

# Medical Treatment of Gastrointestinal Stromal Tumors: State of the Art and Future Perspectives

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**Abstract:** Gastrointestinal Stromal Tumor (GIST) is the most common mesenchymal neoplasm of the gastrointestinal tract, and it is characterized by the occurrence, in > 90 % of cases, of a gain of function mutation in the c-kit proto-oncogene. STI-571 (imatinib mesylate), a selective KIT tyrosine kinase inhibitor, has changed the natural history of this disease, since it has shown high effectiveness in metastatic GIST, and it is currently under investigation also in the adjuvant and neoadjuvant setting. Mechanisms of resistance to imatinib mesylate include both *de novo*, and, more frequently, acquired resistance, which may occur after several months of drug administration and possibly depends, in most cases, upon an acquired second mutation. In order to overcome imatinib mesylate resistance, the addition of other drugs may be considered in patients who have less than an optimal response to imatinib mesylate monotherapy. Investigational agents that are being studied in this setting include the mammalian target of rapamycin (mTOR) inhibitor RAD 001 and the protein kinase C inhibitor PKC412. In addition, other KIT tyrosine kinase inhibitors with anti-VEGF receptor inhibitory activity, such as SU11248, PTK787/ZK787 and AMG 706, are currently being explored as second line monotherapy for imatinib mesylate-resistant GIST. Finally, another new drug, ecteinascidin (ET-743), that blocks cell cycle progression in G2/M phase through a p53-independent apoptotic mechanism, has shown important preclinical and clinical activity against a number of human solid tumors, including GIST.

**Keywords:** Gastrointestinal Stromal Tumors, imatinib mesylate, resistance.

## INTRODUCTION

Gastrointestinal Stromal Tumors (GIST) were described in 1983 by Mazur and Clark as tumors in the gastrointestinal tract and mesentery characterized by a specific histological and immunohistochemical pattern. In the past, these tumors were most often classified as leiomyoma, leiomyosarcoma or leiomyoblastoma [1]. GIST arise predominantly in the stomach (60 %), and small intestine (25 %), but also in the rectum (5%), esophagus (2 %), and a variety of other locations, such as appendix, gallbladder, mesentery, omentum, retroperitoneum and abdominal wall [2-4].

GIST are currently considered to be originated from transformation of interstitial cells of Cajal (ICC), which function as a pacemaker system controlling peristaltic activity [5,6]. Most, if not all, GIST express the KIT, a type III tyrosine kinase, as measured by CD117 immunohistochemistry, which is known to have diverse roles in several major cell systems [7]. Binding of growth factor stem cell factor (SCF) to KIT results in receptor homodimerization, activation of the tyrosine kinase activity, and resultant phosphorylation of a variety of substrates [8]. In many cases, these substrates are themselves kinases and serve as effectors of intracellular signal transduction.

Oncogenic Kit gene mutation, which encode structurally abnormal proteins with a constitutive enzymatic activity [9,

10], is an early event in GIST pathogenesis. Additionally, the frequency of KIT positive is so high (90 to 95%) that it is considered a prerequisite for the histologic diagnosis of GIST [11, 12]. Most of the c-kit mutations occur within the juxtamembrane region, encoded by exon 11. In addition, mutations in other regions of this gene, including exons 9, 13 and 17, have been detected, although they occur at a much lower frequency than those in exon 11 [10, 13, 14]. More recently, Heinrich *et al.* described activating mutations in platelet derived growth factor receptor (PDGFR) in one-third of KIT wild-type GIST [15]. The signal transduction profiles for PDGFR -mutant tumors were indistinguishable from KIT mutant tumors, suggesting that PDGFR can substitute for KIT in GIST oncogenesis.

The advent of STI-571 (imatinib mesylate), a selective KIT tyrosine kinase inhibitor, has changed the natural history of this disease. In fact, until recently, surgery has been the only effective treatment approach for GIST resulting in 5-year survival rates of 48-54% for resectable disease; on the other hand, conventional chemotherapeutic regimens yielded objective responses in only a few cases, thus being devoid of a positive prognostic impact. Despite the excellent overall success of imatinib, many patients can present resistance to this targeted therapy either *de novo*, or more frequently, on an acquired basis, which may occur after several months of drug administration.

