Gastrointestinal Stromal Tumors: A Paradigm for Therapeutic Options in Solid Organ Tumors

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Abstract: Although a relatively rare malignancy with a national incidence of 3500-4000 annually, Gastrointestinal Stromal Tumor (GIST) is of significance in the realm of clinical and pharmacological research. GIST exhibits remarkable uniformity in its pathogenesis and ultrastructure as 95% of cases are linked to constitutive activation and overexpression of a membrane tyrosine kinase, c-KIT (CD117). Although previously refractory to any course of action but surgery, GIST heralded a triumph in targeted cancer therapy when administration of a specific first-generation tyrosine-kinase inhibitor Imatinib mesylate (STI571) was shown to inhibit c-Kit and demonstrated a significant increase in patient survival. Over the subsequent decade, GIST has become a paradigm for the potency of Imatinib in adjuvant or neoadjuvant therapy, showcasing the clinical relevance and rapidity of translational research in the field of targeted molecular therapy. Subsequent to demonstrating the efficacy of Imatinib as a therapeutic agent, GIST has also exposed the limitations of current targeted therapies. Within two years, 50% of GISTs develop secondary mutations that allow resistance to Imatinib. However, extensive research regarding both primary and secondary c-KIT mutations has illuminated the mechanisms of Imatinib resistance and has the potential to ameliorate this therapeutic setback. Current research to this end lies in two primary directions: the development of tyrosine kinase inhibitors (some of which also inhibit other oncongenic agents such as PDGFR, *bcr-abl*, and VEGF) that are either generally more potent than Imatinib or less susceptible to specific mechanisms of resistance; and drugs that target the downstream effectors of the mutant c-KIT kinase, including PKC and mTOR. This paper will systematically review current research on second-generation targeted molecular therapy in the treatment of GIST, and expand upon its value as a model for treatment of other solid organ tumors.

Keywords: Gastrointestinal Stromal Tumor, CD117, c-KIT, targeted therapies, neoadjuvant therapy, adjuvant therapy.

1. BACKGROUND AND EPIDEMIOLOGY

Gastrointestinal stromal tumors are the most common mesenchymal tumor of the GI tract, have only recently gained recognition as a distinct entity. Originally, they were categorized as tumors of smooth muscle derivation, but closer examination of tumor immunophenotype demonstrated this misclassification. Despite their histological similarity to leiomyomas, GISTs express membrane elements commonly found on neuroendocrine cells and neural-derived Interstitial cells of Cajal, the "pacemaker" cells of the gastrointestinal tract. It is from this latter set of cells, or more specifically their stem-cell precursors, that GIST is known to originate [1]. The predominant marker common to most GISTs is hyperexpression of either c-KIT (CD117) and PDGFR, both of which are members of the tyrosine kinase receptor family. The consensus view established by the National Institute of Health declared that definitive diagnosis of GIST should be based on c-KIT positivity [2,3] as eighty to 95% of GISTs stain positive for c-KIT, which is uncommon among other solid neoplasms [4]. Specifically, c-KIT is a type III tyrosine kinase and many elements of cell cycle regulation are within its purview. Under normal circumstances, binding of the ligand stem cell factor (SCF) induces homodimerization of c-KIT and subsequent phosphorylation of downstream products that partake in a diverse array of pathways governing cell growth, differentiation, and proliferation. In GIST, a number of different mutations lead to constitutive activation and proliferation of c-KIT with numerous pathological results. It is the overexpression of c-KIT that has led to the development of these tumors and subsequent therapies against this kinase receptor.

2. CLINICAL PRESENTATION

The clinical presentation of localized GIST is symptomatically heterogeneous, and 20% of patients report no symptoms at all. The type and degree of symptoms vary according to location, tumor size, and gross morphology. Most symptomatic tumors are relatively large (>6cm); those discovered incidentally tend to be smaller (<2cm.) The most common complaint upon presentation is gastrointestinal bleeding, which can be acute or chronic. Most bleeding is due to tumor growth into the gastric or intestinal lumen. Rarely, tumors rupture into the peritoneal cavity, an event which is associated with hemorrhage and a poor prognosis due to tumor seeding. GIST is usually subserosal, but may also be intramural or exhibit polyploid growth into the intes-

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tinal lumen which can lead to intestinal obstruction. This nimiety of gross morphological patterns in larger GISTs correlates to a wide range of symptoms including bloating or nonspecific abdominal discomfort. If a tumor does not cause symptoms, incidental discovery can occur during endoscopy or imaging studies, including computed tomography or magnetic resonance screening [5-10].

The median age of patients with reported GIST is approximately sixty, and pediatric tumors are very rare. The majority (60%-70%) of tumors are gastric in origin; GIST of the small intestine (20-35%), and colon/rectum (<5%), comprise the remainder. Very rarely, tumors are reported in the esophagus, appendix, omentum and mesentery. In the case of discovery of a tumor in the latter two locations, seed metastasis from gastric or intestinal tumors is often implicated [6-9]. Most diagnosed GIST are sporadic, although germ line c-KIT or PDGFR mutations leading have been identified [11].

