CLINICAL INVESTIGATION

Combination therapy for gastrointestinal stromal tumors: evidence from recent clinical trials

Clin. Invest. (2011) 1(6), 825–836

Isabelle Ray-Coquard[†1], Philippe Cassier[1], Intidhar Labidi Galy[1], Pierre Heudel[1], Louis Tassy[1], Maria Chelghoum[1], Amandine Burghas[1], Pierre Méeus[1] & Jean Yves Blay[1]

[1]Département d'Oncologie Médicale, Département de chirurgie, Centre Léon Bérard, 28 rue Laennec, 69373 Lyon, Cedex 08, France
[†]Author for correspondence:
Tel.: +33 478 782 888
Fax: +33 478 782 716
E-mail: ray@lyon.fnclcc.fr

Gastrointestinal stromal tumors (GIST) are neoplasms of mesenchymal origin arising from the GI tract. These tumors are characterized by activating mutations of the receptor tyrosine kinases, either KIT or PDGFRa, which are found in 85% of cases. Despite the use of imatinib in first-line and sunitinib in second-line treatment, patients with metastatic GIST still have a high risk of progression and death. To date, the median overall survival for metastatic disease is close to 5 years. Treatment with imatinib, an inhibitor of KIT and PDGFRa, results in clinical benefit, that is, objective responses or disease stabilization in approximately 85% of patients with unresectable or metastatic GISTs. However, metastatic GIST develop resistance to imatinib at a median time of 24 months, and sometimes more than 8 years after initiation of the treatment. In addition, as many as 15% of patients do not respond initially to imatinib; therefore, novel therapeutic options, new drugs or combination are needed. Genotypic analysis reveals that acquired resistance is frequently caused by secondary missense mutations in KIT exons 13, 14 or 17 (corresponding to the kinase 1 domain and kinase activation loop). Primary resistance is associated with primary activating mutations that are not sensitive to imatinib or have reduced sensitivity to the drug. Considering the molecular heterogeneity of tumors at relapse, the activation of the antiapoptotic pathway and the potential relevance to treating tumor cells and stromal cells, combination treatment targeting different pathways could be relevant in GIST resistant to imatinib. In this article, we will review the trials of combination treatment in advanced GIST after failure of imatinib and sunitinib. These trials are based on the understanding of the biological mechanisms of secondary resistance to imatinib in GIST, in particular the emergence of clones with secondary mutations. The trials exploring combinations of tyrosine kinase inhibitors (TKIs), TKIs with mTOR inhibitors and TKIs with cytotoxic agents will be reviewed and their results presented.

Keywords: combination treatment • gastrointestinal stromal tumor • mTOR inhibitor • resistance to treatment • target therapy

Gastrointestinal stromal tumors (GISTs) are rare tumors of mesenchymal origin, which may develop anywhere along the GI tract. These tumors are thought to originate from the interstitial cells of Cajal and the pace-maker cells of the GI tract, and share with these cells the expression of the CD117 surface antigen, also known as the KIT or stem cell factor receptor, in approximately 95% of cases. Although rare, these tumors represent the most common mesenchymal tumors of the GI tract, with an estimated incidence of 12–15 cases per one million inhabitants in Western countries [1,2]. GIST and their management have been reviewed recently by Rubin *et al.* [3].

FUTURE SCIENCE part of fsg

The management of localized GIST relies on the complete surgical excision of the tumor, avoiding tumor spill during the procedure. GIST is resistant to chemotherapy and probably to radiotherapy. Following the discovery of activating mutations of *KIT* and *PDGFR*α, clinicians have focused on novel agents targeting these kinases. The first agent approved for the treatment of advanced GIST is imatinib mesylate (IM; Gleevec®/Glivec®, Novartis), which produces response rates of approximately 50–70% in patients with advanced GIST, with a median progression-free survival (PFS) of 20–26 months [4;5]. Approximately 9–14% of patients show immediate progression (primary resistance) when treated with IM and 5% of patients are intolerant to the drug. In addition, 50% of patients treated with IM experience disease progression within 2 years of treatment initiation. Patients with IM-resistant or IM-intolerance tumors may be treated with sunitinib (SUTENT®, Pfizer), which has shown activity in this setting in a Phase I/II study [6] and in a placebo-controlled randomized Phase III trial. In this setting, sunitinib produced a Response Evaluation Criteria in Solid Tumors (RECIST) response rate of only 7%, but a median PFS of 27 weeks (~6.2 months), in comparison, patients recieving placebo had a medium PFS of 6 weeks (~1.5 months) for patients receiving placebo [7]. Additional therapies based on the known biology of these tyrosine kinase (TK) receptor targets are being tested alone or in combination with IM. Most combinations are developed on the basis of the understanding of the molecular mechanism of resistance.

Mechanisms of IM & sunitinib resistance

■ Mechanisms of IM resistance

Early progression & primary-IM resistance

The most common identified mechanism of primary resistance to IM is the mutation status. Indeed, most patients who show early progression have either a *KIT* exon 9 mutation, a *PDGFR*α exon 18 D842V mutation or a wild type (WT) genotype for both the *KIT* and *PDGFR*α genes [8–10]. As noted above, some patients without detectable *KIT* or *PDGFR*α mutations show primary resistance to IM. Alterations in the RAS/MEK/ERK pathway may also be involved in the development of IM resistance in GIST, particularly in tumors lacking secondary *KIT* or *PDGFR*α mutations. Agaram and colleagues reported a *BRAF* exon 15 V600E in three of 61 GIST patients, who shared similar clinical features, being 49- to 55-year-old females and having their tumors located in the small bowel [11]. In a patient treated with IM 800 mg/day, simultaneous *KIT* and *PDGFR*α gene amplification was detected by fluorescence *in situ* hybridization analysis [12]. Whether gene amplification is the mechanism underlying resistance in other WT GISTs remains to be determined. Other targets or mutated genes (e.g., *NF1* and *SDH*) are possibly involved in the resistance to IM [13].

Secondary IM resistance

Several mechanisms of secondary resistance to IM have been described in this setting. However, the most documented and frequent mechanism remains the emergence of secondary *KIT* mutations. This later mechanism includes secondary mutations of the kinase domain (exon 13 and 14) or the activating loop (exon 17) [14–23]. Patients with *KIT* exon 11 mutations were more likely to have a secondary *KIT* mutation compared with patients with exon 9 mutations (62.6 vs 40.9%, respectively; $p = 0.021$ using the Chi^2 test), and none of the patients with WT *KIT* developed secondary *KIT* mutations. One of the eight patients with WT *KIT* GIST was found to have *PDGFR*α exon 14 mutations and developed resistance due to a secondary exon 18 mutation (D842V) [10].

In approximately 30–40% of patients, late IM resistance cannot be linked to secondary *KIT* mutations. *KIT* amplification has only been described in a small proportion of GISTs [12,24]. It has been reported as a mechanism of resistance to IM by one group, while other authors have not found *KIT* amplification in patients with IM-resistant GISTs [17]. Debiec-Rychter *et al.* also found IM resistance to be associated with loss of *KIT* expression, which indicates a *KIT*-independent mechanism of resistance [12]. Theou *et al.* found multidrug resistance proteins to be expressed in 21 GISTs samples using immunohistochemistry, however these authors could not find a correlation with clinical outcome, most notably none of those patients had IM-resistant disease [25]. *IGF1R* amplification may represent another mechanism of IM resistance. It is overexpressed in GISTs lacking *KIT*- and *PDGFR*α-activating mutations and in pediatric GISTs, and its inhibition in GIST cell lines results in cell death regardless of *KIT* mutation status [26]. Secondary resistance to IM can also occur due to the involvement of other pathways for tumor progression.

