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Gastrointestinal stromal tumors: what do we know now?

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Gastrointestinal stromal tumors (GISTs) are the most common mesenchymal tumors of the GI tract, arising from the interstitial cells of Cajal, primarily in the stomach and small intestine. They manifest a wide range of morphologies, from spindle cell to epithelioid, but are immunopositive for KIT (CD117) and/or DOG1 in essentially all cases. Although most tumors are localized at presentation, up to half will recur in the abdomen or spread to the liver. The growth of most GISTs is driven by oncogenic mutations in either of two receptor tyrosine kinases: KIT (75% of cases) or PDGFRA (10%). Treatment with tyrosine kinase inhibitors (TKIs) such as imatinib, sunitinib, and regorafenib is effective in controlling unresectable disease; however, drug resistance caused by secondary *KIT* or *PDGFRA* mutations eventually develops in 90% of cases. Adjuvant therapy with imatinib is commonly used to reduce the likelihood of disease recurrence after primary surgery, and for this reason assessing the prognosis of newly resected tumors is one of the most important roles for pathologists. Approximately 15% of GISTs are negative for mutations in KIT and PDGFRA. Recent studies of these so-called wild-type GISTs have uncovered a number of other oncogenic drivers, including mutations in neurofibromatosis type I, RAS genes, BRAF, and subunits of the succinate dehydrogenase complex. Routine genotyping is strongly recommended for optimal management of GISTs, as the type and dose of TKI used for treatment is dependent on the mutation identified.
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Gastrointestinal stromal tumors (GISTs) occur primarily in older patients of either sex, with annual incidences between 11 and 19.6 per million population worldwide.[1–4] This corresponds to between 3300 and 6000 new cases per year in the United States. Following surgical resection, GISTs often recur locally, spread diffusely throughout the serosal surfaces of the abdomen, and/or metastasize to the liver. Advanced disease is associated with metastases to distant sites, including the lung and bone. Before the advent of targeted therapies, the prognosis for advanced GISTs was poor owing to their inherent resistance to both chemotherapy and radiation therapy.

During the past decade, GISTs have served as an important model in the emerging field of molecularly targeted therapies for solid tumors. The nearly simultaneous discovery of oncogenic kinase mutations in GISTs and the introduction of kinase inhibitors have led to a rapid evolution in our understanding of these tumors and the biology that defines them.

Clinical and pathological features

GISTs are most commonly present in the stomach (60%) and small intestine (25%), but they also arise in the colon, rectum, esophagus, mesentery, and omentum (15% together).[5,6] Clinical symptoms associated with GISTs include fatigue, abdominal pain, dysphagia, satiety, and obstruction. The workup often reveals anemia related to mucosal bleeding or intratumoral hemorrhage.

The tumors are generally well circumscribed, have a fleshy pink or tan cut surface, and may show areas of hemorrhagic necrosis and cystic degeneration. They range from 1 cm to more than 40 cm, with an average of ~5 cm. Morphologically, GISTs show a wide spectrum of morphologies, from bland spindle cell proliferations to highly cellular epithelioid tumors with significant nuclear pleomorphism (Figure 1). For these reasons, the morphologic

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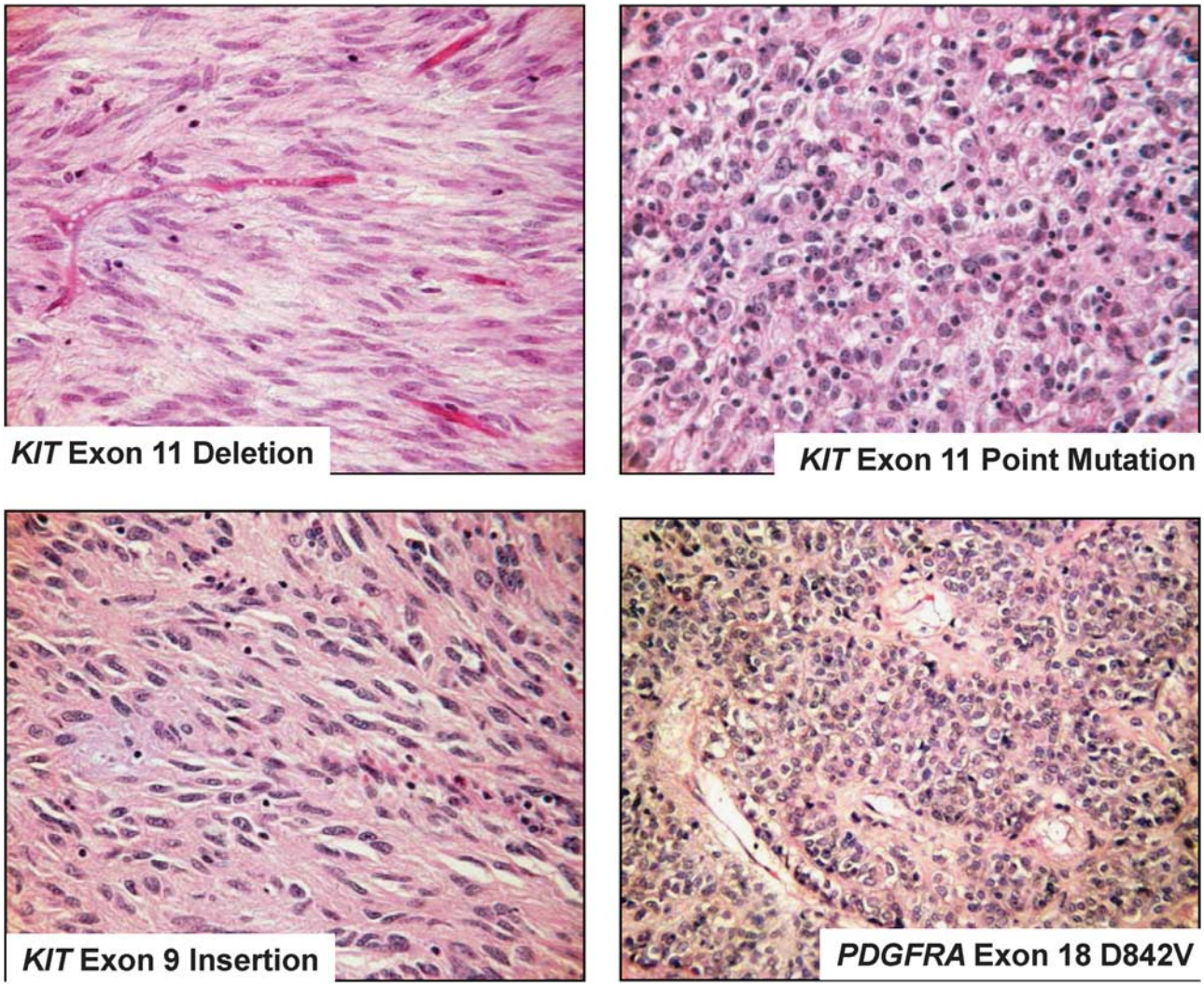

