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Biology of Gastrointestinal Stromal Tumors

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ABSTRACT

Once a poorly defined pathologic oddity, in recent years, gastrointestinal stromal tumor (GIST) has emerged as a distinct oncogenetic entity that is now center stage in clinical trials of kinase-targeted therapies. This review charts the rapid progress that has established GIST as a model for understanding the role of oncogenic kinase mutations in human tumorigenesis. Approximately 80% to 85% of GISTs harbor activating mutations of the KIT tyrosine kinase. In a series of 322 GISTs (including 140 previously published cases) studied by the authors in detail, mutations in the *KIT* gene occurred with decreasing frequency in exons 11 (66.1%), 9 (13%), 13 (1.2%), and 17 (0.6%). In the same series, a subset of tumors had mutations in the *KIT*-related kinase gene *PDGF receptor alpha* (*PDGFRA*), which occurred in either exon 18 (5.6%) or 12 (1.5%). The remainder of GISTs (12%) were wild type for both *KIT* and *PDGFRA*. Comparative studies of *KIT*-mutant, *PDGFRA*-mutant, and wild-type GISTs indicate that there are many similarities between these groups of tumors but also important differences. In particular, the responsiveness of GISTs to treatment with the kinase inhibitor imatinib varies substantially depending on the exonic location of the *KIT* or *PDGFRA* mutation. Given these differences, which have implications both for the diagnosis and treatment of GISTs, we propose a molecular-based classification of GIST. Recent studies of familial GIST, pediatric GIST, and variant forms of GIST related to Carney's triad and neurofibromatosis type 1 are discussed in relationship to this molecular classification. In addition, the role of mutation screening in *KIT* and *PDGFRA* as a diagnostic and prognostic aid is emphasized in this review.

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HISTORICAL OVERVIEW

On the basis of light microscopic descriptions in the 1930s to 1950s, stromal tumors of the gastrointestinal tract were thought to be neoplasms of smooth muscle origin and were, therefore, most often classified as leiomyoma, leiomyosarcoma, or leiomyoblastoma.[1-3] Electron microscopic studies in the late 1960s and early 1970s, however, revealed inconsistent evidence of smooth muscle differentiation.[3] The application of immunohistochemistry to the study of gastrointestinal stromal tumors (GIST), which began in the 1980s, supported the electron microscopic evidence. Expression of muscle markers (actins and desmin) was far more variable than what was observed in smooth muscle tumors arising from the myometrium or vessel wall, and a subset of stromal tumors stained positively for neural crest markers (*S*-100, neuron-specific enolase, and PGP9.5) that were not found in other smooth muscle neoplasms.[1-3] The results of these studies fueled a long-standing debate (largely ignored outside the pathology community) as to the origin and nature of mesenchymal tumors arising within the gut wall.

The term stromal tumor was introduced in 1983 by Mazur and Clark[4] in recognition of the growing evidence that these gastrointestinal tract neoplasms were a clinicopathologically distinct entity. This appellation was not widely adopted, however, until the early 1990s, when it was discovered that most stromal tumors arising in the gastrointestinal tract are positive for CD34.[5,6] As the first relatively specific marker of GISTs, CD34 served in bringing greater recognition to the diagnosis of GIST during the mid-1990s. It should be noted that in the mid-1980s an alternate diagnosis, gastrointestinal autonomic nerve tumor, was put forward for stromal tumors that exhibited significant neural differentiation.[7] It is now established that gastrointestinal autonomic nerve tumor is a morphologic variant of GIST.[8]

KIT TYROSINE KINASE (CD117): A PHENOTYPIC MARKER OF MOST GISTS

The curious overlap of smooth muscle and neural features observed in GISTs by electron microscopy and immunohistochemistry led to speculation that these tumors are related not to muscle cells but rather to little-known populations of spindle cells present in the gut wall, the interstitial cells of Cajal (ICC).[9] GISTs have features in common with the myenteric plexus subtype of ICC that are found in the stomach and intestines, including frequent expression of CD34, embryonic smooth muscle myosin heavy chain, and the intermediate filament nestin.[10,11]

Myenteric plexus ICC cells fail to develop in mice that are deficient in expression of the receptor tyrosine kinase KIT or its ligand, stem-cell factor (SCF), indicating that the KIT-SCF axis is essential to the development of these cells.[12] The observation that ICC cells can be immunohistochemically highlighted with an antibody to KIT (CD117) led to the discovery that KIT is also strongly expressed in most GISTs.[13,14] This discovery not only substantiated the hypothesis that GISTs arise from or share a common stem cell with the ICC, but it also provided a new, more sensitive and specific marker for the diagnosis of GIST. Follow-up studies from a large number of laboratories have established that approximately 95% of GISTs exhibit unequivocal staining for KIT.[15-18]

The use of KIT as an immunohistochemical marker for GISTs has helped to solidify an otherwise untidy field by engendering greater uniformity in both the diagnosis and the comparative study of these tumors. However, no immunohistochemical marker is perfect, and the heavy reliance on KIT staining has created some problems. The first is that there are several commercially available KIT antibodies, and these antibodies are applied using different protocols in different laboratories. The result is disagreement as to the specificity of this marker for GISTs relative to other mesenchymal tumors in the abdomen, including fibromatosis (desmoid tumor), synovial sarcoma, and leiomyosarcoma.[15,16,19,20] From our experience in referral centers for GIST patients, it is apparent that overstaining with inappropriately titered KIT antibodies is a problem in some laboratories. Educational efforts are underway in the United States to help pathology laboratories validate their KIT staining protocols, and improved commercial packages for KIT staining may soon be available. Thus, some of the confusion generated by improper immunohistochemical staining for this marker may abate in coming years.

Perhaps a larger challenge created by the emphasis on KIT staining in GISTs is that recent molecular studies have defined a subset of these tumors that are clearly KIT negative. This new wrinkle, as discussed elsewhere in this review, has implications both for the diagnosis and treatment of GISTs.

PATHOLOGIC FEATURES OF GISTS

Most GISTs are comprised of a fairly uniform population of spindle cells (70% of cases; Fig 1), but some are dominated by epithelioid cells (20% of cases), and the remainder consists of a mixture of these two morphologies. The spindle cells are usually arranged in short fascicles but can be aligned in a strikingly Schwannian pattern with prominent nuclear palisading. Curvilinear collections of extracellular collagen called skeinoid fibers may be seen in either spindle cell or epithelioid tumors. Approximately 5% of cases have prominent myxoid stroma. Occasional tumors have neuroendocrine-like features that resemble paraganglioma or carcinoid. A signet ring-like variant has also been described.[21] Some GISTs have a marked lymphocytic infiltrate, but this is uncommon.[22] Nuclear atypia and multinucleation are more common in epithelioid GISTs; when present, they are often accompanied by other malignant features.

