Biochemical Pharmacology 80 (2010) 575–583

Contents lists available at ScienceDirect

Biochemical Pharmacology

journal homepage: www.elsevier.com/locate/biochempharm

Review

Targeted therapies of gastrointestinal stromal tumors (GIST)—The next frontiers

Stefan Duensing [a,b], Anette Duensing [a,c,*]

[a] Cancer Virology Program, University of Pittsburgh Cancer Institute, Hillman Cancer Center, Pittsburgh, PA, USA
[b] Department of Microbiology and Molecular Genetics, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA
[c] Department of Pathology, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA

ARTICLE INFO

Article history:
Received 31 January 2010
Accepted 5 April 2010

Keywords:
Gastrointestinal stromal tumors
Kinase
Targeted therapy
Small molecular inhibitor
KIT

ABSTRACT

Gastrointestinal stromal tumors (GISTs) are the most common mesenchymal tumors of the gastrointestinal (GI) tract and are caused by activating *KIT* or *PDGFRA* mutations. GISTs can be successfully treated with the small molecule kinase inhibitor imatinib mesylate (Gleevec®, Novartis) with response rates of up to 85%. However, complete responses are rare, and most patients will develop imatinib resistance over time. Recent results have shown that although imatinib effectively stimulates apoptotic cell death in sensitive GIST cells, a considerable proportion of cells does not undergo apoptosis, but instead enters a state of quiescence. Quiescence is characterized by a reversible withdrawal from the cell division cycle, during which the cells remain alive and metabolically active. It is conceivable that quiescence not only plays a pivotal role in the emergence of residual disease but also in creating a pool of tumor cells that survive continuous small molecule therapy and may hence represent the "seeds" for the outgrowth of resistant clones. This review will summarize the current knowledge about GIST biology and treatment response to imatinib including the induction of cellular quiescence in GIST. In addition, we will highlight future strategies to design more effective treatment options to overcome these problems with an aim towards cure of this hitherto untreatable tumor entity.

Contents

1. Introduction

Gastrointestinal stromal tumors (GISTs) are the most common mesenchymal tumors of the gastrointestinal (GI) tract [1]. They can arise throughout the entire GI tract, however, the most commonly affected sites are the stomach (60%) and the small bowel (jejunum and ileum 30%, duodenum 5%) [2]. GISTs are thought to arise from the interstitial cells of Cajal (ICC) or an ICC precursor cell. ICCs are localized between the two muscular layers of the gastrointestinal tract and are involved in the regulation of peristalsis. It has long been known that ICCs are dependent on expression of the KIT receptor tyrosine kinase (RTK) for their proper development and differentiation [3,4]. GISTs are characterized by not only expressing KIT at high levels, but also by the fact that approximately 85% of GISTs harbor mutations in the *KIT* gene [5,6]. Approximately one third of the remaining cases (5–8% of all GISTs) carry mutations in the related RTK, platelet-derived growth factor receptor alpha (*PDGFRA*) [7,8]. Immunohistochemical staining for the KIT protein has become the most important diagnostic parameter for GISTs although a small percentage of GISTs (2–5%) express KIT at very

* Corresponding author at: University of Pittsburgh Cancer Institute, Hillman Cancer Center, Research Pavilion, Suite 1.8, 5117 Centre Avenue, Pittsburgh, PA 15213, USA. Tel.: +1 412 623 5870; fax: +1 412 623 7715.
E-mail address: aduensin@pitt.edu (A. Duensing).

0006-2952/$ – see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.bcp.2010.04.006

low levels or not at all [9–12]. Some, but not all of these tumor are wildtype for *KIT* mutations. Despite these findings, unifying features of most GISTs – irrespective of KIT expression and *KIT*/*PDGFRA* mutational status – are the expression of PKCtheta and DOG1, markers that were originally identified by mRNA expression profiling [13–21].

2. KIT and PDGFRA in GISTs

KIT (CD117) is a 145 kDa, transmembrane receptor tyrosine kinase that belongs to the type III family of RTKs, which includes PDGFRA and PDGFRB, as well as colony-stimulating factor 1 receptor (CSF1R) and FMS-related tyrosine kinase 3 (FLT-3) [22–24]. All type III RTKs are comprised of five extracellular IgG-like loops that encode the ligand-binding and dimerization domains and an intracellular portion, which is divided into the juxtamembrane (JM) domain and a split kinase domain (Fig. 1). KIT is expressed in hematopoietic stem cells, mast cells, melanocytes, germ cells and ICC [25]. Under physiological conditions, the kinase is activated by its ligand stem cell factor (SCF), which leads to receptor dimerization, autophosphorylation and activation (phosphorylation) of downstream signaling cascades that include most prominently the PI3K/AKT/mTOR and the RAS/RAF/MAPK pathways, but also JAK/STAT signaling [26,27,28,29].

Several types of *KIT* mutations can be found in GISTs, eventually affecting different domains of the protein. However, all oncogenic *KIT* mutations cause a constitutive, ligand-independent activation of the kinase thereby driving tumorigenesis [30,31]. Despite the fact that different *KIT* mutations generally lead to the same outcome (constitutive KIT activation), it is likely that downstream signaling pathways that are being activated by different *KIT* genotypes are not exactly the same [29]. Moreover, the type of *KIT* mutation correlates with therapeutic outcome (see below). The most common *KIT* mutations in GIST are detected in *KIT* exon 11 (Table 1), followed by mutations in exons 9, 13 and 17 [1]. Interestingly, a range of various types of mutations can be found in *KIT* exon 11 (insertions, deletions and single base substitutions), whereas exon 9 mutations always comprise of a six-base pair insertion leading to duplication of amino acids 502–503. Mutations in exons 13 and 17 always consist of single base substitutions leading to various missense mutations. Approximately 5–8% of GISTs that do not carry *KIT* mutations harbor activating mutations in the related RTK *PDGFRA* (Table 1) [7,8]. Interestingly, similar types of mutations are found in *PDGFRA* as are in *KIT*—eventually affecting corresponding domains of the protein. However, the frequency distribution of the domains affected differs between *KIT* and *PDGFRA* mutations

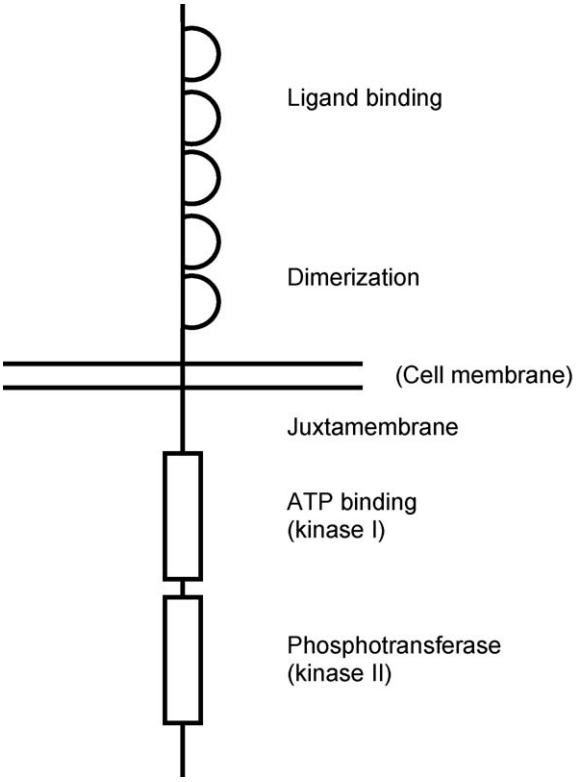

Fig. 1. Stucture of type III receptor tyrosine kinases (RTK). All type III RTKs are comprised of five extracellular IgG-like repeats that are involved in ligand-binding and dimerization. The juxtamembrane domain, just inside the cell membrane, has autoinhibitory function. The kinase domain is split into two parts separating the ATP binding pocket (kinase I) from the activation loop/phosphotransferase domain (kinase II).

[32]. The juxtamembrane domain is most frequently affected by mutations in *KIT*, whereas *PDGFRA* mutations most frequently affect the activation loop of the kinase domain (Table 1). Interestingly, several *KIT*/*PDGFRA* mutation types have a certain predilection with respect to tumor location. For example, GISTs with *KIT* exon 9 mutations are predominantly seen in the small bowel and have been associated with a more aggressive clinical behavior [33,34], whereas an internal tandem duplication in *KIT* exon 11 most frequently occurs in gastric tumors with spindle-cell morphology [35,36]. On the other hand, most gastric GISTs with epithelioid morphology lack *KIT* mutations [34,37,38] and GISTs with *PDGFRA* mutations arise almost exclusively in the stomach [8,39,40].

Table 1
Primary mutations in GIST.

Gene	Exon	Type of mutation	Domain affected	Frequency	
KIT	9	• Insertion (duplication of codons 502/503)	Extracellular (ligand-binding)	12%	84% (75–90%)
	11	• Deletion • Insertion • Single base substitution	Juxtamembrane (auto-inhibitory)	70%	
	13	• Single base substitution	Kinase domain I (ATP binding pocket)	1%	
	17	• Single base substitution	Kinase domain II (phosphotransferase/activation loop)	1%	
PDGFRA	12	• Deletion • Insertion • Single base substitution	Juxtamembrane (auto-inhibitory)	0.7%	7% (5–8%)
	14	• Single base substitution	Kinase domain I (ATP binding pocket)	0.1%	
	18	• Single base substitution	Kinase domain II (phosphotransferase/activation loop)	6%	
WILDTYPE	n/a	n/a	n/a	9%	9% (9–15%)

It is widely believed that *KIT* or *PDGFRA* mutations are the tumor-initiating events in GISTs, and it has been shown that *KIT* and *PDGFRA* mutations are mutually exclusive [32]. Nonetheless, the precise molecular mechanism(s) of mutagenesis as well as the tissue preference remains to be elucidated. It seems to be clear, however, that additional oncogenic events are needed for a clinically symptomatic and more rapidly growing tumor to occur, because very small GIST "tumorlets" can be detected in as many as 30% of the population when examining autopsy specimens [41–44]. Most, if not all of these tumors already contain *KIT* or *PDGFRA* mutations.

