Improved Insight into Resistance Mechanisms to Imatinib in Gastrointestinal Stromal Tumors: A Basis for Novel Approaches and Individualization of Treatment

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Key Words. GIST • Imatinib • Resistance • Sunitinib

LEARNING OBJECTIVES

After completing this course, the reader will be able to:
1. Describe the most important resistance mechanisms to imatinib that are responsible for early and late progression.
2. Discuss the most important systemic treatment options for managing progressive disease under imatinib treatment.
3. Explain how insight into mechanisms conferring sensitivity to imatinib may be used to individualize treatment of patients presenting with GIST.

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ABSTRACT

Gastrointestinal stromal tumor (GIST) is one of the first solid tumor types in which a tyrosine kinase inhibitor, imatinib, has become standard of care for patients with advanced disease. Although imatinib yields antitumor activity in the vast majority of patients, it is likely that all patients eventually experience progressive disease given enough time. In recent years, major progress has been made in the elucidation of mechanisms conferring resistance to imatinib that result in progressive disease. Insight into these resistance mechanisms has already resulted in the availability of strategies that can be applied in cases of progressive disease and it is likely that more approaches will be defined in the next years. Additionally, it can be anticipated that in the near future treatment will be guided according to factors determining sensitivity to imatinib. This review focuses on the factors inducing imatinib resistance that have been elucidated so far, the currently available and potential novel treatment options for patients with progressive disease, and how insight into resistance mechanisms may allow individualized treatment in the near future for patients with advanced GISTs. The Oncologist 2007;12:719–726

Disclosure of potential conflicts of interest is found at the end of this article.
INTRODUCTION
The introduction of imatinib mesylate (Gleevec; Novartis Pharmaceuticals Corporation, East Hanover, NJ) has revolutionized the treatment of gastrointestinal stromal tumor (GIST) patients. Before the availability of imatinib, advanced GIST patients were treated in a way similar to patients with other advanced adult soft tissue sarcomas for which doxorubicin-based chemotherapy is considered standard. Of all soft tissue sarcoma subtypes, it appeared that advanced GIST patients had a poorer outcome to doxorubicin-containing chemotherapy than patients with other subtypes. Following doxorubicin-containing chemotherapy, the 1-year overall survival rates for advanced GIST patients and the whole group of patients with soft tissue sarcomas are approximately 30% and 50%, respectively [1].

In the late 1990s, it was discovered that, in contrast to other soft tissue sarcomas, the malignant behavior of GISTs is a result of gain-of-function mutations in the genes encoding the c-Kit receptor or the platelet-derived growth factor receptor-α (PDGFR-α) [2, 3]. Imatinib is able to specifically inhibit the functions of these mutated gene products and yields spectacular antitumor effects in advanced GIST patients. As a result, the current 5-year overall survival rate is approximately 50% [4]. However, it appears that many, if not all, patients with advanced GISTs eventually experience progressive disease during imatinib treatment. Despite the rarity of GISTs, understanding of the mechanisms that confer resistance to imatinib has rapidly improved in recent years and the importance of this progress is already underlined by the availability of other active systemic therapies against GIST. This review addresses the resistance mechanisms to imatinib that have been elucidated so far and how better insight into these mechanisms can form the basis for novel approaches and individualization of treatment.

