Genetic Aberrations of Gastrointestinal Stromal Tumors

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Gastrointestinal stromal tumor (GIST) is the most common mesenchymal neoplasm in the gastrointestinal tract and is associated with mutations of the *KIT* or *PDGFRA* gene. In addition, other genetic events are believed to be involved in GIST tumorigenesis. Cytogenetic aberrations associated with these tumors thus far described include loss of 1p, 13q, 14q, or 15q, loss of heterozygosity of 22q, numeric chromosomal imbalances, and nuclear/mitochondrial microsatellite instability. Molecular genetic aberrations include loss of heterozygosity of *p16(INK4A)* and *p14(ARF)*, methylation of *p15(INK4B)*, homozygous loss of the *Hox11L1* gene, and amplification of *C-MYC*, *MDM2*, *EGFR1*, and *CCND1*. GISTs in patients with neurofibromatosis type 1 appear to lack the *KIT* and *PDGFRA* mutations characteristic of GISTs and may have a different pathogenetic mechanism. Gene mutations of *KIT* or *PDGFRA* are critical in GISTs, because the aberrant versions not only are correlated with the specific cell morphology, histologic phenotype, metastasis, and prognosis, but also are the targets of therapy with imatinib and other agents. Furthermore, specific mutations in *KIT* and *PDGFR* appear to lead to differential drug sensitivity and may in the future guide selection of tyrosine kinase inhibitors. Activation of the receptor tyrosine kinases involves a signal transduction pathway whose components (mitogen-activated protein kinase, AKT, phosphoinositide 3-kinase, mammalian target of rapamycin, and RAS) are also possible targets of inhibition. A new paradigm of classification, integrating the standard clinical and pathological criteria with molecular aberrations, may permit personalized prognosis and treatment. ***Cancer* 2008;113:1532-43.** *© 2008 American Cancer Society.*

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Sarcomas may be divided into 2 groups based on cytogenetic and molecular genetic characteristics: those with a frequently diploid karyotype and limited chromosomal abnormalities but with frequently observed specific translocations and those with complex karyotypes and multiple cytogenetic and molecular genetic aberrations. Gastrointestinal stromal tumors (GISTs) are generally believed to belong to the former group and are the most common mesenchymal tumors of the gastrointestinal tract. The 2 most important prognostic features of primary GISTs are tumor size and mitotic index, which were the foundation for a consensus approach to risk stratification of GISTs published in 2002. The anatomic location also significantly affects the risk of disease recurrence and progression, and this is noted in the 2007 National Comprehensive Cancer Network (NCCN) risk stratification criteria.[1,2]

Greater than 90% of GISTs harbor a specific *KIT* or *PDGFR-alpha* (*PDGFRA*) gene mutation. These mutations are important for

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TABLE 1
Correlation Between Cytogenetic Aberrations and Clinicopathologic Features in Gastrointestinal Stromal Tumors

Cytogenetic Aberrations	Specific Sites	Potential Clinicopathologic Significance
Loss of 14q	14q11.2-q32.33	Early event in tumorigenesis
LOH of 22q	22q12.2-q13.31	Plays role in early stages of tumorigenesis and late progression
Loss of 1p, 10q, 13q, 15q	—	May harbor tumor suppressor gene(s) related to early recurrence and/or metastasis during malignant transformation
Nuclear and mitochondrial microsatellite instability	—	May play role in pathogenesis
Chromosomal aneuploidy	—	Associated with more aggressive tumor behavior
−14q pathway in oncogenetic tree model	Loss of 14q	Observed in gastric tumors with predominantly stable karyotypes and more favorable outcome
−1p pathway in oncogenetic tree model	Loss of 1p	Characteristic of intestinal GISTs, with increased capacity for cytogenetic complexity and more aggressive clinical course
−22q pathway in oncogenetic tree model	Loss of 22q, 9p; gain of 8q	Associated with increased mitotic activity and recurring disease

LOH indicates loss of heterozygosity; GIST, gastrointestinal stromal tumor.

tumor phenotype, and their presence correlates with benefit from targeted therapy with the kinase inhibitor imatinib mesylate. To our knowledge, Besmer et al were the first to demonstrate that *v-kit* is an oncogene,[3] and it has been demonstrated since then that germline *KIT* mutation leads to imatinib-sensitive GISTs in an autosomal dominant pattern.[4] Moreover, the identification of *KIT* and *PDGFRA* mutations in GISTs has led to development of imatinib, sunitinib, and other tyrosine kinase inhibitors for the treatment of solid tumors. The majority of GISTs have other genetic aberrations in addition to *KIT* and *PDGFRA* mutations. Understanding the genetic aberrations beyond *KIT* and *PDGFRA* may lead to the identification of additional therapeutic targets for GISTs and possibly other cancers. Herein we provide a comprehensive compendium of known cytogenetic and molecular abnormalities in GISTs.