Nevertheless, strong support for a causative role of *KIT*/*PDGFRA* mutations in GIST pathogenesis stems from (i) various *in vitro* transgenic models [5,45,46], (ii) the existence of a familial GIST syndrome in patients with germline *KIT*/*PDGFRA* mutations [47–55], (iii) mouse models that have been established [56–58] and (iv) the finding that most small, incidental GIST (< 1 cm) already carry *KIT* mutations [41,43]. Although most GISTs have detectable *KIT*/*PDGFRA* mutations, 9–15% of GISTs are wildtype for both genes, and no other underlying mutation has been detected yet [9,59]. Some of these cases show overexpression of insulin-like growth factor 1 receptor (IGF1R) [60–62]. GISTs in patients with the familial Carney–Stratakis syndrome (paraganglioma and GIST) have germline mutations in the succinate dehydrogenase (*SDH*) subunits B, C or D leading to enzyme deficiency [63,64]. Although no *SDH* mutations were detected in non-syndromic pediatric patients with *KIT*/*PDGFRA* wildtype tumors, they similarly show a loss of expression and/or function of SDH [65]. Moreover, a few *KIT*/*PDGFRA* wildtype GISTs have been reported to carry activating *BRAF* V600E mutations [66,67]. However, it has not yet formally been determined whether they are the initiating event in these tumors.

3. Targeting KIT with imatinib mesylate (Gleevec®)

Imatinib mesylate (Gleevec®, Novartis) is a small molecule protein tyrosine kinase inhibitor that was originally identified in a screen for PDGFR inhibitors [68]. It competitively binds to the kinase ATP binding pocket, and also effectively inhibits not only PDGFRA/B, but also the KIT and ABL kinases because of structural similarities of their kinase domains [27,45,46]. Imatinib was first clinically used to target the BCR-ABL fusion kinase, which is the underlying oncogenic stimulus in Philadelphia chromosome-positive chronic myeloid leukemia (CML). It was FDA-approved for this indication in 2000. The same year, the first GIST patient was treated imatinib, and the drug gained FDA approval for GIST in 2002 [69,70]. The use of imatinib for treatment of GIST has reversed an untreatable disease into a tumor entity, in which up to 85% of patients that receive the drug achieve disease control [71–73]. The benefits of imatinib treatment are highlighted by a recent retrospective study that calculated the median overall survival for patients with metastatic GIST in the pre-imatinib era at 19 months [74], whereas the current median overall survival under imatinib therapy is estimated to be more than 50 months [72,75]. As mentioned above, the response of GISTs to imatinib treatment correlates with the type of mutation in the primary tumor [76–79]. Tumors with *KIT* exon 11 mutations respond best to imatinib therapy (70–85% objective response—encompassing complete and partial response), whereas *KIT* exon 9 mutant GISTs only showed an 25–48% overall response rate in a recent study [75,76]. The response rates for tumors with kinase domain mutations (exons 13 and 17) or with no *KIT* mutation are even below the response rates of exon 9 mutant GIST. This can be explained by the fact that mutations in the *KIT* kinase domains likely interfere with imatinib binding on one handside, whereas *KIT* wildtype GISTs may not depend on KIT activation as much as GISTs with *KIT* mutations. However, the reason for the difference in response rates for exon 9 mutant GISTs is not exactly clear. Notably, these patients significantly benefit from a dose increase from the standard 400 mg imatinib to 800 mg/day [80]. It is also important for the clinical management of *PDGFRA* mutant GIST that although the most common mutation (D824 V in *PDGFRA* exon 18) is imatinib-resistant *in vitro*, most other *PDGFRA* mutations are imatinib-responsive [77,81,82].

The almost unprecedented success story of the use of a small molecule inhibitor in a solid tumor entity has made the "GIST–imatinib connection" a paradigm for targeted therapy. It has resulted in efforts to find similar therapeutic strategies for other solid tumors to increase remission rates and the quality of positive responses. Unfortunately, the majority of malignancies are not caused by single mutations – but there are nonetheless a plethora of lessons to learn from GISTs and imatinib.

4. The mode of action of imatinib mesylate

Imatinib mesylate binds to the inactivated form of the KIT kinase thereby preventing its activation [27]. It causes a profound and rapid inhibition of active, phosphorylated KIT within minutes, thus underscoring that even the oncogenically activated kinase is in an equilibrium between its active and inactive state, which is merely shifted towards the active conformation. Inhibition of KIT is followed by an equally rapid inhibition of downstream signaling pathways, in particular the PI3K/mTOR/AKT and RAS/RAF/MAPK pathways [29,83]. However, when the dynamics of GIST cell death were analyzed in correlation to the inhibition of KIT kinase activity, it was surprisingly found that apoptosis was considerably delayed and did not peak until approximately 72 h after treatment [29,84]. It is now known that the induction of GIST cell apoptosis is far more complex than a simple shut-down of kinase activity. One example to underscore this notion is the fact that a histone protein of the H2A family, histone H2AX, was found to play an important role in GIST cell death [84,85]. Imatinib causes a massive upregulation of soluble, non-nucleosomal histone H2AX, which drives GIST cells into apoptosis, very likely through an inhibition of ongoing gene transcription. H2AX upregulation was found to correlate with imatinib sensitivity, and depletion of H2AX in GIST cells dampened their apoptotic response to imatinib. Histone H2AX is well known as a key player in the cellular response to DNA damage, but the results obtained from imatinib-treated GIST cells suggest a novel, unexpected function of this protein as a trigger of cell death. Other mechanisms that have been implicated in imatinib-induced GIST cell apoptosis include a downregulation of the protein translation machinery [86] and reduced glucose uptake as evidenced by decreased membrane-bound GLUT4 [87]. In CML, the pro-apoptotic BH3-only proteins BIM and BAD were found to mediate the apoptotic response after imatinib treatment [88,89] and recently, BIM has also been implicated in imatinib-induced apoptosis in GIST [90]. Further dissection of the pro-apoptotic pathways after imatinib treatment is desirable to identify additional molecules that can be targeted for therapeutic purposes (see below).

5. Imatinib triggers gist cell quiescence

Tumor cell quiescence is a major obstacle to conventional anti-tumor therapy since most agents specifically target proliferating cells. Quiescent cells remain metabolically active, but are withdrawn from the cell division cycle, which renders them intrinsically resistant to numerous chemotherapeutic agents [91]. Such "dormant" cells can therefore cause refractory disease and/or relapse, if they harbor changes associated with resistance. It was

hence surprising and disconcerting to find that imatinib can induce molecular changes that lead to GIST cell quiescence [92,93]. Specifically, imatinib was found to downregulate the F-box protein SKP2, thereby causing an accumulation of the CDK inhibitor $p27^{Kip1}$, a major regulator of quiescence. SKP2 itself is regulated by proteolysis, and imatinib was found to disrupt an even higher level of regulation by causing the APC/cyclosome activator CDH1 to relocalize to the nucleus. Here, it may stimulate the degradation of SKP2 and hence initiate the chain of events that ultimately triggers cell cycle exit. The induction of GIST cell quiescence by imatinib reconciles a number of clinical observations including the rarity of complete responses, the fact that some patients remain stable under imatinib therapy in the presence of detectable disease, and, most importantly, the need for continued, long-term drug treatment [94]. Moreover, in the light of the current view that resistance mutations (see below) are pre-existing in most GISTs or evolve during the natural course of the disease even without imatinib treatment [95,96], it is very likely that quiescent cells provide a pool of cells that can lead to resistance at later stages.

The problem of cellular quiescence after imatinib therapy has been focus of many studies in CML and shows parallels to what has been reported in GIST [97]. Studying quiescence in CML is technically facilitated by the fact that it is possible to isolate G_0 subpopulations with the help of flow cytometry [98]. Similar to GIST patients, a full remission (on a molecular level defined as BCR-ABL mRNA levels below the detection level of quantitative real-time PCR in CML) is rarely achieved after imatinib treatment. Cells not eliminated by anti-leukemic therapy were shown to be part of a primitive $CD34^+$ progenitor cell population that has exited the cell division cycle. These $CD34^+$, BCR-ABL^+ CML stem cells are insensitive to imatinib, but also to other apoptotic stimuli [91,99–101]. However, findings using *in vitro* models of CML cells that proliferate at different rates suggest that their resistance to treatment does not necessarily depend on their cell cycle stage, but rather on "other molecular properties" of these cells [102]. Some of these properties may include high expression levels of ATP-binding cassette transporters (ABC), including the multi-drug resistance protein 1 (P-glycoprotein) and ABCG2 [103,104]. Other mechanisms could consist of differential expression levels of genes that are expressed during cellular differentiation as well as a generally low level of transcriptional activity per se [105]. It is important to note, however, that a certain percentage of the quiescent primitive $CD34^+$ progenitor cells already harbors the typical imatinib resistance mutations [106,107]. Attempts to therapeutically target quiescent stem cells in CML have proven to be difficult for the above-mentioned reasons. One approach that yielded promising results in preclinical models included treatment with growth factors like granulocyte-colony stimulating factor (G-CSF) to force quiescent cells to cycle [108]. Future studies are warranted to test whether conceptionally similar approaches can increase the efficiency of targeted therapies in GIST patients.

