Gastrointestinal Stromal Tumor

Advances in Diagnosis and Management

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Gastrointestinal stromal tumors (GISTs) are the most common mesenchymal neoplasms of the gastrointestinal tract. Significant advances in understanding the molecular pathogenesis of these tumors have resulted not only in improved diagnostic accuracy but also identified KIT and PDGFRA as suitable targets for targeted therapies. This review provides a comprehensive discussion of the pathogenesis of GISTs and the rationale for targeted therapy. In addition, the salient clinicopathologic and immunohistochemical features that are useful in differentiating GIST from other mesenchymal tumors arising in the gastrointestinal tract are presented.

CLINICAL ASPECTS OF GIST

Gastrointestinal stromal tumors have been reported in all age groups. However, they occur predominantly in adults older than 50 years, with a median age of 58 years.1 There is no sex predilection. Although GISTs occur in the pediatric population, pediatric GISTs have enough differences in their pathogenesis and clinical behavior that they are best considered a separate clinicopathologic entity (discussed below). There are approximately 5000 new cases of clinically significant GISTs in the United States each year.2 Although GISTs occur throughout the gastrointestinal tract, the most common locations are the stomach (60%), jejunum and ileum (30%), duodenum (5%), and colorectum (<5%). Rare cases have been reported in the esophagus, appendix, and gallbladder.3 Rarely, GISTs can present in the mesentery, omentum, and retroperitoneum and are referred to as extragastrointestinal GISTs.4

Clinically, patients generally present with nonspecific symptoms, such as abdominal pain, bloating, melena, fatigue secondary to anemia, or obstruction, especially if the tumor is located within the tubular gut. Rarely, GISTs can present with an aggressive pattern of disease typified by innumerable intraperitoneal, serosal-based nodules or as liver metastasis,1 but GISTs rarely metastasize to the lymph nodes, lungs, or extra-abdominal sites. Small, incidental GISTs are often discovered during upper or lower endoscopy as submucosal bumps, during surgical resections for unrelated cancers, or during radiologic imaging.5

PATHOLOGIC FEATURES OF GIST

Most GISTs are well-circumscribed lesions arising within the wall of the stomach or intestine (Figure 1). They typically exhibit a tan-white, fleshy cut-surface with foci of cystic degeneration, hemorrhage, or necrosis. Large tumors may show ulceration of the overlying mucosa.

Microscopically, most GISTs demonstrate 3 main histologic subtypes: spindle cell type (most common), epithelioid type, and mixed spindle and epithelioid type.6 In general, GISTs are characterized by a uniform, monotonous appearance with minimal cytologic atypia or mitotic activity. Nuclear pleomorphism is occasionally evident in

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a GIST and, when present, is often admixed with the more conventional cytologic features.

Spindle cell GISTs account for nearly 70% of cases and are composed of cells arranged in short fascicles and whorls (Figure 2, A). The stroma may exhibit areas of myxoid change or, rarely, osseous metaplasia (Figure 2, B and C). The individual cells reveal ill-defined cell borders with ovoid nuclei, fine nuclear chromatin, and inconspicuous nucleoli. The cytoplasm has a pale, eosinophilic, and fibrillary quality. Many gastric spindle cell GISTs show extensive paranuclear vacuolization, originally thought to be a diagnostic feature of smooth muscle tumors (Figure 2, D). The degree of paranuclear vacuolization, however, is much more pronounced in GISTs than it is in smooth muscle tumors. Occasionally, nuclear palisading, similar to Antoni A areas of a schwannoma, are encountered (Figure 2, E). Other schwannoma-like features, such as microcystic degeneration and stromal lymphocytes, may also be seen. Tumors arising in the small bowel are often associated with a peculiar stromal change composed of brightly eosinophilic, hyaline, or fibrillary structures known as skenoid fibers (Figure 2, F).

Epithelioid GISTs account for approximately 20% of cases and are characterized by rounded cells arranged in nests or sheets, with variably eosinophilic to clear cytoplasm and vesicular nuclei (Figure 2, G). Approximately 10% of GISTs show a combination of both epithelioid and spindle cells.

Rare examples of GISTs that show abrupt transformation from conventional KIT-positive tumor cells to KIT-negative cells with marked anaplasia have been documented by Antonescu et al. These tumors have been termed dedifferentiated GISTs (Figure 2, H). Of the 4 patients documented in this study, 3 (75%) did not have either a KIT or PDGFRA mutation in either the conventional or dedifferentiated component, whereas 1 (25%) had a KIT exon 11 deletion in both components. Gene copy number abnormalities, either because of loss of heterozygosity or low-level KIT amplification, were the most common alterations found in the dedifferentiated component.

IMMUNOHISTOCHEMISTRY OF GIST

Strong and diffuse immunoreactivity for KIT (CD117) is seen in about 95% of cases. The high sensitivity and specificity of KIT is a useful marker in differentiating GIST from other mesenchymal tumors of the gastrointestinal tract. Most tumors demonstrate a cytoplasmic staining pattern (Figure 3, A). In some tumors, a coexisting, dotlike, or Golgi staining pattern can also be seen (Figure 3, B). Less commonly, a membranous staining pattern is observed. Because KIT positivity has significant therapeutic implications, it is critical to titrate each new batch of KIT antibody with appropriate positive and negative controls. Fortunately, mast cells in the adjacent nonneoplastic tissue serve as an excellent internal control for evaluating the quality of the KIT staining. Another common marker that is not as sensitive or specific for GIST is CD34. It is expressed in nearly 80% of gastric GISTs, 50% of small intestinal GISTs, and in 95% of GISTs arising in the esophagus and rectum. Immunoreactivity for smooth muscle actin is found in nearly 30% to 40% of GISTs. Caution is advised when interpreting smooth muscle markers (smooth muscle actin and desmin) because entrapped smooth muscle cells from adjacent muscularis propria or muscularis mucosae may be misinterpreted as positive staining within the tumor. This phenomenon usually occurs toward the periphery of the tumor. Variable and weak immunopositivity is also seen with other markers, such as h-caldesmon, S100, desmin, and cytokeratins 8 and 18. Of note, focal desmin staining is more common in epithelioid GISTs arising in the stomach.