In this paper we aim to provide an overview of recent clinical trials with imatinib mesylate which represents a major breakthrough in the treatment of GIST and a new paradigm of targeted cancer therapy. In addition, we want to

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summarize the main completed and ongoing studies with other compounds, and the postulated mechanisms of imatinib mesylate resistance, whose elucidation should provide new insights for its reversal. In this review we considered the published literature (from Pubmed-National Library of Medicine) and recent international meeting data.

### IMATINIB MESYLATE

The tyrosine kinase inhibitor imatinib was recently shown to be highly effective in GIST [16]. The first patient with GIST was started on oral imatinib in February 2000, and he had a major objective response, which was maintained longer than 18 months [16], in spite of his heavy previous treatment.

#### Activity in Advanced/Metastatic Disease

The European Organization for Research and Treatment of Cancer (EORTC) Soft Tissue and Bone Sarcoma Group phase I study of imatinib for patients with advanced soft tissue sarcomas, including GIST, has been published. Forty patients, 36 of whom had GIST, were treated with doses ranging between 400 mg to 1000 mg daily. Substantial activity was seen only in GIST patients, in whom the disease progression rate was only 11 %, while 69 % of patients had an objective response and 19% stable disease [17, 18]. Dose limiting toxicities were nausea, vomiting, edema, and rash; these side effects were seen at the highest dose level (500 mg b.i.d). Therefore, the investigators recommended that a dose of 400 mg twice a day was to be used for phase II/III testing. Confirmatory data have been reported for a multicenter phase II trial which randomized 147 patients with unresectable or metastatic GIST to receive either 400 or 600 mg of imatinib. Seventy-nine patients (53.7%) achieved a partial response, 41 patients (27.9%) had stable disease and only 20 (13.6%) progressed; seven patients (4.8%) could not be evaluated. Mutation of c-kit was found in 86 % of the patients, and patients without mutation were less likely to respond [19]. A prospective phase III study aimed to assess dose dependency of response and progression-free survival with imatinib. A total of 946 patients with metastatic GIST were randomly allocated to receive 400 mg either once or twice a day. A cross-over was possible for the once a day arm. Fifty-two patients (5.5%) achieved a complete response, 442 (47 %) a partial response, and 300 (32%) a stable disease, with no difference between groups. At median follow-up of 760 days, 263 (56%) of 473 patients allocated to imatinib once a day had progressed compared with 235 (50 %) of 473 patients allocated to imatinib twice a day with hazard ratio 0.82 (95 % CI 0.69-0.98) ; p= 0.026). After two years, overall survival was 69 % in patients treated once a day and 74 % in those allocated to twice a day treatment. In conclusion, this trial has shown similar response induction rates for imatinib 400 mg given either once or twice a day, but significantly better progression-free survival for the twice daily regimen [20]. Moreover, cross-over to high dose imatinib (800 mg daily), was feasible in most of the 143 patients (69%) who progressed on low-dose (400 mg daily). Two confirmed partial responses were recorded in this subset of 97 evaluable patients, 30 patients had stable disease, while 65 progressed. Despite increased fatigue and anemia, dose reduction was required in only 31 % of patients within a year

[Zalcberg JR, Verweij J, Casali P, et al. Outcome of patients with advanced gastrointestinal stromal tumors (GIST) crossing over to a daily, imatinib dose of 800 mg (HD) after progression on 400 mg(LD)- an international, intergroup study of the EORTC, ISG and AGITG. *Proc Am Soc Clin Oncol* 2004; 23: 815 (Abstr 9004)]. In a recent subanalysis of this study, the correlation of target genotype with clinical activity of imatinib was investigated. Particularly, in a total of 324 patients, KIT mutations were found in 280 patients (86.4%) and PDGFR mutations in 3 patients (1%) for an overall mutation rate of 87.3%. Patients whose tumor expressed an exon 11 mutant isoform were more likely to have an objective response to therapy (67%) than patients whose tumors expressed a KIT exon 9 mutant isoform (40%) or who had no kinase mutations (39 %; p= 0.0022). In a multivariate analysis, KIT exon 11 was the single best predictor of objective response. Moreover, patients with exon 11 mutant had a significantly longer time to progression (576 days) than patients either exon 9 mutant isoform (308 days) or no detectable kinase mutations (251 days, p=0.0012) [Heinrich MC, Shoemaker JS, Corless CL, et al. Correlation of target kinase genotype with clinical activity of imatinib mesylate (IM) in patients with metastatic GI stromal tumors (GISTs) expressing KIT (KIT +). *Proc Am Soc Clin Oncol* 2005; (abs 7)].