3. STAGING, GRADING, AND PROGNOSIS

Staging of GIST is based on the 6th edition of American Joint Committee on Cancer Staging Manual, section on soft tissue sarcomas. The AJCC system assigns tumors a stage, I-IV, based on TNM criteria. In addition to the classic TNM staging correlating to the size of the primary lesion and the presence or absence nodal metastasis and invasion into adjacent structures or distant metastasis, the AJCC specifies that a grade, G1-4, should be assigned to each tumor based on the degree of histological differentiation [12]. The AJCC system also takes into account both tumor size and mitotic index, or the number of mitoses visible per fifty high-power fields (Table **1**) [2]. It stratifies gastric and intestinal GISTs separately, as the latter is associated with a worse prognosis.

In the case of clinically identifiable and/or symptomatic GIST, risk stratification according to the NIH scheme is the common approach. Approximately 45% of primary localized GISTs are "high risk" at presentation, and these are likely to recur and mestastasize even with complete excision of the primary lesion. "Intermediate risk" and "low risk" GISTs, which comprise 24% and 32% of malignancies respectively, carry a more favorable prognosis. Gastric GISTs are associated with better outcomes relative to GISTs in the small intestine and extra intestinal sites. Regardless of location, GISTs with extensive necrosis, expression of p16, and epithelioid morphology, were correlated with worse prognosis following resection [13-15].

Additionally, analyses of various mutations in c-KIT and PDGFR, which lead to inappropriate activation of tyrosine-kinase receptors, have demonstrated prognostic value. The results of these analyses, and their clinical implications, will be discussed further along in this paper, in the context of their value as predictive tools for GIST response to targeted therapy.

4. TREATMENT OF PRIMARY LESIONS

As with other soft-tissue tumors, resection, whenever possible, is the primary modality in treatment of GIST. For gastric GIST, surgical procedures include wedge resection, distal/subtotal or total gastrectomy, or extended en bloc resection if there is adjacent organ involvement. For intestinal GISTs, segmental or extended resections, (including pancreatoduodectomy for duodenal lesions involving the head of the pancreas) are performed. For colonic, omental, or esophageal GISTs, resections with negative margins are performed. Open surgery is generally preferred to laparoscopy, to reduce the risk of tumor rupture and subsequent peritoneal seeding. Because GISTs usually encroach upon adjacent structures but rarely invade, macroscopically complete resection is achieved in at least 85% of cases. Negative margins should be achieved whenever possible, and there is no proven benefit of wide margins in excision of primary GIST [13,16-21].

Despite the established success of surgical resection alone for primary GIST, local recurrence or metastases are common and the five-year survival rate is a dismal 50%. Between 20 and 30% of patients exhibit metastases or unresectable tumors upon initial diagnosis, and half of patients with successful resection experience local recurrence. In the past, the prognosis for these patients was extremely poor because GIST is refractory to traditional chemotherapy and radiation [10]. The poor response to traditional adjuvant therapies for GIST led to investigations into molecular targets and their receptors, such as c-KIT. The advent of targeted therapy has revolutionized the treatment options for patients with a high likelihood of developing recurrence or with advanced or unresectable disease.

5. TARGETED THERAPY: TREATMENT OF ADVANCED AND UNRESECTABLE GIST

The human genome contains elements coding 700 protein kinases which cleave ATP to ADP, catalyzing the phosphorylation of amino acid residues within the kinase. Subsequent enhancement of kinase catalytic activity or substrate phosphorylation initiates a wide array of cellular events, which include growth, proliferation, and certain features of metabolism and apoptosis. Because of their role as regula-

Table 1. Risk Stratification of GISTs Based on Size and Mitoses

	Gastric GISTs		Intestinal GISTs	
Likely benign	≤5 cm	≤5 M/50HPF*	≤2 cm	≤5 M/50HPF*
Intermediate	5-10	≤5	2-5	≤5
Probably Malignant	>10	>5	>5	>5

*M/50HPF: Mitoses per 50 High Power Field.

Table adapted from Fletcher *et al.*, **2002**.

The commonly accepted system for risk stratification of GISTs is based on tumor size, anatomic location, and number of mitoses observed per high power field. Larger size, intestinal location, and greater rates of proliferation are assosciated with a greater degree of malignancy.

tory proteins and initiators of signal cascades, kinase dysfunction can have grave consequences; in fact kinase mutations are the most common driving force in oncogenesis [22,23].

The causative agent in GIST is almost always inappropriate constitutive activation of either PDGFR or KIT, due to a mutation in receptor composition which normally is activated by ligand binding resulting in homodimerization and initiation of cellular signaling cascades. Approximately 70% of GISTs are due to c-KIT mutations in the regulatory JM domain (exon 11), another 15% are found in the KIT proximal regulatory domain (exon 9), and less than 5% are due to KIT mutations in other sites, including the first and second kinase domains (exons 13 and 17) as depicted in Fig. (**1**). Specifically, mutations on exon 11 consist of an Asp deletion near the 5' end with other mutations compromising deletions or substitutions in this region. These mutations have recently had clinical implications with regard to responses to therapy which will be discussed below. Another 5% of GISTs are due to mutations in PDGFR, 85% of which are found in the second catalytic domain (exon 18), and the remainder of which are located in the first kinase domain (exon 14) and the juxtamembrane domain (exon 12) (Fig. **1**) [24-26]. In GISTs with PDGFR mutations, the most common source of oncogenesis is a point mutation in the activation loop of TK2 with a single A→T substitution (translated to Asp842Val) comprising almost half of PDGFR mutations [24,25]. The remaining 5-10% of GISTs lack mutations in either c-KIT or PDGFR, and are referred to as wild-type GISTs. Wild-type GISTs are common among GIST patients with familial cancer syndromes, including Carney Triad – where GIST is found in concert with extra-adrenal paragangliomas and pulmonary chondromas - and neurofibromatosis.

