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Review

Gastrointestinal stromal tumors: Overview of pathologic features, molecular biology, and therapy with imatinib mesylate

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Summary. Gastrointestinal stromal tumors (GISTs) are the most common mesenchymal tumors of the gastrointestinal tract. These tumors develop at any site but are most commonly reported in the stomach. They originate from the neoplastic transformation of the intestinal pacemaker cell, the interstitial cell of Cajal. GISTs strongly express the receptor tyrosine kinase KIT and have mutations in the *KIT* gene, most frequently in exon 11 encoding the intracellular juxtamembranous region. Expression of KIT is seen in almost all GISTs, regardless of the site of origin, histologic appearance, or biologic behavior, and is therefore regarded as one of the key diagnostic markers.

Distinction from smooth muscle tumors, such as leiomyosarcomas, and other mesenchymal tumors is very important because of prognostic differences and therapeutic strategies. Predicting the biologic behavior of GISTs is often difficult by conventional pathologic examination; tumor size and mitotic rate are the most important prognostic indicators. The prognostic significance of *KIT* mutations is controversial and thus far has not been clearly linked with biologic behavior. *KIT* mutations are associated with tumor development, and cytogenetic aberrations are associated with tumor progression. The pathogenesis of GISTs involves a gain-of-function mutation in the *KIT* proto-oncogene, leading to ligand-independent constitutive activation of the KIT receptor. *KIT*-wild-type GISTs have shown mutually exclusive platelet-derived growth factor receptor (*PDGFR*) mutation and activation. The use of imatinib mesylate (also known as Gleevec or STI-571) has greatly increased the therapeutic efficacy for this otherwise chemotherapy-resistant tumor. GISTs with very low levels of KIT expression may respond to imatinib mesylate therapy if the receptors are activated by specific mechanisms. KIT-activating mutations fall into two groups: the regulatory type and the enzymatic site type. The regulatory type of mutation is conserved at the imatinib binding site, whereas the enzymatic site mutation has a structurally changed drug-binding site, resulting in drug resistance. Resistance to the drug is the major cause of treatment failure in cancer therapy, emphasizing the need for researchers to understand KIT signaling pathways so as to identify new therapeutic targets. This review summarizes the pathologic features of GISTs, recent advances in understanding their molecular and biologic features, and therapy with imatinib mesylate.

Key words: GIST, KIT, imatinib mesylate

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Introduction

Gastrointestinal stromal tumors (GISTs) are receptor tyrosine kinase KIT (CD117)-expressing soft tissue tumors that are the most common mesenchymal tumors of the gastrointestinal tract (Fletcher et al., 2002). GISTs originate from the neoplastic transformation of the intestinal pacemaker cell, the interstitial cell of Cajal (ICC) (Kindblom et al., 1998). The ICCs are a network of innervated cells that coordinate peristaltic activity throughout the gastrointestinal system. There is evidence that the ICC stem cell can differentiate into a smooth muscle or an ICC phenotype under the influence of KIT (Fleischman, 1993; Kluppel et al., 1998).

Classification of GISTs was a source of controversy for many years, but a publication in 1998 by Hirota and colleagues clarified the nature of GISTs. They observed that GISTs strongly express the receptor tyrosine kinase KIT and that mutations of this gene are frequently present in the juxtamembrane domain (exon 11) (Hirota et al., 1998).

The exact incidence of GISTs in the United States is unknown, since the recent classification system of gastrointestinal mesenchymal tumors is different from

the older scheme. Fletcher and colleagues estimated in 2002 that the incidence of GISTs has increased from 300-500 per year to 5000-6000 per year at least partly because of renewed interest and improved diagnosis (Fletcher et al., 2002). About 60-70% of GISTs occur in the stomach, 20-30% in the small intestine, and 10% or fewer in the esophagus, colon, and rectum. Similar tumors sometimes arise in the abdominal cavity such as the omentum, mesentery, and retroperitoneum (Miettinen, 1999, 2000).

The clinical presentation of GISTs is variable. Patients are often asymptomatic until the tumor is large. These tumors may cause nonspecific abdominal pain, discomfort, a palpable mass, or acute hemorrhage into the gastrointestinal (GI) lumen or peritoneal cavity due to tumor rupture (De Silva and Reid, 2003).

A new receptor tyrosine kinase inhibitor drug, imatinib mesylate (Gleevec, formerly STI-571, Novartis, Basel, Switzerland) appears to be therapeutically effective in recurrent and metastatic GISTs (Joensuu et al., 2001). This review summarizes the brief history of GIST classification, pathology, and differential diagnosis, and describes the related advances in the understanding of molecular biology and therapy with imatinib mesylate.

Historical overview

Traditionally, mesenchymal tumors of the GI tract were classified as smooth muscle tumors (leiomyomas, leiomyosarcomas, leiomyoblastomas) because of the irrelevant origin in the muscle layer and the irrelevant morphologic resemblance to smooth muscle tumors at other sites. As early as 1976, Gottlieb and colleagues at The University of Texas M. D. Anderson Cancer Center made the important clinical observation that leiomyosarcomas originating in the GI tract do not respond to conventional chemotherapy, whereas, leiomyosarcomas arising in other organ systems are more likely to respond to doxorubicin-based therapies (Gottlieb et al., 1976). This observation was refined by others, and it became clear that patients with GISTs had poor response rates not only to standard therapy but also to a number of investigational agents.

Electron microscopy, introduced in the late 1960s and early 1970s, and immunohistochemistry, introduced in the early 1980s, revealed that only some of these tumors were of smooth muscle origin and that many lacked the ultrastructural and immunophenotypic features of smooth-muscle differentiation. This discovery led Mazur and Clark in 1983 to introduce the generic designation "stromal tumor" to indicate the lack of markers of specific differentiation in many of these lesions (Mazur and Clark, 1983). However a subset of these lesions with clear ultrastructural evidence of autonomic neuronal differentiation was designated as gastrointestinal autonomic nerve tumors (GANTs) (Walker and Dvorak, 1986). Currently, because of evidence that GANTs share a genetic identity with conventional GISTs, such designation of GANTs as a separate entity may no longer be warranted (Lee et al., 2001).

Immunohistochemical positivity to CD34 antibodies helped further separate GISTs from most true leiomyomas and schwannomas, which are CD34 negative (van de Rijn et al., 1994). However, it became evident that no more than 60-70% of GISTs are CD34 immunoreactive and that Schwann cell neoplasia and true smooth-muscle tumors may also show CD34 positivity. The subsequent observation of KIT expression and mutation in GISTs ultimately led to a major reappraisal of the classification of these tumors. The current scheme suggests that gastrointestinal mesenchymal tumors can be divided into GISTs (KIT positive), true smooth-muscle tumors, and true Schwann cell tumors (Salomo-Rikata et al., 1998). It is now accepted that KIT immunoreactivity, with the clinicopathologic context of mesenchymal lesions of the GI tract, defines a group of tumors showing differentiation toward the interstitial cell of Cajal. As a result of the characterization of these features, the majority of mesenchymal lesions of the GI tract now seem to fit best in this category (Hirota et al., 1998; Sircar et al., 1999: Miettinen et al., 2000; Fletcher et al., 2002).

