**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**
- PCR/sequencing
  - KIT – exons 9, 11, 13, 14, 17, 18
  - PDGFRA – exons 12, 14, 18

**KIT Mutations, Melanoma**
- PCR/sequencing
  - KIT – exons 9, 11, 13, 14, 17, 18
  - PDGFRA – exons 12, 14, 18

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

**CD117 (c-Kit) by Immunohistochemistry 2003806**
- Initial screening test when GIST is suspected based on histology and location of tumor

**DOG1 by Immunohistochemistry 2010168**
- Screening test in tumor that is morphologically and clinically suspicious for GIST when CD117 is negative

**BRAF Codon 600 Mutation Detection by Pyrosequencing 2002498**
- Detect activating BRAF mutations at codon 600
  - Can indicate responsiveness to BRAF inhibitors in melanomas

**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 non-mast cell lineage disease (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**

**Sensitivity**
- Clinical – mutations detected in >85% of GISTs (~70% KIT and ~15% PDGFRA)
- Analytical – 25% mutant alleles (50% tumor)

**Results**
- Detected – KIT mutation detected in exons 9, 11, 13, 14, 17, 18
  - Exon 9
    - Requires an escalated dose of TKI for response
    - Better response to sunitinib than imatinib
  - Exon 11
    - Associated with TKI sensitivity
  - Exon 13
    - Primary (nontherapy associated) – associated with TKI sensitivity
    - Secondary (acquired during therapy) – associated with TKI resistance
  - Exon 14
    - Secondary (acquired during therapy) – associated with TKI resistance
  - Exon 17
    - D816V – associated with TKI resistance
    - Primary – associated with TKI sensitivity
    - Secondary – associated with TKI resistance
  - Exon 18 (D842V and D846V) – associated with TKI resistance
- Detected – PDGFRA mutation detected in exons 12, 14, 18
  - Exon 12 – associated with TKI sensitivity
  - Exon 14 – associated with TKI sensitivity
  - Exon 18 (D842V and D846V) – associated with TKI resistance
- Normal – no mutations detected in KIT or PDGFRA (wild type GISTs)
  - Associated with indolent course if succinate-dehydrogenase (SDH)-deficient GIST
  - Associated with resistance to most TKIs
  - No response to sunitinib

**Limitations**
- Mutations outside of targeted exons are not detected
- Test alone cannot be used for diagnosis of malignancy

**KIT Mutations, Melanoma**

**Analytical sensitivity** – 25% mutant alleles (50% tumor)

**Results**
- Detected – KIT and PDGFRA mutations
  - TKI sensitivity will be interpreted by a pathologist
- Normal – no mutations detected in the designated exons

**Limitations**
- Mutations outside of targeted exons are not detected
### 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* mutation testing is important for
  - Prediction of response to TKI therapy
- Majority of GISTs express the c-kit protein (CD117) which is detectable by immunohistochemistry (IHC)
  - Staining for CD117 – excellent initial screen when histology and tumor location suggest GIST
    - Positive IHC stain – order *KIT* mutation testing
    - Limitations of IHC staining
      - GISTs with *PDGFRA* mutation may have weak KIT IHC staining
      - Does not identify type of mutation, which is crucial for predicting responsiveness to TKI therapy
- Staining for DOG1 – most useful in tumors that stain negative for CD117
  - Does not identify type of mutation, which is crucial for predicting responsiveness to TKI therapy
- ~8% of GISTs have mutations in *PDGFRA*
  - *PDGFRA* and *KIT* mutations are mutually exclusive
  - Majority occur in gastric GIST with epithelioid morphology and weak or negative CD117
- SDH-deficient GISTs account for about half of wild type GISTs (negative for *KIT* and *PDGFRA* mutations)
  - Will stain negatively for SDHB

**Melanoma**
- *KIT* mutation testing
  - Important for determining targeted therapy which may be used in disseminated disease
    - Choice of therapy based on presence of gene mutations – *BRAF, KIT, PDGFRA* (rare)
  - Required to identify mutation
    - *KIT* (CD117) IHC staining does not reliably predict mutation status or sensitivity to TKIs
- TKIs are most useful therapy when TKI-sensitive exon mutation is present
  - Current drug of choice is imatinib mesylate (Gleevec)
- *KIT* mutation
  - Less common than *BRAF* in melanoma
  - Most common in acral and mucosal subtypes

### 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 with an associated clonal hematological nonmast cell lineage disease (SM-AHNMD), mutations other than D816V may be detected
- **GIST**
  - Most commonly located in exon 11
  - Acquired mutations during TKI therapy cluster in exons 13, 14, 17
  - Less common mutations – exons 9, 13, 17, 18
    - Exon 17 D816V mutation rarely seen in GIST; common in other malignancies
- **Melanoma**
  - Most commonly located in exon 11 and less commonly in exons 13, 17, 18
  - Associated with TKI sensitivity
  - Mutation prevalence (Grossmann, 2012)
    - Mucosal melanoma – 6-19%
    - Acral lentiginous melanoma – 11-38%
    - Chronically sun damaged – 17%
  - *KIT* mutations in exons 11 and 13 generally have a favorable response to imatinib
  - *PDGFRA* mutations in exons 12, 14, 18 may be associated with TKI response

**References**