Figure 1 Examples of gastrointestinal stromal tumor (GIST) morphology. Spindle cell GISTs (left panels) often harbor a mutation in *KIT* exon 9 or 11. Epithelioid GISTs (right panels) vary in their genotype, having either (or neither) a *KIT* or *PDGFRA* mutation.

differential diagnosis is necessarily broad (Table 1). Skeinoid fibers are associated with lower-grade lesions as are foci of calcification.

CD34 is expressed in 70% of GISTs and was the first immunohistochemical marker that helped to distinguish these tumors from leiomyomas and leiomyosarcomas of the GI tract. In 1998, reports by two groups that GISTs commonly express KIT (CD117) led to more reliable diagnosis.7,8 The staining may be membranous, diffusely cytoplasmic, or concentrated in a dot-like perinuclear pattern. The addition of DOG1 (ANO1) as another GIST marker has made the diagnosis quite routine,[9] as DOG1 and CD117 each stain >95% of GISTs and, between them, serve to mark essentially all cases. These antigens are only rarely expressed in other mesenchymal tumors. Smooth muscle actin and muscle-specific actin are variably expressed in GISTs, whereas desmin is usually absent. Immunostaining for SDHA and SDHB has recently been shown to be effective in identifying tumors deficient in succinate dehydrogenase activity,[10] the significance of which is discussed below.

Oncogenic mutations in GISTs

KIT

In 1998, Hirota *et al*[7,8] published their breakthrough discovery of KIT mutations in GISTs. Approximately 75% of GISTs harbor a *KIT* gene mutation, and these mutations lead to constitutive activation of the kinase. KIT is a member of the type III receptor tyrosine kinase family that includes platelet-derived growth factor receptors-α and -β (PDGFRA and PDGFRB), as well as the macrophage colony-stimulating-factor receptor (CSF1R) and the Fl cytokine receptor (FLT3).[11] Binding of the KIT ligand (stem cell factor, SCF) to KIT results in receptor homodimerization and kinase activation.

Table 1. Differential diagnosis of GIST

Spindle cell tumors	*Epithelioid tumors*
Fibromatosis (can be weakly CD117 positive)	Epithelioid leiomyoma
Leiomyoma (beware of intermixed CD117-positive ICC cells)	Neuroendocrine tumors
Leiomyosarcoma	Malignant mesothelioma
Schwannoma	Metastatic melanoma (can be CD117 positive)
Malignant peripheral nerve sheath tumor	Angiosarcoma (can be CD117 positive)
Inflammatory myofibroblastic tumor	
Inflammatory fibroid polyp (may have *PDGFRA* mutations)	
Solitary fibrous tumor	
Synovial sarcoma	
Dedifferentiated liposarcoma	
Endometrial stromal sarcoma	
Sarcomatoid carcinoma	

Abbreviations: GIST, gastrointestinal stromal tumor; ICC, interstitial cells of Cajal; PDGFRA, platelet-derived growth factor receptors-α.

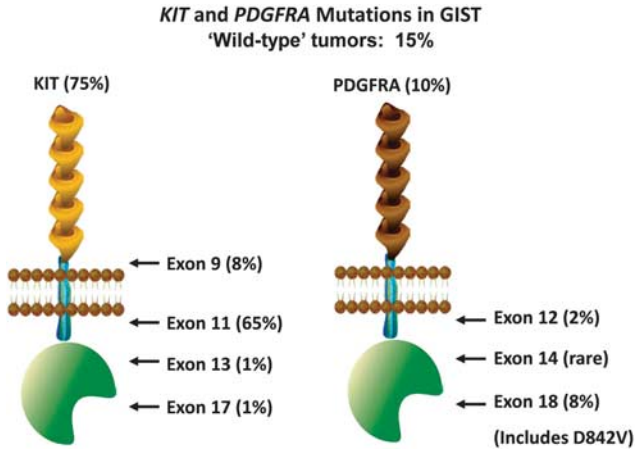

Figure 2 *KIT* and *PDGFRA* mutations in gastrointestinal stromal tumor (GIST).

Oncogenic *KIT* mutations cause ligand-independent kinase activation. The most common mutations in KIT affect the juxtamembrane domain encoded by exon 11 (Figure 2). Two-thirds of GISTs harbor mutations in exon 11 that disrupt the normal juxtamembrane secondary structure that keeps the kinase activation loop from swinging into an active conformation. These mutations include in-frame deletions, insertions, substitutions, or combinations thereof. The deletions are associated with a shorter progression-free and overall survival in comparison with the other exon 11 mutations.[12–18] In particular, deletions involving codons 557 and/or 558 are associated with malignant behavior.[19–21]

Aside from exon 11 mutations, between 7 and 10% of GISTs have a mutation in an extracellular domain encoded by exon 9.[22] These mutations are thought to mimic the conformational change that the extracellular KIT receptor undergoes when ligand is bound. Importantly, the kinase domain in exon 9-mutant KIT is essentially the same as in wild-type KIT, and this influences inhibitor sensitivity. Interestingly, these mutations occur in tumors arising in the small and large intestine, but are very rarely seen in gastric GISTs.

Mutations in the activation loop (encoded by exon 17) of the kinase are uncommon. They stabilize the active conformation.[23] Primary mutations, such as K642E in the ATP-binding region (encoded by exon 13), are also uncommon.[23] The biological basis of kinase activation by this mutation is unknown, but it is speculated that it interferes with normal auto-inhibitory function of the juxtamembrane domain.

