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

Meera Hameed, MD; Christopher Corless, MD, PhD; Suzanne George, MD; Jason L. Hornick, MD, PhD; Sanjay Kakar, MD; Alexander J. Lazar, MD, PhD; Laura Tang, MD; for the Members of the Cancer Biomarker Reporting Committee, College of American Pathologists

The College of American Pathologists offers these templates to assist pathologists in providing clinically useful and relevant information when reporting results of biomarker testing. The College regards the reporting elements in the templates as important elements of the biomarker test report, but the manner in which these elements are reported is at the discretion of each specific pathologist, taking into account clinician preferences, institutional policies, and individual practice.

The College developed these templates as educational tools to assist pathologists in the useful reporting of relevant information. It did not issue them for use in litigation, reimbursement, or other contexts. Nevertheless, the College recognizes that the templates might be used by hospitals, attorneys, payers, and others. The College cautions that use of the templates other than for their intended educational purpose may involve additional considerations that are beyond the scope of this document.

TEMPLATE FOR REPORTING RESULTS OF BIOMARKER TESTING OF SPECIMENS FROM PATIENTS WITH GASTROINTESTINAL STROMAL TUMORS

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.

Accepted for publication December 31, 2014.
Published as an Early Online Release March 2, 2015.

From Surgical Pathology, Department of Pathology, Memorial Sloan-Kettering Cancer Center, New York, New York (Drs Hameed and Tang); the Department of Pathology, Oregon Health & Science University, Portland (Dr Corless); the Center for Sarcoma and Bone Oncology, Dana-Farber Cancer Institute, Boston, Massachusetts (Dr George); the Department of Pathology, Brigham and Women's Hospital, Boston (Dr Hornick); the Department of Pathology, University of California, San Francisco, and the Veterans Affairs Medical Center, San Francisco (Dr Kakar); and the Department of Pathology, Sarcoma Research Center, University of Texas MD Anderson Cancer Center, Houston (Dr Lazar).

Dr George is a consultant for Bayer, ARIAD Pharmaceuticals, Pfizer, Novartis, and Blueprint Medicines. The other authors have no relevant financial interest in the products or companies described in this article.

doi: 10.5858/arpa.2014-0578-CP

Reprints: Meera Hameed, MD, Surgical Pathology, Department of Pathology, Memorial Sloan-Kettering Cancer Center, New York, NY 10065 (e-mail: hameedm@mskcc.org).

BIOMARKER REPORTING TEMPLATE

Gastrointestinal Stromal Tumor (GIST)

Select a single response unless otherwise indicated.

*Note: Use of this template is optional.**

* Reporting on the data elements in this template is not required.

RESULTS

Immunohistochemical Studies (note A)

___ KIT (CD117)†
  ___ Positive
  ___ Negative
___ DOG1 (ANO1)†
  ___ Positive
  ___ Negative
___ SDHB
  ___ Intact
  ___ Deficient
___ SDHA
  ___ Intact
  ___ Deficient
___ Other (specify): _____
  ___ Positive
  ___ Negative

† *Note: Duplicate testing/reporting of KIT (CD117) and DOG is not required if previously performed.*

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

___ Submitted for analysis; results pending
___ Performed, see separate report:
  _____
___ Performed
  Specify method(s) and results:
  _____
___ Not performed

***KIT* Mutational Analysis (note B)**

___ No mutation detected
___ Mutation identified (specify:) _____
___ Cannot be determined (explain):
_____

***PDGFRA* Mutational Analysis (note C)**

___ No mutation detected
___ Mutation identified (specify): _____
___ Cannot be determined (explain):
_____

***BRAF* Mutational Analysis (note D)**

___ 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 E)**

___ No mutation detected
___ Mutation identified (specify): _____
___ Cannot be determined (explain):
_____

***NF1* Mutational Analysis (note F)**

___ No mutation detected
___ Mutation identified (specify): _____
___ Cannot be determined (explain):
_____

METHODS

Dissection Method(s) (select all that apply) (note G)
___ 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**

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**

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 D)**

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 E)**

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 F)**

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 October 29, 2014).

All reported gene sequence variations should be identified following the recommendations of the Human Genome Variation Society (www.hgvs.org; accessed October 29, 2014).

