformation to an acute leukemia is highest in PMF (5%-30%) but can occur in ET and PV.\(^{51,52}\) Because most patients with acute leukemia...
have received prior alkylating chemotherapy, these cases may be better regarded as therapy-related acute myeloid leukemias. Leukocytosis has been shown to be predictive of poorer overall survival and thrombotic risk in ET and PMF.

Although the presence of a \textit{BCR-ABL1} translocation in an MPN is diagnostic for CML, the other MPNs have historically not been linked to an underlying genetic abnormality. This changed in 2005 when 4 separate groups published their findings of a guanine to thiamine transversion at base 1849 in exon 14 of the Janus kinase 2 (\textit{JAK2}) gene on chromosome 9p in large populations of patients with PV, ET, and PMF. Follow-up studies demonstrated that the V617F mutation in \textit{JAK2} is found in approximately 90% to 95% of cases of PV and 50% of both ET and PMF. This mutation, however, is not specific for the Philadelphia chromosome–negative MPNs and is found at lower percentages in a number of other myeloid malignancies, such as systemic mastocytosis, acute...
myeloid leukemia, and chronic myelomonocytic leukemia, but not in lymphomas or solid tumors. The high incidence of the V617F in PV established it as a major criterion in diagnosis; in contrast, its presence is supportive of a diagnosis of ET and PMF but is not required. The prognostic significance of detecting the JAK2 V617F mutation in an MPN is unclear because many conflicting reports on patient survival have been published, and further studies are required. Studies have, however, suggested that cases of PV and ET with a high JAK2 V617F allele burden have an increased risk of thromboses, and that in PMF the mutation is associated with higher WBC counts, splenomegaly, and a higher risk of leukemic transformation.

JAK2 is one of 4 Janus kinases, which are nonreceptor tyrosine kinases that are integral components of cytokine and growth factor signaling. The JAK proteins have 2 adjacent kinase-like domains (JH1 and JH2), of which only the JH1 domain has enzymatic activity. The JH2 domain, or pseudokinase domain, is a negative regulator of kinase activity, and this autoinhibitory function is released by the JAK2 gain-of-function mutation within the JH2 domain. This mutation occurs at a primitive stem cell level and confers cytokine hypersensitivity and cytokine-independent signaling, leading to the downstream activation of multiple signaling cascades, such as the STAT proteins, phosphatidylinositol-3-kinase–AKT pathway, and mitogen-activated protein kinases.

In contrast with BCR-ABL1 in CML, this lesion likely requires additional cooperating genetic aberrations and, in fact, is likely not the disease-initiating event. Although a 5- to 7-fold elevated risk of developing an MPN among first-degree relatives of patients with MPN has been noted, no germline JAK2 V617F mutations have been detected. However, recent studies have shown that a germline single nucleotide polymorphism in JAK2 predisposes people to JAK2 V617F MPNs. Furthermore, leukemic clones that lack the V617F mutation have been shown to arise in a V617F+ MPN. However, the role of previous exposure to known mutagens during chemotherapy may also contribute to these leukemic V617F transformations. In total, these findings favor the possibility that there is a yet unidentified predisposing clonal event. Favoring this belief are the findings of chromosome 20q deletions that predate JAK2 V617F mutations in patients with MPNs. In addition, recent studies suggest that mutations in the TET2 tumor suppressor gene, which have been found in 17% of JAK2 V617F+ Ph− MPNs and 7% of JAK2 V617F− cases, likely precede the acquisition of the JAK2 V617F mutation.

The mechanism of how a single mutation can produce 3 different diseases is not clear. Gene dosage may be relevant because studies in transgenic mice that controlled for the level of expression of the JAK2 V617F allele found that all 3 of the classic Ph− MPNs can be produced with the allele burden increasing from ET to PV to PMF, suggesting that these diseases may represent a continuum of a single disorder. In addition, mitotic recombination results in JAK2 V617F homozygosity in PV, which is rarely experienced in ET. Host genetic modifiers are also likely to influence the MPN phenotype; it has been demonstrated that single nucleotide polymorphisms within JAK2 predispose patients to PV and ET but not PMF.

JAK2 V617F Detection

A number of different modalities have been used to detect the guanine to thymine substitution that produces the V617F mutation, the most common being direct sequencing, restriction fragment length polymorphism, and allele-specific PCR. Furthermore, a variety of methods can then be used to analyze the amplicon, such as melting curve analysis and electrophoresis. These distinct molecular assays obviously carry different analytic sensitivities, with allele-specific PCR being as low as 0.01%, while the sensitivity of direct sequencing ranges between 10% and 40%. The widely used restriction fragment length polymorphism assays are able to detect the mutation if present in greater than 5% of alleles. The source of DNA may also influence the mutational burden; however, a recent report noted similar levels of JAK2 V617F from peripheral blood unfractionated WBCs and bone marrow. Techniques are also available to assess JAK2 status from formalin-fixed, paraffin-embedded tissue by DNA extraction or using surrogate markers of JAK2 activation, such as nuclear STAT5 activation in megakaryocytes.

