Molecular Correlates of Imatinib Resistance in Gastrointestinal Stromal Tumors

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ABSTRACT

Purpose
Gastrointestinal stromal tumors (GISTs) commonly harbor oncogenic mutations of the KIT or platelet-derived growth factor alpha (PDGFRA) kinases, which are targets for imatinib. In clinical studies, 75% to 90% of patients with advanced GISTs experience clinical benefit from imatinib. However, imatinib resistance is an increasing clinical problem.

Patients and Methods
One hundred forty-seven patients with advanced, unresectable GISTs were enrolled onto a randomized, phase II clinical study of imatinib. Specimens from pretreatment and/or imatinib-resistant tumors were analyzed to identify molecular correlates of imatinib resistance. Secondary kinase mutations of KIT or PDGFRA that were identified in imatinib-resistant GISTs were biochemically profiled for imatinib sensitivity.

Results
Molecular studies were performed using specimens from 10 patients with primary and 33 patients with secondary resistance. 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 frequently found in GISTs with secondary resistance (10% vs 67%; P = .002). Evidence for clonal evolution and/or polyclonal secondary kinase mutations was seen in three (18.6%) of 16 patients. Secondary kinase mutations were nonrandomly distributed and were associated with decreased imatinib sensitivity compared with typical KIT exon 11 mutations. Using RNAi technology, we demonstrated that imatinib-resistant GIST cells remain dependent on KIT kinase activity for activation of critical downstream signaling pathways.

Conclusion
Different molecular mechanisms are responsible for primary and secondary imatinib resistance in GISTS. These findings have implications for future approaches to the growing problem of imatinib resistance in patients with advanced GISTs.


INTRODUCTION

Gastrointestinal stromal tumors (GISTs) are the most common type of sarcoma arising in the digestive tract and are generally distinguished from other abdominal sarcomas by the expression of KIT receptor tyrosine kinase. This kinase is important not only as a diagnostic marker for GISTs, but serves as a primary oncogene in approximately 80% of these tumors, as evidenced by activating mutations of the KIT gene.

Imatinib (Gleevec; Novartis Pharmaceuticals, Basel, Switzerland) inhibits the enzymatic activity of several oncogenic tyrosine kinases, including KIT and platelet-derived growth factor alpha (PDGFRA). In clinical studies, 75% to 90% of patients with advanced GISTs treated with imatinib experienced a clinical benefit (stable disease + complete/partial objective response).1-3 These imatinib-induced responses correlated with tumor kinase mutational status.4,5 Patients with KIT exon 11-mutant GIST have a higher response rate and a significantly longer median survival compared with patients with exon 9-mutant GISTs, and those whose GISTs lack KIT or PDGFRA mutations.4

Although imatinib has revolutionized the treatment of advanced GISTs, clinical resistance to this drug has proved to be a significant problem with more prolonged follow-up. Secondary kinase mutations acquired during imatinib treatment have been reported in several small series.6-15 In this study we evaluated molecular
markers of imatinib resistance in a series of well-documented GIST samples from a phase II study of imatinib.

**PATIENTS AND METHODS**

**Patient Materials**

Tumor samples were obtained from patients enrolled in the CSTI571 B2222 phase II trial of imatinib (sponsored by Novartis, Basel, Switzerland) for the treatment of advanced GIST. Of 147 original patients, 92 had documented disease-related treatment failure as of May, 2005. Samples were obtained from 43 progressive-disease patients who consented to analysis of their tumor. Per the clinical protocol, tumor progression was defined according to traditional Southwest Oncology Group response criteria. Specifically, progressive disease was defined as either a 50% or greater increase in the sum of the products of all measurable lesions over the smallest sum observed (or baseline if no decrease was observed); clear worsening from previous examinations of any assessable lesion; reappearance of any lesion that had disappeared; appearance of a new lesion; or the failure to return for evaluation due to death or deteriorating clinical condition. The appearance of a nodule within a mass on follow-up imaging studies was not considered to meet the protocol-specified definition of progression. The study was approved by the local institutional review board of each participating institution, and written informed consent was obtained from each patient. In addition, informed consent for the analysis of tumor-associated genetic alterations was obtained independently of patient consent for participation in the clinical study.

Imatinib response correlates were obtained using paired GIST biopsies taken during the week before initiation of imatinib and biopsies taken during the first week of therapy. Imatinib resistance correlates were evaluated in biopsies taken at the time of GIST progression while patients were still receiving imatinib. Routine pathology review, including KIT immunostaining, was performed on all biopsies.