Pathologic findings

GISTs typically present as unencapsulated but well-circumscribed masses, with a whorled firm or soft fleshy appearance on a cut surface, that appear to arise in the muscularis propria. Larger lesions often show cystic degeneration or central necrosis. Some tumors protrude both into the lumen and to the serosal side of the bowel, with ulceration of the overlying mucosa (De Silva and Reid, 2003). Histologically, they consist mainly of spindle and/or epithelioid cellular features. Familiarity with the spectrum of histologic appearances of GISTs will enable pathologists to develop a high level of suspicion when diagnosing these tumors.

The predominant pattern, seen in 70-80% of GISTs, is of a spindle cell tumor with a fascicular or storiform growth pattern. The cells show less cytoplasmic eosinophilia than do smooth muscle tumors. Some tumors, especially those originating in the stomach, may show striking perinuclear vacuolation, which historically was misinterpreted as a typical feature of smooth-muscle differentiation. Others may show nuclear palisading simulating soft tissue neurilemmomas (nerve sheath tumors). About 20-30% of the tumors are composed mainly of large round or polygonal epithelioid cells with abundant, often eosinophilic or clear cytoplasm. These epithelioid tumors correspond to those previously designated as leiomyoblastomas and epithelioid smooth-muscle tumors and are more common in the stomach. Mixed spindle and epithelioid tumors are common. GISTs originating in the small intestine may contain eosinophilic, hyaline, PAS-positive diastase-resistant,

extracellular material known as skeinoid fibers. Prominent nuclear pleomorphism is unusual in GISTs, which suggests the pathology of leiomyosarcoma rather than GIST (Fletcher et al., 2002; De Silva and Reid, 2003). Although it is unusual for a tumor of the GI tract that reportedly arises from the ICC also to arise outside the gut, Reith and colleagues reported 48 extra-gastrointestinal stromal tumors and found that 39% of them were malignant. Histologically, more than half the tumors were epithelioid, resembling GISTs of the stomach. Most of these tumors were KIT positive (Reith et al., 2000). Miettinen and colleagues found that all the omental GISTs behaved benignly, whereas more than half the mesenteric tumors were malignant (Miettinen et al., 1999). KIT-positive cells were found in the omentum, just beneath the mesothelial lining (Sakurai et al., 2001). Interestingly, similar tumors were observed in the gallbladder (Ortiz-Hidalgo et al., 2000) and in the serosal aspect of the urinary bladder (Lasota et al., 2000).

A familial syndrome of dysphagia with multiple GISTs was recently reported. Family members with the germline *KIT* mutation reported dysphagia, but those without the mutation did not. Family members with the mutation had uncoordinated contractions of the esophagus with abnormal peristalsis. Both tumors and normal tissue contained a mutation at Asp-820 in the tyrosine kinase II domain of the KIT oncogene (Hirota et al., 2002). In a mother and son with multiple gastrointestinal stromal tumors and diffuse hyperplasia of the myenteric plexus layer, a single base mutation resulted in the substitution of Glu for Lys at codon 642 in the kinase I domain (Isozaki et al., 2000). Familial forms of GISTs with cutaneous manifestation (hyperpigmentation of skin and/or urticaria) have also been reported (Beghini et al., 2001; Maeyama et al., 2001). The germline mutation at codon 559 of exon 11 (Val → Ala) occurred in both reports.

Immunohistochemistry

Immunohistochemical evaluation of GISTs using antibodies against KIT has become more common since the linkage of KIT-mutation to the development of GISTs (Sarlomo-Rikala et al., 1998). Expression of KIT is seen in more than 90% of GISTs, regardless of the site of origin, histologic appearance, or biologic behavior, and is therefore regarded as a key marker in the diagnosis of this tumor (Fletcher et al., 2002). KIT is functionally important and is expressed in haematopoietic stem cells, mast cells, germ cells, melanocytic cells, and the ICCs (Fleischman, 1993; Huizinga et al., 1995; Kluppel et al., 1998). Hirota and colleagues showed that GISTs and ICCs stain with antibodies to both CD34 and KIT and postulated that GISTs originate from ICCs (Hirota et al., 1998). KIT expression is commonly manifested as strong, diffuse cytoplasmic and/or membranous staining but may also show perinuclear dot positivity (so called "Golgi pattern") within the cytoplasm with or without a diffuse cytoplasmic pattern (Fletcher et al., 2002). However, it is not known whether these patterns reflect different forms of the *KIT* gene mutation or an epiphenomenon. Most GISTs show KIT positivity in at least 90% of the tumor cells, but a small subset of this tumor type shows focal staining in as few as 5-20% of the tumor cells. This heterogeneity may account for the rare cases that are KIT negative in small biopsies but positive in subsequent excision biopsies. However, despite the use of anti-KIT antibodies as well as refined immunohistochemical analyses, including antigen retrieval to identify GISTs, a small proportion of GISTs (about 5%) shows either a faint expression of KIT or negative staining (Fletcher et al., 2002; De Silva and Reid, 2003). Pathologists sometimes designate these as "GIST-like tumors" or describe them as morphologically similar to GISTs. One author suggests that a diagnosis of GIST in such cases should be rendered only by a pathologist who is highly experienced about GIST pathology (Greenson, 2003). Mast cells and ICCs in a normal bowel wall are useful internal positive controls to ensure that the immunohistochemical stain is working properly. However, it is important to recognize that not all KIT-positive tumors are GISTs. KIT is also expressed by many other tumor types, such as synovial sarcoma, rhabdomyosarcoma, angiosarcoma, Ewing's sarcoma, anaplastic large-cell lymphoma, glioma, germinoma, melanoma, fibromatosis (depending on the antibody used), acute myeloid leukemia (including granulocytic sarcomas), and mastocytosis (Greenson, 2003; Miettinen et al., 2000). However, few of these tumors occur within the gastrointestinal tract, and the faint KIT positivity is more often due to a technical artifact (Hornick and Fletcher, 2002). It should be emphasized that a positive KIT stain must be interpreted in the light of a morphologic and clinical context.

In addition to consistent positivity for KIT, about 60-70% of GISTs show immunopositivity for CD34 (Sarlomo-Rikata et al., 1998; Miettinen et al., 2000). CD34 positivity is seen most consistently in colorectal and esophageal lesions, and its expression is lower in small-intestine GISTs. About 20-40% of GISTs show immunopositivity for smooth-muscle actin (SMA), fewer than 2% express desmin, and approximately 5% stain for the S100 protein. SMA positivity is most often seen in small-intestine tumors (Miettinen et al., 2000; Greenson, 2003).

Differential diagnosis

GISTs are the most common mesenchymal tumor along the GI tract except in the esophagus, where leiomyomas are more common (Miettinen et al., 2000; Fletcher et al., 2002; Greenson, 2003). In general, all gastrointestinal mesenchymal tumors and certain epithelial tumors are included in the differential diagnosis of GISTs. GISTs must be distinguished from smooth-muscle tumors, nerve sheath tumors, and

fibromatosis. Morphologically, smooth-muscle tumors appear less cellular, and their cells contain more eosinophilic cytoplasm. Immunohistochemically, they are consistently positive for desmin and smooth-muscle actin and negative for KIT. About 10-15% of smooth-muscle tumors are positive for CD34 (Miettinen et al., 2000; Fletcher et al., 2002; Greenson, 2003).

Schwannomas most often occur in the stomach but occasionally are found in the colon and esophagus (Greenson, 2003). Schwannomas of the GI tract are composed of spindle cells that may form nuclear palisading and may have a surrounding lymphoid cuff. Immunohistochemically, they are positive for S100 and negative for KIT, and some have focal CD34 positivity. It is very important to distinguish these lesions from GISTs, because unlike GISTs, schwannomas of the gut are generally benign.

