

## Assay Summary

### **Somatic KIT (c-Kit) and PDGFRA Mutation Analysis** **Gastrointestinal stromal tumors (GIST), Melanoma, Systemic Mastocytosis**

#### *Synopsis*

The KIT gene encodes the human homolog of the proto-oncogene c-Kit. Activating mutations of KIT have been reported for 75%-85% of gastrointestinal stromal tumors (GIST), most often in exons 9 and 11<sup>1</sup>. Tumors with mutations in exon 9 of KIT gene are associated with significantly shorter progression-free survival and overall survival compared to tumors with mutations in exon 11<sup>2</sup>. However, tumors expressing an exon 9 mutation in c-kit had improved progression-free survival when given a high dose regimen of imatinib, compared to those with an exon 9 mutation who received regular dosing<sup>2</sup>. Secondary mutations in exons 13, 14, 17, and 18 have also been associated with acquired resistance after imatinib treatment.

Mutations in exon 12, 14 and 18 of the PDGFRA gene may also help predict responsiveness to imatinib and are estimated to be present in 5%-7% GISTs<sup>1</sup>. Specifically, PDGFRA D842V mutation has been associated with resistance to imatinib<sup>1</sup>, whereas other mutations of PDGFRA have been associated with imatinib sensitivity<sup>1</sup>.

KIT mutations have also been reported in melanomas. Particularly, ~15% of acral and ~20% of mucosal melanomas are estimated to have a KIT mutation. Case reports and multiple phase II clinical trials have documented dramatic responses to imatinib in melanoma patients with KIT mutations<sup>3-8</sup>.

For patients with aggressive systemic mastocytosis (SM), the D816V activating mutation of KIT has been reported in greater than 90% of cases and is included as a minor diagnostic criterion by the World Health Organization<sup>9</sup>. The D816V mutation has been associated with poor prognosis in SM and resistance to imatinib<sup>10</sup>. Imatinib is FDA approved as a therapeutic for aggressive systemic mastocytosis of unknown or negative status for the D816V mutation.

#### *Indications for testing*

Molecular testing of the KIT gene should be considered for patients with a diagnosis of GIST or melanoma prior to initiating KIT kinase inhibitor therapies (imatinib, sunitinib). For GIST, it is recommended that a sequential approach to testing be taken with KIT testing first, followed by PDGFRA testing if KIT is negative (KIT with reflex to PDGFRA) as mutations in KIT and PDGFRA gene tend to be mutually exclusive. Testing may also be considered for patients already on imatinib with acquired resistance to therapy. KIT testing may also be indicated to guide diagnosis and therapeutic selection in systemic mastocytosis.

#### *Methodology*

Genomic DNA (gDNA) is extracted from micro-dissected cells from formalin-fixed, paraffin-embedded tissue. A targeted DNA library is generated using the Ion AmpliSeq<sup>™</sup> Cancer Hotspot Panel v2 Kit, and sequenced by semiconductor-based next-generation sequencing technology on an Ion Torrent PGM. The KIT-NGS test targets 136 mutations in exons 2, 9, 11, 13, 14, 17, and 18 of the KIT gene. The PDGFRA-NGS test targets 25 mutations in exons 12, 14, 15, and 18 of the PDGFRA gene.

## ***Performance/Limitations***

The gene is not sequenced in its entirety; only the regions including the targeted mutations are analyzed. The method will not detect gross genetic alterations including large deletions, duplications, and inversions. The minimum detectable mutant allele ratio is approximately 10%.

## ***Specimen Requirements***

### **Formalin-fixed, paraffin-embedded (FFPE) tissue blocks or slides.**

The tissue sample should be large enough to provide at least 3000 tumor cells and at least 30% of tumor cells within the tissue. We prefer to receive FFPE tissue blocks (unused portion will be returned), but slides are also accepted. For slides:

- 10 slides, 10 micron serial sections, unstained, without coverslip.
- 1 representative H&E slide, 4 micron section, with a coverslip.
- If the sample is a needle biopsy or has very little tumor, please send 5 additional slides.
- Slides or blocks should be labeled with the accession number and patient name and accompanied by a copy of the corresponding pathology report.
- Place slides in appropriate container(s) to ensure against breakage.

## ***Test Request Form (TRF)***

A completed CMDL ([TRF](#)) must be submitted with each specimen. Complete testing and billing information must be provided before the specimen is processed

<b><i>Order Codes</i></b>	<b><i>CPT Codes</i></b>	<b><i>TAT</i></b>
KIT-NGS (Targeted analysis for 136 cancer hotspot mutations in exons 2, 9, 11, 13, 14, 17, and 18 of the KIT gene by next generation sequencing)	81404, 88381(Pro), G0452	3-5 days
PDGFRA-NGS (Targeted analysis for 25 cancer hotspot mutations in the PDGFRA gene by next generation sequencing)	81404, 88381, G0452	7-14 days

## ***References***

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