Clinical Utility of the FLT3 Mutation Assay

Acute myeloid leukemia (AML) has in general a poor prognosis. Recent studies have described mutation of the FMS related tyrosine kinase 3 (FLT3) gene to be an important prognostic factor in AML, with FLT3 mutants having a worse outcome and response to standard chemotherapeutic interventions. FLT3 is one of the most commonly mutated genes in AML, occurring in approximately 30% of patients at the time of diagnosis. The highest mutation rates are seen in adult patients with AML and normal- or intermediate-risk cytogenetics, and patients with acute promyelocytic leukemia.

The most prevalent and clinically significant type of FLT3 mutation is an internal tandem duplication (ITD) in the juxtamembrane domain. Many clinical studies have found that FLT3 ITD mutations are associated with higher concentrations of leukemic cells in both blood and bone marrow, increased incidence of relapse and decreased overall survival.

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 and have also been linked to poor overall survival, but to a lesser extent as compared to ITD mutations.

To determine the best treatment options for AML patients, it is recommended that patients with AML be screened for the presence of FLT3 mutations before induction therapy. Induction therapy is unlikely to be altered due to FLT3 mutation status; however, knowing the FLT3 mutation status can help stratify patients into groups that will receive different post-remission treatments. Currently there are several small molecule inhibitors of FLT3 in clinical trials. Identifying patients with FLT3 mutations may make patients eligible for these research therapies.

Indications for Testing

- At initial diagnosis of AML, ALL and MDS
- Stratifying high and low risk AML
- Reoccurrence of leukemia after induction therapy on patients not initially screened for FLT3 mutations

Description of Testing

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. Wild type FLT3 alleles will amplify and produce a product measured at 327±1 bp as generated by the FLT3 Mutation Assay, while alleles that contain ITD mutations will be greater than or equal to 330 bp.

The FLT3 TKD PCR product is digested with EcoRV restriction enzyme and the presence of the mutation is further assessed using capillary electrophoresis.

<table>
<thead>
<tr>
<th>Description of Testing</th>
<th>Turnaround Time</th>
<th>Specimen Requirements</th>
<th>Shipping Conditions</th>
<th>Storage Conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>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. Wild type FLT3 alleles will amplify and produce a product measured at 327±1 bp as generated by the FLT3 Mutation Assay, while alleles that contain ITD mutations will be greater than or equal to 330 bp. The FLT3 TKD PCR product is digested with EcoRV restriction enzyme and the presence of the mutation is further assessed using capillary electrophoresis.</td>
<td>3-7 business days</td>
<td>• 5 mL of peripheral blood in Heparin, EDTA or ACD • 3 mL of bone marrow in Heparin, EDTA or ACD • 1 µg of previously isolated DNA</td>
<td>• Ambient or Cool; Do not freeze</td>
<td>• Room Temp up to 72 hours • 4 °C up to 7 days</td>
</tr>
</tbody>
</table>

Test Code | CPT Code |
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>FLT3</td>
<td>81245, 81246</td>
</tr>
</tbody>
</table>

* FLT3 ITD testing is covered by a United States patent licensed from Takara Bio, Inc.
References