KIT-Negative GIST

Nearly 5% of GISTs are negative for KIT by immunohistochemistry. These tumors seem to have a predilection for stomach or omentum/peritoneum and often exhibit an epithelioid or mixed phenotype. Interestingly, these lesions tend to be either KIT wild-type or to harbor PDGFRA mutations. To improve the diagnostic accuracy for GIST, especially KIT-negative GISTs, several newer markers discovered on gene expression arrays have been studied.

Discovered on GIST 1 (DOG1), a calcium-activated chloride channel composed of 8 transmembrane domains, is one such marker that was found to be highly expressed in GIST. Although ANO1 (also known as TMEM16A and FLJ10261) is the approved gene symbol, DOG1 is the most commonly used name in the diagnostic literature. It is also known as FLJ10261 (hypothetical gene product that was unofficially called DOG1), TMEM16A (transmembrane protein 16A), or ANO1 (anocytosome 1, calcium-activated chloride channel); DOG1 being the most well-accepted name in the diagnostic literature. Recent studies have demonstrated that the overall sensitivity of DOG1 staining in GIST ranges from 75% to 100%, depending on the type of antibody used. Of the 2 antibodies, DOG1.1 (Stanford University Medical Center, Stanford, California) and clone K9 DOG1 (Novocastra antibodies, Leica Microsystems, Wetzlar, Germany), clone K9 DOG1 antibody appears to be more sensitive in detecting both KIT-positive and KIT-negative tumors. DOG1 being the most well-accepted name in the diagnostic literature. It is also more sensitive for detecting tumors with a spindle cell morphology compared with the epithelioid subtype (Figure 4, A). DOG1 can successfully identify most KIT-positive GISTs and up to one-third of KIT-negative GISTs, the latter mostly harboring PDGFRA mutations. Besides GIST, a membranous pattern of DOG1 expression has also been documented in nonmesenchymal tumors, such as head and neck and
Figure 2. Histologic features and patterns of gastrointestinal stromal tumor (GIST). A, Spindle cell GIST composed of fascicles of uniform, bland cells with pale, eosinophilic cytoplasm. B, Spindle cell GIST with myxoid change. C, Another GIST with foci of osseous metaplasia. D, Spindle cell GIST with prominent paranuclear vacuoles. E, Spindle cell GIST with nuclear palisading that is reminiscent of Antoni A areas encountered in a schwannoma. F, Spindle cell GIST arising in the small bowel, displaying numerous bundles of deeply eosinophilic “skenoid fibers.” G, Epithelioid GIST arising in the stomach, composed of cells with abundant, eosinophilic cytoplasm and distinct cell borders. H, Dedifferentiated GIST composed of atypical epithelioid and spindle cells (hematoxylin-eosin, original magnifications \( \times 200 \) [A through C and F through H], \( \times 400 \) [D], and \( \times 100 \) [E]).
esophageal squamous cell carcinomas, lung adenocarcinoma, and hepatocellular carcinoma. Unlike KIT, the newer DOG1 monoclonal antibodies do not highlight mast cells. The interstitial cells of Cajal (ICC) serve as an internal positive control for evaluating DOG1 expression immunohistochemically. Protein kinase C-theta (PKC-\(\theta\)) is another marker that is upregulated in GIST compared with other mesenchymal tumors. It is a member of the serine/threonine family of protein kinases, which are constitutively phosphorylated in GIST, irrespective of KIT immunoreactivity and mutational status. Although most of the earlier studies were based on immunoblot analysis, some immunohistochemical studies have demonstrated that PKC-\(\theta\) has an acceptable level of sensitivity and specificity for diagnosing GIST and may be particularly relevant in the workup of KIT-negative GIST (Figure 4, B).

Carbonic anhydrase II (CAII) is a recently reported diagnostic marker of GIST whose biologic role in GIST has yet to be well characterized. Higher levels of expression have been associated with a better prognosis, compared with tumors with low or no expression, and therefore, it has the potential to serve as a prognostic biomarker as well. Further studies are required to validate CAII as a prognostic biomarker.

**Differential Diagnosis of GIST**

A variety of mesenchymal tumors should be considered in the differential diagnosis of GIST. These include leiomyoma, leiomyosarcoma, schwannoma, fibromatosis, inflammatory myofibroblastic tumor, inflammatory fibroid polyp, and melanoma.

Intramural leiomyomas occur most commonly in the esophagus and are quite rare in the stomach and small intestine. Leiomyomas are generally less cellular than are GISTs, and in contrast to GISTs, they often show more eosinophilic cytoplasm with better-delineated cell borders. Immunohistochemically, leiomyomas are diffusely positive for smooth muscle actin, desmin, and h-caldesmon and are uniformly negative for KIT. Although most GISTs are negative for desmin, rare cases do express desmin, which may lead to misclassification as a smooth muscle tumor. In contrast to leiomyomas, desmin immunoreactivity is focal.