■ Mechanisms of sunitinib resistance

Sunitinib has been approved in the treatment of IM-resistant or -intolerant GISTs. The biological mechanisms underlying sensitivity or resistance to the drug have started to be uncovered. In a Phase I/II trial of sunitinib treatment in patients with IM-resistant GISTs, Heinrich *et al.* reported that patients whose tumor had a *KIT* exon 9 primary mutation or had WT *KIT* and *PDGFR*α were more likely to respond to sunitinib than tumors with a *KIT* exon 11 mutation [27]. The same team showed that sunitinib was effective *in vitro* against *KIT*

exon 13 and 14 mutations encoding for proteins showing IM resistance. Sunitinib was, however, inefficient against *KIT* with exon 17 mutations [28]. In an analysis of post-IM specimens, among all patients with pre-IM *KIT* mutations, the median PFS and overall survival (OS) with sunitinib, was significantly longer for the patients who had secondary *KIT* exon 13 or 14 mutations than for those with secondary exon 17 or 18 mutations. These results correlate with *in vitro* studies showing that sunitinib potently inhibits the phosphorylation of *KIT* double mutants, in which the secondary mutation occurs in the drug–ATP binding pocket, but has little activity versus *KIT* double mutants with secondary mutations in the activation loop [29]. Besides known IM-resistant *KIT* activation-loop mutations, no specific mechanisms of sunitinib resistance have yet been identified.

Alternative pathways involved in IM & sunitinib resistance

Treating patients with combinations in this setting resulted in a different set of observations that identifyed alternative pathways involved in IM and sunitinib resistance.

■ Heterogeneity of tumors during relapse & progression after IM or sunitinib treatment

Several studies have shown that secondary missense mutations occur in a subclonal and nonrandom pattern in GIST tumor masses. These secondary or tertiary mutations involve specific amino acid residues in exons 13, 14 and 17 [8,10,17,18,30] with a wide level of heterogeneity between tumor cells, including in the same metastatic sites. These secondary missense mutations account for approximately 45–65% of cases with acquired resistance. Debiec-Richter and coworkers identified secondary *KIT* mutations in 12 of the 26 patients (46%) who developed progressive disease (PD) after being treated successfully with IM for a median of 77 weeks [12]. Six distinct mutations were found: four patients had V654A mutations, three had T670I mutations, and the remaining patients had D716N, D816G, D820Y/E or N822K mutations. Similarly, Antonescu and colleagues identified secondary mutations in seven of the 15 patients (47%) who acquired resistance to IM: three with N822K mutations, two with D820Y mutations and the others with V654A, T670I or Y823D mutations [17]. One nodule showed loss of heterozygosity of the primary WK557–558 deletion mutation. Wardelmann and coworkers identified secondary mutations in 14 of the 32 patients (44%) with acquired resistance [18]. All secondary mutations were located in exons 13, 14 and 17, and included V654A, T670I and various point mutations between D816 and Y823. In one case, a double-point mutation was detected that resulted in a T670E substitution.

Heinrich and colleagues described the mutational status of 33 patients who acquired resistance to IM after a median of 20.2 months [10]. A total of 22 of the patients had one or more secondary kinase mutations: all secondary *KIT* mutations occurred in patients with underlying primary *KIT* mutations and the lone secondary *PDGFR*α mutation occurred in a patient with an underlying primary *PDGFR*α V561D mutation. The most common secondary *KIT* mutations were V654A, T670I, D820A/E/G, N822K/Y and Y823D. When these mutations were expressed against the WT *KIT* background or one containing a common primary *KIT* exon 11 mutation (V560D) or exon 9 mutation (AY501 insertion), they were associated with moderate-to-high levels of resistance to IM *in vitro* [10].

■ Activation of antiapoptotic pathway

An alternate method of impacting the constitutive activation of KIT and PDGFR is to inhibit downstream components of their signaling pathways. These receptors primarily signal via the phosphoinositide 3-kinase (PI3K)/Akt/mTOR pathway [31]. mTOR is a member of the phosphatidylinositol kinase-related family. It has serine/threonine kinase activity and regulates protein translation, cell-cycle progression and cellular proliferation. Inhibitors of downstream signaling, administered either alone or in combination with IM, may be useful when acquired resistance bypasses KIT and activates other pathways or signaling cascades. Interest in this pathway focusses on the possibility of mTOR inhibition through oral administration of rapamycin derivatives [32]. PI3K inhibitors and AKT inhibitors, in particular perifosine, which has been evaluated in a Phase II study in association with IM in resistant-GIST patients, could be also an interesting model [33].

■ Targeting both tumor cells & the stroma

Gastrointestinal stromal tumors have evidence of VEGF expression by immunohistochemistry, and patients with metastatic tumors have been shown to have increased serum VEGF levels [34]. After therapy with IM, VEGF levels decreased in patients responding to IM [35]. Perfusion MRI has documented decreases in blood flow to tumor in patients treated with IM, associated pathologically with decreased microvessel density and CD31 expression [36]. These data all suggest that VEGF and VEGF receptors are important biologically for GIST survival, and that alterations in VEGF are associated with IM therapy.

Mutant isoforms of the KIT or PDGF receptors expressed by GISTs are considered the therapeutic targets for IM, but case reports of clinical efficacy of IM in GISTs lacking the typical receptor mutations, prompted a search for an alternate mode of action.

Borg *et al*. showed that IM can act on host dendritic cells (DCs) to promote natural killer (NK) cell activation. DC-mediated NK cell activation was triggered *in vitro* and *in vivo* by treatment of DCs with IM as well as by a loss-of-function mutation of KIT [37]. Therefore, tumors that are refractory to the antiproliferative effects of IM *in vitro* responded to IM *in vivo* in a NK cell-dependent manner. Longitudinal studies of IM-treated GIST patients revealed a therapy-induced increase in IFN-γ production by NK cells, correlating with an enhanced antitumor response. These data point to a novel mode of antitumor action for IM [38].

Dendritic cells and NK cells might interact in inflammatory lesions, where chemokines and cytokines recruit both DCs and NK cells, or in the lymph nodes, where cooperation between IL-2-producing $CD4^+$ T cells and NK cells is ongoing. Knowledge of whether the IM-conditioned DC–NK cell crosstalk is mediated *in situ* or at distant sites (lymphoid organs) remains elusive. Nevertheless, in one patient who benefited from therapy with IM for 1 year, DC–NK cell interaction was found in an unusual site (skin undergoing IM-induced lichenoid dermatitis). This side effect regressed after withdrawal of IM, suggesting that the maturation of dermal DCs and/or recruitment of NK cells in the dermis was induced by this TK inhibitor (TKI).

Potential synergy between a cytotoxic agent & an inhibitor of antiapoptotic pathways

In Ewing sarcoma cell lines overexpressing KIT, the addition of doxorubicin to IM showed improved activity compared with IM or doxorubicin alone [39]. In these cases, IM synergistically sensitized Ewing sarcoma cells to doxorubicin treatment by arresting cell cycle and impairing intracellular signaling, mainly through MAPK pathway inhibition [40]. On the basis of these *in vitro* data, it was hypothesized that the combination of doxorubicin with IM could result in clinical activity in patients with GISTs refractory to IM therapy. This question was tested in a Phase II trial performed by the Spanish Group for Sarcoma Research (GEIS) and described in the chapter below.