Cytogenetic Aberrations

Loss of chromosome 14 and/or 22 with minimal recurrent regions in 14q11.2-q32.33 (5 of 7, 71% of tumors) and 22q12.2-q13.31 (7 of 7, 100%) appears to play an important role in early stages of tumor formation as well as in late tumor progression.[5-7] In the report by Wozniak et al, all 66 primary GISTs examined had genomic imbalances, most frequently loss of 14q, 1p, 22q, 15q, or 13q. Furthermore, lower incidence of losses at 14q and higher frequency of losses at 1p and 15q were the most common findings in nongastric GISTs. These findings suggest that loss of 14q is a relatively less frequent genetic event in the development of nongastric GISTs.[8] Chen et al investigated the chromosomal imbalance aberrations in 28 GISTs and found that losses were more common than gains. The median number of chromosomal imbalance aberrations in high-risk GISTs was significantly higher than that in low-risk GISTs, especially for losses. Among the chromosomal imbalance aberrations, losses of 13q, 10q, and 22q suggest that these chromosomal loci were most likely to harbor the tumor suppressor gene(s) that may be related to early recurrence and/or metastasis during malignant transformation of GISTs.[9] Another investigation detected nuclear and mitochondrial microsatellite instability in 3 and 10 of 62 GISTs, respectively, and the chromosomal numerical abnormality in the primary sites was more extensive in GISTs with recurrence and metastasis than in those without. These results indicated that mitochondrial microsatellite instability plays a role in the development of GISTs and that numeric chromosomal abnormalities may be a phenotype associated with more aggressive tumors with a propensity to metastasize and recur locally.[10,11]

As shown in Table 1, the cytogenetics of GISTs in some cases is so complex that an oncogenetic tree model was constructed using comparative genomic hybridization (CGH) data from 203 primary GISTs.[12] The oncogenetic tree model identified 3 major cytogenetic pathways: one is initiated by −14q, the second by −1p, and the third by −22q. The −14q pathway mainly characterized gastric tumors with predominantly stable karyotypes and more favorable clinical course. Conversely, the −1p pathway was more characteristic of intestinal GISTs, with an increased capacity for cytogenetic complexity and more aggressive clinical course. Loss of 22q, which is more closely associated with −1p than −14q, appeared to initiate the critical transition to an unfavorable cytogenetic subpathway.[12] Furthermore,

TABLE 2
Genes Regulated by Mutations of *KIT* and *PDGFRA*

Gene	Target Genes	Downstream Signaling Factors		Signaling Consequences
KIT mutation	*MAPK*	JAK1/2	STAT	Induce progression through cell cycle; prevent apoptosis
		ERK1/2(P44/22)	P90RSK	Inhibit cell differentiation
			MSK	
			ELK-1, STAT	
		p38	PLA2, MNK1, PRAK, Hsp27, STAT1, ELK-1	Inhibit apoptosis
	GRB2	SOS, RAS, RAF, MEK, ERK	P90RSK, MSK, ELK-1, STAT	Inhibit apoptosis
	PI3K	p70S6K	RPS6, ELF-4	Regulate cell growth
		PKB(AKT)↓	mTOR signaling (p70S6K, RPS6, ELF4, STAT)	Regulate cell growth
			RAF, ERK, p70S6K↓	Inhibit apoptosis
			p21, p27	Regulate cell cycle and proliferation
			cyclinD1, p53	
			BAD, FXHR/AFX↓	Induce cell survival
			p53, NF-KB	Modulate cell death
		BAD↓	BCL-XL, BCL-2	Inhibit apoptosis
		NF-KB	IKB, IKK, et al	Inhibit cell differentiation
	SHP1/2	—	—	Negative regulation of KIT signaling pathway
	CBL	—	—	Unknown
	SHC	—	—	Unknown
	EPHA4	—	—	Unknown
	Paxillin	—	—	Unknown
PDGFRA mutation	*MAPK*	JAK1/2, ERK1/2, p38	STAT, P90RSK, MSK, ELK-1, PLA2, MNK1, PRAK, Hsp27	Inhibit apoptosis; inhibit cell differentiation; induce progression through cell cycle
	AKT (with controversy)	mTOR signaling (p70S6K, RPS6, ELF4, STAT)	—	Regulate cell growth
		RAF, ERK, p70S6K	—	Modulate cell growth
		p21, p27	—	Regulate cell cycle and proliferation
		cyclinD1	—	Modulate cell cycle (indirectly)
		BAD, FXHR/AFX	—	Induce cell survival
		p53, NF-KB	—	Modulate cell death (indirectly)
	STAT	—	—	Induce progression through cell cycle; prevent apoptosis

Pylkkanen et al found that allelic losses at 22q were associated with high mitotic activity and recurring disease. Thus, insights into the cytogenetic evolution obtained from oncogenetic tree models may eventually help to improve our understanding of the heterogeneous biological behavior of GISTs.[8,13]