Thus, there are no shortages of options available to clinicians during the workup of a patient for an MPN. Considering the range of analytic sensitivities between the various modalities, this may actually present a source of diagnostic error for patients with a low allele burden. It is not clear if the presence of the JAK2 V617F mutation at low levels has any clinical relevance because the V617F mutation was detected in 10% of healthy donor blood samples using allele-specific PCR. Considering that 10% of the population does not develop a Ph− MPN, there is obviously a subset of healthy people with JAK2 V617F.

The JAK2 V617F mutation is not the only gain-of-function mutation recently identified in the Ph− MPNs, nor is it the only JAK2 mutation. Additional mutations in exon 12 of JAK2 are present in a significant number of JAK2 V617F− PV cases. These cases predominantly display only erythrocytosis, whereas cases with JAK2 V617F also typically have...
leukocytosis or thrombocytosis. MPL, which is the receptor for thrombopoietin and a known interactor of JAK2, also has recently been shown to harbor mutations in a subset of cases of ET and PMF but not PV. The majority of the MPL mutations affect a tryptophan residue at position 515, and, in total, mutations in MPL account for approximately 8% of JAK2V617F– ET cases and approximately 10% of JAK2V617F– PMF cases.

JAK-STAT Targeted Therapy

Current treatment for MPNs involves measures to decrease the risk of thrombotic disease, namely low-dose aspirin, phlebotomy, or drug therapy, including hydroxyurea or interferons. Considering the success of BCR-ABL1 inhibitors for CML, the identification of a common somatic gain-of-function mutation in a tyrosine kinase in ET, PV, and PMF has led many on a quest to generate potent, efficacious, and safe small-molecule JAK2 inhibitors. A series of molecules have been produced and are currently in preclinical and clinical testing. A challenge is to design compounds that are mutant specific considering the importance of wild-type JAK2 signaling in erythropoiesis. Fortunately, there is evidence that JAK2V617F clones may be more sensitive to inhibition, thus widening the therapeutic window. Furthermore, off-target inhibition, most importantly of JAK3, needs to be minimized because blockade of JAK3 function leads to severe combined immunodeficiency. Last, an ideal inhibitor will also be effective against the other known mutations in ET, PMF, and PV, namely JAK2 exon 12 mutations and MPL mutations. Despite these issues, some compounds are showing promise. For example, a JAK2-selective antagonist, TG101348, in a small study selectively inhibited clones with aberrant activation of the JAK-STAT pathway, including not only the JAK2V617F mutation but also MPNs with JAK2 exon 12 and MPL mutations, as well as in a patient with PMF with no known mutation.

Many of the lessons that have been learned from the treatment and monitoring of BCR-ABL1 in CML will in time likely apply to the treatment of PV, ET, and PMF. It is reasonable to assume that if molecules are initially able to block JAK2 activity, resistance will develop and mutant clones will arise, necessitating the production of second-generation inhibitors. Alterations of JAK2 signaling are not disease initiating, and other genetic modifiers are thought to predispose to or aid in the progression of the Ph– classic MPNs, and specific targeting of JAK2 may actually select for JAK2V617F– transformed clones. Once treatment has been initiated with JAK2 inhibitors, monitoring of JAK-STAT activation in treated patients will be required. Many of the issues relevant to BCR-ABL1 monitoring in CML, such as the input source (bone marrow vs peripheral blood), will need to be addressed. It is also likely that a uniform monitoring technique with the ability to assess the mutational burden will be required to monitor response and to ensure consistency among treatment centers. These molecular end points will obviously need to be correlated with clinical response and bone marrow histomorphologic findings.

MPNs With Associated Eosinophilia

MPNs in which eosinophilia is the major component are evaluated by a combination of molecular pathology techniques and bone marrow examination with a tryptase stain. It is important to note that T-cell receptor gene rearrangement studies should be performed to evaluate for reactive eosinophilia associated with a T-cell lymphoma, and other causes of secondary eosinophilia, such as infection, should always be investigated. The other MPNs, namely CML, B-lymphoblastic leukemia/lymphoma, and acute myeloid leukemias with chromosome 16 abnormalities should also be ruled out.