6. Overcoming imatinib-induced quiescence and imatinib resistance

Besides imatinib-associated quiescence, the development of drug resistance remains one of the biggest hurdles for long-term remission and cure of the disease. Approximately 10% of patients experience primary resistance to imatinib, which is defined as progression within the first three to 6 months of therapy [69,73]. Primary resistance can be seen in all mutation types, but *KIT*/*PDGFRA* wildtype tumors as well as tumors harboring a *KIT* exon 9 or *PDGFRA* D842V mutation are more prone to exhibit primary imatinib resistance. In the case of *PDGFRA* D842V this can be explained by the fact of it being localized in the kinase domain presumably affecting imatinib binding. Secondary, or delayed, resistance to imatinib after showing some initial benefit is seen in 40–50% of patients within 2 years of starting imatinib therapy [32,73]. Although resistant/recurring tumors are most often diagnosed by CT scan, a substantial progress in identifying tumors with secondary resistance has been made by the introduction of 18FDG-PET scans [109,110]. By now, it is well established that the leading cause for imatinib resistance are secondary mutations in the *KIT* or *PDGFRA* kinase domain (Table 2) [32,111–120]. Secondary mutations can be detected in two thirds of patients [111–114,121]. They usually occur in the same gene as the primary resistance mutation and have therefore more often been reported in tumors with primary *KIT* mutation. Within *KIT* mutated tumors, secondary kinase mutations are more frequently detected in GISTs with a primary exon 11 mutation than GISTs with a primary exon 9 mutation [116]. Amplification of the *KIT* or *PDGFRA* genes [111,122,123] seem to play a minor role, especially when compared to what has been reported for BCR-ABL amplifications in CML [95,124]. Furthermore, possible pharmacological reasons for imatinib resistance, such as an increased clearance or binding to plasma proteins, have been reported [125,126].

Table 2
Secondary (resistance) mutations in GIST.

Gene	Exon	Mutation	Domain affected
KIT	13	• V654A	Kinase domain I (ATP binding pocket)
	14	• T670I	
	17	• D816A/G/H/V • D820A/E/G/Y • N822H/K • Y823D	Kinase domain II (phosphotransferase/ activation loop)
	18	• A829P	
PDGFRA	18	• D842V	Kinase domain II (phosphotransferase/ activation loop)

Patients who fail to respond to imatinib are currently being treated with sunitinib malate (Sutent®, Pfizer), a second-line therapy for GIST approved by the FDA in 2006 [127,128]. Sunitinib is a multikinase inhibitor that targets not only KIT and the PDGFRs but also VEGFRs 1-3, FLT3 and RET [129]. The response rate to sunitinib as second-line agent was found to be 65% (7% partial response, 58% stable disease) in a phase III placebo-controlled clinical trial [130]. Patients with wildtype *KIT* or *KIT* exon 9 mutations tend to benefit more from sunitinib therapy than patients with *KIT* exon 11 mutations, but resistance is still a problem with this second-line agent [128,130]. This was not entirely unexpected, since – similar to imatinib – sunitinib has also minimal activity against the *KIT* exon 17 and *PDGFRA* exon 18 mutations [131].

These above-mentioned clinical results make it clear that novel approaches to GIST therapy need to focus on (1) making the first line therapy more effective and (2) either avoiding resistance and/or developing innovative approaches to GIST treatment if resistance has occurred.

One approach to make GIST therapy more effective is the use of improved KIT kinase inhibitors, both in terms of higher affinity binding as well as a more favorable activity spectrum. Some examples of these new kinase inhibitors are being discussed below. However, due to the vast number of compounds that are currently being developed or have already entered clinical trials, only the most prominent compounds are being highlighted (Table 3). Nilotinib (Tasigna®, formerly AMN107, Novartis) shows higher affinity kinase binding (for BCR-ABL) and achieves 7–10-fold greater intracellular concentrations than imatinib in GIST [132,133]. It is currently in clinical trials for GIST, but already

Table 3
New therapeutic options for GIST.

Target		Compound	Brand name	Company
Pathway	Molecule(s)			
KIT/PDGFRA	KIT, PDGFRs, ABL	Imatinib mesylate (STI571)	Gleevec®	Novartis
	KIT, PDGFRs, VEGFRs 1-3, FLT3, RET	Sunitinib malate (SU11248)	Sutent®	Pfizer
		Nilotinib (AMN107)	Tasigna®	Novartis
	KIT, PDGFRB, RAF, VEGFR2/3, FLT3, RET	Sorafenib tosylate (BAY 43-9006)	Nexavar®	Bayer
	KIT, PDGFRs, SRC, ABL	Dasatinib (BMS-354825)	Sprycel®	Bristol-Myers Squibb
PI3K	PI3K	BEZ235	–	Novartis
		XL147	–	Exelixis
		XL765*	–	Exelixis
		SF1126	–	Semafore Pharmaceuticals
		PF-04691502*	–	Pfizer
		GDC-0941	–	Genentech
	AKT	Perifosine	–	Keryx Biopharmaceuticals
		XL418	–	Exelixis
	mTOR	Everolimus (RAD001)	Afinitor®	Novartis
		Sirolimus	Rapamune®	Wyeth (now Pfizer)
		Temsirolimus	Torisel®	Wyeth (now Pfizer)
MAPK	RAF	XL281	–	Exilixis
	MEK	RDEA119	–	Ardea Biosciences
	MEK1	GDC-0973 (XL518)	–	Genentech (compound acquired from Exilixis)
HSP90	HSP90	Geldanamycin	n/a	n/a
		17-AAG	Tanespimycin®	Bristol-Myers Squibb
		IPI-504	–	Infinity Pharmaceuticals
		STA-9090	–	Synta Pharmaceuticals
HDAC	HDAC	Vorinostat (SAHA)	Zolinza®	Merck
		Panobinostat (LBH589)	–	Novartis
		Valproic acid	–	n/a
		Trichostatin A	–	n/a
proteasome	26S subunit	Bortezomib	Velcade®	Millennium Pharmaceuticals
KIT	KIT (switch pocket)	(Switch pocket kinase inhibitor)	n/a	Deciphera Pharmaceuticals
transcription	KIT promoter	(Quadruplex–DNA binding)	n/a	n/a

* also inhibits mTOR.

FDA-approved for the treatment of imatinib-resistant BCR-ABL-positive CML. Sorafenib tosylate (Nexavar®, formerly BAY 43-9006, Bayer) is an orally available multikinase inhibitor against RAF, VEGFR2/3, PDGFRB, KIT, FLT3 and RET. It is currently in a Phase II clinical trial for GIST [134]. Dasatinib (Sprycel®, BMS-354825, Bristol-Myers Squibb), mainly marketed as a dual SRC/ABL kinase inhibitor and already FDA-approved for imatinib-resistant CML/AML, also potently inhibits KIT and PDGFR kinase activity and has shown preclinical activity against mutant *KIT* D816 V and mutant *PDGFRA* D842 V [135]. So far, the response rates in these clinical trials seem to be varied, and it will be necessary to take the mutation type as well as other factors of the molecular makeup of those tumors into the equation to be able to better predict the response to therapy.

In addition to the classical direct interaction with the ATP-binding pocket and subsequent inhibition of kinase activity, it may be possible to target KIT or PDGFRA through several unrelated mechanisms. This was the rationale to test HSP90 inhibitors as potential therapeutic agents in GIST. Heat shock proteins are chaperone molecules that prevent the degradation of misfolded proteins, such as proteins derived from a mutated gene. In the context of GIST, mutated *KIT*/*PDGFRA* is presumably misfolded and would be degraded if not protected by chaperone proteins. Therefore, it was reasoned that inhibition of these chaperones could lead to degradation of the mutated *KIT*/*PDGFRA* in GIST (but most likely not the wildtype proteins). This promising strategy has been confirmed in a number of cell line models with several natural (geldanamycin) or synthetic compounds [136]. 17-AAG (Bristol-Myers Squibb) is a geldanamycin-derivative, but is not water-soluble and has hence to be given intravenously [137]. By contrast, IPI-504 (Infinity Pharmaceuticals) was the first water soluble, oral HSP90 inhibitor that entered clinical trials. However, after very promising results in a phase I trial, a phase III clinical trial had to be terminated early because of a higher than anticipated mortality in treated patients versus placebo-treated patients [138]. Nevertheless, new compounds of this class with anticipated less toxicity are in development and/or clinical trials for GIST (for example, STA-9090, Synta Pharmaceuticals).

