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

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| **Version:** GIST Biomarkers 1.0.0.2 | **Template Posting Date:** February 2020 |

**This biomarker template is not required for accreditation purposes but may be used to facilitate compliance with CAP Accreditation Program checklist requirements.**

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Summary of Changes

**V1.0.0.2**

**The following data elements were modified:**

Revised the Explanatory Notes for Immunohistochemistry and Molecular analysis

GIST Biomarker Reporting Template

Template web posting date: February 2020

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.

**GASTROINTESTINAL STROMAL TUMOR (GIST)**

**Select a single response unless otherwise indicated.**

***Note:*** *Use of this template is optional. If some studies were performed on different specimen(s), the specimen number(s) should be provided.*

**RESULTS**

**Immunohistochemical Studies (Note A)**

\_\_\_ KIT (CD117)

\_\_\_ Positive

\_\_\_ Negative

\_\_\_ DOG1 (ANO1)

\_\_\_ Positive

\_\_\_ Negative

\_\_\_ SDHB

\_\_\_ Intact

\_\_\_ Deficient

\_\_\_ SDHA

\_\_\_ Intact

\_\_\_ Deficient

\_\_\_ Other (specify): \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

\_\_\_ Positive

\_\_\_ Negative

**Molecular Genetic Studies (eg, *KIT, PDGFRA, BRAF, SDHA/B/C/D, or NF1* mutational analysis) (Note B)**

\_\_\_ Submitted for analysis; results pending

\_\_\_ Performed, see separate report: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

\_\_\_ Performed

Specify method(s) and results: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

\_\_\_ Not performed

***KIT* Mutational Analysis (Note C)**

\_\_\_ No mutation detected

\_\_\_ Mutation identified (specify:)\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

\_\_\_ Cannot be determined (explain): \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

***PDGFRA* Mutational Analysis (Note D)**

\_\_\_ No mutation detected

\_\_\_ Mutation identified (specify): \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

\_\_\_ Cannot be determined (explain): \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

***BRAF* Mutational Analysis (Note E)**

\_\_ No *BRAF* mutation detected

\_\_\_ *BRAF* V600E (c.1799T>A) mutation

\_\_\_ Other *BRAF* mutation (specify): \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

\_\_\_ Cannot be determined (explain): \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

***SDHA/B/C/D* Mutational Analysis (Note F)**

\_\_\_ No mutation detected

\_\_\_ Mutation identified (specify): \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

\_\_\_ Cannot be determined (explain): \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

***NF1* Mutational Analysis (Note G)**

\_\_\_ No mutation detected

\_\_\_ Mutation identified (specify): \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

\_\_\_ Cannot be determined (explain): \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

**METHODS**

**Dissection Method(s) (select all that apply) (Note H)**

\_\_\_ Laser capture microdissection

\_\_\_ Manual under microscopic observation

\_\_\_ Manual without microscopic observation

\_\_\_ Cored from block

\_\_\_ Whole tissue section (no tumor enrichment procedure employed)

**KIT Mutational Analysis (Note C)**

Exons Assessed (select all that apply)

\_\_\_ Exon 9

\_\_\_ Exon 11

\_\_\_ Exon 13

\_\_\_ Exon 14

\_\_\_ Exon 17

\_\_\_ Other (specify): \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Testing Method(s)*#*

Specify name of method used and exons tested: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

*# Please specify if different testing methods are used for different exons.*

**PDGFRA Mutational Analysis (Note D)**

Exons Assessed (select all that apply)

\_\_\_ Exon 12

\_\_\_ Exon 14

\_\_\_ Exon 18

\_\_\_ Other (specify): \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Testing Method(s)*#*

Specify name of method used and exons tested: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

*# Please specify if different testing methods are used for different exons.*

***BRAF* Mutational Analysis (Note E)**

Exons Assessed

\_\_\_ Exon 15

\_\_\_ Other (specify): \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Testing Method(s)

Specify name of method used and exons tested: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

***SDH A/B/C/D* Mutational Analysis (Note F)**

\_\_\_ Exons assessed (specify): \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Testing Method(s)*#*

Specify name of method used and exons tested: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

*# Please specify if different testing methods are used for different exons.*

***NF1* Mutational Analysis (Note G)**

Exons assessed (specify): \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Testing Method(s)*#*

