Mutation Analysis in Myeloproliferative Neoplasms

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I. Policy Description

Myeloproliferative neoplasms (MPN) are a heterogeneous group of clonal disorders characterized by overproduction of one or more differentiated myeloid lineages (Grinfeld et al., 2017). These include polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF). The majority of MPN result from somatic mutations in the three driver genes, *JAK2*, *CALR*, and *MPL*, which represent major diagnostic criteria in combination with hematologic and morphological abnormalities (Rumi & Cazzola, 2017).

Terms such as male and female are used when necessary to refer to sex assigned at birth.

II. Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" section of this policy document.

1) For the diagnosis of individuals presenting with clinical, laboratory, or pathological findings suggesting classic forms of myeloproliferative neoplasms (MPN)(e.g., polycythemia vera [PV], essential thrombocythemia [ET], or primary myelofibrosis [PMF]), *JAK2, CALR* or *MPL* mutation testing **MEETS COVERAGE CRITERIA** in any of the following situations:
   a) For individuals suspected to have PV who meet at least **one** of the following testing criteria:
      i. Hemoglobin greater than 16.5 g/dL in men or greater than 16.0 g/dL in women; **or** hematocrit greater than 49% in men or greater than 48% in women; **or** increased red cell mass (more than 25% above mean normal predicted value), **and** no other known cause of erythrocytosis, when measured on two separate occasions.
      ii. A bone marrow (BM) biopsy showing hypercellularity for age with trilineage hyperplasia including prominent erythroid, granulocytic, and megakaryocytic proliferation with pleomorphic, mature megakaryocytes (differences in size).
   b) For individuals suspected to have ET who meet at least **one** of the following testing criteria:

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i. Platelet count greater than or equal to 450 × 10^9/L that has persisted for more than three months.

ii. A BM biopsy showing proliferation mainly of the megakaryocyte lineage with increased numbers of enlarged, mature megakaryocytes with hyperlobulated nuclei. No significant increase or left shift in neutrophil granulopoiesis or erythropoiesis and very rarely minor (grade 1) increase in reticulin fibers.

c) For individuals suspected to have PMF who meet at least **one** of the following testing criteria:

i. The individual has demonstrated leukocytosis of greater than or equal to 11 x 10^9/L on two separate occasions in the absence of other conditions that can cause leukocytosis.

ii. The individual has an enlarged spleen.

iii. A BM biopsy shows megakaryocytic proliferation and atypia, without reticulin fibrosis >grade 1, accompanied by increased age-adjusted BM cellularity, granulocytic proliferation, and often decreased erythropoiesis.

iv. A BM biopsy shows presence of megakaryocytic proliferation and atypia, accompanied by either reticulin and/or collagen fibrosis grades 2 or 3.

2) To exclude a diagnosis of chronic myeloid leukemia (CML) for individuals with a suspected MPN, fluorescence in situ hybridization (FISH) or reverse transcriptase polymerase chain reaction (RT-PCR) testing on a peripheral blood sample to detect *BCR::ABL1* transcripts **MEETS COVERAGE CRITERIA**.

3) For individuals with a clinical suspicion of prePMF or overt PMF who have already tested negative for mutations in *JAK2, CALR,* or *MPL* and who do not meet the WHO criteria for BCR-AB1+ CML, PV, ET, myelodysplastic syndromes, or other myeloid neoplasms, screening for mutations in *ASXL1, CBL, DNMT3A, EZH2, IDH1/IDH2, RAS, SRSF2, SFS3B1*, *TET2, TP53,* and *U2AF1* (see Note 1) **MEETS COVERAGE CRITERIA**.

4) For individuals diagnosed with Budd-Chiari Syndrome, *JAK2, CALR,* or *MPL* mutation testing **MEETS COVERAGE CRITERIA**.

5) For individuals with normal blood counts and unexplained splanchnic vein thrombosis, screening for *JAK2* V617F **MEETS COVERAGE CRITERIA**.

6) For individuals suspected to have chronic neutrophilic leukemia, testing for *CSF3R* mutations **MEETS COVERAGE CRITERIA**.

7) For individuals with a clinical suspicion of mastocytosis, screening for *KIT* D816V **MEETS COVERAGE CRITERIA**.

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of an individual's illness.

