LeukoStrat® CDx *FLT3* Mutation Assay

Clinical utility of the LeukoStrat® CDx *FLT3* Mutation Assay

The fms related tyrosine kinase 3 (*FLT3*) is one of the most commonly mutated genes in acute myeloid leukemia (AML), occurring in approximately 30% of patients at the time of diagnosis[1]. Although generally associated with normal cytogenetics where patients have standard risk of relapse, *FLT3* mutations have also been identifi ed in sub-groups of patients with chromosomal abnormalities that are associated with high risk of disease relapse[2-3].

The most prevalent type of *FLT3* mutation is an internal tandem duplication (ITD) in the juxtamembrane domain[4].

The second most common mutation type in the *FLT3* gene is a tyrosine kinase domain (TKD) point mutation in the codon for an aspartate (D835) or an isoleucine (I836) residue. TKD mutations result in constitutive autophosphorylation and activation of *FLT3* [5-6] and have also been linked to poor overall survival, but to a lesser extent as compared to ITD mutations[7].

To determine the best treatment options, it is recommended that patients with AML be screened for the presence of *FLT3* mutations.

Intended Use

The LeukoStrat® CDx *FLT3* Mutation Assay is a PCR-based, in vitro diagnostic test designed to detect internal tandem duplication (ITD) mutations and the tyrosine kinase domain (TKD) mutations D835 and I836 in genomic DNA extracted from mononuclear cells obtained from peripheral blood or bone marrow aspirates of patients diagnosed with acute myelogenous leukemia.

The LeukoStrat® CDx *FLT3* Mutation Assay is used as an aid in the assessment of acute myeloid leukemia patients for whom midostaurin (RYDAPT®) treatment is being considered.

The LeukoStrat® CDx *FLT3* Mutation Assay is to be performed only at Laboratory for Personalized Molecular Medicine (LabPMM) LLC.

Description of Testing	Turnaround Time	Specimen Requirements	Shipping Conditions	Storage Conditions
Primers flanking exons 14, 15 and the activation loop region of exon 20 of the *FLT3* gene are used to amplify DNA extracted from a patient sample. The forward and reverse PCR primers are fluorescently labeled with different fluorophores that serve to confirm the presence of sample signal. The size of the ITD PCR product is determined by capillary electrophoresis and the signal ratio (SR) compares the signal intensity of the mutant to the wild-type. The *FLT3* TKD PCR product is digested with EcoRV and the presence of the mutation is further assessed using capillary electrophoresis and the signal ratio (SR) compares the signal intensity of the mutant to the wild-type.	2-3 business days	• 5 mL of peripheral blood in Heparin • 3 mL of bone marrow in Heparin	• Cool; Do not freeze	• 4 °C up to 7 days

Test Code	CPT Code
FLT3-CDx	81245, 81246

* *FLT3* ITD testing is covered by United States and other jurisdiction patents licensed exclusively to Invivoscribe by Takara Bio, Inc.

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