Molecular Genetic Aberrations of *KIT* and *PDGFRA*

Activation of the KIT receptor tyrosine kinase (RTK) is a central pathogenetic event in most GISTs and generally results from oncogenic, gain-of-function, in-frame deletions or point mutations that can involve either the extracellular juxtamembrane or cytoplasmic domains of the receptor. Oncogenic mutations enable the KIT receptor to phosphorylate various substrate proteins, leading to activation of signal transduction cascades that regulate cell proliferation, survival, chemotaxis, and adhesion (Table 2). *KIT* mutations can be assigned broadly to 1 of 2 groups: 1) those that involve the "regulatory" regions responsible for modulating KIT enzymatic activity and 2) those that involve the enzymatic region itself. Published reports indicate that *KIT* mutations in untreated GISTs are clustered primarily in 4 exons: 9, 11, 13, and 17. Most common are exon 11 (intracellular juxtamembrane domain) mutations that include deletions, point mutations, and duplications of the 3′ region. Exon 13 and 17 mutations are almost exclusively point mutations and occur in several hot spots, and the frequency of exon 13 or exon 17 mutations is no higher than 1% to 2%. Nearly all of the *KIT* exon 13 mutations were the 1945A > G substitution leading to L642G. A majority of the *KIT* exon 17 mutations were the 2487T > A substitution leading to A822L. They may be more commonly present in GISTs of the small bowel, and exon 13 mutation may predict a more aggressive course in gastric GISTs.[14-19] An A842V substitution in exon 18 is the most common *PDGFRA* mutation. GISTs with such mutation are resistant to imatinib. *PDGFRA* mutations are associated with gastric GISTs, epithelioid morphology, and a less malignant course of

TABLE 3
Gene Aberrations Other Than *KIT* and *PDGFRA* Mutations in Gastrointestinal Stromal Tumors

Gene Sites	Related Gene Aberrations	Clinical Correlations
9p21	LOH	More often in high-risk GISTs, absent in low-malignant-potential group, recurrent GISTs showed more frequent deletions than cognate primary tumors
P16 and *p14*	LOH of P16INK4A and p14ARF	May contribute to progression and/or malignant transformation of GISTs
P15	P15INK4B promoter methylation	Loss of tumor suppressor gene expression
PARP2, APEX1, NDRG2, SIVA, ENO1, and *CDKN2A/2B*	Copy number loss	Loss of tumor suppressor gene expression
Hox11L1	Homozygous loss	Has role in tumorigenesis
C-MYC, MDM2, EGFR1, CCND1	Amplification	Associated with clinical and histologic malignancy
FLJ10261	High expression	Involved in tumorigenesis of GISTs
PKC-theta	—	Plays role in development of GISTs
Obscuring, C9orf65	—	Helpful to differentiate GISTs and leiomyosarcoma

LOH indicates loss of heterozygosity; GIST, gastrointestinal stromal tumor.

disease.[18] Recently, with the use of imatinib therapy, a new class of mutations associated with resistance to this treatment has been discovered. These are virtually all point mutations that involve a limited number of codons in exons such as 13, 14, 17, and 18 of *KIT* and sometimes *PDGFRA* and are rarely encountered in untreated GISTs. The primary mutations are retained, but these additional or secondary mutations make the KIT receptor less sensitive to inhibition by imatinib, occasionally by actually affecting binding of the drug to the cytoplasmic tyrosine kinase domain.[19]

GISTs are believed to originate from interstitial cells of Cajal (ICC) or their precursors. Ogasawara et al suggested that ICC undergoing *KIT* mutation as a possible early initiation step in GIST tumorigenesis may thus have preneoplastic potential.[20] Agaimy et al also considered that GIST tumorlets represent the macroscopically recognizable counterpart of sporadic ICC hyperplasia caused by somatic *KIT* or *PDGFRA* mutations.[21] In this recent study, 12 of 19 sporadic ICC hyperplasia lesions were investigated by molecular analysis, and the investigators found 3 KIT exon 11 mutations (2 point mutations and 1 deletion, all involving W557) in 3 cases. Furthermore, the remarkable variation in the incidence of ICC hyperplasia at different gastrointestinal sites suggests an origin from heterogeneous subsets of ICC with various potentials for neoplastic transformation.[22]

Molecular Aberrations of Other Genes

More and more genes, including tumor suppressor genes, have been found to harbor abnormalities in GISTs that are closely correlated with tumorigenesis, such as p16 (Table 3). Sabah et al demonstrated that loss of heterozygosity (LOH) with at least 1 microsatellite marker at the 9p region was a common finding in high-risk GISTs, and recurrent GISTs demonstrated more frequent deletions than their cognate primary tumors. These results suggest that loss of the *p16(INK4A)* gene on 9p may contribute to the progression and/or malignant transformation of GISTs.[23] To assess the involvement of *p14ARF* and *p15INK4B* in addition to *p16INK4A* in GISTs, Perrone et al undertook a molecular and cytogenetic study of the 9p21 locus. The results indicated the loss of *p16INK4A* mRNA expression in 41% of the GISTs studied, mainly because of homozygous deletion of the *p16INK4A* gene. No mutations were found, and promoter methylation was restricted mainly to the *p15INK4B* gene. Alterations in the 9p21 locus were found cumulatively in 54% of the tumors in this series and comprised mainly loss of tumor suppressor gene expression.[3,24] These results were further supported by the investigation of Assamaki et al, which found that most recurrent copy number losses were localized to 14q, 22q, 1p, and 9p, harboring the *PARP2*, *APEX1*, *NDRG2*, *SIVA*, *ENO1*, and *CDKN2A/2B* genes.[25] At the same time, it was demonstrated that the *Hox11L1* gene, which is located on chromosome 2 and exerts a role in proliferation of ICC, had homozygous loss in 7 of 72 GISTs. These data implicate *Hox11L1* in the tumorigenesis of GISTs.[26]