As discussed earlier, approximately 95% of GISTs stain positively for KIT (CD117). Staining for other markers is more variable, including BCL-2 (80%), CD34 (70%), muscle-specific actin (50%), smooth muscle actin (35%), *S*-100 (10%), and desmin (5%).[3,15-18,20,21,23-28] Because GISTs have a relatively broad morphologic spectrum, the differential diagnosis includes a number of mesenchymal, neural, and neuroendocrine neoplasms that occur in the abdomen. These include leiomyoma, leiomyosarcoma, schwannoma, malignant peripheral-nerve sheath tumor, solitary fibrous tumor, inflammatory myofibroblastic tumor, fibromatosis, synovial sarcoma, neuroendocrine tumors (carcinoid and islet cell), gastric glomus tumor, malignant mesothelioma, angiosarcoma, and sarcomatoid carcinoma. Recent success in treating GISTs with imatinib (detailed in the Targeted Therapy of GISTS With Imatinib section) has placed a new priority on making this diagnosis accurately. Fibromatosis and leiomyosarcoma are perhaps the two tumors most frequently mistaken for GIST (Fig 1).

CLINICAL FEATURES AND PROGNOSIS OF GISTS

GISTs arise predominantly in the stomach (60%) and small intestine (25%) but also occur in the rectum (5%), esophagus (2%), and a variety of other locations (5%), including appendix, gallbladder, pancreas, mesentery, omentum, and retroperitoneum.[24,26-33] The recent identification of KIT-positive, ICC-like cells in the omentum suggests that these cells are more widespread than is commonly appreciated, which may account for GISTs arising outside the gut wall.[34]

GIST patients range in age from the teens to the 90s, but peak age is around 60 years. The tumors are generally between 2 and 30 cm in diameter at the time of diagnosis and may cause mass-related symptoms or anemia as a result of mucosal ulceration. Not infrequently, however, GISTs are discovered incidentally during radiologic imaging for an unrelated condition or as a secondary finding in a surgical resection or autopsy specimen.[35]

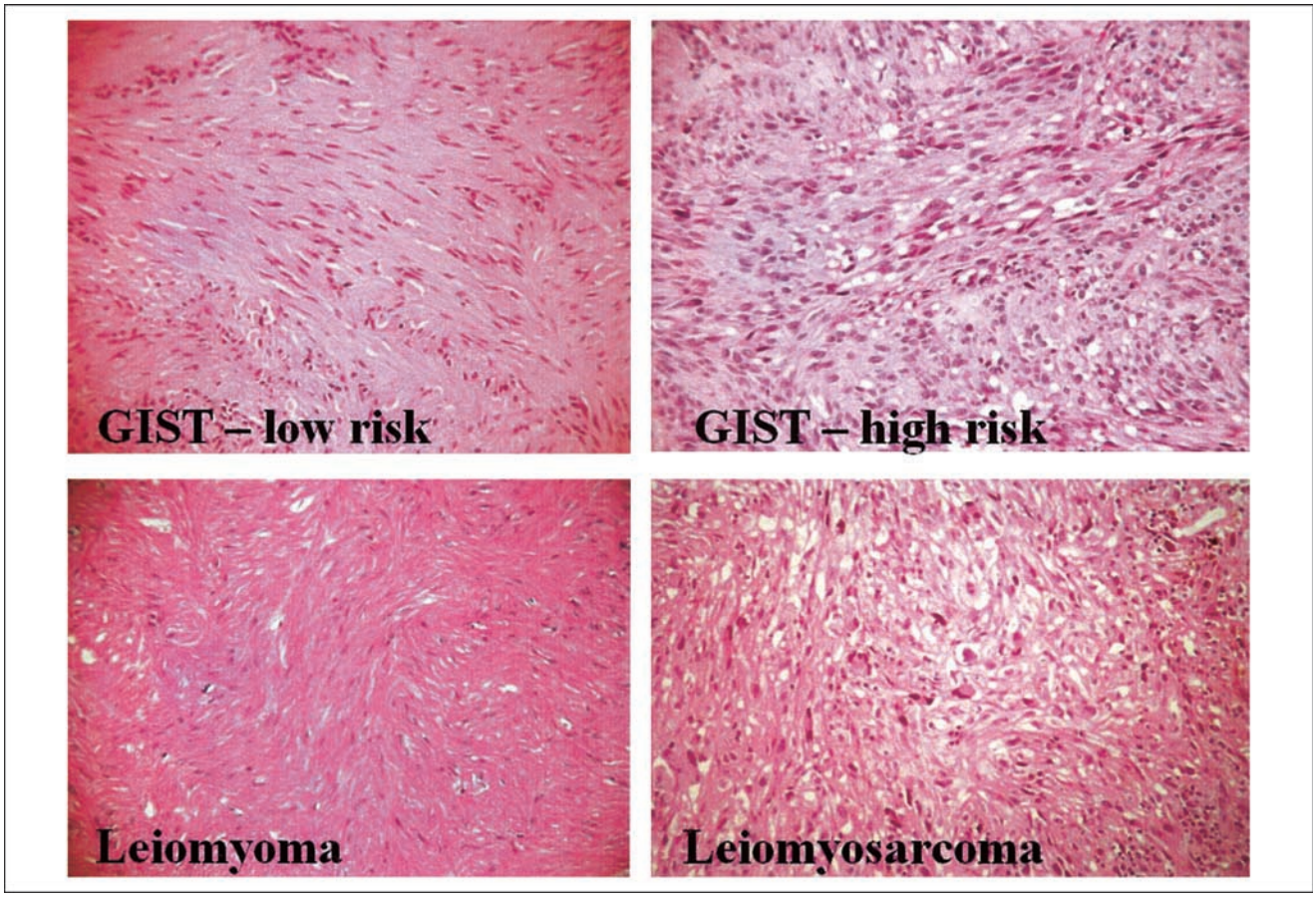

Fig 1. Morphologic similarities of a low-risk gastrointestinal stromal tumor (GIST) and leiomyoma and of a high-risk GIST and leiomyosarcoma. (hematoxylin and eosin stain, original magnification, ×400).

As discussed earlier, GISTs were consistently underrepresented in the older literature, making it difficult to determine their overall frequency. Kindblom et al[36] recently completed a retrospective analysis of all tumors that were potential GISTs identified during the years 1983 to 2000 in a population of 1.5 million in southwestern Sweden. On the basis of this study, which included KIT immunohistochemistry, the annual incidence of GIST in Sweden is estimated at 20 cases per million. Assuming no significant racial differences in GIST frequency, this figure translates to approximately 5,000 new cases in the United States each year.

