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 FISHI) 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 (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 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

- 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 [Last update Feb 2018; Accessed: May 2018]