Intra-abdominal fibromatosis typically originates in the mesentery or retroperitoneum and involves the bowel wall; it may express KIT, depending on the antibody used (Miettinen, 2001). The histologic appearances of the lesions, however, are distinctive, with parallel spindle cells in long sweeping fascicles and abundant keloid-like collagen. Also unlike GISTs, the spindle cells of fibromatosis do not stain with CD34 antibodies (Yantiss et al., 2000) but do express nuclear ß-catenin positivity (Montgomery et al., 2002).

Inflammatory fibroid polyps are typically submucosal and consist of a mixture of small granulation tissue-like vessels, spindle cells, and inflammatory cells, including eosinophils. They often express CD34 but not KIT (Greenson, 2003).

Inflammatory myofibroblastic tumors, which usually appear in childhood, may present as bowel wall involvement. Histologically, these lesions are composed of elongated spindle cells that can mimic GISTs. They can be confused histologically with GIST, but they are positive for desmin and actin and do not express CD34 or KIT (Greenson, 2003).

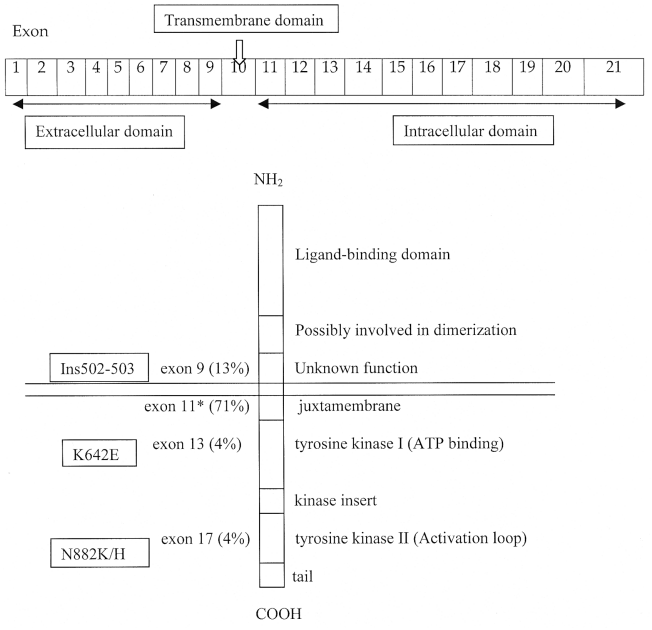
Solitary fibrous tumors occasionally occur in the peritoneal cavity and adhere to the bowel. These tumors can be extremely variegated and CD34 positive and hence may be confused with GISTs. However, they do not stain with KIT (Shidham et al., 2002). Epithelioid GISTs can resemble paragangliomas, but immunohistochemical staining for KIT and S100 readily distinguishes the two. Malignant melanomas are known to metastasize to the gastrointestinal tract and may arise primarily at the anorectum. They may express KIT, but expression of S100 and HMB-45 can distinguish them from GISTs.

Physiology and pathophysiology of KIT

KIT is a type III transmembrane receptor tyrosine kinase (RTK) in which the extracellular domain binds a ligand known as stem-cell factor (also known as Steel factor). An intracellular segment contains the kinase enzymatic domain. KIT is encoded by the KIT proto-oncogene located on chromosome 4q11-12. It is homologous with several other type III RTKs with oncogenic capabilities, including PDGFR-α and PDGFR-ß, CSF1R, and FLT3 (Majumder et al., 1988; Taylor and Metcalfe, 2000). KIT activation normally occurs when two adjacent receptors are brought together by a homodimer ligand (Blume-Jensen et al., 1991; Zhang et al., 2000). This process, known as homodimerization, leads to structural changes in the receptors. The KIT intracellular juxtamembrane region contains a putative alpha-helix, which exerts inhibitory control on the kinase activity of the ligand-unoccupied receptor. Loss of inhibition by ligand binding results in phosphorylation of the KIT kinase domain. The activated kinases then crossphosphorylate tyrosine residues in the opposed homodimer partner, leading to further activation of the receptor (Ma et al., 1999; Heinrich et al., 2002). The phosphotyrosines serve as binding sites for substrates that include various cell-signaling proteins and lead to phosphorylation of tyrosine residue. These steps activate cell-signaling cascades that are important in the regulation of proliferation, apoptosis, adhesion, and differentiation in several cell types, including ICCs. Stem cell factor-KIT interaction is essential for the proper development of melanocytes, hematopoietic progenitors, germ cells, mast cells, and ICCs (Fleischman, 1993; Huizinga et al., 1995). Disruption of KIT (e.g., in mouse models) results in the absence of a functional ICC compartment, manifested by aperistalsis of the gut (Isozaki et al., 1995; Kluppel et al., 1998), whereas mutations that constitutively activate KIT are associated with the pathogenesis of mastocytosis (Furitsu et al., 1993) and gastrointestinal stromal tumors.

The pathogenesis of GIST involves a gain-of-function mutation in the KIT proto-oncogene, leading to ligand-independent constitutive activation of the KIT receptor (Hirota et al., 1998; Nakahara et al., 1998). Somatic mutations that result in constitutive activation of KIT kinase have been reported in a number of studies of GISTs, although the frequencies reported have varied widely (30-92%), possibly because only one segment of exon 11 was evaluated and the study populations in each series were genetically heterogenous (Rubin et al., 2001; Heinrich et al., 2002). Systematic sequencing of the juxtamembrane coding region, coupled with evaluation of the entire KIT coding sequence in GISTs that lack juxtamembrane coding region mutations, reveals oncogenic *KIT* mutations in most GISTs (Heinrich et al., 2002). Mutations are most frequent in exon 11 and are rare in exons 9, 13, 14, and 17 (Moskaluk et al., 1999; Lasota et al., 2000; Hirota et al., 2001; Rubin et al., 2001; Andersson et al., 2002; Corless et al., 2002). Representative report evaluating entire coding region are illustrated in Fig. 1. Exon 11 encodes the juxtamembrane domain, which is the cytoplasmic portion of the receptor. The KIT juxtamembrane domain is pivotal in KIT signal transduction through interactions with various adapter proteins and phosphatases and modulation of KIT