Other compounds that target KIT by other means than competitive ATP binding include so-called quadruplex-binding small molecules [139] and switch pocket inhibitors (developed by Deciphera). Quadruplex-binding small molecules have initially been developed to target quadruplex DNA structures at telomeres with the aim to inhibit of telomerase and disrupt telomere maintenance thereby selectively inhibiting cancer cell growth. However, the same principle can be applied to targeting quadruplex DNA structures in gene promoter regions with subsequent of inhibition of gene transcription. A first compound (a naphthalene diimide derivative) targeting the *KIT* promoter region, which harbors potential quadruplex structures, has shown some promise in preclinical studies [139]. By contrast, switch pocket inhibitors make use of the fact that the so-called kinase switch pocket is usually quite unique in a given kinase or kinase sub-family. This is in contrary to the ATP binding pocket, which is very well conserved over a wide range of kinases. The switch pocket is the space, into which the phosphorylated switch binds upon kinase activation. Inhibiting this binding would therefore presumably also inhibit kinase activity. These novel approaches sound very promising, especially if it is possible to overcome potential hurdles of drug toxicity, pharmacodynamics/-kinetics as well as sufficient tumor distribution.

Taken together, future drug development needs to take into considerations that targeting KIT alone may not be sufficient to completely eliminate GIST cells due to entry into a quiescent state (see above). A deeper understanding of GIST cell quiescence [93,140] and identification of potential drug targets relevant for this process will be instrumental to improve the quality of clinical responses. Here, it will be especially important to understand what factors determine whether a cell responds to imatinib therapy by exiting the cell division cycle or by undergoing apoptosis. One possible factor could be the stage of the cell cycle that this cell is in when imatinib treatment is started. Future experiments are needed to determine how cells can be manipulated to achieve a maximum apoptotic response.

7. Novel targets in GISTs

The search for novel targets outside KIT and PDGFRA is motivated by the fact that most imatinib-resistance mutations occur within the *KIT* or *PDGFRA* genes themselves, which may thwart efforts to target these proteins even with improved compounds. In addition, genotype-phenotype studies have shown that multiple resistant tumor nodules within the same patient oftentimes do not carry the same resistance mutations [111,112,122,141,142], which makes a kinase-centric approach difficult. It may hence be promising to target the KIT/PDGFRA downstream signaling pathways, an approach that has shown some promise in preclinical studies [83]. The most critical signaling axis for GIST cell survival seems to be the PI3K/AKT/mTOR pathway [29,83]. A number of compounds targeting various molecules of these signaling modules are currently in clinical trials including several PI3K inhibitors, AKT inhibitors and mTOR inhibitors (Table 3). Targeting the RAS/RAF/MAPK pathway, for which various compounds are now available (Table 3), may be particularly important for the low percentage of GISTs that present with *BRAF* mutations [66,67] and GISTs developing in NF1 patients [143–145]. However, it has been shown in various *in vitro* models that targeting the PI3K pathway holds more promise in most sporadic GISTs [146]. Compounds targeting KIT/PDGFRA downstream pathways may be especially amenable to combination therapy, either with a KIT/PDGFRA inhibitor or with each other.

An emerging family of target proteins that are not involved in signaling pathways related to KIT/PDGFRA are histone deacetylases. The function of these proteins is to deacetylate histones thereby leading to a conformational change of the chromatin. Histones are the components of nucleosomes, which have long been known as mere packaging units to compact DNA. We now know that histones can be post-transcriptionally modified in many ways, e.g. by phosphorylation, methylation and acetylation, thereby regulating the conformation and accessibility of DNA. Acetylated histones are usually found in regions with open chromatin, thus enabling transcription. Therefore, inhibiting histone deacetylases should promote this relaxed chromatin conformation. It has been hypothesized that this leads to increased transcription of cell cycle inhibitory genes, thereby leading to a therapeutic effect. However, recent reports suggest a more complex mechanism of action that involves increased acetylation of heat shock proteins leading to disruption of their chaperone function [146–148]. A number of HDAC inhibitors are already FDA-approved for lymphomas, and some prominent compounds are trichostatin A (a natural compound), valproic acid, suberoylanilide hydroxamic acid (SAHA, vorinostat, Zolinza®, Merck), and panobinostat (LBH589, Novartis). HDAC inhibitors have recently shown promising results in preclinical models for GIST [146,149] and are currently in clinical trials. The idea of using HDAC inhibitors does not seem to be very specific for targeting KIT in GIST, but they have proven to be quite effective in preclinical models, as mentioned above. As novel functions of HDACs are emerging, we may also learn more about the exact mechanisms of action of HDAC inhibitors.

Another promising novel target in GISTs may be the ubiquitin–proteasome machinery, which is responsible for the degradation of poly-ubiquitylated proteins and therefore protein stability of various regulatory proteins that play a role in GISTs. In a recent study, it was shown that the FDA-approved proteasome inhibitor bortezomib (Velcade®, Millennium) can effectively induce cell death in imatinib-sensitive as well as imatinib-resistant GIST cell lines [150]. Remarkably, the mode of action was an upregulation of the pro-apoptotic histone H2AX and a simultaneous almost complete loss of expression of the KIT kinase. The latter was caused by a transcriptional shutdown in GIST cells. These results not only highlight that bortezomib may be potentially beneficial in GIST patients but also the exquisite dependency of GIST cells on active gene transcription, another promising target in GIST therapy. Similarly to the HDAC inhibitors described above, targeting the proteasome at first glance looks like a rather unspecific therapeutic approach in GIST. However, dissecting the exact mechanism of action of bortezomib in GIST has shown that this compound is able to target two pathways that are crucial for GIST cell survival at the same time.

8. Summary and outlook

Targeted therapy of GISTs has been an extraordinary success story, and up to 85% of patients benefit from single-agent therapy with imatinib. Insights into the mode of action of imatinib as well as a better understanding of the underlying GIST cell biology have revealed pathways that are extremely rich in potential drug targets. In the future, it can be expected that GISTs remain one of the most useful paradigms and model systems for tumor therapy. A concerted effort of clinical and basic science, pathology, drug development and pharmaceutical industry is very likely to succeed in developing innovative approaches to more complete and longer-lasting remissions to improve the quality of life and long-term survival of patients with GISTs.

Acknowledgements

This work was supported by a Research Scholar Grant from the American Cancer Society (RSG-08-092-01-CCG), as well as grants from the GIST Cancer Research Fund and the Life Raft Group (UPCC-AD-100108) to A.D. The authors would like to acknowledge all colleagues as well as members of their laboratory for insightful discussions.

References

[1] Duensing A, Heinrich MC, Fletcher CD, Fletcher JA. Biology of gastrointestinal stromal tumors: KIT mutations and beyond. Cancer Invest 2004;22(1):106–16.
[2] Liegl-Atzwanger B, Fletcher JA, Fletcher CD. Gastrointestinal stromal tumors. Virchows Arch 2010;456(2):111–27.
[3] Isozaki K, Hirota S, Miyagawa J, Taniguchi M, Shinomura Y, Matsuzawa Y. Deficiency of c-kit+ cells in patients with a myopathic form of chronic idiopathic intestinal pseudo-obstruction. Am J Gastroenterol 1997;92(2):332–4.
[4] Isozaki K, Hirota S, Nakama A, Miyagawa J, Shinomura Y, Xu Z, et al. Disturbed intestinal movement, bile reflux to the stomach, and deficiency of c-kit-expressing cells in Ws/Ws mutant rats. Gastroenterology 1995;109(2):456–64.
[5] Hirota S, Isozaki K, Moriyama Y, Hashimoto K, Nishida T, Ishiguro S, et al. Gain-of-function mutations of c-kit in human gastrointestinal stromal tumors. Science 1998;279(5350):577–80.
[6] Kindblom LG, Remotti HE, Aldenborg F, Meis-Kindblom JM. Gastrointestinal pacemaker cell tumor (GIPACT): gastrointestinal stromal tumors show phenotypic characteristics of the interstitial cells of Cajal. Am J Pathol 1998;152(5):1259–69.