EXPLANATORY NOTES

A: Immunohistochemical Analysis.—Because of the advent of small-molecule kinase inhibitor therapy for 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. Approximately 95% of GISTs are immunoreactive for KIT (CD117).[3] Most KIT$^-$ GISTs are gastric or omental tumors that harbor mutations in platelet-derived growth factor receptor A (*PDGFRA*).[4] KIT immu-

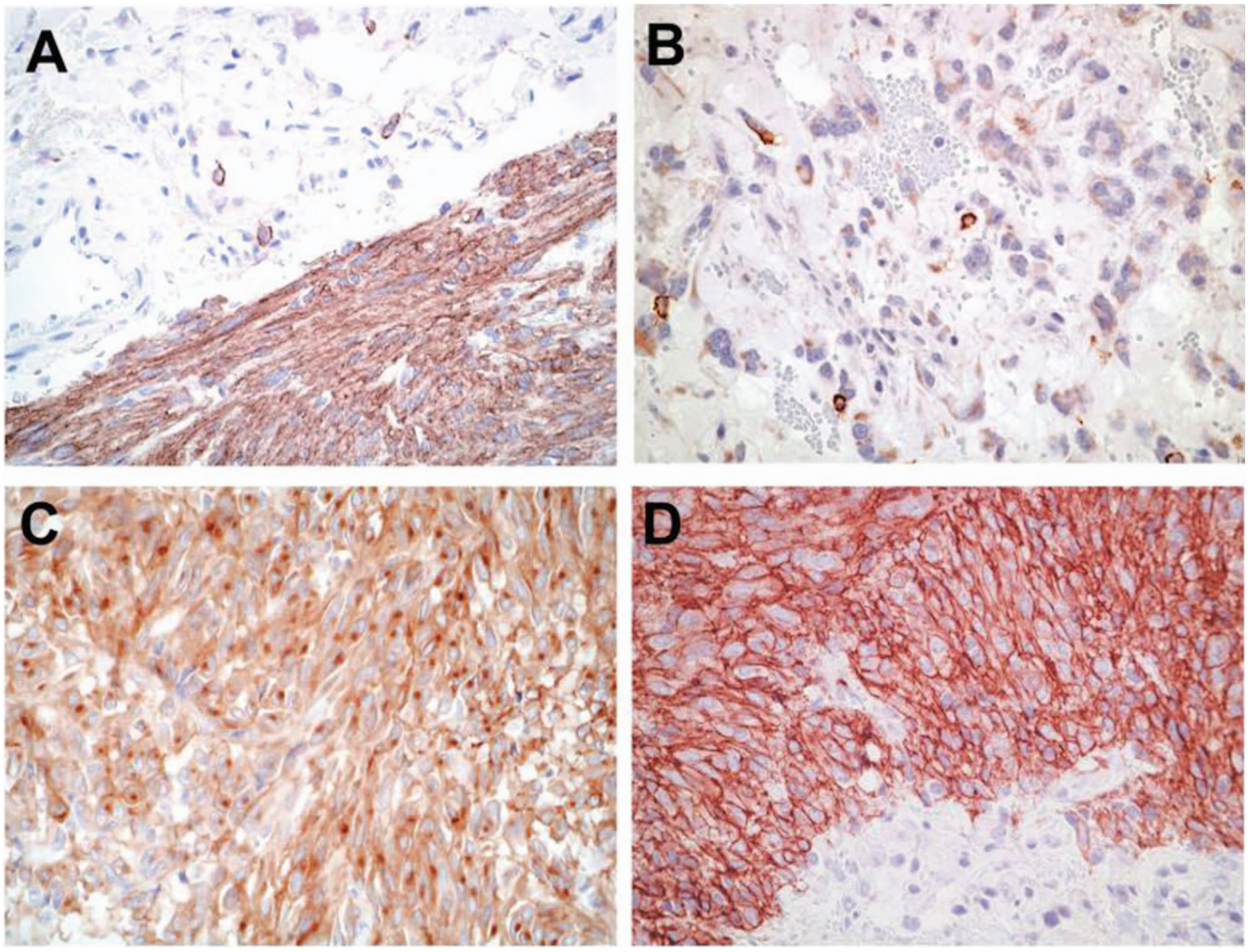

Figure 1. *Patterns of KIT staining in gastrointestinal stromal tumor (GIST). A, Diffuse and strong immunoreactivity in a typical GIST. B, Focal and weak pattern in an epithelioid gastric GIST with a* PDGFRA *mutation. C, Dotlike perinuclear staining. D, Membranous pattern (original magnification ×400 [A through D]). Reprinted from Rubin BP, Blanke CD, Demetri GD, et al; Cancer Committee, College of American Pathologists. Protocol for examination of specimens from patients with gastrointestinal stromal tumor.* Arch Pathol Lab Med. *2010;134(2):165–170 with permission from the* Archives of Pathology & Laboratory Medicine. *Copyright 2010. College of American Pathologists.*

noreactivity is usually strong and diffuse but can be more limited in extent in some cases (Figure 1, A and B). It is not unusual for GISTs to exhibit dotlike perinuclear staining (Figure 1, C), whereas, less commonly, some exhibit membranous staining (Figure 1, D). These patterns do not clearly correlate with mutation type or response to therapy. DOG1 is another highly sensitive and specific marker for GIST, which was discovered by gene expression profiling.[5,6] DOG1 (also known as anoctamin 1, ANO1) is particularly useful for KIT^{-} tumors and those with limited KIT expression; DOG1 is more sensitive than KIT for gastric epithelioid GISTs.[7] Approximately 70% of GISTs are positive for CD34, 30% to 40% are positive for smooth muscle actin, 5% are positive for S100 (usually focal), 5% are positive for desmin (usually focal), and 1% to 2% are positive for keratin (weak/focal).[8]