The functional importance of *KIT* mutations in GIST development is supported by several lines of evidence. First, phosphorylated (activated) KIT is detectable in GIST tumor extracts. Second, mutant KIT is oncogenic, supporting the growth of stably transfected BA/F3 cells in nude mice.[7,24] Third, when expressed in transfected cell lines, mutant forms of KIT show constitutive kinase activity in the absence of SCF, as evidenced by autophosphorylation and activation of downstream signaling pathways.[7,25] Finally, mice engineered to express KIT with mutations of the type found in human GISTs develop diffuse ICC hyperplasia within the muscular wall of the stomach and intestine.[26,27] These mice also develop GIST-like tumors. This histologic picture is similar to that seen in individuals with inherited KIT-activating mutations.[28,29]

Tumor extracts from KIT-mutant GISTs demonstrate evidence of activation of downstream signaling pathways, including the MAP kinase pathway (RAF, MEK, and ERK), the PI3 kinase/AKT pathway, and STAT3 (Figure 3a).[24,30–32] The MAP kinase pathway upregulates important transcriptional regulators such as MYC, ELK, and CREB, and can stimulate the cell cycle through FOS. AKT activation through PI3 kinase and PDK1 leads to increased protein translation, downregulation of the cell cycle inhibitor $p27^{KIP}$, and anti-apoptotic effects. Recent studies have shown that ETV1 is an important regulator of GIST-specific gene expression during tumorigenesis.[33] KIT signaling through the MAP kinase pathway serves to maintain ETV1 activity.

In general, GISTs are heterozygous for a given mutation; however, in ~15% of tumors the remaining wild-type *KIT* allele is lost and this is associated with malignant behavior.[29,34–36] In serial samples from individual patients, Chen *et al*[36] have provided evidence that this occurs through mitotic non-dysjunction, ie, failure of a chromosome 4 pair bearing the wild-type *KIT* allele to separate during mitosis, leaving one daughter cell with a single chromosome 4 containing the mutant *KIT* allele (uniparental monosomy). This correlates with increased mitotic activity and topoisomerase II expression.

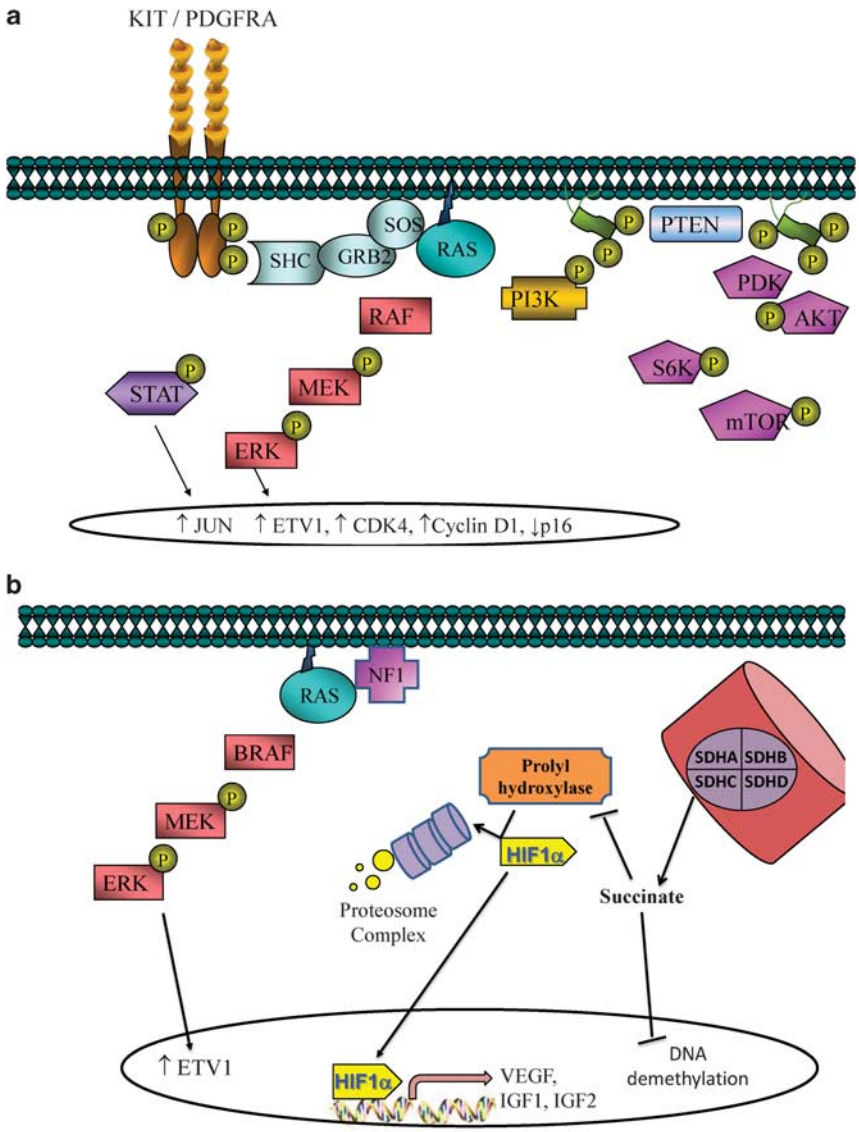

Figure 3 (**a**) *KIT* and *PDGFRA* cell signaling pathways. Dimerization of KIT or platelet-derived growth factor receptors-α (PDGFRA) leads to signaling through the mitogen-activated protein (MAP) kinase pathway (RAF, MEK and ERK) and the phosphoinositide 3-kinase (PI3K) pathway (AKT, mammalian target of rapamycin (mTOR), S6 kinase). In addition, signal transducer and activator of transcription 3 (STAT3) is activated. The collective impact favors an increase in cell metabolism, cell cycle progression, and a decreased sensitivity to apoptosis. (**b**) Cell signaling in 'wild-type' gastrointestinal stromal tumors (GISTs). Mutations in neurofibromatosis type I (NF1), RAS genes, or BRAF lead to increased signaling through the MAP kinases MEK and ERK, promoting cell growth. Loss of the mitochondrial succinate dehydrogenase complex through mutations in *SDHA*, *SDHB*, *SDHC*, or *SDHD*, leads to an accumulation of succinate, which inhibits prolyl hydroxylase-mediated degradation of hypoxia-inducible factor-1α (HIF1α) through the proteosome complex. This results in upregulated transcription of a number of genes, including *VEGF*, *IGF1*, and *IGF2*. Succinate also inhibits demethylation of DNA by TET2 through increasing α-ketoglutarate levels.

