Template for Reporting Results of Biomarker Testing of Specimens From Patients With Gastrointestinal Stromal Tumors

Version: 1.1.0.0
Protocol Posting Date: December 2022
This biomarker template is not required for accreditation purposes but may be used to facilitate compliance with CAP Accreditation Program Requirements

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Accreditation Requirements
Completion of the template is the responsibility of the laboratory performing the biomarker testing and/or providing the interpretation. When both testing and interpretation are performed elsewhere (eg, a reference laboratory), synoptic reporting of the results by the laboratory submitting the tissue for testing is also encouraged to ensure that all information is included in the patient's medical record and thus readily available to the treating clinical team.
Summary of Changes
v 1.1.0.0

- General format updates to harmonize with other biomarker reporting protocols
Reporting Template
Protocol Posting Date: December 2022
Select a single response unless otherwise indicated.

CASE SUMMARY: (GIST Biomarker Reporting Template)
Completion of the template is the responsibility of the laboratory performing the biomarker testing and / or providing the interpretation. When both testing and interpretation are performed elsewhere (eg., a reference laboratory), synoptic reporting of the results by the laboratory submitting the tissue for testing is also encouraged to ensure that all information is included in the patient’s medical record and thus readily available to the treating clinical team.
Fixative type, time to fixation (cold ischemia time), and time of fixation should be reported if applicable in this template or in the original pathology report.
Gene names should follow recommendations of The Human Genome Organization (HUGO) Nomenclature Committee (www.genenames.org; accessed October 18, 2022). (Note A)
All reported gene sequence variations should be identified following the recommendations of the Human Genome Variation Society (www.hgvs.org/mutnomen/; accessed October 18, 2022). (Note A)

GIST BIOMARKER TESTS

Immunohistochemical Studies (Note B)
+KIT (CD117)
___ Positive
___ Negative
+DOG1 (ANO1)
___ Positive
___ Negative
+SDHA
___ Intact
___ Deficient
+SDHB
___ Intact
___ Deficient

Other IHC Studies
+Test Name (repeat this question as needed): _________________
___ Positive
___ Negative
___ Other (specify): _________________

Molecular Genetic Studies (Note C)
+KIT Mutational Analysis (Note D)
___ No mutation detected
___ Mutation identified (specify): _________________
___ Cannot be determined (explain): _________________

+KIT Exons Assessed (Note D) (select all that apply)
___ Exon 9
___ Exon 11
___ Exon 13
___ Exon 14
___ Exon 17
___ Other (specify): _________________
+PDGFRA Mutational Analysis (Note E)
  ___ No mutation detected
  ___ Mutation identified (specify): ____________________
  ___ Cannot be determined (explain): ____________________
+PDGFRA Exons Assessed (Note E) (select all that apply)
  ___ Exon 12
  ___ Exon 14
  ___ Exon 18
  ___ Other (specify): ____________________
+SDH A / B / C / D Mutational Analysis (Note G)
  ___ No mutation detected
  ___ Mutation identified (specify): ____________________
  ___ Cannot be determined (explain): ____________________
+NF1 Mutational Analysis (Note H)
  ___ No mutation detected
  ___ Mutation identified (specify): ____________________
  ___ Cannot be determined (explain): ____________________
+BRAF Mutational Analysis (Note F) (select all that apply)
  ___ No BRAF mutation detected
  ___ BRAF V600E (c.1799T>A, exon 15) mutation
  ___ Other BRAF mutation (specify): ____________________
  ___ Cannot be determined (explain): ____________________
+BRAF Fusion Gene Analysis (Note I)
  ___ No fusion detected
  ___ Fusion identified (specify): ____________________
  ___ Cannot be determined (explain): ____________________
+FGFR1 Fusion Gene Analysis (Note I)
  ___ No fusion detected
  ___ Fusion identified (specify): ____________________
  ___ Cannot be determined (explain): ____________________
Other Gene Mutational Analysis
  +Mutation Name (repeat this question as needed): ____________________
    ___ No mutation detected
    ___ Mutation identified (specify): ____________________
    ___ Cannot be determined (explain): ____________________
Other Fusion Gene Event Analysis
  +Fusion Name (repeat this question as needed): ____________________
    ___ No fusion detected
    ___ Fusion identified (specify): ____________________
    ___ Cannot be determined (explain): ____________________

COMMENTS

Comment(s): ____________________
Explanatory Notes

A. Reporting Nomenclature
Consistent gene mutation nomenclature is essential for efficient and accurate reporting.¹ The following are examples as recommended by Human Genome Variation Society (HGVS) for description of variant changes.² It is also preferred that protein alterations are mentioned in the report in addition to genomic coordinates.