New strategies in the management of IM- & sunitinib-resistant GIST: considering combination therapy

The understanding of the mechanisms responsible for IM and/or sunitinib resistance has guided the treatment options. GISTs with secondary missense mutations involving the KIT kinase 1 domain, ATP-binding site or kinase activation loop typically remain dependent on constitutive KIT-mediated activation and signaling. In such cases, the first choice is to escalate the IM dose from 400 to 800 mg/day, and only if dose escalation is ineffective should patients be switched to an alternative KIT-targeted kinase inhibitor. The same approach is appropriate if resistance results from *KIT* or *PDGFRα* gene amplification or drug-transport pump upregulation.

When resistance is caused by activation of other signaling pathways, tumor progression is no longer entirely dependent on constitutive KIT activation. In such cases, consideration may be given to use of novel agents either alone or in combination with KIT-based TKIs.

Several molecules in the ever-expanding family of TKIs are effective inhibitors of KIT and PDGFRα. These molecules can be divided into different classes: broad-scale TKIs with VEGFR2, such as sunitinib, sorafenib, AMG 706, regorafenib and dovitinib, and second-generation selective inhibitors of KIT such as nilotinib, masitinib or dasatinib. Everolimus, a potent mTOR inhibitor, has been tested in a Phase I/II trial in combination with IM [32], with prolonged tumor control in some patients. Another molecule with interesting activity is PKC412, which is a protein kinase C (PKC) inhibitor with broad activity as a kinase inhibitor. In the present paragraph, we review the currently available data on these molecules used in combination with other TKIs (Table 1).

■ Combination of TKIs targeting KIT &/or PDGFRa

Nilotinib (Tasigna®, Novartis) is a second generation TKI targeting KIT and PDGF receptors in addition to the Bcr-Abl kinase [41]. It has shown *in vitro* activity against KIT, PDGFRα, FMS, as well as some of the IM-resistant variants of KIT and PDGFRα [42]. Studies in GIST cell lines have shown that nilotinib reduces cell viability to a similar extent as IM and has antiproliferative activity against both IM-sensitive and -resistant forms of KIT [43]. A recent preclinical study by Pantaleo *et al.* evaluated the association of IM and nilotinib in a GIST xenograft model [44]. Nilotinib has a unique mechanism of intracellular transport leading to a seven–tenfold higher intracellular concentration in IM-sensitive and -resistant cell lines, respectively [43]. It is thought that this differential cellular uptake may make nilotinib less susceptible to cellular transport-driven IM resistance. Tolerance of nilotinib in patients with IM-resistant GIST was investigated in a Phase I/II trial. Preliminary results were presented at the 2007 American Society of Clinical Oncology meeting and republished in 2008 [45,46]. Patients with IM-resistant GIST received nilotinib alone (400 mg orally twice a day [b.i.d.]) or escalating doses of nilotinib (200 mg/day, 400 mg/day or 400 mg b.i.d.) in combination with IM (400 mg orally b.i.d.) or nilotinib 400 mg b.i.d. plus IM 400 mg/day. A total of 53 patients received nilotinib, alone (n = 18, five IM-intolerant) or in combination

Table 1. Summary of combination treatments.

Drug combination	Patients (n)	Previous lines	Objective response	Stable disease	Progression-free survival	Overall survival	Toxicities	Ref.
Imatinib + nilotinib	35	>1	3%	26%	203 days	NR	44% SAE GI two pts Cutaneous rash three pts No DDI reported	[45]
Imatinib + RAD01	42 28 47	>1 >1 >2	NR 0 2%	55% 36% 43%	NR 1.9 months 3.5 months	12 months 14.9 months 10.7 months	All cohorts: 65% SAE G3 14% diarrhea 10% altered PS 10% nausea 10% vomiting 29% anemia 26% hypo K^+ 10% hypo Na^+ Both cytochromes P450, CYP 3A4 no DDI reported	[32]
Imatinib + PKC412	19	>1	0	4/5 pts	NR	NR	Four pts G3 toxicity Hyperglycemia Hyperamylasemia Hypercalcemia Transaminitis Hyperthyroidism DDI reported	[54]
Sirolimus + PKC412	4	>1	3/5 pts	1/4 pts	NR	NR	One pt skin toxicity G3 DDI not evaluated	[55]
Imatinib + pegIFN	8	>1	8 pts	0	365–900 days	NR	Two pts cutaneous rash G3 DDI not evaluated	[57]
Imatinib + doxorubicin	26	>1	3 pts/22	5 pts/22	100 days	390 days	SAE grade 3–4: Three anemia Two transaminitis One anorexia Three asthenia	[58]

DDI: Drug–drug interaction; G3: OMS grade 3; GI: Gastrointestinal; NR: Not reported; PS: Performance status; pts: Patients; SAE: Serious adverse event.

with IM (n = 35). A total of 39 patients (74%) were resistant, refractory and/or intolerant to one or more prior therapies. A total of 16 patients received combination therapy at the nilotinib 400 mg b.i.d./IM 400 mg/day dose. Median duration of therapy in this combination arm was 217 days (range: 27–492 days) versus 186 days for nilotinib alone (range: 8–718 days). Grade 3/4 adverse effects were experienced by nine (50%) patients in the nilotinib alone arm and seven (44%) patients in the niltinib 400 b.i.d./IM 400/day arm. The most common of these was grade 3 gastrointestinal disorders, which occurred in eight (44%) patients in the nilotinib alone arm and two (12.5%) patients in the niltinib 400 b.i.d./IM 400/day arm. A total of one patient on nilotinib alone experienced dose-limiting hyperbilirubinemia; three patients on niltinib 400 b.i.d./IM 400/day experienced dose-limiting rash. No patients experienced QTcF > 500 msec. A total of two patients, one on nilotinib alone and one in the niltinib 400 b.i.d./IM 400/day arm, achieved partial response (PR) lasting 197 and 176 days, respectively. A total of 13 patients (72%) and nine patients (56%) in the nilotinib alone and niltinib 400 b.i.d./IM 400/day cohorts, respectively, experienced stable disease (SD). Median PFS was 168 days for nilotinib alone and 203 days for niltinib 400 b.i.d./IM 400/day. Median duration of disease control (complete response, PR or SD) was 158 days for nilotinib alone and 259 days for the niltinib 400 b.i.d./IM 400/day cohort [45]. Nilotinib, alone and in combination with IM, has significant activity in patients with GIST who are resistant to prior TKIs. A randomized Phase III trial testing this question has been completed and is currently under submission for publication testing nilotinib versus 'current

treatment options' in patients with IM- and sunitinib-resistant GIST. No statistical differences in PFS or OS for niltinib versus best supportive care (usually with continuation of TKI therapy) were demonstrated in the intent to treat population. The mixed patient population entering the study (multiple lines of previous therapy, lack of documented failure to prior therapy) and investigator choice to include TKI continuation in the best supportive care control make the outcomes difficult to interpret. Given the almost 2-month improvement in median OS in the intent to treat population and 4-month improvement in true third-line patients, further study of niltinib activity in well-defined GIST patient populations is warranted.