These results are confirmed by a phase III study (S0033), that had the same design of the former. The data from 320 patients demonstrated that survival estimates at 2 years were 78 % (95 % CI : 73 – 82 %) for the imatinib 400 mg arm versus 73 % (95 % CI: 68-77 %) for imatinib 800 mg arm. Moreover, 164 patients treated at the initial dose of 400 mg daily had disease progression; and 88 of them crossed over the other arm of treatment. Following crossover, 5 of 68 evaluable patients had partial response and 20 had stable disease. Median progression free survival and overall survival following crossover were 4 and 19 months, respectively [Rankin C, von Mehren M, Blake C, et al. Dose effect of imatinib (IM) in patients (pts) with metastatic GIST – Phase III Sarcoma Group Study S0033. *Proc Am Soc Clin Oncol* 2004; 22: (Abs 9005)].

Taken together, these two studies demonstrated that imatinib at either 400 or 800 mg daily prolongs survival. There is no significant statistical difference in disease control between patients who begin imatinib at standard dose compared to high dose. In addition, following disease progression on the standard dose arm, a subset of patients achieves clinical benefit following cross-over to the higher dose of 800 mg daily.

#### Continuous Versus Intermittent Schedule

The schedule of imatinib administration is a matter of investigation since it might influence imatinib resistance. In a phase III prospective study, after 1 year of treatment with imatinib, patients were randomized to imatinib continuation (arm 1) or interruption (arm 2) followed by restart upon progression. Forty-six patients were randomized in total (23 in each arm); 0 (0%) and 5 (21%) patients have a documented progression at 3 months in the arm 1 and arm 2, respectively. However, imatinib reintroduction in the arm 2, enabled tumor control in all patients [Le Cesne A, Perol d,

Ray-Coquard *et al.* Interruption of imatinib (IM) in GIST patients with advanced disease: Updated results of the prospective French Sarcoma Group randomized phase III trial on survival and quality of life. *Proc Am Soc Clin Oncol* 2005; 23: (Abstr 9031)].

### Adjuvant/Neoadjuvant Setting

The role of imatinib mesylate in the adjuvant setting is under active investigation. This adjuvant strategy might be able to eradicate microscopic disease and lead to cure, but it might also facilitate the emergence of imatinib-resistant cell clones. The ideal patients for clinical studies in this setting are, of course, those who have a substantial risk of relapse, such as tumor size >10 cm, tumor rupture, tumor hemorrhage or multifocal (more than five) tumors. Recently, the safety results were available of a phase II trial (Z9000), in which the patients at high risk after complete surgery for GIST were prescribed imatinib 400 mg daily for 1 year. Among 106 enrolled patients, 87 (82%) patients completed one year of therapy. Among them, 72 (68%) tolerated full dose without dose-modification, 8 had a temporary dose reduction and 7 were permanently switched to a lower dose. Further follow-up is required to determine the effect of adjuvant imatinib on survival [DeMatteo RP, Antonescu CR, Chardaram V, *et al.* Adjuvant imatinib mesylate in patients with primary high risk gastrointestinal stromal tumor (GIST) following complete resection: Safety results from U.S. Intergroup Phase II trial ACOSOG Z9000. *Proc Am Soc Clin Oncol* 2005(abstract 9009)]. A phase III intergroup trial led by the American College of Surgeons Oncology Group (trial Z9001) will randomize 380 patients to receive either 400 mg imatinib for 1 year or placebo.

At present, two adjuvant studies are open in Europe. In the first phase III trial conducted by the EORTC Soft Tissue and Bone Sarcoma Group 400 GIST patients are randomized to receive either two-years of imatinib (400 mg daily) or no further therapy after complete surgery. The second trial is a randomized phase II study conducted by the Scandinavian Sarcoma Group, in which 80 patients at high risk of disease recurrence or rendered free from metastatic disease by surgery are randomized to either 12 or 36 months of imatinib (400 mg daily).