Consensus guidelines for GIST prognosis, assembled during an National Institutes of Health/National Cancer Institute–sponsored workshop in April 2001 (Table 1), emphasize tumor size and mitotic index for risk stratification of primary tumors.[37] This is supported by the work of DeMatteo et al,[38] who performed a retrospective analysis of 200 patients with surgically resected GISTs and found that the 5-year disease-specific survival after removal of a primary tumor larger than 10 cm was approximately 20%, whereas the survival for tumors less than 5 cm was approximately 60%. Similar observations with regard to tumor size were published by Singer et al[39] in an analysis of 48 GISTs. Mitotic index, whether assessed by direct counting or immunohistochemistry for a cell cycle marker (PCNA and Ki-67), has been linked to prognosis in a large number of studies and should be included in the evaluation of any primary tumor.[24,39-43] Aneuploidy is a negative prognostic factor in GISTs, but ploidy analysis by flow cytometry is not generally used in routine diagnosis.[41,44] Another

Table 1. Prognosis of Primary GIST

Risk	Size (cm)	Mitotic Count (per 50 HPF)
Very low risk	< 2	< 5
Low risk	2-5	< 5
Intermediate risk	< 5	6-10
	5-10	< 5
High risk	> 5	> 5
	> 10	> Any mitotic rate
	Any tumor	> 10

NOTE. From Fletcher et al.[37]
Abbreviations: GIST, gastrointestinal stromal tumor; HPF, high-power field.

prognostic feature is tumor location. In several studies, it has been noted that patients with primary gastric tumors fare significantly better than those with small bowel and rectal primary tumors.[17,42,45]

Up to 30% of newly diagnosed GISTs are overtly malignant or have features that connote a high malignant potential.[45] Progression of such tumors follows a characteristic course that includes recurrence at the site of resection, intra-abdominal spread on serosal surfaces, and the development of liver metastases. Lymph node metastases are uncommon, and disease outside the abdomen is seen only in advanced cases. It should be noted that, even among low-risk GISTs, recurrences have been reported up to 20 years after surgical resection.[3] For this reason, most experts do not regard any GIST as truly benign; instead, tumors are stratified for risk of malignant behavior (Table 1).

Surgical resection is the mainstay of therapy for GIST. Outcomes are relatively good for patients with low-risk or intermediate-risk tumors, but recurrence is almost inevitable after resection of high-risk tumors.[38,39,46] In patients with recurrent disease, either localized or disseminated, the results of secondary surgery and other forms of localized salvage therapy have been uniformly poor.[38,47] Single-agent and combination chemotherapy trials have consistently failed to yield partial response rates greater than 5%.[48] These disappointing results are possibly related to high-level expression of BCL-2 and multidrug resistance proteins in many GISTs.[49-52] Fortunately, the historically grim prospects for patients with locally advanced or metastatic GIST are now much improved with the advent of imatinib therapy, as detailed in the Targeted Therapy of GISTS With Imatinib section.

ONCOGENIC MUTATIONS OF KIT ARE COMMON IN GISTS

KIT is a 145-kD transmembrane glycoprotein that serves as the receptor for SCF and has tyrosine kinase activity.[53,54] A member of the subclass III family of receptor tyrosine kinases, KIT is closely related to the receptors for PDGF, macrophage colony-stimulating factor, and FLT3 ligand.[55] KIT function is critical to the development of the ICC, as well as to the development of hematopoietic progenitor cells, mast cells, and germ cells.[56] Binding of SCF to KIT results in receptor homodimerization, activation of the tyrosine kinase activity, and resultant phosphorylation of a variety of substrates.[57] In many cases, these substrates are themselves kinases and serve as effectors of intracellular signal transduction.

Mutations of the Juxtamembrane Domain (exon 11)

In a landmark 1998 publication, Hirota et al[13] documented not only that GISTs express KIT protein but also that mutations in the juxtamembrane domain (exon 11) of the *KIT* gene can be found in these tumors. Five of six tumors examined had exon 11 mutations; four were in-frame deletions, and the fifth was a point mutation that resulted in substitution of a single amino acid. The mutant KIT isoforms demonstrated constitutive kinase activity when expressed in vitro; that is, their kinase domains were active even in the absence of SCF. In addition, all five KIT mutants were shown to transform Ba/F3 cells in a nude mouse tumorigenesis assay, whereas wild-type KIT did not. These results suggested that oncogenic activation of KIT plays an important role in the growth and survival of GISTs.

The juxtamembrane region of KIT (exon 11) functions to inhibit receptor dimerization in the absence of SCF. Mutagenesis studies of KIT have demonstrated that small in-frame deletions and insertions or point mutations of this domain disrupt this function, allowing ligand-independent receptor dimerization.[58-60] The reported frequency of exon 11 mutations in GISTs varies over a wide range (20% to 92%), but the highest yields have come from studies based on cDNA prepared from frozen tumor samples. For example, Hirota et al[13] found exon 11 mutations in five (83%) of six tumors, whereas Rubin et al[61] uncovered 34 exon 11 mutations in 48 tumors (71%).[13,61] In most studies that have used genomic DNA extracted out of paraffin-embedded tumor tissue, the frequency has been lower (20% to 57%).[39,62-66]

Although the discrepancy in *KIT* exon 11 mutation frequencies between the cDNA-based and paraffin/genomic-based studies could reflect population differences, technical issues are the more likely the culprit. In some paraffin-based studies, the polymerase chain reaction primers used to amplify exon 11 did not allow for analysis of the entire exon. In addition, most groups have relied on single strand conformation polymorphism to screen for the presence of a deletion or point mutation, but this technique is not as sensitive as other approaches.