To determine whether known oncogenes take part in genomic rearrangements and to investigate the potential clinical significance of their amplifications, the oncogenes *C-MYC*, *MDM2*, *GLI1*, *CDK4*, *HER2*, *EGFR1*, *CCND1*, *FGF3*, and *EMS* were analyzed by fluorescent in situ hybridization on a tissue microarray containing 94 primary GISTs. Amplification was found for *C-MYC* in 3 of 90 cases, for *MDM2* in 5 of 94, for *EGFR1* in 5 of 94, and for

CCND1 in 7 of 79. Amplifications of *MDM2* and *CCND1* were associated with clinical and histologic malignancy. The data show that gene amplification does occur in a subset of GISTs. *MDM2/CCND1* amplification may represent a molecular feature important in the pathogenesis of some GISTs.[27] West et al also characterized gene expression patterns in GISTs and found that the gene *FLJ10261* (*DOG1*, discovered on GIST-1), encoding a hypothetical protein, was specifically expressed in GISTs. Immunoreactivity for *DOG1* was found in 136 of 139 (97.8%) GISTs; all 7 GIST cases with a *PDGFRA* mutation were *DOG1* positive, whereas most of these were *KIT* negative. These findings suggested the *DOG1* may be involved in GIST tumorigenesis.[28,29] Other genes such as *PKC-theta* have also proven to be useful markers and may play roles in the development of GISTs; expression of the *obscurin* and *prune2* genes can be helpful in differentiating GISTs and leiomyosarcomas.[30,31]

Molecular Genetic Aberrations and Clinicopathologic Features

Reports showing that molecular genetic aberrations in GISTs are correlated with specific cell morphologies and histologic phenotypes are accumulating. Wardelmann et al provided evidence that GISTs may be divided into distinctive entities with different genetic, biological, and phenotypic features. They found *PDGFRA*-mutated tumors were preferentially located in the stomach, whereas GISTs with exon 9 and 13 *KIT* mutations occurred predominantly in the small bowel. Furthermore, GISTs carrying *PDGFRA* mutations displayed an epithelioid or mixed phenotype, whereas *KIT*-mutated GISTs almost always exhibited a spindled or mixed histologic pattern.[32] The investigators also found that some mutations were located in the second kinase domain of *PDGFRA*, including 16 point mutations and 4 larger deletions of 9 to 12 bp. The occurrence of *PDGFRA* mutations was significantly associated with a higher frequency of epithelioid or mixed morphology and gastric location. These data indicate that GISTs can be conceptualized as distinctive subsets, differing in genetic, biologic, and morphologic features.[33] Additional studies have confirmed these findings.[16,19,34] The correlations between mutation and primary site by examining expression of *KIT* and *PDGFRA* in a large series of primary GISTs also confirmed the results. GISTs with *KIT* mutation had a significantly higher expression of KIT and at the same time a significantly lower expression of PDGFRA than GISTs with *PDGFRA* mutation. With regard to the site of the primary tumor, gastric GISTs had a significantly higher expression of *PDGFRA* and a significantly lower expression of KIT than intestinal GISTs. Although GISTs with higher *PDGFRA* expression constitute only a minority of gastric cases, the higher *PDGFRA* expression may contribute to the site-dependent clinical behavior of these tumors.[34,35]

In summary, exclusive gain-of-function *KIT* or *PDGFRA* mutations occur in a majority of GISTs; these include in-frame deletions, point mutations, duplications, and insertions. Mutation of the *KIT* juxtamembrane domain is the most common mutation site independent of the site of the primary tumor. Conversely, *KIT* extracellular domain *Ala502-Tyr503* duplication appears to be relatively specific for intestinal GISTs. Mutations in *PDGFRA* have been identified in juxtamembrane and tyrosine kinase domains, mostly in gastric GISTs and the epithelioid histologic variants.