As tumor genotyping became increasingly common, there was an effort to determine the relationship between kinase mutation and prognosis. Initially, researchers believed that c-KIT exon 9 mutations were indicative of a poor prognosis, but multivariate analyses showed that this correlation was due to the fact that the majority of exon 9 mutations have an intestinal location abrogating the prognostic significance of the mutation. A statistically significant difference between c-KIT exon 11 deletions and substitutions was established, however, with the former displaying a tendency towards more aggressive and malignant behavior when compared to the latter [7,10,24-28]. And although exon 11 mutations have demonstrated a tendency toward a more aggressive phenotype, the clinical importance of primary c-KIT and PDGFR mutations is due to their influence on tumor response to targeted therapy which will be discussed below.

Most importantly, prior to a discussion about specific targeted therapies, we must define our target, strategies, and limitations. Increased knowledge of the molecular structure of protein kinases, *via* genomic analysis and x-ray crystallography, has enabled the development of drugs that specifically target kinases and block the catalytic site and prevent ATP binding and cleavage. This approach was first attempted with tyrosine kinases, a family consisting of ninety known unique members, 58 of which are receptor proteins and the remainder of which are cytosolic. Unfortunately, the process of drug discovery for kinase inhibitors, which could ultimately lead to advances in our treatment of cancer, was complicated by similarities amongst ATP-binding pockets of

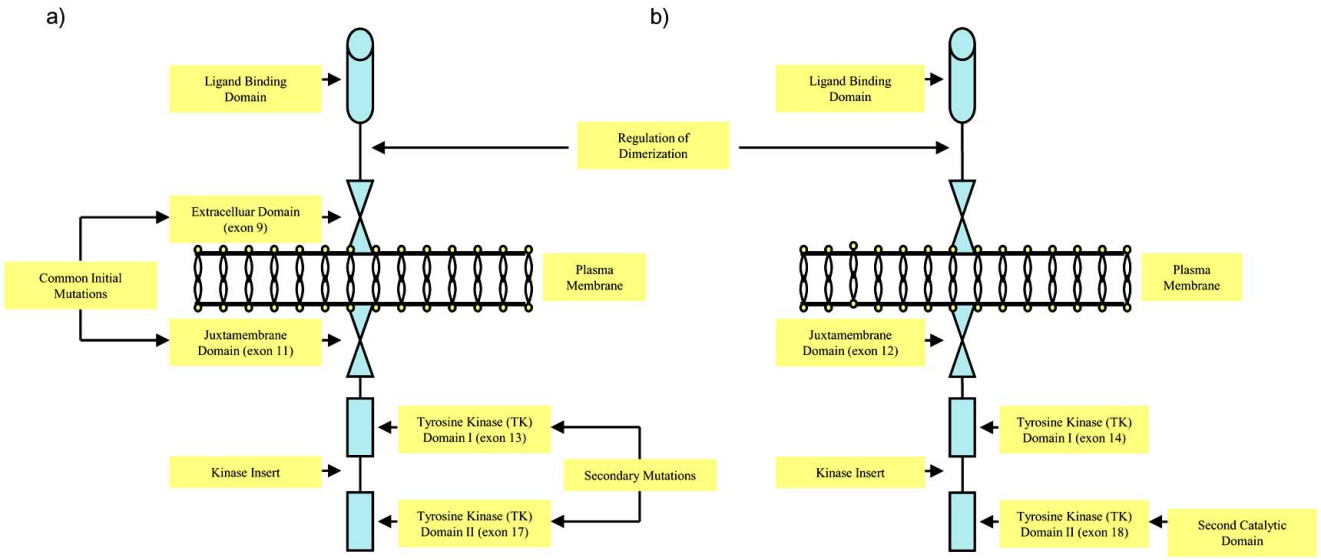

Fig. (1). c-Kit, PDGFR tyrosine kinase structure schematic. The tyrosine kinase receptor c-Kit (**a**) is illustrated with specific domains and exons indicated. Seventy percent of c-Kit mutations are found within exon 11 while another 15% involve exon 9. Only rarely are primary mutations found in exons 13 and 17, but they are common sites of secondary mutations that can promote resistance to Imatinib. Mutations in the PDGF receptor (**b**) are most frequently located on the second catalytic domain (exon 18) with the remainder located mutations on exons 14 and 12.

many human tyrosine kinases. Although the binding site of the triphosphate group has a highly conserved sequence, the adenosine recognition motif was more unique. Ultimately, this unique motif became the focus of most targeted therapy research [29,30] with the advantages of this scheme being multifold. Agents that inhibit a specific receptor, or set of receptors, could potentially have a far more innocuous and limited side effect profile than traditional chemotherapy while maintaining a high degree of efficacy, especially with certain tumors that significantly express the target. The first breakthrough was Imatinib mesylate, with a number of other agents following soon therafter.