Combination of IM & sunitinib

Imatinib and sunitinib are currently being tested in combination in a Phase I/II trial in patients with GIST (NCT00573404) [101].

Combination of mTOR inhibitors & IM

A trial of inhibitors of downstream signaling, administered either alone or in combination with IM, may be appropriate when acquired resistance bypasses KIT and activates other pathways or signaling cascades. This resistance may occur because of the development of additional *KIT* mutations, genomic amplification of *KIT* or activation of alternative oncogenic signalling mechanisms, including the PI3K/Akt/mTOR pathway. Based on these observations, a combination of IM and everolimus (Afinitor®, Novartis) was tested in a Phase I/II trial in patients with IM-resistant GIST. In this trial patients received IM 600 mg/day and everolimus 20 mg/week. After the first 12 patients were accrued, the pharmacokinetic study showed a major interaction between IM and everolimus, resulting in the increase of everolimus concentrations. Given the acceptable toxicity profile of the combination, the study was extended with everolimus at 2.5 or 5.0 mg/day. A total of 31 patients had been treated. Grade 3–4 dose-limiting toxicities (e.g., stomatitis, thrombocytopenia, gastritis and hemorrhagic gastritis) occurred in three of five patients treated with everolimus 5 mg/day. Thus the cohort of patients receiving everolimus 2.5 mg/day was expanded to include 13 patients. Overall, seven patients had at least SD for >4 months, one of them having a PR. In the Phase I/II trial, administration of everolimus, primarily at doses of 2.5 mg/day, in combination with IM 600 mg/day, produced SD lasting >4 months in eight of 31 patients (26%) with IM-refractory GIST, with improvement to PR in two of three patients still receiving combination therapy at the time of this report. In stratum one, 36% had SD and 54% PD, while in stratum two, 2% had PR, 43% SD and 32% PD. Predetermined efficacy criteria were met in both strata [32]. The combination of everolimus and IM after failure on IM and sunitinib merits further investigation in GIST.

Interestingly, *in vitro* treatment of IM-resistant cell lines with everolimus only resulted in a modest reduction in proliferation and failed to induce caspase activation. Additive effects were seen for the combination of IM and everolimus *in vitro* in IM-sensitive cell lines, but not or only marginally in IM-resistant cell lines. These data further highlight the limits of our understanding of the role played by mTOR as a therapeutic target.

Other signaling pathways that may be candidates for inhibition include the phosphoinositide-3-kinase pathway, RAS pathway and PI3 kinase pathway downstream of FLT3 and mTOR [47].

Combination of AKT inhibitors & IM

Perifosine inhibits activation of the Akt pathway, which results in apoptosis and blocks cancer cell proliferation. Since AKT is a molecule downstream of KIT, its inhibition may overcome KIT-dependent IM resistance. A Phase II trial to assess antitumor activity of perifosine in patients with advanced GIST, who were refractory to IM mesylate, was reported at the 2009 ASCO meeting by Conley *et al.* [33]. Patients with KIT-positive advanced GIST who have PD on IM were eligible. Patients continued their current dose of IM and were randomized to one of two dosing schedules of perifosine (arm A: 100 mg/day orally × 28 plus daily IM or arm B: 900 mg [300 mg three-times a day orally] weekly plus daily IM). From August 2005 to July 2008, 41 patients were accrued. After one patient exclusion and two crossovers, 22 patients were in arm A and 18 patients in arm B. No complete response was identified but the PR rate was four out of 36 (11%) by Choi (four PR, nine SD) but zero out of 36 by RECIST (16 SD). A total of four out of five (80%) of patients with WT *KIT* appeared to benefit (Choi: one PR, three SD; RECIST: four SD). Median PFS and OS for 40 patients were 2.2 and 18.3 months. No difference in PFS was noted for the two schedules. Toxicity was assessed in 39 patients; 46 grade 3 events and four grade 4 events (e.g., ALT elevation, blurred vision, fatigue and mood alteration) were noted. Finally, the authors concluded that the addition of perifosine to IM has minimal activity in IM-refractory GIST, although its activity in GIST with WT KIT may be further investigated.

Combination of Src inhibitor & IM

The Src familly kinases, Src and Lyn, were active in GIST and, surprisingly, IM treatment stimulated their phosphorylation/activation. Rossi *et al.* reported recently that integrin signaling activates focal adhesion kinase and, consequently, SFKs in GIST, and that IM enhances

integrin signaling, implying a role for the extracellular matrix and integrin signaling in tumor maintenance and IM resistance [48]. Dasatinib, an inhibitor of SFKs and KIT, inhibited SFK and focal adhesion kinase activation in GIST, but also inhibited KIT and KIT-dependent downstream signaling pathways, including phosphoinositide 3-kinase and mitogen-activated protein kinase, but not signal transducer and activator of transcription (STAT) signaling. Whereas dasatinib and IM alone both produced a minimal histopathologic response, combination therapy improved their efficacy, leading to increased necrosis in GIST. Therefore, combination treatment of GIST with receptor TKIs that target different effectors of KIT signaling may improve clinical efficacy in the treatment of GIST. These results highlight the importance of SFK and STAT signaling in GIST and suggest that the clinical efficacy of IM may be limited by the stimulation of integrin signaling [48].

■ Combination of deacetylases, acetylate HSP90 & IM

Histone deacetylase inhibitors (HDACIs) have been shown to enhance IM activity in IM-resistant chronic myelogenous leukemia. Against this background, Bauer *et al.* explored whether HDACI might provide an alternative therapeutic strategy to KIT/PDGFRα kinase inhibitors in GIST [49]. Inhibition of cell proliferation by HDACI was seen in KIT-positive but not in KIT-negative GIST cell lines, suggesting that HDACI activity is mainly conferred by targeting oncogenic KIT. KIT activity, expression and activation of downstream pathways were strongly inhibited by several HDACI (e.g., suberoylanilide hydroxamic acid, LBH589, valproic acid, trichostatin A and sodium butyrate). Suberoylanilide hydroxamic acid and LBH589 induced apoptosis in KIT-positive GIST, and, at low concentrations, strong synergism with IM was observed. These results provide preclinical evidence for a disease-specific effect of HDACI in KIT-positive GIST, which could translate into therapeutic activity [50].

Furthermore, Dewaele and collegues evaluated new compounds for resistant GIST cell lines. Primary IM-resistant tumor cells and cell lines expressing IM-resistant *PDGFRα*^{D842V} or IM-sensitive *PDGFRα*$^{\Delta DIM842-844}$ mutants were treated with different concentrations of dasatinib, sorafenib, nilotinib and IPI-504. All inhibitors tested exhibited a high efficacy toward the *PDGFRα*$^{\Delta DIM842-844}$ mutant. By contrast, *ex vivo* and *in vitro* assays revealed that only dasatinib potently inhibited the *PDGFRα*^{D842V} isoform. Sorafenib and nilotinib were significantly less efficacious against this mutation, inhibiting the PDGFRα kinase activity. IPI-504 treatment potently inhibited PDGFRα kinase activity by inducing the degradation of *PDGFRα*^{D842V} and *PDGFRα*$^{\Delta DIM842-844}$. Treatment with dasatinib or the heat shock protein 90 inhibitor IPI-504 may provide a therapeutic alternative for GIST patients whose tumors carry the IM-resistant *PDGFRα*^{D842V} mutant isoform [51].