catalytic activity (Kozlowski et al., 1998; Heinrich et al., 2002). Exon 9 encodes the extracellular domain, and exons 13 and 14 encode the kinase domains. The mutations vary from single base-pair substitutions to complex deletions and insertions. These activating mutations can transform cells in vitro and induce aggressive behavior of the cells in vivo (Nakahara et al., 1998). A recent evaluation has shown that activation (phosphorylation) of KIT is always demonstrated in GISTs that lack detectable KIT mutations (Rubin et al., 2001; Heinrich et al., 2002). Mechanisms that might account for KIT activation in mutation-negative GISTs are undetected mutations, inactivation of KIT-inhibitory phosphatases, up-regulation of the KIT ligand, and KIT heterodimerization with other activated receptor-tyrosine kinase proteins (Heinrich et al., 2002). KIT mutations in tumors that are small (10 mm or less), clinically incidental, or morphologically benign are similar to those identified in larger malignant lesions. In addition, the overall frequency of mutations (85%) in the incidental tumors is not significantly different from that seen in advanced and metastatic GISTs. These observations suggest that activating mutations in KIT occur early in the pathogenesis of GISTs (Corless et al., 2002). Recently, Heinrich and colleagues discovered a small subset of GISTs that are KIT wild type (WT) and have highly activated PDGFR detected by immunoprecipitation with polyclonal antisera (panRTK antisera) against peptides from regions of strong sequence conservation across the family of RTKs. These GISTs showed mutually exclusive phospho-KIT and phospho-PDGFR-α expression. Heinrich et al. also evaluated PDGFR-α genomic mutations in exons 10, 12, 14, and 18 that corresponded to the KIT exons containing oncogenic mutations in many GISTs and found PDGFR-α mutations in 11 of 37 (29.7%) KIT-WT GISTs but not in 36 KIT- mutant GISTs. In the above study, protein kinase Cq (antibody used in western blotting) was used for confirmation of GIST diagnoses in immunohistochemically KIT-negative, KIT-WT GISTs (Heinrich et al., 2003). On the basis of these results, GISTs can be schematically classified as KIT-high expression with KIT-mutation (about 80%), KIT-high expression without KIT-mutation (about 10%), KIT-low expression with KIT-mutation (rare), KIT-low or nonexpression with PDGFR-mutation (about 3%), and KIT-low or no expression and so far undetermined pathogenesis.



*Examples of exon 11 mutation.
c-KIT : 549 QKPMYEVQWKVVEEINGNNYVYIDPTQLPYDHKWEFPR 586
Point mutation: V559D/A, V560E/D/G, L576P, W557R/G
Deletion: 549-558, 550-558, 551-555, 551-556, 552-553, 553-556, 553-558, 555-558, 555-560, 555-571, 557-558, 557-561, 558-560, 558-564, 559-560, 560, 560-562, 562, 564-578, 568-574, 570, 570-577, 570-578, 579
Insertion/Duplication: 572-588, 574-586, 576-584, 576-585, 576-586
Complex: K550N + Del 552-556, Del579 + K581Q, M552T + Del 553-558, Y568C + Del 570-576, Q556P + Del 557 + K558Q, Del 557-558 + V559I, Del 557-558 + V559F, Del 558-565R, Del 567-576KV, Del 563-577 T, Del 557-559C, Del 551-554H, Del 552-555I, Del 552-558T, Del557-559F/I, Del557-558S, Del557-573S, Del 559-560 + Del 562-569D, K558N + Ins 559P
(References: Hirota et al., 1998, Lasota et al., 1999, Allander et al., 2001; Corless et al., 2002; Choi et al., 2003, Schneider-Stock et al., 2003).

Fig. 1. Schematic illustration of the KIT receptor and oncogenic mutations involving the extracellular, juxtamembrane, and kinase domains (Rubin et al., 2001). Almost all reported mutations of exon 9 are insertion/duplication 552-553 (AY), exon 13 are K642E, and exon 17 are N822K or H. Exampes of exon 11 mutation are listed separately.

GISTs occasionally develop in families, with germline mutations of KIT in exons 11 and 13. Family members develop diffuse hyperplasia of the ICC, which is considered a preneoplastic lesion (Nishida et al., 1998; Isozaki et al., 2000; Beghini et al., 2001; Maeyama et al., 2001).

KIT-activating mutations can be divided into two groups (Longley et al., 2001; Heinrich et al., 2002). The first group has mutations in regions forming the active kinase pocket and thus directly affect the enzymatic site structure. These types of mutations can be called enzymatic pocket or enzymatic site mutations. The second group of mutations involves regulatory portions of the KIT protein. These regulatory-type mutations differ from enzymatic site mutations in that they preserve the normal structure of the enzymatic site. The distinction between regulatory and enzymatic site mutation mechanisms is important clinically, because KIT inhibition by some small molecule compounds depends in part on these mutation types. Enzymatic site mutations of amino acids that participate directly in binding to therapeutic inhibitors often render the inhibitors totally ineffective (Ma et al., 2002). Regulatory-type KIT mutations are found in most GISTs. The best-characterized KIT regulatory region is the intracellular juxtamembrane region, encoded by exon 11. A broad range of mutational events affecting this region can result in constitutive KIT activation. An essential feature of these regulatory-type mutations is

that the KIT oncoproteins have the same active enzymatic site as that in normal KIT. Therefore, kinase inhibitors such as imatinib mesylate, which binds well to the enzymatic site of normal KIT, are also effective in this type of mutation. In contrast to regulatory-type mutations, enzymatic site-type mutations, which activate KIT by altering the structure of the enzymatic site, might not be inhibited by these kinase inhibitors.

The exact signaling pathways activated by a mutant KIT differ from those activated by normal KIT (Piao et al., 1996; Linnekin, 1999; Chian et al., 2001; De Miguel et al., 2002). For example, ligand-induced KIT activation generally leads to phosphorylation of the signal transducers and activates of transcription (STAT), protein kinaseB (AKT), and mitogen-activated protein kinase (MAPK) proteins, which have antiapoptotic and proliferation-inducing roles. In contrast, STAT, AKT, and MAPK phosphorylation are occasionally inconspicuous in GISTs expressing constitutively activated oncogenic KIT proteins. An oncogenic KIT mutation induces a degradation of SHP-1, a physiological attenuator of the KIT signaling pathway, in murine mastocytoma cell line P815, resulting in prolonged activation of KIT. Elucidation of the oncogenic KIT signaling pathways is clinically very important. Some GISTs may ultimately become resistant to KIT-inhibitor therapies, as has happened in chronic myelogenous leukemia (CML). Thus, essential downstream signaling proteins must be identified that can serve as alternative therapeutic targets to more effectively silence KIT signaling in GISTs (Heinrich et al., 2002).

Prognostic factors

It is often difficult to predict the behavior of GISTs using the features of the pathologic examination alone. Tumor stage, size, histologic type, degree of necrosis, cellularity, nuclear pleomorphism, and mitotic activity evaluated in the context of tumor location constitute the clinicopathological prognostic parameters (Fletcher et al., 2002; De Silva and Reid, 2003). Gastric GISTs are less aggressive than those originating in the esophagus, small intestine, or colon. The most consistent pathomorphologic features used to predict aggressiveness are tumor size and mitotic index (Fletcher et al., 2002; De Silva and Reid, 2003) .

It is now thought that categorizing GISTs into low, intermediate, and high-risk tumors on the basis of an estimation of their potential for recurrence and metastasis is more appropriate than categorizing as benign or malignant. Classification systems based on tumor size and mitotic rate are detailed elsewhere (Fletcher et al., 2002; De Silva and Reid, 2003).

Studies at M. D. Anderson Cancer center of mostly gastric and small intestine GISTs found that patients with tumors 5-10 cm and those with tumors >10 cm in size (19 and 17 months, respectively) had similarly poor survival rates. Conversely, patients with tumors <5 cm in size had a 36-month median disease free survival time (Ng et al., 1992). However, tumors <5 cm are not necessarily benign. In fact, some pathologists are reluctant to use the term "benign" because GISTs are so unpredictable (Fletcher et al., 2002). For example, at M. D. Anderson, we have occasionally seen very small (even <3 cm) lesions that metastasize to the peritoneum or liver.