5.1. Imatinib Mesylate

Imatinib mesylate (Gleevac®, Fig. **2**) is the result of a series of high-throughput screenings for molecular action against cancer cells. Its potential as a tyrosine kinase inhibitor first came to light with the discovery that its parent compound, 2-phenylaminopyrimidine was active against Bcr-Abl, the oncogenic fusion protein that is the causative agent in chronic myelogenous leukemia (CML). Replacement of the imidazole with a benzamido group increased the potency of the compound and methylation ortho to the pyrimidinyl-amino group increased selectivity towards cAbl.

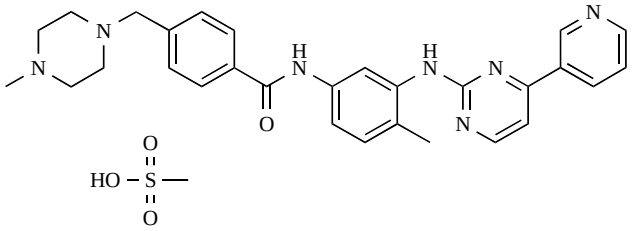

Fig. (2). Imatinib mesylate.

Imatinib mesylate, IUPAC name 4-[(4-Methyl-1-piperazinyl)methyl]-N-[4-methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl] amino]-phenyl]benzamide methanesulfonate, empirical formula $C_{29}H_{31}N_7O$, molecular mass 493.603 g/mol, was originally marketed as a selective inhibitor of the cytosolic Bcr-Abl in CML, and subsequent research demonstrated that it is both effective and generally well-tolerated for this malignancy. Clinical trials have shown that treatment with Imatinib achieves complete hematologic response rates in over 90% of patients with a significant cytologic response as well. Imatinib also exhibited the ability to slow disease progression to blast phase and induce tumor cell apoptosis [31-36]. Further laboratory work demonstrates that Imatinib inhibition is not exclusive to Bcr-Abl but may include a number of other proteins, most notably tyrosine kinase family member's c-KIT and PDGFR [37]. As depicted in Table **2**, Imatinib's IC_{50} is above 10,000 nm for other human tyrosine kinases ensuring a minimal side effect profile. Crystallographic studies suggest that selectivity is due, at least in part, to an enlarged hydrophobic pocket unique to these kinases, which are not strictly conserved among other members of the tyrosine kinase family. Imatinib binding in this pocket inactivates the kinase, and in the case of Bcr-Abl, renders it unable to complete nuclear transport and engage in anti-apoptotic activity [37,38].

Table 2. Inhibitory Concentration of Imatinib Mesylate on Membrane Receptors

Kinase	Gleevac IC_{50}nm
cAbl	188 +-18
KIT	413 +-23
PDGFR-B	386 +-111

This table (adapted from Manley *et. al*, 2002) shows the inhibitory concentration of Imatinib against cAbl, KIT, and PDGFR with ATP concentrations optimized for each kinase.

In vitro, Imatinib binds to the inactive conformation of either c-KIT or PDGFR and inhibits ATP binding and slows tumorgenesis, raising hope for patients with metastatic or unresectable GIST who were universally refractory towards standard chemotherapy [10,37]. In subsequent clinical trials, Imatinib demonstrated efficacy *in vivo* as well. In patients with unresectable or metastatic GIST, Imatinib achieved response rates in 50% of patients, with another 30% showing stable disease [38-43]. Long term follow up studies of the Phase II trials showed a dramatic increase in overall survival rates for metastatic and unresectable GIST from an average of 1.5 years in the pre-Imatinib era to as high as 4.5 years with Imatinib therapy [39,40,44] with minimal side-effects and prohibitive toxicities in only a small number of patients which is a clear advantage over traditional chemotherapy [39,40,42].

Although mutational status has demonstrated a clinical correlation with regard to tumor behavior, phase clinical trials have also reported a significant correlation of mutational status and response to therapy. The most common mutations, those in exon 11 of c-KIT, demonstrated the best results for Imatinib, with 85% of patients responding. Exon 9 mutants showed a less promising response (approximately 45%), and the remaining mutations showed even more limited responses to Imatinib. The most common PDGFR mutation, described above, was completely refractory to Imatinib therapy [24,25,27,28,45]. Conformational changes of each receptor may provide an explanation for this range of sensitivities. Because Imatinib binds c-KIT or PDGFR in the inactive conformation, mutations that favor the active conformation of either receptor, such as the Asp842Val PDGFR kinase domain mutation, are intrinsically Imatinib resistant. This mechanism is responsible for the generalized therapeutic failure of Imatinib in systemic mastocytosis, where an inherently resistant KIT Asp816Val is responsible for tumorgenesis [25,46].

In Phase II clinical trials, investigators began examining the possible use of Imatinib as adjuvant therapy for completely resected high-risk GISTs. The results were impressive; one study showed disease free survival at 1, 2, and 3 year of 94, 73, and 61% respectively, where a comparable study from the pre-Imatinib era reported 1, 2 and 5 year recurrence free rates of 85.2, 53.8, and 43.7% [17,47,48]. Other small trials demonstrate similar dramatic results in favor of adjuvant Imatinib, with documented recurrence free survival rate of 97% with adjuvant Imatinib vs. 83% placebo at one year [49-51]. Although adjuvant Imatinib is still con-

sidered investigational, current NCCN guidelines recommend twelve months of treatment for any tumor stratified as high risk after biopsy.