Primary leiomyosarcomas of the gastrointestinal (GI) tract are extremely rare. They tend to occur in older
patients and most often arise in the colon, compared with the rest of the GI tract. In contrast to GISTs, leiomyosarcomas are composed of spindle-shaped cells, with brightly eosinophilic cytoplasm, accompanied by focal or diffuse nuclear pleomorphism and high mitotic activity. Moreover, KIT positivity in leiomyosarcomas is extremely rare.\textsuperscript{15}

Gastrointestinal schwannomas are uncommon tumors that occur in the stomach (60\%–70\%) or colon (20\%–30\%) and are rare in the rest of the GI tract.\textsuperscript{31,32} In the stomach, they exhibit a dense, peripheral cuff of lymphocytes with or without germinal centers and are composed of cells that display strong immunoreactivity for S100 and glial fibrillary acid protein. They are negative for KIT and CD34. They usually lack well-defined nuclear palisading, Verocay bodies, foamy histiocytes, and hyalinized vessels that are typical of schwannomas elsewhere. In fact, nuclear palisading and perivascular hyalinization are seen in up to 33\% of GISTs.\textsuperscript{15} In addition to morphologic differences, GI schwannomas appear to be genetically distinct compared with non-GI schwannomas. They rarely demonstrate NF2 gene deletions.\textsuperscript{33}

Mesenteric fibromatosis or intra-abdominal desmoid fibromatosis can be confused with GIST because of its gross appearance as well as its location. It can arise either sporadically or in the setting of Gardner syndrome. Microscopically, it is characterized by an infiltrative growth pattern, as opposed to rounded, expansile borders of GISTs. It is composed of long, sweeping fascicles of spindle to stellate fibroblasts within a collagenous or keloidal stroma. The stroma in GISTs tends to surround nests of cells rather than individual cells as is evident in fibromatosis. Another important histologic feature that helps to differentiate these entities is the vascular pattern. Desmoid tumors typically exhibit small, muscular arteries and dilated, thin-walled veins throughout the lesion. Nuclear \(\beta\)-catenin immunoreactivity can be demonstrated in 75\% to 90\% of cases of fibromatosis, whereas it is completely negative in GIST.\textsuperscript{34-36} Different studies have shown KIT positivity in 0\% to 75\% cases of fibromatosis\textsuperscript{34,37-39}; however, that is caused by variability in the immunostaining technique as well as by the type of antibody used, and with optimization, KIT immunoreactivity in desmoid tumors is infrequent.

Inflammatory myofibroblastic tumor occurs in children and young adults and may present as a mesenteric mass.\textsuperscript{40} Histologically, it has a more heterogeneous composition with spindle cell areas admixed with a predominantly plasma cell-rich inflammatory infiltrate. These tumors are typically negative for KIT.\textsuperscript{41} The diagnostic marker for this tumor is anaplastic lymphoma kinase; it is, however, expressed in less than 50\% of cases.\textsuperscript{42}

Inflammatory fibroid polyp is a submucosal mesenchymal tumor that has a predilection for the stomach (particularly, the antrum) and small intestine. Morphologically, there is a proliferation of stellate and spindle cells that tend to form whorls around blood vessels. The stroma often has a granulation tissue-like appearance and is enriched with eosinophils, lymphocytes, and plasma cells. Inflammatory fibroid polyps can be confused with GISTs because of their CD34 positivity. However, they are negative for KIT and DOG1.\textsuperscript{20,31,44} More recently, mutations in PDGFR\(\alpha\) of exactly the same type that are present in GISTs have been described in inflammatory fibroid polyps as well.\textsuperscript{45,46}

Epithelioid GISTs must be distinguished from melanoma, carcinoma, germ cell tumors, seminoma, glomus tumor, and clear cell sarcoma. Melanomas exhibit a variety of morphologic patterns, including spindle and epithelioid cell forms, and, therefore, enter into the differential diagnosis of GIST. This is further complicated by their KIT-positivity. Expression of melanoma markers, such as HMB-45, Melan-A, or S100, easily helps to resolve this differential. Interestingly, the KIT immunoreactivity in metastatic melanomas may be much weaker than it is in primary tumors.\textsuperscript{47} Glomus tumors are rare in the GI tract and are found almost exclusively in the stomach. They are characterized by a uniform population of cells with pale eosinophilic cytoplasm and round nuclei that exhibit strong immunoreactivity for smooth muscle actin and lack expression of desmin, S100, or KIT.\textsuperscript{48} Similarly, clear cell sarcomas of the GI tract are most often negative for HMB-45 and are characterized by EWSR1-ATF1 or EWSR1-CREB1 gene fusions.\textsuperscript{49,50} Besides their distinctive morphology of large epithelioid cells with abundant pale eosinophilic to clear cytoplasm and lymphocyte-rich stroma, seminomas can be distinguished from GISTs by their characteristic immunoreactivity for placental alkaline phosphatase and transcription factor OCT4.\textsuperscript{47} They may, however, show diffuse immunoreactivity with KIT leading to a potential diagnostic pitfall. The most common differential diagnoses and the immunohistochemical markers that can be used to distinguish them from GISTs are summarized in Table 1.