Approximately 8% of gastric GISTs are characterized by dysfunction of the mitochondrial succinate dehydrogenase (SDH) complex, known as *SDH-deficient GISTs*.[9] This clinically and pathologically distinctive subset of GISTs, which can be recognized by multinodular/plexiform architecture, has a predilection for children and young adults, is usually dominated by epithelioid cytomorphology, often metastasizes to lymph nodes (an exceeding rare occurrence in conventional GIST), and pursues a relatively indolent clinical course when metastatic.[10] Approximately 50% of patients with SDH-deficient GISTs have mutations in one of the SDH subunit genes (see the following). The diagnosis of SDH-deficient GIST can be confirmed by demonstrating loss of expression of SDHB by immunohistochemistry, which is observed irrespective of the presence of an identifiable SDH mutation (or the particular mutation type). Other genetic groups of GIST (eg, those with mutations in *KIT* or *PDGFRA*) show granular cytoplasmic staining for SDHB.[11] Mutations in *SDHA* are detected in 30% of SDH-deficient GISTs; *SDHA* is the most commonly mutated gene in this class of tumors (see below). Loss of expression of SDHA specifically identifies tumors with *SDHA* mutations[12,13]; other SDH-deficient GISTs show normal (intact) cytoplasmic staining for SDHA. Immunohistochemistry for SDHB and SDHA can, therefore, be used to triage patients for genetic testing.

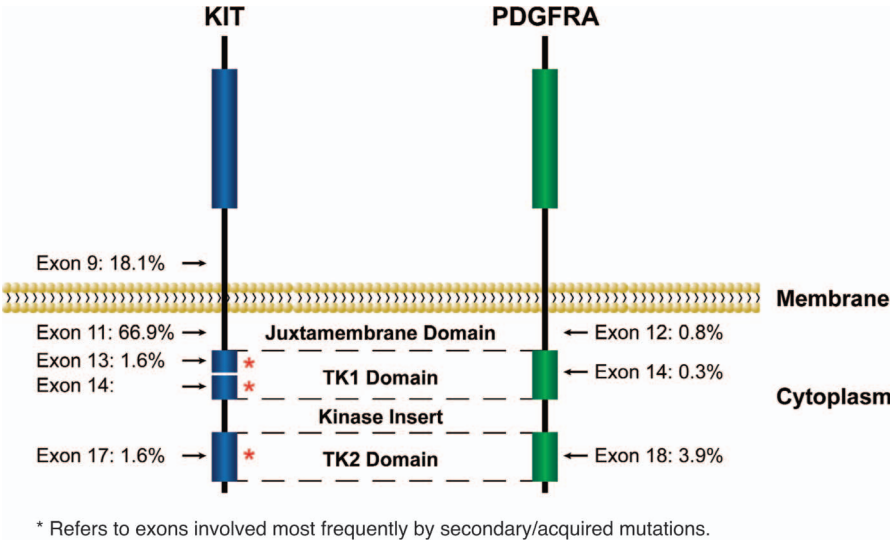

Figure 2. *Locations and frequency of activating* KIT *and* PDGFRA *mutations in gastrointestinal stromal tumor. Adapted with permission from Heinrich et al, 2003.*[14] *Copyright 2003 by the American Society of Clinical Oncology. All rights reserved.*