PATHOGENESIS OF GIST
The pathogenesis of GIST has recently been reviewed in several papers and is, therefore, only briefly discussed [5, 6]. In 1998, Hirota et al. [2] showed that GISTs frequently harbor mutations in the c-kit gene, which is also known as the stem cell factor receptor, or CD117. c-Kit can activate several signal transduction pathways through phosphorylation, in which the tyrosine kinase domain of c-Kit plays a crucial role. Signaling pathways that can be induced by c-Kit include the Ras/Raf/extracellular signal–related kinase, Janus kinase/signal transducer and activator of transcription, phosphatidylinositol 3′ kinase (PI3K)/Akt/mammalian target of rapamycin (mTOR), and Src kinase pathways [6], and it is likely that, in the near future, additional pathways will be revealed. Activation of these pathways leads to proliferation and decreased susceptibility to apoptotic triggers. While under normal circumstances the tyrosine kinase domain of c-Kit is only activated after stem cell factor binding, the ligand of the c-Kit receptor, this domain is constitutively activated in c-Kit receptors encoded by gain-of-function mutations as found in GISTs [2]. These activating mutations can occur at several sites in the c-kit gene. The most frequently mutated site is exon 11 in 70% of the cases, but mutations have also been found in exon 9, 13, and 17, each with a frequency of about 5% [7–9]. In patients with wild-type c-kit, gain-of function mutations in the gene for PDGFR-α that induce similar signaling pathways as c-Kit, can also underlie GISTs. These are seen in approximately 5% of GIST patients [3].

MECHANISM OF ACTION OF IMATINIB AND CLINICAL RESULTS
The recognition that the malignant behavior of GISTs was driven by c-Kit or PDGFR-α prompted the search for compounds targeting these factors. Imatinib is such a drug that targets tyrosine kinase activity of several proteins including c-Kit, PDGFR-α, Abl, and Lck [10]. It exerts its antitumor activity through binding to the tyrosine kinase domains of these proteins, thereby abrogating the binding of ATP, which is required for phosphorylation of substrate proteins. As a consequence, activation of downstream signaling cascades by c-Kit or PDGFR-α is inhibited.

The first to treat an advanced GIST patient with imatinib were Joensuu et al. [11]. Daily administration of imatinib resulted in a rapid response in this patient previously failing several lines of cytotoxic treatment. Since then, several clinical trials have rapidly been conducted, which clearly demonstrate the efficacy of imatinib in advanced GIST patients [1, 12–14]. Based on a randomized phase III study [1], imatinib at a dose of 400 mg is regarded as standard, given the equivalent overall survival in patients treated with 400 mg or 800 mg. In patients with advanced disease, imatinib yields clinical improvement in 80% of the patients; complete response, partial response, and stable disease according to Response Evaluation Criteria in Solid Tumors are achieved in 5%, 45%–50%, and 25%–30% of patients, respectively [1]. Responses are durable, with a median progression-free survival duration of >2 years [1] and a median overall survival time of 5 years [4].

RESISTANCE MECHANISMS TO IMATINIB
It is generally believed that all GIST patients treated with imatinib for advanced disease will inevitably develop progressive disease. It appears that there are in fact two kinds of progression, early and late progression. They are represented in the progression-free survival curves by a rapid decline in the first months of treatment (early progression) and
a more gradual decrease thereafter (late progression). These two distinct patterns of progression have different underlying mechanisms of resistance (Table 1).

**Early Progression**
Early progression is defined as progression occurring in patients who have never shown any response to imatinib. In these patients, progression is in general encountered within 6 months after initiation of imatinib, although some studies define progression within 3 months after imatinib start as early progression. Early progression concerns approximately 10%–15% of all patients. It is mainly a result of factors that are already present prior to treatment start, so-called intrinsic or primary tumor cell resistance. So far, the most common primary resistance mechanism identified is the initial mutational status. Almost all patients showing early progression have tumors bearing either a c-kit mutation in exon 9, a D842V mutation in PDGFR-α, or a wild-type genotype in both c-kit and PDGFR-α [7, 15, 16]. Response rates and overall survival are also poorer in GIST patients with tumors harboring wild-type genes or an exon 9 mutation than in patients with tumors expressing an exon 11 c-kit mutation, the most frequently occurring mutant [7]. The exact underlying molecular mechanisms of why these subtypes may respond poorly to imatinib and progress early are not clear. In vitro, activity of wild-type c-kit and exon 9 and exon 11 mutants can be inhibited at similar concentrations of imatinib [7]. This is in contrast to the observation that GIST patients with exon 9 mutations have a longer progression-free survival duration during imatinib treatment at 800 mg than at the standard dose of 400 mg, while for other mutants there is no difference between 400 mg and 800 mg [9]. Furthermore, there are patients with exon 9–mutated GISTs who respond very well to imatinib, also when given the standard dose [9]. This clearly illustrates that factors other than mutational status also affect initial sensitivity to imatinib. Patients whose tumors have a D842V mutation in PDGFR-α may also progress early [7, 16]. This is consistent with in vitro data showing that, for inhibition of D842V-mutated PDGFR-α activity, 10- to 20-fold higher levels of imatinib are required compared with wild-type PDGFR-α [7].