A high Ki-67 index and high expression of Bcl-2, p53, and c-Myc proteins are frequently associated with poor prognosis (Panizo-Santos et al., 2000; Al-Bozom, 2001; Hasegawa et al., 2002). Vascular endothelial growth factor (VEGF) expression has been studied with respect to tumor size, liver metastasis, Ki- 67 labeling index, and microvascular density and is associated with poor prognosis (Takahashi et al., 2003). Alterations in $p16^{INK4}$, a cyclin-dependent kinase4 inhibitor gene, is associated with poor prognosis. Moreover, genetic alterations in $p16^{INK4}$ have been correlated with the immunohistochemical expression of $p16^{INK4}$, which is frequently not expressed in the case of promoter methylation, the presence of inactivating mutations, and the loss of heterozygosity at the *p16* locus (Schneider-Stock et al., 2003). The prognostic significance of *KIT* mutations is controversial. In a large series (124 patients) described by Taniguchi and colleagues, exon 11 mutations were identified in 57%of the GISTs and seemed to correlate with a poor prognosis (Taniguch et al., 1999). Several other studies have also shown a correlation between exon 11 KIT mutations and poor prognosis, and suggested that exon 11 mutations may be one of the strongest prognostic factors (Ernst et al., 1998; Lasota et al., 1999). Contrary to these reports, however, some authors have reported that KIT mutations are not restricted preferentially to higher-grade tumors and that in fact, they are frequently found in pathologically low-risk GISTs (Sakurai et al., 1999; Rubin et al., 2001; Corless et al., 2002). Recently, Choi and colleagues reported that high mobility group box 1 (HMGB1) was strongly expressed in GISTs having a KIT mutation, more than three times higher than the maximum observed in the GISTs that did not have KIT mutation. However, prognostic significance and correlation with outcome were not reported (Choi et al., 2003). The cytogenetic aberrations in GISTs appear to be a secondary event since they are commonly found in only a few neoplastic cells. Moreover, some GISTs have normal karyotypes but have KIT-activating mutations. Therefore, it is possible that KIT mutations involve the initiation of the neoplastic process in most GISTs, whereas cytogenetic aberrations may be involved in the progression of those tumors (Heinrich et al., 2002). The most common abnormalities reported are loss of chromosome 14 and/or 22. Loss of 9p and 1p, chromosome 15, 3p, and gain of chromosome 4 and 5 preferentially occur in high-risk tumors (Gunawan et al., 2002). Andersson and colleagues reported similar cytogenetic changes, but they did not report the prognostic significance (Andersson et al., 2002).

Comparative genomic hybridization-based studies have shown that low-risk GISTs contain significantly fewer DNA copy number changes (mean, 2.6 aberrations per tumor) than malignant primary GISTs (mean, 7.5) or metastatic GISTs (mean, 9). Gains and high-level amplifications at 5p and 20q and losses in 9p were seen only in malignant primary and metastatic GISTs (El-Rifai et al., 2000). Although these and other studies have shown certain patterns related to genetic mutations, the behavior of GISTs, in contrast to other sarcomas, cannot be clearly predicted.

Treatment

In general, GISTs have not been responsive to chemotherapy or radiotherapy. The impact of imatinib mesylate on long-term survival and the possibility that this drug is curative are subjects of ongoing studies. Today, however, the standard of care for treatment of GISTs is complete surgical resection with negative margins. Because GISTs rarely metastasize to lymph nodes, lymph node dissection and biopsy are not routinely employed. DeMatteo and colleagues reported that in a series of 200 GISTs, the median survival for patients who underwent complete resection was 66 months, compared with 22 months for those who underwent incomplete resection (Dematteo et al., 2000). However, with surgery alone, the overall prognosis is relatively poor.

Before imatinib mesylate, there were little or no benefits to drug therapy in locally recurrent or metastatic cases (Dematteo et al., 2002). Doxorubicin and ifosfamide, the two most active agents used for treating sarcoma and the centerpiece for most regimens used to treat soft-tissue sarcomas, have very limited activity in patients with GIST.

The recent introduction of imatinib mesylate has provided an effective treatment for recurrent or metastatic GISTs. Its use in the treatment of cancer represents a major paradigm shift in cancer therapy targeting specific molecules crucial to the etiology of a cancer. Imatinib mesylate selectively inhibits the ABL, BCR-ABL, ARG, KIT, and PDGFR tyrosine kinases, thereafter selectively inhibiting the growth of tumor cells that highly express these kinases (Buchdunger et al., 2000; Savage and Antman, 2002). Initially, imatinib was developed for CML, which has a BCR-ABL translocation and therefore highly expresses ABL kinase. Application of this agent to GISTs was logical since KIT is an RTK, much like BCR-ABL is in CML. Tuveson and colleagues demonstrated that the inhibition of mutant KIT in GIST by imatinib mesylate leads to growth arrest and eventual apoptosis in cultured human GIST cell lines (Tuveson et al., 2001). The first patient with a GIST treated with imatinib mesylate was in Finland (Joensuu et al., 2001). The patient, whose tumor expressed KIT and contained an exon 11 mutation in the KIT gene, had progressive, widely metastatic disease after failing to improve with extensive previous therapy. Within a few weeks of starting daily oral administration of imatinib mesylate, the patient showed a major objective clinical response that has been maintained for more than 18 months at the time of publication. The response was confirmed by the absence of tumor metabolic activity as shown by 18FDG-positron emission tomography scanning. Subsequent biopsies revealed that the tumor had been largely replaced by myxoid degeneration and fibrosis.

The preliminary trials of imatinib for treating GIST were so successful that sarcoma investigators met at the National Cancer Institute in November 2000 to discuss the results and design a study to expand access to this agent for other GIST patients who might benefit from it. With more than 600 patients enrolled, this current study is designed to test whether imatinib 800 mg/day is more effective than imatinib 400 mg/day. A European study conducted by the Soft Tissue and Bone Sarcoma Group of the European Organization for Research and Treatment of Cancer (EORTC) confirmed the preliminary results found in the United States (Van Oosterom et al., 2001). Of 36 patients treated with imatinib, the rate of disease progression was only 11%, with 69% of the patients having a major or minor response. An additional 19% had stable disease.

Moreover, in recent clinical trials, the majority of patients with malignant GISTs have shown a benefit to treatment with imatinib (Demetri et al., 2002). A total of 147 patients received 400 mg or 600 mg of imatinib daily. Overall, 54% had a partial response, 28% had a stable disease, and no patients had complete response.

Imatinib mesylate is a great development for patients who suffer from metastatic or recurrent GISTs. Before its use, there was no known effective therapy. In February 2002, the United States Food and Drug Administration approved imatinib mesylate for use in KIT-positive, unresectable and/or metastatic GISTs.

Recently, Bauer and colleagues reported a patient whose metastatic GIST responded well to imatinib mesylate treatment despite the tumor's near absence of KIT expression (Bauer et al., 2003). The tumor was morphologically typical of GIST, stained positive for CD34, and had an in-frame deletion mutation in *KIT* exon 11. These findings suggest that even GISTs with very low levels of *KIT* expression may respond to imatinib mesylate.

In CML, resistance to imatinib in patients with advanced blast crisis has been associated with BCR-ABL gene amplification and/or the development of new mutations in the kinase domain (Gorre et al., 2001). These events presumably diminish the binding ability of imatinib to the KIT enzymatic site and results in treatment failure.

It will be critical to design new drugs for the GIST patients whose tumors are resistant to imatinib either initially or after drug administration. Knowledge of the mechanisms of imatinib resistance will facilitate the development of new drugs that are not resistant (Dematteo et al., 2002).