**Reagents and Cell Lines**

The GIST430 and GIST48 cell lines were established from imatinib-resistant GISTs. Site-directed mutagenesis was used to generate relevant single, double, or triple mutations of KIT or PDGFRA cDNA. Experiments involving recombinant DNA were performed using biosafety level 2 conditions in accordance with National Institutes of Health Guidelines for Research Involving Recombinant DNA Molecules. Imatinib mesylate was purchased from the Oregon Health & Science University Hospital pharmacy (Portland, OR).

**KIT Short Hairpin RNA Studies**

A KIT lentiviral short hairpin RNA (shRNA) was obtained from William Hahn, MD, PhD (Dana-Farber Cancer Institute, Boston, MA, and Broad Institute RNAi consortium, Cambridge, MA). This shRNA was assembled by ligating KIT forward 5'-CCGGCCATAAGGTTTCGTTACTCGAGTACAGAAACGGAAGAGCCGAAGGACAGAC-3' and reverse 5'-AATTCCTCAAGGTTTCGTTACTCGAGTACAGAAACGGAAGAGCCGAAGGACAGAC-3'.

**Immunoblotting Studies**

Whole cell lysates were prepared from GIST biopsies or GIST cell lines. The quality of each lysate was determined by immunoblotting for the GIST markers KIT and PDGFRA, as described previously. Immunoblotting was performed as described previously. In addition, hamster monoclonal anti-bcl-2 (the kind gift of Stan Korsmeyer, MD, Dana-Farber Cancer Institute) was utilized in the current report. Protein expression studies of mutant KIT or PDGFRA isoforms were performed as previously described by transient transfection of Chinese Hamster ovary cells. The transfected cells were treated with various concentrations of imatinib before preparation of protein lysates. KIT protein was assayed for activation status (phosphorylation) by Western blot analysis and detection using appropriate antibodies.

### Table 1. PCR Primers Used for Detection of Mutations of KIT or PDGFRA

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Abbreviations: PDGFRA, platelet-derived growth factor alpha; D-HPLC, denaturing high-performance liquid chromatography; K, KIT exon; NA, not available; P, PDGFRA exon.
immunoprecipitation using an anti-KIT antibody, followed by sequential immunoblotting for phosphoKIT (using antiphosphotyrosine antibody) or total KIT (anti-KIT monoclonal antibody). In the case of PDGFRA mutations, an identical methodology was used except for substitution of an anti-PDGFRA antibody for the immunoprecipitation and total PDGFRA immunodetection steps. IC_{50} (50% inhibitory concentration) was measured by densitometry of the phosphoKIT bands and normalization using total KIT expression (to correct for variations in loading of KIT protein in the various lanes).\textsuperscript{4,17,18}

**Mutation Detection Methods**

Mutational analyses were performed on genomic DNA extracted from paraffin embedded or fresh frozen tumor tissue using a combination of polymerase chain reaction (PCR) amplification, denaturing high-performance liquid chromatography (D-HPLC) screening, and automated sequencing, as described previously.\textsuperscript{4,17,22} PCR primer pairs and D-HPLC conditions are listed in Table 1.

**PCR Evaluations of the Allelic Relationships of Primary and Secondary Mutations**

Allelic relationships of primary and secondary KIT mutations were evaluated by reverse transcriptase-PCR (exons 8-18) or genomic PCR (exons 11 to 13) using primer pairs listed in Table 1. The resultant PCR products were cloned and individual bacterial colonies were bidirectionally sequenced.\textsuperscript{23}

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**RESULTS**

**Description of Patient Population**

The study population consisted of 147 patients treated in a randomized, phase II study of imatinib for patients with advanced GISTs.\textsuperscript{2,24} As of May 2005, the median time to treatment failure for the entire phase II study population was 19.3 months and the median survival was estimated to be 57 months. Late treatment failure was primarily due to disease progression rather than discontinuation of therapy related to medication intolerance.

**Secondary Resistance**

Treatment failures were divided into two groups. Patients who never achieved a partial remission and showed continued tumor growth during the first 180 days of treatment were defined as having primary imatinib resistance. Disease progression after a minimum of 6 months of partial remission or stable disease was defined as secondary imatinib resistance. This study is based on comparative analyses of tumor samples obtained from both these groups before and after treatment failure.