Another molecular mechanism that may account for early progression is the development of new secondary mutations in c-kit that are less sensitive to imatinib. This mechanism is thought to play a role in approximately 10% of the patients encountering early progression [16]. It is much more common in patients with late progression and is discussed below.

In a study exploring imatinib at 400 mg versus 800 mg [1], other factors independently associated with early progression within 3 months after imatinib initiation were the presence of pulmonary metastases, low baseline hemoglobin, and the absence of liver metastases [17]. The association of early progression with the presence of pulmonary and absence of liver metastases was possibly a result of inclusion of patients wrongly diagnosed as having GIST. In contrast to GISTs, non-GIST soft tissue sarcomas frequently metastasize to the lungs and rarely to the liver, while they are not sensitive to imatinib [14]. Why a low baseline hemoglobin level is associated with early progression is not known, but it may involve a more aggressive tumor or more advanced disease, or may reflect an impact of hemoglobin on imatinib pharmacokinetics [17]. It should be noted that, in this latter study, mutational status was not available.

**Late Progression**
Late progression is defined as progression occurring in those patients who initially had a response or a progression-free survival interval exceeding 3 months, in some studies, and 6 months in most studies, after starting imatinib treatment. Late progression is a result of secondary or acquired resistance mechanisms that develop under imatinib pressure. Probably the most important event is the occurrence of secondary c-kit mutations next to the initial mutation. This has been found in 50%–70% of the patients showing late progression. Secondary mutations predominantly occur in exon 13, 14, 17, or 18 of the c-kit gene, all encoding regions in the vicinity of the ATP-binding site or the kinase activating loop of c-Kit [15, 16, 18, 19]. These secondary mutations cause changes in c-Kit conformation, which hinder the binding of imatinib, thereby rendering these proteins less sensitive to imatinib [20]. For yet unknown reasons,
secondary mutations develop more often in tumors with a primary exon 11, rather than exon 9 mutated c-kit, 60% and 20% of the cases, respectively [15, 18]. The observation that distinct secondary mutations can be found in different tumor lesions in one patient [16, 18, 19] demonstrates the heterogeneity and genetic instability of GISTs.

Another potential mechanism yielding late progression is genomic amplification of c-kit. Theoretically, this may lead to an increased number of c-Kit molecules to be inhibited, outweighing the inhibitory capacity of imatinib. Whether this mechanism is really clinically relevant is questionable [16, 18].

A phenomenon that is likely to contribute to late progression indeed is activation of factors other than c-Kit or PDGFR-α that share similar downstream transduction pathways yielding uncontrolled proliferation. This will render tumor cells independent of c-Kit or PDGFR-α, as a consequence of which imatinib will be ineffective [21]. It remains, however, to be elucidated which factors are precisely involved in such cases.

Clinical parameters found to be independently associated with late progression are tumor size, a nongastric primary tumor site, high baseline granulocyte count, and allocation to the lower dose in the study randomly comparing 400 mg with 800 mg imatinib [17]. Nongastric primary tumors frequently express c-kit mutations other than the imatinib-sensitive exon 11 [22], and in this study mutational status was not available. Therefore, a nongastric primary site is likely to be eliminated as an independent factor after correction for mutational status. Size as an independent factor is probably a result of the fact that larger tumors have a higher likelihood of developing secondary mutations. The reason for the association with high baseline granulocyte levels is unclear [17].