Platelet-Derived Growth Factor Receptor-α

Immunoblots of GIST extracts from tumors lacking *KIT* gene mutations sometimes show high levels of phosphorylation of the α-receptor for platelet derived growth factor (PDGFRA), which is a close homolog of KIT.[37] PDGFRA is activated in GISTs harboring mutations in the juxtamembrane domain (exon 12), the ATP-binding domain (exon 14), or the activation loop (exon 18) (Figure 2 and Table 2). Consistent with their extensive functional overlap, *KIT* and *PDGFRA* mutations are mutually exclusive in GISTs.

Observations supporting the significance of *PDGFRA* mutations in GIST parallel to those for *KIT* mutations. When expressed in transfected cell lines, mutant forms of PDGFRA have constitutive kinase activity in the absence of their ligand, PDGF-A,[37,38] the activated downstream pathways are identical to those in *KIT*-mutant GISTs,[37,39] and PDGFRA is also stabilized by HSP90.[40] In addition, both types of tumors are immunopositive

Table 2 Molecular Classification of GISTs

Genetic type	*Relative frequency (%)*	*Anatomic distribution*	*Germline example*
KIT mutation	75		
Exon 8	Rare	Small bowel	One kindred
Exon 9 ins AY502–503	8	Small bowel, colon	None
Exon 11 (deletions, single nucleotide substitutions, and insertions)	65	All sites	Several kindreds
Exon 13 K642E	1	All sites	Three indreds
Exon 17 D820Y, N822K, and Y823D	1	All sites	Five kindreds
PDGFRA mutation	10		
Exon 12 (eg, V561D)	1	All sites	Two kindreds
Exon 14 N659K	Rare	Stomach	None
Exon 18 D842V	6	Stomach, mesentery, omentum	None
Exon 18 (eg, del IMHD 842-846)	2	All sites	One kindred
KIT and *PDGFRA* wild type	15	All sites	
BRAF V600E	~2		None
SDHA/B/C/D mutations	~6	Stomach and small bowel	Carney–Stratakis
HRAS, *NRAS*, and *PIK3CA* mutation	<1		None
Pediatric/Carney triad	~1	Stomach	Not heritable
NF1-related	<1	Small bowel	Numerous

Abbreviations: GIST, gastrointestinal stromal tumor; ICC, interstitial cells of Cajal; PDGFRA, platelet-derived growth factor receptors-α; NF1, neurofibromatosis type I.

for the markers DOG1 and protein kinase C-θ (PKCθ).[9,41,42] These markers are highly selective for GISTs over other mesenchymal tumors. Further, as discussed below, both genotypes are associated with cytogenetic changes that are distinctive for GIST.[37,43]

Despite these molecular similarities, *PDGFRA*-mutant GISTs do show features distinctive from *KIT*-mutant GISTs, including differences in gene expression profile,[39,44] a striking predilection for the stomach, variable (sometimes negative) expression of KIT,[20,41,45–48] and a generally lower potential for malignancy.[49] Morphologically, however, *PDGFRA*-mutant GISTs are not reliably distinguishable from *KIT*-mutant GISTs (Figure 1).

Other Driver Mutations

Approximately 15% of GISTs do not have a detectable mutation in either *KIT* or *PDGFRA*. In other respects, these so-called 'wild-type' GISTs are clinically indistinguishable from KIT- or PDGFRA-mutant GISTs, having identical morphology, expressing high levels of KIT, and occurring anywhere in the GI tract. Phosphorylated KIT is detectable in these tumors, suggesting that KIT is still activated,[30] but the mechanism of this activation is unclear. However, recent studies have revealed that wild-type GISTs are a heterogeneous group and display various oncogenic mutations (Table 2). For example, the *BRAF* V600E substitution common in papillary thyroid carcinoma and melanoma is present in up to 13% of wild-type GISTs.[50] *HRAS*, *NRAS* and *PIK3CA* gene mutations also occur, but are much more rare. As BRAF and the RAS proteins are constituents of the MAP kinase signaling cascade, they can result in KIT-independent growth stimulation (Figure 3b) and are possible causes for resistance to KIT/PDGFRA kinase inhibitors.

It is estimated that 7% of patients with neurofibromatosis type I (NF1) develop one or more GISTs.[51–56] Most arise in the small intestine and they do not readily metastasize.[51] The majority of these GISTs are wild type for *KIT* and *PDGFRA*, but as expected they do show either somatic mutation or loss of the remaining wild-type *NF1* allele, resulting in signaling through the MAP kinase cascade (Figure 3b).[51,54,55,57]

SDH-Deficient GISTs

Approximately half of all wild-type GISTs show loss of respiratory chain complex II enzymatic activity.[10,58] This complex comprises four subunits (SDHA, SDHB, SDHC, and SDHD) and serves to oxidize succinate to fumarate as part of the mitochondrial Krebs cycle. Loss of any of these subunits through gene mutation or post-transcriptional downregulation destabilizes the complex and causes the accumulation of succinate. This results in increased transcription of HIF1α-regulated genes and decreased DNA demethylation (Figure 3b).[59] Indeed, SDH-deficient GISTs show a global increase in DNA methylation similar to that seen in gliomas and leukemias with IDH1 and IDH2 mutations.[60]

Germline mutations in *SDHA*, *SDHB*, or *SDHC* increase the risk not only for the development of one or more SDH-deficient GISTs but also for paragangliomas (Carney–Stratakis syndrome).[10,61] The GISTs in affected patients show either loss or somatic mutation (second hit) of the remaining

wild-type allele. The absence of immunostaining for SDHA, as well as SDHB, is helpful in identifying GISTs with possible SDH gene mutations.[58,58,62–66]

Approximately 50% of wild-type GISTs demonstrate high expression of IGF1R.[67] In SDH-deficient GISTs, upregulation of IGF1 and IGF2 may activate IGF1R in an autocrine manner (Figure 3b), resulting in signaling through both the MAP kinase and PI3 kinase/AKT pathways.[68]