8) For all other situations not described above, *JAK2* tyrosine kinase, *CALR*, and *MPL* mutation testing **DOES NOT MEET COVERAGE CRITERIA**.

NOTES:

Note 1: For 5 or more gene tests being run on the same platform, please refer to AHS-R2162 Reimbursement Policy.

III. Table of Terminology

Term	Definition
ABL	*Abelson murine leukemia viral oncogene*
aCML	Atypical chronic myeloid leukemia
ARMS	Amplification refractory mutation system
ASXL1	*additional sex combs like 1, transcriptional regulator*
BCR	Breakpoint cluster region
BPGM	*Bisphosphoglycerate mutase*
BSH	British Society for Haematology
CALR	*Calreticulin*
CBL	*Casitas B-lineage lymphoma* proto-oncogene
CELNOS	Chronic eosinophilic leukemia, not otherwise specified
CML	Chronic myeloid leukemia
CMML	Chronic myelomonocytic leukemia
CNL	Chronic neutrophilic leukemia
CSF3R	*Colony stimulating factor 3 receptor*
DNM3TA	*DNA methyltransferase 3 alpha*
EASL	European Association for the Study of the Liver
ELN	European Leukemia Net
ESMO	European Society of Medical Oncology
ET	Essential thrombocythemia
EZH2	*Enhancer of zeste 2 polycomb repressive complex 2 subunit*
FLT3	*Fms related receptor tyrosine kinase 3*
FLT3-ITD	*FLT3*- internal duplications
HBA1	*Hemoglobin subunit alpha 1*
HBA2	*Hemoglobin subunit alpha 2*
HBB	*Hemoglobin subunit beta*
HSC	Hematopoietic stem cell
HTLV	Human T-lymphotropic virus type 1
IDH1	*Isocitrate dehydrogenase (NADP(+)) 1*

IDH2	*Isocitrate dehydrogenase (NADP(+)) 2*
IR	Ionizing radiation
JAK2	*Janus Kinase 2*
JMML	Juvenile myelomonocytic leukemia
LNK	*Lymphocyte adapter protein*
MONU	MPN unclassifiable
MPL	*MPL proto-oncogene, thrombopoietin receptor*
MPN	Myeloproliferative neoplasms
NCCN	National Comprehensive Cancer Network
PCR	Polymerase chain reaction
PMF	Primary myelofibrosis
PV	Polycythemia vera
RAS	*Rat sarcoma virus* gene
SETBP	*SET binding protein*
SF3B1	*Splicing factor 3b subunit 1*
SH2B3	*SH2B adaptor protein 3*
SRSF2	*Serine and arginine rich splicing factor 2*
STAT	Signal transducer and activator of transcription
TET2	*Tet methylcytosine dioxygenase 2*
TP53	*Tumor protein p53*
U2AF1	*U2 small nuclear RNA auxiliary factor 1*
WHO	World Health Organization

IV. Scientific Background

Myeloproliferative neoplasms, including polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF), arise from somatic mutation in hematopoietic stem cell (HSC) that clonally expand resulting in single or multilineage hyperplasia (Vainchenker & Kralovics, 2017). They are relatively rare, affecting 0.84 (PV), 1.03 (ET), and 0.47 (PMF) per 100,000 people worldwide; however, these may not be reflective of its true incidence due to the high heterogeneity of MPN (Titmarsh et al., 2014).

Myeloproliferative neoplasms share features of bone marrow hypercellularity, increased incidence of thrombosis or hemorrhage, and an increased rate of progression to acute myeloid leukemia. Abnormalities in cytokine signaling pathways are common and usually lead to increased JAK-STAT signaling (Grinfeld et al., 2017). PV is characterized by erythrocytosis with suppressed endogenous erythropoietin production, bone marrow panmyelosis, and *JAK2* mutation leading to constitutive activation. ET is defined by thrombocytosis; bone marrow megakaryocytic proliferation; and presence of *JAK2*, *CALR*, or *MPL* mutation. PMF is characterized by bone marrow megakaryocytic proliferation; reticulin and/or collagen fibrosis; and presence of *JAK2*, *CALR*, or *MPL* mutation (Rumi & Cazzola, 2017). Mutations in other genes involved in signal transduction (*CBL, LNK/SH2B3*), chromatin modification

