UNC Myeloid Mutation Panel: For Acute Myeloid Leukemia (AML), Myelodysplastic Syndrome (MDS), or Myeloproliferative neoplasms (MPN)

The UNC Molecular Genetics Laboratory performs a myeloid mutation panel targeting selected regions of multiple genes using next-generation sequencing to facilitate disease classification and to guide selection of therapy.

Rationale for testing:
Testing for the presence of somatic gene mutations may assist in diagnosis, prognosis, and therapy selection for myeloid disorders. All regions listed are analyzed in both the AML and MDS/MPN panel. The AML panel includes *FLT3*-ITD/TKD testing and RNA extraction for possible quantitative *NPM1* testing.

1) **AML:** Mutational information impacts World Health Organization classification and prognosis in AML. For instance, AML with *NPM1* mutation or biallelic *CEBPA* mutation are recognized as distinct entities, which generally have favorable prognosis. To identify patients who may benefit from targeted therapy with midostaurin, a separate *FLT3*-ITD/TKD panel is included with this order. For specimens that test positive for *NPM1* mutation, the *NPM1* Q-rtPCR assay will be ordered to determine whether that test may be used for minimal residual disease (MRD) monitoring.

2) **MDS:** The presence of a somatic mutation may assist diagnosis by supporting the presence of a clonal process. In patients with confirmed MDS, *SF3B1* mutation may confer a favorable prognosis, whereas mutation in *ASXL1, BCOR, CBL, ETV6, EZH2, NRAS, PPM1D, RUNX1, SETBP1, SRSF2, STAG2, TP53,* or *U2AF1* is associated with less favorable outcome.

3) **MPN:** The presence of a somatic mutation may assist diagnosis by supporting the presence of a clonal process. In addition, many *BCR-ABL1*-negative MPNs are associated with characteristics mutations, such as *JAK2* mutation polycythemia vera, *JAK/CALR/MPL* mutation in essential thrombocythemia and primary myelofibrosis, and *CSF3R* mutation chronic neutrophilic leukemia. Mutations in additional genes on this panel have prognostic significance in MPNs.

Clinical Indications for Myeloid Mutation Panel testing:
1) **For AML:** Refine classification and prognosis.
2) **For MDS:** Demonstrate clonality to assist in diagnosis and refine prognosis.
3) **For MPN:** Assist in diagnosis and prognosis of polycythemia vera, essential thrombocythemia, primary myelofibrosis, chronic neutrophilic leukemia, and other *BCR-ABL1*-negative MPNs.

Specimen Requirements for the Myeloid Mutation Panel:
Bone marrow aspirate (1 mL, EDTA) or peripheral blood (3mL, EDTA) having at least 30% myeloid cells, and refrigerated for up to 72 hours. Unacceptable sample types include: fresh, frozen, or paraffin embedded tissue. The assay is sensitive to variants above 5% allele frequency (10% clonal cells). This test is NOT appropriate for MRD monitoring. For patients undergoing repeat testing, previously detected variants will be reported to 3% VAF.

Gene Regions Tested – These regions are covered by both the AML and MDS/MPN Panel

ABL1 (exons 4-9)
ASXL1 (exons 8-12)
BCOR (exons 2-15)
BRAF (exon 15)
CALR (exon 9)
CBL (exons 8,9)
CEBPA (full coverage)
CSF3R (exons 4-17)
DNMT3A (all exons)
ETV6 (all exons)
EZH2 (all exons)
FLT3 (exons 13,14,15,20)
HRAS (exons 2,3)
IDH1 (exon 4)
IDH2 (exons 4,5)
JAK2 (all exons)
KIT (exons 2,8-13,17-19)
KRAS (exons 2,3)
MPL (exon 10)
MYD88 (all exons)
NOTCH1 (all exons)
NPM1 (exons 11,12)
NRAS (exons 2,3)
PPM1D (all exons)
PTPN11 (exons 3, 7-13)
RUNX1 (all exons)
SETBP1 (exon 4)
SF3B1 (exons 10-16)
SRSF2 exons (all exons)
STAG2 (all exons)
TET2 (all exons)
TP53 (all exons)
U2AF1 (exons 2,6)
WT1 (exons 6-10)
ZRSR2 (all exons).

Limitations:
Gene amplifications, translocations, and insertions or deletions over 90 bases in length are not reliably detected by this assay. 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. Presence of clonality does not establish a diagnosis of malignancy. 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:

1. National Comprehensive Cancer Network (NCCN) Clinical Practice Guidelines in Oncology: Acute Myeloid Leukemia, www.nccn.org
2. Mrózek K, et al. Prognostic significance of the European LeukemiaNet standardized system for reporting cytogenetic and molecular alterations in adults with acute myeloid leukemia. J Clin Oncol. 2012; 30(36):4515-23. PMID: 22987078
3. Levis M. FLT3 mutations in acute myeloid leukemia: what is the best approach in 2013? Hematology Am Soc Hematol Educ Program. 2013;2013:220-6. PMID: 24319184
4. Chen W et al. Nucleophosmin gene mutations in acute myeloid leukemia. Arch Pathol Lab Med. 2006;130:1687.
5. Cazzola M, Kralovics R. From Janus kinase 2 to calreticulin: the clinically relevant genomic landscape of myeloproliferative neoplasms. Blood. 2014 Apr 30, PMID: 24786775
6. Tefferi A, Pardanani A. Genetics: CALR mutations and a new diagnostic algorithm for MPN. Nat Rev Clin Oncol. 2014 Mar;11(3):125-6. PMID: 24514146
7. Makishima H, et al. Somatic SETBP1 mutations in myeloid malignancies. Nat Genet. 2013 Aug;45(8):942-6. PMID: 23832012
8. Maxson JE, et al. Oncogenic CSF3R mutations in chronic neutrophilic leukemia and atypical CML. N Engl J Med. 2013 May 9;368(19):1781-90. PMID: 23656643
9. Bejar R, et al. Clinical effect of point mutations in myelodysplastic syndromes. N Engl J Med. 2011 Jun 30;364(26):2496-506. PMID: 21714648
10. Bejar R, et al. Clinical and genetic predictors of prognosis in myelodysplastic syndromes. Haematologica. 2014 Jun;99(6):956-64. PMID:24881041
11. Malcovati L, et al. Clinical significance of SF3B1 mutations in myelodysplastic syndromes and myelodysplastic/myeloproliferative neoplasms. Blood. 2011 Dec 8;118(24):6239-46. PMID: 21998214
12. McClure, Rebecca F. et al. Clinical Significance of DNA Variants in Chronic Myeloid Neoplasms. The Journal of Molecular Diagnostics , Volume 20 , Issue 6 , 717 - 737

Questions?
Call the Molecular Genetics Lab at (984) 974-1825 or Dr. Nathan Montgomery at 919-445-6414 E-mail Nathan.Montgomery@unchealth.unc.edu

Website= http://labs.unchealthcare.org/directory/molecular_pathology/index_html