Denaturing high-performance liquid chromatography (D-HPLC) is a method that is highly sensitive for both deletions and point mutations. We used this method to screen for *KIT* gene mutations in a group of 127 paraffin-embedded malignant GIST cases analyzed as part of a clinical trial, and we found exon 11 mutations (sequence confirmed) in 66.9%.[67] We also examined 13 very low–risk GISTs using this approach and found exon 11 mutations in 10 of them (76.9%).[35] An additional 182 GISTs, ranging from low risk to malignant, have since been analyzed in our laboratories using D-HPLC. When added to our 140 published cases, the exon 11 mutation frequency for the entire series of 322 GISTs is 66.1%. The spectrum of mutations identified in our series, represented in the histogram shown in Figure 2, is similar to that reported by other groups. Deletions and insertions tend to affect the first part of the exon, particularly codons 557 to 559. Point mutations are limited to just four codons within the exon (557, 559, 560, and 576), whereas internal tandem duplications are observed near the end of the exon. A subset of the tumors (17.8%) were either

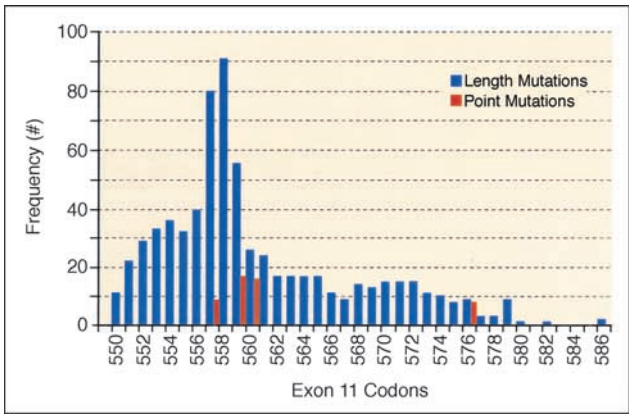

Fig 2. Frequency of involvement of *KIT* exon 11 codons by mutations in 322 gastrointestinal stromal tumors. Data from Corless et al,[35] Heinrich et al, [67] and authors' additional unpublished cases.

hemizygous or homozygous for the observed mutation, suggesting that there is selective pressure against expression of the wild-type KIT allele in exon 11–mutant tumors. This is supported by in vitro data demonstrating that a peptide corresponding to the wild-type juxtamembrane domain is inhibitory to activated isoforms of KIT.[60]

Mutations in the Extracellular Domain (exon 9)

Lux et al[68] were the first to describe a mutation in the extracellular domain of KIT (exon 9). An insertion of six nucleotides that results in duplication of Ala^{501} and Tyr^{502} was found in six GISTs that lacked an exon 11 mutation. Hirota et al[69] confirmed that this mutation occurs in a subset of GISTs and showed that the resulting KIT isoform has a constitutively active kinase. Other groups have also observed this mutation and noted its preferential association with small intestinal origin.[70-72] Among 127 malignant GISTs that we analyzed, 23 (18.1%) had exon 9 mutations, and only one of these was hemizygous/homozygous.[67] All were the AY501-502 duplication/insertion, with the exception of a single FAF506-508 duplication/insertion. Among 322 GISTs that we have studied (including 140 published cases) the frequency of exon 9 mutations was somewhat lower (10.2%); as discussed in the KIT Mutations and Tumor Prognosis section, these mutations seem to be relatively overrepresented among malignant tumors. On the basis of our cases and other published examples of exon 9–mutant GISTs, 95% of these tumors are associated with the small intestine. The mechanism of action has not yet been determined, but it is hypothesized that exon 9 mutations disrupt an antidimerization motif in the extracellular domain.

Mutations in the Kinase I Domain (exon 13)

A point mutation in *KIT* exon 13, K642E, was first identified in GIST by Lux et al[68] and has since been observed by several other investigators.[70,73] The frequency of this mutation is consistently low, ranging from 0.8% to 4.1%. Our series of 322 GISTs (including 140 published cases) yielded just four of these exon 13 mutations (1.2%).[67] There is evidence that this mutation results in ligand-independent activation of the receptor, although it is unclear whether spontaneous receptor homodimerization is the mechanism.[67]

Mutations in the Activation Loop (exon 17)

Mutations involving the activation loop of KIT are rare in GISTs. Rubin et al[61] reported an N822K and an N822H mutation in one case each. We have observed two additional N822K mutants (one in a published series of 127 malignant GISTs) among 322 GISTs (0.6%), but no such mutations were observed by Kinoshita et al[73] among 124 GISTs. As discussed in the Familial GIST section, a germline D820Y substitution has been related to familial GIST[74]; however, this mutation has not been reported in sporadic tumors. The N822K and D820Y mutations cause constitutive activation of the kinase domain, although the mechanisms remain unclear.[67,74] It is interesting that a nearby codon in exon 17 (Asp^{816}) is commonly mutated in other human malignancies, including mast cell disease, seminoma and dysgerminoma, acute myelogenous leukemia, and sinonasal natural killer and T-cell lymphoma.[75-78] Although mutations of this codon are highly activating, they have not been observed in more than 700 GISTs published to date. Conversely, activating mutations of *KIT* exon 11 have been found in only five cases of human mastocytosis. The implication of these observations is that the stem cells that give rise to GISTs have different transforming requirements than those that give rise to mastocytosis, and these requirements may be reflected in alternative signaling initiated by the various *KIT* mutations.

KIT *Mutations and Tumor Prognosis*

Several groups have reported an association between the presence of an exon 11 mutation and more aggressive clinical behavior of GISTs.[39,62-64,66] These studies, however, were based on single strand conformation polymorphism and/or direct sequence analyses of exon 11 amplimers prepared from paraffin-extracted DNA and may have underestimated the true frequency of *KIT* mutations. In an initial application of D-HPLC, we analyzed 13 incidentally discovered GISTs that were 1 cm or less in size and devoid of mitoses (very low–risk tumors, Table 1). Ten of the tumors (76.9%) had exon 11 mutations of the same type that were reported by others to be associated with malignant behavior, suggesting that *KIT* mutations are acquired early in GIST development.[35] This is supported by the observations that germline exon 11 mutations predispose to the development of GISTs in both humans and mice, as discussed in the Familial GIST section. Among 275 fully risk-stratified GISTs that we have studied (including 140 published cases), the exon 11 mutation frequency in the low-risk group (< 5 cm and < 5 mitoses/50 high-power field) was 87.1%. Studies by Rubin et al[61] and Wardelmann et al[18] have also yielded high frequencies of exon 11 mutations in low-risk

GISTs.[18,61] Further studies are needed to determine whether specific subtypes of exon 11 mutations confer a higher risk of malignant behavior, as recently suggested by Wardelmann et al[79] and Antonescu et al.[72]

In contrast to exon 11 mutations, the frequency of exon 9 mutations in our series of 275 GISTs was higher among malignant tumors (17.3%) than among high-risk (3.0%) and low-risk tumors (2.5%). These mutations seem to support altered intracellular signaling compared with exon 11–mutant tumors (Fletcher et al, manuscript in preparation). Thus, the biology of exon 9–mutant tumors is inherently different and perhaps more aggressive than that of other GISTs.