**KIT Kinase Is Inhibited by Imatinib in Responding GISTs, but Not in Tumors With Primary Resistance**

Activation of KIT was evaluated in fresh frozen metastatic GIST lesions from two patients (11 and 34) who underwent biopsy at the week before starting imatinib therapy and again after 5 or 7 days of treatment (Fig 1 and Table 2). Both patients had GISTs with KIT exon 11 mutations. KIT phosphorylation was evident in the pretreatment biopsies, but became nearly undetectable during the first few days of imatinib therapy. KIT inhibition was accompanied by dramatic decreases in the phosphorylated forms of mTOR, AKT, and mitogen-activated protein kinase (MAPK). Imatinib therapy also decreased expression of a proliferation marker (PCNA) and a critical antiapoptotic protein (bcl-2).

We analyzed the activation status of KIT-dependent signaling pathways in biopsies obtained from two patients whose GISTs had a KIT exon 9 mutation and showed primary imatinib resistance (Fig 2A and Table 3). Frozen tumor samples were not available from either patient pretreatment; nevertheless, on-treatment biopsies contained phosphorylated KIT protein in quantities equivalent to that typically present in untreated GIST specimens.\textsuperscript{21} The pattern of intracellular signaling was similar to that seen in untreated GISTs (Fig 1 and Duensing et al\textsuperscript{21}), indicating that primary resistance is associated with persistent KIT phosphorylation and activation of downstream AKT and MAPK pathways.

**Reactivation of KIT and KIT-Dependent Signaling in Secondary Resistance**

Two patients who had objective responses to imatinib underwent tumor biopsy after documented progression at 18 and 32 months. Matched pretreatment biopsies were available for both patients (Fig 2B and Table 2). Patient 1 had a primary KIT exon 11 deletion. Patient 14 had two activating KIT mutations in multiple pretreatment specimens (K642E and N822K). This is the only such example of double mutations in our series of more than 1,000 imatinib-naïve patients with GISTs.\textsuperscript{17,25} We speculate that one mutation was the initiating mutation and the other developed during malignant progression. The imatinib-resistant tumors contained abundant amounts of activated KIT. Notably, the ratio of phosphorylated-AKT (P-AKT) to AKT was markedly increased in the progression samples.
compared with matched pretreatment samples. In contrast, MAPK was only significantly activated in one of the progression samples compared with matched pretreatment samples. In contrast, MAPK was only significantly activated in one of the progression samples.

**Secondary Kinase Mutations Are Common in Secondary but Not Primary Imatinib Resistance**

To determine whether imatinib resistance is associated with acquired (secondary) kinase mutations, we screened the cytoplasmic domain exons of KIT and PDGFRα in DNA prepared from 79 tumor samples obtained from 43 patients with primary or secondary drug resistance (Tables 2 and 3). In 90.7% of patients (39 of 43), the pretreatment kinase genotype had been previously determined.

In 10 patients with primary resistance the median time to treatment failure was 3.6 months (range, 0.8 to 5.8; Table 3). No secondary mutations of KIT or PDGFRα kinase domain were found in specimens obtained from these patients at the time of progression. Patient 39 had primary progression on a dose of 400 mg of imatinib. No secondary kinase mutations were found at the time of progression on 400 mg, and cross-over to 600 mg led to disease stabilization. Twenty-three months later, the patient had tumor progression, and ultimately underwent surgical debulking. Specimens from this procedure had multiple secondary kinase mutations (Table 2 and Fig 3). This frequency is distinctly higher than that in patients with primary resistance (10% vs. 67%; Table 2 and Fig 3).

In thirty-three patients with secondary resistance, the median time to treatment failure was 20.2 months (range, 7.2 to 52.7). Sixty-seven percent of 33 patients with secondary resistance had one or more secondary kinase mutations (21 KIT and 1 PDGFRα; Table 2 and Fig 3). This frequency is distinctly higher than that in patients with primary resistance (10% vs. 67%; 𝑃 = 0.002). Notably, all secondary KIT kinase mutations were found in GISTs with an
underlying primary KIT mutation and these secondary mutations were not present in pretreatment specimens. No secondary mutations were identified in one GIST lacking a primary KIT or PDGFRA mutation. In addition, the only secondary PDGFRA mutation identified was in a GIST with a primary PDGFRA V561D mutation. The secondary KIT kinase mutations were nonrandom and involved either the adenosine triphosphate (ATP) binding pocket of the kinase domain (V654A, T670I) or the kinase activation loop (C809G, D816H, D820A/E/G, N822K/Y, Y823D). Figure 3 summarizes the spectrum and frequency of secondary KIT mutations in this series and other published reports.