(*TET2*, *EZH2*, *IDH1/2, ASXL1*, *DNM3TA*), RNA splicing (*SF3B1*, *SRSF2*, *U2AF1*), and tumor suppressor function (*TP53*) have also been reported and are considered "high-risk" (NCCN, 2019, 2023)

The gene *JAK2*, which stands for "Janus Kinase 2," is a gene whose mutation is responsible for a significant amount of MPNs. It is a mutation that causes hypersensitivity of hematopoietic progenitor cells to other cytokines, and this mutation typically appears on red blood cells or bone marrow cells. This mutation is often found on exon 12 or 14, and the exon 14 mutation results in a cytokine-independent activation of several regulatory pathways. *JAK2* mutations contribute to at least 95% of PV cases, about 50-65% of ET cases, and 60-65% of PMF cases (Tefferi, 2022a, 2023a, 2023b).

The gene *MPL*, which encodes a thrombopoietin receptor, also contributes to MPNs. *MPL* mutations result in a similar phenotype to *JAK2* mutations; both result in cytokine-independent growth of their targets. However, *MPL* mutations are not nearly as common as *JAK2* and *CALR* mutations, casting doubt on the clinical utility for testing. *MPL* mutations comprise up to 4% of ET cases and 5% of PMF cases (Tefferi, 2022a, 2023a, 2023b).

The gene *CALR* encodes calreticulin (or calregulin), which is a Ca^{2+} binding protein. The mutation typically involves the creation of the incorrect Ca^{2+} binding region, thereby not allowing the protein to perform its regular duties such as maintaining calcium homeostasis. This results in a similar phenotype to the *JAK2* mutation, which is the cytokine-independent activation of regulatory pathways. *CALR* mutations contribute to approximately 15-25% of ET cases and 20-25% of PMF cases, and about 70% of ET or PMF patients without a *JAK2* or *MPL* mutation have this mutation (Tefferi, 2022a, 2023a, 2023b).

The significance of *JAK2*, *MPL*, *CALR* and other mutations in the genesis of the MPNs as well as their roles in determining phenotype are unclear (Tefferi, 2022b). However, integrated genomic analyses suggest that regardless of diagnosis or *JAK2* mutational status, MPNs are characterized by upregulation of JAK-STAT target genes, demonstrating the central importance of this pathway in the pathogenesis (Rampal et al., 2014). This may lead to development of novel *JAK2* therapeutics (Silvennoinen & Hubbard, 2015). Thus, mutation analysis at the time of diagnosis has value for determining prognosis as well as individual risk assessment and guide treatment-making decisions (Hussein et al., 2013; Tefferi, 2022b).

Neutrophilia, an increase in peripheral blood neutrophils at least two standard deviations above the mean, can be associated with any the MPNs. In chronic neutrophilic leukemia (CNL), *CSF3R* mutations have been discovered in most patients with CNL (Coates, 2022; Tefferi et al., 2014). A study released in 2013 reported 16 of 27 patients with CNL or atypical chronic myeloid leukemia (aCML) had activating mutations in *CSF3R* (Maxson et al., 2013). *SETBP1* has also been used as a part of comprehensive mutation profiling in distinguishing aCML and chronic myelomonocytic leukemia (CMML). A 2019 NGS study reports significant differences in the profiles of patients with aCML or CMML when comparing *TET2*, *SETBP1*, and *CSF3R*. The researchers conclude, "differential mRNA expression could be detected between both cohorts in a subset of genes (*FLT3*, *CSF3R*, and *SETBP1* showed the strongest correlation). However, due to high variances in the mRNA expression, the potential utility for the clinic is limited" (Faisal et al., 2019).

Proprietary Testing

In 2017 the FDA approved ipsogen® *JAK2* RGQ PCR Kit (FDA, 2017b) to detect Janus Tyrosine Kinase 2 (*JAK2*) gene mutation G1849T (V617F) with an allele-specific, quantitative, polymerase chain reaction (PCR) using an amplification refractory mutation system (ARMS). The device marketing authorization was based on data from a clinical study of 473 suspected patients with MPNs, 276 with suspected PV, 98 with suspected ET, and 99 with suspected PMF. The study compared results from the ipsogen *JAK2* RGQ PCR Kit to results obtained with independently validated bi-directional sequencing. The study found that the ipsogen *JAK2* RGQ PCR Kit test was in 96.8% agreement with the reference method, 100% in positive agreement, and 95.1% in negative agreement, with 458 samples in agreement out of 473. The concordance with each condition was also high; agreement of 90.8% within the ET samples (89/98), 94.9% agreement within the PMF samples (94/99), and 99.6% within the PV samples (275/276). All three conditions had positive agreements of 100%. The authors went on to note that the 15 samples with disagreeing results had mutation levels under the detection capability of bi-directional sequencing. To validate these 15 samples, an independently validated NGS panel was used to compare results with the kit, and all 15 samples were found to test positive, thereby agreeing with the kit. The authors concluded that the kit was accurate for any mutation levels at or above 1% (FDA, 2017a).

