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SUPPLEMENT (SPECIAL ISSUE)

Molecular basis for primary and secondary tyrosine kinase inhibitor resistance in gastrointestinal stromal tumor

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Abstract Small molecule kinase inhibitors have irrevocably altered cancer treatment. March 2010 marks the 10th anniversary of using imatinib in gastrointestinal stromal tumors (GIST), a cardinal example of the utility of such targeted therapy in a solid tumor. Before imatinib, metastatic GIST was frustrating to treat due to its resistance to standard cytotoxic chemotherapy. Median survival for patients with metastatic GIST improved from 19 to 60 months with imatinib. In treating patients with GIST, two patterns of tyrosine kinase inhibitor resistance have been observed. In the first, ~9–14% of patients have progression within 3 months of starting imatinib. These patients are classified as having primary or early resistance. Median progression-free survival (PFS) on imatinib is approximately 24 months; patients with later progression are classified as having secondary or acquired resistance. Primary studies and a meta-analysis of studies of imatinib in GIST patients have identified prognostic features that contribute to treatment failure. One of the strongest predictors for success of therapy is *KIT* or *PDGFRA* mutational status. Patients with *KIT* exon 11 mutant GIST have better response rates, PFS, and overall survival compared to other mutations. A great deal has been learned in the last decade about sensitivity and resistance of GIST to imatinib; however, many unanswered questions remain about secondary resistance mechanisms and clinical management in the third- and fourth-line setting. This review will discuss the role of dose effects, and early and late resistance to imatinib and their clinical implications. Patients intolerant to imatinib (5%) and those who progress on imatinib are treated with sunitinib. The mechanism of resistance to sunitinib is unknown at this time but is also appears related to growth of clones with secondary mutations in *KIT*. Third- and fourth-line treatments of GIST and with future treatment strategies are also discussed.

Keywords GIST · Primary tyrosine kinase inhibitor resistance · Secondary tyrosine kinase inhibitor resistance · Nilotinib

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Introduction

While GISTs are found associated with the GI tract from esophagus to anus, and rarely in the peritoneum free of the bowel wall, they are not found in other parts of the body, indicating a unique cell giving rise to this disease. GISTs appear to arise from interstitial cells of Cajal (ICC) or their precursors. ICCs are the neural pacemaker cells responsible for gut peristalsis [1, 2]. The developmental origin of both ICC and GIST has been greatly debated among pathologists since the beginning of the twentieth century. ICC cells have been variably thought to be of neural, muscle, or fibroblastic origin. Similarly, GIST was thought to be a smooth muscle neoplasm; however, variable and inconsistent IHC staining for smooth muscle markers (desmin, actin, myosin) along with a subtype of GIST that stains for neural markers (S-100, neuron-specific enolase) casts doubt about its smooth muscle origins [3]. ICC cells were shown to have neurotransmitting properties along with evidence that they arise from ventral neural tube that proved their neural origin [3]. In 1995, Huizinga et al. reported that a knockout mice model of *KIT* failed to express ICC cells, leading to hypothesize that *KIT* was essential for the development of ICC cells [4, 5]. Prior mouse models had shown that *KIT* knockout mice were deficient in hematopoiesis and in melanocyte and germ cell

development. *KIT* encodes a 145-kilodalton (kDA) receptor tyrosine kinase and is the normal cellular homolog of *v-KIT*, a viral oncogene found in the genome of the Hardy-Zuckermann 4 feline sarcoma virus [6, 7]. Coincidentally, an antibody against KIT (also termed CD117) was shown to be a sensitive and specific marker for GIST. CD117 is positive in 95% of GIST specimens and is an important component in diagnosing GIST [8–10]. It is noteworthy that ~5% of patients with GIST do not express KIT [1, 11]. A seminal paper by Hirota et al. reported that GISTs exhibit a gain-of-function mutation in *KIT* exon 11, which resulted in growth advantage by constitutive, ligand-independent activation of the receptor tyrosine kinase [12]. Importantly, *KIT* has been shown to be the key oncogenic driver in GIST that is essential for growth and survival [13]. Chromosomal abnormalities tend to accumulate with increasing tumor grade: simple GISTs exhibit normal karyotype, while aggressive ones have sequential accumulations of chromosomal aberrations. About two-thirds of GISTs exhibit monosomy 14 or partial loss of 14q, and 50% have loss of the long arm of chromosome 22 [14, 15]. Aberrations in chromosome 14 or 22 are associated with borderline malignant disease. An aggressive biology is noted with loss of chromosome 1p, 9p (spanning *CDKN2A* or *p16INK4A*), and 11p regions. Gain in chromosomal segments is noted in 8q and 17q regions which is associated with metastatic potential. The contribution of these chromosomal abnormalities toward tumorigenesis remains unknown [15, 16].

Initially, GISTs without any evidence of *KIT* mutations were classified as "wild type" (WT). However, in 2003, Heinrich and colleagues analyzed WT GIST and reported novel mutations in the alpha chain of the platelet-derived growth factor receptor (*PDGFRA*) that resulted in constitutive, PDGF-independent activation of downstream STAT3 and PI3 K pathways [17]. Currently, *PDGFRA* mutations account for 5–10% of known mutations in GIST; two large phase III trials reported only 1.3–2.9% *PDGFRA* mutations. Approximately, 9–15% of all GISTs do not exhibit mutations in either *KIT* or *PDGFRA* and are termed "wild type" (WT) [18].

GISTs arise from interstitial cells of Cajal (ICC),

KIT is essential for the development and function of hematopoietic cells, ICC, melanocytes, and germ cells.

KIT (also termed CD117) is structurally related to PDGFRA and encodes a 145-kDA RTK.

95% of GIST tumors express KIT (or CD117).

KIT mutations are noted in approximately 85% of GIST. PDGFRA accounts for 5–10%, and remaining 10–15% have no mutations (WT).

KIT and PDGF tyrosine kinase receptors

The proto-oncogene *KIT* encodes a 145-kDa transmembrane type III receptor tyrosine kinase that is structurally related to the platelet-derived growth factor receptor, colony-stimulating factor 1 receptor (CSF1R), kinase insert domain receptor (KDR or VEGFR-2), and the Fms-like tyrosine kinase receptor (FLT3). The sequence and functional similarities between the human protein kinases superfamily have been well described [19]. A modified kinase dendrogram adapted from Manning et. al. depicts the relationships between KIT and other closely related receptor tyrosine (Fig. 1, Reprinted with permission from AAAS). *KIT* and *PDGFRA*, both located on chromosome 4q12, have well-defined role in the oncogenic pathway regulating GIST [1, 20, 21]. The KIT transmembrane receptor is composed of an extracellular, immunoglobulin-like motif (IGM), a transmembrane hinge, a juxta-membrane domain (JM), and an intracellular domain with two tyrosine kinase domains.

The tyrosine kinase domain consists of two regions separated by a kinase insert domain (KID) that has an undefined function (Fig. 2). The proximal kinase domain functions as an ATP-binding region, and the distal region contains the

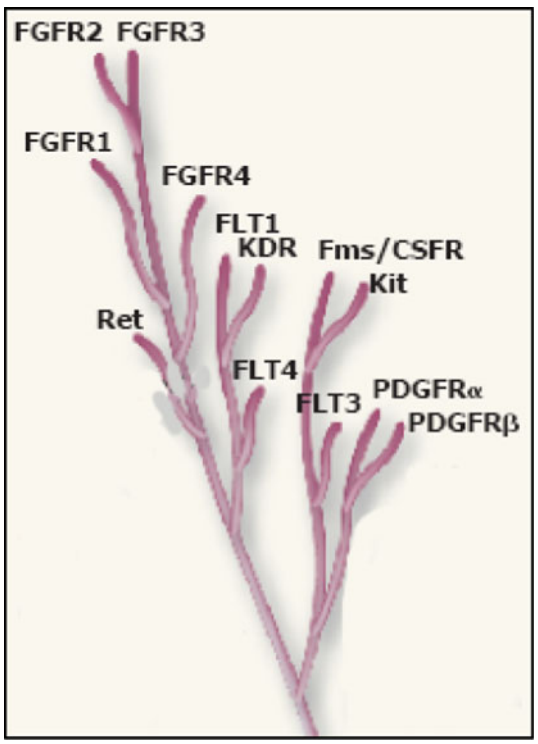

Fig. 1 Relatedness of catalytic sequence and functional similarities of KIT to other receptor tyrosine kinases. The distance between kinases is proportional to their similarity