### Table 1. Immunohistochemistry in Differential Diagnosis of Gastrointestinal Stromal Tumor (GIST)\textsuperscript{a}

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>KIT</th>
<th>Smooth Muscle</th>
<th>Desmin</th>
<th>S100</th>
<th>CD34</th>
<th>Keratin</th>
</tr>
</thead>
<tbody>
<tr>
<td>GIST</td>
<td>+++</td>
<td>+ (40)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leiomyoma</td>
<td>–</td>
<td>+++</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>±</td>
</tr>
<tr>
<td>Leiomyosarcoma</td>
<td>–</td>
<td>+++</td>
<td>+ to +++ (80)</td>
<td>–</td>
<td>+ (10)</td>
<td>(25)</td>
</tr>
<tr>
<td>Schwannoma</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>±</td>
<td>–</td>
</tr>
<tr>
<td>Fibromatosis</td>
<td>–</td>
<td>+ to +++ (metaplastic/sarcomatoid)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+ to +++</td>
</tr>
<tr>
<td>Carcinoma</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Melanoma</td>
<td>+ (50)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>±</td>
<td>–</td>
</tr>
</tbody>
</table>

Abbreviations: –, no cells positive by immunohistochemistry; ±, sometimes weak positive, sometimes negative by immunohistochemistry; +, \(<25\%\) of cells positive by immunohistochemistry; ++, \(25\%–50\%\) of cells positive by immunohistochemistry; ++++, \(>50\%\) of cells positive by immunohistochemistry.

\(\textsuperscript{a}\) Parenthetical numbers indicate approximate percentage of cases that are positive.
KIT is a transmembrane growth factor receptor for a stem cell factor that belongs to the family of receptor tyrosine kinases. KIT is expressed strongly in hematopoietic stem cells, melanocytes, mast cells, germ cells, and ICC. The KIT receptor is composed of a ligand-binding extracellular domain, a transmembrane domain, a juxtamembrane domain, and 2 cytoplasmic tyrosine kinase domains (TK I and TK II) (Figure 5). The binding of the KIT ligand stem cell factor (also known as steel factor) brings together 2 KIT molecules, which trigger a cascade of events, including receptor dimerization, cross-phosphorylation of cytoplasmic tyrosines and activation of several cell signaling pathways that regulate cell proliferation, adhesion, apoptosis, and differentiation.

The function of KIT was discovered by observational studies in murine models with loss-of-function KIT mutations. For years, researchers have studied the original “white spot” mutant mice that carried a dominant-acting loss-of-function KIT mutation. It was not until 1995 that it was realized that mice with loss-of-function KIT mutations demonstrated loss of ICC as well. This discovery ultimately linked KIT to GIST—the so-called KIT connection.

Interstitial cells of Cajal are a network of cells distributed throughout the GI tract that serve as pacemakers by transducing signals from the nervous system. They form an intimate network within the myenteric plexus of the intestinal wall and thus facilitate coordinated peristalsis. It is now widely accepted that GISTs arise from ICC or from an ICC precursor. Because KIT deficiency caused a loss of ICC, Hirota and colleagues wondered whether gain of function KIT mutations might result in ICC tumors. They hypothesized that GISTs would be a good candidate for ICC tumors because the phenotype of GISTs did not seem to fit with a known line of differentiation. They examined GISTs and found that they expressed KIT strongly and had activating KIT mutations. This seminal study was followed by many subsequent studies that confirmed this finding.
**KIT MUTATIONS**

KIT mutations found in GIST cause constitutional activation of the receptor tyrosine kinase pathway, in the absence of their ligands. Four different regions of KIT, namely exon 9, exon 11, exon 13, and exon 17, are most often mutated in sporadic GISTs. Most KIT mutations (nearly 65%) involve the juxtamembrane domain (exon 11), followed by extracellular domain (exon 9; 9%), and the TK I, ATP-binding pocket (exon 13), and TK II, kinase activation loop (Figure 5).61,62 Very rarely, mutations have been found in exons 8, 12, 14, and 18 in primary GISTs.

Exon 11 mutations most commonly are composed of in-frame deletions of one or more codons; some of these (codons 557–558) are typically associated with poor clinical outcome.63 Missense point mutations are the next most common type of mutations. In gastric GISTs, these mutations are associated with a better prognosis; no such correlation has been documented with small intestinal GISTs.12,15

Virtually all exon 9 mutations are characterized by a 6-nucleotide duplication encoding alanine and tyrosine at amino acid residues 502 and 503.64 These mutations are associated with small intestinal GISTs and a worse prognosis.12

Exon 13 and exon 17 mutations are rare (<1%–2%). Exon 13 mutations are usually a substitution of aspartate for lysine at residue 642. Exon 17 mutations are mostly substitutions, occur predominantly in small intestinal GISTs, and do not appear to have any prognostic implication.65

**PDGFRA MUTATIONS**

PDGFRA is a type III receptor tyrosine kinase, and similar to KIT, activating mutations cause downstream activation of multiple cell-signaling cascades that control vital cellular functions. Both KIT and PDGFRA are located on the long arm of chromosome 4.66 Exon 18, exon 12, and exon 14 are the 3 PDGFRA regions that are mutated in GISTs. They correspond to the exon 17, exon 11, and exon 13 mutations of KIT (Figure 5). Overall, nearly 7% of GISTs harbor PDGFRA mutations, and more than 80% of those mutations are missense mutations in exon 18. They have a strong predilection for either gastric or extrabdominal locations, exhibit an epithelioid phenotype and myxoid stroma, and characteristically have multinucleated and rhabdoid cells (Figure 6, A). KIT and PDGFRA mutations are mutually exclusive.67 PDGFRA-mutant GISTs tend to be faintly positive or completely negative for KIT by immunohistochemistry (Figure 6, B).