Neoadjuvant imatinib is an even more recent approach. Bauer *et al.* [21] evaluated the role of resection of residual disease in patients with metastatic GIST responding to treatment with imatinib; the drug was able to allow a complete resection in 11 out of 90 patients. A prospective phase II trial of neoadjuvant imatinib in primary GIST is being developed by Radiation Therapy Oncology Group.

The results of these trials will not be available for several years, and, for the time being, adjuvant/neoadjuvant imatinib should be regarded as experimental forms of therapy.

### Mechanisms of Resistance

Treatment with imatinib as a single agent has to face an inherent weakness of monotherapy, i.e. the emergence of tumor resistance [22]. Resistance mechanisms in the treatment of GIST still represent a fairly obscure field. Primary resistance is generally defined as progression within

the first 6 months of imatinib treatment. This subgroup of GIST usually express either wild-type KIT, or mutations in exon 9 of KIT or mutated PDGFR. Moreover, secondary resistance occurs according to two different patterns: partial and multifocal resistance. In the first subgroup, in which one or a limited number of metastases show an enlargement, but the other sites remain controlled by imatinib, a multidisciplinary approach is considered, and possible strategies include the combination of locoregional approaches and either an increased dosing of imatinib or an alternative experimental targeted therapy. On the other hand, in the second subgroup, increasing the dose of imatinib or an alternative targeted therapy are both potentially suitable strategies, while surgery or radiofrequency ablation are less useful [23].

Central to the success of imatinib is the vulnerability of tumor cells to the disruption of dominant oncogenic pathways, a phenomenon referred to as "oncogene dependence" [24]. Oncogene dependence is hypothesized to result from the subversion and reorganization of normal cellular signaling networks by an introduced dominant oncogene; removal or modulation of the oncogene leads to alterations in the aberrant signaling network that are detrimental to cell proliferation and/or survival. Preclinical evidence supporting this model of oncogene dependence has been provided by both cell culture experiments and transgenic mouse tumors models. Particularly, Druker *et al.* demonstrated that M07 human megakaryocyte cells, IL-3 dependent and imatinib resistant, subsequently became IL-3 independent and imatinib sensitive following transfection with BCR-ABL [25].

Interestingly, in some imatinib-resistant chronic myeloid leukemia patients, neither mutation nor amplification of the BCR-ABL locus can be detected, indicating that other forms of resistance must also occur *in vivo* [26]. In analogy to these findings, it is known that expression of KIT is seen in almost all of the GIST, with the exception of a small proportion of tumors with morphological features of GIST that are essentially KIT negative. Heinrich *et al.* have shown that a proportion of GIST lacking KIT mutations have activating mutations in the related receptor tyrosine kinase PDGFR and that KIT and PDGFR mutations appear to be alternative and mutually exclusive oncogenic mechanisms in these tumors [15]. Additionally, Bauer *et al.* have reported the existence of GIST patients who responded well to imatinib mesylate despite having near complete absence of KIT expression [27]. Taken together, this information indicated that the mutational status of these oncoproteins is predictive of clinical response to imatinib. In a prospective study, 127 GIST patients were examined for mutations of KIT, or PDGFR, and their correlation with clinical outcome. KIT mutation was found in 112 patients (88.2%) and PDGFR mutation in 6 patients (4.7%). A better partial response rate (83.5%) was observed in presence of exon 11 KIT- mutation with respect to that observed in patients with exon 9 (47.8%) and no KIT or PDGFR mutations (0%) [28]. These results are in keeping with those of other studies [12] and suggest that there are probably differences in downstream signaling in exon 9 versus exon 11 KIT-mutant GIST and such biologic differences might influence the susceptibility of the tumor cells to apoptosis in response to kinase suppression by imatinib.

Other hypothesis of resistance include the development of secondary point mutations affecting the ATP pocket of the KIT receptor that most likely produces allosteric conformational changes that alter the conformation of KIT kinase domain and relative affinity of KIT to imatinib [29, and *Tamborini E, Prici S, Negri T, et al. Biochemical analyses and molecular modeling of two aminoacidic substitutions detected in two gastrointestinal stromal tumor (GIST) patients showing acquired resistance to imatinib. Proc Amer Assoc Cancer Res 2004; 46 (Abstr LB-286)*].