Recent phase III trials emphasized the importance of biopsy results and the prognostic value of exon mutations for GIST. (Table **3**) These trials were conducted on a global scale, consisting of two large groups comprising roughly the Eastern and Western hemispheres. In both studies, patients were randomized to either 400mg or 800mg of Imatinib daily, with patients of the lower dose given the opportunity to cross over to 800 mg on disease progression [39,40,43]. Although these studies demonstrated more toxicity on the higher initial dose, with an overall statistically insignificant increase in progression-free survival [39,40,43,52,53], the patients whose GIST was related to a mutation on exon 9 of the c-KIT extracellular domain, had a therapeutically beneficial response to the higher initial dose of Imatinib. These results highlight the important clinical implications of mutational analysis.

Neoadjuvant (preoperative) Imatinib for marginally resectable or unresectable tumors is also under investigation. Because Imatinib reduces tumor size and density, it is able to decrease tumor size and provide an option for surgical resection or debulking. Phase II trials indicate that this therapeutic route is promising, and a number of case studies offer anecdotal evidence in support of this direction as well [41,54-57]. Despite its demonstrated success in treatment of metastatic disease, as well as its possible benefits in the adjuvant and neoadjuvant setting, Imatinib is by no means a universal remedy. Aside from the primary resistance described earlier, as many as 50% of GISTs treated with Imatinib develop resistance to therapy within a two year period. Research has determined that acquired resistance to Imatinib usually occurs along two distinct routes; either acquisition of a second mutation that confers resistance, or *via* genomic amplification of the original mutated receptor [10,58].

In the case of the former, the secondary acquired mutation is usually found in the kinase domain and creates a physical impediment to Imatinib binding in a manner analogous to the intrinsically resistant primary mutations discussed earlier. A point mutation resulting in a V654A substitution is the most common example of this type of acquired resistance, and mirrors mechanisms of acquired resistance in CML patients treated with Imatinib. Patients that develop this mutation show subsequent rapid progression in spite of treatment with Imatinib [58,59]. In order to address both primary and secondary Imatinib resistance, the development of other pharmacological agents is underway along two different routes which include designing other tyrosine kinase inhibitors that may possess enhanced potency against certain mutations and agents that enhance degradation of mutated tyrosine kinases or inhibit their downstream effectors.

5.2 Second-Generation Tyrosine Kinase Inhibitors

Sunitinib

Sunitinib malate (Sutent®, Fig. **3**) IUPAC name *N*-[2-(diethylamino)ethyl]-5-[(*Z*)-(5-fluoro-1,2-dihydro-2-oxo-3*H*-indol-3-ylidine)methyl]-2,4-dimethyl-1*H*-pyrrole-3-carboxamide, empirical formula $C_{22}H_{27}FN_4O_2$, molecular mass 398.474 g/mol is a rationally-designed small molecule tyrosine kinase inhibitor that binds to the inactive conformation of a tyrosine kinase, preventing ATP binding and catalysis. Although its mechanism of action is homologous to Imatinib, its structure and range of kinase selectivity are different. Where Imatinib is active against KIT, Bcr-Abl and PDGFR, Sunitinib is capable inhibition of additional tyrosine kinase receptors, including flt-3 (another type III tyrosine kinase) and members of the vascular endothelial growth factor receptor (VEGFR) family *in vitro* [60,61].

Phase I and II trials of Sunitinb in GIST patients who progressed on Imatinib demonstrated efficacy *in vivo* [62]. The results of a large phase III trial were dramatic enough to cause unblinding of the study during an interim analysis so

Table 3. GIST Phase III Studies with Imatinib Mesylate

Gene	Approximate % of GISTs	Response to Imatinib in North American Phase III Trials	Response to Imatinib in EORTC phase III trials
KIT: Exon 11 mutations	70	Favorable response compared to exon 9, wild type. CR/PR (complete response/partial response) 71.7 %	Overall response rate of approximately 90%
KIT: Exon 9 mutations	15	Less favorable CR/PR 44.4%	Overall response rates of approximately 70%
KIT: Exon 13 mutations	<5		Full response in all 9 patients
KIT: exon 17 mutations	<5		Partial response in some mutants, primary resistance in others.
PDGFR: D842V	4	No response to Imatinib	No response to Imatinib
Other PDGFR	1	Limited response reported	Limited Response to Imatinib
Wild Type	5-10	CR/PR 44.6% (response comparable to exon 9 response)	Relatively high response; partial response in 23% with stable disease in another 50%.

Table based on reported results from Phase III studies [27,92]. The phase III studies demonstrated that the efficacy of GIST varies with the c-KIT or PDGFR mutation implicated in tumor growth.

that patients on placebo could begin Sunitinib immediately. Before unblinding of the randomized study, 7% of the patients showed objective response to Sunitinib and an additional 58% demonstrated stable disease, with only 19% showing progressive disease. In the placebo group, comparable rates were 0%, 48%, and 37%. Overall, it seemed that Sunitinib conferred a significant benefit of at least an additional 5 months until disease progression [63]. This demonstration of therapeutic value was significant enough to approve Sunitinib as the second line pharmacological treatment of GIST.