[7] Heinrich MC, Corless CL, Duensing A, McGreevey L, Chen CJ, Joseph N, et al. PDGFRA activating mutations in gastrointestinal stromal tumors. Science 2003;299(5607):708–10.
[8] Hirota S, Ohashi A, Nishida T, Isozaki K, Kinoshita K, Shinomura Y, et al. Gain-of-function mutations of platelet-derived growth factor receptor alpha gene in gastrointestinal stromal tumors. Gastroenterology 2003;125(3):660–7.
[9] Medeiros F, Corless CL, Duensing A, Hornick JL, Oliveira AM, Heinrich MC, et al. KIT-negative gastrointestinal stromal tumors: proof of concept and therapeutic implications. Am J Surg Pathol 2004;28(7):889–94.
[10] Debiec-Rychter M, Wasag B, Stul M, De Wever I, Van Oosterom A, Hagemeijer A, et al. Gastrointestinal stromal tumours (GISTs) negative for KIT (CD117 antigen) immunoreactivity. J Pathol 2004;202(4):430–8.
[11] Miettinen M, Lasota J, Sobin LH. Gastrointestinal stromal tumors of the stomach in children and young adults: a clinicopathologic, immunohistochemical, and molecular genetic study of 44 cases with long-term follow-up and review of the literature. Am J Surg Pathol 2005;29(10):1373–81.
[12] Kontogianni-Katsarou K, Lariou C, Tsompanaki E, Vourlakou C, Kairi-Vassilatou E, Mastoris C, et al. KIT-negative gastrointestinal stromal tumors with a long term follow-up: a new subgroup does exist. World J Gastroenterol 2007;13(7):1098–102.
[13] Nielsen TO, West RB, Linn SC, Alter O, Knowling MA, O'Connell JX, et al. Molecular characterisation of soft tissue tumours: a gene expression study. Lancet 2002;359(9314):1301–7.
[14] Allander SV, Nupponen NN, Ringner M, Hostetter G, Maher GW, Goldberger N, et al. Gastrointestinal stromal tumors with KIT mutations exhibit a remarkably homogeneous gene expression profile. Cancer Res 2001;61(24):8624–8.
[15] Duensing A, Joseph NE, Medeiros F, Smith F, Hornick JL, Heinrich MC, et al. Protein Kinase C theta (PKCtheta) expression and constitutive activation in gastrointestinal stromal tumors (GISTs). Cancer Res 2004;64(15):5127–31.
[16] Lee HE, Kim MA, Lee HS, Lee BL, Kim WH. Characteristics of KIT-negative gastrointestinal stromal tumours and diagnostic utility of protein kinase C theta immunostaining. J Clin Pathol 2008;61(6):722–9.
[17] Blay P, Astudillo A, Buesa JM, Campo E, Abad M, Garcia-Garcia J, et al. Protein kinase C theta is highly expressed in gastrointestinal stromal tumors but not in other mesenchymal neoplasias. Clin Cancer Res 2004;10(12 Pt 1):4089–95.
[18] Motegi A, Sakurai S, Nakayama H, Sano T, Oyama T, Nakajima T. PKC theta, a novel immunohistochemical marker for gastrointestinal stromal tumors (GIST), especially useful for identifying KIT-negative tumors. Pathol Int 2005;55(3):106–12.
[19] West RB, Corless CL, Chen X, Rubin BP, Subramanian S, Montgomery K, et al. The novel marker, DOG1, is expressed ubiquitously in gastrointestinal stromal tumors irrespective of KIT or PDGFRA mutation status. Am J Pathol 2004;165(1):107–13.
[20] Espinosa I, Lee CH, Kim MK, Rouse BT, Subramanian S, Montgomery K, et al. A novel monoclonal antibody against DOG1 is a sensitive and specific marker for gastrointestinal stromal tumors. Am J Surg Pathol 2008;32(2):210–8.
[21] Miettinen M, Wang ZF, Lasota J. DOG1 antibody in the differential diagnosis of gastrointestinal stromal tumors: a study of 1840 cases. Am J Surg Pathol 2009;33(9):1401–8.
[22] Qiu FH, Ray P, Brown K, Barker PE, Jhanwar S, Ruddle FH, et al. Primary structure of c-kit: relationship with the CSF-1/PDGF receptor kinase family—oncogenic activation of v-kit involves deletion of extracellular domain and C terminus. EMBO J 1988;7(4):1003–11.
[23] Besmer P, Murphy JE, George PC, Qiu FH, Bergold PJ, Lederman L, et al. A new acute transforming feline retrovirus and relationship of its oncogene v-kit with the protein kinase gene family. Nature 1986;320(6061):415–21.
[24] Chabot B, Stephenson DA, Chapman VM, Besmer P, Bernstein A. The proto-oncogene c-kit encoding a transmembrane tyrosine kinase receptor maps to the mouse W locus. Nature 1988;335(6185):88–9.
[25] Majumder S, Brown K, Qiu FH, Besmer P. c-kit protein, a transmembrane kinase: identification in tissues and characterization. Mol Cell Biol 1988; 8(11):4896–903.
[26] Nocka K, Buck J, Levi E, Besmer P. Candidate ligand for the c-kit transmembrane kinase receptor: KL, a fibroblast derived growth factor stimulates mast cells and erythroid progenitors. EMBO J 1990;9(10):3287–94.
[27] Mol CD, Dougan DR, Schneider TR, Skene RJ, Kraus ML, Scheibe DN, et al. Structural basis for the autoinhibition and STI-571 inhibition of c-Kit tyrosine kinase. J Biol Chem 2004;279(30):31655–63.
[28] Mol CD, Lim KB, Sridhar V, Zou H, Chien EY, Sang BC, et al. Structure of a c-kit product complex reveals the basis for kinase transactivation. J Biol Chem 2003;278(34):31461–4.
[29] Duensing A, Medeiros F, McConarty B, Joseph NE, Panigrahy D, Singer S, et al. Mechanisms of oncogenic KIT signal transduction in primary gastrointestinal stromal tumors (GISTs). Oncogene 2004;23(22):3999–4006.
[30] Tsujimura T, Hashimoto K, Kitayama H, Ikeda H, Sugahara H, Matsumura I, et al. Activating mutation in the catalytic domain of c-kit elicits hematopoietic transformation by receptor self-association not at the ligand-induced dimerization site. Blood 1999;93(4):1319–29.
[31] Rubin BP, Singer S, Tsao C, Duensing A, Lux ML, Ruiz R, et al. KIT activation is a ubiquitous feature of gastrointestinal stromal tumors. Cancer Res 2001; 61(22):8118–21.
[32] Gramza AW, Corless CL, Heinrich MC. Resistance to tyrosine kinase inhibitors in gastrointestinal stromal tumors. Clin Cancer Res 2009;15(24):7510–8.
[33] Lasota J, Wozniak A, Sarlomo-Rikala M, Rys J, Kordek R, Nassar A, et al. Mutations in exons 9 and 13 of KIT gene are rare events in gastrointestinal stromal tumors. A study of 200 cases. Am J Pathol 2000;157(4):1091–5.