■ Combination of PKC412 & IM

PKC412 is a PKC that has broad kinase-inhibiting activity. Combined with IM, complex drug–drug interactions occur, which precluded the development of this combination. The clinical data regarding PKC are not extensive. A Phase I trial was conducted several years ago in unselected patients with solid malignancies and subsequently the recommended dose for Phase II trials was 150 mg/day, with very few grade 3 events reported [52]. Preclinical data from several teams indicate that PKC is a potent inhibitor of *KIT* WT, as well as several *KIT* mutants [12,53] and the IM-resistant exon 18 mutant *PDGFRα* isoforms.

Administration of PKC412 in combination with IM in a study of 19 patients with disease progression on IM 600 mg/day or higher, provided some clinical benefit [54]. A total of 19 patients have been entered so far at doses ranging from IM 600–1000 mg/day in combination with PKC 200 mg/day. Frequently reported common toxicity criteria grade 1–2 adverse events in this heavily pretreated group of patients included nausea, vomiting, anorexia, diarrhoea, oedema, fatigue, dyspepsia, candidiasis, sweating, skin toxicity and anemia. Four grade 3 adverse effects were attributed to the investigational combination: hyperglycaemia, transient asymptomatic hyperamylasemia, hypocalcaemia and transaminitis, all reported previously for PKC alone. Notably, hyperthyroidism manifested by elevated TSH (grade 2, manageable) was seen in four out of 12 patients. Complete pharmacokinetic data for the first 12 (33%) patients treated with PKC 100 mg b.i.d. in combination with IM 600 mg/day from 2 to 5 months showed that PKC trough plasma concentrations increased ~twofold over that seen in acute myeloid leukemia studies with PKC alone. IM exposure decreased ~70% after 1 month of co-administration with PKC412, either due to enzyme induction or protein-binding interactions. The study was therefore amended to allow for inter-cohort dose escalation of IM and temporary dose reduction of PKC412 to decrease peak levels, which resulted in reduced toxicity and increased IM PK trough levels equal to IM 600 mg/day. Two out of five (40%) patients evaluable for response had SD at 4 months, with characteristic CT findings. Preliminary evidence in this study indicates activity of the combination of PKC412 and IM in IM-resistant GIST. However, the addition of PKC412 to IM results in a strong drug–drug interaction on both combination partners:

- PKC412 causes a decrease in IM PK levels, possibly due to enzyme induction of CYP3A4;
- IM causes an increase in PKC412 levels, possibly due to a inhibition of CYP3A4 compensating the extent of induction via PKC412.

In the original study, rapid progression in the majority of patients occurred early, possibly due to decrease of IM drug levels below activity. The amended regimen minimized the rapid progression by maintaining IM levels above 1 µg/ml, suggesting the necessity for maintaining IM in radiotherapy of patients' with IM-resistant disease. There is some evidence of preliminary clinical activity of the combination of PKC412 and IM in IM-resistant GIST.

Combination of PKC412 & sirolimus

The exon 18 *PDGFRα-D842V* mutation is the most common *PDGFRα* mutation in GIST. This mutation appears to be resistant to IM, but preclinical data have reported its sensitivity to PKC412 [53]. The combination of mTOR inhibitor and IM has been previously explored in IM-resistant GIST, based on the involvement of the AKT/mTOR pathway in GIST oncogenic signalling mechanisms. Palassini and colleagues reported the use of PKC412 and sirolimus, an mTOR inhibitors, in a patient with a *PDGFRα-D842V* metastatic GIST, progressing on PKC412 as a single agent [55]. A 54-year old woman with a *PDGFRα-D842V* GIST arising from the omentum was treated with surgery, then IM 400 mg/day at relapse. After 3 months, it was interrupted due to PD and PKC412 100 mg/day was started, but after 6 weeks, on a CT scan this too showed PD. Then sirolimus 2 mg/day was added to PKC412. After 4 weeks of combined treatment, SD according to Choi's and RECIST criteria was observed. It was maintained at 11 weeks. At the time of the reporting, treatment is ongoing, in the lack of severe or unexpected toxicities. In this patient, progressing on PKC412, a SD was obtained by adding sirolimus to the latter. Since August 2007, the same Italian group proposed to treat three *PDGFRα-D842V* GIST patients (age: 54, 56 and 63 years) with sirolimus (2–3 mg/day) in combination to TKI (PKC412 in one patient and IM in two patients). A total of two patients were progressing on IM, while the third patient was treated with IM plus sirolimus upfront. Two patients had a Choi's response 6 and 3 months, respectively, after starting the combination. The last patient, undergoing IM plus sirolimus, has a short follow-up at the time of the publication. Disease was stable at 2 months, although clinical improvement was noticed and therapy is ongoing. The first patient underwent surgery of residual peritoneal disease after 13 months and is now continuing therapy with surgically no evidence of disease. The other patient had a tumor progression after 9 months from starting therapy and 6 months from tumor response (with a negative PET scan). Treatment was relatively well tolerated, with skin toxicity in one patient. The authors concluded for signs of antitumor activity in three patients with this mutation, by combining TKI (IM or PKC412) with sirolimus [56].

Nevertheless, potential drug–drug interaction of this formerly unexplored combination, through CYP3A4 common metabolic pathway, would require, for any future cases, formal dose-limiting toxicity and reciprocal drug-level assessment within a proper clinical trial setting.

Combination of IM with immunotherapy (pegylated IFN-α2b)

Major barriers to durable remission in cancer are drug-resistant clones and tumor stem cells. We sought to harness and enhance endogenous antitumor immunity in GIST by combining IM with pegylated IFN-α2b (PegIntron; pegIFN-α2b). Chen *et al.*, reported at the 2010 ASCO meeting, patients with primary tumor ≥6 cm or metastatic IM-sensitive GIST receiving IM at 400 mg/day for *KIT* exon 11 mutation GIST and 800 mg/day for the rest, plus pegIFN-α2b [57]. The first cohort (three patients) received PegIFN-α2b at 4 µg/kg/week and developed granulocytopenia, so the dose was reduced to 3 µg/kg/week × 4 doses then 1.5 µg/kg/week × 18 dose. A total of eight patients were enrolled (age range: 42–89 years). The combination treatment was well tolerated; grade 3 skin rash/dermatitis occurred in two patients. All eight patients showed PR by Choi and RECIST criteria and seven patients showed PR and one inevaluable (not fluorodeoxiglucose avid) by PET-CT. The oldest patient died of unrelated causes while in remission. PFS of seven evaluable patients ranges from 365 to >900 days, six patients exceeded historical controls and one will reach it in 1 month (PFS of historical control of *KIT* exon 11 mutation GIST: 687 days; *KIT* exon 9 mutation: 200 days; WT: 82 days). When pegIFN-α2b was restarted, a second PR was induced, with one target lesion showing SUV reduction from 22.8 to 9.5 and another from 28 to 18.2. Combination of IM and pegIFN-α2b showed promising efficacy in eight GIST patients. Owing to the excellent results, the trial was closed early in anticipation of a larger future study [57].

Combination of doxorubicin plus IM

In KIT-expressing Ewing sarcoma cell lines, the addition of doxorubicin to IM increases apoptosis, compared with IM or doxorubicin alone. On the basis of these *in vitro* data, the GEIS group conducted a Phase I–II trial of doxorubicin with IM in patients with GISTs

refractory to high-dose IM therapy [58]. The aim of the study is to evaluate a metronomic strategy with doxorubicin, to improve potential IM interactions, minimize toxicities and reverse resistance.