Finally, “biological resistance” (in which the cancer cells are no longer dependent on the targeted oncogene) has to be considered. Biological resistance may occur in the presence of additional dominant oncogenes in the tumor cells that are not inhibited by targeted therapy (secondary oncogene dependence). There is evidence for activation of downstream pathways in GIST including MAPK, Akt, p70/85S6K, STAT1 and STAT3 [30, and *Noma K, Naomoto Y, Haisa M, et al. Analysis of signal transduction in oncogenesis of gastrointestinal stromal tumor. Proc Amer Assoc Cancer Res 2004; 46 (Abstr 5528)*]. Additionally in a study in 12 GIST it was demonstrated that the expressions of phospho-STAT-3 and phospho-Akt were more intense in the GIST with KIT mutation than in those with PDGFR mutation, while expression of phospho-Erk-1/2 was stronger in GIST with PDGFR [Kang HJU, Nam SW, Rhee H, et al. *Different protein activation in gastrointestinal stromal tumors with the KIT and PDGFR mutation. Proc Amer Assoc Cancer Res 2004; 46 (Abstr 5539)*]. Recently, in the retrospective analyses of 21 GIST, with a long follow-up, the role of PTEN, a tumor suppressor gene often altered in human cancer, was evaluated by immunohistochemistry. It was seen that PTEN downregulation is implied in GIST progression [31]. In the retrospective study, the expression of the serine-threonine protein kinase PKC- $\delta$ , analyzed by immunohistochemistry in a panel of 26 GIST was recently identified. It was demonstrated that PKC- $\delta$ , a novel isotype, was expressed in GIST, whereas this protein was undetectable in other mesenchymal or epithelial tumors. So this protein is a sensitive and specific marker for these tumors [32]. Finally, these findings emphasize that molecular subclassification of GIST is crucial in identifying patients who are at high risk for early treatment failure (primary-resistance).

Indeed, preclinical data have suggested the presence of additional mechanism for the lack of response to targeted therapies, i.e. “pharmacokinetic resistance”, which might occur by a variety of mechanisms that decrease drug delivery to cancer cells [33]. Imatinib is primarily metabolized in the liver by CYP3A4 whose inducers, such as St. Jones Wort and rifampicin, may increase metabolism and decrease imatinib concentration, thus significantly reducing drug exposure. On the other hand, imatinib can increase plasma concentration of other CYP3A4 substrates. Caution is therefore required when giving imatinib in combination with CYP3A4 substrates with a narrow therapeutic window [34]. Future studies should consider pharmacokinetic evaluation in serum and target tissue.

Taken together, these findings demonstrate that GIST are heterogenous tumors with differential activation and

expression of some genes. Some of these genes could be useful as a promising independent prognostic factor and might represent a possible target for treatment. Improved tailoring of effective imatinib-therapy, according to KIT or PDGFR status and other biological predictive markers, has to be considered a major breakthrough.

### Novel Compounds Under Investigation

There are complex interactions between the KIT or PDGFR receptors and their downstream effectors. Preliminary studies of signal transduction in GIST demonstrate a somewhat homogenous pattern of signal transduction activation. KIT-mutant GIST have strong KIT phosphorylation, including the GRB2 and PI3K. Particularly, there is evidence for activation of downstream pathways including MAPK, AKT, STAT1 and STAT3, while the JNK and STAT5 pathways are not activated [12, 15]. Using specific inhibitors of KIT, MEK, PI3K or mTOR, it has been shown that activation of the PI3K/mTOR, but not the MEK/MAPK, is essential to KIT mediated-oncogenic signaling in GIST.

Correspondingly, selective inhibition of the PI3K/mTOR pathway reduces proliferation and increases apoptosis [35]. Similar pathways of signal transduction activation were observed in PDGFR - mutant GIST. So newer targeted therapy alone or in combination to imatinib could be used in patients with GIST refractory to imatinib.

In addition, there are other selected drugs with a novel mode of action that are used after development of imatinib - resistance.

There is a clear indication that additional investigational agents will be able to overcome some of these unknown resistance mechanisms. Some of the other newer molecules are described:

#### SU 11248

SU 11248, a multi-targeted tyrosine kinase inhibitor, is a small molecule that potently inhibits PDGFR and VEGFR-1, VEGFR-2, and KIT, and therefore has both direct antitumor and antiangiogenic properties [36]. Its potent activity was evaluated *in vitro* and *in vivo* [37].