GISTs that arise in pediatric patients (~1–2% of all GISTs) are predominantly SDHB immunonegative, but rarely harbor an *SDH* gene mutation. These tumors, which often metastasize but tend to grow slowly, have a different gene expression signature from KIT/PDGFRA-mutant GISTs.[69–71] Some pediatric-type GISTs are accompanied by pulmonary chondromas and/or paragangliomas, referred to as Carney triad, a non-heritable syndrome, the genetic cause for which has yet to be determined.[72]

The origin of GISTs

Interstitial Cells Of Cajal

During the 1990s, a number of investigators noted similarities between GISTs and a population of cells in the GI tract called the interstitial cells of Cajal (ICCs), which serve as pacemakers for peristaltic contractions. These observations led to the hypothesis that ICCs could be the cell-of-origin of GISTs. Mice engineered to express KIT with mutations of the type found in human GISTs develop diffuse ICC hyperplasia within the muscular wall of the stomach and intestine.[26,27] These mice also develop GIST-like tumors. Diffuse ICC hyperplasia has been described in several kindreds with heritable mutations in *KIT* (Table 2), and is associated with dysphagia and the development of multiple GISTs,[26,29,53,73–78] although many of the tumors do not follow a malignant course.

The relationship between GISTs and ICCs is further supported by parallels in gene expression. For example, high levels of PKCθ, nestin, and DOG1 are expressed in both GISTs and ICCs. In addition, the ETS family transcription factor ETV1 is highly expressed in both GISTs and in the specific subpopulations of ICCs (myenteric and intramuscular, as opposed to submucosal) thought to give rise to GISTs.[33]

The observation that some *KIT* and *PDGFRA* mutations in GISTs correlate closely with anatomical location (Table 2) might be explained by their ICC origin. For example, GISTs with a *KIT* exon 9 mutation, which arise primarily in the intestines, may derive from a different subgroup of ICCs than those with a *PDGFRA* D842V mutation, which occur only in the stomach, mesentery, and omentum. The more common *KIT* mutations, by contrast, can be found in GISTs throughout the GI tract, perhaps deriving from a more ubiquitous ICC subtype.

Micro-GISTs

Minute growths (1–10 mm) of ICC/GIST-like cells are present in between 2.9 and 35% of the stomachs thoroughly examined after surgical removal or at autopsy.[79–82] These so-called micro-GISTs are mitotically inactive and often partially calcified, suggesting tumorigenic arrest. In contrast to the diffuse ICC hyperplasia observed in the presence of a germline *KIT* mutation, micro-GISTs appear to represent a nodular form of ICC overgrowth caused in most cases by local, somatic acquisition of a *KIT* mutation. The type and frequency of *KIT* mutations in micro-GISTs is essentially the same as in clinically significant tumors.[83] Subcentimeter GISTs with *PDGFRA* mutations have also been reported.[79] These observations on micro-GISTs suggest that kinase gene mutations occur very early in GIST tumorigenesis; however, the mutations are probably not sufficient for progression to an oncologically threatening lesion (Figure 4). The large pool of micro-GISTs in the general population likely explains the multiple reported cases in which two or more genotypically distinct GISTs are found in a patient during a single surgical procedure.[53,79,84]

Chromosomal and Molecular Alterations During GIST Progression

Although oncogenic kinase mutations have a significant role in the development of GISTs, other genetic events are important in their clinical progression (Figure 4). Approximately two-thirds

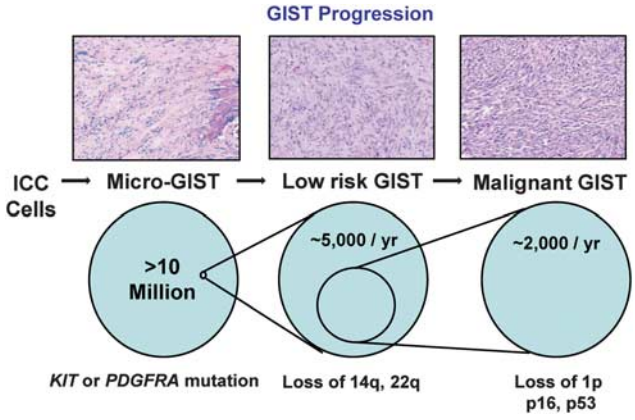

Figure 4 Origin and progression of gastrointestinal stromal tumors (GISTs). Under the influence of a KIT or platelet-derived growth factor receptors-α (PDGFRA) mutation, a clonal outgrowth of interstitial cells of Cajal (ICCs) may form a discrete nodule (micro-GIST) measuring <1 cm and showing essentially no mitotic activity. A conservative estimate for the prevalence of micro-GISTs in the US population would exceed 10 million lesions. With the accumulation of additional mutations and chromosomal changes, a micro-GIST may progress to a larger lesion that is clinically significant. Approximately 5000 such lesions are identified each year in the United States, of which 2000 are of high risk or are malignant.

of GISTs demonstrate either monosomy of chromosome 14, or partial loss of 14q.[37,85–89] Interestingly, these chromosome 14 abnormalities are observed in both *KIT*-mutant and *PDGFRA*-mutant GISTs.[37,43] Deletions of 14q11.2 include the genes *PARP2*, *APEX1*, and *NDRG2*, whereas deletions of 14q32 include the *SIVA* gene.[90] Loss of the long arm of chromosome 22 is observed in ~50% of GISTs.[37,43,85,86,89,91]

Losses on chromosomes 1p, 9p, 11p, and 17p are successively less common than 14q and 22q losses, but are more significantly associated with malignancy (Figure 4).[37,43,85,89,91–94] Losses on chromosomes 10, 13q, and 15q have also been reported in GISTs.[43,91] Gains on chromosomes 8q (including *MYC*), 3q (including *SMARCA3*), and 17q are associated with metastatic behavior.[86,95]

In a recent array-based analysis of gene copy number in 42 GISTs (23 with recurrence or metastasis), the tumors were separated into four groups reflecting their accumulated chromosomal changes. The overall survival of group 1 (loss of 22q, 19, and 1p distal) and group 2 (additional loss of 14q) was significantly better than that of group 3 (added losses of 15q and 1p proximal) and group 4 (additional loss of 10). Specific genes implicated in this analysis included *OXA1L* on 14q, as well as *AKAP13* and *C15orf5* on 15q.

