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

Types of Variation	*Examples*
Substitution	c.123A>G
Deletion	c.123delA, c.586_591delTGGTCA or c.586_591del6
Duplication	c.123dupA, c.586_591dupTGGTCA or c.586_591dup6
Insertion	c.123_124insC, c.1086_1087insGCGTGA
Frame shift	p. Arg83 fs or p. Arg83Ser fsX15
Deletion/insertions “indel”	c.112_117delAGGTCAinsTG

References

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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.[1,2] 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%).[3,4,5] 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 (*PDGFRA*) mutation.[6] 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.[4,5] 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).[7]

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.

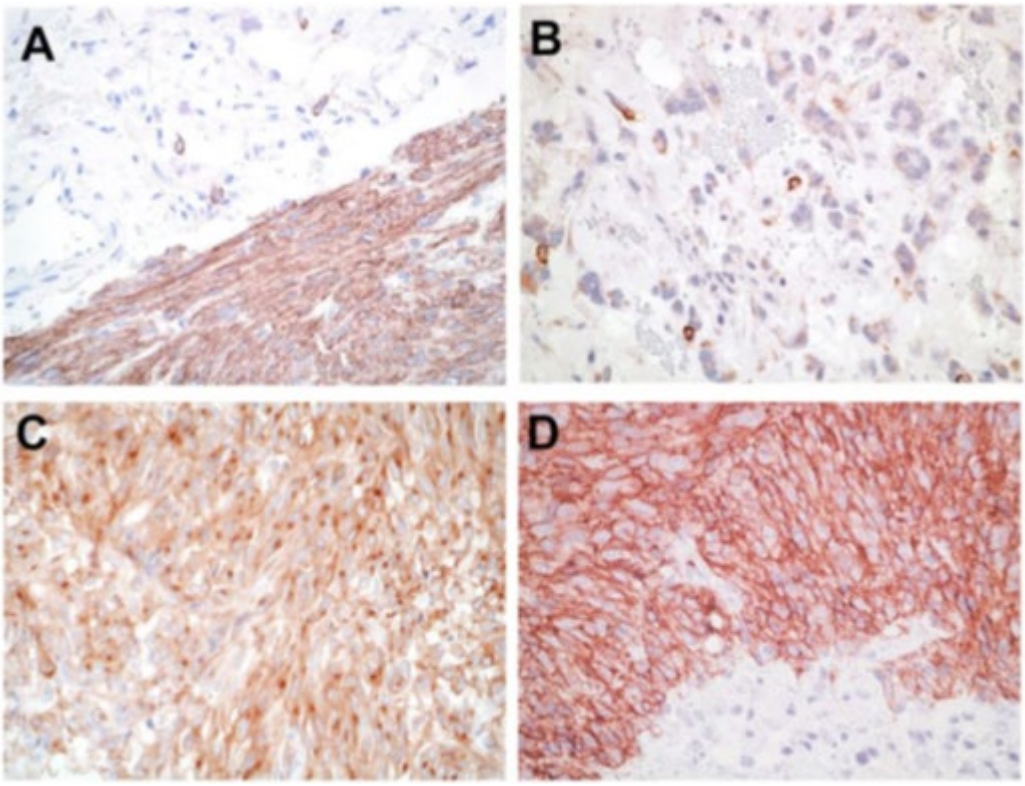

Figure 1. Patterns of KIT staining in gastrointestinal stromal tumor (GIST). A, Diffuse and strong immunoreactivity in a typical GIST. B, Focal and weak pattern in an epithelioid gastric GIST with a *PDGFRA* mutation. C, Dot-like perinuclear staining. D, Membranous pattern. (Original magnification X400)

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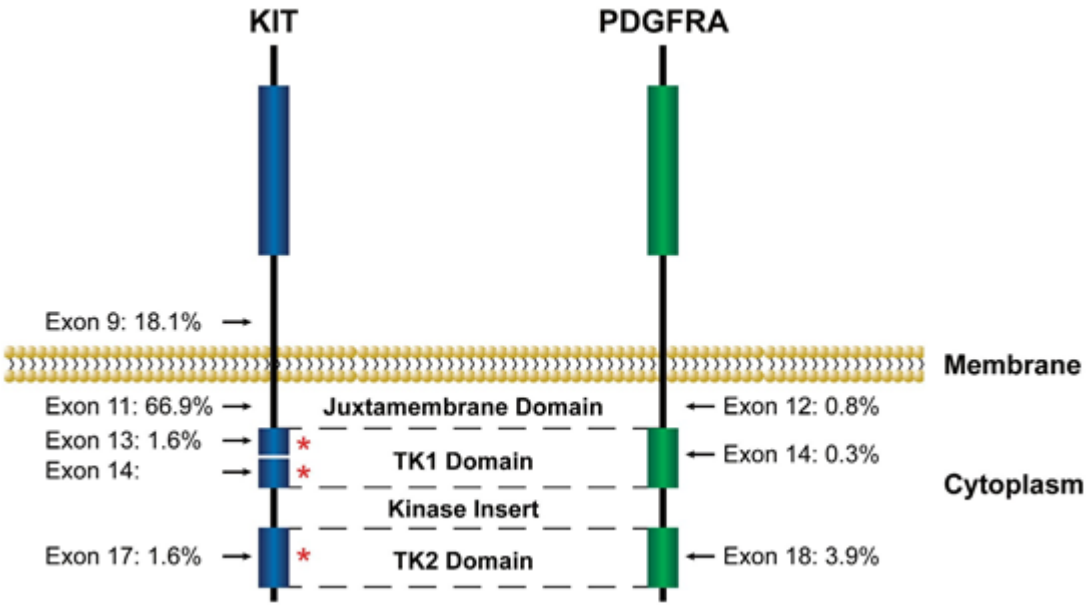
C. Molecular Analysis

Approximately 75% of GIST possess activating mutations in the *KIT* gene, whereas another 10% have activating mutations in the *PDGFRA* gene.[1,2,3,4] 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 (SHD)-competent and SDH-deficient GIST, both of which can arise in the sporadic or familial setting.[6,7] SDH-competent GIST include tumors with mutations of *KIT* and *PDGFRA* as well of 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 exclusive 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.[1] 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.[14,15,16,17] SDH-deficient GIST is usually resistant to imatinib but may have a higher probability of response to sunitinib.[6,18] 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 [19] because this may influence which drug the patient receives.[1] Secondary resistance mutations may also affect drug selection as their significance is further defined.

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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.[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).[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.[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.[8] *KIT* exon 8 mutations are extremely rare (0.15%).[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).[10]

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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.[1,2] *PDGFRA*-mutated GIST tends to have a lower risk of recurrence.[1,3] 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).[1,2] Mutations include

substitutions and deletions. Primary resistance to imatinib is seen with the most common *PDGFRA* exon 18 *D842V* mutation.[1]

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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]

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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]

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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.[1,2] 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.[2,3,4,5]

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I. 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.[1,2,3,4]

References

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