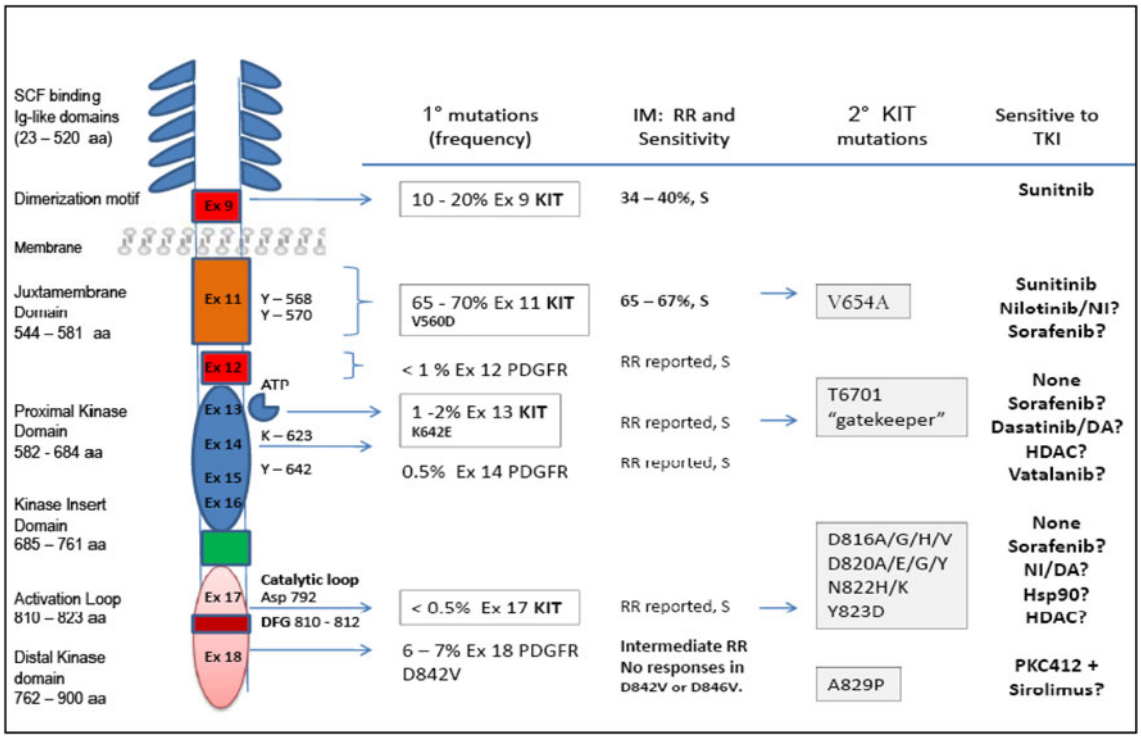

Fig. 2 Structure and mutations of KIT or PDGF receptor with TKI sensitivity. Schematic representation of KIT molecule along with primary and secondary mutations, frequency of mutations, and response to TKI

activation loop (AL) that stabilizes the activated receptor [22, 23]. The ligand stem cell factor (SCF) is a soluble transmembrane protein which upon interaction with KIT induces receptor homo-dimerization, kinase activation, and phosphorylation of tyrosine residues which share a Src homology motif. Primary mutations in *KIT/PDGFRA* result in constitutive, ligand (SCF)-independent activation that results in the activation of downstream pathways Ras/MAPK, JAK/STAT3, and PI3 K/Akt, of which the latter two are thought to play an important role in cell proliferation and inhibition of apoptosis [13, 24–26] (Fig. 3).

An important observation is that immunohistochemical detection of KIT on cell surface is independent of underlying mutations in *KIT*. In about 90% of GISTs, the underlying genetic change is a gain-of-function mutation in *KIT* (85%) or *PDGFRA* (5%) that leads to constitutive activation. The remaining GISTs lack mutations in *KIT* or *PDGFRA* and therefore termed WT (WT) [1]. In addition, mutant KIT proteins are not expressed on the cell surface and instead retained in intracellular endoplasmic compartment. The vast majority of *KIT* mutations are found in exon 11 coding for JM (66–71%), exon 9 coding for extracellular IGM (13%), exon 13 coding proximal KD (1–3%), and exon 17 coding for distal KD and activation loop (1–3%). PDGFR mutations include 5–7% of GISTs mostly affecting exon 18 (5–6%) and followed by exon 12 (1–1.5%) and exon 14 (0.3%). In two large phase III trials, the distribution of mutations was slightly different: KIT exon 11 (66 vs. 77%), exon 9 (8 vs. 16%), PDGFR (1.3 vs. 2.9%), and WT (17 vs. 13%) [27].

Gain-of-function mutations in KIT involve exon 11 (70%), exon 9 (approx 15%), exon 13 (2%), and exon 17 (1%). PDGFR mutations involve exon 18 (5%), exon 12 (1%), and exon 14 (<0.5%).

Imatinib is a competitive inhibitor for the ATP-binding domain. Imatinib binds the inactivated form of KIT and prevents conformational shift to the active form.

Primary and secondary resistance to imatinib results in a conformational shift in the kinase domain of KIT that favors the activated state.

Imatinib mesylate (STI571, Gleevec, Glivec; Novartis Pharma, Basel, Switzerland) is a potent inhibitor of the ATP-binding kinase domain of BCR-Abl, a fusion gene product that confers a proliferative drive in chronic myelogenous leukemia [28, 29]. Imatinib received FDA approval in 2001 for treatment of CML. As early as 1999, in vitro studies showed that imatinib also inhibited KIT kinase activity in both WT and KIT GIST cell lines

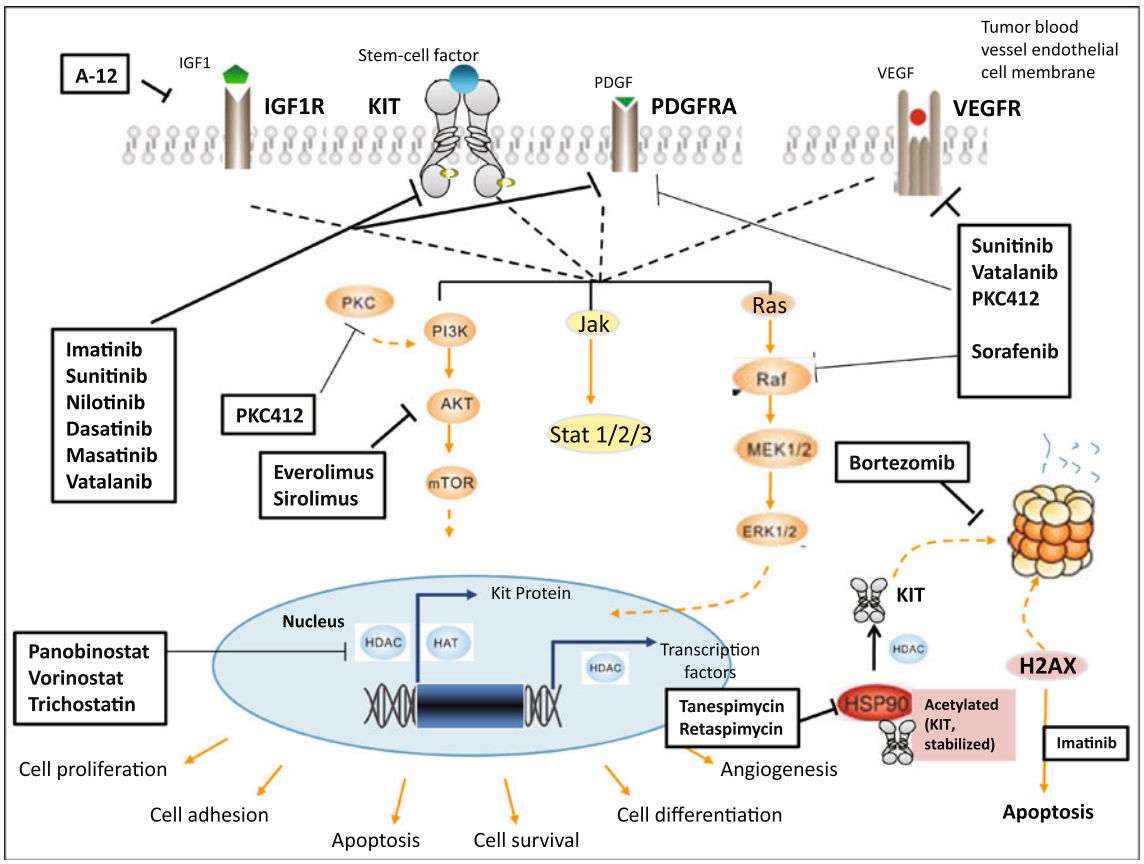

Fig. 3 Schema of some signaling pathways in GIST and potential targets for inhibition. *IGF1R* insulin-like growth factor 1 receptor, *MAPK* mitogen-activated protein kinase, *Erk1/2* extra-cellular-signal-related kinases, *PKC* protein kinase C, *PI3K* phophotidylinositol 3 kinase, *mTOR* mammalian target of rapamycin, *Jak* Janus kinase, *STAT* signal transducers and activators of transcription, *HDAC* histone deacetylase, *Hsp 90* heat-shock protein

resulting in growth arrest and apoptosis, foreshadowing the clinical trials that were to rapidly follow [30]. Crystal structures show that imatinib is a competitive inhibitor for ATP at the kinase-binding domain. The three-dimensional structural knowledge is essential not only for understanding the mechanisms of primary and secondary mutations but also to develop the next-generation drugs to overcome resistance [31].