Genetic Aberrations of Extragastrointestinal Stromal Tumors

Extragastrointestinal stromal tumor (EGIST) is a unique tumor that occurs outside the gastrointestinal tract. EGISTs have a histologic appearance similar to that of GISTs. Yamamoto et al examined the clinicopathologic features, prognostic factors, and *KIT* and *PDGFRA* mutations in 39 cases of EGIST. The *KIT* mutations were found in exon 11 in 12 of 29 cases and in exon 9 in 2 of 29 cases. The *PDGFRA* gene mutation was found at exon 12 and 18 in 1 case each. The pattern of *KIT* and *PDGFRA* mutation in EGISTs was essentially similar to that in GISTs, albeit at a lower frequency.[36]

Genetic Aberrations of GISTs in Other Tumors

GIST has an apparent association with other cancers, such as neurofibromatosis type 1 (NF1). NF1 is caused by mutations of the *NF1* gene, and patients with such mutations have an elevated risk of developing a GIST. In study of Kinoshita et al, none of the 29 GISTs derived from NF1 patients had detectable *KIT* mutations, and none of the 10 GISTs derived from patients without NF1 had detectable *NF1* mutations.[37] In what to our knowledge is the largest case analysis performed to date, by Miettinen et al, it was demonstrated that, in 45 patients who had NF1 and GISTs, none of the 16 tumors from 15 patients had a *KIT* mutation in exon 9, 11, 13, or 17 or a *PDGFRA* mutation in exon 12 or 18 as is typical in sporadic GISTs. These data clearly indicate that GISTs in NF1 patients have a different pathogenesis than sporadic GISTs.[38] Data from others confirm this, and they suggest that the molecular event underlying GIST development in this patient group may be a somatic inactivation, such as LOH of the wild-type *NF1* allele.

This leads to inactivation of neurofibromin and subsequent activation of the MAP-kinase pathway. Interestingly, the JAK-signal transducer activator of transcription 3 and phosphoinositide 3-kinase-AKT pathways were less activated in NF1-related GISTs than in sporadic GISTs.[39,40] Recently, Pasini et al reported a patient who had a unique combination of multiple fibrous polyps and lipomas of the small intestine and several gastric GISTs. The patient was found to carry a germline *PDGFRA* mutation (V561D) in the heterozygous state, which to our knowledge has been seen only rarely before and only in sporadic GISTs. CGH identified losses of chromosomal regions 1p33-36, 9q12-24, 11q13, and 16q.[41] Carney triad is an extremely rare syndrome with 3 types of tumors present, including GISTs, extra-adrenal paragangliomas, and pulmonary chondromas. Incomplete Carney triad cases have 2 of the 3 tumor types present, usually the GISTs and chondromas. Agaimy et al evaluated GISTs from 3 women with incomplete Carney triad for *KIT* and *PDGFRA* mutations and found all cases had wild-type *KIT* exons 9, 11, 13, and 17 and *PDGFRA* exons 12 and 18. CGH revealed 14 aberrations, including 11 gains (X, 1q, 5p, 8q, 9p, 12p, 13q, 18p, and 19q), 2 amplifications (1q, 19p), and 1 loss (13q). Carney triad-related GISTs not only lack conventional *KIT* and *PDGFRA* mutations, but they also lack the nonrandom loss of 14q and 22q characteristic of their sporadic counterparts, suggesting an origin through a distinct pathogenetic pathway.[42]

Prognostic Value of Genetic Aberrations

Singer et al evaluated the prognostic relevance of *KIT* mutations in a series of GISTs and determined that particular *KIT* mutation types are associated with prognosis. The independent predictors of disease-free survival were the presence of deletion/insertion exon 11 mutations, mixed histologic patterns, and male sex. These results suggest that *KIT* mutation and activation are important in GIST pathogenesis and also may provide important prognostic information.[15] In a population-based series involving long-term follow-up of 177 GIST patients not treated with imatinib, investigators found that *KIT* exon 11 deletions adversely affected outcome. It was suggested that *KIT* exon 11 deletion is an independent adverse prognostic factor in patients with GISTs.[43] Cho et al and others also reported that *KIT* mutation-positive GISTs showed more frequent liver metastases and higher mortality than *KIT* mutation-negative GISTs, which indicates that *KIT* mutations, especially deletions in exon 11, are markers of poor prognosis for preimatinib gastric GISTs.[44] Miettinen et al examined 906 patients with GISTs of the jejunum and ileum and found similar results.[45] Deletions affecting codons 557 to 558 are also relevant for the prognosis in GIST patients. This genetic alteration could be considered in prognostic stratification of GIST patients for randomized trials exploring imatinib mesylate in the adjuvant setting.[46]

Kikuchi et al suggested that LOH of the *KIT* gene is an important event that leads to imatinib resistance and metastatic progression of GISTs, which played an important role in aggressive tumor behavior and perhaps the process of liver metastasis.[47,48] What is more, Lasota et al documented shifting from heterozygosity to homozygosity of *KIT* exon 11 mutations during tumor progression in metastases, but not in primary GISTs.[49] At the same time, a small subset of GISTs with otherwise typical clinicopathologic and cytogenetic features did not express detectable KIT protein. When compared with KIT-positive GISTs, these KIT-negative GISTs are more likely to have epithelioid cell morphology, express *PDGFRA* oncogenic mutations, and arise in the omentum/peritoneal surface. Notably, some KIT-negative GIST contained imatinib-sensitive *KIT* or *PDGFRA* mutations; therefore, patients with KIT-negative GISTs should not, a priori, be denied imatinib therapy.[50]