In contrast to primary resistance, two of three KIT exon 9 mutant GISTs were found to have additional KIT kinase mutations in biopsies obtained after the development of secondary imatinib resistance. However, the frequency of secondary KIT mutations in KIT exon 9 mutant GISTs was not obviously different between cases with primary resistance versus secondary resistance (1 of 7 v 2 of 3; P = .18).

Evidence for Clonal Evolution and/or Polyclonal Secondary Resistance Mutations in Individual Patients

Multiple biopsy and/or resection specimens were available from 16 patients with progressive disease on imatinib. In three of these
patients (3 of 16; 18.8%), we found evidence of clonal evolution and/or polyclonal secondary kinase mutations (Tables 2 and 4).

Patient 5 had a baseline KIT V560D substitution on one allele and a single adenine base insertion in KIT exon 13 of the other allele, resulting in a frameshift and protein truncation. Thus, this patient’s tumor was functionally homozygous due to loss of the wild-type allele. In specimens obtained at the time of imatinib resistance, a V654A mutation was present in two biopsy specimens and D816H in a third specimen. All three specimens had the original preimatinib KIT exon 11 and 13 mutations.

**Allelic Distribution of Secondary Kinase Mutations**

Most KIT mutant GISTs (>90%) are heterozygous for the original activating mutation and therefore have one wild-type and one mutant KIT allele. Without exception, all secondary mutations were on the same allele as the primary mutation (Table 4). As noted above, patient 14 (Tables 2 and 4) had a GIST with two monoallelic preimatinib KIT mutations (K642E and N822H). An acquired C809G substitution found at the time of imatinib resistance was on the same allele as the K642E and N822H mutations.

**Secondary Kinase Mutations Lead to Imatinib Resistance In Vitro**

To determine if secondary kinase mutations were the cause of clinically observed imatinib resistance, we tested the in vitro sensitivity of selected single, double, or triple mutant kinases to imatinib. Because GISTs manifest a wide range of mutations in exon 11 (Table 2), we chose a representative KIT exon 11 point mutation, V560D, as the standard for comparing imatinib sensitivity of single and double mutant KIT kinases. Secondary mutations of interest (for example, V654A) were then engineered into the V560D isoform as well as into a wild-type cDNA, allowing comparative analyses of the effects of secondary mutations on imatinib sensitivity.

Several of the secondary KIT mutations identified in our series have been previously identified in human cancers: D816H, D820A, N822H/K, and Y823D. In contrast, there are no reports of V654A, T670I, or C809G mutations occurring as a primary mutation. The isolated KIT V560D mutation was very sensitive to imatinib (IC\textsubscript{50} = 100 nmol/L; Fig 4). Both V654A and T670I resulted in strong imatinib resistance, with IC\textsubscript{50} of 5 and 10 \(\mu\text{mol/L}\), respectively—either in isolation or when coexpressed with KIT V560D. Similar results were obtained using KIT exon 9 as the primary mutation (Fig 4).

A number of amino acids located in or near the KIT activation loop were mutated in imatinib-resistant tumors (Fig 3 and Table 2). In vitro profiling of isolated activation loop mutations revealed a spectrum of imatinib sensitivity ranging from relatively sensitive (IC\textsubscript{50} of 100-200 nmol/L, N882K, Y823D) to highly resistant (IC\textsubscript{50} > 5,000 nmol/L, D816H). When coexpressed with V560D, all of the double mutant kinases were extremely resistant to imatinib, with the exception of V560D + N822K which was only moderately imatinib resistant (Fig 5A). Similar results were obtained using KIT exon 9 as the primary mutation (Fig 5B). Notably, both primary mutations were intrinsically imatinib sensitive, but
coexpression of D820G with KIT exon 9 resulted in moderate imatinib resistance. Coexpression of the imatinib-sensitive K642E mutation with D816H resulted in extreme imatinib resistance.

A doubly mutant (K642E + N822H) kinase modeled on patient 14 was moderately resistant to imatinib (IC_{50} of \approx 2 \mu mol/L). Notably, patient 14 received a dose of 600 mg daily of imatinib and experienced an objective partial response that lasted 500 days. The C809G mutation found at the time of progression proved resistant in vitro (IC_{50} > 5 \mu mol/L), both in isolation and when coexpressed with KIT K642E + N822H.

