**KIT Molecular Testing**

### Indications for Ordering

Provide diagnostic, prognostic, and predictive information for
- Acute myeloid leukemia (AML) associated with inv(16) or t(8;21)
  - Also known as core-binding factor (CBF) AML
- Mastocytosis
- Gastrointestinal stromal tumors (GIST)
- Melanoma

### Test Description

**KIT Mutations in AML by Fragment Analysis and Sequencing**
- Polymerase chain reaction (PCR)/fragment analysis/sequencing
- Capillary electrophoresis to detect insertions/deletions on exon 8
- Sequencing of exon 17

**KIT (D816V) Mutation by PCR**
- PCR
- Allele specific PCR of exon 17

**Gastrointestinal Stromal Tumor Mutation**
- Genomic DNA isolated from microscopically guided dissection of tumor tissue
- Enrichment for the following regions of interest:
  - KIT (NM_002253.2) – exons 9, 11, 13, 14, 17, 18
  - PDGFRA (NM_006206.4) – exons 12, 14, 18
- Mutation status determined by massively parallel sequencing (next generation sequencing)

**KIT Mutations, Melanoma**
- Genomic DNA isolated from microscopically guided dissection of tumor tissue
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  - PDGFRA (NM_006206.4) – exons 12, 14, 18
- Mutation status determined by massively parallel sequencing (next generation sequencing)

### Tests to Consider

**Primary tests**

**KIT Mutations in AML by Fragment Analysis and Sequencing 2002437**
- Prognostication in CBF AML

**KIT (D816V) Mutation by PCR 3000440**
- Aid in the diagnosis of mastocytosis
- Provide prognostic and predictive information for tyrosine kinase inhibitor (TKI) therapy planning

**Gastrointestinal Stromal Tumor Mutation 2002674**
- Detect activating mutations in KIT and PDGFRA
- Predict response to targeted therapy

**KIT Mutations, Melanoma 2002695**
- Detect activating mutations in KIT and PDGFRA
- Predict response to targeted therapy

**Related tests**

**Myeloid Malignancies Mutation Panel by Next Generation Sequencing 2011117**
- Assess for single gene mutations, including substitutions and insertions and deletions, that may have diagnostic, prognostic, and/or therapeutic significance

**Acute Myeloid Leukemia Panel by FISH 2011132**
- Identify prognostically important abnormalities in newly diagnosed AML
- Monitor response to therapy with specific probes (CHR FISH) or progression of disease with probe panel

**Eosinophilia Panel by FISH 2002378**
- Identify prognostically important inv(16) in AML associated with eosinophilia

**BRAF Codon 600 Mutation Detection by Pyrosequencing 2002498**
- Detect activating BRAF mutations at codon 600
- Predict response to targeted therapy in melanomas and colorectal cancers
- Assess prognosis of certain thyroid cancers

**BRAF V600E Mutation Detection in Circulating Cell-Free DNA by Digital Droplet PCR 2013921**
- Determine BRAF V600E mutation status in patients with solid tumors to select candidates for targeted therapy with kinase inhibitors (BRAF and/or MEK)
- Monitor response to therapy and disease progression in patients carrying BRAF V600E mutation
### Test Interpretation

**KIT Mutations in AML by Fragment Analysis and Sequencing**

**Analytical sensitivity**
- Detects mutations in exon 17 in specimens with at least 30% AML cells carrying the mutation
- Detects mutations in exon 8 in specimens with at least 5% AML cells carrying the mutation

**Results**
- Detected – KIT exon 8 or 17 mutation
  - Associated with less favorable outcome
  - TKIs may be useful in conjunction with standard chemotherapy
- Not detected – no mutation in KIT exon 8 or 17

**Limitations**
- Not intended to detect minimal residual disease
- Mutations outside of exons 8 and 17 are not detected
- Mutations below analytical sensitivity will not be detected

**KIT (D816V) Mutation by PCR**

**Sensitivity**
- Clinical – occurs in >80% of systemic mastocytosis (SM) cases
- Analytical – 0.3% allelic burden

**Results**
- Detected – KIT (D816V) point mutation
  - Supports a diagnosis of SM or SM-associated clonal hematologic nonmast cell lineage disease (SM-AHNMD) in the correct clinical context
  - Therapeutic implications
    - Imatinib – ineffective if mutation is present
    - Dasatinib and Nilotinib – uncertain clinical efficacy
- Not detected – no KIT (D816V) point mutation

**Limitations**
- Mutations other than the D816V mutation are not detected, including other D816 variants
- Mutations below analytical sensitivity will not be detected