It will also be important not to overly generalize the successes of imatinib therapy in treating GIST since GISTs appear to have relatively homogeneous pathogenetic mechanisms and a remarkably distinct and uniform expression profile on the basis of cDNA microarrays (Allandar et al., 2001). Most other common human malignancies are the end result of complex multistep carcinogenesis; therefore, targeting specific molecules for their treatment will require more complex treatment schemas than required for GISTs.

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References

Al-Bozom I.A. (2001). p53 expression in gastrointestinal stromal tumors. Pathol. Int. 51, 519-523.

Allander S.V., Nupponen N.N., Ringner M., Hostetter G., Maher G.W., Goldberger N., Chen Y., Carpten J., Elkahloun A.G. and Meltzer P.S. (2001). Gastrointestinal stromal tumors with KIT mutations exhibit a remarkably homogeneous gene expression profile. Cancer Res. 61, 8624-8268.

Andersson J., Sjogren H., Meis-Kindblom J.M., Stenman G., Aman P. and Kindblom L.G. (2002). The complexity of KIT gene mutations and chromosome rearrangements and their clinical correlation in gastrointestinal stromal (pacemaker cell) tumors. Am. J. Pathol. 160, 15-22.

Bauer S., Corless C.L., Heinrich M.C., Dirsch O., Antoch G., Kanja J., Seeber S. and Schutte J. (2003). Response to imatinib mesylate of a gastrointestinal stromal tumor with very low expression of KIT. Cancer Chemother. Pharmacol. 51, 261-265.

Beghini A., Tibiletti M.G., Roversi G., Chiaravalli A.M., Serio G., Capella C. and Larizza L. (2001). Germline mutation in the juxtamembrane domain of the kit gene in a family with gastrointestinal stromal tumors and urticaria pigmentosa. Cancer 92, 657-662.

Blume-Jensen P., Claesson-Welsh L., Siegbahn A., Zsebo K.M., Westermark B. and Heldin C.H. (1991). Activation of the human c-kit product by ligand-induced dimerization mediates circular actin reorganization and chemotaxis. EMBO J. 10, 4121-4128.

Buchdunger E., Cioffi C.L., Law N., Stover D., Ohno-Jones S., Druker B.J. and Lydon N.B. (2000). Abl protein-tyrosine kinase inhibitor STI571 inhibits in vitro signal transduction mediated by c-kit and platelet-derived growth factor receptors. J. Pharmacol. Exp. Ther. 295, 139-145.

Chian R., Young S., Danilkovitch-Miagkova A., Ronnstrand L., Leonard E., Ferrao P., Ashman L. and Linnekin D. (2001). Phosphatidylinositol 3 kinase contributes to the transformation of hematopoietic cells by the D816V c-Kit mutant. Blood 98, 1365-1373.

Choi Y.R., Kim H.K., Kang H.J., Kim N.G., Kim J.J., Park K.S., Paik Y.K., Kim H.O. and Kim H. (2003). Overexpression of high mobility group box 1 in gastrointestinal stromal tumors with KIT mutation. Cancer Res. 63, 2188-2193.

Corless C.L., McGreevey L., Haley A., Town A. and Heinrich M.C. (2002). KIT mutations are common in incidental gastrointestinal stromal tumors one centimeter or less in size. Am. J. Pathol. 160, 1567-1572.

Demetri G.D., von Mehren M., Blanke C.D., Van den Abbeele A.D., Eisenberg B., Roberts P.J., Heinrich M.C., Tuveson D.A., Singer S., Janicek M., Fletcher J.A., Silverman S.G., Silberman S.L., Capdeville R., Kiese B., Peng B., Dimitrijevic S., Druker B..J., Corless C., Fletcher C.D. and Joensuu H. (2002). Efficacy and safety of imatinib mesylate in advanced gastrointestinal stromal tumors. N. Engl. J. Med. 347, 472-480.

De Miguel M.P., Cheng L., Holland E.C., Federspiel M.J., and Donovan P.J. (2002). Dissection of the c-Kit signaling pathway in mouse primordial germ cells by retroviral-mediated gene transfer. Proc. Natl. Acad. Sci. USA 99, 10458-10463.

De Silva C.M. and Reid R. (2003). Gasrointestinal stromal tumors (GIST): C-kit mutations, CD117 expression, differential diagnosis and targeted cancer therapy with imatinib. Pathol. Oncol. Res. 9, 13-19.

DeMatteo R.P., Lewis J.J., Leung D., Mudan S.S., Woodruff J.M. and Brennan M.F. (2000). Two hundred gastrointestinal stromal tumors: Recurrence patterns and prognostic factors for survival. Ann. Surg. 231, 51-58.

Dematteo R.P., Heinrich M.C., El-Rifai W.M. and Demetri G. (2002). Clinical management of gastrointestinal stromal tumors: Before and after STI-571. Hum. Pathol. 33, 466-477.

El-Rifai W., Sarlomo-Rikala M., Andersson L.C., Knuutila S. and Miettinen M. (2000). DNA sequence copy number changes in gastrointestinal stromal tumors: Tumor progression and prognostic significance. Cancer Res. 60, 3899-3903.

Ernst S.I., Hubbs A.E., Przygodzki R.M., Emory T.S., Sobin L.H. and O'Leary T.J. (1998). KIT mutation portends poor prognosis in gastrointestinal stromal/smooth muscle tumors. Lab. Invest. 78, 1633-1636.

Fleischman R.A. (1993). From white spots to stem cells: The role of the KIT receptor in mammalian development. Trends Genet. 9, 285-290.

Fletcher C.D., Berman J.J., Corless C., Gorstein F., Lasota J., Longley B.J., Miettinen M., O'Leary T.J., Remotti H., Rubin B.P., Shmookler B., Sobin L.H. and Weiss S.W. (2002). Diagnosis of gastrointestinal stromal tumors: A consensus approach. Hum. Pathol. 33, 459-465.

Furitsu T., Tsujimura T., Tono T., Ikeda H., Kitayama H., Koshimizu U., Sugahara H., Butterfield J.H., Ashman L.K. and Kanayama Y. (1993). Identification of mutations in the coding sequence of the proto-oncogene c-kit in a human mast cell leukemia cell line causing ligand-independent activation of c-kit product. J. Clin. Invest. 92, 1736-1744.

Greenson J.K. (2003). Gasrointestinal stromal tumors and other mesenchymal lesions of the gut. Mod. Pathol. 16, 366-375.

Gorre M.E., Mohammed M., Ellwood K., Hsu N., Paquette R., Rao P.N. and Sawyers C.L. (2001). Clinical resistance to STI-571 cancer therapy caused by BCR-ABL gene mutation or amplification. Science 293, 876-880.

Gottlieb J.A., Benjamin R.S., Baker L.H., O'Bryan R.M., Sinkovics J.G., Hoogstraten B., Quagliana J.M., Rivkin S.E., Bodey G.P. Sr, Rodriguez V., Blumenschein G.R., Saiki J.H., Coltman C. Jr, Burgess M.A., Sullivan P., Thigpen T., Bottomley R., Balcerzak S. and Moon T.E. (1976) Role of DTIC (NSC-45388) in the chemotherapy of sarcomas. Cancer Treat Rep. 60, 199-203.

Gunawan B., Bergmann F., Hoer J., Langer C., Schumpelick V., Becker H. and Fuzesi L. (2002). Biological and clinical significance of cytogenetic abnormalities in low-risk and high-risk gastrointestinal

stromal tumors. Hum. Pathol. 33, 316-321.