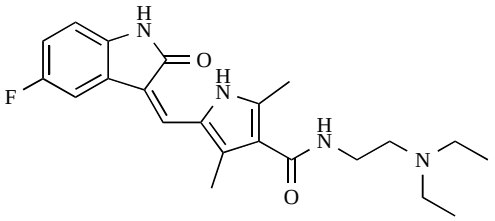

Fig. (3). Sunitinib malate.

Notably, success of treatment with Sunitinib appeared to follow predictable patterns based on the KIT or PDGFR mutation implicated. GISTs with a primary mutation in exon 9 appeared to have a much stronger Sunitinib response than those with an exon 11 mutation [60]. It should be noted, however, that there is a possibility that this statistical relationship would be abrogated if the high rate of secondary mutations leading to Imatinib resistance in exon 11 mutants is taken into account. In addition, it was discovered that Sunitinib showed a higher efficacy against GISTs with secondary mutations in exons 13 and 14, compared to secondary mutations in exons 17 and 18 [62,64].

Regardless of mutational status, however, all GISTs eventually progress. Secondary mutations in the kinase domain arise in response to Sunitinib analogous to those that cause Imatinib resistance [65]. Subsequently, Sunitinib should be realistically regarded as offering prolongation, not cure, and the search for alternative therapies for refractory GIST should continue.

Although its action against GIST is finite, Sunitinib is also approved to treat a number of other solid tumors. Phase III trials show it is effective against renal cell carcinoma [63]. Phase II trials are also underway to test the potential therapeutic use of Sunitinib in colon cancer, melanoma, and squamous cell carcinoma [61].

Dasatinib

Dasatinib (Sprycel®, Fig. **4**), IUPAC name *N*-(2-chloro-6-methylphenyl)-2-[[6-[4-(2-hydroxyethyl)-1-piperazinyl]-2-methyl-4-pyrimidinyl]amino]-5-thiazole carboxamide monohydrate, empirical formula $C_{22}H_{26}ClN_7O_2S$, molecular mass 488.01 g/mol, is a potent inhibitor of Bcr-Abl. Unlike Imatinib and Sunitinib, Dasatinib binds to the active conformation of the kinase, and is therefore more effective against mutations within its domain [66].

Dasatinib demonstrated 90% response rates in chronic phase CML patients, and was approved to treat this malignancy in 2006, with phase III trials to determined appropriate dosage underway [66-68]. In GIST, Dasatinib was exceptionally active against the PDGFR D842V mutant that showed intrinsic resistance to both Imatinib and Sunitinib [69]. For patients expressing this mutation, Dasatinib is a critical therapeutic alternative. There is also evidence that Dasatinib may be useful against a wide range of other solid-organ tumors [70]. Molecular studies demonstrated efficacy against Src, a tyrosine kinase that plays a role in the progression of prostate, breast, pancreatic, and some lung and skin cancers [71-73]. Mouse models showed that Dasatinib was capable of halting proliferation of pancreatic tumor cells *in vitro*, offering a potential alternative for a malignancy that remains dauntingly difficult to treat [74].

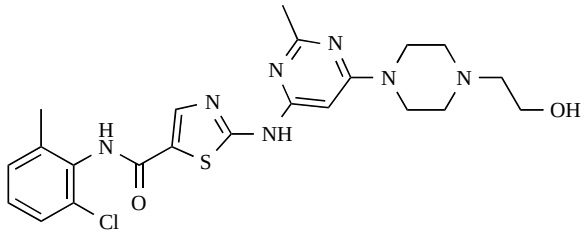

Fig. (4). Dasatinib.

Sorafenib

Sorafenib tosylate (Nexavar®, Fig. **5**) IUPAC name 4-(4-{3-[4-Chloro-3-(trifluoromethyl)phenyl]ureido}phenoxy)-N^2-methylpyridine-2-carboxamide 4-methylbenzenesulfonate, empirical formula $C_{21}H_{16}ClF_3N_4O_3$ x $C_7H_8O_3S$, molecular mass 637 g/mol, was originally developed as a specific inhibitor of serine–threonine kinase RAF.

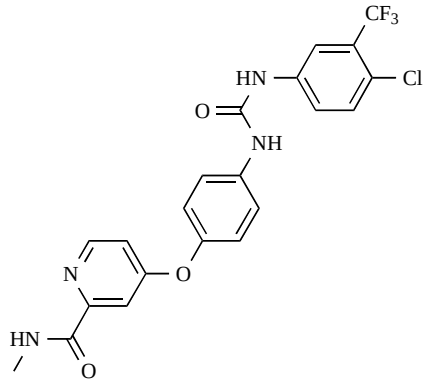

Fig. (5). Sorafenib tosylate.

RAF has been demonstrated to be a downstream effector of many tyrosine kinase receptors involved in tumor development. Sorafenib not only exhibits activity against Raf kinase MEK/Erk pathway, it also exhibits activity against other tyrosine kinase receptors (including both KIT and PDGFR). This discovery raised the possibility of Sorafenib acting against a wide range of maliganancies including GIST [75,76]. A current phase II trial is exploring the action of Sorafenib in patients resistant to both Imatinib and Sunitinib in GIST. Initial results from this trial show partial response in 13% of patients and stable disease in an additional 58%. Sorafenib was well-tolerated in these patients, and merits consideration as a third line treatment (behind Imatinib and Sunitinib) in metastatic or unresectable GIST. Sorafenib has

demonstrated clinical success against hepatocellular and renal cell carcinoma, and was officially approved to treat the latter in 2005 [77,78].