KIT exists in equilibrium between activated and inactivated form (Fig. 3). The three-dimensional structure of KIT is similar to most that of RTKs. It exists as a bilobed structure composed of an extracellular (N lobe) and an intracellular portion (C lobe). The extracellular portion and the transmembrane hinge are responsible for ligand binding and trans-phosphorylation. The intracellular C lobe is responsible for ATP catalysis and downstream signaling. The intracellular domain consists of a juxta-membrane domain (JM) and two kinase domains (KD), proximal and distal portions that are split by a kinase insertion domain (KID). The proximal kinase domain is involved in anchoring and orienting ATP. The distal kinase domain is responsible for binding and phosphorylating downstream substrates. The distal kinase domain also contains an activation loop (AL) that stabilizes the activated KIT receptor. The three-dimensional space created by the proximal and distal kinase is the catalytic domain of the kinase. This is a dynamic space that when "open/on" allows ATP to bind and its "closed/off" conformation allows substrates to be phosphorylated. The catalytic cleft of the receptor is formed when amino acid residues found on three separate (KDD: Lysine 623, Aspartate792, Aspartate 810) but highly conserved regions of the protein come together in a three-dimensional configuration. Lysine 623 found on the proximal kinase domain forms salt bridges with ATP. Aspartate 792 is part of a catalytic loop (different from the catalytic domain KDD) that is highly conserved (HR**D**LAARN) in all RTK [32, 33]. Aspartate 810 is also part of the highly conserved **D**FG motif (Aspartate810-Phenylalanine811-Glycine812) found at the start of all RTK activation loops (AL) [3, 22, 23, 31, 34].

In the unbound state, the juxta-membrane (JM) domain prevents dimerization by creating steric hinderance at the ATP-binding site. Ligand (SCF) binding causes receptor homo-dimerization and trans-phosphorylation of two tyrosine residues in JM (Y568 and Y570). This leads to three important events: (1) a steric shift that allows ATP and other substrates to bind to the ATP-binding domain, (2) release of the activation loop (AL), and (3) phosphorylation of Tyr823 in AL that stabilizes the KIT receptor in an active form.

Imatinib binds the ATP-binding domain in the inactive or unbound state of the KIT receptor. Imatinib inserts itself into the dynamic cleft formed by the proximal and distal kinase domains and binds to the amino acids of the DFG motif (D810 and F811), the hinge region (C673), and the proximal kinase domain (Glu640). Imatinib thus prevents the conformational shift to the active form. However, a secondary exon 11 mutation (V654A) in JM results in ligand-independent dimerization, loss of auto-inhibitory function, and concomitant release of the activation loop. Exon 9 mutations affect the extracellular domain which disrupts an inhibitory dimerization motif. Mutations in *KIT* exon 13 or 17 result in a conformational shift where activity of the KIT receptor is no longer dependent on the two kinase domains (KD).

Clinical activity

Based on these and other pre-clinical data indicating the activity of imatinib in a GIST cell line, imatinib was tested in a compassionate use setting in a 50-year-old woman with widely metastatic GIST who was refractory to surgery, several lines of cytotoxic therapies, thalidomide, and interferon alpha [30, 35]. This patient had a dramatic PET response and myxoid degeneration of the tumor within 1 month of starting treatment. A mixed response (CR and PR) was seen in several metastatic lesions at the 8-month follow-up [35].

Phase I imatinib

The remarkable findings in the index patient prompted an EORTC phase I trial with 36 GIST patients with varying doses and schedule of imatinib. Dose-limiting toxicity was observed at 500 mg twice daily, and the MTD was established as 400 mg twice daily. Inhibition of tumor growth was seen in 32 of 36 patients, and there were 19 RECIST partial responses, 6 patients with unconfirmed RECIST PR, 7 patients with stable disease, and 4 with progressive disease [36, 37].

Phase II imatinib

A follow-up phase II study by EORTC that enrolled 27 GIST patients on 400 mg twice daily of imatinib showed 4% complete remission (CR), 67% PR, 19% SD, and 11% PD. A US-Finnish phase II study evaluated 147 patients who were randomly assigned to 400 mg or 600 mg of Imatinib daily [38]. Patients who progressed on 400 mg daily were assigned to the 600-mg cohort. In this study, 54% had PR, 28% with SD, and 13% with PD. Of the nine patients who had progressive disease on the lower dose and underwent crossover, three patients had PR or SD. The duration of response and stable disease lasted more than 6 months at the time of initial publication. A recent update on this trial reported long-term follow-up of up to 71 months [39]. Objective response rates were higher at longer follow-up (68%), and CR was noted in 2 patients who crossed over to the higher drug arm. The median OS was 57 months (19 months in historical controls). The study was not powered to compare the two arms, but no difference in response rates, median progression-free survival, and overall survival was noted between the two arms [39]. Based on these pivotal phase II studies, imatinib gained FDA approval in 2002 for metastatic or unresectable GIST [40].

Phase III randomized studies

Following this, two large randomized phase III trials were initiated to compare the efficacy of imatinib at 400 mg and 800 mg daily in metastatic or unresectable GIST [41, 42]. The trial design was intentionally similar in order to combine the results in a meta-analysis. The SWOG/CALGB/NCI-sponsored trial (S0033 or US-CDN) measured OS as the primary endpoint, while the EORTC/ISG/AGITG trial (62,005) measured progression-free survival. The two trials were identical in their outcome. The S0033 trial randomized 746 patients to standard-dose or high-dose imatinib and showed that both arms had identical response rates and overall survival. Patients who progressed on 400 mg of imatinib were allowed to crossover to the higher dose arm, and this resulted in 31% response rates (SD and few PR). In the EORTC study, 946 patients were similarly randomized to 400 mg or 800 mg daily of imatinib. Again, there was no difference between response rates or overall survival between the two arms. At initial analysis, there was a small, but significant, improvement in median PFS at 25 months in the higher dose arm, but this diminished with a median follow-up of 40 months. Similarly, patients who received high dose on progression had a 29% response rate (PR + SD) [41–43].

Summary of clinical trials in imatinib	
Phase I	36 patients with 19/36 (53%) with PR and 6/36 (17%) with minor response. MTD was established as 400 mg twice daily.
Phase II	147 patients randomized to 400 mg or 600 mg IM daily and noted 67% PR and 17% with minor response and SD. No difference in outcomes between the two dose levels. In EORTC study, 27 GIST patients on 800 mg daily and noted to have 4% CR, 67% PR, and 18% minor response and SD.
Phase III	EORTC study randomized 946 patients to 400 mg or 800 mg daily IM and noted 5% CR, 45% PR, and 32% with minor response and SD. US study randomized 746 patients to 400 mg or 800 mg IM and noted 2% CR, 46% PR, and 26% with minor response and SD. Both trials allowed crossover to higher dose at progression. No difference in RR or OS in any of the arms. PFS slightly longer in the higher dose EORTC study.

Genotype and clinical response to imatinib

The first definitive evidence linking *KIT* or *PDGFRA* mutations with response to imatinib was demonstrated in the US-Finnish phase II study where 127 of 147 patient tumor specimens were analyzed to correlate with clinical response [44]. This revealed activating mutations in 112 (88%) KIT, 6 (5%) PDGFRA mutations, and 9 (7%) with no mutations in KIT or PDGFR (WT). *KIT* mutations included 85 exon 11 mutations with 71 in-frame deletions (del557–558) and 14 point mutations (V560G). The remaining *KIT* mutations included exon 9 mutations ($n = 22$) involving in-frame duplications and insertions at codon 502, exon 13 ($n = 2$, K642E), and exon 17 ($n = 2$, N822H, N822K). PDGFRA mutations included point mutations in the JM domain ($n = 1$, V561D) and point mutations ($n = 3$, D842V) and deletions ($n = 2$, DIMH842–845 and I843) in the activation loop. In vitro kinase assays with imatinib showed nanomolar inhibition in all KIT and PDGFRA mutants when compared to ligand-activated wild-type KIT. The only exception was the PDGFR D842 V mutation, which was insensitive to IM [44].

An early analysis of the *US-Finnish phase II study* showed a statistically significant correlation between radiographic response (PR) and mutational status: *KIT* exon 11 (83%), *KIT* exon 9 (48%), *PDGFRA*-sensitive (67%), exon 13 (100%), exon 17 (50%), *PDGFRA* resistant (0%, D842V), and WT (0%). Subgroup analysis showed significantly longer event-free survival in patients with *KIT* exon 11 (57.2 months), exon 9 (16.6 mo), and wild type (6.8 mo). Overall survival also correlated with *KIT* exon 11 patients having a significantly better outcome than exon 9 or WT mutations. The most recent update from this phase II study confirmed earlier finding that kinase genotype is predictive for both response rates and survival. Response rates and median OS were 86% and 63 months with *KIT* exon 11 mutations, 48% and 44 months with exon 9 and 0%, and 26 months for WT or other mutations [44].