A phase I/II study of SU11248 evaluated the safety, tolerability, and preliminary activity of this new drug administered to patients with metastatic GIST resistant to imatinib-mesylate [Manning WC, Bello CL, Deprimo SE, et al. *Pharmacokinetic and pharmacodynamic evaluation of SU11248 in a phase I clinical trial of patients with imatinib-resistant stromal tumors. Proc Am Soc Clin Oncol 2003; 22: (Abstr 768)*, and *Demetri GD, Desai J, Fletcher JA, et al. SU11248, a multi-targeted tyrosine kinase inhibitor, can overcome imatinib (IM) resistance caused by diverse genomic mechanisms in patients (pts) with metastatic gastrointestinal stromal tumor. (GIST) Proc Am Soc Clin Oncol 2004; 22: (Abstr 3001)*]. The recommended dose/schedule for phase II was SU11248 50 mg orally once daily for 4 weeks, followed by a 2 week period off drug in each 6 weeks cycle. 92 patients were evaluable for response. Partial response was observed in 7 patients (8%) and stable disease in 53 patients (58%), for an overall benefit achieved

in 60/92 patients (65%). With median treatment time over 1 year, 26 patients remain on study, while 6 patients developed progressive disease [Maki RG, Fletcher JA, Baum C, et al. Results from a continuation trial of SU11248 in patients (pts) with imatinib-resistant gastrointestinal stromal tumor (GIST) Proc Am Soc Clin Oncol 2005; (Abs 9011)]. For 22 patients with RECIST-evaluable disease neither median time to progression nor overall survival have been reached. Grade 3 and 4 adverse events for this cohort of patients included: hypertension (16%), asymptomatic lipase elevation (15%), neutropenia (14%), fatigue (11%). This study also evaluated the activity of SU11248 by KIT and PDGFR mutation analysis in 57 patients with > 6 months of follow-up and samples available for genotype analysis. This subanalysis found that there were one mutation in KIT only in 22 patients (39%); one mutation in PDGFR only in 1 patient (2%); no detectable mutations (wild type) in 9 patients (16%); 2 mutation in KIT in 25 patients (44%). 12 out of the 15 patients with exon 9 KIT mutation (80%) had an objective benefit (response + > 6 months stable disease); 1 patient with single PDGFR mutation had an objective benefit as well. Finally, there were other two groups of 9 patients with wild-type KIT + PDGFR, and 16 patients with secondary mutation of KIT exon 13 or 14, for which the objective benefit was achieved in 5/9 (55%) and 9/16 (56%), respectively. All of the above patients were reported as highly sensitive to the drug. On the other hand, the groups of patients with exon 11 KIT mutation and secondary exon 17 KIT mutations were reported as being less sensitive to SU11248. In this study, compared with baseline, tumors from patients with clinical benefit had a significant decrease of 15% in phosphorylated PDGFR-beta activity in the tumor cell compartment, whereas tumors in non-responders had an increase of 11%. [McConkey DJ, Heymach JV, Desai J, et al. Pharmacodynamic analysis of target receptor tyrosine kinase activity and apoptosis in GIST tumors responding to therapy with SU11248. Proc Am Soc Clin Oncol 2005; (abs 3006)]. An international, multicenter, randomized phase III study of SU11248 is ongoing.

### PTK 787/ZK787 and AMG 706

PTK 787/ZK787 (PTK/ZK) is a novel oral, small molecule, angiogenesis inhibitor targeting all known VEGF receptor tyrosine kinases, including VEGFR-1/flt-1, VEGFR-2/KDR, VEGFR-3/flt-4 and also PDGFR, KIT [38]. In preclinical studies, PTK/ZK has been shown to inhibit growth and reduce microvasculature in subcutaneously implanted human tumor xenografts in nude mice [38, 39].

In a phase I study, 43 patients with advanced cancers (two of whom with GIST) received PTK/ZK at doses of 150 to 1000 mg daily. Dose-related grade 3 fatigue and vomiting were observed. The maximum-tolerated oral dose was 750 mg twice daily [40].

At present, a phase II study in metastatic GIST is open (CPTK787 A2401/300267). This study will evaluate the activity of PTK/ZK (1250 mg/die), in patients with metastatic GIST resistant to imatinib mesylate.