Examples of DNA, RNA, and Protein Nomenclature
DNA: A, G, C, T (example: c.957A>T)
RNA: a, g, c, u (example: r.957 a>u)
Protein: 3-letter amino acid code, X= Stop codon (example: p. Glu78Gln)

Examples of Nomenclatures for Types of Sequence Variants

<table>
<thead>
<tr>
<th>Types of Variation</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Substitution</td>
<td>c.123A&gt;G</td>
</tr>
<tr>
<td>Deletion</td>
<td>c.123delA, c.586_591delTGGTCA or c.586_591del6</td>
</tr>
<tr>
<td>Duplication</td>
<td>c.123dupA, c.586_591dupTGGTCA or c.586_591dup6</td>
</tr>
<tr>
<td>Insertion</td>
<td>c.123_124insC, c.1086_1087insGCGTGA</td>
</tr>
<tr>
<td>Frame shift</td>
<td>p. Arg83 fs or p. Arg83Ser fsX15</td>
</tr>
<tr>
<td>Deletion/insertions “indel”</td>
<td>c.112_117delAGGTCAinsTG</td>
</tr>
</tbody>
</table>

References

B. Immunohistochemical Analysis
Because small-molecule kinase inhibitor therapy is highly effective in the treatment of GIST, it has become imperative to distinguish GIST from its histologic mimics, mainly leiomyoma, leiomyosarcoma, schwannoma, and desmoid fibromatosis.¹² Immunohistochemistry is instrumental in the workup of GIST. For the initial workup of GIST, a basic immunohistochemical panel including CD117 (KIT), DOG1 (Ano1), Desmin, S100 protein, and CD34 is recommended. GIST is immunoreactive for KIT (CD117) (approximately 95%) and/or DOG1 (>99%).³⁴ KIT immunoreactivity is usually strong and diffuse but can be more focal in unusual cases (Figure 1, A, and B). It is not unusual for GIST to exhibit dot-like perinuclear staining (Figure 1, C), while less commonly, some cases exhibit membranous staining (Figure 1, D). These patterns do not clearly correlate with mutation type or response to therapy. Most KIT-negative/DOG1 positive GIST is gastric or extra-visceral GIST and almost invariably harbor a platelet-derived growth factor receptor A (PDGFRα) mutation.⁵ DOG1 expression is not related to mutational status in GIST, and it may be a useful marker to identify a subset of patients with CD117-negative GIST, who might benefit from targeted therapy.⁶⁷ Approximately 70% of GIST are positive for CD34, 30% to 40% are positive for smooth muscle actin, 5% are positive for S100 protein (usually focal), 5% are positive for desmin (usually focal), and 1% to 2% are positive for keratin (weak/focal).²
Note: PanTrk immunohistochemistry may be positive in GIST, a tumor typically negative for NTRK fusion and this immunostain is not recommended.

Since succinate dehydrogenase (SDH)-deficient GIST may be familial, have specific implications (see the following), it is recommended that all gastric GIST be screened for loss of SDH by immunohistochemistry, best accomplished by immunostaining for SDHB, which is lost in all independent of the SDH-subunit that is inactivated. \(^8\)\(^9\)\(^10\)\(^11\) Mutations in SDHA are detected in 30% of SDH-deficient GIST and loss of expression of SDHA specifically identifies tumors with SDHA mutations; other SDH-deficient GIST show normal (intact) cytoplasmic staining for SDHA.\(^12\)\(^13\) Patients with SDH-deficient GIST should be referred to a genetic counselor for appropriate workup.