[34] Antonescu CR, Sommer G, Sarran L, Tschernyavsky SJ, Riedel E, Woodruff JM, et al. Association of KIT exon 9 mutations with nongastric primary site and aggressive behavior: KIT mutation analysis and clinical correlates of 120 gastrointestinal stromal tumors. Clin Cancer Res 2003;9(9):3329–37.
[35] Antonescu CR, Viale A, Sarran L, Tschernyavsky SJ, Gonen M, Segal NH, et al. Gene expression in gastrointestinal stromal tumors is distinguished by KIT genotype and anatomic site. Clin Cancer Res 2004;10(10):3282–90.
[36] Lasota J, Dansonka-Mieszkowska A, Stachura T, Schneider-Stock R, Kallajoki M, Steigen SE, et al. Gastrointestinal stromal tumors with internal tandem duplications in 3' end of KIT juxtamembrane domain occur predominantly in stomach and generally seem to have a favorable course. Mod Pathol 2003;16(12):1257–64.
[37] Wardelmann E, Neidt I, Bierhoff E, Speidel N, Manegold C, Fischer HP, et al. c-kit mutations in gastrointestinal stromal tumors occur preferentially in the spindle rather than in the epithelioid cell variant. Mod Pathol 2002;15(2):125–36.
[38] Debiec-Rychter M, Lasota J, Sarlomo-Rikala M, Kordek R, Miettinen M. Chromosomal aberrations in malignant gastrointestinal stromal tumors: correlation with c-KIT gene mutation. Cancer Genet Cytogenet 2001; 128(1):24–30.
[39] Lasota J, Dansonka-Mieszkowska A, Sobin LH, Miettinen M. A great majority of GISTs with PDGFRA mutations represent gastric tumors of low or no malignant potential. Lab Invest 2004;84(7):874–83.
[40] Wardelmann E, Hrychyk A, Merkelbach-Bruse S, Pauls K, Goldstein J, Hohenberger P, et al. Association of platelet-derived growth factor receptor alpha mutations with gastric primary site and epithelioid or mixed cell morphology in gastrointestinal stromal tumors. J Mol Diagn 2004;6(3):197–204.
[41] Corless CL, McGreevey L, Haley A, Town A, Heinrich MC. KIT mutations are common in incidental gastrointestinal stromal tumors one centimeter or less in size. Am J Pathol 2002;160(5):1567–72.
[42] Kawanowa K, Sakuma Y, Sakurai S, Hishima T, Iwasaki Y, Saito K, et al. High incidence of microscopic gastrointestinal stromal tumors in the stomach. Hum Pathol 2006;37(12):1527–35.
[43] Agaimy A, Wunsch PH, Hofstaedter F, Blaszyk H, Rummele P, Gaumann A, et al. Minute gastric sclerosing stromal tumors (GIST tumorlets) are common in adults and frequently show c-KIT mutations. Am J Surg Pathol 2007;31(1):113–20.
[44] Chetty R. Small and microscopically detected gastrointestinal stromal tumours: an overview. Pathology 2008;40(1):9–12.
[45] Heinrich MC, Griffith DJ, Druker BJ, Wait CL, Ott KA, Zigler AJ. Inhibition of c-kit receptor tyrosine kinase activity by STI 571, a selective tyrosine kinase inhibitor. Blood 2000;96(3):925–32.
[46] Tuveson DA, Willis NA, Jacks T, Griffin JD, Singer S, Fletcher CD, et al. STI571 inactivation of the gastrointestinal stromal tumor c-KIT oncoprotein: biological and clinical implications. Oncogene 2001;20(36):5054–8.
[47] McWhinney SR, Pasini B, Stratakis CA. Familial gastrointestinal stromal tumors and germ-line mutations. N Engl J Med 2007;357(10):1054–6.
[48] Li FP, Fletcher JA, Heinrich MC, Garber JE, Sallan SE, Curiel-Lewandrowski C, et al. Familial gastrointestinal stromal tumor syndrome: phenotypic and molecular features in a kindred. J Clin Oncol 2005;23(12):2735–43.
[49] O'Riain C, Corless CL, Heinrich MC, Keegan D, Vioreanu M, Maguire D, et al. Gastrointestinal stromal tumors: insights from a new familial GIST kindred with unusual genetic and pathologic features. Am J Surg Pathol 2005;29(12):1680–3.
[50] Chompret A, Kannengiesser C, Barrois M, Terrier P, Dahan P, Tursz T, et al. PDGFRA germline mutation in a family with multiple cases of gastrointestinal stromal tumor. Gastroenterology 2004;126(1):318–21.
[51] Beghini A, Tibiletti MG, Roversi G, Chiaravalli AM, Serio G, Capella C, et al. Germline mutation in the juxtamembrane domain of the kit gene in a family with gastrointestinal stromal tumors and urticaria pigmentosa. Cancer 2001;92(3):657–62.
[52] Hirota S, Nishida T, Isozaki K, Taniguchi M, Nishikawa K, Ohashi A, et al. Familial gastrointestinal stromal tumors associated with dysphagia and novel type germline mutation of KIT gene. Gastroenterology 2002; 122(5):1493–9.
[53] Isozaki K, Terris B, Belghiti J, Schiffmann S, Hirota S, Vanderwinden JM. Germline-activating mutation in the kinase domain of KIT gene in familial gastrointestinal stromal tumors. Am J Pathol 2000;157(5):1581–5.
[54] Maeyama H, Hidaka E, Ota H, Minami S, Kajiyama M, Kuraishi A, et al. Familial gastrointestinal stromal tumor with hyperpigmentation: association with a germline mutation of the c-kit gene. Gastroenterology 2001;120(1):210–5.
[55] Nishida T, Hirota S, Taniguchi M, Hashimoto K, Isozaki K, Nakamura H, et al. Familial gastrointestinal stromal tumours with germline mutation of the KIT gene. Nat Genet 1998;19(4):323–4.
[56] Sommer G, Agosti V, Ehlers I, Rossi F, Corbacioglu S, Farkas J, et al. Gastrointestinal stromal tumors in a mouse model by targeted mutation of the Kit receptor tyrosine kinase. Proc Natl Acad Sci USA 2003;100(11): 6706–11.
[57] Rubin BP, Antonescu CR, Scott-Browne JP, Comstock ML, Gu Y, Tanas MR, et al. A knock-in mouse model of gastrointestinal stromal tumor harboring kit K641E. Cancer Res 2005;65(15):6631–9.
[58] Nakai N, Ishikawa T, Nishitani A, Liu NN, Shincho M, Hao H, et al. A mouse model of a human multiple GIST family with KIT-Asp820Tyr mutation generated by a knock-in strategy. J Pathol 2008;214(3):302–11.
[59] Janeway KA, Liegl B, Harlow A, Le C, Perez-Atayde A, Kozakewich H, et al. Pediatric KIT wild-type and platelet-derived growth factor receptor alpha-wild-type gastrointestinal stromal tumors share KIT activation but not