Other proprietary tests are available for mutational analysis in MPN. IntelliGEN® Myeloid is a NGS assay that analyzes fifty genes for somatic mutations that could be useful in providing diagnostic or prognostic information for patients with MDS, AML, or MPN (labcorp, 2023). The LeukoVantage® Myeloid Neoplasm Mutation Panel detects myeloid neoplasm-associated mutations in 48 genes associated with AML, MDS, and MPN. The LeukoVantage AML panel can be used to assess AML subclass and prognosis based on genetic abnormalities in *NPM1, CEBPA*, and *RUNX1* (Quest_Diagnostics, 2020). NeoGenomics offers two tests which include the MPN Reflex Test and NeoTYPE® Myeloid Disorders Profile. The MPN Reflex Test is a sequential testing panel for qualitative detection of *JAK2* V617F*, JAK2 Exon 12-14, CALR exon 9*, and *MPL exon 10 (NeoGenomics, 2022a).* NeoTYPE® Myeloid Disorders Profile is a 63 gene panel that targets known mutations associated with AML, MPN, MDS, CML, chronic myelomonocytic leukemia (CMML) and juvenile myelomonocytic leukemia (JMML) (NeoGenomics, 2022b). Centogene has released a Myeloid Tumor Panel which targets 35 genes that are associated with myeloid malignancies which also include AML, MPN, MDS, CML, CMML, and JMML (Centogene, 2022).

Analytical Validity

Poluben et al. (2019) analyzed the characteristics of myeloproliferative neoplasms (MPN) in patients exposed to ionizing radiation (IR) from the 1986 Chernobyl accident. 281 patients (90 exposed to radiation, 181 unexposed) were included. *JAK2*, *MPL*, and *CALR* mutations were identified. IR-exposed patients had several different genetic features compared to the unexposed cohort: lower rate of *JAK2* V617F mutations (58.4% vs 75.4%), higher rate of type 1-like *CALR* mutations (12.2% vs 3.1%), higher rate of triple-negative cases (27.8% vs 16.2%), and higher rate of “potentially pathogenic” sequence variants (4.8 vs 3.1). The authors suggested IR-exposed patients as a cohort with “distinct” genomic characteristics (Poluben et al., 2019).

Rosenthal et al. (2021) studied the analytical validity of a 48-gene NGS panel for detecting mutations in myeloid neoplasms. The panel detects detect single nucleotide variations (SNVs), insertions/deletions, and *FLT3* internal tandem duplications (*FLT3*-ITD). 184 samples were analyzed using the 48-gene panel and compared to those identified by a 35-gene hematologic neoplasms panel using an additional 137 samples. Analytical validation yielded 99.6% sensitivity and 100% specificity. Concordance of variants detected by the 2 tested panels was 100%. "Among patients with suspected myeloid neoplasms, 54.5% patients had at least one clinically significant mutation: 77% in AML patients, 48% in MDS, and 45% in MPN." The authors conclude that "the assay can identify mutations associated with diagnosis, prognosis, and treatment options of myeloid neoplasms even in technically challenging genes" (Rosenthal et al., 2021).

Clinical Utility and Validity

An Argentinean study focusing on establishing the frequency of *JAK2*, *MPL*, and *CALR* mutations and comparing their clinical and hematological features corroborates this importance. Mutations of *JAK2*V617F, *JAK2* exon 12, *MPL* W515L/K and *CALR* were analyzed in 439 patients with *BCR-ABL1*-negative MPN, and it was demonstrated that these mutations were present in 94.9% of the cases of polycythemia vera (PV), 85.5% in patients with essential thrombocythemia (ET), and 85.2% with primary myelofibrosis, leading the researchers to conclude that "the combined genetic tests of these driver mutations are essential for accurate diagnoses of *BCR-ABL1*-negative MPN" (Ojeda et al., 2018).

