**FLT3 Internal Tandem Duplication (ITD) and Tyrosine Kinase Domain (TKD) Mutation Testing**

*FLT3* mutation status helps refine prognosis and guide therapy in patients with acute myeloid leukemia (AML). Two basic categories of mutation are recognized in *FLT3*: (1) internal tandem duplication (ITD) within the *FLT3* juxtamembrane domain and (2) activating missense mutations affecting the tyrosine kinase domain (TKD). Both categories of mutation cause constitutive activity of the mutant *FLT3* protein. The resulting constitutive *FLT3* kinase activity is thought to promote cell proliferation and anti-apoptotic signaling via the JAK/STAT, PI3K/AKT and RAS pathways.

*FLT3* ITD results from an in-frame insertion in exons 14 and 15 of the juxtamembrane domain of *FLT3* that can vary in size (3–400 nucleotides). *FLT3* ITD mutations are observed in approximately 15% of pediatric, 30% of young adult/adult, and 25% of older adult AML patients, more commonly in the presence of normal cytogenetics.

*FLT3* TKD mutations occur at exon 20 of the kinase domain and can result from point mutations, insertions or deletions. This assay identifies 90% of reported mutations in the *FLT3* tyrosine kinase domain by interrogating two commonly mutated codons- Asp835 (D835) and Ile836 (I836)- associated with response to midostaurin. TKD mutations have been reported in 7–10% of AML patients.

The presence of either a *FLT3* ITD or TKD mutation may be associated with response to the tyrosine kinase inhibitor, midostaurin.1 In addition, *FLT3*-ITD mutations are associated with inferior prognosis in patients with AML, particularly when the allelic ratio (mutant allele:wild-type allele ratio) is elevated.2 The threshold for a high allelic ratio has varied in publications, with cut-offs ranging from 0.5 to 0.8.

**Orderable tests and clinical indications:**
1. **Combined FLT3 (ITD) and FLT3 (TKD) DNA assay panel:** Indicated in new AML patients to refine prognosis and to identify those patients likely to respond to the tyrosine kinase inhibitor, midostaurin. The combined *FLT3*-ITD/TKD assay is included as a reflex order with the Myeloid Mutation Panel- AML. For samples positive for a *FLT3*-ITD mutation, the ratio of the mutant to wild-type allele is reported (*FLT3*-ITD allelic ratio).

2. **Stand-alone FLT3 (ITD) DNA analysis:** Indicated in patients with relapsed or persistent AML, which was previously shown to harbor a *FLT3*-ITD mutation. Stand-alone *FLT3*-ITD testing may be utilized to confirm that the persistent clone continues to harbor an ITD mutation. When positive, an allelic ratio is reported.

**Turn around time:**
Less than 7 days (the assay is run twice per week).

**Specimen Requirements:**
Bone marrow aspirate (1 mL, EDTA) and peripheral blood (3mL, EDTA) having at least 10% neoplastic cells and refrigerated up to 24 hours. However, Wright-stained or unstained bone marrow aspirate smears are also accepted. The assay is generally sensitive to variants above 5% allele fraction (10% clonal cells). Therefore, a minimum percentage of 10% neoplastic cells is required. This test is NOT appropriate for monitoring minimal residual disease. Results are reported as positive or negative to a sensitivity of 5% of DNA.

**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. Nathan Montgomery at (919) 445-6414. E-mail: nathan.montgomery@unchealth.unc.edu.