AMG 706 is a potent oral, multi-kinase inhibitor with anti-angiogenic and anti-tumor activity. In a preclinical

study, AMG 706 produced a statistically significant reduction in vascular blood flow in a human tumor xenograft [Jackson E, Esparza-Coss E, Bankson A, et al. The effect of AMG 706, a novel multi-kinase inhibitor, on vascular permeability and blood flow as assessed by dynamic contrast enhanced magnetic resonance imaging in an in vivo preclinical tumor model. Proc Am Soc Clin Oncol, 2005(abs 3134)]. Safety and pharmacokinetic of AMG706 have been recently evaluated in a phase I trial in advanced solid tumors. AMG706 was well tolerated up to the dose of 125 mg once daily using the intermittent (21 days of therapy followed by 7 days without dosing) and continuous dosing schedule. The most frequent adverse events were hypertension, fatigue, and headache, but the treatment was generally well tolerated. Among 56 evaluable patients, best responses were 4% partial responses and 61% stable disease. Vascular changes were demonstrated by dynamic contrast enhanced magnetic resonance imaging [Rosen L, Kurzrock R, Jackson E, Wathen L, et al. Safety and pharmacokinetics of AMG706 in patients with advanced solid tumors. Proc Am Soc Clin Oncol, 2005(abs 3013)].

Recently, a clinical trial of AMG706 was started in three Italian centers; it will evaluate the activity of this drug in patients with metastatic GIST resistant to imatinib.

### mTOR and Protein Kinase C (PKC) Inhibitors

The mammalian target of rapamycin, also known as FRAP/RAFT/mTOR, is a serine/threonine protein kinase which acts downstream of the PI3K/Akt signaling pathway. It has been implicated in the control of a variety of metabolic and transcriptional processes that lead to cell growth and/or proliferation [41, 42]. mTOR can also regulate the stability of some cell cycle regulatory proteins, such as cyclin D and p27 [43].

The development of anticancer agents which act as rapamycin analogue (CCI-779, RAD 001 or everolimus) has been undertaken [44, 45]. RAD 001 has shown antitumor activity *in vitro* in GIST cells. Furthermore, a synergism between imatinib and everolimus, in slowing proliferation and inducing apoptosis in primary GIST cells resistant to imatinib has been demonstrated *in vitro*. A phase I/II clinical study that tested the feasibility of the combination of RAD001 and imatinib in GIST patients refractory to imatinib was undertaken. Patients with metastatic GIST were eligible if progressing after at least 4 months of imatinib therapy at optimal doses. The combination was well tolerated. A pharmacokinetic evaluation was performed in this study, and it showed that imatinib increased the levels of everolimus, but everolimus did not affect the levels of imatinib or of its main metabolite [Van Oosterom AT, Dumez H, Desai J, et al. Combination signal transduction inhibition: a phase I/II trial of the oral mTOR – inhibitor everolimus (E, RAD 001) and imatinib mesylate (IM) in patients (pts) with gastrointestinal stromal tumor (GIST) refractory to IM. Proc Am Soc Clin Oncol 2004; abst 3002]. The increase in RAD001 bioavailability could be *via* competition with imatinib for CYP3A4 and/or PgP. The most frequent adverse events were grade 1-2 fatigue, diarrhea, vomiting, nausea, anemia and skin rash. The median duration of treatment was 14 weeks (range 3-60). Stable disease was observed in 8 patients for

more than 4 months (1 patients at 20 mg/week, 5 patients at 2.5 mg daily and 2 patients at 5 mg/day); in two patients (2.5 mg/day) it improved to partial response. Molecular analyses are ongoing to characterize responding patients [van Oosterom A, Reichardt P, Blay J-Y, et al. *A phase I/II trial of the oral mTOR-inhibitor everolimus (E) and imatinib mesylate (IM) in patients (pts) with gastrointestinal stromal tumors (GIST) refractory to IM: Study update. Proc Am Soc Clin Oncol 2005 (abs9033)*].

PKC 412 is a staurosporine derivative with activity against several tyrosine kinases, such as PKC, KIT, PDGFR [46, 47, 48]. The activity of PKC 412 against imatinib resistant mutants has been characterized in progressive GIST [49]. This study has shown the high frequency of KIT/PDGFR kinase domain mutations in patients with secondary resistance and define genomic amplification of KIT/PDGFR as an alternative cause of resistance to the drug. These findings showed the sensitivity of the imatinib-resistant KIT-T670I and KIT-V654A and of PDGFR-D842V mutants to PKC and lent support to clinical evaluation of the combination.