Patients with metastatic GIST resistant to IM at 400 mg b.i.d. orally were eligible for this multicenter study and received IM (400 mg/day orally) concomitantly with doxorubicin 15–20 mg/m^2/week for four cycles (monthly cycles), followed by IM (400 mg/day orally) maintenance in nonprogressive patients. Spiral computed tomography and PET with F18-fluorodeoxyglucose were done basally and after 2 months of therapy to evaluate response. An *in vitro* study assessed the effect of combining IM and doxorubicin. A total of 26 patients with progressive GIST were entered in the study. Treatment was well tolerated. Three (14%) of 22 evaluable patients had PRs per RECIST and eight (36%) had clinical benefit (PR or SD for ≥6 months). Median PFS was 100 days (95% CI: 62–138) and median survival was 390 days (95% CI: 264–516). Interestingly, PFS was 211 days (95% CI: 52–370) in patients with WT KIT and 82 days (95% CI: 53–111) in non-WT patients (ten mutants, six not assessed). A synergistic effect on cell-line proliferation and apoptosis was found with IM and doxorubicin combination. Low-dose chemobiotherapy combination showed promising activity in heavily pretreated GIST patients, especially in those with WT-*KIT* genotype.

The reasons for the activity of the combination are not clear. Recent *in vivo* preclinical data show that the association of cytotoxic metronomic therapies with dual VEGF receptor and PDGF receptor (PDGFR) inhibitor (IM or sunitinib) further enhanced efficacy. In this model, the pericyte detachment induced by PDGFR inhibition sensitized the endothelial cells to metronomic chemotherapy [59]. Therefore, we can not rule out the possibility that the combined therapy could also have an antiangiogenic effect.

■ Combination of bevacizumab plus IM

Imatinib has been shown to cause down regulation of PDGFR phosphorylation in tumor vasculature. In addition, some have hypothesized that part of the clinical activity of sunitinib is by VEGFR inhibition. Based on these data, a Phase III trial testing the combination of IM with bevacizumab, a fully humanized monoclonal antibody that binds VEGF, compared with single-agent IM in patients with previously untreated metastatic disease was scheduled but finally not activated.

Conclusion & future perspective

Despite the approval of sunitinib, the management of patients with advanced IM-resistant GIST remains a therapeutic challenge. No medical treatment is currently approved for the treatment of IM and sunitinib-resistant GIST. Several drugs have shown promising activity in this setting: nilotinib, sorafenib, masatinib, possibly dasatinib and midostaurin in selected molecular subsets. Combinations of these agents in patients failing the only two registered agents have been tested in Phase I/II trials with occasional long-term tumor control. Combinations with IM and everolimus or with cytotoxic have also been reported to yield substantial antitumor activity in small uncontrolled Phase I/II trials. However, the majority of these patients still progress rapidly under combination treatment and novel agents and/or combinations are urgently needed. In the future, other TKIs and various agents that block signaling cascades, may emerge as new treatment options. Some of these drugs are already available although not approved. It is likely, however, that most of the drugs that have shown activity in second- or third-line treatment of patients with advanced GIST in Phase II trials will not be further developed for this indication for economic reasons. On the other hand, since GIST is a model for molecular targeted cancer therapy, it is likely that new investigational agents will be available, at least for clinical trial participant and maybe as new approved drugs for this disease.

This article reported different combinations (TKI vs TKI plus other compounds) able to benefit some selected patients, however, any clear evidence could be delineated today concerning a real improvement for the survival of patients with such combination. Although, the improvement in terms of OS from the included patients in the first-line clinical trials with IM is very long (>3 years) compared with historical data. Thus, the fact that the median PFS with IM in first-line is not more than 24 months is surprising. This observation suggests a real benefit of followed treatment after first line with sunitinib, surgery and also new compounds such as nilotinib, sorafenib, masatinib and mTOR inhibitors, used alone or in combination. Concerning the benefit of combined schema versus sequential, the reported Phase I or II trials seem to suggest some benefit for the combination for some patients resistant to monotherapy. In addition, owing to the possible drug interactions, we had to wait the results of ongoing trials (as doxorubicin plus IM or bevacizumab plus IM) before to propose such association in routine practice. Another approach could be to propose use of these drugs sequentially before any appearance of progression signs, than waiting the resistance and then to modify the treatment. This schema to introduce a new compound as maintenance treatment (then the patient is not progressing under the previous treatment) could be considered as a switch maintenance therapy. This hypothesis needs to be confirmed in clinical trials but has been validated for

other metastatic cancer models such as non-small-cell carcinoma [60]. Most combinations tested associated the two agents given at the same time, with the aim to overcome the emergence of resistant clones frequently observed at the time of IM and sunitinib resistance. However, the analysis of the mechanisms of emergence of resistant clones in the chronic myeloid leukemia model suggests that alternative strategies could be proposed, using sequential combinations of noncross-resistant agents. Indeed, it was shown in chronic myeloid leukemia that the emergence of IM-resistant clone was facilitated by the maintenance of therapeutic pressure by IM. Conversely, treatment interruption resulted in a reduction of the selection pressure and lead to the 'deselection' of clones expressing a drug resistant kinases [61]. In view of these observations, the exploration of therapeutic strategies with rotation of TKIs with a different spectrum of activity on the primary and secondary mutated kinases may be worth investigating in advanced GIST. Such trials may use as primary end points, the time to onset of resistance to the two agents. Using sequential approach, treatment one would be given until progression followed by a switch to treatment two, then an attempt to reintroduce treatment one; in a rotating approach, treatment one and two may be given for a fixed period of time (e.g., every 2–3 months), and rotated systematically until the potential emergence of resistant clones. The comparison of the time to resistance to drug one and two would then be the primary end point.

Better characterization of the molecular mechanisms involved in the development of primary and secondary TKI resistance is also needed to assist with the selection of appropriate alternative therapies for patients with advanced GIST who exhibit refractory genotypes.

Financial & competing interests disclosure

The authors have received funding for research in sarcoma from INCA and ligue départementale de lutte contre le cancer de l'AIN, Roche SA, Novartis SA, Merck Sereno SA and DAM'S association. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

Executive summary

- Secondary resistance in gastrointestinal stromal tumors occurs through the emergence of resistant clones with additional *KIT* mutations.
- Primary and secondary *KIT* mutations have predictive value for response to imatinib and sunitinib.
- Mutated KIT proteins have differential sensitivity to tyrosine kinase inhibitors.
- Combination of targeted treatment may enable treatment of tumor clones with different sensitivity profiles.
- Clinical trials exploring rotation of tyrosine kinase inhibitors are warranted.

Bibliography

1 Nilsson B, Bumming P, Meis-Kindblom JM *et al.* Gastrointestinal stromal tumors: the incidence, prevalence, clinical course, and prognostication in the preimatinib mesylate era – a population-based study in western Sweden. *Cancer* 103, 821–829 (2005).

2 Cassier PA, Ducimetiere F, Lurkin A *et al.* A prospective epidemiological study of new incident GISTs during two consecutive years in Rhone Alpes region: incidence and molecular distribution of GIST in a European region. *Br. J. Cancer* 103, 165–170 (2010).