Insights into the molecular pathogenesis of MPNs with associated eosinophilia led to the establishment of a new distinct category of myeloid and lymphoid neoplasms with eosinophilia in the 2008 WHO classification. These neoplasms are molecularly characterized by abnormalities involving the PDGFR (PDGFA or PDGFRB) or the fibroblast growth factor receptor (FGFR1). The most common alteration of PDGFR involves a cryptic deletion at 4q12 that results in a FIP1L1-PDGFR translocation. Other rare translocation partners have been described, including BCR and ETV6. Neoplasms involving PDGFRB most frequently manifest as chronic eosinophilic leukemia, but have also been described in acute myeloid leukemias and lymphoblastic T-cell lymphomas. In contrast, myeloid neoplasms involving PDGFRB, located at 5q31-33, frequently mimic chronic myelomonocytic leukemia. ETV6-PDGFRB, t(5;12), is the most common abnormality, but a number of other fusion genes have been described. For PDGFRB alterations, the causal deletion is only 800 kb in size and, thus, is frequently detected only by FISH or RT-PCR, whereas abnormalities involving PDGFRB can frequently be detected by conventional cytogenetics. Last, neoplasms involving FGFR1 at 8p11 involve a number of fusion gene partners and can manifest with features of an MPN, but also may manifest as an acute myeloid leukemia or lymphoblastic lymphoma.

The importance in correctly establishing the diagnosis of these molecularly characterized myeloid and lymphoid neoplasms cannot be overemphasized because the PDGFA and PDGFRB fusion oncogenes are extremely sensitive to tyrosine kinase inhibitors such as imatinib. Similar to CML, imatinib resistance secondary to acquired mutations occurs, and treatment with other kinase inhibitors such as sorafenib has shown some success. In contrast, tyrosine kinase inhibition has not proven effective for the FGFR1-derived neoplasms, and, thus, prognosis is unfavorable.
plasma cell myelomas. When present with eosinophilia, this entity should be differentiated by molecular techniques from the myeloid neoplasms associated with PDGFR abnormalities. The D816V gain-of-function mutation in c-KIT is identified in the majority of cases of systemic mastocytosis and can be detected by PCR-based modalities. Recent studies have shown that additional mutations, such as loss of function mutations in the TET2 tumor suppressor gene, may also contribute to mast cell neoplasms, especially those associated with monocytosis. Although other activating mutations in c-KIT such as the juxtamembrane mutations frequently observed in gastrointestinal stromal tumors are sensitive to treatment with imatinib, the mutations in the activation loop of c-KIT seen in mastocytosis are resistant. New generation kinase inhibitors are currently being evaluated.

Chronic Neutrophilic Leukemia

Chronic neutrophilic leukemia (CNL) is an exceedingly rare entity, which, in contrast with the other MPNs, is without a known molecular alteration. It is characterized by peripheral leukocytosis exceeding 25,000/μL (25 × 10^9/L), of which segmented neutrophils and band forms constitute more than 80% of peripheral WBCs. The diagnosis of CNL is made only after exclusion of reactive conditions, especially infectious or inflammatory conditions that may cause a leukemoid reaction with sustained neutrophilia, and other MPNs, such as CML. CNL lacks a BCR-ABL1 fusion gene.

Mastocytosis

MPNs characterized by a proliferation of morphologically abnormal mast cells have been shown to be clonal stem cell neoplasms linked to activating mutations in KIT (CD117) and, hence, are now included as an MPN subcategory in the WHO classification. Various subtypes, depending on the tissue distribution, are recognized. Bone marrow examination in systemic mastocytosis will show mast cell clusters of greater than 15 mast cells, and at least 25% will be spindle shaped, which can be highlighted with Leder histochemical analysis and immunohistochemical analysis for tryptase, CD117, and CD25. Bone marrow examination will also aid in the identification of other clonal hematologic disorders associated with mastocytosis (systemic mastocytosis with associated clonal hematologic non–mast cell lineage disease), which can include chronic myelomonocytic leukemia and acute leukemias and plasma cell myelomas. When present with eosinophilia, this entity should be differentiated by molecular techniques from the myeloid neoplasms associated with PDGFR abnormalities.

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Mastocytosis. A, Bone marrow aspirate with increased mast cells (Wright-Giemsa, ×1,000). B through E, Bone marrow core biopsy sample with large cluster of mast cells identified by H&E (B) and Leder (C) staining and immunohistochemical analysis for CD117 (D) and tryptase (E) (B–E, ×400).
and chronic myeloproliferative disorders with the p230 form of BCR-ABL1 are now categorized as neutrophilic CML rather than CNL.143,144

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