The safety and pharmacokinetics profiles of PKC412 have been determined in phase I clinical trials [50, 51]. The safety and the tolerability of the combination of imatinib (dose 600 to 1000 mg daily) and PKC412 (200 mg daily) in patients refractory to imatinib was evaluated in a phase I/II study, which is still ongoing. [Cohen PS, Wang Y, YU R, et al. *A phase I/II trial of the oral PKC-inhibitor PKC412 (PKC) in combination with imatinibmesylate (IM) in patients (pts) with gastrointestinal stromal tumor (GIST) refractory to IM. Proc Am Soc Clin Oncol 2005, (abs 3016)*]. Patients with metastatic GIST were eligible if they had progressed after at least 4 months on imatinib therapy. Nineteen patients have been entered up to now, and 2 out of 5 patients evaluable for response had stable disease at 4 months. Four grade 3-4 adverse events were attributed to the combination, namely hyperglycemia, transient asymptomatic hyperamylasemia, hypercalcemia and transaminitis. Complete pharmacokinetic data for the first twelve patients show that PKC trough plasma concentrations increased about 2-fold over that seen in AML studies with PKC. Imatinib exposure decreased about 70% after one month of co-administration with PKC, either due to enzyme induction, or protein binding interactions [52].

### **Ecteinascidin-743 (ET-743)**

Ecteinascidin-743 (ET-743) (or trabectedin; Yondelis) is a marine-derived compound isolated from the Caribbean tunicate *Ecteinascidia turbinata* with a novel mechanism of action [53]. It is known that this drug is a unique DNA-interacting agent with covalent binding to the DNA minor groove [54]. ET-743 blocks cell cycle progression in G2/M phase through a p53-independent apoptotic process [55] and inhibits the transcriptional activation of inducible genes [56]. Trabectedin has a potent antitumor activity demonstrated *in vitro* and *in vivo* in several solid tumors, including ovarian, breast cancer, melanoma, and sarcoma [57, 58]. Of major importance, ET-743 showed antiproliferative activity on drug-resistant tumors, with minimal or no cross-resistance to several conventional chemotherapeutic agents [53, 59].

Based on these preclinical findings, 163 patients were included in five phase I studies assessing six different schedules of administration with doses of ET-743 ranging from 0.05 mg/m<sup>2</sup> to 1.9 mg/m<sup>2</sup> [60 and Forouzeh B, Hidalgo M, Denis L, et al. *Phase I and pharmacokinetic study of ET-743, a minor groove DNA binder administered weekly to patients with advanced cancer. Proc Am Soc Clin Oncol 2001; 20: (Abstr 373)*].

The recommended dose/schedule of ET-743 for phase II studies was 1.5 mg/m<sup>2</sup> per course as a 24 hour continuous intravenous infusion every 3 weeks. [61, 62, 63]. A phase II study of ET-743 was conducted by the EORTC Soft Tissue and Bone Sarcoma Group in 28 patients with GIST previously untreated with cytotoxic chemotherapy before imatinib era. The best response was stable disease in 9 (33%) patients, and disease progression in 18 patients (67%) with a median time to disease progression and overall survival of 51 and 589 days, respectively [64]. In conclusion, ET-743 is one of most promising cytotoxic agents tested in the last two decades in sarcomas, and probably in imatinib-resistance GIST.

### **CONCLUSIONS**

Targeted therapies promise to revolutionize the care of cancer patients. The most dramatic examples of the potential power of this approach come from success with the small molecule imatinib-mesylate, that effectively inhibits the aberrant signalling properties and malignant consequences of oncogenic BCR-ABL, KIT and PDGFR in patients with chronic myelogenous leukaemia and GIST, respectively. Additionally, future studies should consider the KIT and PDGFR receptors and their downstream signaling components. This type of analysis may also identify patient populations that would benefit from imatinib-mesylate alone or in combination with other tyrosine-kinase inhibitors. In fact, new methods are focused on the changes in gene expression patterns between normal and tumoral tissue. This would allow for the elucidation or alteration of specific signaling pathways that could envision personalized patient therapies. There is also considerable hope that this success will serve as a paradigm for the development of the therapy for other, more common tumors.

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