In theory, the progression of GISTs might be related to the accumulation of secondary mutations in *KIT*. For example, if a tumor harboring an exon 11 point mutation subsequently acquired an exon 9 deletion, it might have an additional growth advantage. This hypothetical phenomenon has not been observed in tumor samples. Among 127 malignant GISTs that we examined in detail for *KIT* exons 9, 11, 13, and 17, none had more than a single mutation.[67]

PDGF RECEPTOR ALPHA (*PDGFRA*) IS AN ALTERNATIVE ONCOGENE IN GISTS

In a recent study of GISTs that were negative for a *KIT* gene mutation (*KIT*-wild type [*KIT*-WT]), the authors of this review searched for other activated kinases using a novel methodology.[80] A cocktail of antibodies to epitopes shared by a wide range of receptor tyrosine kinases was used to immunoprecipitate kinases from extracts of *KIT*-WT tumors. Western blotting of the immunoprecipitates with a phosphotyrosine-specific antibody revealed a novel band that was subsequently identified as PDGFRA. Phosphorylated PDGFRA was detectable in a subset of *KIT*-WT tumors but was not present in extracts of tumors with known KIT mutations. Conversely, extracts of KIT-mutant tumors had phosphorylated KIT but were negative for phosphorylated PDGFRA. These results suggested that PDGFRA is the active kinase in some *KIT*-WT tumors.

Examination of genomic DNA from *KIT*-WT tumors yielded a variety of mutations in the juxtamembrane (exon 12) and activation loop (exon 18) domains of the *PDGFRA* gene.[80] When cloned and transfected into Chinese hamster ovary cells, the mutant PDGFRA isoforms were found to be constitutively phosphorylated in the absence of PDGF-AA ligand, which was consistent with oncogenic activation. The morphology of the *PDGFRA*-mutant tumors was generally epithelioid, and many of them expressed KIT only weakly or not at all (Fig 3). The signal transduction profiles for the *PDGFRA*-mutant tumors were indistinguishable from *KIT*-mutant tumors, suggesting that PDGFRA can substitute for KIT in GIST oncogenesis.[80] Correspondingly, activation of the two genes seems to be mutually exclusive.[67,80] Six (4.7%) of 127 malignant GISTs were found to harbor *PDGFRA* mutations.[67] The frequency of these mutations was slightly higher (7.1%) in our series of 322 tumors, which included 182 previously unpublished cases. Recently, Hirota et al[81] confirmed the presence of *PDGFRA* mutations in five of the eight *KIT*-WT GISTs examined. The impact of *PDGFRA* mutations on the diagnosis and treatment of GISTs is considered in later sections.

FAMILIAL GIST

Several kindreds with heritable mutations in the juxtamembrane region (exon 11) of the *KIT* gene have been identified. The first to be reported was a Japanese family in which a deletion of one of two consecutive valine residues (codon 559 or 560, GTTGTT) was traced through three generations. Affected individuals had hyperpigmentation of perineal skin and suffered the development of multiple benign and malignant GISTs.[82] Interestingly, mice engineered to express an equivalent isoform of KIT (murine $KIT^{\Delta 558}$) by a knock-in approach develop KIT-positive stromal tumors of the cecum.[83] A V559A substitution has been described in a kindred in Italy and in another kindred from Japan.[84,85] Affected members in both kindreds had pigmented macules involving the skin of the perineum, axilla, hands, and face (but not lips or buccal mucosa), as well as evidence of skin mastocytosis (urticaria pigmentosa) on biopsy. In addition, patients in both families developed multiple GISTs of the stomach and small bowel as early as age 18 years. Resected tumors were accompanied by diffuse ICC hyperplasia in the adjacent gut wall. We have recently identified another kindred with the germline V559A *KIT* mutation manifesting in heritable skin pigmentation and multiple GISTs (Li et al, manuscript in preparation). Earlier reports of individuals or families with multiple intestinal leiomyomas associated with skin hyperpigmentation and/or mast cell disease may represent additional examples of germline exon 11 mutations in the *KIT* gene.[86]

Multiple gastrointestinal autonomic nerve tumors were reported in a 69-year-old mother and her 52-year-old daughter from North America, both of whom also had diffuse neuronal hyperplasia of the small intestine.[87] Subsequent studies in the laboratory of Dr. Seichi Hirota demonstrated that the tumors of these two patients were strongly positive for KIT by immunohistochemistry. Furthermore, a germline W557R mutation in *KIT* exon 11 was found in both patients.[88] There was no mention of skin pigmentation or mast cell proliferations.[87]

A germline mutation in the kinase I domain of KIT was reported in a 67-year-old mother and her 40-year-old son from France. Both patients had more than a dozen duodenal and jejunal GISTs, and both were found to have a constitutional K642E substitution in exon 13 of the *KIT* gene.[89] The tumors taken from these patients were uni-

