Classic *BCR-ABL1*-negative Myeloproliferative Neoplasms

Indications for Ordering

Diagnose and manage classic *BCR-ABL1*-negative myeloproliferative neoplasms (MPNs)
- Polycythemia vera (PV)
- Essential thrombocythemia (ET)
- Primary myelofibrosis (PMF)
- Other myeloproliferative disorders

Test Descriptions

JAK2 Gene, V617F Mutation, Qualitative
- Polymerase chain reaction (PCR)

JAK2 Gene, V617F Mutation, Quantitative
- PCR
- Mutant-to-wild type allele ratio is reported

CALR (Calreticulin) Exon 9 Mutation Analysis by PCR
- PCR/capillary electrophoresis

Myeloid Malignancies Mutation Panel by Next Generation Sequencing
- Massively parallel sequencing

JAK2 Exon 12 Mutation Analysis by PCR
- Allele-specific PCR amplification with subsequent sequencing of positive products to detect single and multiple base substitutions within exon 12 of the *JAK2* gene

MPL codon 515 Mutation Detection by Pyrosequencing, Quantitative
- PCR/pyrosequencing
- Wild type-to-mutant allele ratio is reported

Myeloproliferative Disorders Panel by FISH
- Fluorescence in situ hybridization

Tests to Consider

JAK2 Gene, V617F Mutation, Qualitative 0051245
- Reasonable screening test for routine evaluation of
  - Unexplained erythrocytosis
  - Thrombocytosis
  - BCR-ABL1-negative granulocytosis
  - Bone marrow (BM) fibrosis
- Appropriate screen in patients with symptoms characteristic for *BCR-ABL1*-negative MPN
  - Unusual thrombotic events (eg, splanchnic vein thrombosis, Budd-Chiari)
  - Aquagenic pruritus
  - Unexplained splenomegaly

JAK2 Gene, V617F Mutation, Quantitative 0040168
- May be useful in assessment of residual disease after stem cell transplant
- Enhances diagnostic certainty in cases with low mutant allelic burden

CALR (Calreticulin) Exon 9 Mutation Analysis by PCR 2010673
- Use for diagnostic and prognostic information in patients with myeloproliferative neoplasms (MPNs) when *JAK2* testing is negative

Myeloid Malignancies Mutation Panel by Next Generation Sequencing 2011117
- Assess for single gene mutations, including substitutions and insertions and deletions that may have diagnostic, prognostic, and/or therapeutic significance

JAK2 Exon 12 Mutation Analysis by PCR 2002357
- Use when MPN suspected and *JAK2* V617F testing is negative

MPL codon 515 Mutation Detection by Pyrosequencing, Quantitative 2005545
- May be useful when essential thrombocythemia or idiopathic myelofibrosis is suspected in *JAK2* V617F-negative individuals

JAK2 Gene, V617F Mutation, Qualitative with Reflex to JAK2 Exon 12 Mutation Analysis by PCR 2012085
- Use when diagnosis of PV is suspected

JAK2 Gene, V617F Mutation, Qualitative with Reflex to CALR (Calreticulin) Exon 9 Mutation Analysis by PCR with Reflex to MPL codon 515 Mutation Detection by Pyrosequencing, Quantitative 2012084
- Use when diagnosis of ET or PMF is suspected

Myeloproliferative Disorders Panel by FISH 2002360
- Detect specific recurrent genomic aberrations in suspected MPNs
  - *BCR/ABL1*
  - *PDGFRA*
  - *PDGFRB*
  - *FGFR1*

Disease Overview

Incidence – 5/100,000 worldwide for combined PV, ET, and PMF

PV

- Must exclude secondary causes of erythrocytosis
- **WHO diagnostic criteria** (2008) – must meet both major and 1 minor criteria or the first major and 2 minor criteria
- **Major criteria**
  - Evidence of increased RBC volume, including ≥1 of the following
    - Hgb>18.5 g/dL (men), >16.5 g/dL (women)
    - Hgb or Hct >99th percentile of reference
    - Red cell mass >25% above mean predicted
    - Hgb>17 g/dL (men), Hgb>15 g/dL (women) if associated with sustained increase ≥2 g/dL not attributed to correction of iron deficiency anemia
  - Presence of *JAK2* (V617F) or *JAK2* exon 12 mutations
- **Minor criteria**
  - BM trilineage proliferation
  - Subnormal serum erythropoietin level
  - Endogenous erythroid colony (EEC) growth in vitro

ET

- Must exclude secondary causes of thrombocytosis
- **WHO diagnostic criteria** (2008) – must meet all 4 criteria
  - Sustained platelet count ≥450 x 10^9/L before treatment
  - BM proliferation of enlarged, mature megakaryocytes
  - Does not meet WHO criteria for *BCR-ABL1*-positive CML, PV, PMF, MDS, or myeloid malignancy
  - Demonstration of *JAK2* (V617F), *MPL* gene mutations (eg, W515L and W515K), or no evidence of reactive thrombocytosis

PMF

- **WHO diagnostic criteria (**2008) – must meet all 3 major and 2 minor criteria
- **Major criteria**
  - Megakaryocyte proliferation with atypia in association with reticulin and/or collagen fibrosis **or**, in the absence of reticulin fibrosis, an increase in BM cellularity associated with granulocyte proliferation and often decreased erythrocyte proliferation
  - Does not meet the WHO criteria for *BCR-ABL1*-positive CML, PV, ET, MDS, or myeloid malignancy
  - Presence of *JAK2* V617F or other clonal marker **or** no evidence of reactive marrow fibrosis
- **Minor criteria**
  - Leukoerythroblastosis
  - Increased serum LDH
  - Anemia
  - Palpable splenomegaly