Immunohistochemistry for SDHB/SDHA need not be performed on all GISTs but only to confirm the diagnosis in a resection of a gastric GIST with multinodular architecture and to screen small biopsies of gastric GISTs with epithelioid cytomorphology (particularly in younger patients).

Molecular Analysis

Most GISTs are driven by oncogenic mutations in one of 2 receptor tyrosine kinases, *KIT* (75%) and *PDGFRA* (10%).[14,15] These mutations result in constitutive ligand-independent activation of full-length proteins. Mutations cluster within "hot spot" exons 9, 11, 13, 17 in *KIT* and exons 12, 14, 18 in *PDGFRA* (Figure 2). *KIT* and *PDGFRA* mutations are mutually exclusive. Multiple phase I, II, and international phase III trials have established the efficacy of tyrosine kinase inhibitors, such as imatinib, sunitinib, and regorafenib, in metastatic tumors and in the adjuvant setting.[16–20] Imatinib was originally granted accelerated approval for the treatment of advanced or metastatic GIST in 2002. In 2012, the US Food and Drug Administration approved the use of imatinib for GIST in the adjuvant setting. The most recent National Comprehensive Cancer Network task force on GIST strongly encourages that *KIT* and *PDGFRA* mutational analysis be performed if imatinib therapy is begun for unresectable or metastatic disease and that mutational analysis be considered for patients with primary disease, particularly those with high-risk tumors. In the setting of long-term imatinib therapy, secondary or acquired mutations occur in *KIT* exons 13, 14, and 17 and *PDGFRA* exon 18.[21]

B: *KIT* Mutational Analysis.—The most common mutations affect the juxtamembrane 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.[22–25] About 7% to 10% of the tumors harbor mutations in the extracellular domain encoded by exon 9 (most commonly insAY502-503).[26] Primary mutations in the activation loop (exon 17) and ATP binding region (exon 13) are uncommon (1%). Most of these mutations are substitutions.[27] *KIT* exon 8 mutations are extremely rare (0.15%).[28] Secondary or resistance mutations occur commonly in tumors harboring primary exon 11 mutations. The newly acquired secondary mutations are always located in exons encoding tyrosine kinase domain (exons 13, 14, 17).[29]

C: *PDGFRA* Mutational Analysis.—More than 80% of *KIT*$^-$ GISTs have *PDGFRA* mutations. Activation of PDGFRA is seen in GISTs harboring mutations in juxtamembranous domain (exon 12), the ATP-binding domain (exon 14), or the activation loop (exon 18).[30] Mutations include substitutions and deletions. Primary resistance to imatinib is seen with the most common *PDGFRA* exon 18 D842V mutation.

D: *BRAF* Mutational Analysis.—Activating mutations of *BRAF* (V600E) has been identified in a small subset (7%) of *KIT*/*PDGFRA* wild-type GISTs. These tumors show a predilection for small bowel location.[31]

E: *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 gastric 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*. Although most mutations are substitutions, deletions, splice-site mutations, frame shifts, and duplications have also been reported.[9,11,13,32]

F: Neurofibromatosis Type 1 (*NF1*) Mutational Analysis.—Neurofibromatosis type 1 is an inherited, autosomal-

Table 1. Examples of DNA, RNA, and Protein Nomenclatures

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)

Table 2. 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

dominant disease characterized by multiple café au lait spots, Lisch nodules, freckling, and development of neurofibromas. The GISTs in patients with NF1 arise predominantly from the small intestine, can be multicentric, and lack *KIT* and *PDGFRA* mutations. Until now, no specific genetic alterations have been found in NF1-related GIST.[32]

G: Dissection Method.—Although in most cases GIST samples show tumor percentage (%) well above the analytic sensitivity of Sanger sequencing (>50% neoplastic cell percentage per 20% to 25% mutant allele percentage), in cases of mutation analysis of treated samples, careful macrodissection or microdissection may be necessary to avoid false-negative results.