\_\_\_ Sanger

\_\_\_ Next-generation sequencing (NGS)

\_\_\_ Other (specify): \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Specify name of method used: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

*# Please specify if different testing methods are used for different exons.*

**COMMENT(S)**

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

*Note: 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 Organisation (HUGO) Nomenclature Committee (www.genenames.org; accessed February 16, 2015). (Note I)*

*All reported gene sequence variations should be identified following the recommendations of the Human Genome Variation Society (www.hgvs.org/mutnomen/; accessed February 16, 2015). (Note I)*

Explanatory Notes

**A. Immunohistochemical Analysis**

Because of the advent of small-molecule kinase inhibitor therapy in the treatment of GIST (see the following), 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 work up of GIST, a basic immunohistochemical panel including CD117 (KIT), DOG1 (Ano1), Desmin, S100 protein and CD34 is recommended. GISTs are immunoreactive for KIT (CD117) (approximately 95%) and/or DOG1(>99%).3-5 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 GISTs 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 GISTs are gastric or extra-visceral GISTs and almost invariably harbor a *platelet-derived growth factor receptor A (PDGFRA)* mutation.6 DOG1 expression is not related to mutational status in GISTs, and it may be a useful marker to identify a subset of patients with CD117-negative GISTs, who might benefit from targeted therapy 4,5. Approximately 70% of GISTs 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

Since succinate dehydrogenase (SDH)-deficient GISTs have specific implications (see the following), it is recommended to screen all gastric GISTs for loss of SDH by immunohistochemistry, usually best accomplished by staining for SDHB, which is loss in all subtypes of SDH-deficient GISTs. 8-11 Mutations in SDHA are detected in 30% of SDH-deficient GISTs and loss of expression of SDHA specifically identifies tumors with SDHA mutations; other SDH-deficient GISTs show normal (intact) cytoplasmic staining for SDHA.12,13 Patients with SDH-deficient GIST should be referred to a genetic counselor for appropriate work up.



**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

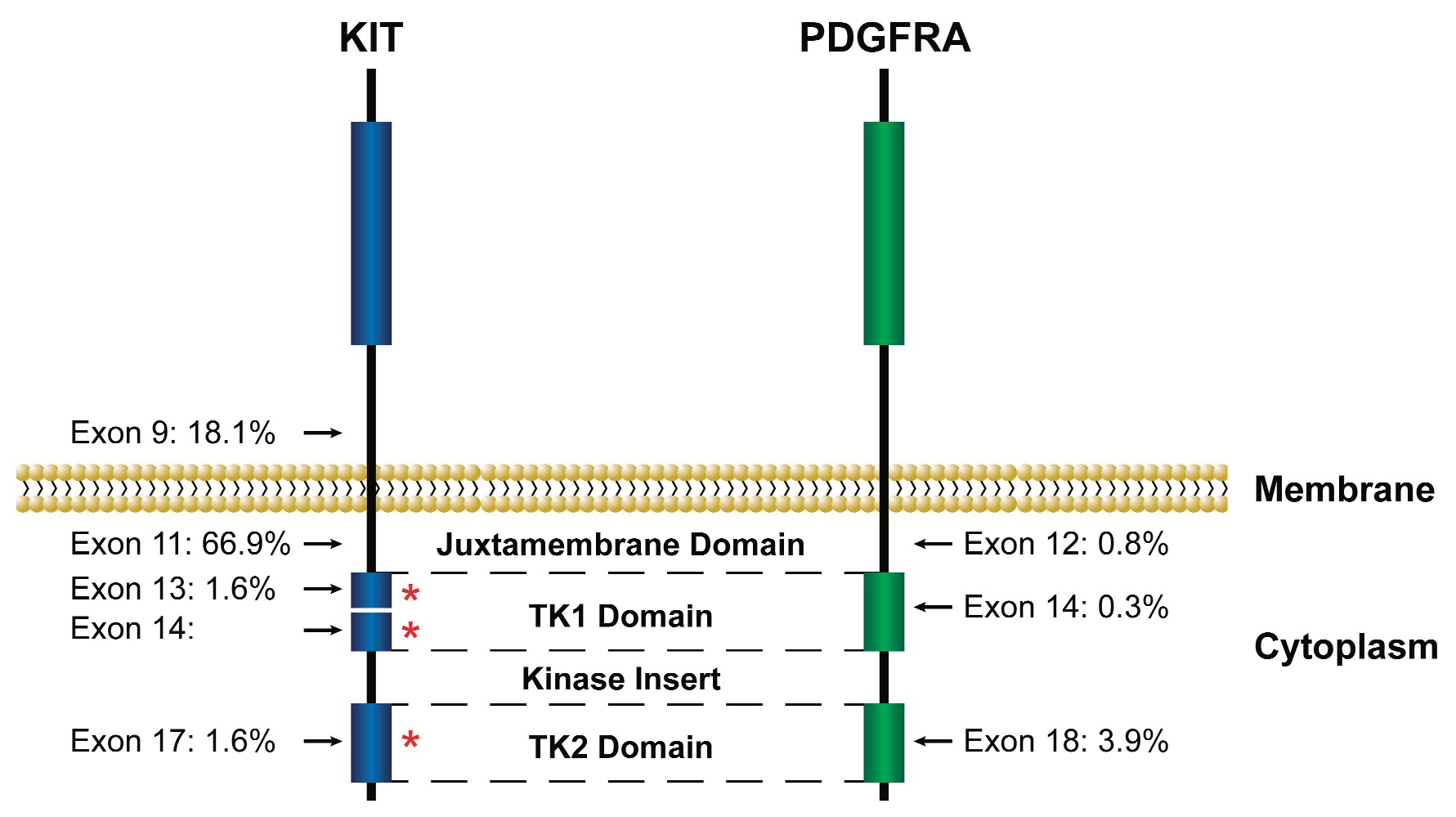
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9. Gill AJ. Succinate dehydrogenase (SDH) and mitochondrial driven neoplasia. Pathology. 2012 Jun;44(4):285-92.
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12. Wagner AJ, Remillard SP, Zhang YX, et al. Loss of expression of SDHA predicts SDHA mutations in gastrointestinal stromal tumors. *Mod Pathol*. 2013;26(2):289-294.12.
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**B. Molecular Analysis**