3 Rubin BP, Heinrich MC, Corless CL. Gastrointestinal stromal tumour. *Lancet* 369, 1731–1741 (2007).

4 Verweij J, Casali PG, Zalcberg J *et al.* Progression-free survival in gastrointestinal stromal tumours with high-dose imatinib: randomised trial. *Lancet* 364, 1127–1134 (2004).

5 Blanke CD, Demetri GD, Von Mehren M *et al.* Long-term follow-up of a Phase II randomized trial in advanced gastrointestinal stromal tumor (GIST) patients (pts) treated with imatinib mesylate. *ASCO Meeting Abstracts* 24, 9528 (2006).

6 Heinrich MC, Maki RG, Corless CL *et al.* Sunitinib (SU) response in imatinib-resistant (IM-R) GIST correlates with KIT and PDGFRα mutation status. *ASCO Meeting Abstracts* 24, 9502 (2006).

7 Demetri GD, Van Oosterom AT, Garrett CR *et al.* Efficacy and safety of sunitinib in patients with advanced gastrointestinal stromal tumour after failure of imatinib: a randomised controlled trial. *Lancet* 368, 1329–1338 (2006).

8 Heinrich MC, Corless CL, Demetri GD *et al.* Kinase mutations and imatinib response in patients with metastatic gastrointestinal stromal tumor. *J. Clin. Oncol.* 21, 4342–4349 (2003).

9 Corless CL, Schroeder A, Griffith D *et al.* *PDGFRα* mutations in gastrointestinal stromal tumors: frequency, spectrum and *in vitro* sensitivity to imatinib. *J. Clin. Oncol.* 23, 5357–5364 (2005).

10 Heinrich MC, Corless CL, Blanke CD *et al.* Molecular correlates of imatinib resistance in gastrointestinal stromal tumors. *J. Clin. Oncol.* 24, 4764–4774 (2006).

11 Agaram NP, Wong GC, Guo T *et al.* Novel V600E BRAF mutations in imatinib-naive and imatinib-resistant gastrointestinal stromal tumors. *Genes Chromosomes Cancer* 47, 853–859 (2008).

12 Debiec-Rychter M, Cools J, Dumez H *et al.* Mechanisms of resistance to imatinib mesylate in gastrointestinal stromal tumors and activity of the PKC412 inhibitor against imatinib-resistant mutants. *Gastroenterology* 128, 270–279 (2005).

13 Cassier P, Pissaloux D, Alberti L, Ray-Coquard I, Blay JY. Targeted treatment of rare connective tissue tumors and sarcomas. *Bull. Cancer* 97, 693–700 (2010).

14 Chen LL, Trent JC, Wu EF *et al.* A missense mutation in KIT kinase domain 1 correlates with imatinib resistance in gastrointestinal stromal tumors. *Cancer Res.* 64, 5913–5919 (2004).

15 Tamborini E, Gabanti E, Lagonigro MS *et al.* KIT/Val654 Ala receptor detected in one imatinib-resistant GIST patient. *Cancer Res.* 65, 1115 (2005).

16 McLean SR, Gana-Weisz M, Hartzoulakis B *et al.* Imatinib binding and cKIT inhibition is abrogated by the cKIT kinase domain I missense mutation Val654Ala. *Mol. Cancer Ther.* 4, 2008–2015 (2005).

17 Antonescu CR, Besmer P, Guo T *et al.* Acquired resistance to imatinib in gastrointestinal stromal tumor occurs through secondary gene mutation. *Clin. Cancer Res.* 11, 4182–4190 (2005).

18 Wardelmann E, Merkelbach-Bruse S, Pauls K *et al.* Polyclonal evolution of multiple secondary KIT mutations in gastrointestinal stromal tumors under treatment with imatinib mesylate. *Clin. Cancer Res.* 12, 1743–1749 (2006).

19 Haller F, Detken S, Schulten HJ *et al.* Surgical management after neoadjuvant imatinib therapy in gastrointestinal stromal tumours (GISTs) with respect to imatinib resistance caused by secondary KIT mutations. *Ann. Surg. Oncol.* 14, 526–532 (2007).

20 Utsunomiya T, Okamoto M, Yano S *et al.* Secondary c-kit mutation in a recurrent gastrointestinal stromal tumor under long-term treatment with imatinib mesylate: report of a case. *Surg. Today* 38, 65–67 (2008).

21 Heinrich MC, Corless CL, Blanke CD *et al.* Molecular correlates of imatinib resistance in gastrointestinal stromal tumors. *J. Clin. Oncol.* 24, 4764–4774 (2006).

22 Desai J, Shankar S, Heinrich MC *et al.* Clonal evolution of resistance to imatinib in patients with metastatic gastrointestinal stromal tumors. *Clin. Cancer Res.* 13, 5398–5405 (2007).

23 Grabellus F, Ebeling P, Worm K *et al.* Double resistance to imatinib and AMG 706 caused by multiple acquired KIT exon 17 mutations in a gastrointestinal stromal tumour. *Gut* 56, 1025–1026 (2007).

24 Tabone S, Theou N, Wozniak A *et al.* KIT overexpression and amplification in gastrointestinal stromal tumors (GISTs). *Biochim. Biophys. Acta* 1741, 165–172 (2005).

25 Theou N, Gil S, Devocelle A *et al.* Multidrug resistance proteins in gastrointestinal stromal tumors: site-dependent expression and initial response to imatinib. *Clin. Cancer Res.* 11, 7593–7598 (2005).

26 Agaram NP, LaQuaglia MP, Ustun B *et al.* Molecular characterization of pediatric gastrointestinal stromal tumors. *Clin. Cancer Res.* 14, 3204–3215 (2008).

27 Heinrich MC, Maki RG, Corless CL *et al.* Sunitinib (SU) response in imatinib-resistant (IM-R) GIST correlates with KIT and PDGFRα mutation status. *ASCO Meeting Abstracts* 24, 9502 (2006).

28 Heinrich MC, Corless CL, Liegl B *et al.* Mechanisms of sunitinib malate (SU) resistance in gastrointestinal stromal tumors (GISTs). *ASCO Meeting Abstracts* 25, 10006 (2007).

29 Gramza AW, Corless CL, Heinrich MC. Resistance to tyrosine kinase inhibitors in gastrointestinal stromal tumors. *Clin. Cancer Res.* 15, 7510–7518 (2009).

30 Wardelmann E, Thomas N, Merkelbach-Bruse S *et al.* Acquired resistance to imatinib in gastrointestinal stromal tumours caused by multiple KIT mutations. *Lancet Oncol.* 6, 249–251 (2005).

31 Duensing A, Medeiros F, McConarty B *et al.* Mechanisms of oncogenic KIT signal transduction in primary gastrointestinal stromal tumors (GISTs). *Oncogene* 23, 3999–4006 (2004).

32 Schoffski P, Reichardt P, Blay JY *et al.* A Phase I-II study of everolimus (RAD001) in combination with imatinib in patients with imatinib-resistant gastrointestinal stromal tumors. *Ann. Oncol.* 21, 1990–1998 (2010).

33 Conley A, Araujo D, Ludwig DL *et al.* A randomized Phase II study of perifosine (P) plus imatinib for patients with imatinib-resistant gastrointestinal stromal tumor (GIST). *J. Clin. Oncol.* 27(Suppl. 15) Abstr. 10563 (2009).

34 Takahashi R, Tanaka S, Kitadai Y *et al.* Expression of vascular endothelial growth factor and angiogenesis in gastrointestinal stromal tumor of the stomach. *Oncology* 64, 266–274 (2003).