H: Reporting Nomenclature.—Consistent gene mutation nomenclature is essential for efficient and accurate reporting.[33] Tables 1 and 2 are examples as recommended by the Human Genome Variation Society for description of variant changes.[34] It is also preferred that protein alterations be mentioned in the report, in addition to genomic coordinates.

References

1. Hornick JL, Fletcher CD. Immunohistochemical staining for KIT (CD117) in soft tissue sarcomas is very limited in distribution. *Am J Clin Pathol*. 2002;117(2):188–193.
2. Miettinen M, Sobin LH, Sarlomo-Rikala M. Immunohistochemical spectrum of GISTs at different sites and their differential diagnosis with a reference to CD117 (KIT). *Mod Pathol*. 2000;13(10):1134–1142.
3. Sarlomo-Rikala M, Kovatich AJ, Barusevicius A, Miettinen M. CD117: a sensitive marker for gastrointestinal stromal tumors that is more specific than CD34. *Mod Pathol*. 1998;11(8):728–734.
4. Medeiros F, Corless CL, Duensing A, et al. KIT-negative gastrointestinal stromal tumors: proof of concept and therapeutic implications. *Am J Surg Pathol*. 2004;28(7):889–894.
5. West RB, Corless CL, Chen X, et al. The novel marker, DOG1, is expressed ubiquitously in gastrointestinal stromal tumors irrespective of KIT or PDGFRA mutation status. *Am J Pathol*. 2004;165(1):107–113.
6. Espinosa I, Lee CH, Kim MK, et al. A novel monoclonal antibody against DOG1 is a sensitive and specific marker for gastrointestinal stromal tumors. *Am J Surg Pathol*. 2008;32(2):210–218.
7. Miettinen M, Wang ZF, Lasota J. DOG1 antibody in the differential diagnosis of gastrointestinal stromal tumors: a study of 1840 cases. *Am J Surg Pathol*. 2009;33(9):1401–1408.
8. Miettinen M, Wang ZF, Sarlomo-Rikala M, et al. Succinate dehydrogenase-deficient GISTs: a clinicopathologic, immunohistochemical, and molecular genetic study of 66 gastric GISTs with predilection to young age. *Am J Surg Pathol*. 2011;35(11):1712–1721.
9. Doyle LA, Hornick JL. Gastrointestinal stromal tumours: from KIT to succinate dehydrogenase. *Histopathology*. 2014;64(1):53–67.
10. Doyle LA, Nelson D, Heinrich MC, Corless CL, Hornick JL. Loss of succinate dehydrogenase subunit B (SDHB) expression is limited to a distinctive subset of gastric wild-type gastrointestinal stromal tumours: a comprehensive genotype-phenotype correlation study. *Histopathology*. 2012;61(5):801–809.
11. Wagner AJ, Remillard SP, Zhang YX, Doyle LA, George S, Hornick JL. Loss of expression of SDHA predicts SDHA mutations in gastrointestinal stromal tumors. *Mod Pathol*. 2013;26(2):289–294.
12. Dwight T, Benn DE, Clarkson A, et al. Loss of SDHA expression identifies SDHA mutations in succinate dehydrogenase-deficient gastrointestinal stromal tumors. *Am J Surg Pathol*. 2013;37(2):226–233.
13. Fletcher CD, Berman JJ, Corless C, et al. Diagnosis of gastrointestinal stromal tumors: a consensus approach. *Hum Pathol*. 2002;33(5):459–465.
14. 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.
15. Heinrich MC, Corless CL, Duensing A, et al. PDGFRA activating mutations in gastrointestinal stromal tumors. *Science*. 2003;299(5607):708–710.
16. Hirota S, Isozaki K, Moriyama Y, et al. Gain-of-function mutations of c-kit in human gastrointestinal stromal tumors. *Science*. 1998;279(5350):577–580.
17. Joensuu H, Roberts PJ, Sarlomo-Rikala M, et al. Effect of the tyrosine kinase inhibitor ST1571 in a patient with metastatic gastrointestinal stromal tumor. *N Engl J Med*. 2001;344(14):1052–1056.