Hasegawa T., Matsuno Y., Shimoda T. and Hirohashi S. (2002). Gastrointestinal stromal tumor: Consistent CD117 immunostaining for diagnosis and prognostic classification based on tumor size and MIB-1 grade. Hum. Pathol. 33, 669-676.

Heinrich M.C., Rubin B.P., Longley B.J. and Fletcher J.A. (2002). Biology and genetic aspects of gastrointestinal stromal tumors: KIT activation and cytogenetic alterations. Hum. Pathol. 33, 484-495.

Heinrich M.C., Corless C.L., Duensing A., McGreevey L., Chen C.J., Joseph N., Singer S., Griffith D.J., Haley A., Town A., Demetri G.D., Fletcher C.D., Fletcher J.A. (2003). DGFRA activating mutations in gastrointestinal stromal tumors. Science 299, 708-710.

Hirota S., Isozaki K., Moriyama Y., Hashimoto K., Nishida T., Ishiguro S., Kawano K., Hanada M., Kurata A., Takeda M., Muhammad Tunio G., Matsuzawa Y., Kanakura Y., Shinomura Y. and Kitamura Y. (1998). Gain-of-function mutations of c-kit in human gastrointestinal stromal tumors. Science 279, 577-580.

Hirota S., Nishida T., Isozaki K., Taniguchi M., Nakamura J., Okazaki T. and Kitamura Y. (2001). Gain-of-function mutation at the extracellular domain of KIT in gastrointestinal stromal tumours. J. Pathol. 193, 505-510.

Hirota S., Nishida T., Isozaki K., Taniguchi M., Nishikawa K., Ohashi A., Takabayashi A., Obayashi T., Okuno T., Kinoshita K., Chen H., Shinomura Y. and Kitamura Y. (2002). Familial gastrointestinal stromal tumors associated with dysphagia and novel type germline mutation of KIT gene. Gastroenterology 122, 1493-1499.

Hornick J.L. and Fletcher C.D. (2002). Immunohistochemical staining for KIT (CD117) in soft tissue sarcomas is very limited in distribution. Am. J. Clin. Pathol. 117, 188-193.

Huizinga J.D., Thuneberg L., Kluppel M., Malysz J., Mikkelsen H.B. and Bernstein A. (1995). W/kit gene required for interstitial cells of Cajal and for intestinal pacemaker activity. Nature 373, 347-349.

Isozaki K., Hirota S., Nakama A., Miyagawa J., Shinomura Y., Xu Z., Nomura S. and Kitamura Y. (1995). Disturbed intestinal movement, bile reflux to the stomach, and deficiency of c-kit-expressing cells in Ws/Ws mutant rats. Gastroenterology 109, 456-464.

Isozaki K., Terris B., Belghiti J., Schiffmann S., Hirota S. and Vanderwinden J.M. (2000). Germline-activating mutation in the kinase domain of KIT gene in familial gastrointestinal stromal tumors. Am. J. Pathol. 157, 1581-1585.

Joensuu H., Roberts P.J., Sarlomo-Rikala M., Andersson L.C., Tervahartiala P., Tuveson D., Silberman S., Capdeville R., Dimitrijevic S., Druker B. and Demetri G.D. (2001). Effect of the tyrosine kinase inhibitor STI571 in a patient with a metastatic gastrointestinal stromal tumor. N. Engl. J. Med. 344, 1052-1056.

Kindblom L.G., Remotti H.E., Aldenborg F. and Meis-Kindblom J.M. (1998). Gastrointestinal pacemaker cell tumor (GIPACT): Gastrointestinal stromal tumors show phenotypic characteristics of the interstitial cells of Cajal. Am. J. Pathol. 152, 1259-1269.

Kluppel M., Huizinga J.D., Malysz J. and Bernstein A. (1998). Developmental origin and KIT-dependent development of the interstitial cells of Cajal in the mammalian small intestine. Dev. Dyn. 211, 60-71.

Kozlowski M., Larose L., Lee F., Le D.M., Rottapel R. and Siminovitch K.A. (1998). SHP-1 binds and negatively modulates the c-Kit receptor by interaction with tyrosine 569 in the c-Kit juxtamembrane domain. Mol. Cell. Biol. 18, 2089-2099.

Lasota J., Carlson J.A. and Miettinen M. (2000). Spindle cell tumor of urinary bladder serosa with phenotypic and genotypic features of gastrointestinal stromal tumor. Arch. Pathol. Lab. Med. 124, 894-897.

Lasota J., Jasinski M., Sarlomo-Rikala M. and Miettinen M. (1999). Mutations in exon 11 of c-Kit occur preferentially in malignant versus benign gastrointestinal stromal tumors and do not occur in leiomyomas or leiomyosarcomas. Am. J. Pathol. 154, 53-60.

Lee J.R., Joshi V., Griffin J.W. Jr., Lasota J. and Miettinen M. (2001). Gastrointestinal autonomic nerve tumor: Immunohistochemical and molecular identity with gastrointestinal stromal tumor. Am. J. Surg. Pathol. 25, 979-987.

Linnekin D. (1999). Early signaling pathways activated by c-Kit in hematopoietic cells. Int. J. Biochem. Cell. Biol. 31, 1053-1074.

Longley B.J., Reguera M.J. and Ma Y. (2001). Classes of c-KIT activating mutations: proposed mechanisms of action and implications for disease classification and therapy. Leuk. Res. 25, 571-576.

Ma Y., Cunningham M.E., Wang X., Ghosh I., Regan L. and Longley B.J. (1999). Inhibition of spontaneous receptor phosphorylation by residues in a putative alphahelix in the KIT intracellular juxtamembrane region. J. Biol. Chem. 274, 13399-13402.

Ma Y., Zeng S., Metcalfe D.D., Akin C., Dimitrijevic S., Butterfield J.H., McMahon G. and Longley B.J. (2002). The c-kit mutation causing human mastocytosis is resistant to STI-571 and other KIT kinase inhibitors: Kinases with enzymatic site mutations show different inhibitor sensitivity profiles than wild-type kinases and those with regulatory-type mutations. Blood 99, 1741-1744.

Maeyama H., Hidaka E., Ota H., Minami S., Kajiyama M., Kuraishi A., Mori H., Matsuda Y., Wada S., Sodeyama H., Nakata S., Kawamura N., Hata S., Watanabe M., Iijima Y. and Katsuyama T. (2001). Familial gastrointestinal stromal tumor with hyperpigmentation: Association with a germline mutation of the c-kit gene. Gastroenterology 120, 210-215.

Majumder S., Brown K., Qiu F.H. and Besmer P. (1998). c-kit protein, a transmembrane kinase: Identification in tissues and characterization. Mol. Cell. Biol. 8, 4896-4903.

Mazur M.T. and Clark H.B. (1983). Gastric stromal tumors: Reappraisal of histogenesis. Am. J. Surg. Pathol. 7, 507-519.

Miettinen M. (2001). Are desmoid tumors KIT positive? Am. J. Surg. Pathol. 25, 549-550.

Miettinen M., Monihan J.M., Sarlomo-Rikala M., Kovatich A.J., Carr N.J., Emory T.S. and Sobin L.H. (1999). Gastrointestinal stromal tumors/smooth muscle tumors (GISTs) primary in the omentum and mesentery: Clinicopathologic and immunohistochemical study of 26 cases. Am. J. Surg. Pathol. 23, 1109-1118.