V. Guidelines and Recommendations

World Health Organization (WHO)

The 2017 edition of the World Health Organization's classification of myeloid neoplasm and acute leukemia proposed the following criteria for the diagnosis of PV, ET and PMF.

WHO Criteria for PV
Diagnosis of PV requires meeting either all three major criteria, or the first two major criteria and the minor criterion:

Major Criteria

1. Hemoglobin >16.5 g/dL in men; Hemoglobin >16.0 g/dL in women, or
   Hematocrit >49% in men; Hematocrit >48% in women, or
   Increased red cell mass (More than 25% above mean normal predicted value)
2. Bone marrow biopsy showing hypercellularity for age with trilineage growth (panmyelosis) including prominent erythroid, granulocytic, and megakaryocytic proliferation with pleomorphic, mature megakaryocytes (differences in size)
3. Presence of *JAK2V617F* or *JAK2* exon 12 mutation

Minor Criteria
Subnormal serum erythropoietin level

WHO Criteria for ET
Diagnosis of ET requires meeting all four major criteria or the first three major criteria and the minor criterion:

Major Criteria

1. Platelet count ≥450 × 10^9/L
2. Bone marrow biopsy showing proliferation mainly of the megakaryocyte lineage with increased numbers of enlarged, mature megakaryocytes with hyperlobulated nuclei. No significant increase or left shift in neutrophil granulopoiesis or erythropoiesis and very rarely minor (grade 1) increase in reticulin fibers
3. Not meeting WHO criteria for BCR-ABL1+ CML, PV, PMF, myelodysplastic syndromes, or other myeloid neoplasms
4. Presence of *JAK2*, *CALR*, or *MPL* mutation

Minor Criteria

Presence of a clonal marker or absence of evidence for reactive thrombocytosis

WHO Criteria for PrePMF

Diagnosis of prePMF requires meeting all three major criteria, and at least one minor criterion:

Major Criteria

1. Megakaryocytic proliferation and atypia, without reticulin fibrosis >grade 1, accompanied by increased age-adjusted BM cellularity, granulocytic proliferation, and often decreased erythropoiesis
2. Not meeting the WHO criteria for BCR-ABL1$^+$ CML, PV, ET, myelodysplastic syndromes, or other myeloid neoplasms
3. Presence of *JAK2*, *CALR*, or *MPL* mutation or in the absence of these mutations, presence of another clonal marker (e.g., *ASXL1*, *EZH2*, *TET2*, *IDH1/IDH2*, *SRSF2*, *SF3B1*), or absence of minor reactive BM reticulin fibrosis

Minor Criteria (presence of one of the following):

1. Anemia not attributed to a comorbid condition
2. Leukocytosis ≥11 × 10^9/L
3. Palpable splenomegaly
4. LDH increased to above upper normal limit of institutional reference range

The minor criteria must be confirmed in two consecutive determinations.

WHO Criteria for Overt PMF

Diagnosis of overt PMF requires meeting all three major criteria, and at least 1 minor criterion

Major Criteria

1. Presence of megakaryocytic proliferation and atypia, accompanied by either reticulin and/or collagen fibrosis grades 2 or 3
2. Not meeting WHO criteria for ET, PV, BCR-ABL1$^+$ CML, myelodysplastic syndromes, or other myeloid neoplasms

3. Presence of *JAK2*, *CALR*, or *MPL* mutation or in the absence of these mutations, presence of another clonal marker (e.g., *ASXL1*, *EZH2*, *TET2*, *IDH1/IDH2*, *SRSF2*, *SF3B1*), or absence of reactive myelofibrosis

<u>Minor Criteria</u>

1. Anemia not attributed to a comorbid condition
2. Leukocytosis ≥11 × 10^9/L
3. Palpable splenomegaly
4. LDH increased to above upper normal limit of institutional reference range
5. Leukoerythroblastosis (Barbui, Thiele, et al., 2018)

These guidelines also list four additional "clinicopathologic entities" for MPNs: "chronic myeloid leukemia (CML), chronic neutrophilic leukemia (CNL), chronic eosinophilic leukemia, not otherwise specified (CELNOS) and MPN, unclassifiable (MPN-U)". The guidelines note that although *CSF3R* mutations are "specific" to WHO-defined CNL, they also remark that "the presence of a membrane proximal *CSF3R* mutation in a patient with neutrophilic granulocytosis should be sufficient for the diagnosis of CNL, regardless of the degree of leukocytosis" (Barbui, Thiele, et al., 2018).