The *EORTC phase I/II study* similarly analyzed genotype and clinical response. The conclusions were similar to the US-Finnish phase II study; however, the outcomes of patients with exon 9 and wild-type mutations were slightly better [45]. These differences may be explained in part by differences in gene mutational frequencies between the two studies, and importantly, the phase I dose (400 mg daily, 300 mg twice daily and 500 mg twice daily) and phase II dose (400 mg twice daily) of imatinib were higher in the EORTC study.

Of the 67 patients enrolled in the EORTC phase II trial, 37 tumor samples were evaluable for mutational studies. Mutations were found in *KIT* ($n = 29$, 78%) and *PDGFRA* ($n = 2$, 5%) and no mutations in remaining 6 samples. The frequency of *KIT* mutations was as follows: exon 11 ($n = 24$, 83%), exon 9 ($n = 4$, 14%), and exon 13 ($n = 1$, 3%). Patients with exon 11 mutations had an 83% PR compared to 23% with other mutations. All patients with exon 9 mutations responded to imatinib, and surprisingly, the two patients with *PDGFRA* D842V maintained stable disease, and WT patients demonstrated stable disease or PR. The event-free survival for the group (37 patients) was 705 days, but subgroup analysis showed improved survival in exon 11 patients (849 days) versus 327 days with other mutations. The authors conclude that these data support the first-line use of imatinib in all patients and mutational status should not guide this decision [45].

The *EORTC phase III study* group reported their analysis of mutational status, drug dose, and clinical benefit. In a study of 946 patients, tumor samples were collected, centrally evaluated, and genotyped in 377 patients [46]. *KIT* mutations were found in 315 patients (84%) in the following distribution: exon 11 ($n = 248$, 66%), exon 9 ($n = 58$, 15%), exon 13 ($n = 6$, 2%), and exon 17 ($n = 3$, 1%). *KIT* exon 11 mutations predominantly involved in-frame deletion (55%), point mutations, and the rest with a variety of complex mutations (20%). Other mutations were KIT exon 9 in-frame deletions involving AY 501–502, missense substitutions involving exon 13 ATP-binding domain, and exon 17 activation loop. No *KIT* mutations were found in 20% of specimens, but further analysis showed *PDGFRA* mutations in 16% of samples, with both imatinib-resistant (D842, D846V) and imatinib-sensitive (D561V, IMH843–845, DIM842–844, and DIMH842–845) mutations observed. Clinical correlation showed that patients with exon 9 mutations had significantly worse PFS and OS when compared to exon 11 mutations but no difference when compared to the WT group. No PFS or OS differences were noted when comparing deletions, point

mutations, heterozygous, or homozygous exon 11 mutations. The response rates in these groups were 69% for *KIT* exon 11, 34% with exon 9, and 25% with WT genotypes. There was no primary resistance observed in *KIT* exon 13 or 17 tumors. Not surprisingly, *PDGFRA* mutants with D842 or D846V did not respond to imatinib [45, 46].

Correlation of kinase genotype and clinical outcome was reported in the *SWOG/CALGB phase III* study (S0033). In a study of 746 patients, tumor samples were obtained, confirmed for CD117 status, and genotyped in 368 patients [41, 44, 47]. Mutations in *KIT* exon 11 were found in 71% of tumor samples and followed by exon 9 (8%), *KIT* exon 13 (1%), *PDGFRA* exon 18 (1%), and *KIT* exon 17 (1%). Further analysis of each mutation revealed some rare and novel mutations, and clinical correlation was evaluated although in vitro response to imatinib was not assessed. This included *KIT* exon 8 (PR, TTP = 8.1 mo), *KIT* exon 9 reduplication of codons 506–508 (TTP = 10.6 mo), and deletion of 484–487 (TTP = 46.9 mo, OS = 59.3 mo). The predominant mutation in exon 9 was the AY 502–503 internal tandem duplication. Eight patients had *PDGFRA* exon 18 mutation whose deletion/substitution IMHDS 843–847M, deletion DIMH842–845 ($n = 3$), and the imatinib-resistant D842V substitution ($n = 4$). Three of the D842V patients had PFS of less than 2 months, and the fourth patient had a PFS of 34 months with an OS of 9.7 months for the group. Consistent with all other studies, patients with *KIT* exon 11 mutations had a higher ORR of 71% compared to 44 and 45% in patients with *KIT* exon 9 and wild type, respectively. A similar trend was seen in the median TTP and OS: *KIT* exon 11 (24.7 mo and 60 mo), exon 9 (16.7 mo, 38.4 mo), and wild type (12.8 mo and 49 mo). There was no difference in ORR, TTP, or OS between *KIT* exon 9 and wild-type patients. An important result of this study was the finding that *KIT* exon 9 patients had significantly higher response rates but not TTP (9.4 mo vs. 18 mo) when treated with 800 mg (ORR = 67%) versus 400 mg (ORR = 17%) imatinib. This difference was not seen in *KIT* exon 11 or wild-type patients.

Meta-analysis of GIST

The meta-GIST study pooled the two large studies and evaluated 1640 patients in the two arms [48]. The pooled data showed a small (4 months) but significant increase in median PFS in the high-dose arm, but this advantage diminished after 2 years. The authors suggest that this short advantage may be due to frequent dose reductions in the high-dose arm along with various mutations that govern early (<3 months), late (>3 months), and very late recurrence (>18 months). The pooled analysis showed identical overall survival in both arms. The study identified seven significant adverse prognostic factors that predicted overall survival. These include high granulocyte count (strongest), poor performance status (strongest), advanced age (OS), low albumin level at entry (OS), prior chemotherapy, large tumor size (OS), and male gender (OS and PFS). Tumor site was not prognostic. It is important to recognize that these clinical variables were derived from a cohort where all patients received IM; therefore, whether this is an appropriate prognostic indicator in untreated patients or only applicable for imatinib-treated patients is unknown. Mutational status and clinical outcome was identical in both arms. Consistent with the phase III trials, the meta-analysis concluded that RR, PFS, and OS were improved in patients with *KIT* exon 11 mutations (80%, 26, 60 months) when compared to *KIT* exon 9 (40%, 13, 31 months), wild-type *KIT* (16, 43 months), and other mutations (11, 34 months). The study concluded that for most patients, the recommended daily dose is 400 mg daily. An important exception was for those who harbor *KIT* exon 9 mutation in whom high dose has better response rates and prolongs PFS, but not OS [48].

Summary: Clinical response and mutational status

Phase II	Correlation of radiographic response and median OS with mutational status: 86% and 63 months for *KIT* exon 11, 48% and 44 months with exon 9, and 0% and 26 months for WT or other mutations (US-Finnish). EORTC reported similar outcomes, but patients with exon 9 and wild-type mutations had slightly better outcomes.
Phase III	EORTC study reported response rates of 69% for *KIT* exon 11, 34% with exon 9, and 25% with WT genotypes. SWOG reported response rates, median TTP, and OS as follows: exon 11 (71%, 24.7 mo and 60 mo), exon 9 (44%, 16.7 mo and 38.4 mo), and WT (45%, 12.8 mo and 49 mo).
Meta-GIST	A 4-month increase in median PFS in the high-dose arm but benefit diminished after 2 years. RR, PFS, and OS are as follows: *KIT* exon 11 mutations (80%, 26 months, 60 months) when compared to *KIT* exon 9 (40%, 13, 31 months), wild-type *KIT* (16, 43 months), and other mutations (11, 34 months).
Meta-analysis	*KIT* exon 9 mutations treated with imatinib 800 mg daily had better response rates and prolongs PFS, but not OS.

Summary: Prognostic factors that predict PFS and OS with IM therapy

High granulocyte count (strongest), poor performance status (strongest), advanced age (OS), low albumin level at entry (OS), prior chemotherapy, large tumor size (OS), and male gender (OS and PFS). Tumor site was not prognostic.

Mechanism of early resistance

"Innate", "Early", or "primary" resistance are terms to describe patients in whom progression is noted within 3–6 months of initiating imatinib [27, 49, 50]. As noted above, approximately 15–20% of patients fall in this category. The US-Finnish phase II study reported that 14% of patients had PD within 3 months, while 5% showed resistance within 2 months [38]. EORTC phase I-II studies reported 9–13% early resistance [36, 37].

There are several mechanisms of early resistance to therapy [51]. The most important factor is the underlying mutational status of KIT and PDGFR. Although early resistance is reported with all mutations, it is most often seen in KIT exon 9, PDGFR, and wild-type GIST [41–43, 46, 48]. The EORTC group analyzed KIT exon 11 mutations to clinical outcomes showed that mutation or deletion of codon 565 or 579 had shorter PFS.