Miettinen M., Sobin L.H. and Sarlomo-Rikala M. (2000). Immunohistochemical spectrum of GISTs at different sites and their differential diagnosis with a reference to CD117 (KIT). Mod. Pathol. 13, 1134-1142.

Montgomery E., Torbenson M.S., Kaushal M., Fisher C. and Abraham S.C. (2002). b-catenin immunohistochemistry separates mesenteric fibromatosis from gastrointestinal stromal tumor and sclerosing mesenteritis. Am. J. Surg. Pathol. 26, 1296-1301.

Moskaluk C.A., Tian Q., Marshall C.R., Rumpel C.A., Franquemont D.W. and Frierson H.F. Jr. (1999). Mutations of c-kit JM domain are found in a minority of human gastrointestinal stromal tumors. Oncogene 18, 1897-1902.

Nakahara M., Isozaki K., Hirota S., Miyagawa J., Hase-Sawada N., Taniguchi M., Nishida T., Kanayama S., Kitamura Y., Shinomura Y. and Matsuzawa Y. (1998). A novel gain-of function mutation of c-kit

gene in gastrointestinal stromal tumors. Gastroenterology 115, 1090-1095.

Ng E.H., Pollock R.E., Munsell M.F., Atkinson E.N. and Romsdahl M.M. (1992). Prognostic factors influencing survival in gastrointestinal leiomyosarcomas. Implications for surgical management and staging. Ann. Surg. 215, 68-77.

Nishida T., Hirota S., Taniguchi M., Hashimoto K., Isozaki K., Nakamura H., Kanakura Y., Tanaka T., Takabayashi A., Matsuda H. and Kitamura Y. (1998). Familial gastrointestinal stromal tumours with germline mutation of the KIT gene. Nat. Genet. 19, 323-324.

Ortiz-Hidalgo C., de Leon Bojorge B. and Albores-Saavedra J. (2000). Stromal tumor of the gallbladder with phenotype of interstitial cells of Cajal. A previously unrecognized neoplasm. Am. J. Surg. Pathol. 24, 1420-1423.

Panizo-Santos A., Sola I., Vega F., de Alava E., Lozano M.D., Idoate M.A. and Pardo-Mindan J. (2000). Predicting metastatic risk of gastrointestinal stromal tumors: Role of cell proliferation and cell cycle regulatory proteins. Int. J. Surg. Pathol. 8, 133-144.

Piao X., Paulson R., van der Geer P., Pawson T. and Bernstein A. (1996). Oncogenic mutation in the Kit receptor tyrosine kinase alters substrate specificity and induces degradation of the protein tyrosine phosphatase SHP-1. Proc. Natl. Acad. Sci. USA 93, 14665-14669.

Reith J.D., Goldblum J.R., Lyles R.H. and Weiss S.W. (2000). Extragastrointestinal (soft tissue) stromal tumors: An analysis of 48 cases with emphasis on histological predictors of outcome. Mod. Pathol. 13, 577-585.

Rubin B.P., Singer S., Tsao C., Duensing A., Lux M.L., Ruiz R., Hibbard M.K., Chen C.J., Xiao S., Tuveson D.A., Demetri G.D., Fletcher C.D. and Fletcher J.A. (2001). KIT activation is a ubiquitous feature of gastrointestinal stromal tumors. Cancer Res. 81, 8118-8121.

Sakurai S., Fukasawa T., Chong J.M., Tanaka A. and Fukayama M. (1999). C-kit gene abnormalities in gastrointestinal stromal tumors (tumors of interstitial cells of Cajal). Jpn. J. Cancer Res. 90, 1321-1328.

Sakurai S., Oguni S., Hironaka M., Fukayama M., Morinaga S. and Saito K. (2001). Mutations in c-kit gene exons 9 and 13 in gastrointestinal stromal tumors among Japanese. Jpn. J. Cancer Res. 92, 494-498.

Sarlomo-Rikala M., Kovatich A.J., Barusevicius A. and Miettinen M. (1998). CD117: A sensitive marker for gastrointestinal stromal tumors that is more specific than CD34. Mod. Pathol. 11, 728-734.

Savage D.G. and Antman K.H. (2002). Imatinib mesylate - a new oral targeted therapy. N. Engl. J. Med. 346, 683-693.

Schneider-Stock R., Boltze C., Lasota J., Miettinen M., Peters B., Pross M., Roessner A. and Gunther T. (2003). High prognostic value of p16INK4 alterations in gastrointestinal stromal tumors. J. Clin. Oncol. 21, 1688-1697.

Shidham V.B., Chivukula M., Gupta D., Rao R.N. and Komorowski R. (2002). Immunohistochemical comparison of gastrointestinal stromal tumor and solitary fibrous tumor. Arch. Pathol. Lab. Med. 126, 1189-1192.

Sircar K., Hewlett B.R., Huizinga J.D., Chorneyko K., Berezin I. and Riddell R.H. (1999). Interstitial cells of Cajal as precursors of gastrointestinal tumors. Am. J. Surg. Pathol. 23, 377-389.

Takahashi R., Tanaka S., Kitadai Y., Sumii M., Yoshihara M., Haruma K. and Chayama K. (2003). Expression of vascular endothelial growth factor and angiogenesis in gastrointestinal stromal tumor of the stomach. Oncology 64, 266–274.

Taniguchi M., Nishida T., Hirota S., Taniguchi M., Nishida T., Hirota S., Isozaki K., Ito T., Nomura T., Matsuda H. and Kitamura Y. (1999). Effect of c-kit mutation on prognosis of gastrointestinal stromal tumors. Cancer Res. 59, 4297-4300.

Taylor M.L. and Metcalfe D.D. (2000). Kit signal transduction. Hematol Oncol Clin. North Am. 14, 517-535.

Tuveson D.A., Willis N.A., Jacks T., Griffin J.D., Singer S., Fletcher C.D., Fletcher J.A. and Demetri G.D. (2001). STI571 inactivation of the gastrointestinal stromal tumor c-KIT oncoprotein: Biological and clinical implications. Oncogene 20, 5054-5058.

Van de Rijn M., Hendrickson M.R. and Rouse R.V. (1994). The CD34 expression by gastrointestinal stromal tymors. Hum. Pathol. 25, 766-771.

Van Oosterom A.T., Judson I., Verweij J., Stroobants S., Donato di Paola E., Dimitrijevic S., Martens M., Webb A., Sciot R., Van Glabbeke M., Silberman S. and Nielsen O.S. European Organisation for Research and Treatment of Cancer Soft Tissue and Bone Sarcoma Group. (2001). Safety and efficacy of imatinib (STI571) in metastatic gastrointestinal stromal tumors: A phase I study. Lancet 358, 1421-1423.

Walker P. and Dvorak A.M. (1986). Gastrointestinal autonomic nerve (GAN) tumor: Ultrastructural evidence for a newly recognized entity. Arch. Pathol. Lab. Med. 110, 309-316.

Yantiss R.K., Spiro I.J., Compton C.C. and Rosenberg A.E. (2000). Gastrointestinal stromal tumor versus intra-abdominal fibromatosis of the bowel wall. Am. J. Surg. Pathol. 24, 947-957.

Zhang Z., Zhang R., Joachimiak A., Schlessinger J. and Kong X.P. (2000). Crystal structure of human stem cell factor: Implication for stem cell factor receptor dimerization and activation. Proc. Natl. Acad. Sci. USA 97, 7732-7737.

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