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

The UNCH Molecular Genetics Laboratory performs a myeloid mutation panel targeting selected 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 below are analyzed in both the AML and MDS/MPN panel. The AML panel additionally includes *FLT3*-ITD/TKD testing and RNA extraction for possible quantitative *NPM1* testing.

1) **AML:** Mutational information impacts World Health Organization (and International Consensus) Classification and prognosis. For instance, AML with *NPM1* mutation or AML with *CEBPA* mutation are recognized as distinct entities, which generally have favorable prognosis. To identify patients with *FLT3* mutations (ITD or TKD), which impacts prognosis and may qualify a patient for targeted therapy, a separate *FLT3*-ITD/TKD panel is included with this order. For specimens that test positive for *NPM1* mutation, a *NPM1* quantitative RT-PCR assay will be ordered to determine whether that more sensitive 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. Given the dynamic clonal architecture of MDS, repeat testing may be used in certain settings to assess clonal evolution.

3) **MPN:** The presence of a somatic mutation may assist diagnosis by supporting the presence of a clonal myeloid process. In addition, many *BCR-ABL1*-negative MPNs are associated with characteristic mutations, such as *JAK2* mutation in polycythemia vera, *JAK2* or *CALR* or *MPL* mutation in essential thrombocythemia or primary myelofibrosis, and *CSF3R* mutation in chronic neutrophilic leukemia. Suspected MPNs lacking mutations in these four genes may benefit from a broader MDS/MPN panel.

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), peripheral blood (3mL, EDTA), or formalin-fixed, paraffin-embedded bone marrow clot sections (10 unstained slides, minimum area of marrow = 4mm^2) having at least 30% myeloid cells. The assay is sensitive to 5% variant allele fraction (VAF; 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 in fresh samples (5% in FFPE samples).

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.

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

Genes	RNA	Exons	Genes	RNA	Exons
ASXL1	NM_015338.5	All	*MPL*	NM_005373.2	10
BCOR	NM_001123385.1	All	*NF1*	NM_001042492.2	All
BCORL1	NM_001184772.2	All	*NPM1*	NM_002520.6	10-11
BRAF	NM_004333.4	15	*NRAS*	NM_002524.4	All
CALR	NM_004343.3	8-9	*PHF6*	NM_001015877.1	All
CBL	NM_005188.3	All	*PIGA*	NM_002641.3	All
CDKN2A	NM_058195.3	All	*PPM1D*	NM_003620.3	All
CEBPA	NM_004364.4	All	*PRPF8*	NM_006445.3	All
CSF3R	NM_000760.3	All	*PTEN*	NM_000314.6	All
CUX1	NM_181552.3	All	*PTPN11*	NM_002834.4	All
DDX41	NM_016222.3	All	*RAD21*	NM_006265.2	All
DNMT3A	NM_022552.4	All	*RRAS2*	NM_012250.5	All
ETV6	NM_001987.4	All	*RUNX1*	NM_001754.4	All
EZH2	NM_001203247.1	All	*SETBP1*	NM_015559.2	All
FLT3	NM_004119.2	All	*SF3B1*	NM_012433.3	10-16
GATA2	NM_032638.4	All	*SH2B3*	NM_005475.2	All
GNAS	NM_001077490.2	All	*SMC3*	NM_005445.3	All
HRAS	NM_176795.4	All	*SRSF2*	NM_003016.4	1
IDH1	NM_005896.3	4	*STAG2*	NM_001042750.1	All
IDH2	NM_002168.3	4	*TET2*	NM_001127208.2	All
JAK2	NM_004972.3	All	*TP53*	NM_000546.5	All
JAK3	NM_000215.3	All	*U2AF1*	NM_006758.2	2,6
KIT	NM_000222.2	2,8-11,13,17-19	*WT1*	NM_024426.4	6-10
KMT2A	NM_001197104.1	All	*ZRSR2*	NM_005089.3	All
KRAS	NM_033360.3	All			

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Questions?

Call the Molecular Genetics Lab at (984) 974-1825 or Dr. Jonathan Galeotti at 984-974-8321, E-mail Jonathan.Galeotti@unchealth.unc.edu

Website= https://www.uncmedicalcenter.org/mclendon-clinicallaboratories/directory/molecularpathology-and-genetics/