Haller et al examined the prognostic relevance of the CDKN2A tumor suppressor pathway in GIST and found that low mRNA expression of the *CDKN2A* transcripts *p16* and *p14* but high mRNA expression of *CDK4*, *RB1*, *MDM2, TP53*, and *E2F1* was associated with aggressive clinical behavior and unfavorable prognosis. Univariate analysis revealed high expression of E2F1 to be associated with mitotic count, proliferation rate, *KIT* mutation, and aggressive clinical behavior. The findings implicate differential regulation schemes of the CDKN2A tumor suppressor pathway converging to up-regulation of E2F1 as a critical link to increased cell proliferation and adverse prognosis in GISTs.[51] Not only do the molecular genetic aberrations correlate with prognosis, but cytogenetic aberrations also have prognostic value in preimatinib GIST patients (Table 4).[26,52]

However, findings regarding the prognostic value of mutations of *KIT* and *PDGFRA* genes present different opinions. In 1 study of 134 Taiwanese GIST patients, *KIT*/*PDGFRA* mutations, 99% in *KIT* and 1% in *PDGFRA*, regardless of the location (exon 9 vs 11) and type (missense, insertion, or deletion, including deletion specifically involving codons 557 and 558), were not found to be significantly associated with a poor progression-free survival rate. Comparing overall survival in imatinib-treated patients, there was no significant difference between patients with exon 11

TABLE 4
Genetic Aberrations and Their Possible Significance in Gastrointestinal Stromal Tumors

Gene	Specific Aberrations	Potential Clinical Significance and Prognosis
KIT	Exon 11 deletions	Independent adverse prognostic factor in patients with GISTs
	Exon 11 mutations	Independent predictors for disease-free survival, mixed histologic pattern; more frequent liver metastasis; poor prognosis for gastric GISTs (preimatinib)
	Exon 11 duplication	Characteristic site: almost exclusively in gastric GISTs
	Exon 9 mutation	Characteristic site: almost exclusively in small intestinal GISTs
	Deletion of 557-558 codon	Prognostic value in choosing imatinib therapy
	LOH	Possible role in liver metastasis
PDGFR	Exon 18 mutation	Imatinib resistance
CDKN2A tumor suppressor pathway	p16, p14 low mRNA expression	Associated with aggressive clinical behavior and unfavorable prognosis
	CKK4, RB1, MDM2, TP53, E2F1 high mRNA expression	Associated with aggressive clinical behavior and unfavorable prognosis
	Up-regulation of E2F1	Increased cell proliferation and adverse prognosis in GISTs
P16	Negative expression	Worse prognosis and 2.3-fold relative increased risk of dying of disease
Hox11L1	LOH	Worse tumor-specific and recurrence-free survival rates

GIST indicates gastrointestinal stromal tumors; LOH, loss of heterozygosity.

mutation and those with exon 9 mutations.[53] This study raises the question of the prognostic significance of racial differences, which requires further investigation.

Genetic Alterations and Targeted Therapy

Because most GISTs have an activating mutation of *KIT* or *PDGFRA* tyrosine-kinase receptors, imatinib, a selective inhibitor of ABL, KIT, and PDGFR tyrosine kinases, provides a clinical benefit in most patients with advanced GISTs.[54,55] The proteins inhibited by imatinib and other inhibitors of specific tyrosine kinases are shown in Table 5. Imatinib treatment markedly inhibited KIT, MAP, and Akt phosphorylation in all transfectants, leading to reduced glucose uptake via decreased levels of plasma membrane-bound Glut4 and induction of apoptosis and/or growth arrest.[56] Long-term results from a randomized phase 2 trial by Blanke et al demonstrated that nearly 50% of patients with advanced GISTs who were treated with imatinib survived for >5 years, regardless of whether a 400-mg/day or 600-mg/day starting dose was used.[57] Chen et al found the effect of imatinib on KIT(820Tyr) was weaker than that on KIT(del559-560) or KIT(642Glu), indicating various biologic effects of imatinib on GISTs that have different *KIT* and *PDGFRA* mutational settings.[58] In the recently published randomized European Organization for Research and Treatment of Cancer phase III trial study, tumors with mutation in *KIT* exon 11 showed response rates of up to 80%, whereas <50% of tumors with mutation in *KIT* exon 9 responded.[59] The presence of exon 9-activating mutations in *KIT* was the strongest adverse prognostic factor for response to imatinib, increasing the relative risk of progression by 171% and the relative risk of death by 190% when compared with *KIT* exon 11 mutants. Similarly, the relative risk of progression was increased by 108% and the relative risk of death by 76% in patients without detectable *KIT* or *PDGFRA* mutations. In patients whose tumors expressed an exon 9 KIT oncoprotein, treatment with the high-dose regimen (800 mg/d) resulted in a significantly superior progression-free survival, with a reduction of the relative risk of 61%. GISTs without detectable *KIT* mutation in either of these exons often are resistant to imatinib.[59] From these data, it is apparent that therapeutic insight can be gained by genotyping of *KIT* and *PDGFRA* before imatinib therapy.