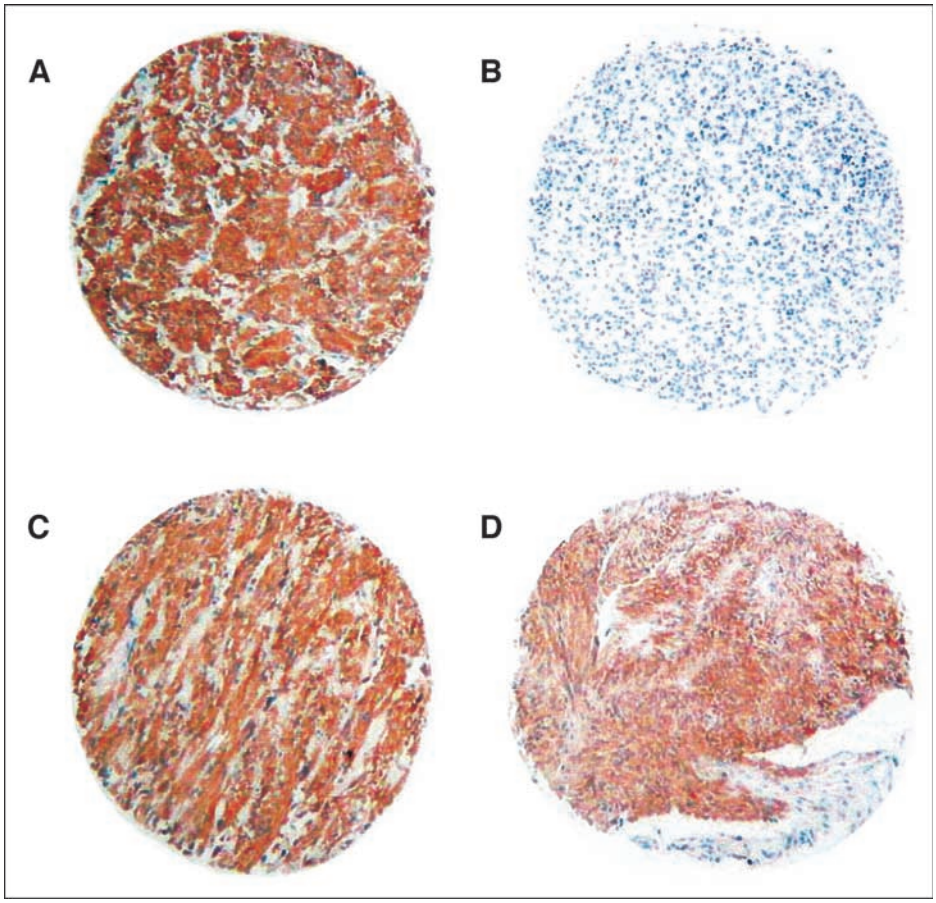

Fig 3. Immunohistochemistry for KIT in gastrointestinal stromal tumors (GISTs) harboring *KIT* versus *PDGFRA* mutations. Strong staining is observed in three samples of *KIT*-mutant GIST on a tissue microarray, whereas a neighboring *PDGFRA*-mutant GIST sample is negative (original magnification, ×100). (A) *KIT* exon 11 deletion; (B) *PDGFRA* exon 12 deletion; (C) *KIT* exon 11 deletion; (D) *KIT* exon 11 point mutation.

formly low grade and were accompanied by marked hyperplasia of myenteric plexus ICCs. Interestingly, neither patient showed skin pigmentation or evidence of mastocytosis, suggesting that the KIT K642E mutation does not support melanocyte or mast cell proliferation.

A mutation in the activation loop of KIT was recently described by Hirota et al[74] in a kindred with multiple gastric and small bowel GISTs. The D820Y mutation found in affected family members caused diffuse ICC hyperplasia and GIST formation but was not associated with skin hyperpigmentation or mast cell disease. Curiously, complaints of dysphagia in these patients were related to measurable abnormalities in esophageal peristalsis rather than obstruction by tumor.[74]

MOLECULAR PROGRESSION OF GISTS

As discussed earlier, patients harboring germline activating mutations of the *KIT* gene develop multiple GISTs, but their tumors are not clinically manifest until early adulthood. Clearly, mutations in other genes are necessary for a stromal tumor to emerge from a background of ICC hyperplasia. Clues to the whereabouts of some of these genes have been provided by cytogenetic studies. For example, karyotypes from approximately two thirds of GISTs demonstrate either monosomy 14 or partial loss of 14q.[80,90-94] On the basis of loss of heterozygosity and comparative genomic hybridization studies, at least two regions of this chromosome, 14q11.1-q12 and 14q22-24, seem to be hot spots for deletions and represent likely sites for tumor suppressor genes that play a role early in GIST formation.[91,95]

Loss of the long arm of chromosome 22 is observed in approximately 50% of GISTs and is associated with progression to a borderline or malignant lesion.[80,90,91,94] Losses on chromosomes 1p, 9p, and 11p are successively less common than 14q and 22q losses but are more significantly associated with malignancy.[80,90,94,96,97] One target on chromosome 9p is the *CDKN2A* ($p16^{INK4A}$) gene, which is inactivated through several mechanisms in a significant fraction of malignant GISTs.[98] Gene amplifications are also reported in GIST karyotypes. Gains on chromosomes 8q and 17q, as revealed by karyotypes and comparative genomic hybridization, are associated with metastatic behavior.[91,95,99]

In summary, a simplified pathway for the genetic changes observed in the development and progression of GISTs is as follows: *KIT* or *PDGFRA* mutation → 14q deletion → 22q

deletion → 1p deletion → 8p gain → 11p deletion → 9p deletion → 17q gain.[93] Additional studies are needed to determine the identity of the progression genes in this pathway.

GENE EXPRESSION AND SIGNALING PATHWAYS IN GISTS

Nielsen et al[52] compared the gene expression profiles of eight GISTs with 33 other soft tissue tumors using cDNA microarrays. Although the *KIT* and *PDGFRA* mutational status of these eight GISTs was not reported, their expression profiles were tightly clustered away from the other tumors, and *KIT* was a prominent discriminator. Included in the GIST-associated expression cluster was the gene for protein kinase C theta (*PKCθ*). Other notable genes in this cluster were members of the superfamily of adenosine triphosphate–binding cassette transporters (*ABCB1* and *ABCC4*), as well as *bcl-2*, *Sprouty1*, and *Sprouty4*.[52]

Allander et al[51] reported the gene expression profile of 13 *KIT*-mutant GISTs compared with six extra-abdominal tumors with spindle cell morphology that lacked KIT expression. Similar to the report by Nielsen et al, all 13 GISTs were tightly clustered with a relatively homogeneous pattern of gene expression, and the most highly ranked gene on the discriminator list was *KIT*. Closely associated with KIT were the expression of a G-coupled receptor (*GPR20*) and *PKCθ*. Larger gene profiling studies of GISTs are indicated to test for shared or unique gene expression profiles in *KIT*-mutant versus *PDGFRA*-mutant versus wild-type GISTs.

PKCθ has been confirmed as a useful marker of GIST in immunoblotting experiments comparing lysates from GISTs and other spindle cell neoplasms of the abdominal cavity. In addition, activation of PKCθ, as evidenced by strong phosphorylation of threonine 358, is observed in GISTs.[80,100] Studies are in progress to determine whether PKCθ immunohistochemistry will be useful in the routine diagnosis of GIST cases, especially those with low or absent KIT expression. Another potential immunohistochemical marker of GISTs to be identified through gene expression analyses is FLJ10261 (DOG-1), a novel protein with unknown function (R. West and M. van de Rijn, personal communication).

Preliminary studies of signal transduction in GISTs demonstrate a somewhat homogeneous pattern of signal transduction activation. Notably, *KIT*-mutant GISTs have strong KIT phosphorylation, including the GRB2 and PI3K binding sites (tyrosines 703 and 721, respectively). There is evidence for activation of downstream pathways including mitogen-activated protein kinase (extracellularly regulated kinases 1 and 2), AKT, p70/85S6K, STAT1, and STAT3. In contrast, the JNK and STAT5 pathways are not activated.[80,101] Using specific inhibitors of KIT, MEK1/2, PI3K, or mTOR, it has been shown that activation of the PI3K/mTOR, but not the MEK/mitogen-activated protein kinase pathway, is essential to KIT-mediated oncogenic signaling in GISTs. Correspondingly, selective inhibition of the PI3K/mTOR pathway reduces proliferation and increases apoptosis.[102] Similar pathways of signal transduction activation were observed in *PDGFRA*-mutant GISTs. These data will doubtless be useful in the development of new targeted therapies for GISTs.