Mutations in exon 9 result in mutation in the extracellular domain that normally inhibits receptor dimerization. Some of the resistance in this genotype is a result of a steric hinderance that does not allow imatinib to bind strongly to the catalytic domain. While some patients with this mutation respond to 400 mg daily, others may require a higher dose (600 mg or 800 mg) at progression [41–43, 46, 48]. Mutations in exon 13 and 17 are rare, and some mutations are associated with response (exon 13: K642E), while other are associated with resistance (exon 13: V654A).

PDGFRA mutations are rare, and definite conclusions regarding response to imatinib are difficult to make. In the limited cases, mutations in exon 12 and 14 are noted to be sensitive, while early resistance is seen with D842V PDGFRA mutations. This is not surprising since this substitution in the activation loop (similar to KIT AL) favors the kinase in the active conformation which is not conducive for imatinib binding. Importantly, this mutation results in release of the activation loop away from the KIT molecule and facilitates constitutive downstream phosphorylation [31]. An unexplained phenomenon is the occasional response noted in patients with D842V mutation. Few cases of wild-type GIST have mutations in *BRAF* exon 15 [52].

Drug metabolism: An important mechanism of early resistance is the emerging effect of imatinib drug levels and clinical response [53]. In a phase II B2222 study, patients in the lowest plasma quartile of trough imatinib (<1,100 ng/ml) had median TTP of 11.3 months, while those in higher quartiles had a median PFS of 30 months ($P = 0.0029$). When the data were stratified according to mutational status, patients with *KIT* exon 11 mutation and >1,100 ng/ml had a 100% disease control rate (CR + PR + SD). This was a subgroup analysis consisting of 79/147 patients, and some patients in the lower quartile of drug level were noted to have long-lasting response [53]. While the data are hypothesis-forming and imatinib drug level testing is now widely available, currently there is no clear role for titrating dose of imatinib in drug level, pending the completion of a clinical trial to examine this question. For example, if a lower dose of imatinib is insufficient to engender a clinical response initially, a higher dose can be employed to achieve better clinical benefit later, without penalty to the patient (at least according to the two large randomized clinical trials in metastatic disease).

Mechanism of early resistance

Early resistance: 20% of all patients.

Seen with all mutations, but most often with *KIT* exon 9, *PDGFRA*, and WT. Primary mutations in *KIT* exon 13 and 17 are rare and response noted in exon 13 (K642E) but resistant with V654A.

PDGFRA mutations are rare, and definite conclusions regarding response to imatinib are difficult to make. *PDGFRA* mutations are rare, and exon 12 and 14 are sensitive while D842 V *PDGFRA* mutations are resistant (rare exceptions).

Level of plasma imatinib level may correlate with early resistance.

Mechanism of delayed resistance

"Delayed", "Acquired", or "Secondary" resistance is seen in patients who develop progression of disease after an initial response or stable disease on imatinib [49–51, 54–56]. Patients develop this resistance between 6 and 24 months of starting therapy and are classified as "late" (>6 months) and "very late" (>24 months) [27]. In GIST, progressing tumors often demonstrate an unusual pattern that does not fit progression by RECIST. Radiological images of tumors show one or more areas of increased vascularity within a previously responding or stable lesion. These lesions can be seen up to several months before documented progression by RECIST. Biopsy and mutational analysis of individual nodules within a dominant mass show multiple clonal origins of each nodule, each with distinct mutational changes. In a study where patients were treated with imatinib alone or followed by sunitinib, 83% of samples had at least one secondary mutation, 67% had two to five different secondary mutations in different tumor samples, and 34% had more than two mutations in the same clone [57]. This finding is the strongest evidence that argues against repeat biopsy and genotyping at progression [27].

Secondary mutations have only been found in patients who initially were found to have primary *KIT* mutations

and rarely in those with primary *PDGFRA* mutations. Secondary mutations are most often found in the ATP-binding pocket of the kinase domain (exon 13 and 14) or in the kinase activation loop (exon 17 and 18). No secondary *KIT* or *PDGFRA* mutations have been documented in wild-type tumors [44, 46, 49, 50, 54, 55]. This is not surprising since imatinib binding seems to provide the selective pressure.

In the phase II B2222 trial patients, 67% of samples in patients who progressed contained one or more secondary mutations. In a phase I/II trial of sunitinib, 49% of patients had a secondary *KIT* mutation. This cohort also contained patients who were intolerant of imatinib and therefore unlikely to have secondary mutations. Secondary mutations were found in patients who initially harbored *KIT* exon 11 mutations (73%) compared to *KIT* exon 9 mutations (19%) [55]. Further analysis showed that the most common mutation was the T670I, which is termed the "gatekeeper" mutation, previously observed in some patients with BCR-Abl-positive CML who develop resistance to imatinib. In normal KIT, threonine 670 forms a hydrogen bond to stabilize imatinib and thus allows the molecule to enter a hydrophobic pocket. However, when threonine is replaced by a bulky isoleucine, imatinib is unable to form this stabilizing bond or enter this hydrophobic pocket. Similarly, substitution of valine by alanine at position 654 (Val654Ala) prevents formation of hydrophobic bonds with imatinib [31]. Another secondary mutation in *KIT* involves the activation loop where tyrosine is replaced by aspartate (Tyr823Asp). Aspartate is negatively charged and mimics phosphorylated tyrosine and thus results in an active conformation, that is prevents to binding of imatinib. Mutations in exon 11 L576P have also been reported [56, 58]. These have poor clinical response to imatinib, and in vitro studies confirmed a twofold less sensitivity to imatinib when compared to the highly sensitive KIT-V599D mutation. It is thought that the L576P mutation shifts the KIT molecule toward the active conformation and thus decreases imatinib affinity.

Alternate mechanisms of resistance

In GISTs lacking *KIT* mutations (WT), gene amplification of *KIT* and/or *PDGFRA* is implicated in early and delayed resistance. In some cases, the genome loses the remaining wild-type allele and becomes homozygous for *KIT* mutation. Activation of alternate growth pathways is also implicated in resistance to imatinib. Overexpression of AXL (RTK) with downregulation of KIT is noted in few cases. In a D820Y imatinib-resistant cell line, upregulation of focal adhesion kinase (FAK) and AKT, and downregulation of KIT have been implicated as a less common mechanism of imatinib resistance. Insulin-like growth factor 1 receptor (IGF1R) is frequently overexpressed in wild-type GIST, and inhibition of this pathway results in cell death and discussed later in this review. Other genetic mechanisms that have been postulated are levels of intratumoral VEGF expression. High levels of VEGF were correlated with poor PFS in 53 patients treated with imatinib for advanced GIST. Other groups have also reported similar findings in resected GIST. EORTC investigators have also evaluated levels of p53, p16, BCL2, and CHK2 expression and reported that high expression of p53 and low levels of p16 to correlate with a shorter PFS in a multivariate analysis.

In conclusion, the major mechanisms of delayed IM resistance are (1) secondary mutation in KIT or PDGFR in addition to the initial mutation and rarer events that include (2) overexpression of KIT as evidenced by genomic amplification, (3) activation of alternate pathways and loss of KIT, and (4) functional resistance defined as mutations in regions of KIT or PDGFR that are not bound by imatinib. ABC transporter overexpression could also provide another obvious method for tumors cells to become resistant to tyrosine kinase inhibitors such as imatinib.

Mechanism of delayed resistance (6–24 months)

Biopsy and mutational analysis of individual nodules show multiple clonal origins with distinct mutational changes.

Secondary mutations are found in 50–70% of patients who progress and only in patients with initial *KIT/PDGFRA* mutations especially *KIT* exon 11.

T670I "gatekeeper" is the most common secondary mutation. Other mutations are in exon 14, 17, and 18. No secondary mutations have been documented in wild-type tumors.

Major mechanisms of imatinib resistance are (1) secondary mutation in *KIT* or *PDGFRA* in addition to the initial mutation, (2) overexpression of KIT as evidenced by genomic amplification, (3) activation of alternate pathways and loss of KIT, and (4) functional resistance, defined as mutations in regions of KIT or PDGFR that are not bound by imatinib.

Patients who progress on 400 mg may benefit with 800 mg of imatinib.

Clinical implications of imatinib resistance

Patients with PD within 6 months can be titrated to a higher imatinib dose (up to 800 mg daily). Clinical trials have consistently demonstrated that this strategy results in ORR in 33% of patients (7% PR). The underlying mechanism may be related to higher imatinib drug levels and robust inhibition of *KIT* exon 9 or *KIT*-amplified tumors. In patients who have low burden of disease and show progression in one to three lesions, then surgical resection, radiofrequency ablation, arterial embolization, or cryotherapy can be considered

as reasonable options. In small case series with carefully selected patients, this modality was shown to improve median PFS and OS.