Another mechanism accounting for resistance may be overexpression of drug-efflux pumps, such as P-glycoprotein [23] and breast cancer resistance protein [24], by tumor cells. Imatinib has been reported to be a substrate for these pumps, resulting in reduced intracellular drug levels and, consequently, efficacy [23]. The majority of untreated GISTs expresses P-glycoprotein or multidrug resistance protein-1 [25]. However, to our knowledge, there are no studies in humans that have assessed whether or not this mechanism actually yields late progression.

Pharmacological factors may also impair the efficacy of imatinib. High levels of the blood protein α1-acid glycoprotein have been found to bind imatinib and to reduce availability of the drug [26]. In addition, it has been demonstrated that systemic drug levels decline over time in imatinib-treated patients [27]. This decrease is considerable given that the area under the curve (AUC) of imatinib in patients treated for >1 year is approximately 50% of the AUC measured after 1 month of treatment. This phenomenon may be explained by increased expression of drug efflux pumps on gastrointestinal mucosal cells potentially leading to decreased imatinib resorption or increased clearance [24]. However, for these pharmacological factors as well, the exact relationship with progression is currently unknown.

So, numerous resistance mechanisms have been identified in recent years and it is likely that in the next years the spectrum of mechanisms conferring resistance will be expanded. Improved understanding of these mechanisms is of utmost importance, as they will form the basis for developing more active treatments.

**Systemic Treatment Options for Managing Progressive Disease**

In recent years, several approaches have become available that can be applied in patients progressing during imatinib treatment (Table 2). A strategy that has been proven to benefit a subset of patients with progression is dose escalation of imatinib. In contrast to other genotypes in which response rates and progression-free survival times were equivalent for imatinib administered at 400 mg or 800 mg, it appeared that the progression-free survival duration of patients with an exon 9–mutated tumor was longer in patients allocated to the higher dose [9]. This observation, that some mutants may respond better to higher doses of imatinib, forms a rationale for dose escalation. Other patients who may benefit from dose escalation are patients in whom lower imatinib systemic levels with time or genomic ampli-

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**Table 2. Systemic treatment options for managing imatinib-resistant gastrointestinal stromal tumors**

<table>
<thead>
<tr>
<th>Established options</th>
<th>Options under study</th>
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<tbody>
<tr>
<td>Dose escalation from 400 mg imatinib to 800 mg</td>
<td>Other c-Kit–targeting tyrosine kinase inhibitor</td>
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<tr>
<td></td>
<td>Combination of imatinib with other c-Kit–targeting tyrosine kinase inhibitor</td>
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<tr>
<td></td>
<td>Combination of imatinib with other drug targeting c-Kit–mediated transduction pathways (e.g., mammalian target of rapamycin inhibitor)</td>
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<td>Drug resulting in c-Kit degradation</td>
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<td></td>
<td>Combination of c-Kit (and preferably also vascular endothelial growth factor receptor) targeting tyrosine kinase inhibitor with conventional chemotherapy</td>
</tr>
<tr>
<td></td>
<td>Combination of c-Kit–targeting tyrosine kinase inhibitor and Bcl-2 inhibitor</td>
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fication of c-kit underlie progression. After dose escalation to 800 mg, which is the maximum-tolerated dose in GIST patients [12], 29% of the patients progressing at 400 mg obtained a secondary clinical benefit in terms of a partial response (2%) or prolonged stable disease (27%). At 1 year, 18% of the patients were still progression free [28]. Success rates of activation of kinase-dependent factors other than c-Kit or PDGFR-α. Sunitinib (SU11248) is such a drug, with activity against imatinib-resistant c-kit mutants in vitro [30] and targeting a wider spectrum of kinases than imatinib [10]. These include, next to c-Kit and PDGFR-α, vascular endothelial growth factor receptors and Ret [10]. At an interim analysis of a placebo-controlled, double-blind study in patients failing imatinib because of progressive disease or intolerance, sunitinib produced a longer median time to progression (27.3 weeks versus 6.4 weeks; hazard ratio [HR], 0.33; 95% confidence interval [CI], 0.23–0.47) and overall survival (HR, 0.49; 95% CI, 0.29–0.83) [31]. Benefits from sunitinib were seen regardless of the prior dose of imatinib or whether or not progression was observed within 6 months after starting imatinib [31]. Similar to what is seen using imatinib, sensitivity to sunitinib differs among the distinct c-kit mutants. There are indications that tumors initially bearing an exon 9 mutation have a high chance to respond to sunitinib [32]. The same holds true for some of the most common secondary mutations. Exon 13 and 14 mutants are considered sunitinib sensitive whereas exons 17 and 18 mutants are resistant in vitro [32]. Accordingly, the progression-free survival time during sunitinib treatment was significantly longer in patients with tumors bearing exon 13 or 14 mutations than in patients with exon 17 or 18 c-kit mutations in a small study [32]. Next to sunitinib, several other c-Kit–targeting tyrosine kinase inhibitors are in different phases of clinical testing in imatinib-refractory GIST, such as valatinib (PTK 787/ZK222584) [33], nilotinib (AMN107) [34], sorafenib [35], and AZD2171 [35].

