MEMORANDUM #27

TO: UNCHCS Attending Physicians, Housestaff, Clinical Nurse Coordinators, Department Heads and Supervisors

FROM: Nathan Montgomery, MD, PhD, Director, Molecular Hematopathology
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SUBJECT: Changes to Molecular Testing for Myeloid Malignances at UNC

DATE: March 25, 2019

Effective March 31, 2019, the methodology and gene content will be updated for Myeloid Mutation Panels at UNC.

What is changing on the panel?

1. The assay will transition from amplicon-based to capture-based sequencing methodology.
2. The total number of genes tested will increase from 26 to 34, with addition of *BRAF, CBL, HRAS, KRAS, MYD88, NOTCH1, PPMD1,* and *PTPN11* to the panel
3. For patients with either myelodysplastic syndrome (MDS) or a myeloproliferative neoplasm (MPN), the current UNC Myeloid Mutation Panel – MDS and UNC Myeloid Mutation Panel – MPN orders will be combined into a single 34-gene panel, orderable as **Myeloid Mutation Panel (MDS & MPN)**. The full list of genes is included below.
4. For patients with acute myeloid leukemia, the current **Myeloid Mutation Panel (AML) with *FLT3* testing** will expand to 34 genes. In addition, it will continue to include rapid *FLT3*-ITD/TKD testing and an RNA extract & hold order.
5. A new 3 gene **Myeloproliferative neoplasm (MPN) hotspot panel (*JAK2, CALR, MPL*)** will become available for more narrow molecular testing in patients being evaluated for essential thrombocythemia or primary myelofibrosis.
6. Variant allele fractions will be reported for all mutations, except for *FLT3* internal tandem duplication mutations, which will be separately reported as an allelic ratio.

Why are these changes occurring?

1. Methodologic changes will improve detection of larger insertion/deletion mutations and coverage of technically challenging regions, including *CEBPA*.
2. Recent publications have emphasized the clinical utility of broader panels in MPNs.
3. Variant allele fraction reporting is now recommended by professional guidelines.

Will stand alone *JAK2* V617F testing continue to be offered?

Yes. *JAK2* V617F quantitative mutation analysis is offered separately and recommended for (1) *JAK2* testing in cases with low suspicion for polycythemia vera, where separate exon 12 testing may not be necessary and (2) quantitative monitoring of *JAK2* V617F allele burden. See the McLendon Laboratory website for more details on this testing.

Genes tested on the Myeloid Mutation Panel (AML and MDS/MPN):

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

^ - Exon 11 is the site of the common type A mutation. This exon is frequently referred to as exon 12 in the literature.

Specimen Requirements: Bone marrow aspirate (1mL EDTA) or peripheral blood (3mL, EDTA) having at least 30% myeloid cells and refrigerated for up to 72 hours.

Questions: Email Nathan Montgomery (**Nathan.montgomery@unchealth.unc.edu**), or call the UNC Molecular Genetics Lab at **(984) 974-1825.**

Website: https://www.uncmedicalcenter.org/mclendon-clinical-laboratories/directory/molecular-pathology-and-genetics/.