European LeukemiaNet (ELN)

ELN guidelines also recommend "strict adherence" to these guidelines for the three categories of Philadelphia-negative MPNs, (i.e. ET, PV, and MF) (Barbui, Tefferi, et al., 2018).

However, they also recommend "searching" for complementary clonal markers such as *ASXL1*, *EZH2*, *IDH1/2*, and *SRSF2* for patients that tested negative for the three driver mutations and have bone marrow features as well as a clinical phenotype consistent with myelofibrosis (Barbui, Tefferi, et al., 2018).

National Comprehensive Cancer Network (NCCN)

The NCCN Guidelines Version 1.2024 Myeloproliferative Neoplasms recommends molecular testing for *JAK2* V617F mutations as part of an initial workup for all patients. If *JAK2* mutation testing is negative, molecular testing for *CALR* and *MPL* mutations should be performed for patients with suspected ET and MF, and molecular testing for *JAK2* exon 12 should be done for patients who test negative for *JAK2* but are suspected of PV. An NGS panel including *JAK2*, *CALR*, and *MPL* may also be used for the workup of all patients. The NCCN follows the 2017 edition of the WHO diagnostic criteria for all three conditions. The NCCN does state that NGS "may be useful to establish clonality in selected circumstances (e.g., triple negative non-mutated *JAK2*, *MPL*, and *CALR*)". The NCCN includes a list of somatic mutations with prognostic significance in individuals with MPN that includes the *ASXL1*, *EZH2*, *RAS, IDH1/2*, *SRSF2*, *TP53*, *U2AF1* Q157, *DNMT3A,* and *CBL*.

For individuals suspected of MPN, the NCCN recommends excluding a diagnosis of chronic myeloid leukemia: "Fluorescence in situ hybridization (FISH) or a multiplex reverse transcriptase polymerase chain reaction (RT-PCR), if available, on peripheral blood to detect *BCR::ABL1* transcripts and exclude the diagnosis of CML is especially recommended for patients with left-shifted leukocytosis and/or thrombocytosis with basophilia" (NCCN, 2023).

British Society for Haematology (BSH)

The BSH recommends testing for *CALR* for patients suspected of ET and PMR, as *CALR* mutations account for most patients without either a *JAK2* or *MPL* mutation. The authors found that as many as one third of ET and PMF patients had a mutation in exon 9 of the *CALR* gene (Harrison et al., 2014).

The BSH also published guidelines on the diagnosis of polycythaemia vera (PV). In it, they divide PV into *JAK2*-positive and *JAK2*-negative PV. For *JAK2*-positive PV, the only two diagnostic criteria are as follows:

- "High haematocrit (>0·52 in men, >0·48 in women) OR raised red cell mass (>25% above predicted)"
- "Mutation in *JAK2*"

For *JAK2*-negative PV, the diagnostic criteria are as follows (requiring A1-A4, as well as another "A" criteria or two "B" criteria).

- "A1 Raised red cell mass (>25% above predicted) OR haematocrit ≥0·60 in men, ≥0·56 in women"
- "A2 Absence of mutation in *JAK2*"
- "A3 No cause of secondary erythrocytosis"
- "A4 Bone marrow histology consistent with polycythaemia vera"
- "A5 Palpable splenomegaly"
- "A6 Presence of an acquired genetic abnormality (excluding BCR-ABL1) in the haematopoietic cells"
- "B1 Thrombocytosis (platelet count >450 × 10^9 /l)"
- "B2 Neutrophil leucocytosis (neutrophil count >10 × 10^9 /l in non-smokers, ≥12.5 × 10^9 /l in smokers)"
- "B3 Radiological evidence of splenomegaly"
- "B4 Low serum erythropoietin"

The guidelines also note that investigation of erythrocytosis should be undertaken to properly identify the diagnosis. The BSH remarks that EPO receptor mutations may be a primary cause for erythrocytosis and that *EGNL1*, *VHL*, and *EPAS1* mutations may be a secondary cause. Other hemoglobinopathies caused by mutations in genes such as *HBA1*, *HBA2*, *HBB*, or *BPGM* may also be a factor (McMullin et al., 2019).