Other systemic treatment strategies for imatinib-refractory patients are all in early clinical testing. These include phase I trials exploring the safety and feasibility of combinations of imatinib with other kinase inhibitors such as PKC412 [36] or AMN107 [34]. Such combinations aim to target a broad range of c-kit mutations, which is important given the heterogeneity of mutants and their differences in sensitivity to the different compounds.

Because it is unlikely that tyrosine kinase inhibitors in combination or as single agents will effectively inhibit all imatinib-resistant mutants, other approaches that target c-Kit–mediated pathways at a level other than c-Kit itself are worthwhile to explore. An example of such an approach is the combination of imatinib with everolimus (RAD001), an inhibitor of the mTOR. mTOR is a factor involved in the PI3K/Akt/mTOR signaling pathway that is thought to be important in c-Kit–driven malignant behavior. The combination of imatinib and everolimus showed synergistic interaction against imatinib-resistant GIST cell lines in vitro, and they can be safely combined in humans [37].

Another strategy for imatinib-resistant GIST may be enhanced degradation of c-Kit. c-Kit is stabilized and protected from proteasome-mediated degradation by heat shock protein (hsp)-90. An attractive feature of hsp-90 inhibitors is that they may exert antitumor activity irrespective of the specific c-kit mutation, because all c-Kit, mutated or not, need hsp-90 for stabilization. Accordingly, the hsp-90 inhibitor 17-allylamino-18-demethoxy-geldanamycin (17-AAG) inhibited c-Kit expression, phosphorylation of downstream factors, and proliferation in several imatinib-resistant cell lines [38]. A phase I study exploring the toxicity of IPI-504, a 17-AAG analogue, in patients with imatinib-resistant GISTs is ongoing [35].

Combinations of c-Kit–targeting tyrosine kinase inhibitors and conventional cytotoxic agents are theoretically attractive as well. GISTs are resistant to cytotoxic drugs in view of the response rates being <5% and a 2-year survival rate of 20% after doxorubicin-based treatment [1, 5]. The underlying mechanisms for this chemoresistance are not exactly known, but it is likely that c-Kit–regulated factors are involved, such as Bcl-2 [39]. Bcl-2, which is frequently overexpressed in GISTs, is known to confer resistance against conventional chemotherapy by preventing apoptosis. Consequently, inhibition of c-Kit activity may reduce Bcl-2 levels, rendering tumor cells more sensitive to cytotoxic drugs. Another factor that might be responsible for the chemoresistance of GISTs is vascular endothelial growth factor (VEGF) expression. Increased VEGF expression in soft tissue sarcoma cell lines induces resistance to chemotherapeutic drugs [40]. VEGF expression has been found in a substantial number of untreated GISTs and is associated with a poor outcome [41]. Several compounds that inhibit c-Kit target one or more of the VEGF receptors (VEGFRs)
as well [10], and may thereby sensitize tumor cells to conventional cytotoxic drugs. In addition, inhibition of VEGFR has been shown to decrease the interstitial fluid pressure of tumors, resulting in enhanced drug uptake, which will probably enhance chemotherapy-induced anti-tumor effects [42]. Hence, there are several reasons why tyrosine kinase inhibitors that target c-Kit, and preferentially VEGFR as well, potentially interact synergistically with chemotherapy against GISTs, rendering such combinations attractive to explore in GIST patients.