As more experience with imatinib has accumulated, primary and secondary resistance to this agent is becoming a major clinical challenge. As many as 40% of patients with GISTs develop secondary resistance to imatinib, which often is because of secondary *KIT* mutations occurring in addition to the primary mutation.[56] Chen et al reported, for what to our knowledge is the first time, the presence, after imatinib treatment, of an additional specific and novel *KIT* mutation in imatinib-resistant GIST. They studied 12 GIST patients with initial near-complete response to imatinib. Seven harbored mutations in *KIT* exon 11, and 5 harbored mutations in exon 9. Within 31 months, 6 rapidly progressive, imatinib-resistant peritoneal implants developed in 5 of these patients. All 6 imatinib-resistant implants demonstrated an identical novel *KIT* missense mutation,

TABLE 5
Proteins Regulated by Imatinib and Other Inhibitors of Specific Tyrosine Kinases and Downstream Effects

Target Protein	Downstream Signaling Factors		Potential Functions
BCR-ABL↓	PI3K, AKT	BAD, MYC	Promote apoptosis
	BAD	BCL-XL, BCL-2	Induce apoptosis
	JAK2	STAT	Prevent cell proliferation
	GRB-2	SOS, RAS, RAF, MEK, ERKs, P90RSK, MSK, ELK-1, STAT	Prevent cell proliferation
	STAT	—	Prevent cell proliferation
Caspase-3↑	PARP, DNA-PK, SRE/BP, rho-GDI, et al	—	Cell apoptosis
p38↑	PLA2, MNK1, PRAK, Hsp27, STAT1, ELK-1	—	Inhibit proliferation and induce apoptosis by activation of p38 MAPK pathways
Exogenous IL-6↓	—	—	Inhibit KIT signaling pathway activated by exogenous interleukin-6
ERK1/2↓	p90RSK, MSK, ELK-1, PLA2, MNK1, PRAK, Hsp27, STAT	—	Induce apoptosis and inhibit proliferation by down-regulation of ERK pathways
ERK5↓	—	—	Inhibit proliferation and induce apoptosis by down-regulation of ERK5 pathways
Kit↓	Decreased autophosphorylation by competing at ATP-binding site	—	Down-regulation of activation of ERK1/2 and AKT, induce apoptosis
PDGFRA↓	—	—	Down-regulation of activation of ERK1/2 and AKT, induce apoptosis
C-ABL↓	—	—	Prevent cell proliferation, induce apoptosis
SPRY4A, FZD8, PDE2A, RTP801, FLJ20898, and ARHGEF2↓	—	—	Prevent cell proliferation, induce apoptosis
MAFbx↑	—	—	Prevent cell proliferation, induce apoptosis

1982T → C, which resulted in V654A in *KIT* tyrosine kinase domain 1. This novel mutation was not present in preimatinib or postimatinib residual quiescent GISTs, and was strongly correlated with imatinib resistance. Allele-specific sequencing data indicated that this new mutation occurred in the allele that harbored the original activating mutation of *KIT*, suggesting that resistance emerged under the selective pressure of imatinib.[60] Tamborini et al also reported a novel point mutation in *KIT*, in exon 14, which resulted in T670I substitution. Functional analyses showed that *KIT* T670I is insensitive to imatinib, and that introduction of this mutation into a receptor responding to imatinib subverted its sensitivity to the drug.[61] Debiec-Rychter et al performed a cytogenetic analysis and screened for mutations of the *KIT* and *PDGFRA* kinase domains in 26 resistant GISTs. Six distinct secondary *KIT* mutations were detected in 12 progressive tumors; of these, V654A and T670I were frequent. One progressive tumor was found to have an acquired *PDGFRA* D842V mutation. GIST cells carrying *KIT*-del557-558/T670I or *KIT*-insAY502-503/V654A mutations were resistant to imatinib; PKC412 significantly inhibited autophosphorylation of these mutants. Resistance to imatinib and sensitivity to PKC412 of *KIT* T670I and *PDGFRA* D842V mutants was confirmed using Ba/F3 cells.[62]

Multiple studies indicate several point mutations involving secondary mutation in the kinase domain of *KIT*, including T670I, T823A, V654A, and other sites of exons 13, 14, 17, and 18, conferring imatinib resistance in GISTs. Furthermore, secondary mutations T670I and V654A confer imatinib-acquired resistance, and the former is more resistant to imatinib than the latter.[63-69] Heinrich et al found that imatinib-resistant tumors had levels of activated KIT that were similar to or greater than those typically found in untreated GISTs. Secondary kinase mutations were rare in GISTs with primary resistance but frequent in GISTs with secondary resistance. Evidence for clonal evolution and/or polyclonal secondary kinase mutations was noted in 3 of 16 patients. Secondary kinase mutations were nonrandomly distributed and were significantly more associated with decreased imatinib sensitivity than typical *KIT* exon 11 mutations. By using RNA interference technology, these investigators demonstrated that imatinib-resistant GIST cells remain dependent on KIT kinase activity for activation of critical downstream signaling pathways. From these data, it is clear that different molecular mechanisms are usually responsible for primary and secondary imatinib resistance in GISTs.[70]