18. Van Oosterom AT, Judson I, Verweij J, et al. ST1571, an active drug in metastatic gastrointestinal stromal tumors (GIST) an EORTC phase 1 study [abstract 37]. *Proc Am Soc Clin Oncol*. 2001;20:1a.
19. Demetri GD, von Mehren M, Blanke CD, et al. Efficacy and safety of imatinib mesylate in advanced gastrointestinal stromal tumors. *N Engl J Med*. 2002;347(7):472–480.
20. Blanke CD, Rankin C, Demetri GD, et al. Phase III randomized intergroup trial assessing imatinib mesylate at two dose levels in patients with unresectable or metastatic gastrointestinal stromal tumors expressing the kit receptor tyrosine kinase: S0033. *J Clin Oncol*. 2008;26(4):626–632.
21. Verweiji J, Casali PG, Zaleberg J, et al. Progression–free survival in gastrointestinal stromal tumors with high-dose imatinib: randomized trial. *Lancet*. 2004;364(9440):1127–1134.
22. Gastrointestinal Stromal Tumor Meta-Analysis Group (MetaGIST). Comparison of two doses of imatinib for the treatment of unresectable or metastatic gastrointestinal stromal tumor: a meta-analysis of 1,640 patients. *J Clin Oncol*. 2010;28(7):1247–1253.
23. Heinrich MC, Corless CL, Blanke CD, et al. Molecular correlates of imatinib resistance in gastrointestinal stromal tumors. *J Clin Oncol*. 2006;24(29):4764–4774.
24. Andersson J, Bumming P, Meis-Kindblom JM, et al. Gastrointestinal stromal tumors with KIT exon 11 deletions are associated with poor prognosis. *Gastroenterology*. 2006;130(6):1573–1581.
25. Liu XH, Bai CG, Xie Q, Feng F, Xu ZY, Ma DL. Prognostic value of KIT mutation in gastrointestinal stromal tumors. *World J Gastroenterol*. 2005;11(25):3948–3952.
26. Wardelmann E, Losen I, Hans V, et al. Deletion of Trp-557 and Lys-558 in the juxtamembrane domain of the c-kit protooncogene is associated with metastatic behavior of gastrointestinal stromal tumors. *Int J Cancer*. 2003;106(6):887–895.
27. Lux ML, Rubin BP, Biase TL, et al. KIT extracellular and kinase domain mutations in gastrointestinal stromal tumors. *Am J Pathol*. 2000;156(3):791–795.
28. Huss S, Künstlinger H, Wardelmann E, et al. A subset of gastrointestinal stromal tumors previously regarded as wild-type tumors carries somatic activating mutations in KIT exon 8 (p.D419del). *Mod Pathol*. 2013;26(7):1004–1012.
29. Lasota J, Corless CL, Heinrich MC, et al. Clinicopathologic profile of gastrointestinal stromal tumors (GISTs) with primary KIT exon 13 or 17 mutations: a multicenter study of 54 cases. *Mod Pathol*. 2008;21(4):476–484.
30. LaCosta J, Miettinen M. Clinical significance of oncogenic KIT and PDGFRA mutations in gastrointestinal stromal tumors. *Histopathology*. 2008;53(3):245–266.
31. 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.
32. Nannini, M, Biasco B, Astolfi A, Pantaleo MA. An overview on molecular biology of KIT/PDGFRA wild type (WT) gastrointestinal stromal tumours (GIST). *J Med Genet*. 2013;50(10):653–661.
33. Ogino S, Gulley M, den Dunneb JT, Wilson RB; Association for Molecular Pathology Training and Education. Standard mutation nomenclature in molecular diagnostics: practical and educational challenges [published correction appears in *J Mol Diagn*. 2009;11(5):494].*J Mol Diagn*. 2007;9(1):1–6.
34. den Dunnen JT, Antonarakis SE. Mutation nomenclature. *Curr Protoc Hum Genet*. doi:10.1002/0471142905.hg0713s37. 2003;(chapter 7, Unit 7.13). http://www.currentprotocols.com/WileyCDA/CPUnit/refId-hg0713.html. Accessed October 29, 2014.