**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

### Tests to Consider

#### Primary tests

**KIT Mutations in AML by Fragment Analysis and Sequencing 2002437**
- Prognostication in CBF AML (NCCN, 2011)

**KIT (D816V) Mutation by PCR 0040137**
- 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
- Insurance providers may require documentation of drug-sensitive activating mutation for TKI reimbursement

#### 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

### Test Description

**KIT Mutations in AML by Fragment Analysis and Sequencing**
- Peripheral blood or bone marrow (BM) is acceptable
  - 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**
- Peripheral blood, BM, or tissue (fresh, frozen, or formalin-fixed, paraffin-embedded [FFPE]) is acceptable
  - Allele specific PCR of exon 17

**Gastrointestinal Stromal Tumor Mutation**
- Requires FFPE tissue block or five unstained 5-micron slides
  - PCR amplification/Sanger sequencing
    - KIT – exons 9, 11, 13, 14, 17, 18
    - PDGFRA – exons 12, 14, 18

**KIT Mutations, Melanoma**
- Requires FFPE tissue block or five unstained 5-micron slides
  - PCR amplification/Sanger sequencing
    - KIT – exons 9, 11, 13, 14, 17, 18
    - PDGFRA – exons 12, 14, 18
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 (rare)
    - Secondary (acquired during therapy) – 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 it is a succinate-dehydrogenase-deficient GIST
  - Associated with resistance to most TKIs
  - No response to sunitinib

**Limitations**
- Mutations other than the 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 systemic mastocytosis)
  - 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
  - Succinate dehydrogenase (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
- >500 described
- A variety of 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)

SM
- 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

GISTs
- 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