Myeloid Mutation Panel for Classifying Acute Myeloid Leukemia, Myelodysplastic Syndrome, or Myeloproliferative Neoplasia

A panel targeting selected genes facilitates diagnosis, classification, and selection of therapy in patients with acute myeloid leukemia (AML), myelodysplastic syndrome (MDS), or myeloproliferative neoplasia (MPN). Mutation hotspots in up to 26 genes are examined using massive parallel sequencing (next-generation sequencing) in neoplastic blood or bone marrow specimens. The method of testing and rationale for testing depends on which of three myeloid tumors is under consideration:

1) **Acute Myeloid Leukemia/Myelodysplastic Syndrome**: A 21-gene hotspot panel informs prognosis of MDS or AML. In patients with potential MDS, presence of a mutation provides evidence of clonality to help confirm neoplasia in concert with marrow morphology. In patients with AML, we automatically perform a test for FLT3 internal tandem duplication to refine prognosis. When NPM1 mutation is detected, we automatically perform NPM1 Q-rtPCR to determine whether the patient is a candidate for minimal residual disease monitoring.

2) **Myeloproliferative Neoplasm**: A 5-gene hotspot panel informs diagnosis and management of MPN. JAK2, MPL or CALR mutation is detected in >95% of polycythemia vera, 90% of primary myelofibrosis, and 80% of essential thrombocythemia cases. JAK2 inhibitor drugs are available for primary myelofibrosis patients. Risk of thrombosis is impacted by genotype in essential thrombocythemia patients. SETBP1 mutation is detected in 25% of patients with atypical chronic myeloid leukemia. CSF3R mutation is detected in the majority of patients with chronic neutrophilic leukemia.

**Clinical Indications for Myeloid Mutation Panel testing:**

1) **For AML**: Refine classification and prognosis of newly diagnosed AML.
2) **For MDS**: Demonstrate clonality to assist in diagnosis, and refine prognosis.
3) **For MPN**: In an adult with persistently elevated blood counts, demonstrate clonality to assist in diagnosis, and to refine prognosis.

**Specimen Requirements for the Myeloid Mutation Panel:**

Bone marrow aspirate (1 mL, EDTA) or peripheral blood (3mL, EDTA) having at least 10% neoplastic cells and refrigerated for up to 24 hours. Unacceptable sample types include: fresh or frozen solid tissue or paraffin embedded tissue. The assay is generally sensitive to variants above 5% allele frequency (10% clonal cells). Therefore, a minimum blast percentage (or neoplastic cell burden) of at least 10% is required. This test is NOT appropriate for monitoring minimal residual disease.

**Gene Regions Tested** – DNA is sequenced using Illumina TruSight reagents on a MiSeq instrument:

### For AML and MDS:

- **ASXL1**: exon 12
- **BCOR**: all exons
- **CEBPA**: exon 1 (partial)*
- **DNMT3A**: all exons
- **ETV6/TEL**: all exons
- **EZH2**: all exons
- **FLT3**: exons 14,15,20**
- **IDH1**: exon 4
- **IDH2**: exon 4
- **KIT**: exons 2, 8-11,13,17
- **NPM1**: exon 11***
- **NRAS**: exons 2,3
- **RUNX1**: all exons
- **SF3B1**: exon 13-16
- **SRSF2**: exon 1
- **STAG2**: exons 3-6, 8-35****
- **TET2**: exons 3-11
- **TP53**: exons 2-11
- **U2AF1**: exons 2,6
- **WT1**: exons 7,9

### For MPN:

- **CALR**: exon 9
- **CSF3R**: exons 14-17
- **JAK2**: exons 12,14
- **MPL**: exon 10
- **SETBP1**: exon 4 (partial)

* CEBPA N-terminal domain and bZIP region includes ~70% of reported mutations
** FLT3 internal tandem duplication test is reflexively ordered for patients with AML.
***Canonical mutations often mistakenly described in literature as occurring in exon 12 of NPM1.
****Regions encoding amino acids 522 – 527 of STAG2 are not covered by this assay.
Limitations:

Gene amplifications, translocations, and insertions or deletions over 25 bases in length are not detectable by this assay, although the most common deletion variants in the CALR gene are detected. Variants predicted to be non-deleterious (such as synonymous coding changes and population variants) are not reported. Lack of mutation does not exclude myeloid neoplasia. Given that clonal mutation is common in older individuals without myeloid malignancy, correlation with clinicopathologic information is required. Normal tissue is not tested to determine whether a gene variant is somatic (acquired) or germline (heritable). If the patient has evidence of a heritable cancer syndrome (e.g., different tumor types, early age of onset, family history), genetic counseling is recommended.

References:


To consult a pathologist about indications for testing or the significance of a result, call the Molecular Genetics Lab at (984) 974-1825 or Dr. Patel at (984) 974-1454. E-mail: nirali_patel@med.unc.edu


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