Myeloproliferative Neoplasms: New Approaches to Diagnosis and Disease Monitoring

John L Frater, MD
Jeffery M Klco, MD, PhD
Department of Pathology and Immunology
Washington University School of Medicine
St Louis, Missouri
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. myelopoiesis, 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
Chronic Myelogenous Leukemia Blast Phase (WHO)

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

BCR-ABL Wildtype   BCR-ABL T315I Mutant

Kaplan-Meier survival curves for patients with mutations

Presumptive CML

Conventional Cytogenetics

- t(9;22)+ (~95%)
  - Molecular
    - BCR-ABL1+

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

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
BCR-ABL MRD Testing

• Quantitative RT-PCR analysis is technically feasible, reproducible with excellent intra-laboratory agreement, useful in assessing response to therapy
• Current recommendations – serial assessment of BCR-ABL levels at 3 month intervals in patients treated with imatinib
• 4 transcript level patterns: continual decline, undetectable, stable/ plateau, rising
• MMR (major molecular response): therapeutic goal (IRIS study), ≥3 log reduction in BCR-ABL transcript compared to the standardized baseline
• IRIS study: patients with MMR + CCR (complete cytogenetic response) at 12 months have 100% rate of progression free survival
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 **

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

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

Failure to Respond

- No CHR
- <CHR, No CR
- <PaCR
- <CCR
- <MMR


Suboptimal Response

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
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 (18-24g/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

Evolution → Manifestation → Transformation

10-15% mimic “ET”

Fibrosis

Post-polycythemic myeloid Metaplasia ~20%

10-15 years

Splenomegaly

Post-PV MF with blastic transformation <10%

Pre-polycythemic stage

Polycythemic stage

Terminal phase

Definite increase in RBC mass

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
Primary Myelofibrosis
Fibrotic Phase: Laboratory Features

• Peripheral blood
  – Dacrocytes (teardrop RBCs)
  – Nucleated RBCs
  – Immature granulocytes
  – Anemia/ leukopenia/ thrombocytopenia
  – Increased LAP

• Bone marrow
  – Obliterative fibrosis
  – Osteosclerosis
Primary Myelofibrosis
Natural History

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

Essential Thrombocythemia

• Clonal neoplasm derived from pluripotential hematopoietic stem cell
• Marked hyperplasia of bone marrow megakaryocytes
• Peripheral thrombocytosis
Essential Thrombocytethemia
Clinical Manifestations

• Thrombocytosis/ bleeding due to platelet dysfunction
• Splenomegaly due to extramedullary hematopoiesis
Essential Thrombocythemia
Laboratory Features, Peripheral Blood

- Thrombocytosis >1,000,000/mm$^3$
- Abnormal platelet morphology
- Abnormal platelet function
- Leukocytosis 15-40,000/mm$^3$
- Eosinophilia
- Basophilia
Essential Thrombocythemia
Laboratory Features, Bone Marrow

- Hypercellular bone marrow
- Marked megakaryocytic hyperplasia
- Variable hypercellularity of granulocytic and erythroid lineages
- Minimal bone marrow fibrosis
Essential Thrombocythemia
Natural History

• Episodic bleeding and/or thrombosis
• <1% progress to acute leukemia
Essential Thrombocythemia

Classic Myeloproliferative Neoplasms

Chronic Myelogenous Leukemia \[\rightarrow\] BCR-ABL1 (Philadelphia Chromosome)

Polycythemia vera (PV)

Essential thrombocythemia (ET)

Primary myelofibrosis (PMF)

?
Acquired mutation of the tyrosine kinase JAK2 in human myeloproliferative disorders


letters to nature

A unique clonal JAK2 mutation leading to constitutive signalling causes polycythaemia vera

Chloé James1, Valérie Ugo2,3, Jean-Pierre Le Couédic1,4, Judith Staerk5, François Delhommeau1,3, Catherine Lacout1, Loïc Garçon6, Hana Raslova6, Roland Berger7, Annelise Benaîne-Grisell6, Jean Luc Villeval1, Stefan N. Constantinescu8, Nicole Casadevall1,2 & William Vainchenker1,2

The NEW ENGLAND JOURNAL of MEDICINE

A Gain-of-Function Mutation of JAK2 in Myeloproliferative Disorders

Robert Kralovics, Ph.D., Francesco Passamonti, M.D., Andreas S. Buser, M.D., Soon-Siong Teo, B.S., Ralph Tiedt, Ph.D., Jakob R. Passweg, M.D., Andre Tichelli, M.D., Mario Cazzola, M.D., and Radek C. Skoda, M.D.

