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 sguided 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)

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 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
- 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

Analytical Sensitivity			
Variant Class	**No. Variant Tested**	**Positive Percent Agreement (PPA)**	**PPA, 95% Tolerance at 95% Reliability**
SNV	177	100%	98.9-100.0%
MNVs	42	95%	85.6-99.0%
Small insertions and duplications[a]	42	100%	95.6-100.0%
Medium insertions and duplications[b]	10	100%	82.9-100.0%
Large insertions[c]	1	100%	22.9%-100.0%
Small deletions[a]	80	100%	97.6-100.0%
Medium deletions[b]	14	100%	71.2%-99.2%
Large deletions[d]	22	64%	42.9%-81.1%

[a]≤21 bp
[b]22-60 bp
[c]≥ 61 bp and ≤ 64bp
[d]≥ 61 bp and ≤ 13547bp
bp, base pair

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

KIT Mutations, Melanoma

Analytical Sensitivity			
Variant Class	**No. Variant Tested**	**Positive Percent Agreement (PPA)**	**PPA, 95% Tolerance at 95% Reliability**
SNV	177	100%	98.9-100.0%
MNVs	42	95%	85.6-99.0%
Small insertions and duplications[a]	42	100%	95.6-100.0%
Medium insertions and duplications[b]	10	100%	82.9-100.0%
Large insertions[c]	1	100%	22.9%-100.0%
Small deletions[a]	80	100%	97.6-100.0%
Medium deletions[b]	14	100%	71.2%-99.2%
Large deletions[d]	22	64%	42.9%-81.1%

[a] ≤21 bp
[b] 22-60 bp
[c] ≥ 61 bp and ≤ 64bp
[d] ≥ 61 bp and ≤ 13547bp

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

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]
- NCCN Clinical Practice Guidelines in Oncology, Melanoma. National Comprehensive Cancer Network. Fort Washington, PA [Last update Jan 2018; Accessed: June 2018]

  NCCN Clinical Practice Guidelines in Oncology, Soft Tissue Sarcoma. National Comprehensive Cancer Network. Fort Washington, PA [Last update Mar 2018; Accessed: June 2018]