In 2021, the BSH published guidelines on the use of genetic tests to diagnose and manage patients with myeloproliferative neoplasms. The following recommendations were made:

1. "Molecular screening for *JAK2*, *CALR* and *MPL* variants as appropriate is recommended in patients with persistent erythrocytosis or thrombocytosis (GRADE 1B).
2. Screening for *JAK2* V617F is recommended in cases with normal blood counts and unexplained splanchnic vein thrombosis (GRADE 1B) and may be considered in selected patients with unexplained cerebral vein thrombosis (GRADE 2C).

3. Screening for *CALR* variants may be considered in patients with splanchnic vein thrombosis or cerebral vein thrombosis (GRADE 2C).
4. Screening for *JAK2*, *CALR* and *MPL* variants should be considered for patients with arterial or unprovoked venous thrombosis who have a mildly or variably elevated haematocrit or platelet count that persists for 2–3 months (GRADE 2C).
5. *BCR–ABL1* should be excluded in cases with persistent thrombocytosis negative for *JAK2, CALR* and *MPL* variants or with atypical features (GRADE 1B).
6. Younger patients (e.g., under 60 years) with bone marrow histology typical of ET [or myeloproliferative neoplasm, unclassifiable (MPN-U) or suspected prefibrotic MF] where confirmation of a clonal disorder would be useful in view of the patient's likely long-term disease course and ideally where a broad panel that covers non-canonical variants in *JAK2* and *MPL* and a range of other driver genes is available.
7. Patients with significant thrombocytosis (e.g., platelet count > 600 × 10^9/l), no reactive cause and borderline bone marrow histology, where cytoreduction would be indicated if there was convincing evidence of a clonal disorder. Examples would include those with an unexplained thrombotic event, particularly younger patients. For older patients without thrombosis, testing may be considered but results must be interpreted with caution in view of the possibility of incidental CH.
8. A myeloid gene panel and cytogenetic analysis (or equivalent) is recommended for patients with bone marrow histology and clinical features consistent with PMF (+/− suggestive features of MDS or MDS/MPN) who test negative for *JAK2*/*CALR*/*MPL* (GRADE 1B).
9. A myeloid gene panel and cytogenetic analysis (or equivalent) is not recommended for most patients with *JAK2*/*CALR*/*MPL*-negative erythrocytosis or thrombocytosis but may be considered in individual cases (GRADE 2C).
10. Myeloid gene panel testing is recommended for MPN cases who test positive for *JAK2*/*CALR*/*MPL* mutations and have additional cytopenias(s) at diagnosis, unexplained ring sideroblasts or other dysplasia, increased blasts (including blastic transformation), peripheral-blood monocytosis or atypical clinical features (GRADE 1B).
11. Myeloid gene panel testing and conventional karyotyping are recommended for all patients with PMF, post-PV or post-ET MF who are candidates for allogeneic stem cell transplant (GRADE 1B).
12. Myeloid gene panel testing should be considered for other patients if the additional genomic data will guide clinical management (GRADE 2C).
13. High-sensitivity assays of mutant allele burden are recommended following post-allogeneic stem cell transplant to monitor for residual disease (GRADE 1C).
14. Quantitative assays of mutant allele burden are not recommended for most MPN patients but may be considered where demonstration of molecular response would influence clinical management (GRADE 2C).
15. Patients with persistent eosinophilia should be investigated initially for *FIP1L1–PDGFRA* by FISH and/or nested RT-PCR (GRADE 1B).
16. BM cytogenetics or FISH is recommended to screen for other fusion genes, which must then be confirmed by molecular methods (GRADE 1B).
17. Myeloid gene panel and *KIT* D816V testing should be considered for patients with persistent unexplained eosinophilia who test negative for fusion genes (GRADE 2B).