mechanisms of genetic progression with adult gastrointestinal stromal tumors. Cancer Res 2007;67(19):9084–8.
[60] Tarn C, Rink L, Merkel E, Flieder D, Pathak H, Koumbi D, et al. Insulin-like growth factor 1 receptor is a potential therapeutic target for gastrointestinal stromal tumors. Proc Natl Acad Sci USA 2008;105(24):8387–92.
[61] Agaram NP, Laquaglia MP, Ustun B, Guo T, Wong GC, Socci ND, et al. Molecular characterization of pediatric gastrointestinal stromal tumors. Clin Cancer Res 2008;14(10):3204–15.
[62] Janeway KA, Zhu MJ, Barretina J, Perez-Atayde A, Demetri GD, Fletcher JA. Strong expression of IGF1R in pediatric gastrointestinal stromal tumors without IGF1R genomic amplification. Int J Cancer 2010; [Epub ahead of print].
[63] Pasini B, Matyakhina L, Bei T, Muchow M, Boikos S, Ferrando B, et al. Multiple gastrointestinal stromal and other tumors caused by platelet-derived growth factor receptor alpha gene mutations: a case associated with a germline V561D defect. J Clin Endocrinol Metab 2007;92(9):3728–32.
[64] Pasini B, McWhinney SR, Bei T, Matyakhina L, Stergiopoulos S, Muchow M, et al. Clinical and molecular genetics of patients with the Carney-Stratakis syndrome and germline mutations of the genes coding for the succinate dehydrogenase subunits SDHB, SDHC, and SDHD. Eur J Hum Genet 2008;16(1):79–88.
[65] Janeway MD, Katherine A, Liegl B, Nose V, Hornick JL, Barretina J, et al. Complete loss of succinate dehydrogenase B (SDHB) protein in pediatric gastrointestinal stromal tumors (GIST). In: Annual Meeting, Connective Tissue Oncology Society; 2009 [abstract 39408].
[66] Agaram NP, Wong GC, Guo T, Maki RG, Singer S, Dematteo RP, et al. Novel V600E BRAF mutations in imatinib-naive and imatinib-resistant gastrointestinal stromal tumors. Genes Chromosomes Cancer 2008;47(10):853–9.
[67] Agaimy A, Terracciano LM, Dirnhofer S, Tornillo L, Foerster A, Hartmann A, et al. V600E BRAF mutations are alternative early molecular events in a subset of KIT/PDGFRA wild-type gastrointestinal stromal tumours. J Clin Pathol 2009;62(7):613–6.
[68] Buchdunger E, Cioffi CL, Law N, Stover D, Ohno-Jones S, Druker BJ, et al. Abl protein-tyrosine kinase inhibitor STI571 inhibits in vitro signal transduction mediated by c-kit and platelet-derived growth factor receptors. J Pharmacol Exp Ther 2000;295(1):139–45.
[69] Demetri GD, von Mehren M, Blanke CD, Van den Abbeele AD, Eisenberg B, Roberts PJ, et al. Efficacy and safety of imatinib mesylate in advanced gastrointestinal stromal tumors. N Engl J Med 2002;347(7):472–80.
[70] Joensuu H, Roberts PJ, Sarlomo-Rikala M, Andersson LC, Tervahartiala P, Tuveson D, et al. Effect of the tyrosine kinase inhibitor STI571 in a patient with a metastatic gastrointestinal stromal tumor. N Engl J Med 2001; 344(14):1052–6.
[71] van Oosterom AT, Judson IR, Verweij J, Stroobants S, Dumez H, Donato di Paola E, et al. Update of phase I study of imatinib (STI571) in advanced soft tissue sarcomas and gastrointestinal stromal tumors: a report of the EORTC Soft Tissue and Bone Sarcoma Group. Eur J Cancer 2002;38(Suppl 5):S83–7.
[72] Blanke CD, Rankin C, Demetri GD, Ryan CW, von Mehren M, Benjamin RS, et al. Phase III randomized, intergroup trial assessing imatinib mesylate at two dose levels in patients with unresectable or metastatic gastrointestinal stromal tumors expressing the kit receptor tyrosine kinase: S0033. J Clin Oncol 2008;26(4):626–32.
[73] Verweij J, Casali PG, Zalcberg J, LeCesne A, Reichardt P, Blay JY, et al. Progression-free survival in gastrointestinal stromal tumours with high-dose imatinib: randomised trial. Lancet 2004;364(9440):1127–34.
[74] Gold JS, van der Zwan SM, Gonen M, Maki RG, Singer S, Brennan MF, et al. Outcome of metastatic GIST in the era before tyrosine kinase inhibitors. Ann Surg Oncol 2007;14(1):134–42.
[75] Blanke CD, Demetri GD, von Mehren M, Heinrich MC, Eisenberg B, Fletcher JA, et al. Long-term results from a randomized phase II trial of standard- versus higher-dose imatinib mesylate for patients with unresectable or metastatic gastrointestinal stromal tumors expressing KIT. J Clin Oncol 2008;26(4):620–5.
[76] Debiec-Rychter M, Sciot R, Le Cesne A, Schlemmer M, Hohenberger P, van Oosterom AT, et al. KIT mutations and dose selection for imatinib in patients with advanced gastrointestinal stromal tumours. Eur J Cancer 2006;42(8): 1093–103.
[77] Heinrich MC, Corless CL, Demetri GD, Blanke CD, von Mehren M, Joensuu H, et al. Kinase mutations and imatinib response in patients with metastatic gastrointestinal stromal tumor. J Clin Oncol 2003;21(23):4342–9.
[78] Debiec-Rychter M, Dumez H, Judson I, Wasag B, Verweij J, Brown M, et al. Use of c-KIT/PDGFRA mutational analysis to predict the clinical response to imatinib in patients with advanced gastrointestinal stromal tumours entered on phase I and II studies of the EORTC Soft Tissue and Bone Sarcoma Group. Eur J Cancer 2004;40(5):689–95.
[79] Heinrich MC, Owzar K, Corless CL, Hollis D, Borden EC, Fletcher CD, et al. Correlation of kinase genotype and clinical outcome in the North American Intergroup Phase III Trial of imatinib mesylate for treatment of advanced gastrointestinal stromal tumor: CALGB 150105 Study by Cancer and Leukemia Group B and Southwest Oncology Group. J Clin Oncol 2008;26(33):5360–7.
[80] Gastrointestinal Stromal Tumor Meta-Analysis Group (MetaGIST). Comparison of two doses of imatinib for the treatment of unresectable or metastatic gastrointestinal stromal tumors: a meta-analysis of 1,640 patients. J Clin Oncol 2010;28(7):1247–53.
[81] Corless CL, Schroeder A, Griffith D, Town A, McGreevey L, Harrell P, et al. PDGFRA mutations in gastrointestinal stromal tumors: frequency, spectrum and in vitro sensitivity to imatinib. J Clin Oncol 2005;23(23):5357–64.
[82] Weisberg E, Wright RD, Jiang J, Ray A, Moreno D, Manley PW, et al. Effects of PKC412, nilotinib, and imatinib against GIST-associated PDGFRA mutants with differential imatinib sensitivity. Gastroenterology 2006;131(6):1734–42.
[83] Bauer S, Duensing A, Demetri GD, Fletcher JA. KIT oncogenic signaling mechanisms in imatinib-resistant gastrointestinal stromal tumor: PI3-kinase/AKT is a crucial survival pathway. Oncogene 2007;26(54):7560–8.
[84] Liu Y, Tseng M, Perdreau SA, Rossi F, Antonescu C, Besmer P, et al. Histone H2AX is a mediator of gastrointestinal stromal tumor cell apoptosis following treatment with imatinib mesylate. Cancer Res 2007;67(6):2685–92.
[85] Rogakou EP, Pilch DR, Orr AH, Ivanova VS, Bonner WM. DNA double-stranded breaks induce histone H2AX phosphorylation on serine 139. J Biol Chem 1998;273(10):5858–68.
[86] Rossi F, Ehlers I, Agosti V, Socci ND, Viale A, Sommer G, et al. Oncogenic Kit signaling and therapeutic intervention in a mouse model of gastrointestinal stromal tumor. Proc Natl Acad Sci USA 2006.
[87] Tarn C, Skorobogatko YV, Taguchi T, Eisenberg B, von Mehren M, Godwin AK. Therapeutic effect of imatinib in gastrointestinal stromal tumors: AKT signaling dependent and independent mechanisms. Cancer Res 2006;66(10): 5477–86.
[88] Kuroda J, Puthalakath H, Cragg MS, Kelly PN, Bouillet P, Huang DC, et al. Bim and Bad mediate imatinib-induced killing of Bcr/Abl+ leukemic cells, and resistance due to their loss is overcome by a BH3 mimetic. Proc Natl Acad Sci USA 2006;103(40):14907–12.
[89] Ito T, Tanaka H, Kimura A. Establishment and characterization of a novel imatinib-sensitive chronic myeloid leukemia cell line MYL, and an imatinib-resistant subline MYL-R showing overexpression of Lyn. Eur J Haematol 2007;78(5):417–31.
[90] Gordon PM, Fisher DE. Role for the pro-apoptotic factor BIM in mediating imatinib-induced apoptosis in a c-KIT dependent gastrointestinal stromal tumor cell line. J Biol Chem 2010; [Epub ahead of print].
[91] Holtz MS, Forman SJ, Bhatia R. Nonproliferating CML CD34+ progenitors are resistant to apoptosis induced by a wide range of proapoptotic stimuli. Leukemia 2005;19(6):1034–41.
[92] Liu Y, Perdreau SA, Chatterjee P, Wang L, Kuan SF, Duensing A. Imatinib mesylate induces quiescence in gastrointestinal stromal tumor cells through the CDH1-SKP2-p27Kip1 signaling axis. Cancer Res 2008;68(21):9015–23.
[93] Parry JA, Brown MF, Seneviratne DS, Duensing S, Duensing A. Imatinib mesylate modulates regulators of tumor cell quiescence in gastrointestinal stromal tumor cells. In: Annual Meeting, Connective Tissue Oncology Society; 2009 [abstract 39394].
[94] Blay JY, Le Cesne A, Ray-Coquard I, Bui B, Duffaud F, Delbaldo C, et al. Prospective multicentric randomized phase III study of imatinib in patients with advanced gastrointestinal stromal tumors comparing interruption versus continuation of treatment beyond 1 year: the French Sarcoma Group. J Clin Oncol 2007;25(9):1107–13.
[95] Milojkovic D, Apperley J. Mechanisms of resistance to imatinib and second-generation tyrosine inhibitors in chronic myeloid leukemia. Clin Cancer Res 2009;15(24):7519–27.
[96] Sakamoto K, Sakurai S, Kanda T, Sakuma Y, Hishima T, Hironaka M et al. Pleomorphic phenotypes of gastrointestinal stromal tumors at metastatic sites with or without imatinib treatment. Cancer Sci 2010; [Epub ahead of print].
[97] Barnes DJ, Melo JV. Primitive, quiescent and difficult to kill: the role of non-proliferating stem cells in chronic myeloid leukemia. Cell Cycle 2006;5(24):2862–6.
[98] Holyoake T, Jiang X, Eaves C, Eaves A. Isolation of a highly quiescent subpopulation of primitive leukemic cells in chronic myeloid leukemia. Blood 1999;94(6):2056–64.
[99] Graham SM, Jorgensen HG, Allan E, Pearson C, Alcorn MJ, Richmond L, et al. Primitive, quiescent, Philadelphia-positive stem cells from patients with chronic myeloid leukemia are insensitive to STI571 in vitro. Blood 2002;99(1):319–25.
[100] Holtz MS, Slovak ML, Zhang F, Sawyers CL, Forman SJ, Bhatia R. Imatinib mesylate (STI571) inhibits growth of primitive malignant progenitors in chronic myelogenous leukemia through reversal of abnormally increased proliferation. Blood 2002;99(10):3792–800.
[101] Goto T, Nishikori M, Arlin Z, Gee T, Kempin S, Burchenal J, et al. Growth characteristics of leukemic and normal hematopoietic cells in Ph' + chronic myelogenous leukemia and effects of intensive treatment. Blood 1982;59(4): 793–808.
[102] La Rosee P, Shen L, Stoffregen EP, Deininger M, Druker BJ. No correlation between the proliferative status of Bcr-Abl positive cell lines and the proapoptotic activity of imatinib mesylate (Gleevec/Glivec). Hematol J 2003;4(6):413–9.
[103] Wang E, Casciano CN, Clement RP, Johnson WW. The farnesyl protein transferase inhibitor SCH66336 is a potent inhibitor of MDR1 product P-glycoprotein. Cancer Res 2001;61(20):7525–9.
[104] Jordanides NE, Jorgensen HG, Holyoake TL, Mountford JC. Functional ABCG2 is overexpressed on primary CML CD34+ cells and is inhibited by imatinib mesylate. Blood 2006;108(4):1370–3.
[105] Goldman J, Gordon M. Why do chronic myelogenous leukemia stem cells survive allogeneic stem cell transplantation or imatinib: does it really matter? Leuk Lymphoma 2006;47(1):1–7.
[106] Chu S, Xu H, Shah NP, Snyder DS, Forman SJ, Sawyers CL, et al. Detection of BCR-ABL kinase mutations in CD34+ cells from chronic myelogenous leuke-