Tumors that do not harbor either the KIT or PDGFRA mutations account for about 10% to 15% of GISTs and have been designated as KIT-PDGFRA wild-type GISTs.68,69 Recently, 7% to 13% of wild-type GISTs in adults were shown to harbor BRAF V600E mutations at exon 15.70–72

The response to therapy (imatinib mesylate [Gleevec, Novartis Pharmaceuticals, East Hanover, New Jersey] or sunitinib malate [Sutent, Pfizer Pharmaceuticals, New York, New York]) appears to correlate with specific KIT or PDGFRA mutations (discussed later in the section on “Therapeutic Implications of Activating KIT Mutations”).

**FAMILIAL GIST AND OTHER GIST SYNDROMES**

Germline mutations in KIT and PDGFRA have been documented. Since their original description by Nishida et al.,20 additional families with familial GIST have been identified. These mutations are identical to those found in sporadic tumors and are inherited in an autosomal-dominant pattern.23–25 Every family member who harbors a germline KIT mutation will develop one or more GISTs, usually at a younger age than those with sporadic tumors. Clinically, many members of familial GIST syndrome kindreds manifest with cutaneous findings that include hyperpigmentation (especially perineal), increased numbers of nevi, and mast cell disease in the form of urticaria pigmentosa or even systemic mastocytosis.26,27 Morphologically, these tumors are indistinguishable from sporadic GISTs. Because most reported familial GIST kindreds are small, it is difficult to accurately compare the behavior of GISTs arising in the familial setting with sporadic GISTs. Although some reports suggest that these tumors appear to be indolent, in a study by Kleinbaum et al.,72 2 patients presented with metastases at the time of diagnosis despite having variable mitotic activity. The authors,75 therefore, believed that patients with familial GISTs should be categorized as being at high risk for metastasis, irrespective of the tumor size and mitotic activity. The results from that study72 also suggest that individuals from known GIST kindreds require close surveillance.

Carney triad and Carney-Stratakis syndrome are 2 other syndromes that are predisposed toward GISTs. The finding of multiple GISTs is a common feature of both these syndromes. Carney triad usually occurs in young women who present with a combination of gastric GIST, paraganglioma, and pulmonary chondroma.26–30 A study by Zhang et al.81 recently confirmed that Carney triad-associated GISTs are pathologically and clinically different from sporadic GISTs. These lesions tend to exhibit an epithelioid morphology and appear to be at a higher risk for metastasis, particularly to lymph nodes. Neither KIT nor PDGFRA mutations have been found in a subset of tumors that have been analyzed thus far.

In contrast to Carney triad-associated GISTs, which are thought to be sporadic rather than familial, Carney-Stratakis syndrome-related GISTs occur because of germline mutations in the enzyme succinate dehydrogenase (SDH) subunits SDHB, SDHC, or SDHD.44 Clinically, these patients present with multifocal GISTs, paragangliomas, and pheochromocytomas. The SDH is an enzyme complex located within the inner mitochondrial membrane that participates in the citric acid cycle and the electron transport chain. Recent studies45,46 have evaluated the utility of SDHB immunohistochemistry in specifically separating Carney-Stratakis syndrome-related GISTs from KIT-PDGFRA–mutated GISTs and sporadic GISTs. It appears that lack of immunoreexpression of SDHB has been observed not only in patients with GISTs with a syndromic association and pediatric GISTs but also in some individuals with wild-type GISTs.87 Additionally, Carney-Stratakis syndrome-related GISTs tend to be localized to the stomach and demonstrate an epithelioid morphology, akin to pediatric GISTs.

In a large series of Carney triad-associated GISTs, Zhang et al.80 demonstrated that, compared with sporadic GISTs, these tumors show slow growth, frequently metastasize (especially to lymph nodes), and demonstrate either no response or an equivocal response to imatinib therapy. Despite metastatic disease, their clinical behavior is unpredictable, and there appears to be no
correlation between conventional risk assessment and clinical behavior.

Multiple GISTs also arise in the setting of neurofibromatosis type I (NF1). This syndrome results from germline mutation of the NF1 gene, which encodes for a GTPase-activating protein, neurofibromin. Approximately 7% of patients with NF1 have GISTs. NF1-related GISTs most commonly occur as multiple small tumors in the small bowel and exhibit spindle cell morphology. They are replete with skenoid fibers. Most of these tumors do not harbor the KIT or PDGFRA mutations. They do, however, express KIT; a subset of them also show S100 immunoreactivity. Clinically, they usually exhibit a benign behavior; rare cases of malignant GISTs associated with numerous benign tumor nodules have been reported.

PEDIATRIC GISTS

Pediatric GISTs usually present during the second decade of life, the median age of presentation being 13 and 14.5 years in some of the larger series to date. They have a marked female predominance, are commonly located in the stomach, and most often display an epithelioid morphology. The presence of multiple tumor foci is another distinctive feature of pediatric GISTs. Although they uniformly express KIT, at a molecular level, pediatric GISTs rarely have KIT or PDGFRA mutations. Additionally, comparative genomic hybridization analysis has revealed that most pediatric GISTs that lack the KIT or PDGFRA mutation have minimal, large-scale chromosomal changes compared with adult GISTs. The histomorphologic and clinical features of pediatric GISTs are remarkably similar to Carney-triad–associated GISTs. A subset of patients with pediatric GIST have Carney-triad, and therefore, GIST in a child should trigger evaluation for potential Carney triad.

