Testing Algorithm for Myeloproliferative Neoplasms

**Concern for PV**
- **JAK2 p.V617F**
  - If neg
    - **JAK2 exon 12**
      - If neg
        - **CALR (exon 9) and MPL p.W515**
          - 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**

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

**Concern for CNL**
- **CSF3R**
  - If neg
  - **Consider panel testing for other diagnostic or prognostic signatures**

**Concern for CEL (vs HES or myeloid/lymphoid neoplasms)**
- **FISH for PDGFR, PDGFRB, FGFR1, ABL1, JAK2, or FLT3**
  - 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.
### Genes to Test in Myeloproliferative Neoplasms

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

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<th>Biomarker</th>
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| **BCR/ABL1**    | Philadelphia (Ph) Chromosome  
Usually p210 fusions - e13a2 and e14a2  
Rarely p190 fusion - e1a2 or p230 fusion - e19a2 or fusions involving a3 | 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 | Diagnostic of CML in the correct clinical context.  
Also found in some ALLs and AMLs.  
Reported in International Scale (% IS), used for monitoring CML |
| **JAK2**        | p.V617F activating hotspot  
Exon 12 activating substitution mutations | Diagnosis:  
p.V617F is first line single gene testing for PV (95%), ET (50-70%), and PMF (30-50%)  
Exon 12 mutations (~5% of PV)  
Therapy: JAK2 inhibitors | 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 (>75%) is associated with disease progression |
| **CALR**        | Exon 9 frameshift mutations | Diagnosis:  
CALR exon 9 frameshift in ET (25-30%) and PMF (30-35%) | Exon 9 frameshift not specific for a single disease but are useful to support a diagnosis of ET or PMF |
| **MPL**         | p.W515K/L and SS05N/A activating hotspot | Diagnosis:  
MPL p.W515K/L and SS05N/A in ET (5%) and PMF (5-10%) | p.W515K/L and SS05N/A not specific for a single disease but are useful to support a diagnosis of ET or PMF |
| **CSF3R**       | p.T618I activating hotspot  
Other activating substitutions, predominantly exon 17 | Diagnosis:  
CNL (100%), other myeloid neoplasms  
Therapy:  
P.T618I - possible ruxolitinib sensitivity  
P.S783fs* - possible dasatinib sensitivity | Mutated in nearly all cases of CNL as well as in other myeloid neoplasms  
Specific mutations suggest therapy |
| **PDGFRA/variou** partners | Most common: *FIP1L1::PDGFRA*, cryptic deletion involving CHIC2 AT 4q21 | Diagnosis: identify specific subtype of neoplasms  
Therapy response:  
PDGFRA/B: excellent response to TKI  
FGFR1: high-rate of response to FGFR1 inhibitor, especially in chronic phase  
JAK2: limited response to ruxolitinib  
FLT3: various response to FLT3 inhibitors  
ETV6::ABL1: various response to 2nd generation TKI | Fusions involving PDGFRA/B, FGFR1, JAK2, FLT3, and ETV6::ABL1 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 |
| **PDGFRB/variou** partners | Most common: ETV6 | Diagnosis: define specific subtype of neoplasms  
Therapy response:  
PDGFRA/B: excellent response to TKI  
FGFR1: high-rate of response to FGFR1 inhibitor, especially in chronic phase  
JAK2: limited response to ruxolitinib  
FLT3: various response to FLT3 inhibitors  
ETV6::ABL1: various response to 2nd generation TKI | Fusions involving PDGFRA/B, FGFR1, JAK2, FLT3, and ETV6::ABL1 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 |
| **FGFR1/variou** partners | Most common: *ZMYM2::FGFR1* | Diagnosis: identify specific subtype of neoplasms  
Therapy response:  
PDGFRA/B: excellent response to TKI  
FGFR1: high-rate of response to FGFR1 inhibitor, especially in chronic phase  
JAK2: limited response to ruxolitinib  
FLT3: various response to FLT3 inhibitors  
ETV6::ABL1: various response to 2nd generation TKI | Fusions involving PDGFRA/B, FGFR1, JAK2, FLT3, and ETV6::ABL1 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 |
| **JAK2/variou** partners | Most common: *PCM1-JAK2* | Diagnosis: identify specific subtype of neoplasms  
Therapy response:  
PDGFRA/B: excellent response to TKI  
FGFR1: high-rate of response to FGFR1 inhibitor, especially in chronic phase  
JAK2: limited response to ruxolitinib  
FLT3: various response to FLT3 inhibitors  
ETV6::ABL1: various response to 2nd generation TKI | Fusions involving PDGFRA/B, FGFR1, JAK2, FLT3, and ETV6::ABL1 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 |
| **FLT3**        | Most common: ETV6::FLT3 | Diagnosis: identify specific subtype of neoplasms  
Therapy response:  
PDGFRA/B: excellent response to TKI  
FGFR1: high-rate of response to FGFR1 inhibitor, especially in chronic phase  
JAK2: limited response to ruxolitinib  
FLT3: various response to FLT3 inhibitors  
ETV6::ABL1: various response to 2nd generation TKI | Fusions involving PDGFRA/B, FGFR1, JAK2, FLT3, and ETV6::ABL1 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 |
| **ETV6::ABL1**  | t(9;12(q34;p13.2)) / ETV6::ABL1 | Diagnosis: identify specific subtype of neoplasms  
Therapy response:  
PDGFRA/B: excellent response to TKI  
FGFR1: high-rate of response to FGFR1 inhibitor, especially in chronic phase  
JAK2: limited response to ruxolitinib  
FLT3: various response to FLT3 inhibitors  
ETV6::ABL1: various response to 2nd generation TKI | Fusions involving PDGFRA/B, FGFR1, JAK2, FLT3, and ETV6::ABL1 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 |

**Panel testing including JAK2, CALR, MPL, CSF3R, ASXL1, TET2, EZH2, IDH1/2, DNMT3A, TP53, SF3B1, SRSF2, U2AF1, CBL, N/KRAS, SETBP1, others**  
More prognosis and/or therapy related genes are accrued  
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  
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)  
Some related to therapy, such as IDH1/2

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**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 (v2.2023) and Myeloproliferative Neoplasms (v1.2023)

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