Nilotinib

Nilotinib (Tasigna®, Fig. **6**), IUPAC name 4-methyl-*N*-[3-(4-methyl-1*H*-imidazol-1-yl)-5(trifluoromethyl)phenyl]-3-[(4-pyridin-3-ylpyrimidin-2-yl) amino]benzamide, empirical formula $C_{28}H_{22}F_3N_7O$, molecular mass 529.516 g/mol, was originally designed to target Bcr-Abl, with binding ability and subsequent inhibitory properties many times more potent than Imatinib.

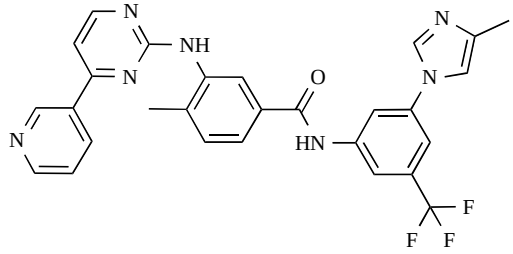

Fig. (6). Nilotinib.

In vitro, Nilotinib is active against Bcr-Abl, c-KIT, and PDGFR [79]. Clinical trials against chronic CML patients who had previously demonstrated resistance or intolerance to Imatinib showed dramatic response levels to Nilotinib, with complete response in 31% of patients, and partial response in an additional 16%. In addition, Nilotinib was well-tolerated, and subsequently is likely to become an established second or even first line treatment for Bcr-Abl positive CML [80]. Phase I trials of Nilotinib in patients with Imatinib resistant GIST compared Nilotinib alone vs. Nilotinib with concurrent Imatinib. Sixty-eight percent of patients on Nilotinib alone had stable disease for 6 weeks to 6 months, providing evidence that Nilotinib may extend progression-free survival. Current Phase II trials are exploring the action of Nilotinib as a first line treatment in Imatinib-naïve patients with metastatic or unresectable GIST. Phase III trials comparing the action of Nilotinib vs. 800mg Imatinib in patients who have progressed on 400 mg Imatinib are also underway.

Vatalanib

Vatalanib (Fig. **7**), IUPAC Name *N*-(4-chlorophenyl)-4-(pyridin-4-ylmethyl)phthalazin-1-amine, empirical formula $C_{20}H_{15}ClN_4$, molecular mass 346.813 g/mol, represents the very latest of tyrosine kinase inhibitors. It has action *in vitro* against all members of the VEGF receptor family, as well as KIT and Bcr-Abl, and demonstrated clinical efficacy for treatment of CML [81].

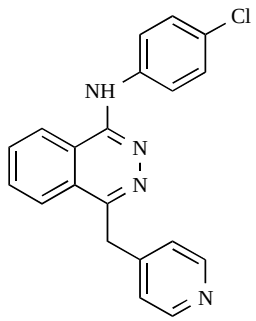

Fig. (7). Vatalanib.

A small Phase II study of Vatalanib in fifteen patients with Imatinib resistant GIST showed partial response in 13% of patients and stable disease for greater than three months in an additional 53% [82]. Vatalanib was well-tolerated in all patients, and additional trials of Vatalanib as monotherapy, as well as Vatalanib in concert with downstream inhibitors of KIT, are underway.

5.3 Other Agents

Heat Shock Protein 90 Inhibitors

Heat Shock protein 90 is a chaperone protein that may protect KIT from apoptosis; inhibition of this protein may enable increased degradation of mutated KIT, preventing it from complexing with both regulatory and catalytic subunits. *In vitro*, HSP90 inhibitor IPI-504 (Retaspimycin®, Fig. **8**), IUPAC name 18, 21-Didehydro-17-demethoxy-18, 21-dideoxo-18, 21-dihydroxy-17-(2-propenylamino) geldanamycin, empirical formula $C_{31}H_{45}N_3O_8$, molecular weight 587.7 g/mol, is active against KIT-positive GISTs when used alone.

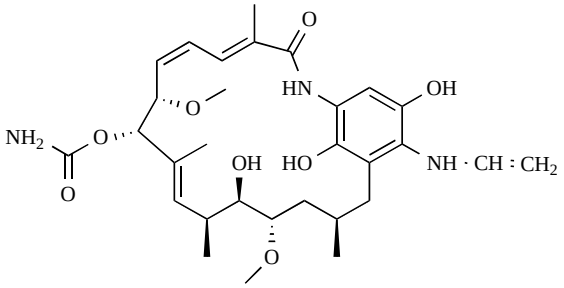

Fig. (8). IPI-504.

In addition, IPI-504 may help to prevent the development of secondary mutations that lead to tyrosine kinase resistance by enabling degradation of mutated proteins before positive selection is possible [69,83]. Preliminary results from a phase I trial of IPI-504 in patients with tyrosine kinase-resistant GIST report stable disease in 78% of patients by RECIST criteria (the international standard for tumor response as determined by various forms of imaging), including an observable partial response in 22% by positron emission tomography (PET) [84].