OTHER GIST VARIANTS

Carney[103] reported an association of gastric leiomyosarcoma, paraganglioma, and pulmonary chondromas in seven unrelated young woman, two of whom had all three lesions. The genetic basis for this rare association, known as Carney triad, is not known, although it is sporadic rather than familial. Virtually all reported patients have had one or more gastric tumors that were morphologically and immunophenotypically consistent with GIST.[103] In most instances, the tumors are diagnosed before the patient reaches age 30 years. As is true for nontriad GISTs, the tumors are insensitive to chemotherapy and radiation therapy.[103] Preliminary studies of Carney triad–associated GISTs suggest that they do not harbor *KIT* or *PDGFRA* mutations (unpublished data).

Only 15% of Carney triad patients are male.[103] In contrast, males are equally represented in a new syndrome of familial gastric stromal sarcoma and paraganglioma recently defined by Carney and Stratakis.[104] In this syndrome, which seems to be autosomal dominant, patients develop multiple paragangliomas (frequently functional) of the neck, mediastinum, and/or retroperitoneum, as well as multifocal GIST in the stomach. The genetic locus for this syndrome is unknown.

Gastric GISTs are occasionally diagnosed in pediatric patients outside of Carney triad.[105] A malignant GIST of the stomach from one patient was recently screened for mutations in *KIT* exons 9, 11, and 13 and found to be negative.[106] Preliminary studies of additional nonsyndromic pediatric GISTs indicate that *KIT* and *PDGFRA* mutations are much less common than in adult GISTs (unpublished data). Further studies are needed because insight into the molecular origin of these tumors may also shed light on the subset of adult GISTs that are wild type for *KIT* and *PDGFRA*.

The occurrence of gastric, intestinal, and/or colonic GISTs in a subset of patients with neurofibromatosis type I (von Recklinghausen's neurofibromatosis) is another intriguing observation. The gastrointestinal tumors in such patients are frequently multifocal and have been described as autonomic nerve tumors, stromal tumors with skeinoid fibers, or leiomyomatosis in the older literature.[107] That these tumors are true GISTs rather than some variant of neurofibroma is supported by a recent report documenting strong KIT positivity.[43] Based on a Swedish study of 70 neurofibromatosis type I patients, the incidence of GISTs in this population is approximately 7%.[108] Why the tumors arise in only a minority of these patients and yet are multifocal remains an interesting mystery.

TARGETED THERAPY OF GISTS WITH IMATINIB

Imatinib mesylate (STI571, Gleevec; Novartis Pharma, Basel, Switzerland) is a 2-phenylpyrimidine derivative that blocks the binding of adenosine triphosphate to ABL kinase. Developed by Dr. Brian Druker in collaboration with Novartis Pharma, this drug has received worldwide attention for its effectiveness against chronic myelogenous leukemia (CML). The *BCR-ABL* fusion gene product of the Philadelphia chromosome in CML is responsible for driving tumor proliferation. This mutant form of ABL is inhibited by imatinib, and more than 85% of chronic-phase CML patients taking one oral dose of imatinib per day achieve a complete hematologic response; many patients also have a complete cytogenetic remission.[109] Several comprehensive reviews on the development and use of imatinib in the treatment of CML have been published.[110]

Imatinib is not entirely specific for ABL and has significant inhibitory activity against related tyrosine kinases ARG (ABL-related kinase), PDGFRA, PDGFRB, and KIT. Two important observations made in 1999 suggested that imatinib might be effective against GISTs. The first was that imatinib could block the in vitro kinase activity of both wild-type KIT and a mutant KIT isoform commonly found in GISTs (point mutation in exon 11).[111] The second observation was that imatinib inhibited the growth of a GIST cell line containing a *KIT* gene mutation.[112] In part, on the basis of these preclinical findings, a patient with GIST metastatic to the liver was granted compassionate use of imatinib mesylate in March 2000. Within a matter of weeks, metastases in this patient decreased in size by up to 75%, and six of 28 hepatic lesions were no longer detectable on follow-up MRI scans after 8 months of therapy. This clinical response correlated with a near complete inhibition of [^{18}F]fluorodeoxyglucose uptake on positron emission tomography scan, and a posttreatment biopsy showed a marked decrease in tumor cellularity and extensive myxoid degeneration. Imatinib was well tolerated in this patient, and all cancer-related symptoms disappeared.[113]

The success in treating the first GIST patient with imatinib quickly led to a multicenter trial (CSTIB2222) that included the Dana-Farber Cancer Institute, Fox-Chase Cancer Center, Oregon Health & Science University Cancer Institute, and the University of Helsinki.[114] In all, 147 patients with advanced, unresectable, KIT-positive GIST were enrolled. Patients were randomly assigned to either 400 mg or 600 mg per day in a single oral dose; patients on 400 mg were allowed to go to 600 mg if their tumor progressed. With a minimum follow-up of 6 months, partial responses were observed in 54% of patients, and an additional 28% had stable disease. Disease progression was seen in only 14% of patients during initial follow-up.

Similar results were reported for the European Organization for Research and Treatment of Cancer Soft Tissue and Sarcoma Group phase I study of imatinib for patients with advanced soft tissue sarcomas, including GISTs.[115] Forty patients, of whom 36 had GISTs, were treated with dose levels from 400 mg to 1,000 mg daily, with therapy continuing until progression, unacceptable toxicity, or patient refusal to proceed. A dose of 500 mg bid resulted in dose-limiting toxicities (mostly nausea and vomiting) in five of eight patients. Substantial activity was seen only in the GIST patients, with 19 (53%) of 36 patients having a partial response and only seven failing therapy during 9 months of follow-up. By contrast, none of the four patients with non-GIST sarcomas had a demonstrable response to imatinib.