For risk stratification and prognosis, the system used in adults does not seem to be predictive in children. Although many patients may develop recurrence or metastasis, in general, pediatric GISTs follow an indolent course. In a meta-analysis of 113 pediatric GISTs with a mean follow-up period of 5.7 years, 68% patients (n = 77) were alive without disease, 21% were alive with disease (n = 24) and 11% (n = 12) died of disease. It has been suggested that treatment with receptor tyrosine kinase inhibitors should be restricted to patients with metastasis or unresectable tumors. Pediatric KIT-wild-type GISTs exhibit KIT activation at levels comparable with KIT-mutant pediatric and adult GISTs, although they do not have KIT or PDGFRA mutations. As a result, it has been suggested that the therapies for pediatric GISTs should focus on inhibitors of KIT activation or signaling molecules downstream of KIT with an emphasis on second-generation receptor tyrosine kinase inhibitors, such as sunitinib and nilotinib, that have been shown to inhibit wild-type GISTs.

DO GISTS ARISE FROM PRECURSOR LESIONS?

Microscopic foci of KIT-positive spindle cell hyperplasia are commonly found in patients with germline KIT or PDGFRA mutations or NF1 mutations. They have also been described adjacent to sporadic GISTs. They are common incidental findings in gastroesophageal resections (9%–35%). These lesions have been variably designated as sporadic Cajal cell hyperplasia, microscopic GISTs, GIST tumorlets, or “seedling” GISTs. Although nearly 85% of incidental microscopic lesions harbor KIT mutations, based on statistics, only a small proportion (1%) progress to clinically significant GISTs. Therefore, these microscopic lesions require additional genetic events to transform into clinically significant neoplasms. Cyto genetic analyses and comparative genomic hybridization studies have revealed compelling correlations between the biologic progression of GISTs and chromosomal alterations. These include 1q deletion, 22q deletion, 1p deletion, 8p gain, 11p deletion, 9p deletion, and 17q gain. Loss of 9p is associated with an aggressive behavior and appears to correspond to loss of CDKN2A (p16INK4A), a tumor suppressor gene that is inactivated in many mesenchymal tumors of the gastrointestinal tract.

Both aggressive and/or metastatic behavior have been associated with gains of chromosomes 5p, 20q, 8q, and 17q. The presence of hyalinization and dystrophic calcification serve as an indication that small, incidental GISTs have limited growth potential.

MANAGEMENT OF LOCALIZED AND ADVANCED DISEASE

Gastrointestinal stromal tumors are often discovered incidentally during computed tomography or endoscopic investigations. Although preoperative biopsy is not recommended for lesions that are highly suspicious for GIST, lesions of indeterminate type are often evaluated by fine-needle aspiration technique or needle core biopsies. Surgical resection with preservation of the pseudocapsule is the primary therapy for localized GISTs. These tumors should be carefully handled to avoid tumor rupture, which leads to a very high risk of intra-abdominal dissemination. Because nonsyndromic adult GISTs rarely metastasize to lymph nodes, routine lymphadenectomy is not recommended.

In the preimatinib era, patients with metastasis within the peritoneal cavity or liver typically had a median survival of 18–24 months. These tumors responded poorly to all forms of chemotherapy or radiation therapy. It was only when a patient with widespread disease responded well to imatinib in a compassionate use protocol that clinical trials to treat advanced disease were initiated.

THERAPEUTIC IMPLICATIONS OF ACTIVATING KIT MUTATIONS

Imatinib mesylate and sunitinib malate are competitive inhibitors of the ATP-binding pocket of KIT and PDGFRA that were US Food and Drug Administration–approved for first and second line GIST treatment, respectively. They bind to and stabilize the inactivated form of the receptor tyrosine kinases, leading to inhibition of autophosphorylation and activation, resulting in inhibition of downstream KIT signaling. Both drugs differ in their binding targets as well as inhibitory activity. Imatinib binds to amino acid residues within the ATP-binding pocket as well as the activation loop, whereas sunitinib interacts with different amino acid residues in the ATP-binding pocket. Sunitinib also possesses activity against vascular endothelial growth factor receptors 1/3 and thus has antiangiogenic properties as well.

Although a significant difference was observed in the median overall survival between KIT-positive tumors and KIT-negative tumors (53 months versus 31 months), it
is the KIT mutation genotype rather than KIT protein expression that appears to drive the therapeutic response to imatinib.110–112 In one of the randomized clinical trials (US-Finnish B2222 phase 2 trial), GISTs with exon 11 mutations demonstrated the most favorable objective response compared with GISTs with exon 9 mutations and wild-type GISTs (83.5% versus 48% versus 0%, respectively).113 Debiech-Rychter and colleagues114 found that increasing the dosage from 400 mg to 800 mg per day specifically improved progression-free survival of exon 9–mutant GISTs. Interestingly, KIT mutation status also predicts response to sunitinib and is opposite to what is seen with imatinib. KIT exon 9 and KIT-PDGFRα wild-type GISTs appear to respond better to sunitinib than do KIT exon 11–mutant GISTs. Although some PDGFRA mutations respond to imatinib or sunitinib, the most common PDGFRA mutant, exon 18 D842V, is highly resistant to both imatinib and sunitinib.115–117