In patients who progress with high-dose imatinib or are intolerant to imatinib should be started on sunitinib as second-line therapy. Currently, neither genotyping of new lesions nor measuring trough imatinib drug levels is recommended. Third- and fourth-line agents include sorafenib, nilotinib, and dasatinib.

GIST and sunitinib

Sunitinib was approved by FDA on Jan 26, 2006, for patients with GIST who are resistant to or intolerant of imatinib (www.fda.gov/bbs/topics/news/2006/NEW01302.html). Sunitinib, an oral TKI, is a potent inhibitor of KIT, PDGFRs, VEGFR 1, 2, 3, FLT3, and RET [59, 60]. Both imatinib and sunitinib bind to the ATP-binding domain of KIT/PDGFRA in the inactive configuration; however, their binding properties differ, and in addition, sunitinib has potent anti-angiogenic properties that may play a role in GIST [60, 61]. Pre-clinical studies in imatinib-resistant GIST cell lines harboring KIT-V654A, KIT-T670I, or PDGFRA-D842 V mutant isoforms were evaluated for sensitivity to sunitinib by IHC and proliferation assay. Only KIT-T670I (gatekeeper) and V654A were sensitive to sunitinib, while PDGFRA-D842 V (activation loop) remained resistant [62]. Phase I studies in solid tumors determined 50 mg/day (4 weeks on, 2 weeks off) as an appropriate MTD for further studies [63]. Responses in phase I trial were seen in IM-resistant GIST, RCC, gastrointestinal, and neuroendocrine tumors. Phase I/II study of sunitinib at 50 mg/day (4 on, 2 off) in 97 imatinib-resistant (median 78 weeks of imatinib therapy) or imatinib-intolerant patients was conducted [64]. Most patients (78/97) enrolled in this study underwent biopsy before initiation and at progression. Primary mutations were 83% *KIT*, 5% *PDGFRA,* and 12% WT, with the following distributions: *KIT* exon 11 (69%), *KIT* exon 9 (30%), *KIT* exon 13 (2%), and few *PDGFRA* (exon 12 and 18). Radiological benefit (RB: defined as RECIST PR or SD $>$ 6 months) was noted in all major GIST subtypes as follows: *KIT* exon 11 (42 pts, RB 36%, PR 5%), *KIT* exon 9 (19 pts, RB 42%, PR 37%), *PDGFRA* (4 pts, RB 25%), and WT (9 pts, RB 56%). Both PFS and OS were significantly improved in patients with both *KIT* exon 9 mutations (19.4 and 26.9 mo) and WT (19 and 30.5 mo) when compared to exon 11 mutations (5.1 and 12.3 mo).

Analysis of pre-sunitinib, tumor samples revealed secondary mutations in 50% of patients (33/67 patients) which included exons 13, 14, 17, and 18. Secondary *KIT* mutations were more common in patients who initially harbored exon 11 (79%) versus exon 9 (19%). One secondary *PDGFRA* mutation was found in exon 18; however, patients initially harboring D842V mutation lacked any secondary mutations. No mutations were found in WT patients. Subgroup analysis of secondary mutations and outcomes (RB, PFS, and OS) showed that patients with exon 13 or 14 mutations (61%, 7.8 and 13 mo, respectively) had significantly improved outcomes than those with exon 17 or 18 (15%, 2.3 and 4 mo, respectively). This finding is not surprising given that sunitinib inhibits the ATP-binding kinase domain coded by exon 13 or 14 and has no activity against the activation loop coded by exon 17 or 18. This study suggests that sunitinib may be more effective against *KIT* exon 9 and WT rather than exon 11 GIST; however, it is important to note that all patients in this study had significantly longer exposure to imatinib, and many of the exon 11 cohort had secondary *KIT* mutations. This study also provided primary data that discontinuation of imatinib is deleterious even in the setting of disease progression; those patients who were allowed to crossover to sunitinib after receiving placebo still had a higher rate of mortality than patients who received sunitinib initially as part of the study. These data suggest that future studies examining new tyrosine kinase inhibitors should compare the new agent to continuation of the prior tyrosine kinase inhibitor (or another one) and similarly point out a significant weakness in randomized discontinuation clinical trial designs.

Sunitinib gained FDA approval based on *phase III data* where imatinib-resistant (400 mg daily) or intolerant patients were randomized (2:1) to sunitinib (50 mg, 4 on, 2 off) or placebo with an option to crossover at progression. In this trial, 310 patients were randomized to either sunitinib ($n = 205$) or placebo ($n = 105$). Partial response and stable disease was seen in 7% and 58% of patients in the sunitinib arm, while no responses were seen with placebo. Median PFS in the treatment arm was 6.3 mo compared to 1.5 mo in the placebo arm. An interesting biomarker in this study included measurement of serum KIT levels. Investigators showed in a separate report that serum KIT levels dropped in the sunitinib arm when compared to placebo [65]. Interestingly, rise of serum KIT level after 12 weeks of treatment was correlated with poor outcome.

More recently, in a phase II study, 60 patients with imatinib-resistant or -intolerant GIST were treated with a daily continuous dosing of sunitinib at 37.5 mg and showed PFS and OS of 8.5 and 28 months, respectively [66]. This study reported that with treatment, serum VEGF levels increased while soluble KIT and VEGFR (2 and 3) decreased. While there was a trend toward improvement in PFS and OS was noted in patients whose serum KIT levels dropped from baseline, this was not significant until cycle 6 of treatment.

To date, no clear picture has emerged to describe the mutations in patients who fail SU. Recently, Antonescu et al. described secondary mutations in the activation loop (exon 17: D820Y, D820E, and N822 K) of three patients who initially harbored primary *KIT* exon 9 mutations and progressed on sunitinib after >12 months of therapy. Stable cell lines containing primary *KIT* exon 9 and secondary mutations in *KIT* exon 13 (sunitinib-sensitive) and exon 17 (sunitinib-resistant) were established, and different TKI were tested for kinase inhibition and apoptosis. Not surprisingly, both imatinib and sunitinib had minimal meaningful activity. Dasatinib and nilotinib had modest *KIT* kinase inhibition (<100 nmol/L) and even weaker apoptosis of cell lines (<1,000 nmol/L). In double mutant cell lines, sorafenib inhibited kinase activity at 100–1,000 nmol/L range; however, this was not associated with significant apoptosis [67].

Summary: Sunitinib: Approved 2nd line agent with imatinib resistance or intolerance

KIT T670I ("gatekeeper") and V654A-sensitive to sunitinib while D842 V resistant.

Phase III: 310 patients who progressed on imatinib randomized to sunitinib or placebo. Partial response and stable disease seen in 7 and 58% of patients in the sunitinib arm while none in placebo. Median PFS was 6.3 mo with sunitinib. Improvement in OS when a novel statistical analysis was employed to account for crossover at progression.

Responses and improved PFS and OS noted in patients with WT, primary KIT exon 9, and secondary KIT exon 13 or 14.

Discontinuation of imatinib is deleterious even in the setting of disease progression.

No well-recognized mutations to explain resistance with sunitinib.

Other tyrosine kinase inhibitors

Nilotinib

Nilotinib, a second-generation oral TKI, is a potent inhibitor of KIT, PDGFRA, and BCR-Abl is approved for imatinib-resistant or -intolerant chronic myelogenous leukemia. Pre-clinical studies in imatinib-resistant and -sensitive GIST cell lines with nilotinib (AMN107) showed more potent kinase inhibition and growth inhibition with nilotinib than with imatinib [68]. In another study, intracellular concentration of nilotinib was seven- to tenfold greater than imatinib, while inhibitory kinase activity was comparable [69]. A large retrospective study reported the results of 52 patients with imatinib- or sunitinib-resistant/intolerant GIST who received nilotinib 400 mg twice daily as part of a compassionate use program in Europe [70]. Nilotinib was intolerable in 12% of patients and discontinued. In an intention to treat analysis, the study reported 10% PR, 37% SD, median PFS of 12 weeks, and median OS of 34 weeks. In a case report, nilotinib was used in a single patient who had progressed on both imatinib and sunitinib. The patient had mild-moderate response on PET performed at 3 months after treatment. CT showed overall stable disease [71].

A phase I study evaluated the efficacy and long-term safety of nilotinib alone and in combination with IM [70, 72]. A total of 53 patients with imatinib-resistant GIST were randomized to three arms: nilotinib alone ($n = 18$, 400 mg twice daily), escalating doses of nilotinib with high-dose imatinib (400 mg bid), and high-dose nilotinib (400 mg bid) along with standard dose imatinib (400 mg daily). Nilotinib was administered after failure of 74% of patients failed second-line therapies (sunitinib, AMG-706, dasatinib, or RAD001). One durable PR was seen in nilotinib alone in a patient with KIT exon 11 mutation. Stable disease was seen in 36 patients (68%), and median overall PFS was 134 days.