In patient 7, a primary PDGFRA V561D mutation (known to be sensitive to imatinib) was accompanied by a PDGFRA D842V mutation at the onset of progression. The doubly mutant V561D + D842V kinase proved imatinib-resistant with an IC_{50} similar to the isolated D842V isoform (Fig 6).

**GIST Cell Lines With Secondary Kinase Mutations Have In Vitro Imatinib Resistance**

To further validate our biochemical studies, we generated cell lines from two patients with secondary imatinib resistance (neither patient was part of the phase II study). The GIST48 cell line has a combination of mutations (homozygous V560D + heterozygous D820A) similar to that documented in samples from patient 19. Concentrations of imatinib higher than 1 \mu mol/L were required for complete inhibition of KIT activation in this cell line (Fig 7A). This concentration is 10-fold greater than that necessary to block exon 11 mutant isoforms of KIT in GIST cells.8,21,31 Activation of AKT was partially but not completely inhibited by imatinib doses of 0.1 to 5 \mu mol/L.

The GIST430 cell line is heterozygous for a KIT exon 11 deletion mutation and the V654A substitution (both on the same allele). Eight samples from imatinib-resistant tumors in our study had a similar combination of KIT exon 11 and V654A mutations. The doubly mutant KIT isoform expressed by GIST430 cells was imatinib resistant (IC_{50} of \approx 2.5 \mu mol/L; IC_{90} of > 5 \mu mol/L). AKT activation in this cell line was only partially inhibited by imatinib doses higher than 2.5 \mu mol/L (Fig 7A).

We used RNAi to determine whether KIT expression was still required for activation of signaling pathways in imatinib-resistant GIST cells. shRNA knockdown of total KIT expression in the previously described imatinib-sensitive GIST882 cell line (homozygous KIT K642E mutation)31 resulted in parallel decreases in phospho-KIT, phospho-AKT, phospho-p70S6K, and the proliferation marker cyclin A expression (Fig 7B). In contrast, there was no change in the expression of p70S6K, AKT, or PI3K. Comparable findings were obtained after KIT shRNA knockdown in GIST430 cells, demonstrating that activation of proliferation/survival signaling pathways remains KIT dependent in this imatinib-resistant cell line. KIT knockdown in the cell lines also induced flow-cytometric evidence for G1 block, decreased S phase, and markedly increased apoptosis (data not shown).

**DISCUSSION**

We analyzed genomic mechanisms of imatinib resistance in a cohort of patients that were part of a randomized phase II study of imatinib for treatment of advanced, unresectable GIST. Forty-three (46.7%) of 92 patients with disease-related treatment failures consented to studies of their tumor samples. The resulting collection of 83...
specimens from well-monitored patients receiving standardized imatinib treatment is the largest examined to date for molecular mechanisms related to drug resistance.

Imatinib-resistant GISTs show activation of the same signaling pathways that are operative in untreated GISTs. Interestingly, primary imatinib resistance is infrequently associated with secondary kinase mutations (1 of 10; 10%). PDGFRA D842V and KIT exon 9 mutations were significantly over-represented in this group compared with secondary imatinib resistance. GISTs with these genotypes are less responsive to imatinib therapy than GISTs with an associated KIT exon 11 mutation. The PDGFRA D842V mutation has moderate to high level in vitro resistance to imatinib explaining why this mutation was responsible for primary imatinib resistance in the two patients with a primary PDGFRA D842V mutation. This conclusion is strengthened by our

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**Fig 5.** Biochemical characterization of secondary mutations involving the KIT activation loop. Secondary mutations involving the KIT activation loop were tested for in vitro sensitivity to imatinib when expressed in (A) isolation or when coexpressed with the KIT exon 11 V560D or (B) in isolation or coexpressed with KIT exon 13 K642E or KIT exon 9 mutations. P-, phosphorylated.

**Fig 6.** Platelet-derived growth factor alpha (PDGFRA) D842V mutation is associated with primary and secondary imatinib resistance. Imatinib potently inhibits the phosphorylation of the primary PDGFRA exon 12 V561D mutation (patient 17, pretreatment), but not PDGFRA D842V—either expressed in isolation (patients 1 and 10) or when coexpressed with V561D (patient 17, progression specimen).
observation that the PDGFRA D842V mutation was associated with secondary imatinib resistance in a GIST harboring a primary PDGFRA V561D mutation. Debiec-Rychter et al also reported a case of imatinib-resistant GIST associated with acquisition of a PDGFRA D842V mutation in a GIST with a primary KIT exon 11 mutation.