In the study of Desai et al, a unique "resistant clonal nodule" pattern (defined as a new enhancing

nodular focus enclosed within a pre-existing tumor mass) was observed in 23 of 48 patients and was believed to represent the emergence of clones resistant to imatinib. This investigation revealed new activating kinase mutations in 80% (8 of 10) of the examined tumors. The resistant clonal nodule is a unique pattern of disease progression observed in patients with GISTs after an initial response to imatinib and reflects emergence of imatinib-resistant clones. A new enhancing nodule growing within a pre-existing tumor mass should be classified as a new lesion and be regarded as at least partial progression of GIST.[71]

All of these data highlight the potential mechanisms of resistance to imatinib and would be useful in clinical treatment, but to our knowledge the precise molecular mechanisms of this drug resistance are not well understood. Mahadevan et al found that the imatinib-resistant GIST cell line (GIST-R) developed from the imatinib-sensitive (GIST-S) GIST882 cell line acquires imatinib resistance by overexpressing the oncogenic RTK AXL in a "kinase switch." Real-time polymerase chain reaction and Western blot analysis of the GIST-S and GIST-R cells confirmed the switch from KIT to AXL. This switch is associated with a morphologic change from spindle to epithelioid histologic pattern. Molecular modeling of the kinase domain of mutant *KIT* exon 13 (V654A) and AXL showed no binding to imatinib.[72] From the present reports regarding drug resistance of GIST, Tarn et al considered that the phenomenon of resistance to treatment, which arises primarily through selection for secondary mutations in GISTs, could also involve amplification or activation of other RTKs.[73] Alteration of drug sensitivity can be fought by specific RTK inhibitors, and RTK activation involves a transduction pathway whose components (mitogen-activated protein kinase, AKT, phosphoinositide 3-kinase, mammalian target of rapamycin, and RAS) are possible targets of new molecular treatment. A new paradigm of classification integrating the classic pathologic criteria with the molecular changes may eventually facilitate more personalized prognosis and treatment.[74] Furthermore, there are patients with primary GISTs who lack mutations in either *KIT* or *PDGFRA*, or express "imatinib-resistant" mutations in these genes. These tumors typically do not respond well to imatinib therapy. The use of "second-generation" KIT and PDGFRA inhibitors is still in its early stages, but is promising, because some of these drugs use alternative molecular mechanisms that may be able to counter primary or secondary resistance to imatinib. The use of rational combinations of inhibitors to prevent or slow the development of resistance may be useful. This may not be the complete solution, and identifying additional genetic factors that contribute to the pathogenesis of GISTs, independent of KIT and PDGFRA, may be important in developing additional anti-GIST therapies. New drugs that can serve as alternative therapies in imatinib-resistant GISTs or that can be used in combination with imatinib are needed.

Conclusions

GIST is the most common malignant mesenchymal tumor in the gastrointestinal tract and is typically characterized by specific *KIT* or *PDGFRA* gene mutations. In addition to *KIT* and/or *PDGFRA* mutation, other genetic events are likely involved in tumorigenesis. Described cytogenetic aberrations include loss of 1p, 13q, 14q, 15q, and 22q; chromosomal numerical and imbalance aberrations; and nuclear/mitochondrial microsatellite instability. Other molecular genetic aberrations include LOH of *p16(INK4A)* or *p14(ARF)*; methylation of *p15(INK4B)*; homozygous loss of *Hox11L1*; amplification of *C-MYC*, *MDM2*, *EGFR1*, and *CCND1*; and others. GISTs in patients with NF1 seem to lack *KIT* and *PDGFRA* mutations and appear to have a different molecular mechanism. Molecular genetic aberrations of the *KIT* or *PDGFRA* gene are correlated not only with the specific cell morphology, metastasis, and prognosis, but also with the efficacy of targeted therapy, especially imatinib. Because the biologic effects of imatinib vary with the site of *KIT* and *PDGFRA* mutation in GISTs, genotyping can be helpful in guiding aspects of therapy with imatinib or other related inhibitors. The mechanisms of acquired resistance to imatinib in GISTs include secondary mutation of *KIT* and *PDGFR* and possibly amplification or activation of other RTKs. Primary or secondary mutations in the kinase domain of *KIT* involving imatinib resistance include V654A, T670I, T823A, del557-558,[74] insAY502-593, and other sites of exon 9 (partial resistance), 13 and 17 in the kinase domain of *KIT*, and D842V in *PDGFRA*. Alteration of imatinib sensitivity can be fought by specific RTK inhibitors, and a new paradigm of classification integrating the classic pathologic criteria with the molecular changes will facilitate personalized prognosis and treatment.[17,73]

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