18. Testing for *CSF3R* variants, preferably as part of wider myeloid panel, is recommended for all patients with suspected CNL (Grade 2B).
19. Sensitive testing for *KIT* D816V is recommended for all patients with a clinical suspicion of mastocytosis (GRADE 1B).
20. If negative for *KIT* D816V, screening for other *KIT* mutations should be considered for adults (but is recommended for children) (GRADE 1B).
21. Myeloid panel analysis is recommended for patients with advanced SM who are candidates for allogeneic stem cell transplantation (GRADE 1B).
22. Myeloid panel analysis may be considered for other SM patients if the apparent aggressiveness of the disease might influence options for therapy (GRADE 2B).
23. Myeloid panel and/or BM cytogenetics should be considered to characterise the AHN component of SM-AHN (GRADE 2B).
24. *BCR–ABL1* should be excluded in all cases of suspected MDS/MPN, and rearrangements associated with MLN-eo should be excluded in cases with eosinophilia (GRADE 1B).
25. Myeloid gene panel analysis and BM cytogenetics or SNP array is recommended for patients diagnosed with MDS/MPN and for cases with suspected MDS/MPN but with indeterminate morphology (GRADE 1B)" (Cross et al., 2021).

European Association for the Study of the Liver (EASL)

For myeloproliferative neoplasms, the EASL recommends testing for *JAK2* V617F mutations in splanchnic vein thrombosis patients, as well as patients with normal peripheral blood cell counts. If the *JAK2* mutation test is negative, a calreticulin mutation test should be performed, and if both are negative, a bone marrow histology analysis should be performed (EASL, 2016).

European Society of Medical Oncology (ESMO)

The ESMO recommends that anyone with a suspected MPN be tested for the three driver mutations (*JAK2*, *CALR*, *MPL*) and that genotyping should be obtained at diagnosis. However, the ESMO states that it is not recommended to repeat testing in follow-up or assessing response to treatment, except for "allogeneic stem-cell transplantation and possibly interferon treatment". For these two assessments a detection limit of ≤1% is recommended. The ESMO also notes that conventional sequencing methods (PCR, melting analysis) may be used for detecting mutations (Vannucchi et al., 2015).

VI. Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website. For the most up-to-date Medicaid policies and coverage, visit the applicable state Medicaid website.

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Food and Drug Administration (FDA)

On July 28, 2017 the FDA approved ipsogen® JAK2 RGQ PCR Kit (FDA, 2017b) to detect Janus Tyrosine Kinase 2 (JAK2) gene mutation G1849T (V617F) with an allele-specific, quantitative, polymerase chain reaction (PCR) using an amplification refractory mutation system (ARMS). This is the first FDA-authorized test intended to help physicians in evaluating patients for suspected Polycythemia Vera (PV). However, the FDA specifically states that this test is not intended for a stand-alone diagnosis of an MPN, nor can it detect less common mutations for MPN such as an exon 12 mutation (FDA, 2017a).

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA ’88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

VII. Important Reminder

The purpose of this Medical Policy is to provide a guide to coverage. This Medical Policy is not intended to dictate to providers how to practice medicine. Nothing in this Medical Policy is intended to discourage or prohibit providing other medical advice or treatment deemed appropriate by the treating physician.
Benefit determinations are subject to applicable member contract language. To the extent there are any conflicts between these guidelines and the contract language, the contract language will control.

This Medical Policy has been developed through consideration of the medical necessity criteria under Hawaii's Patients' Bill of Rights and Responsibilities Act (Hawaii Revised Statutes §432E-1.4) or for QUEST members, under Hawaii Administrative Rules (HAR 1700.1-42), generally accepted standards of medical practice and review of medical literature and government approval status.

HMSA has determined that services not covered under this Medical Policy will not be medically necessary under Hawaii law in most cases. If a treating physician disagrees with HMSA's determination as to medical necessity in a given case, the physician may request that HMSA reconsider the application of the medical necessity criteria to the case at issue in light of any supporting documentation.

Genetic testing is covered for level 1 or 2A recommendations of the National Comprehensive Cancer Network (NCCN and in accordance with Hawaii’s Patients’ Bill of Rights and Responsibilities Act (Hawaii Revised Statutes §432E-1.4) or for QUEST members, the Hawaii Administrative Rules (HAR 1700.1-42).

VIII. Evidence-based Scientific References

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IX. Policy History

Action Date	Action
June 01, 2023	Policy created
December 03, 2024	Policy approved by Medical Directors
December 20, 2024	Policy approved at UMC
February 01, 2025	Policy effective date following notification period