Dasatinib (BMS-354825)

Src family kinase (sarcoma, SFK) is an essential signaling pathway implicated in growth, survival, motility, and adhesion [73]. Dasatinib (BMS-354825) is a TKI which inhibits Src, KIT, PDGFRA, EPHA2, and BCR-Abl kinase. In vitro studies show potent kinase inhibition and induction of apoptosis in cell lines with WT and mutated KIT, including those harboring imatinib-resistant KIT activation loop mutations [74]. Dasatinib at 70 mg twice daily is FDA-approved for second line in CML and Ph + ALL. A phase I, open-label, dose escalation study was conducted in 67 patients with advanced, treatment-refractory solid tumors, which was conducted to determine schedule, MTD, DLT, and RP2D [75]. There were 19 GIST patients in this cohort who were either on a twice daily, 5 days on/2 days off schedule or a continuous, twice daily dosing. No objective response was observed in this study; however, 3/19 (16%) of GIST patients had stable disease (resolution of ascites) lasting for >3 months in one of these patients. No DLT was observed. The recommended phase II dose of dasatinib is 120 mg twice daily on the 5D2 schedule and 70 mg twice daily in the CDD schedule.

Sorafenib

Sorafenib (SOR) (BAY43–9006) is a TKI that inhibits KIT, PDGFR, and VEGFR 1, 2 and 3 and in addition also inhibits serine/threonine kinases along the RAS/RAF/MEK/ERK pathway [76]. In vitro studies in Ba/F3 cell lines harboring either imatinib-sensitive or imatinib-resistant

mutations showed sorafenib inhibited kinase activity and induced apoptosis in all cell lines including imatinib-resistant cells with gatekeeper secondary mutation (T670I) [77]. Nilotinib was noted to be more potent than sorafenib and dasatinib in inhibiting imatinib-resistant KIT (V560del/V654A) and KIT(V559D/D820Y) cell lines; however, only sorafenib was able to inhibit KIT 9WK557-8del/T670I cells [78, 79]. However, a phase II trial of 26 patients (6 imatinib-resistant, 20 imatinib/sunitinib-resistant) evaluated sorafenib 400 mg bid and reported 13% RECIST PR, 58% SD, and 71% disease control rate. Median PFS was 5.3 mo, and median OS was 13 mo [80]. A phase II study, placebo-controlled, randomized discontinuation trial with sorafenib at 400 mg bid was conducted in 26 patients with soft tissue sarcoma. In this cohort, there were 3/26 (12%) GIST patients. One patient had a minor response, and the remaining two patients progressed within 12 weeks of starting [81]. A retrospective study from Europe showed similar activity of sorafenib in fourth line in patients with otherwise refractory GIST [82]. Twenty-four patients with GIST who progressed on IM, SU, and NI were treated with sorafenib 400 mg twice daily. At a median follow-up of 93 days, 20% of patients had PR and 50% of patients had SD.

Masatinib (AB1010)

Masatinib (AB1010) is a novel TKI that inhibits KIT, PDGFR (α and β), Lyn, fibroblast growth factor receptor 3, and to a lesser extent the focal adhesion kinase (FAK) pathway. Importantly, it inhibits both wild-type and activated KIT (JM mutation). In vitro, it was found to have more potent kinase inhibition (lower IC_{50}) and increased cell death than imatinib [83]. Similar inhibition was noted with PDGFR, Lyn kinase, and to a lesser extent FGFR3. However, no activity was noted against ABL, Fms, and other kinases, thus distinguishing itself as a potent and highly selective TKI [83]. Pre-clinical studies in animals showed it was well tolerated and had lesser cardiotoxicity. A phase I, dose escalation study in 40 patients with advanced solid tumors (19 GIST) reported 12 mg/kg/day as an acceptable dose [84]. An MTD was not formally reached; however, 38/40 patients had grade 1–2 toxicities. One of two GIST patients with imatinib intolerance had a PR, and 29% of imatinib-resistant GIST had stable disease. A phase II trial of oral masatinib at 7.5 mg/kg/d was studied in thirty imatinib-naïve, advanced GIST patients [85]. Best RECIST responses were CR (3%), PR (50%), SD (43%), and PD (3%). Median time to response was 5.6 months (0.8–3.8 mo). Estimated median PFS was 41.3 months with PFS rate of 60 and 55% at 2 and 3 years, respectively. The OS at 2 and 3 years was stable at 90%.

Vatalanib (PTK787/ZK222584, Novartis-Schering)

Vatalanib (PTK787/ZK222584, Novartis-Schering) is an oral multi-targeted TKI that inhibits VEGFR1, 2 and 3, KIT, and PDGFRβ. It has no activity against epidermal growth factor receptor, fibroblast growth factor receptor-1, MET, and Tie-2, or intracellular kinases such as Src, Abl, and protein kinase C-α. In vitro studies in renal cell carcinoma showed that vatalanib exerts potent anti-angiogenic effects by inhibiting VEGF- and PDGF-induced tumor angiogenesis [86]. Phase I studies in solid tumors identified 750 mg orally bid as the MTD with PK and biological activity achieved at $\leq$1,000 mg total daily dose [87, 88]. A very small phase II trial of 15 GIST patients who had progressed on imatinib (11 with high dose) was conducted with Vatalanib at 1,250 mg orally daily [89]. The study reported two PR (13%), eight SD for $\geq$3 months (53%), and five PD (33%). Median time to progression was 8.5 months (0.0–24.8 mo).

Other non-TKI based therapies

Hsp90 inhibitors

Heat-shock proteins (Hsp) normally function to stabilize the cellular machinery during stressful conditions such as hypoxia, acidemia, temperature elevation, and oxidative damage [90]. An important and parallel function of Hsp is their role in protein folding, ubiquitination, and degradation. Hsp are also upregulated in malignant cells as a means to "protect oncoproteins" and thus maintain homeostasis in an otherwise toxic tumor microenvironment characterized by hypoxia and acidosis. There is biological rationale to target these proteins in order to tip the balance toward stress-induced apoptosis. Nakatani et al. first demonstrated that imatinib inhibits the clustering of KIT on the cell surface membrane and in addition prevents the interaction of KIT and molecular chaperone protein 90 (hsp90) which is thought to play a role in WT and mutated KIT [91]. Bauer et al. showed that Hsp90 protects KIT from proteasome-mediated degradation [92]. In vitro studies with 17-AAG (17-allylamino-18-demethoxy-geldanamycin), a specific Hsp90 inhibitor in imatinib-sensitive and imatinib-resistant GIST cell lines, showed inhibition of KIT tyrosine phosphorylation, protein expression, kinase activation, and cellular proliferation. GIST cell lines that were not KIT-dependent did not exhibit these changes. Molecular analysis with tissue microarrays in 306 GIST patients revealed Hsp90 overexpression correlated with an aggressive biology (larger size, non-gastric origin, high mitotic index, unfavorable RTK genotype, and high Ki-67) and poor overall outcome [93]. A phase I trial of retaspimycin

(IPI-504), an hsp90 inhibitor, was studied in 54 patients with TKI-resistant GIST ($N = 38$) or soft tissue sarcoma ($n = 16$). Patients were treated with retaspimycin (intravenous, twice weekly, different schedules) and showed PET PR (22%), RECIST PR (0%), PET SD (66%), and RECIST SD (78%) [94]. However, a phase III study with retaspimycin was closed for toxicity. Other oral hsp90 inhibitors are currently in clinical trials.

HDAC

Histone deacetylases (HDAC) are enzymes that remove acetyl groups on lysine residues found on histones [95]. Hyper-acetylated chromatin is transcriptionally active. HDAC inhibitors cause hypo-acetylation of chromatin which results in both silencing of growth promotion genes and expression of cell cycle inhibitory genes. In addition, HDAC inhibitors also play an important role in acetylation of lysine residues in non-histone proteins (p53, Fas, TNFα, HIF1) which result in growth arrest and apoptosis [95]. In vitro data suggest that HDAC enzymes acetylate heat-shock proteins and disrupt their chaperone function [96–98]. Liu et al. reported that imatinib triggers apoptosis in GIST cell lines by upregulating H2AX, a soluble histone, which mediates its activity through a still poorly understood interaction between KIT, PI3 K, and the proteasome pathway. Imatinib-induced H2AX upregulation results in aberrant chromatin aggregation, transcriptional block, and apoptosis [97]. Panobinostat, an HDAC inhibitor, showed remarkable activity in mouse xenografts implanted with tumor implants from patients who progressed on imatinib and also in a xenograft model with a GIST cell line harboring *KIT* exon 13 mutations [99]. Trials with HDAC inhibitors in soft tissue sarcomas are currently ongoing.

Bortezomib

Bauer and colleagues tested whether bortezomib, a proteasome inhibitor, can disable the ubiquitin–proteasome machinery and indirectly cause upregulation of H2AX and thereby trigger apoptosis [100]. When GIST cell lines (imatinib-resistant and -sensitive) were treated with bortezomib, rapid apoptosis was noted along with upregulation of H2AX and downregulation of KIT protein. Bortezomib in combination with other agents is currently in clinical trials for solid tumors and soft tissue sarcomas.