Activating mutation in the tyrosine kinase JAK2 in polycythemia vera, essential thrombocythemia, and myeloid metaplasia with myelofibrosis

Ross L. Levine,1,2,11 Martha Wadleigh,2,11 Jan Cools,6 Benjamin L. Ebert,2,8 Gerlinde Wernig,1 Brian J.P. Huntly,1 Titus J. Boggon,4 Iwona Wlodarska,6 Jennifer J. Clark,1 Sandra Moore,1 Jennifer Adelsperger,1 Sumin Koo,1 Jeffrey C. Lee,8 Stacey Gabriel,8 Thomas Mercher,1 Alan D’Andrea,3 Stefan Fröhling,1 Konstanze Döhner,7 Peter Marynkin,5 Peter Vandenberghe,6 Ruben A. Mesa,9 Ayalew Teweri,9 James D. Griffin,2 Michael J. Eck,4 William R. Sellers,2,8 Matthew Meyerson,2,8 Todd R. Golub,3,8,10 Stephanie J. Lee,2,8 and D. Gary Gilliland1,2,10,*
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 domains (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>MPN</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 Thrombocytethemia</td>
<td>55%</td>
</tr>
</tbody>
</table>

- JAK2 V617F is not specific for MPNs
  - CMML: 3-9%
  - MDS: 3-5%
  - AML: <5%
  - Not associated with solid tumors or NHL

- JAK2 translocations have been described in hematologic malignancies
  - TEL-JAK2: ALL
  - PCM1-JAK2: acute myeloid leukemia, T cell lymphoma
  - BCR-JAK2: atypical MPD

- Jak family mutations are found in other hematologic malignancies
  - Jak1: AML
  - Jak3: M7 AML (megakaryoblastic leukemia)
  - SOCS mutations: Hodgkin lymphoma, primary mediastinal B-cell lymphoma (PMBL)

Adapted from Tefferi, Leukemia 2010
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
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.

### Gene Frequency in MPN (PV, PMF, ET) Other myeloid disorders

<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, MDS, CMML</td>
</tr>
<tr>
<td>IDH1/IDH2</td>
<td>&lt;5%</td>
<td>AML, BP-MPN, MDS, CMML</td>
</tr>
<tr>
<td>DNMT3a</td>
<td>5-10%</td>
<td>AML, BP-MPN, MDS, CMML</td>
</tr>
<tr>
<td>EZH2</td>
<td>~5%</td>
<td>AML, BP-MPN, MDS, CMML</td>
</tr>
<tr>
<td>LNK</td>
<td>rare</td>
<td>BP-MPN</td>
</tr>
<tr>
<td>ASXL1</td>
<td>~5%</td>
<td>AML, BP-MPN, MDS, 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 panmyelosis
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 Thrombocytethemia

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 erythroids
   - 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 PDGFRA
- 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

Adapted from Practical Diagnosis of Hematologic Disorders
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 PDGFRA, PDGFRB 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]

Dr. Shashi Kulkarni
Department of Pathology and Immunology
Washington University School of Medicine
nuc ish(PDGFRBx2)(5'PDGFRB sep 3'PDGFRBx1)[181/200]

Dr. Shashi Kulkarni
Department of Pathology and Immunology
Washington University School of Medicine
<table>
<thead>
<tr>
<th>Alteration</th>
<th>Age/Gender</th>
<th>Disease Presentation</th>
<th>Histologic Features</th>
<th>Translocation partners</th>
<th>Molecular confirmation</th>
<th>Gleevec Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>PDGFRA</td>
<td>M&gt;&gt;F (20:1)</td>
<td>Chronic eosinophilic leukemia</td>
<td>Tissue infiltration by eosinophils; +/- atypia</td>
<td>FIP1L1-PDGFR; rare variants have been reported</td>
<td>Karyotype-no FISH/PCR</td>
<td>Yes</td>
</tr>
<tr>
<td>PDGFRB</td>
<td>M&gt;F (2:1); 4th-5th decade</td>
<td>CMML with eosinophilia</td>
<td>Hypercellular BM with granulocytic hyperplasia; Increased mast cells</td>
<td>ETV6-PDGFRB/t(5;12) Over 20 possible partners</td>
<td>Karyotype, FISH or PCR</td>
<td>Yes</td>
</tr>
<tr>
<td>FGFR1 (aka 8p11 syndrome)</td>
<td>Slight male predominance; 3rd decade</td>
<td>MPN, AML, T-ALL, B-ALL, mixed phenotype AL</td>
<td>Varies depending on presentation</td>
<td>ZNF198-FGFR1; CEP110-FGFR1</td>
<td>Karyotype, FISH or PCR</td>
<td>No</td>
</tr>
</tbody>
</table>

Adapted from WHO 2008
Persistent eosinophilia (secondary conditions ruled out)

- T cell receptor studies
- Bone marrow biopsy
  - Tryptase stain to evaluate for mast cell disease
  - Molecular studies to evaluate for PDGFRA, PDGFRB and FGFR1 rearrangements
  - If above studies negative-chronic eosinophilic leukemia vs hypereosinophilic syndrome

Adapted from WHO 2008
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>
</tr>
</thead>
<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 Thrombocythemia/Primary Myelofibrosis</td>
<td>JAK2 V617F, MPL W515</td>
</tr>
<tr>
<td>Mast Cell Disease</td>
<td>KIT D816V</td>
</tr>
<tr>
<td>Myeloid diseases associated with clonal eosinophilia</td>
<td>PDGFRA/PDGFRB/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