Another combination that is currently being explored in GIST patients is the combination of imatinib with oblimersen [35]. Oblimersen is an antisense directed towards Bcl-2, an important resistance factor against apoptotic triggers, which is overexpressed in GIST.

For up-to-date information regarding ongoing studies in patients with imatinib-resistant GIST, the reader is referred to internet Websites such as http://www.clinicaltrials.gov.

**INDIVIDUALIZED TREATMENT IN THE NEAR FUTURE?**

One of the major challenges in oncology is individualization of treatment, and GIST may be one of the first diseases in which this could become reality. In particular, the insight that c-Kit mutants differ in their sensitivity to different doses of drugs or to different compounds will contribute to this.

The most logical parameter that can serve as a predictive factor is the initial c-kit mutational status. As previously mentioned, patients whose tumors bear a c-kit mutation in exon 9, a D842V mutation in PDGFR-α, or no mutation in either c-kit or PDGFR-α have a lower chance of benefiting from imatinib than patients with exon 11–expressing tumors [7, 9, 16]. Even in the subgroup of patients with exon 11–mutated tumors, several distinct groups can be identified according to the exact site of mutation. Patients with a mutation/deletion at codon 565 or codon 579 do poorly compared with patients with tumors that have a mutation in exon 11 at another site [9].

In the future, different treatment strategies may be applied for the different predictive groups classified according to c-kit mutation status. Already, at present, treatment with 800 mg imatinib instead of 400 mg can be considered for patients with a GIST harboring an exon 9 c-kit mutation given the longer progression-free survival duration [9]. However, it is not known yet whether this also results in better overall survival. In addition, it should be kept in mind that this advantage is at the cost of more toxicity and that some patients with exon 9 mutations respond well to imatinib at 400 mg.

Furthermore, it may be that in the near future not only the dose, but also the drug applied, will depend on the mutation found. There are indications that sunitinib is in particular active against exon 9 mutations [32]. Dasatinib (BMS-354825) is a compound in early clinical testing that inhibits several c-kit mutants more potently than imatinib in vitro [29]. As a result, it can be assumed that in the near future the treatment of GIST, in terms of which agent and at which dose, will be guided by the mutation found. Since sunitinib in second-line treatment is less active against tumors with secondary exon 17 and 18 mutations compared with exon 13 or 14 mutants, such an approach is unlikely to be limited to first-line therapy alone but may be applied in second-line therapy, and maybe even in the adjuvant setting as well. It should be emphasized, however, that data from randomized studies supporting such an approach are currently lacking. Therefore, randomized studies comparing different tyrosine kinase inhibitors should be stratified or specifically designed for the mutations involved.

A major concern remains the heterogeneity of mutations that can be encountered in a single patient. Hsp-90 inhibitors or combination regimens are therefore likely to gain an important role in treatment. However, these strategies should also preferably be tailored according to predictive factors, stressing the need for further identification of such factors.

**CONCLUSIONS**

After the relatively recent introduction of imatinib in the treatment of GIST, rapid progress has been made in the elucidation of mechanisms conferring resistance to this drug. Based on these mechanisms, several strategies are already in use for patients with imatinib-refractory disease and it can be expected that other active systemic treatments will be developed on the basis of this improved insight.

Next to initiating the development of other active treatments, resistance mechanisms may also serve as predictive factors, enabling the identification of several distinct patient categories that should be treated differently in the future. Therefore, randomized studies that are exploring the efficacy of different treatment strategies in GIST patients should be stratified or specifically designed for mutational status, the most important predictive factor identified so far. In addition, further elucidation of factors affecting sensitivity to systemic therapies is warranted. Both can only be realized by close collaboration and treating GIST patients in the context of clinical trials.

**DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST**

The authors indicate no potential conflicts of interest.
REFERENCES