Approximately 75% of GISTs possess activating mutations in the *KIT* gene, whereas another 10% have activating mutations in the *PDGFRA* gene.1-4 These mutations result in virtually full-length KIT proteins that exhibit ligand-independent activation. *KIT* and *PDGFRA* eachcontain 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 GISTs 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 GISTs recognized two major subgroups : succinate dehydrogenase (SHD)-competent and SDH-deficient GISTs, both of which can arise in the sporadic or familiar setting.6.7 SDH-competent GISTs include tumors with mutations of KIT and PDGFRA as well of a subset of wild-type GISTs with mutations mainly in NF1 and BRAF genes. On the other hand, SDH-deficient GISTs include tumors with a genetic alteration in any of the SDH subunits leading to SDH dysfunction.

SDH-deficient GISTs represent approximately 8% of GISTs and comprise some sporadic cases, the majority of pediatric GISTs, and two forms of syndromic GISTs (Carney triad and Carney-Stratakis syndrome).6 SDH is a mitochondrial enzyme comprising four subunits (SDHA, SDHB, SDHC and SDHD) that is 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 GISTs arise almost exclusive in the stomach, affect predominantly female patients and tend 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 SHD subunits can lead to paragangliomas/pheochromocytomas, SDH-deficient renal cell carcinoma and pituitary tumors in addition to GISTs. Since SDH-deficient GISTs typically require germline genetic testing possibly including family members as well as possible surveillance for paragangliomas/pheochromocytomas, it is recommended that all gastric GISTs be screened for loss of SDHB by immunohistochemistry. All patients with SDH-deficient GISTs identified by loss of SDHB stain 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. 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 two compounds of this class, imatinib mesylate (Gleevec, Novartis Pharmaceuticals, Basel, Switzerland) and sunitinib malate (Sutent, Pfizer Pharmaceuticals, New York, New York), have shown efficacy in clinical trials and have been approved by the US Food and Drug Administration for the treatment of GIST.8-10 SDH-deficient GISTs are usually resistant to imatinib but may have a higher probability of response to sunitinib.6 Because different tyrosine kinase inhibitors (TKIs) may have more efficacy in genetic subsets of GIST, oncologists may want to know the mutation status of each GIST, because this may impact which drug each patient should receive.1,11 Secondary resistance mutations may also affect drug selection as their significance is further defined.