Mutational analysis of primary tumors is not routinely recommended at this time. Per the National Comprehensive Cancer Network (NCCN) Task Force Report, it may be performed in select cases, such as to confirm the diagnosis of KIT-positive tumors with atypical morphology or clinical features; in KIT-negative GISTs, to differentiate GIST from other mesenchymal tumors; or to identify patients at higher risk for recurrence in the setting of postoperative imatinib therapy. Similarly, in advanced disease, it may be considered for gastric GISTs that are nonresponders to imatinib and for small-bowel GISTs because exon 9 mutations are shown to respond better to higher-dose imatinib.115

**MECHANISMS OF IMATINIB RESISTANCE AND MANAGEMENT**

Nearly 50% of GISTs treated with imatinib therapy will demonstrate resistance in the first 2 years. Resistant lesions often present as new nodules within the main lesion, the so-called nodule within a nodule appearance, and most of them are radiologically detectable as areas of high density on computed tomography.115

Resistance is categorized as either primary resistance or secondary resistance. Primary resistance to imatinib is defined by lack of stabilization or decrease in the size of the tumors within the first 6 months of treatment and is most commonly seen in patients with KIT exon 9, PDGFRA exon 18, and wild-type KIT genotypes.116 These patients have the same mutations pretherapy and posttherapy with imatinib and do not develop new mutations. At a molecular level, this is characterized by continuous phosphorylation/activation of KIT and PDGFRA in the presence of imatinib.

Tumors exhibiting secondary resistance, defined by initial stabilization or decrease in the size of tumor for a period of 6 months on imatinib followed by progression, commonly develop one or more new mutations in KIT or PDGFRA.108 At the biochemical level, the phenomenon of secondary resistance is characterized by an initial loss of KIT or PDGFRA phosphorylation/activation followed by phosphorylation/activation of these proteins as a result of mutations that are almost exclusively expressed in tumor nodules undergoing progression.117 Individuals may have “polyclonal resistance,” where newly acquired mutations in the same patient differ within different, resistant tumor nodules.117 Genetic analysis has shown that nearly 67% of resistant tumors show mutations in exons 13 and 14 (ATP-binding domain) and exons 16, 17, and 18 (activating loop domain)118 (Figure 5). Mutations in exon 13 (V654A) account for nearly 40% of secondary mutations that decrease the binding capacity of imatinib. Approximately 10% of resistant GISTs do not demonstrate secondary mutations. Instead, they show an increase in copy number of mutated KIT.119

In January 2006, sunitinib malate was approved by the US Food and Drug Administration as a second-line therapy for advanced disease based on an overall survival benefit provided by sunitinib in patients with imatinib-resistant or imatinib-intolerant tumors. Long-term intake is, however, associated with serious adverse effects in nearly 20% of patients. In addition to fatigue, diarrhea, hand-foot syndrome, hypertension, and myelosuppression, these patients need to be carefully monitored for development of hypothyroidism.120 Other drugs being tested for patients progressing on imatinib and sunitinib are the second-generation tyrosine kinase inhibitors, such as sorafenib, dasatinib, and nilotinib. Preliminary data from phase 1 and phase 2 trials show that sorafenib and nilotinib result in improved performance status including symptom relief in patients pretreated with imatinib and sunitinib.121,122

**TREATMENT-RELATED CHANGES IN GIST**

Following treatment, GISTs usually demonstrate hypo-cellularity and prominent stromal changes in the form of sclerosis, calcifications, myxoid change, and necrosis (Figure 7).119,123 In some cases, they may change their phenotype from spindled to epithelioid type and may lose KIT immunoreactivity. This can be challenging diagnostically, especially in biopsy samples sent for histologic analysis. Mutational analysis of these tumors shows retention of the same genotype following treatment.124

**PROGNOSTIC FACTORS AND RISK ASSESSMENT IN PRIMARY GIST**

Based on long-term follow-up study of more than 1600 patients performed at the Armed Forces Institute of Pathology, Miettinen et al. issued guidelines for risk stratification based on mitotic index, size, and anatomic location (Table 2). These guidelines have been recommended by both NCCN and the College of American Pathologists.105,125 In contrast to many soft tissue neoplasms where mitotic activity is expressed per 10 high-power fields (HPFs), the mitotic index in GISTs is typically expressed per 5 mm² (approximately 20–25 HPFs using a ×40 magnification field on a modern microscope).121,125 Mitotic counts performed in the original studies required counting 50 HPFs, compared with the currently recommended 20 to 25 HPFs. This change is due to the current wide-field microscopes that have approximately twice the ocular field size compared with older microscopes, which were used in the original analysis. To accurately report mitotic counts, the following calculations must be performed: (1) determine the ocular field diameter of the ×40 magnification field using a slide micrometer and determine the radius of the field, for example, for a ×40 magnification field, if the ocular field diameter is 0.56 mm, the calculated radius is 0.28 mm; (2) calculate the area of a single HPF using the formula πr², for example, 3.1416 × 0.28 × 0.28 = 0.25 mm²; and (3) divide that value by the Armed Forces Institute of Pathology’s recommended 5 mm², which yields 20 HPFs.
Mitotic index is by far the most important prognostic feature of GISTs.\textsuperscript{126} Anatomic site is also important because gastric GISTs tend to behave better than GISTs that arise elsewhere.\textsuperscript{9} Finally, size is also predictive of aggressive behavior because larger GISTs have a worse prognosis than smaller GISTs.

To predict postoperative recurrence of localized GIST, Gold et al\textsuperscript{27} devised a nomogram that assigns points based on tumor size, mitotic rate, and tumor location. The total score predicts the likelihood of recurrence at 2 years and 6 years after surgical resection of the primary GIST. The nomogram predictions have a higher concordance probability compared with the modified Armed Forces Institute of Pathology–Miettinen et al\textsuperscript{4} risk-stratification system with minimal, statistically insignificant differences between the two. The nomogram appears to be better calibrated to the actual recurrence-free survival compared with any of the other risk stratification systems.

