

# 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

#### [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 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 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

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### 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

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### 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

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- Grossmann AH, Grossmann KF, Wallander ML. Molecular testing in malignant melanoma. *Diagn Cytopathol.* 2012 Jun;40(6):503-10
- NCCN Clinical Practice Guidelines in Oncology, Acute Myeloid Leukemia. National Comprehensive Cancer Network. Fort Washington, PA [Accessed: Mar 2017]