Downstream Pathway Inhibitors

Although inappropriate expression and constitutive activation of KIT kinase is the hallmark of GIST, hyperactivity of the various signaling pathways downstream from KIT are directly responsible for maladaptive cellular proliferation and tumor growth. Inhibition of these pathways has therapeutic potential, particularly when the KIT receptor itself is refractory to treatment. Various signal transduction molecules, including P13-K and mTOR (mammalian target of rapamycin), have been specifically implicated in these pathways.

RAD001 (Everolimus®, Fig. **9**), IUPAC name dihydroxy-12-[(2*R*)-1-[(1*S*,3*R*,4*R*)-4-(2-hydroxyethoxy)-3-methoxycyclohexyl]propan-2-yl]-19,30-dimethoxy-15,17,21,23,29,35-hexamethyl-11,36-dioxa-4-azatricyclo[30.3.1.$0^{4,9}$]hexatriaconta-16,24,26,28-tetraene-2,3,10,14,20-pentone, empirical formula $C_{53}H_{83}NO_{14}$, molecular weight 958.224 g/mol, is an mTOR inhibitor that has been evaluated for treatment of a variety of malignancies [85,86].

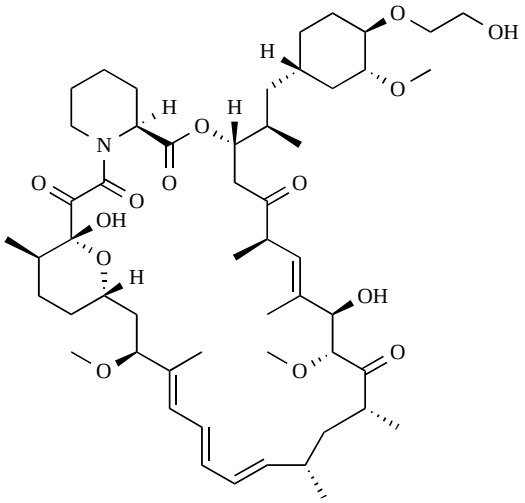

Fig. (9). RAD001.

In vitro, RAD001 effectively abrogates the downstream effects of constitutively activated c-KIT [87]. Furthermore, it has been shown to potentiate the inhibitory effect of Sunitinib *in vitro* [88]. A Phase I/II study showed that RAD001 is effective in synergy with Imatinib and second-generation tyrosine kinase inhibitors, and suggests that further randomized trials should be conducted [84]. Trials of RAD001 in concert with both Vatalanib and Dasatinib are also underway.

DISCUSSION

In the field of cancer research in general, and targeted therapy in particular, the treatment of gastrointestinal stromal tumors represents a significant accomplishment and an opportunity. The exhaustive typification of the underlying framework of GIST oncogenesis creates a unique canvas for illustration of the kinase mutation-dependent efficacy of targeted therapy. Each targeted agent has a range of efficacy correlated to the mutation implicated in GIST tumors, which in some cases determines the individual response to treatment.

With time, it should be possible to utilize this information in an anticipatory manner. If each GIST patient is subjected to mutational analysis at diagnosis, the knowledge garnered will enable physicians to tailor subsequent treatment to the individual. Examples where this approach would be of value are already in evidence. Patients with KIT exon 9 mutations should be considered for a higher inductive dose of Imatinib. Patients with mutations that favor the active mutation of KIT, such as those with the common PDGFR D842V mutation, may fare better with Dasatinib as initial monotherapy in place of Imatinib. In addition, knowledge regarding the molecular basis of Imatinib binding may enable the development of more potent agents, such as Nilotinib.

In addition to the tyrosine kinase inhibitors themselves, agents that have potential value in combination therapy are under study. To prevent the development of secondary mutations that lead to tyrosine kinase inhibitor resistance, agents that enhance ubiquitination of KIT, such as heat shock protein inhibitors, should be considered. In order to potentiate the effects of tyrosine kinase inhibitors, downstream actors such as mTOR inhibitors may also be of value.

The importance of investigation along these avenues extends far beyond the field of GIST alone. Protein kinase dysfunction is a major cause of oncogenesis, and many of the agents mentioned above are already under study in the realm of other malignancies. Aside from CML and GIST, Imatinib was recently approved for treatment of Dermatofibrosarcoma Protuberans [89]. The use of Sunitinib and Sorafenib for treatment of renal cell carcinoma provides evidence that targeted therapy may be applicable in direct treatment of more common malignancies. Targeted therapies are also under investigation and in common use as potential anti-angiogenesis agents in both colorectal and non-small cell lung cancer [90,91].

As the vast spectrum of potential applications for targeted therapies becomes evident, the ability to use them efficiently and effectively becomes increasingly important. By virtue of its uniquely well characterized genetic basis, and documented response to individual agents, GIST functions as a near-ideal model for the relative success of targeted therapy as a whole. Enhanced knowledge of structural mutations and their relationship to drug efficacy enables physicians to tailor the therapy to the individual tumor, paving the way for more precise, efficacious, treatment. The GIST model furthers the advancement of targeted therapy development, which continues to revolutionize the treatment of cancer at large.

ACKNOWLEDGEMENT

We thank Dr. Gary E. Gallick, PhD, University of Texas-MD Anderson Cancer Center, Genitourinary Medical Oncology Department, for his admirable mentoring and support.

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Received: January 17, 2010 Revised: April 02, 2010 Accepted: April 03, 2010