None of the above karyotypic changes is present in pediatric-type GISTs, which remain near-diploid, again emphasizing the different biology of these tumors. In contrast, GISTs arising in NF1 patients often show losses of 14q and 22q.[96]

On the basis of gene expression profiling of high-risk versus low-risk GISTs, the high-risk tumors show significant changes in genes regulating the cell cycle, including genes influenced by the PI3 kinase pathway and genes involved in the G2/M checkpoint.[97] A significant fraction of malignant GISTs show inactivation of the tumor suppressor gene *CDKN2A* (which encodes the cell cycle regulatory protein $p16^{INK4A}$) through chromosome 9p21 deletion, either biallelic or in combination with mutation or promoter methylation.[98–101] *TP53* mutations and decreased p53 immunostaining also correlate with a poor prognosis.[102–104] Likewise, amplifications of *MDM2* and *CCND1* (cyclin D1), although uncommon in GISTs, are associated with malignancy.[105]

Kinase mutations and tyrosine kinase inhibitor therapy

Until the year 2000, treatment options for patients with advanced GIST were poor. The response rate to conventional chemotherapy was <5% and median survival for patients with advanced disease was ~18 months.

The tyrosine kinase inhibitor (TKI) imatinib was developed in the early 1990s as a treatment for chronic myelogenous leukemia because of its ability to inhibit the fusion oncoprotein BCR–ABL.[106] The observation that ABL shares structural similarity with KIT, and several other tyrosine kinases led to experiments showing that imatinib can inhibit the growth of cells expressing mutant forms of KIT.[25] In addition, imatinib showed potent activity against a KIT-mutant GIST cell line. Imatinib inhibits KIT kinase by directly binding to the ATP pocket and competitively inhibiting ATP binding. The KIT receptor is normally in equilibrium between active and inactive conformations. The latter is favored by steric hindrance conferred by the juxtamembrane domain (exon 11), which prevents the activation loop from assuming the conformation required for kinase activation.

With the knowledge that imatinib inhibits KIT signaling, imatinib was first used clinically to treat a 50-year-old female with metastatic GIST, and a dramatic response was observed.[107] Promising results from phase I and II trials led to two international phase III trials, each using similar protocols that would allow a subsequent meta-analysis. The phase III trials compared 400 versus 800 mg daily doses of imatinib. Overall, imatinib achieved disease control in 70–85% of patients with advanced KIT-immunopositive GISTs, and the median progression-free survival was 20–24 months.[108–112] Currently, the median survival for patients with advanced disease treated with frontline imatinib is 5 years, with 34% of patients surviving more than 9 years.[113]

A meta-analysis of the phase III trials proved that patients with exon 9-mutant GIST had a significantly longer progression-free survival if they were treated with 800 mg of imatinib; hence, this is now the target dose for these patients. In contrast, only 400 mg of imatinib is needed to manage exon 11-mutant tumors.[112]

The success with imatinib in patients with metastatic disease quickly led to trials of the drug in the adjuvant setting. It was initially shown that 12 months of imatinib after primary resection of a GIST significantly delayed disease recurrence versus a placebo.[114] A subsequent trial proved that 36 months of adjuvant imatinib was superior to 12 months.[115]

Assessing GIST prognosis

FDA approval for the use of imatinib in the adjuvant setting has made it imperative that the prognosis of a newly resected GIST be predicted as accurately as possible. In a 2-year prospective study of sarcomas in France, >85% of GISTs presented as localized lesions.[116] Thus, the question of whether to use adjuvant therapy must be considered for the great majority of newly diagnosed GISTs. Kinase genotype does not factor into overall survival once a GIST becomes metastatic. Thus, a *KIT* or *PDGFRA* mutation may set the initial course of a GIST, but the prognosis at the time of clinical presentation is

Table 3 Assessing GIST prognosis

	Size (cm)	*Gastric (n = 1055), %*	*Jejunum/ileum (n = 629), %*	*Duodenum (n = 144), %*	*Rectum (n = 111), %*
Mitotic index $\leq 5/5\,mm^2$	≤ 2	0	0	0	0
	$>2\leq 5$	1.9	4.3	8.3	8.5
	$>5\leq 10$	3.6	24	Insufficient data	Insufficient data
	>10	10	52	34	57
Mitotic index $>5/5\,mm^2$	≤ 2	None	High	Insufficient data	54
	$>2\leq 5$	16	73	50	52
	$>5\leq 10$	55	85	Insufficient data	Insufficient data
	>10	86	90	86	71

Abbreviations: GIST, gastrointestinal stromal tumor.
The risk of disease recurrence or metastasis can be estimated based on three parameters defined in this table: (1) the mitotic index (either ≤ 5 mitoses/5 mm^2 or >5 mitoses/5 mm^2); (2) tumor size (largest diameter); and (3) tumor site of origin. The table is based on data published by Miettinen *et al*.[117,118,150]

clearly influenced by other genetic events. Unfortunately, our knowledge of these additional mutations remains limited, and current recommendations for assessing prognosis are based on three simple parameters: tumor size, tumor location, and mitotic index (mitoses/5 mm^2). A number of risk assessment schemes have been published; the most widely used scheme was developed at the Armed Forces Institute of Pathology by Miettinen *et al*[117–119], whose considerable efforts in studying the outcome of patients before the advent TKIs have provided the most complete data available (Table 3). It should be noted that tumor rupture, either before or during surgery, is another important negative prognostic factor. Incomplete resection, particularly in the area of the rectum, is also associated with a higher risk of recurrence.[120]

As a group, PDGFRA-mutant GISTs appear to be less aggressive than KIT-mutant GISTs,[49] yet PDGFRA-mutant tumors can still progress and kill patients. These tumors are assessed using the same criteria as other GISTs.

Responses to TKI therapy

Clinical Disease Persistence

Clinical data suggest that even long-term TKI treatment fails to eradicate GIST cells, resulting in disease persistence. In an attempt to determine the optimal duration of imatinib therapy for advanced, unresectable GIST, an interesting trial randomized patients who had continuous control of their disease during 3 years of imatinib treatment to either continue or to discontinue treatment.[121] For those continuing treatment progression-free survival over the next 2 years was 80%, but for those who stopped therapy it was only 16%. Patients who relapsed after discontinuation of therapy did so because of persistent disease. In contrast, the progression that developed in some of the patients who maintained therapy was due to resistant disease.