**Figure 1.** Patterns of KIT staining in gastrointestinal stromal tumor (GIST). A, Diffuse and strong immunoreactivity in a typical GIST. B, Focal and weak pattern in an epithelioid gastric GIST with a PDGFRA mutation. C, Dot-like perinuclear staining. D, Membranous pattern. (Original magnification X400)

**References**


C. Molecular Analysis

Approximately 75% of GIST possess activating mutations in the KIT gene, whereas another 10% have activating mutations in the PDGFRA gene.1234 These mutations result in virtually full-length KIT proteins that exhibit ligand-independent activation. KIT and PDGFRA each contain 21 exons. However, mutations cluster within “hotspots”: exons 9, 11, 13, and 17 in KIT and exons 12, 14, and 18 in PDGFRA (Figure 2). About 5% to 10% of GIST appear to be negative for both KIT and PDGFRA mutations. The most recent NCCN Task Force on GIST strongly encourages that KIT and PDGFRA mutational analysis be performed if tyrosine kinase inhibitors (TKIs) are considered as part of the treatment plan for unresectable or metastatic disease and that mutational analysis be considered for patients with primary disease, particularly those with high-risk tumors. KIT and PDGFRA mutation status can be determined easily from paraffin-embedded tissue. Secondary or acquired mutations can be associated with development of tumor resistance in the setting of long-term imatinib mesylate treatment. These are usually point mutations that occur most commonly in KIT exons 13, 14, and 17.5 The clinical utility of these mutations is an evolving concept, but it is important not to confuse them with the primary or initial mutation in GIST.

Recent studies focusing on the molecular classification of GIST recognized two major subgroups: succinate dehydrogenase (SDH)-competent and SDH-deficient GIST, both of which can arise in the sporadic or familial setting.52 SDH-competent GIST include tumors with mutations of KIT and PDGFRA as well as a subset of wild-type GIST with mutations mainly in NF1 and BRAF genes or rarely fusion gene events involving FGFR1 or BRAF.8,9,10,11,12,13 On the other hand, SDH-deficient GIST includes tumors with a genetic alteration in any of the SDH subunits leading to SDH dysfunction.

SDH-deficient GIST represents approximately 8% of GIST, although these may arise sporadically. The majority of pediatric GIST arise in Carney triad and Carney-Stratakis syndrome and are SDH-deficient. SDH is a mitochondrial enzyme comprising four subunits (SDHA, SDHB, SDHC, and SDHD) that are
involved in the Krebs cycle. Genetic alteration of any of the four subunits results in SDH dysfunction and subsequent loss of SDHB expression by immunohistochemistry. SDH-deficient GIST arises almost exclusively in the stomach, affects predominantly female patients, and tends to manifest at a young age. Pathologic features associated with SDH-deficient tumors include multinodular and/or plexiform growth pattern, epithelioid morphology, lymphovascular invasion, nodal involvement, and frequent metastasis to the liver and peritoneum. Importantly, germline mutations in the genes coding for any of the SDH subunits can lead to paragangliomas/pheochromocytomas, SDH-deficient renal cell carcinoma, and pituitary tumors in addition to GIST. It is recommended that all gastric GIST be screened for loss of SDHB by immunohistochemistry. All patients with SDH-deficient GIST identified by loss of SDHB immunostain should be referred to a genetic counselor.

* Refers to exons involved most frequently by secondary/acquired mutations.

Figure 2. Locations and frequency of activating KIT and PDGFRA mutations in GIST. They were adapted with permission from Heinrich et al. Copyright 2003 by the American Society of Clinical Oncology. All rights reserved.

KIT and PDGFRA are excellent targets for small-molecule tyrosine kinase inhibitors, and compounds of this class, imatinib mesylate (Gleevec, Novartis Pharmaceuticals, Basel, Switzerland), sunitinib malate (Sutent, Pfizer Pharmaceuticals, New York, New York), avapritinib (Ayvakit, PDGFRA D842V (exon 18) mutant, may be resistant to standard therapy), regorafenib (3rd line), and ripretinib (4th line, Qinlock) have shown efficacy in clinical trials and have been approved by the US Food and Drug Administration for the treatment of GIST. SDH-deficient GIST is usually resistant to imatinib but may have a higher probability of response to sunitinib. Because different tyrosine kinase inhibitors (TKIs) may have differential efficacy depending on the type of mutation present in GIST, oncologists may want to know the mutation status of each GIST because this may influence which drug the patient receives. Secondary resistance mutations may also affect drug selection as their significance is further defined.