References

1. Heinrich MC, Corless CL, Demetri GD, et al. Kinase mutations and imatinib response in patients with metastatic gastrointestinal stromal tumor. *J Clin Oncol*. 2003;21(23):4342-4349.

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11. Corless CL, Schroeder A, Griffith D, et al. PDGFRA mutations in gastrointestinal stromal tumors: frequency, spectrum and in vitro sensitivity to imatinib. *J Clin Oncol*. 2005;23(23):5357-5364.

**C.  *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.1-6 The vast majority of exon 11-mutated GISTs are located in the stomach. 5 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 GISTs arise predominantly in the small bowel and have 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

References

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**D.  *PDGFRA* Mutational Analysis**

More than 80% of *KIT*-negative GISTS have *PDGFRA* mutations. The majority of *PDGFRA*-mutated GISTs arise in the stomach, usually with epithelioid or mixed epithelioid and spindle cell morphology and often with myxoid stromal changes. 1,2 *PDGFRA*-mutated GISTs tend to have a lower risk of recurrence. 1,3 Activation of PDGFRA is seen in GISTs 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

References

1. Mei L, Smith SC, Faber AC, et al. Gastrointestinal Stromal Tumors: The GIST of Precision Medicine. *Trends Cancer*. 2018;4:74-91.

2. LaCosta J, Miettinen M. Clinical significance of oncogenic KIT and PDGFRA mutations in gastrointestinal stromal tumors. *Histopathology*. 2008;53(3):245-266.

3. Barnett CM, Corless CL, Heinrich MC. Gastrointestinal stromal tumors: molecular markers and genetic subtypes*. Hematol Oncol Clin North Am*. 2013 Oct;27(5):871-88.

**E. *BRAF* Mutational Analysis**

Activating mutations of *BRAF* (V600E) have been identified in a small subset (7%) of *KIT*/*PDGFRA* wild-type GISTs. 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 GISTsshow primary resistance to imatinib but may respond to *BRAF* inhibitors.2

References

1. Agaram NP, Wong GC, Guo T, et al. Novel V600E BRAF mutations in imatinib-naive and imatinib-resistant gastrointestinal stromal tumors. *Genes Chromosomes Cancer.* 2008;47(10):853–859.

2. Mei L, Smith SC, Faber AC, et al. Gastrointestinal Stromal Tumors: The GIST of Precision Medicine. *Trends Cancer*. 2018;4:74-91.

**F. *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 GISTs (all lacking mutations in *KIT* and *PDGFRA*) are caused by dysfunction of the SDH complex ("SDH-deficient GISTs"). Around 50% of patients affected by such tumors harbor germline mutations in one of the SDH subunit genes (*SDHA/B/C* or *D*). *SDHA*-inactivating mutations are most common, detected in about 30% of SDH-deficient GISTs. 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, frame shift, and duplications have also been reported.1-4

References

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2. Wagner AJ, Remillard SP, Zhang YX, et al. Loss of expression of SDHA predicts SDHA mutations in gastrointestinal stromal tumors. *Mod Pathol.* 2013;26(2):289-294.

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**G. 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. GISTs in NF1 patients arise 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 NF-1 patients develop *NF1*-mutated GISTs, therefore, molecular testing for canonical mutations in *KIT* and *PDGFRA* is recommended for GISTs arising in the setting of neurofibromatosis.2

References

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2. Mei L, Smith SC, Faber AC, et al. Gastrointestinal Stromal Tumors: The GIST of Precision Medicine. *Trends Cancer*. 2018;4:74-91.

**H. Dissection Method**:

While in majority of cases GIST samples show tumor percentage (%) well above the analytical sensitivity of Sanger sequencing (>50% neoplastic cell percentage/20% to 25% mutant allele percentage), in cases of mutation analysis of treated samples, careful macro/microdissection may be necessary to avoid false negative results.

**I. Reporting Nomenclature**

Consistent gene mutation nomenclature is essential for efficient and accurate reporting.1 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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