Persistence of GIST cells during TKI treatment could be due to the failure of these drugs to eradicate mature GIST cells and/or the failure to eliminate GIST stem cells. Current evidence suggests that both mechanisms underlie GIST persistence in the face of prolonged TKI therapy. Agaram *et al*[122] examined a series of 43 clinically responsive GISTs lesions from 28 patients. Histological responses in these resected tumors after 1–31 months of imatinib treatment ranged from $<10\%$ to $>90\%$ reduction in tumor cellularity, but tumor cells persisted in all lesions. Three quarters of the lesions showed an absence of mitoses and a proliferative index of 0% by Ki-67 staining, indicating biological quiescence. Interestingly, some of the tumor cells showed transdifferentiation. Indeed, examples of rhabdomyoblastic, cartilaginous, and osseous differentiation have all been observed in cases of treated GIST (Figure 5). Thus, under imatinib suppression, GIST cells may avoid apoptosis by exiting the cell cycle and expressing genes associated with a differentiated phenotype. Whether these cells proliferate again when a TKI is discontinued, or whether GIST stem cells are the source of renewed growth, has not been determined.

In rare cases, GISTs may progress to high grade, anaplastic sarcomas that lose CD117 expression. This has been observed in both imatinib-treated and TKI-naive tumors.[123]

Resistance to TKI therapy

Primary Resistance

Resistance to treatment with KIT/PDGFRA inhibitors such as imatinib can be divided into two types: primary and secondary. Approximately 10% of patients with GIST have primary resistance, defined as progression within the first 6 months of treatment. On the basis of data from phase II and phase III trials, tumor response to imatinib correlates with the underlying kinase genotype.[34,41,112,124,125] The

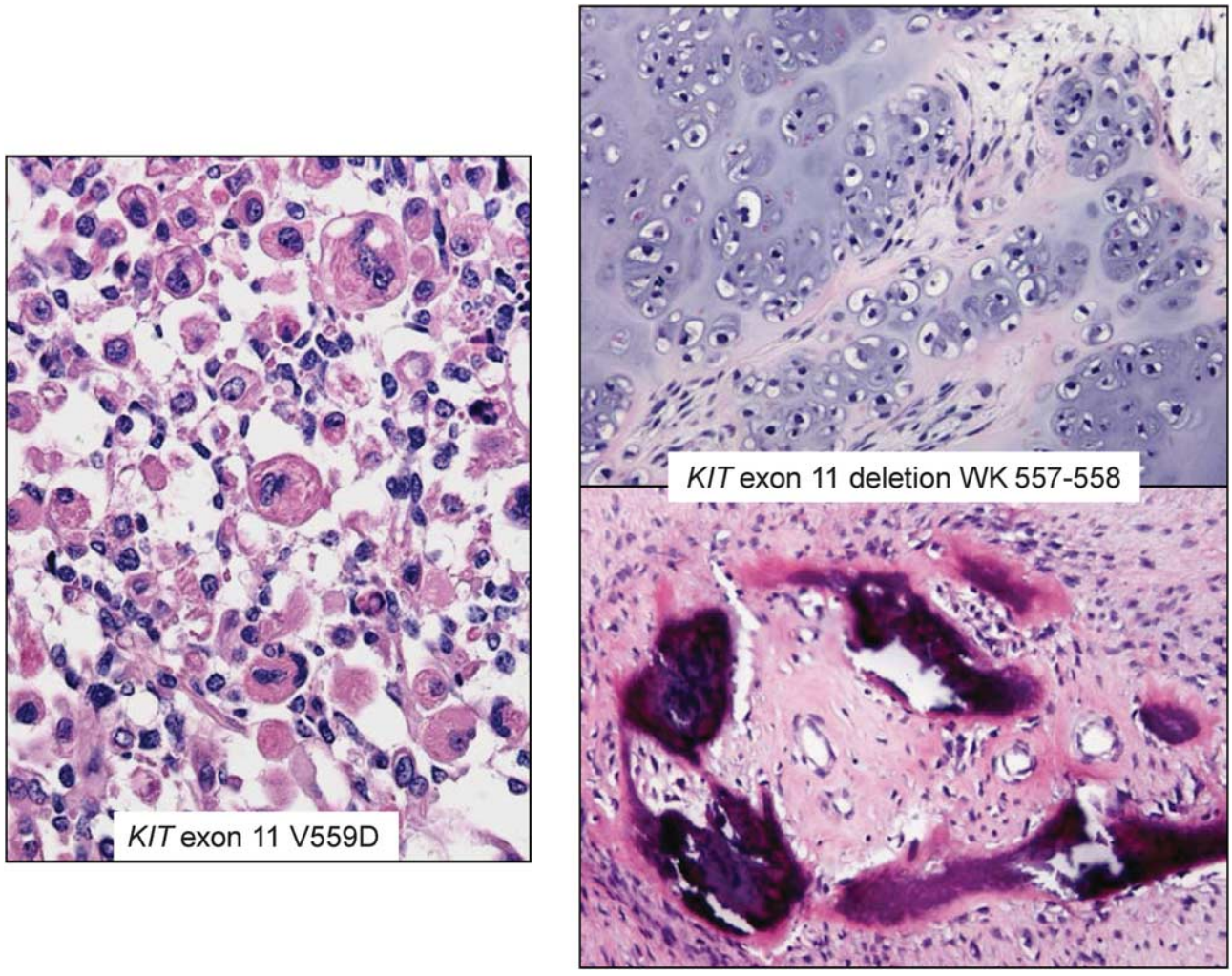

Figure 5 Differentiation of gastrointestinal stromal tumor (GIST) cells under suppression of a tyrosine kinase inhibitor (TKI). In some cases, imatinib treatment may lead to rhabdomyoblastic differentiation (left panel), or else chondroid or osseous differentiation (right panels).

probability of primary resistance to imatinib for *KIT* exon 11, *KIT* exon 9, and wild-type GISTs is 5, 16 and 23%, respectively.[34] These findings likely reflect the underlying sensitivities of different KIT genotypes. Exon 11-mutant KIT is highly sensitive to imatinib, with an IC_{50} of <100 nM, whereas exon 9-mutant KIT and wild-type KIT are less sensitive to the drug (IC_{50} $\sim$1000 nM for each).[126] Thus, underdosing of imatinib in patients with exon 9 mutations probably accounts for some of the apparent resistance.[112]

On the basis of *in vitro* data, the most common PDGFRA mutation in GISTs, D842V, is fully resistant to the effects of imatinib.[34,38,127,128] This mutation favors the active conformation of the kinase domain and consequently disfavors imatinib binding.[34,129,130] This is corroborated by clinical results, as patients with PDGFRA D842V-mutant GIST have low response rates and very short progression-free and overall survivals during imatinib treatment. Crenolanib is a TKI that has activity versus D842V and is now being tested in a clinical trial. There are, however, other PDGFRA mutations that are sensitive to imatinib *in vitro* and patients with these mutations have shown durable responses to imatinib.