The molecular mechanisms underlying primary resistance in GISTs with KIT exon 9 mutations are as yet unidentified, but may be related to differences in the underlying biology of this subset of GISTs. We speculate that some KIT exon 9 mutant GISTs have an alternative mechanism of KIT activation that does not require the enzymatic activity of KIT. Notably, imatinib only binds to the inactive form of KIT. Therefore,
KIT-independent mechanisms of KIT phosphorylation could stabilize the protein in an active, imatinib-resistant conformation. GISTs with KIT exon 11 mutations commonly become imatinib resistant due to acquisition of secondary kinase mutations located on the same allele as the original gain of function KIT mutation. These secondary kinase mutations appear to be the predominant mechanism for late imatinib resistance in KIT exon 11 mutant GISTs. Fluorescence in situ hybridization assays (data not shown) revealed low-level KIT amplification (3 to 4 copies per cell), accompanied by KIT protein overexpression, in only two patients, suggesting that genomic KIT amplification has only a minor role in GIST imatinib resistance. We believe that the difference in the frequency of secondary mutations between GISTs with underlying KIT exon 11 versus KIT exon 9 mutations is reflective of the underlying biology of these tumors—namely, that KIT exon 11 mutant GISTs are more addicted to KIT signaling than KIT exon 9 mutant GISTs. In this model, KIT exon 9 mutant GISTs are able to use alternative signaling mechanisms to drive cellular proliferation. In contrast, KIT exon 11 mutant GISTs are more restricted in their signaling requirements and the most common mechanism of resistance is expansion of a clone with an imatinib-resistant kinase mutation. Using RNAi KIT knockdown, we demonstrated that imatinib-resistant GIST cell lines with secondary kinase mutations remain dependent on KIT signaling for growth and survival. These findings have implications for future approaches to the growing problem of imatinib resistance in patients with advanced GISTs.

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REFERENCES

Authors’ Disclosures of Potential Conflicts of Interest

Although all authors completed the disclosure declaration, the following authors or their immediate family members indicated a financial interest. No conflict exists for drugs or devices used in a study if they are not being evaluated as part of the investigation. For a detailed description of the disclosure categories, or for more information about ASCO’s conflict of interest policy, please refer to the Author Disclosure Declaration and the Disclosures of Potential Conflicts of Interest section in Information for Contributors.

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Dollar Amount Codes

(A) < $10,000 (B) $10,000-99,999 (C) = $100,000 (N/R) Not Required

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GLOSSARY

AKT: A transforming serine-threonine kinase involved in cell survival.

bcl2: First discovered as a translocated locus [t(14;22)] in B-cell leukemias, bcl-2 is an antiapoptotic protein that protects cells from programmed cell death by preventing the activation of proapoptotic caspase proteins.

KIT: A member of the PDGFR family, c-kit is a tyrosine kinase receptor that dimerizes following ligand binding and is autophosphorylated on intracellular tyrosine residues.

MAPK (mitogen-activated protein kinase): MAPKs are a family of enzymes that form an integrated network influencing cellular functions such as differentiation, proliferation, and cell death. These cytoplasmic proteins modulate the activities of other intracellular proteins by adding phosphate groups to their serine/threonine amino acids.

PDGFRA (platelet-derived growth factor alpha): The receptor for PDGF exists distinctively as the dimeric αα or ββ form. All dimer combinations of PDGF A and B signal through PDGFR-αα; PDGF BB signals through PDGFR-ββ; PDGF CC signals through the αα and αβ receptors; and PDGF DD signals through the ββ and αβ receptors.

PKC theta (PRKCQ): A member of the protein kinase C family. PKC theta is highly expressed by T cells, interstitial cells of cajal, and gastrointestinal stromal tumors.

RNAi: Post-transcriptional gene silencing that regulates gene expression.

shRNA (short hairpin RNA; short interfering hairpin): shRNA contains sense and antisense sequences from a target gene connected by a loop, and is expressed in mammalian cells from a vector by a pol III-type promoter. The shRNA is transported from the nucleus into the cytoplasm, where Dicer processes it. Once in the cell, the shRNA can decrease the expression of a gene with complementary sequences by RNAi.

Tyrosine kinase: Generic name for enzymes that transfers a terminal phosphate group from ATP to specific protein tyrosine residues. Tyrosine kinases are a member of the family of protein kinases.