KIT Molecular Testing

Molecular testing for *KIT* mutations is relevant for various types of cancer and can provide diagnostic, prognostic, and predictive information for systemic mastocytosis (SM), gastrointestinal stromal tumors (GIST), melanoma, and acute myeloid leukemia (AML) associated with inv(16) or t(8;21), also known as core-binding factor (CBF) AML. For GIST and melanoma, *PDGFRA* testing is also often relevant.

Disease Overview

Core-Binding Factor Acute Myeloid Leukemia

- *KIT* mutation testing is important for prognostication[1]
- *KIT* mutations are associated with higher incidence of relapse and lower survival[1]
- *KIT* mutations may be detected in[1]:
  - 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 targeted therapy

Gastrointestinal Stromal Tumors

- *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
- Mutation presence and type determine 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
- 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

Genetics

Gene

KIT

Tests to Consider

KIT Mutations in AML by Fragment Analysis and Sequencing 2002437

Method: Polymerase Chain Reaction/Fragment Analysis/Sequencing

Prognostication in CBF AML

KIT (D816V) Mutation by ddPCR, Quantitative 3002956

Method: Droplet Digital Polymerase Chain Reaction

- Aids in the diagnosis of mastocytosis in peripheral blood and bone marrow specimens
- The D816V mutation is a diagnostic marker for systemic mastocytosis
- The D816V mutation confers resistance to imatinib

Gastrointestinal Stromal Tumor Mutation 2002674

Method: Massively Parallel Sequencing

- Detects activating mutations in *KIT* and *PDGFRA*
- Predicts response to targeted therapy

KIT Mutations, Melanoma 2002695

Method: Massively Parallel Sequencing

- Detects activating mutations in *KIT* and *PDGFRA*
- Predicts response to targeted therapy

Related Tests

Myeloid Malignancies Mutation Panel by Next Generation Sequencing 2011117

Method: Massively Parallel Sequencing

Assesses for multiple gene mutations, including substitutions and smaller insertions and deletions that may have prognostic and/or therapeutic significance

Acute Myeloid Leukemia Panel by FISH 2011132

Method: Fluorescence in situ Hybridization (FISH)

- Identifies prognostically important abnormalities in newly diagnosed AML

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 (exons 8, 9), and kinase domain (exons 13, 17). These mutations are commonly detected in patients with:

- CBF AML
  - Detected in ~20% of AML with *KIT* mutations, including inv(16)
  - Detected in 20-25% of AML with t(8;21) (particularly the D816V mutation)
- Mastocytosis
  - Adults
    - D816V mutation detected in 95% of patients with SM
    - Rare juxtamembrane mutations
  - Children
    - D816V mutation detected in 30-40% of patients with SM
    - ~40% carry *KIT* mutations that reside outside exon 17 (mainly exons 8 and 9)
  - Mutations other than D816V may be detected in SM-associated hematologic neoplasm (AHN)
- GIST
  - *KIT* mutations 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
  - *PDGFRA* mutations present in ~5% of cases
    - Primary mutations most common in exon 18 (~5% of cases)
    - Primary mutations much less common in other exons
- Melanoma
  - *KIT* mutations present in 2-8% of cases overall (more common in mucosal and acral melanomas)
  - Most common in:
    - Exon 11 (70% of *KIT* mutated cases)
    - Exon 13 (20% of *KIT* mutated cases)
  - Much less common in other exons

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 harboring the mutation
- Exon 8 in specimens with at least 5% AML cells harboring the mutation

Results

- Detected: *KIT* exon 8 or 17 mutation
  - Associated with less favorable outcome in in CBF AML

- Aids in classification of AML including subtypes with recurrent genetic abnormalities
- Monitors response to therapy with specific probes (CHR FISHI) or progression of disease with probe panel

Eosinophilia Panel by FISH 2002378

Method: Fluorescence in situ Hybridization (FISH)

Aids in diagnosis and classification of hematologic neoplasms presenting with prominent eosinophilia

CD117 (c-Kit) by Immunohistochemistry 2003806

Method: Immunohistochemistry

Initial screening test when GIST or mastocytosis is suspected based on histology and location of tumor

DOG1 by Immunohistochemistry 2010168

Method: Immunohistochemistry

- Screening test in tumor that is morphologically and clinically suspicious for GIST when CD117 is negative
- Performed as a stain and return (technical) service only

BRAF Codon 600 Mutation Detection by Pyrosequencing 2002498

Method: Polymerase Chain Reaction/Pyrosequencing

- Detects activating *BRAF* mutations at codon 600
- Predicts response to targeted therapy in melanomas and colorectal cancers
- Assesses prognosis of certain thyroid cancers

BRAF V600E Mutation Detection in Circulating Cell-Free DNA by Digital Droplet PCR 2013921

Method: Polymerase Chain Reaction

- Determines *BRAF* V600E mutation status in patients with solid tumors to select candidates for targeted therapy with kinase inhibitors (BRAF and/or MEK)
- Monitors response to therapy and disease progression in patients carrying *BRAF* V600E mutation

- TKIs may be useful in conjunction with standard chemotherapy
- Not detected: no detectable 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 ddPCR, Quantitative

Sensitivity

- Clinical: occurs in >80% of SM cases
- Analytical: 0.03% variant allele frequency (VAF)

Results

- Detected VAF: *KIT* (D816V) point mutation; allele specific amplification of the c.2447 C>T (D816V)
- Results are reported as a percent mutated allele
  - 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 detectable *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 Mutations and *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%

[a]≤21 bp

[b]22-60 bp

[c]≥61 bp and ≤64bp

[d]≥61 bp and ≤13547bp

bp, base pair; MNVs, multiple nucleotide variants; SNV, single nucleotide variant

Variant Class	No. Variant Tested	Positive Percent Agreement (PPA)	PPA, 95% Tolerance at 95% Reliability
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; MNVs, multiple nucleotide variants; SNV, single nucleotide variant

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% VAF 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

References

1. Swerdlow S, Campo E, Harris N, et al. WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues, 4th ed. Lyon, France: International Agency for Research on Cancer, 2008.

Additional Resources

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