35 Bono P, Krause A, von Mehren M *et al.* Serum KIT and KIT ligand levels in patients with gastrointestinal stromal tumors treated with imatinib. *Blood* 103, 2929–2935 (2004).

36 McAuliffe JC, Lazar AJ, Yang D *et al.* Association of intratumoral vascular endothelial growth factor expression and clinical outcome for patients with gastrointestinal stromal tumors treated with imatinib mesylate. *Clin. Cancer Res.* 13, 6727–6734 (2007).

37 Borg C, Terme M, Taieb J *et al.* Novel mode of action of c-kit tyrosine kinase inhibitors leading to NK cell-dependent antitumor effects. *J. Clin. Invest.* 114, 379–388 (2004).

38 Menard C, Blay JY, Borg C *et al.* Natural killer cell IFN-γ levels predict long-term survival with imatinib mesylate therapy in gastrointestinal stromal tumor-bearing patients. *Cancer Res.* 69, 3563–3569 (2009).

39 Scotlandi K, Manara MC, Strammiello R *et al.* C-kit receptor expression in Ewing's sarcoma: lack of prognostic value but therapeutic targeting opportunities in appropriate conditions. *J. Clin. Oncol.* 21, 1952–1960 (2003).

40 Gonzalez I, Andreu EJ, Panizo A *et al.* Imatinib inhibits proliferation of Ewing tumor cells mediated by the stem cell factor/KIT receptor pathway, and sensitizes cells to vincristine and doxorubicin-induced apoptosis. *Clin. Cancer Res.* 10, 751–761 (2004).

41 Giles FJ, Rosti G, Beris P *et al.* Nilotinib is superior to imatinib as first-line therapy of chronic myeloid leukemia: the ENESTnd study. *Expert Rev. Hematol.* 3, 665–673 (2010).

42 Weisberg E, Manley P, Mestan J, Cowan-Jacob S, Ray A, Griffin JD. AMN107 (nilotinib): a novel and selective inhibitor of BCR-ABL. *Br. J. Cancer* 19(94), 1765–1769 (2006).

43 Reichardt P, Blay J, Gelderblom H *et al.* Phase III trial of nilotinib in patients with advanced gastrointestinal stromal tumor (GIST): first results from ENEST g3. *J. Clin. Oncol.* 28(Suppl. 15), Abstr. 10017^ (2010).

44 Pantaleo MA, Nicoletti G, Nanni C *et al.* Preclinical evaluation of KIT/PDGFRA and mTOR inhibitors in gastrointestinal stromal tumors using small animal FDG PET. *J. Exp. Clin. Cancer Res.* 29, 173 (2010).

45 Von Mehren M, Reichardt P, Casali PG *et al.* A Phase I study of nilotinib alone and in combination with imatinib (IM) in patients (pts) with imatinib-resistant gastrointestinal stromal tumors (GIST) – study update. *J. Clin. Oncol.* 25, 10023 (2007).

46 Blay JY, Casali PG, Reichardt P *et al.* A Phase I study of nilotinib alone and in combination with imatinib in patients with imatinib-resistant gastrointestinal stromal tumors (GIST): study update. *J. Clin. Oncol.* 26, Abstr. 10553 (2008).

47 Cools J, Maertens C, Marynen P. Resistance to tyrosine kinase inhibitors: calling on extra forces. *Drug Resist. Updat.* 8, 119–129 (2005).

48 Rossi F, Yozgat Y, de StanchinaE *et al.* Imatinib upregulates compensatory integrin signaling in a mouse model of gastrointestinal stromal tumor and is more effective when combined with dasatinib. *Mol. Cancer Res.* 8, 1271–1283 (2010).

49 Bauer S, Yu LK, Demetri GD, Fletcher JA. Heat shock protein 90 inhibition in imatinib-resistant gastrointestinal stromal tumor. *Cancer Res.* 66(18), 9153–9161 (2006).

50 Muhlenberg T, Zhang Y, Wagner AJ *et al.* Inhibitors of deacetylases suppress oncogenic KIT signaling, acetylate HSP90, and induce apoptosis in gastrointestinal stromal tumors. *Cancer Res.* 69, 6941–6950 (2009).

51 Dewaele B, Wasag B, Cools J *et al.* Activity of dasatinib, a dual SRC/ABL kinase inhibitor, and IPI-504, a heat shock protein 90 inhibitor, against gastrointestinal stromal tumor-associated PDGFRαD842V mutation. *Clin. Cancer Res.* 14, 5749–5758 (2008).

52 von Mehren M. Recent advances in the management of gastrointestinal stromal tumors. *Curr. Oncol. Rep.* 5, 288–294 (2003).

53 Weisberg E, Wright RD, Jiang J *et al.* Effects of PKC412, nilotinib, and imatinib against GIST-associated *PDGFR*α mutants with differential imatinib sensitivity. *Gastroenterology* 131, 1734–1742 (2006).

54 Reichardt P, Pink D, Lindberg DA *et al.* A Phase I/II trial of the oral PKC-inhibitor PKC412 (PKC) in combination with imatinib mesylate (IM) in patients (pts) with gastrointestinal stromal tumor (GIST) refractory to IM. *J. Clin. Oncol.* 23(Suppl. 16) (2005).

55 Palassini E, Fumagalli E, Coco P *et al.* Combination of PKC412 and sirolimus in a metastatic patient with PDGFRα-D842V gastrointestinal stromal tumor (GIST). *J. Clin. Oncol.* 26, Abstr. 21515 (2008).

56 Piovesan C, Fumagalli E, Coco P *et al.* Response to sirolimus in combination to tirosine kinase inhibitors (TKI) in three cases of PDGFRα-D842V metastatic gastrointestinal stromal tumor (GIST). *J. Clin. Oncol.* 27, Abstr. 10565 (2009).

57 Chen L, Chen HX, Gouw L *et al.* Combination of targeted therapy (imatinib) with immunotherapy (peginterferon α-2b) to overcome drug resistance and tumor stem cells in gastrointestinal stromal tumors (GIST). *J. Clin. Oncol.* 28, Abstr. 10035 (2010).

58 Maurel J, Martins AS, Poveda A *et al.* Imatinib plus low-dose doxorubicin in patients with advanced gastrointestinal stromal tumors refractory to high-dose imatinib: a Phase I-II study by the Spanish Group for Research on Sarcomas. *Cancer* 116, 3692–3701 (2010).

59 Pietras K, Hanahan D. A multitargeted, metronomic, and maximum-tolerated dose 'chemo-switch' regimen is antiangiogenic, producing objective responses and survival benefit in a mouse model of cancer. *J. Clin. Oncol.* 23, 939–952 (2005).

60 Ciuleanu T, Brodowicz T, Zielinski C *et al.* Maintenance pemetrexed plus best supportive care versus placebo plus best supportive care for non-small-cell lung cancer: a randomised, double-blind, Phase 3 study. *Lancet* 374, 1432–1440 (2009).

61 Hochhaus A, Schenk T, Erben P, Ernst T, La RP, Muller MC. Cause and management of therapy resistance. *Best Pract. Res. Clin. Haematol.* 22, 367–379 (2009).

Website

101 Clinicaltrial.gov: imatinib mesylate and sunitinib in treating patients with gastrointestinal stromal tumors www.clinicaltrials.gov/ct2/show/NCT00573404