Some authors have proposed the use of proliferation markers, such as Ki-67, as a more objective parameter for risk assessment. Although multivariate analyses in several studies do indicate that Ki-67 index can be independently used as an outcome predictor, those studies do not take into account the type of surgical procedure, follow-up, or histologic type and location of the tumors.\textsuperscript{126-130} Other markers, such as down-regulation of p16,\textsuperscript{131,132} loss of Raf kinase inhibitory protein,\textsuperscript{133} ezrin overexpression,\textsuperscript{134} BCL2 overexpression,\textsuperscript{135-136} and predominantly fetal-expressed tetramerization domain (pfetin, a potassium channel protein) expression,\textsuperscript{137} have been associated with high-risk behavior. However, these markers are still investigational and per NCCN guidelines, tumor size, mitotic activity, and anatomic location are the criteria that should be used for predicting clinical behavior.

In routine practice, the College of American Pathologists checklist provides a comprehensive list of components to be included while reporting a resected GIST.\textsuperscript{125} These include type of procedure, tumor site, tumor size, tumor fociality, histologic subtype, mitotic rate, percentage of necrosis, histologic grade, risk assessment (per modified Armed Forces Institute of Pathology–Miettinen et al\textsuperscript{4} risk-stratification system), and margins. Information about ancillary studies, such as immunohistochemical analysis and molecular genetic studies, should also be included. Although the diagnosis of GIST can be made with a high level of certainty on fine-needle aspirates, immunohistochemical analysis on the cell-block preparation is important to confirm the diagnosis.\textsuperscript{138} On biopsy samples, every attempt should be made to provide the following information: tumor site, histologic subtype (spindle cell, epithelioid, or combined spindle cell and epithelioid), mitotic rate, as well as the immunohistochemical profile of the tumor. Overall, it is best to avoid risk stratification based on limited samples such as fine-needle aspirates or needle core biopsies.\textsuperscript{139}

### SUMMARY AND FUTURE DIRECTIONS

During the past decade, basic and translational research advances have provided a detailed understanding of the molecular pathogenesis of gastrointestinal stromal tumors. We now understand that most GISTs have KIT or PDGFR\textsubscript{A} mutations and respond to specific small-molecule tyrosine kinase KIT inhibitors with promising clinical results. It has, therefore, become critical to diagnose GISTs accurately and to provide prognostic information based on the guidelines suggested by the NCCN and College of American Pathologists. We now, however, face challenges related to primary and secondary drug resistance. This has resulted in the birth of second-generation tyrosine-kinase inhibitors, which have shown activity in imatinib-resistant and sunitinib-resistant GISTs. Additionally, current research is now focused on investigating other molecular mechanisms that lead to tumor progression with the hope of providing alternative therapeutic modalities to reduce recurrence and to prolong survival in patients diagnosed with GIST.

### Table 2. Risk Stratification of Primary Gastrointestinal Stromal Tumor (GIST) by Mitotic Index, Size, and Site\textsuperscript{a,b}

<table>
<thead>
<tr>
<th>Tumor Parameters</th>
<th>Stomach</th>
<th>Jejunum/Ileum</th>
<th>Duodenum</th>
<th>Rectum</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mitotic Rate</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤5 per 50 HPF</td>
<td>≤2</td>
<td>None (0)</td>
<td>None (0)</td>
<td>None (0)</td>
</tr>
<tr>
<td>&gt;2, ≤5</td>
<td>≤2</td>
<td>Very low (1.9)</td>
<td>Low (4.3)</td>
<td>Low (8.3)</td>
</tr>
<tr>
<td>&gt;5, ≤10</td>
<td>Low (3.6)</td>
<td>Moderate (24)</td>
<td>Insufficient data</td>
<td>Insufficient data</td>
</tr>
<tr>
<td>&gt;10</td>
<td>Moderate (12)</td>
<td>High (52)</td>
<td>High (54)</td>
<td>High (57)</td>
</tr>
<tr>
<td>≤5 per 50 HPF</td>
<td>None\textsuperscript{d}</td>
<td>High\textsuperscript{b}</td>
<td>Insufficient data</td>
<td>Insufficient data</td>
</tr>
<tr>
<td>&gt;2, ≤5</td>
<td>Moderate (16)</td>
<td>High (73)</td>
<td>High (50)</td>
<td>High (52)</td>
</tr>
<tr>
<td>&gt;5, ≤10</td>
<td>High (55)</td>
<td>High (85)</td>
<td>Insufficient data</td>
<td>Insufficient data</td>
</tr>
<tr>
<td>&gt;10</td>
<td>High (86)</td>
<td>High (90)</td>
<td>High (86)</td>
<td>High (71)</td>
</tr>
</tbody>
</table>

Abbreviation: HPF, high-power field.

\textsuperscript{a} Adapted from Miettinen and Lasota.\textsuperscript{4} Copyright 2006 with permission from Elsevier.

\textsuperscript{b} Data are based on long-term follow-up of 1055 patients (54.4%) with gastric GISTs, 629 patients (32.4%) with small-intestine GISTs, 144 patients (7.4%) with duodenal GISTs, and 111 patients (5.7%) with rectal GISTs.

\textsuperscript{c} Defined as metastasis or tumor-related death.

\textsuperscript{d} Denotes small number of cases.

### References

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