Classic **BCR-ABL1**-negative Myeloproliferative Neoplasms

### Indications for Ordering

Diagnose and manage classic **BCR-ABL1**-negative myeloproliferative neoplasms (MPNs)
- Polycythemia vera (PV)
- Essential thrombocytopenia (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 Mutation Detection by Capillary Electrophoresis 2005545**
- May be useful when essential thrombocytopenia 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 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**
  - PDGFR
  - 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 greater than or equal to one 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 four 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 three major and two 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

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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 S505N – rare

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 Mutation Detection by Capillary Electrophoresis
Clinical sensitivity
- ET – 3-4%
- PMF – 8-10%
- PV – very rare

Results
- Detected
  - Meets one diagnostic criterion for JAK2 MPN
  - MPL mutations present (W515K, W515L, W515A, and S505N)
  - 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