mia patients in complete cytogenetic remission on imatinib mesylate treatment. Blood 2005;105(5):2093–8.
[107] Branford S, Rudzki Z, Walsh S, Parkinson I, Grigg A, Szer J, et al. Detection of BCR-ABL mutations in patients with CML treated with imatinib is virtually always accompanied by clinical resistance, and mutations in the ATP phosphate-binding loop (P-loop) are associated with a poor prognosis. Blood 2003;102(1):276–83.
[108] Jorgensen HG, Copland M, Allan EK, Jiang X, Eaves A, Eaves C, et al. Intermittent exposure of primitive quiescent chronic myeloid leukemia cells to granulocyte-colony stimulating factor in vitro promotes their elimination by imatinib mesylate. Clin Cancer Res 2006;12(2):626–33.
[109] Papaetis GS, Syrigos KN. Targeted therapy for gastrointestinal stromal tumors: current status and future perspectives. Cancer Metastasis Rev;29(1):151–70.
[110] Van den Abbeele AD. The lessons of GIST–PET and PET/CT: a new paradigm for imaging. Oncologist 2008;13(Suppl 2):8–13.
[111] Debiec-Rychter M, Cools J, Dumez H, Sciot R, Stul M, Mentens N, et al. Mechanisms of resistance to imatinib mesylate in gastrointestinal stromal tumors and activity of the PKC412 inhibitor against imatinib-resistant mutants. Gastroenterology 2005;128(2):270–9.
[112] Antonescu CR, Besmer P, Guo T, Arkun K, Hom G, Koryotowski B, et al. Acquired resistance to imatinib in gastrointestinal stromal tumor occurs through secondary gene mutation. Clin Cancer Res 2005;11(11):4182–90.
[113] Chen LL, Trent JC, Wu EF, Fuller GN, Ramdas L, Zhang W, et al. A missense mutation in KIT kinase domain 1 correlates with imatinib resistance in gastrointestinal stromal tumors. Cancer Res 2004;64(17):5913–9.
[114] Tamborini E, Pricl S, Negri T, Lagonigro MS, Miselli F, Greco A, et al. Functional analyses and molecular modeling of two c-Kit mutations responsible for imatinib secondary resistance in GIST patients. Oncogene 2006;25(45):6140–6.
[115] Wardelmann E, Thomas N, Merkelbach-Bruse S, Pauls K, Speidel N, Buttner R, et al. Acquired resistance to imatinib in gastrointestinal stromal tumours caused by multiple KIT mutations. Lancet Oncol 2005;6(4):249–51.
[116] Heinrich MC, Maki RG, Corless CL, Antonescu CR, Harlow A, Griffith D, et al. Primary and secondary kinase genotypes correlate with the biological and clinical activity of sunitinib in imatinib-resistant gastrointestinal stromal tumor. J Clin Oncol 2008;26(33):5352–9.
[117] Negri T, Pavan GM, Virdis E, Greco A, Fermeglia M, Sandri M, et al. T670X KIT mutations in gastrointestinal stromal tumors: making sense of missense. J Natl Cancer Inst 2009;101(3):194–204.
[118] Roberts KG, Odell AF, Byrnes EM, Baleato RM, Griffith R, Lyons AB, et al. Resistance to c-KIT kinase inhibitors conferred by V654A mutation. Mol Cancer Ther 2007;6(3):1159–66.
[119] Foster R, Griffith R, Ferrao P, Ashman L. Molecular basis of the constitutive activity and STI571 resistance of Asp816Val mutant KIT receptor tyrosine kinase. J Mol Graph Model 2004;23(2):139–52.
[120] Gajiwala KS, Wu JC, Christensen J, Deshmukh GD, Diehl W, DiNitto JP, et al. KIT kinase mutants show unique mechanisms of drug resistance to imatinib and sunitinib in gastrointestinal stromal tumor patients. Proc Natl Acad Sci USA 2009;106(5):1542–7.
[121] Lim KH, Huang MJ, Chen LT, Wang TE, Liu CL, Chang CS, et al. Molecular analysis of secondary kinase mutations in imatinib-resistant gastrointestinal stromal tumors. Med Oncol 2008;25(2):207–13.
[122] Liegl B, Kepten I, Le C, Zhu M, Demetri GD, Heinrich MC, et al. Heterogeneity of kinase inhibitor resistance mechanisms in GIST. J Pathol 2008;216(1):64–74.
[123] Miselli FC, Casieri P, Negri T, Orsenigo M, Lagonigro MS, Gronchi A, et al. c-Kit/PDGFRA gene status alterations possibly related to primary imatinib resistance in gastrointestinal stromal tumors. Clin Cancer Res 2007;13(8):2369–77.
[124] Heinrich MC, Corless CL, Blanke CD, Demetri GD, Joensuu H, Roberts PJ, et al. Molecular correlates of imatinib resistance in gastrointestinal stromal tumors. J Clin Oncol 2006;24(29):4764–74.
[125] Judson I, Ma P, Peng B, Verweij J, Racine A, di Paola ED, et al. Imatinib pharmacokinetics in patients with gastrointestinal stromal tumour: a retrospective population pharmacokinetic study over time. EORTC Soft Tissue and Bone Sarcoma Group. Cancer Chemother Pharmacol 2005;55(4):379–86.
[126] Delbaldo C, Chatelut E, Re M, Deroussent A, Seronie-Vivien S, Jambu A, et al. Pharmacokinetic-pharmacodynamic relationships of imatinib and its main metabolite in patients with advanced gastrointestinal stromal tumors. Clin Cancer Res 2006;12(20 Pt 1):6073–8.
[127] Rock EP, Goodman V, Jiang JX, Mahjoob K, Verbois SL, Morse D, et al. Food and drug administration drug approval summary: sunitinib malate for the treatment of gastrointestinal stromal tumor and advanced renal cell carcinoma. Oncologist 2007;12(1):107–13.
[128] Goodman VL, Rock EP, Dagher R, Ramchandani RP, Abraham S, Gobburu JV, et al. Approval summary: sunitinib for the treatment of imatinib refractory or intolerant gastrointestinal stromal tumors and advanced renal cell carcinoma. Clin Cancer Res 2007;13(5):1367–73.
[129] Faivre S, Delbaldo C, Vera K, Robert C, Lozahic S, Lassau N, et al. Safety, pharmacokinetic, and antitumor activity of SU11248, a novel oral multitarget tyrosine kinase inhibitor, in patients with cancer. J Clin Oncol 2006;24(1):25–35.
[130] Demetri GD, van Oosterom AT, Garrett CR, Blackstein ME, Shah MH, Verweij J, et al. Efficacy and safety of sunitinib in patients with advanced gastrointestinal stromal tumour after failure of imatinib: a randomised controlled trial. Lancet 2006;368(9544):1329–38.
[131] Liegl B, Fletcher JA, Corless CL, Fletcher CD, Raut CP, Donsky R, et al. Correlation between KIT mutations and sunitinib (SU) resistance in GIST. In: ASCO Gastrointestinal Cancers Symposium; 2008 [abstract 92].
[132] Prenen H, Guetens G, de Boeck G, Debiec-Rychter M, Manley P, Schoffski P, et al. Cellular uptake of the tyrosine kinase inhibitors imatinib and AMN107 in gastrointestinal stromal tumor cell lines. Pharmacology 2006;77(1):11–6.
[133] Montemurro M, Schoffski P, Reichardt P, Gelderblom H, Schutte J, Hartmann JT, et al. Nilotinib in the treatment of advanced gastrointestinal stromal tumours resistant to both imatinib and sunitinib. Eur J Cancer 2009;45(13):2293–7.
[134] Wiebe LKK, Maki RG, D'Adamo DR, Chow WA, Wade JL, Agamah E, et al. Activity of sorafenib (SOR) in patients (pts) with imatinib (IM) and sunitinib (SU)-resistant (RES) gastrointestinal stromal tumors (GIST): a phase II trial of the University of Chicago phase II consortium. J Clin Oncol 2008;26:10502.
[135] Dewaele B, Wasag B, Cools J, Sciot R, Prenen H, Vandenberghe P, 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 PDGFRAD842V mutation. Clin Cancer Res 2008;14(18):5749–58.
[136] Bauer S, Yu LK, Demetri GD, Fletcher JA. Heat shock protein 90 inhibition in imatinib-resistant gastrointestinal stromal tumor. Cancer Res 2006;66(18):9153–61.
[137] Fumo G, Akin C, Metcalfe DD, Neckers L. 17-Allylamino-17-demethoxygeldanamycin (17-AAG) is effective in down-regulating mutated, constitutively activated KIT protein in human mast cells. Blood 2004;103(3):1078–84.
[138] Demetri GD, Le Censne A, von Mehren M, Chmielowski B, Bauer S, Chow WA, et al. Rodenas E. Final results from a phase III study of IPI-504 (retaspimycin hydrochloride) versus placebo in patients (pts) with gastrointestinal stromal tumors (GIST) following failure of kinase inhibitor therapies. In: ASCO Gastrointestinal Cancers Symposium; 2010 [abstract 64].
[139] Gunaratnam M, Swank S, Haider SM, Galesa K, Reszka AP, Beltran M, et al. Targeting human gastrointestinal stromal tumor cells with a quadruplex-binding small molecule. J Med Chem 2009;52(12):3774–83.
[140] Michor F, Hughes TP, Iwasa Y, Branford S, Shah NP, Sawyers CL, et al. Dynamics of chronic myeloid leukaemia. Nature 2005;435(7046):1267–70.
[141] Desai J, Shankar S, Heinrich MC, Fletcher JA, Fletcher CD, Manola J, et al. Clonal evolution of resistance to imatinib in patients with metastatic gastrointestinal stromal tumors. Clin Cancer Res 2007;13(18 Pt 1):5398–405.
[142] Wardelmann E, Merkelbach-Bruse S, Pauls K, Thomas N, Schildhaus HU, Heinicke T, et al. Polyclonal evolution of multiple secondary KIT mutations in gastrointestinal stromal tumors under treatment with imatinib mesylate. Clin Cancer Res 2006;12(6):1743–9.
[143] Andersson J, Sihto H, Meis-Kindblom JM, Joensuu H, Nupponen N, Kindblom LG. NF1-associated gastrointestinal stromal tumors have unique clinical. phenotypic, and genotypic characteristics. Am J Surg Pathol 2005;29(9):1170–6.
[144] Maertens O, Prenen H, Debiec-Rychter M, Wozniak A, Sciot R, Pauwels P, et al. Molecular pathogenesis of multiple gastrointestinal stromal tumors in NF1 patients. Hum Mol Genet 2006;15(6):1015–23.
[145] Miettinen M, Fetsch JF, Sobin LH, Lasota J. Gastrointestinal stromal tumors in patients with neurofibromatosis 1: a clinicopathologic and molecular genetic study of 45 cases. Am J Surg Pathol 2006;30(1):90–6.
[146] Muhlenberg T, Zhang Y, Wagner AJ, Grabellus F, Bradner J, Taeger G, et al. Inhibitors of deacetylases suppress oncogenic KIT signaling, acetylate HSP90, and induce apoptosis in gastrointestinal stromal tumors. Cancer Res 2009;69(17):6941–50.
[147] Kovacs JJ, Murphy PJ, Gaillard S, Zhao X, Wu JT, Nicchitta CV, et al. HDAC6 regulates Hsp90 acetylation and chaperone-dependent activation of glucocorticoid receptor. Mol Cell 2005;18(5):601–7.
[148] Bali P, Pranpat M, Bradner J, Balasis M, Fiskus W, Guo F, et al. Inhibition of histone deacetylase 6 acetylates and disrupts the chaperone function of heat shock protein 90: a novel basis for antileukemia activity of histone deacetylase inhibitors. J Biol Chem 2005;280(29):26729–34.
[149] Floris G, Debiec-Rychter M, Sciot R, Stefan C, Fieuws S, Machiels K, et al. High efficacy of panobinostat towards human gastrointestinal stromal tumors in a xenograft mouse model. Clin Cancer Res 2009;15(12):4066–76.
[150] Bauer S, Parry JA, Muhlenberg T, Brown MF, Seneviratne D, Chatterjee P et al. Proapoptotic activity of bortezomib in gastrointestinal stromal tumor cells. Cancer Res 2010;70(1):150–9.