Genetics

Gene – *JAK2*

Structure/function

- Located on 9p24
- Nonreceptor tyrosine kinase involved in cytokine receptor signaling

Mutations

JAK2 V617F mutation

- Present in classic MPNs
  - PV – 90-95%
  - ET – ~50%
  - PMF – 50%
- Mutations rarely found in non-MPNs such as AML, MDS
- Mutation is common in the rare MDS/MPN subtype RARS-T (refractory anemia with ring sideroblasts and thrombocytosis)

JAK2 exon 12 mutation

- Present in the majority of patients with PV who are *JAK2* V617F negative
- Rarely occurs in PMF, ET

Gene – *CALR*

Structure/function

Chromosome 19p13.3-13.2

- *CALR* encodes a calcium-binding chaperone protein involved in glycoprotein folding and calcium homeostasis

Mutations

Exon 9 mutations

- A variety of insertions/deletions (indels) have been identified
- Net result of the exon 9 indels is a frame shift

Prevalence

- Essential thrombocythemia (ET) – 67% of *JAK2* and *MPL* negative cases (Nangalia, 2013)
- Primary myelofibrosis (PMF) – 88% of *JAK2* and *MPL* negative cases

Diagnostic issues

- *CALR* somatic mutations are seen in a subset of patients with MPNs who lack *JAK2* and *MPL* mutations (Nangalia, 2013)
- *CALR* mutations appear restricted to patients with ET and PMF and are not found in patients with PV (Klampfl, 2013; Nangalia, 2013)

Prognostic issues

- *CALR* mutated ET and PMF are associated with increased overall survival when compared with *JAK2* and *MPL* mutated ET/PMF (Rotunno, 2013)
- *CALR* mutated ET is associated with a decreased incidence of thrombosis when compared to *JAK2* mutated ET (Rotunno, 2013)

Gene – *MPL*

Structure/function

- Located on 1p34
- Thrombopoietin receptor; mutation causes downstream signaling and contributes to megakaryocytic myeloproliferation

Mutations

Several acquired mutations in exon 10 have been reported

- W515L and W515K – most common
  - ET – 3-4%
  - PMF – 8-10%
  - PV – rare
- W515A and W515R – rare

Test Interpretation

***JAK2* Gene, V617F Mutation, Qualitative**

Clinical sensitivity

- PV – 95%
- ET – 50%
- PMF – 50%

Results

- Positive – meets a diagnostic criterion for *JAK2* MPN
- Not detected – no evidence of the *JAK2* V617F point mutation

Limitations

- Only one point mutation is detected
- Limit of detection is 0.5% mutant allele

***JAK2* Gene, V617F Mutation, Quantitative**

Clinical sensitivity

- PV – 95%
- ET – 50%
- PMF – 50%

Results

- The percentage of *JAK2* alleles positive for the V617F mutation (allele burden) is reported
  - 0% – no evidence of the *JAK2* V617F mutation
- Lower *JAK2* V617F allele burden in PMF is associated with worse outcome

Limitations

- Only the one point mutation is detected
- Limit of detection is 0.2% mutant allele

***CALR* (Calreticulin) Exon 9 Mutation Analysis by PCR**

Results

- Positive – a mutation in *CALR* exon 9 is detected
  - Highly suggestive of MPN in conjunction with appropriate clinical and histological findings
- Negative – a mutation in *CALR* exon 9 is not detected

Limitations

- Detects only exon 9 indel mutations and does not detect mutations in other regions of the *CALR* gene
- Results must be interpreted in the context of morphological and other relevant data
- Test should not be used alone to diagnose malignancy

***JAK2* Exon 12 Mutation Analysis by PCR**

Clinical sensitivity

- PV – 2%

Results

- Positive – meets a diagnostic criterion for *JAK2* MPN
- Not detected – no evidence of the *JAK2* exon mutation

Limitations

- Only exon 12 mutations are detected
- Limit of detection is 1/1,000 cells

***MPL* codon 515 Mutation Detection by Pyrosequencing, Quantitative**

Clinical sensitivity

- ET – 3-4%
- PMF – 8-10%
- PV – very rare

Results

- Detected
  - Meets a diagnostic criterion for *JAK2* MPN
  - *MPL* codon 515 mutation present (W515K or W515L)
  - Mutant allele burden is reported
- Not detected
  - *MPL* codon 515 mutation not detected

Limitations

- Does not detect mutations in other locations within the *MPL* gene
- Limit of detection for this test is 5% mutant allele

References

- Nangalia J, Massie CE, et al. Somatic CALR mutations in myeloproliferative neoplasms with nonmutated JAK2. N Engl J Med. 2013 Dec 19;369(25):2391-2405
- Klampfl T, Gisslinger H, et al. Somatic mutations of calreticulin in myeloproliferative neoplasms. N Engl J Med. 2013 Dec 19;369(25):2379-2390
- Rotunno G, Mannarelli C, et al. Impact of calreticulin mutations on clinical and hematological phenotype and outcome in essential thrombocythemia. Blood. 2014 Mar 6;123(10):1552-5