### Gastrointestinal Stromal Tumor Mutation

<table>
<thead>
<tr>
<th>Variant Class</th>
<th>No. Variant Tested</th>
<th>Positive Percent Agreement (PPA)</th>
<th>PPA, 95% Tolerance at 95% Reliability</th>
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</thead>
<tbody>
<tr>
<td>SNV</td>
<td>177</td>
<td>100%</td>
<td>98.9-100.0%</td>
</tr>
<tr>
<td>MNVs</td>
<td>42</td>
<td>95%</td>
<td>85.6-99.0%</td>
</tr>
<tr>
<td>Small insertions and duplications</td>
<td>42</td>
<td>100%</td>
<td>95.6-100.0%</td>
</tr>
<tr>
<td>Medium insertions and duplications</td>
<td>10</td>
<td>100%</td>
<td>82.9-100.0%</td>
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<tr>
<td>Large insertions</td>
<td>1</td>
<td>100%</td>
<td>22.9%-100.0%</td>
</tr>
</tbody>
</table>

### KIT Mutations, Melanoma

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</tr>
<tr>
<td>Large insertions</td>
<td>1</td>
<td>100%</td>
<td>22.9%-100.0%</td>
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<tr>
<td>Small deletions</td>
<td>80</td>
<td>100%</td>
<td>97.6-100.0%</td>
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<tr>
<td>Medium deletions</td>
<td>14</td>
<td>100%</td>
<td>71.2%-99.2%</td>
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<tr>
<td>Large deletions</td>
<td>22</td>
<td>64%</td>
<td>42.9%-81.1%</td>
</tr>
</tbody>
</table>

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Results

- Detected – KIT mutation detected in exons 9, 11, 13, 14, 17, 18
- Detected – PDGFRA mutation detected in exons 12, 14, 18
- Not detected – no mutations detected in KIT and PDGFRA

Limitations

- Mutations outside of targeted exons are not detected
- Test alone cannot be used for diagnosis of malignancy
- Variants below the limit of detection (LOD) of 5% variant allele frequency may not be detected
- 10 ng input DNA from extracted tissue sample is minimally required, but 50 ng input DNA is recommended for optimal results
- Large variants (>60bp) may not be detected
- Not intended to detect minimal residual disease
- Does not distinguish between somatic and germline variants

Disease Overview

CBF AML
- KIT mutation testing is important for prognostication
  - KIT mutations are associated with higher incidence of relapse and lower survival
- KIT mutations may be detected in
  - inv(16) or t(16;16) AML
  - t(8;21) AML

Mastocytosis
- KIT mutation testing is important for
  - Diagnosis (presence of mutation is a minor criteria for SM)
  - Prediction of response to TKI therapy

GIST
- KIT and PDGFRA mutation testing is important for prediction of response to targeted therapy and should be performed in all patients considered for targeted therapy
- Not only the presence but also type of mutation determines if the patient will benefit from targeted therapy
- Detection of secondary resistance mutations in patient already treated with targeted therapy may guide the use of other therapeutic agents
- For specific treatment recommendations please refer to NCCN Clinical Practice Guidelines in Oncology, Soft Tissue Sarcoma (Gastrointestinal Stromal Tumors section) [www.nccn.org]
- Mutation testing may be occasionally used to aid in establishing GIST diagnosis in difficult cases (unusual location, morphology, or immunoprofile)
- Immunohistochemistry for c-kit (CD117) is useful for diagnostic purposes but should not be used to predict response to targeted therapy

Melanoma
- KIT mutation testing is important for prediction of response to targeted therapy
- Immunohistochemistry for c-kit (CD117) should not be used to predict response to targeted therapy
- For specific treatment recommendations please refer to NCCN Clinical Practice Guidelines in Oncology, Melanoma [www.nccn.org]

Genetics

Gene – KIT

Structure/function
- Maps to 4q12
- Receptor tyrosine kinase (type III)
  - Important in hematopoiesis for regulation of cell proliferation and maturation

Mutations
- A variety of >500 mutations have been described, most commonly in
  - Juxtamembrane region (exon 11)
  - Extracellular region (exon 9)
  - Kinase domain (exons 13, 17)
- CBF AML
  - Detected in ~30% of AML with inv(16)
  - Detected in 20-25% of AML with t(8;21) (particularly the D816V mutation)
- Mastocytosis
  - Adults
    - D816V mutation detected in 95%
    - Rare juxtamembrane mutations
  - Children
    - D816V mutation detected in 30-40%
    - ~40% carry KIT mutations that reside outside exon 17 (mainly exons 8 and 9)
  - In SM-AHNMD, mutations other than D816V may be detected
- GIST
  - Mutations in KIT are present in ~85% of cases
    - Primary mutations most common in exon 11 (~70% of cases) and exon 9 (~10-15% of cases); much less common in other exons
    - Secondary resistance mutations occur in exons 13, 14, 17, and 18
  - Mutations in PDGFRA are present in ~5% of cases
    - Primary mutations most common in exon 18 (~5% of cases); much less common in other exons
- Melanoma
  - Mutations in KIT are present in 2-8% of cases overall (more common in mucosal and acral melanomas)
    - Most common in exon 11 (70% of KIT mutated cases) and exon 13 (20% of KIT mutated cases); much less common in other exons

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References