On the basis of the results of the CSTIB2222 trial and the European Organization for Research and Treatment of Cancer trial, imatinib was approved by the US Food and Drug Administration for the treatment of unresectable and metastatic GIST on February 1, 2002. Preliminary reports from ongoing phase III trials of imatinib for GIST treatment in both Europe and the United States confirm the phase II results.[116,117] Trials of adjuvant and neoadjuvant treatment of GISTs with this drug are also underway. There are several excellent reviews that provide additional details on the use of imatinib in the clinical management of GISTs.[48,118,119]

KIT AND *PDGFRA* MUTATION STATUS PREDICTS RESPONSE TO IMATINIB

One of the questions addressed by the CSTIB2222 trial of imatinib therapy for advanced GIST was whether there is a relationship between target kinase mutations and tumor response. Genomic tumor DNA from 127 of the patients enrolled on the trial was screened for mutations in *KIT* exons 9, 11, 13, and 17 and *PDGFRA* exons 12 and 18 by the combination of D-HPLC and direct sequencing.[67] With a median follow-up of approximately 19 months, there were unexpected outcome differences among the tumor subsets. Patients with exon 11–mutant tumors had a significantly better overall partial response rate (83.5%, n = 85) than patients whose tumor harbored an exon 9 mutation (48.7%, n = 23) or had no detectable mutation (0%, n = 9). These differences translated into significantly longer event-free and overall survival among the exon 11–mutant group versus the other two groups. Thus, even though wild-type and exon 9–mutant forms of KIT are equally sensitive to imatinib in vitro, tumors with these genotypes are less responsive to treatment than are exon 11–mutant tumors.

Another interesting observation in the study was that none of the three patients in whom a PDGFRA D842V point mutation was present showed a response to imatinib therapy. This was consistent with in vitro data showing relative resistance of this isoform to imatinib.[67,81] Further studies of the correlation of *KIT* and *PDGFRA* mutation status with drug response are underway for a large cohort of GIST patients being treated in a phase III trial of imatinib.

MECHANISMS OF IMATINIB RESISTANCE

In a high percentage of CML patients who have leukemic progression after an initial response to imatinib, secondary mutations are detectable in the *ABL* domain of the *BCR-ABL* oncogene.[120-122] Moreover, many patients have more than one such mutation, and the mutations can be found at low levels before the emergence of clinical resistance. Amplification of the *BCR-ABL* oncogene is also observed in imatinib-resistant CML cells, although this seems to be less common.[121] These observations suggest that ongoing mutagenesis in CML cells can lead to the development of drug resistance during routine monotherapy with imatinib.

Preliminary studies of GISTs suggest the following four mechanisms for drug failure, with parallels to imatinib resistance in CML[123]: (1) acquisition of a secondary point mutation in *KIT* or *PDGFRA* that confers drug resistance; (2) genomic amplification of *KIT* and resultant kinase overexpression; (3) activation of an alternate, yet unknown, receptor tyrosine kinase with loss of KIT oncoprotein expression; (4) functional resistance in tumors expressing kinases that are imatinib sensitive in vitro (eg, *KIT* exon 9 and *KIT*-WT). Fortunately, there is progress in the development of new kinase-targeted small molecule inhibitors that provides hope in the growing battle against imatinib resistance.[124]

KIT-NEGATIVE GISTS

Immunohistochemical studies from a number of different groups indicate that a subset of GISTs (variably estimated at 2% to 10%) have little or no KIT expression. By all other criteria, including clinical presentation, anatomic location, morphology, and immunophenotypic markers, these tumors qualify as GISTs. On the basis of our experience, such KIT-low/negative GISTs are a heterogeneous group, comprised in part by tumors containing *PDGFRA* mutations and in part by tumors with *KIT* mutations (Fletcher et al, submitted for publication). The vast majority of *PDGFRA*-mutant GISTs express little or no KIT, perhaps because downregulation of the wild-type *KIT* gene is advantageous to these tumors (Fig 3).[80] It would be tempting to use the absence of KIT expression as the key identifying feature of *PDGFRA*-mutant tumors, but GISTs that are immunohistochemically very weak or negative for KIT may still harbor a *KIT* exon 11 mutation and respond well to imatinib therapy.[125] To complicate things further, loss of KIT expression has been observed in advanced GISTs that have become imatinib resistant.[123] Rather than relying on equivocal immunohistochemical staining in such cases, we advocate screening for *KIT* and *PDGFRA* mutations as an alternative means of defining a tumor as a GIST. Approximately 90% of KIT-low/negative patients will have a mutation in one of these two genes, the presence of which will not only confirm the diagnosis but will provide prognostic information on the likelihood of imatinib response.

SUMMARY: MOLECULAR CLASSIFICATION OF GISTS

In less than half a decade, GISTs have emerged from historical anonymity to become an important focal point in trials of targeted therapeutics. From the studies presented in this review, it is clear that these tumors do not constitute a single, uniform entity, but rather, they represent a group of closely related neoplasms. For this reason, we propose that GISTs be classified according to the scheme outlined in Table 2, which emphasizes the molecular context of the tumor and provides a quick reference for other syndromes with which it may be associated. To the extent that this classification is useful in identifying patients in whom initial imatinib therapy is likely to fail or in identifying kindreds with possible germline *KIT* mutations, it is obvious

Table 2. Molecular Classification of GISTs

GIST Type	Comments
Sporadic GIST	
KIT mutation	
Exon 11	Best response to imatinib
Exon 9	Intermediate response to imatinib
Exon 13	Sensitive to imatinib in vitro; clinical responses observed
Exon 17	Sensitive to imatinib in vitro; clinical responses observed
PDGFRA mutation	
Exon 12	Sensitive to imatinib in vitro; clinical responses observed
Exon 18	D842V has poor response to imatinib; other mutations are sensitive
Wild type	Poor response to imatinib
Familial GIST	
KIT exon 11 (V559A, delV559, W557R)	Skin pigmentation, urticaria pigmentosa, mastocytosis
KIT exon 13 (K642E)	No skin pigmentation or mastocytosis
KIT exon 17 (D820Y)	No skin pigmentation or mastocytosis; abnormalities in esophageal peristalsis
GIST with paraganglioma	Autosomal dominant; endocrine symptoms common
Pediatric GIST	
Sporadic	*KIT* mutations much less frequent than in adults
Carney's triad	Gastric GIST with pulmonary chondroma and/or paraganglioma; female to male ratio = 7:1; no *KIT* mutations identified
NF1-related GIST	No *KIT* mutations identified

Abbreviations: GIST, gastrointestinal stromal tumor; NF1, neurofibromatosis type 1.

that there will be an increasing role for mutation screening in newly diagnosed GISTs. Further progress in defining the oncogenic pathway(s) in wild-type GISTs and in the development of new therapeutics will certainly bring revisions to this classification, but in the meantime, it may be helpful in interpreting the results from ongoing and future clinical trials of new targeted therapies for GIST.

Authors' Disclosures of Potential Conflicts of Interest

The following authors or their immediate family members have indicated a financial interest. No conflict exists for drugs or devices used in a study if they are not being evaluated as part of the investigation. Performed contract work within the last 2 years: Michael C. Heinrich, Novartis. Received more than $2,000 a year from a company for either of the last 2 years: Michael C. Heinrich, Novartis.

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