Testing Algorithm for Myeloproliferative Neoplasms

Concern for PV
- JAK2 p.V617F
  - If neg
  - JAK2 exon 12
    - If neg
    - Consider CALR, MPL, or panel testing and rethink your diagnosis

Concern for ET or PMF
- JAK2 p.V617F
  - If neg
  - CALR (exon 9) and MPL p.W515
    - If neg
    - Consider panel testing for other diagnostic or prognostic signatures (e.g., ASXL1, TP53)

Concern for CML
- Quantitative BCR/ABL1 at minimum for e13a2 and e14a2
  - If neg
  - FISH for BCR/ABL1
    - If neg
    - Consider panel testing and rethink your diagnosis

Concern for CNL
- CSF3R
  - If neg
  - Consider panel testing for other diagnostic or prognostic signatures (ASXL1, TP53)
    - If neg
    - Consider panel testing for other markers of clonality and poor prognostic signatures (e.g., ASXL1, TP53)

Concern for CEL (vs HES or myeloid/lymphoid neoplasms)
- FISH for FIP1L1/PDGFRα, PDGFRβ, FGFR1, or PCM1-JAK2
  - If neg
  - Consider panel testing for other markers of clonality and poor prognostic signatures (e.g., ASXL1, TP53)

Abbreviations: PV – Polycythemia Vera; ET – Essential Thrombocythemia; PMF – Primary Myelofibrosis; CML – Chronic Myeloid Leukemia; CNL – Chronic Neutrophilic Leukemia; CEL – Chronic Eosinophilic Leukemia; HES – hypereosinophilic syndrome; MPN – myeloproliferative neoplasm; MDS – myelodysplastic syndrome; RS – ring sideroblast; T – thrombocytosis; VAF – variant allele frequency

NOTES:
- Blue lines/box if using only NGS panel testing, green boxes for sequential single gene testing.
- Many of these diagnoses also require proving the absence of BCR/ABL1 by any method.
- A karyotype is also a standard part of the work-up in all cases.

Prepared by the Association for Molecular Pathology Training and Education Committee
For more educational resources, see: www.amp.org/AMPEducation

Revised 10/21
## Genes to Test in Myeloproliferative Neoplasms

### Samples to Test: Peripheral Blood or Bone Marrow  **Abbreviations:** See Opposite Side of Card

<table>
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<tr>
<th>Biomarker</th>
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<th>Result Interpretation/Significance</th>
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<tr>
<td><strong>BCR/ABL1</strong></td>
<td>Philadelphia (Ph) Chromosome Usually p210 fusions - e13a2 and e14a2 Rarely p190 fusion - e1a2 or p230 fusion - e19a2 or fusions involving a3</td>
<td>Diagnosis and monitoring of CML Diagnosis of other MPNs and MDS/MPNs which require exclusion of CML by negative BCR/ABL1 Therapy: ABL1 kinase inhibitors</td>
<td>Diagnostic of CML in the correct clinical context. Also found in some ALLs and AMLs. Reported in International Scale (% IS), used for monitoring CML</td>
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<tr>
<td><strong>JAK2</strong></td>
<td>p.V617F activating hotspot Exon 12 activating substitution mutations</td>
<td>Diagnosis: p.V617F for first line single gene testing for PV (95%), ET (50-70%), and PMF (30-50%) Exon 12 mutations (~5% of PV) Therapy: JAK2 inhibitors</td>
<td>p.V617F not specific for a single disease can be found in a wide range of myeloid neoplasms, associated with thrombosis and erythrocytosis High VAF (&gt;75%) is associated with disease progression</td>
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<td><strong>CALR</strong></td>
<td>Exon 9 frameshift mutations</td>
<td>Diagnosis: CALR exon 9 frameshift in ET (25-30%) and PMF (30-35%)</td>
<td>Exon 9 frameshift not specific for a single disease but are useful to support a diagnosis of ET or PMF</td>
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<td><strong>MPL</strong></td>
<td>p.W515K/L activating hotspot</td>
<td>Diagnosis: MPL p.W515K/L in ET (5%) and PMF (5-10%)</td>
<td>p.W515K/L not specific for a single disease but are useful to support a diagnosis of ET or PMF</td>
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<td><strong>CSF3R</strong></td>
<td>p.T618I activating hotspot Other activating substitutions, predominantly exon 17</td>
<td>Diagnosis: CNL (100%), other myeloid neoplasms Therapy: p.T618I - possible ruxolitinib sensitivity p.S783fs* - possible dasatinib sensitivity</td>
<td>Mutated in nearly all cases of CNL as well as in other myeloid neoplasms Specific mutations suggest therapy</td>
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<tr>
<td><strong>FIP1L1/PDGFR</strong></td>
<td>Usually cryptic deletion involving CHIC2</td>
<td>Diagnosis: identify specific subtype of neoplasms Therapy response: PDGFR/β fusions are typically responsive to kinase inhibitors such as imatinib FGFR1 fusions are typically non-responsive to imatinib and may need newer agents</td>
<td>Fusions involving PDGFR/β, FGFR1, and JAK2 are diagnostic of a specific subgroup of lymphoid and myeloid neoplasms with eosinophilia that are considered in a distinct category from MPNs, but may be considered in the differential of CEL</td>
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<tr>
<td><strong>PDGFRB</strong></td>
<td>Various fusion partners, most commonly ETV6</td>
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<tr>
<td><strong>FGFR1</strong></td>
<td>Various fusion partners</td>
<td></td>
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<tr>
<td><strong>PCM1-JAK2</strong></td>
<td>Also includes other partners of JAK2 (e.g., ETV6 or BCR)</td>
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<td><strong>Panel testing</strong></td>
<td>Includes other partners of JAK2 (e.g., JAK2, CALR, MPL, CSF3R, ASXL1, TET2, EZH2, IDH1/2, DNMT3A, TP53, SF3B1, SRSF2, U2AF1, CBL, N/KRAS, SETBP1, others)</td>
<td>Clonality may be used to support a diagnosis of an MPN in the correct clinical/pathologic context (e.g., triple negative MPNs, CEL vs HES) Specific patterns may suggest other entities SF3B1 mutations may suggest MDS-RS or MDS/MPN-RS-T CBL mutations may suggest CMML</td>
<td>Provides diagnostic information to support single gene testing Adds additional prognostic information (e.g., ASXL1 or TP53 mutations or high number of mutations portend poor prognosis) May suggest pending progression (e.g., NRAS mutations, elevated JAK2 VAF)</td>
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</table>

**Where to Test:** Testing should be performed in the laboratories that are certified under Clinical Laboratory Improvement Amendments of 1988 (CLIA-88) as qualified to perform high complexity (molecular pathology) testing.

**References:** NCCN Guidelines for Chronic Myeloid Leukemia (v1.2018) and Myeloproliferative Neoplasms (v2.2018)

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