References


D. KIT Mutational Analysis
The most common mutations affect the juxta membrane domain encoded by exon 11 (two-thirds of GIST). These mutations include in-frame deletions, substitutions, and insertions. Deletions (in particular codon 557 and/or 558) are associated with shorter progression-free and overall survival.\textsuperscript{1,2,3,4,5,6} The vast majority of exon 11-mutated GIST is located in the stomach. About 7% to 10% of the tumors harbor mutations in the extracellular domain encoded by exon 9 (most commonly insAY502-503).\textsuperscript{5,7} Exon 9-mutant GIST arises predominantly in the small bowel and has reduced sensitivity to imatinib which could be overcome by using higher doses.\textsuperscript{5} Primary mutations in the activation loop (exon 17) and ATP binding region (exon 13) are uncommon (1%). The majority of these mutations are substitutions.\textsuperscript{8} KIT exon 8 mutations are extremely rare (0.15%).\textsuperscript{5,9} Secondary or resistance mutations occur commonly in tumors harboring primary exon 11 mutations. These newly acquired secondary mutations are always located in exons encoding tyrosine kinase domain (exons 13, 14, 17).\textsuperscript{10}

References

E. PDGFRA Mutational Analysis
More than 80% of KIT-negative GIST have PDGFRA mutations. The majority of PDGFRA-mutated GIST arises in the stomach, usually with epithelioid or mixed epithelioid and spindle cell morphology and often with myxoid stromal changes.\textsuperscript{12} PDGFRA-mutated GIST tends to have a lower risk of recurrence.\textsuperscript{13} Activation of PDGFRA is seen in GIST harboring mutations in juxta membranous domain (exon 12), the ATP binding domain (exon 14), or the activation loop (exon 18).\textsuperscript{14} Mutations include
substitutions and deletions. Primary resistance to imatinib is seen with the most common PDGFRA exon 18 D842V mutation.1

References

F. BRAF Mutational Analysis
Activating mutations of BRAF (V600E) have been identified in a small subset (7%) of KIT/PDGFRA wild-type GIST. These tumors show a predilection for small bowel location, arise in middle-aged females, exhibit a high mitotic rate, and are associated with early metastasis.1,2 BRAF-mutated GIST shows primary resistance to imatinib but may respond to BRAF inhibitors.2

References

G. SDH A/B/C/D Mutational Analysis
The succinate dehydrogenase (SDH) complex (mitochondrial complex II) participates in both the Krebs cycle and the electron transport chain of oxidative phosphorylation. About 8% of GIST (all lacking mutations in KIT and PDGFRA) is caused by dysfunction of the SDH complex (‘SDH-deficient GIST’). Around 50% of patients affected by such tumors harbor germline mutations in one of the SDH subunit genes (SDH A/B/C or D). SDHA-inactivating mutations are most common, detected in about 30% of SDH-deficient GIST. Mutations involve exons 2, 3, 5, 6, 7, 8, 9, 10, 11, 13, 14 of SDHA; exons 1, 2, 3, 4, 6, 7 of SDHB; exons 1, 4, 5 of SDHC; and exons 4 and 6 of SDHD. While the majority of the mutations are substitutions; deletions, splice-site mutations, frameshift, and duplications have also been reported.1,2,3,4

References

H. Neurofibromatosis Type 1 (NF1) Mutational Analysis
NF1 is an inherited, autosomal dominant disease characterized by multiple café au lait spots, Lisch nodules, freckling, and development of neurofibromas. GIST in NF1 patients arises predominantly from
the small intestine, including duodenum, can be multicentric, lack KIT and PDGFRA mutations and are associated with Cajal cell hyperplasia. Only a minority (approximately 7%) of NF1 patients develop NF1-mutated GIST, therefore, molecular testing for canonical mutations in KIT and PDGFRA is recommended for GIST arising in the setting of neurofibromatosis.

References

1. FGFR1 or BRAF Fusion Gene Analysis
Rare cases of GIST lacking other driver events (e.g., KIT, PDGFRA, SDH, RAS, or NF1 mutations; quadruple wild-type GIST) have recently been reported to harbor fusion genes involving FGFR1 or BRAF. Identification of these alterations may impact therapeutic considerations.

References