Wild-type GISTs include tumors with mutations downstream of KIT;[10,50,131,132] hence, these subsets of wild-type GISTs might respond better to other targeted agents, such as VEGFR inhibitors for pediatric/SDH-deficient GIST and BRAF/MEK inhibitors for BRAF and RAS mutant GIST.[133]

Secondary Resistance

After an initial response to imatinib, the vast majority of patients eventually develop disease progression. This secondary resistance may manifest in a number ways, including growth of a nodule within a pre-existing, clinically quiescent lesion, the development of one or more new lesions, or

widespread disease throughout the liver or abdominal cavity. It is now established that acquired mutations in *KIT* or *PDGFRA* account for most secondary resistance, and that these mutations occur almost exclusively in the same gene and allele as the primary oncogenic driver mutation.[35,134–140]

In a phase II imatinib study for advanced GIST, 67% of the patients whose tumor showed imatinib resistance had a new, or secondary, mutation in KIT. Notably, these mutations were common among tumors with a primary exon 11 mutation, but were not observed in wild-type GIST samples.[137] Unlike primary mutations that activate KIT, which are predominantly in the juxtamembrane regions encoded by exons 9 and 11, the secondary mutations were concentrated in two regions of the KIT kinase domain. One is the ATP-binding pocket, encoded by exons 13 and 14, mutations of which directly interfere with drug binding. The second is the activation loop (exons 17 and 18), where mutations can stabilize KIT in the active conformation and thereby hinder drug interaction. Drug resistance has also been observed in PDGFRA-mutant GISTs, in which the most common one is an acquired D842V mutation (activation loop).[135,137]

Additional studies using more sensitive assays have identified secondary mutations in >80% of drug-resistant GIST lesions.[141–143] More sobering is that there is significant heterogeneity of resistance across different lesions, and even within different areas of the same lesion. For example, there are reports of up to five different drug resistance mutations in different portions of an individual lesion and up to seven different secondary resistance mutations across multiple tumors in the same patient.[141] This heterogeneity of resistance significantly impacts the efficacy of salvage TKI therapy after frontline imatinib, because the diversity of resistant, minority clones precludes the systemic suppression of GIST cells by any particular TKI.

Approaches to imatinib-resistant GIST

Most GIST patients who develop secondary resistance to imatinib will not respond to dose escalation, forcing a switch to an alternative KIT/PDGFRA TKI. Such salvage agents include sunitinib, sorafenib, regorafenib, vatalanib, masatinib, nilotinib, and dasatinib, as well as other investigational inhibitors. Although all of these agents are KIT/PDGFRA inhibitors, the majority of them (in contrast to imatinib) also target VEGFR1/2.[144] Whether the disease stabilization that can be seen with these salvage agents is related to VEGFR1/2 inhibition and a consequent decrease in angiogenesis remains unclear.

Sunitinib is FDA-approved for the treatment of GIST patients with progression on imatinib,[145] but biochemical evidence suggests that its activity against secondary imatinib-resistant kinase mutations is suboptimal. KIT ATP-binding pocket mutations are extremely sensitive to sunitinib *in vitro*; however, the activation loop mutations are strongly cross-resistant. Given the approximately equal frequency of these different classes of mutations in imatinib-resistant lesions and the multiplicity of lesions in a typical patient, it is not surprising that mixed responses are common during sunitinib therapy.[124,146]

Most recently, the FDA has approved regorafenib for the third-line treatment of patients with GIST that is resistant to imatinib and sunitinib. In a phase III trial, the progression-free survival on regorafenib was 4.8 months.[147] Thus, even with newer drugs such as regorafenib, resistance develops over time, suggesting that escape from ATP-competitive inhibitors of KIT and PDGFRA is inevitable. A new class of non-ATP mimetic kinase inhibitors (switch pocket kinase inhibitors, such as DP-2976) have shown high potency when tested *in vitro* and represent hope in the fight against TKI resistance.[148,149]

Summary and future directions

Achievements in the treatment of GISTs during the past decade are the direct result of a growing understanding of their molecular biology. The high frequency of primary KIT and PDGFRA mutations in these tumors makes them sensitive to kinase inhibitors such as imatinib, and this drug is FDA-approved for use in both the adjuvant and advanced disease settings. Accurate assessment of the risk of disease recurrence is essential in determining the use of adjuvant imatinib after resection of a primary tumor. Current recommendations for assessing recurrence risk are based on tumor size, tumor location, and mitotic index (mitoses/5 mm^2).[117–119]

Resistance to imatinib develops in the majority of advanced cases of GIST. An immediate research goal is the development of new inhibitors that have activity against secondary mutations in the activation loop. In addition, the development of effective combination therapy is likely to improve tumor control. Ongoing high-throughput genomic studies may identify additional drivers/modifiers of GIST biology that can be targeted.

Clinical genotyping of GISTs is important to identify KIT exon 9-mutant tumors that require a higher dose of imatinib for optimal disease control. Further, there are other molecular subtypes of GIST that do not respond well to conventional KIT inhibitors, but may be better treated with other agents (eg, SDH-deficient GIST and PDGFRA D842V GIST). Thus, the GIST genotyping is critical in personalizing the care of patients who need TKI therapy.

In summary, new insights into the origin and progression of GISTs are setting the stage for further therapeutic innovations, with the goal not just to

control disease growth, but to eliminate all tumor cells at the time of initial therapy. Going forward, the challenge will be to move from a paradigm of tumor suppression to true cancer cure.

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