Everolimus (RAD001)

Everolimus is a derivative of sirolimus and inhibitor of mTOR (mammalian target of rapamycin). The role of MAPK and PI3 K/AKT/mTOR pathway in GIST is well known, and therefore, inhibition of mTOR which is downstream of P13 K makes a rationale target. In addition, in vitro studies demonstrated synergy in resistant GIST cell lines treated with both everolimus (RAD001) and imatinib. A phase I/II trial evaluated the combination of everolimus and imatinib in patients who progressed after ≥4 months of optimal therapy with imatinib [101]. Pharmacokinetic studies of the combination reported that imatinib increased the AUC and Cmax of RAD001 via the CYP3A4 and/or P-glycoprotein. The phase II study evaluated the combination of imatinib 600 mg/day and RAD001 2.5 mg/day in two cohorts [102]. One arm had patients who progressed after 4 months of imatinib (strata 1) and another arm where patients progressed on both imatinib and sunitinib (strata 2). The PFS at 4 months was 17.4 and 37.1% in strata 1 and strata 2, respectively. Further evaluation of this approach seems appropriate for what appears to be at least modest activity.

PKC412

PKC412 is a derivative of staurosporine, a non-selective inhibitor of protein kinase (including PKC), FLT-3, VEGF-R2, PDGFR, and KIT [103]. Extensive pre-clinical work in leukemia showed that PKC412 is effective in leukemic cells that develop imatinib resistance after acquiring secondary mutations. Clinical trials with PKC412 are ongoing in hematological malignancies. Debiec-Rychter and colleagues reported 3 recurrent secondary mutations (PDGFR D842V, KIT V654A, and KIT T670I) in tumor samples that progressed on imatinib [104]. They went on to describe in vitro studies where cell lines with above mutations had attenuated auto-phosphorylation of KIT when treated with PKC412; however, there was no effect on KIT signaling with imatinib. A phase I/II trial of PKC 412 in combination with imatinib was conducted in 19 patients with imatinib-resistant GIST [105]. Stable disease at 4 months was noted in 2 of 5 evaluable patients. Reversible grade 1–2 toxicities were reported. PK showed that the combination of PKC412 and imatinib was noted to decrease the effective plasma concentration of imatinib and double the AUC of PKC412. A case report described a 54-year-old woman with PDGFRA-D842 V metastatic GIST who had progressed on imatinib and subsequently on single agent PKC412 [106]. At this point, sirolimus 2 mg/day was added to PKC412, and this intervention resulted in stable disease noted at 11 weeks. Several phosphatidyl inositol 3 kinase (PI3 K), PKC, Akt, and mTOR inhibitors are currently in clinical trials.

Insulin-like growth factor axis (IGF)

The IGF axis consists of insulin-like growth factor (1 and 2), IGF receptors 1 and 2, and IGF-binding proteins. This axis

is triggered by growth factor (GH) and is crucial in embryonic development, adult homeostasis, and oncogenesis. In vitro studies in GIST cell lines identified upregulation of insulin-like growth factor-binding protein-3 (IGFBP-3), in imatinib-sensitive cell lines, but not in resistant cell lines [107]. Analysis of tumor biopsy from patients who had FDG-PET response showed a sevenfold upregulation of IGFBP-3; meanwhile, a 12-fold reduction in IGFBP-3 was noted in patients who did not have a PET response to imatinib. Other investigators analyzed 8 tumor samples (2 WT and 6 KIT mutants) and reported overexpression of IGF1R at mRNA and protein level in WT but not in KIT mutant samples. In cell lines, inhibition of IGF1R using small interfering RNA or a small molecule inhibitor such as NVP-AWE541 resulted in apoptosis. This finding posits an alternative oncogenic pathway in WT and pediatric tumors that lack KIT mutations [108]. Subsequently, Braconi and colleagues reported IHC expression of IGF1, IGF2, and IFGR-1 in 94 GIST patient samples and correlated with clinical outcome [109]. IGFR-1 was overexpressed in all samples, while expression of IGF1 and 2 was variable. IGF1 and 2 expressions correlated with aggressive tumor biology with worse disease-free survival. A similar study by Steigen et al. analyzed IGF2 levels and patient outcomes [110]. High IGF2 level was correlated with worse median overall survival in the pre-imatinib era. Patients with GIST located in the stomach were found to have low IGF2 levels, and within this cohort, the median survival was 4 and 6.9 years in patients with high and low levels of tumor IGF2. Clinical trials with inhibitors of IGF1R are currently ongoing in solid tumors.

Succinate dehydrogenase

SDH complex consists of four subunits (A, B, C D) and plays a fundamental role in Krebs cycle and in the electron transport chain. Germline loss of this gene is lethal in mice. Germline mutations result in decreased life span, mitochondrial encephalopathy, and malignancies of chromaffin cells. Germline mutations in SDH have been described in familial paragangliomas and pheochromocytomas [111, 112]. Carney–Stratakis syndrome is a dyad consisting of GIST and paragangliomas inherited in an autosomal dominant pattern with incomplete penetrance. Germline mutations involving allelic loss of SDH subunits B, C, and D were described in patients with Carney–Stratakis syndrome [113, 114]. There were no mutations noted in *KIT* or *PDGFRA*. Evaluation of pediatric GIST shows that it differs from adult GIST in that only 15–20% of tumors have mutations in *KIT* or *PDGFRA*. Not surprisingly, response to TKIs is limited in this population. Evaluation of pediatric GIST with KIT mutations or wild-type tumors

Drug	Description of study	Results
Nilotinib (NI)	$N = 52$, retrospective.	Intolerable in 12%. 10% PR, 37% SD, median PFS 12 weeks and OS 34 weeks.
	Imatinib- and/or sunitinib-resistant.	
	$N = 53$. After failure of second-line SU. Phase I study. NI alone (400 mg bid, $n = 18$) versus escalating doses of NI + IM (400 mg bid) versus high-dose NI (400 mg bid) + IM 400 mg daily.	Stable disease 68% and median PFS 134 days. 1 durable PR.
Dasatinib	Phase I. $n = 19$. Two schedules: 5 days on/2 days off or continuous twice daily.	No ORR. 3/19 patients with SD > 3 months and resolution of ascites in 1 patient. Phase II dose: 120 mg twice daily 5on/2off or 70 mg twice daily (CDD).
Sorafenib	Phase II. $N = 26$. 6 IM-resistant and 20 IM- and SU-resistant. 400 mg oral bid.	13% RECIST PR, 58% SD and 71% disease control rate. Median PFS: 5.3 mo and median OS: 13 mo
	Phase II: placebo-controlled, randomized discontinuation study. $N = 3/26$ with GIST.	1 patient with minor response. 2 patients with PD < 12 weeks.
	Retrospective study. $N = 24$. Sorafenib 400 mg oral bid. After failure of IM, SU and NI.	Median days of treatment = 87 days. 20% PR and 50% SD. PFS at 4 months: 25% and OS not reached.
Masatinib	Phase I. $n = 19/40$ GIST.	No MTD reached. 12 mg/kg/day dose.
	Phase II: $n = 30$ GIST. IM naïve patients.	1 patient with IM intolerance with PR and 29% of IM-resistant with SD. CR (3%), PR (50%), SD (43%) and PD (3%).
		Median time to response: 5.6 months. Median PFS: 41.3 months and OS of 90% at 2 and 3 years.

continued

Drug	Description of study	Results
Vatalanib	Phase II. $N = 15$ GIST progressed on IM. Dose = 1,250 mg daily.	2 PR (13%), eight SD for >/= 3 months (53%) and five PD (33%). Median time to progression: 8.5 months (0.0–24.8 mo).
Retaspimycin (IPI-504), Hsp inhibitor	Phase I. $n = 38/54$ TKI-resistant GIST.	No ORR response, only SD. PET response seen.
	Phase III study.	Closed for toxicity.
Everolimus (RAD001)	Phase I/II. Everolimus + IM	IM increased the AUC and Cmax of RAD001 via the CYP3A4 and/or P-glycoprotein
	Phase II. IM + RAD001. Evaluated patients who progressed after IM or after IM and SU.	PFS at 4 months was 17.4% (IM only) and 37.1% (IM and SU).
PKC412	Phase I/II trial. $N = 19$ with IM-resistant GIST. Combination of IM and PKC412.	2/5 evaluable patients with SD at 4 months. PKC decreased dose plasma concentration of PKC412.

revealed loss of function and protein expression of SDH subunit B in WT GIST.[115]. No germline or somatic mutations of the SDHB gene were noted. The authors hypothesize that epigenetic silencing may be a mechanism of decreased protein expression.

Conclusions

There is every indication that GIST tries to maintain signaling through KIT or PDGFR in GIST that bear mutations in one of these genes. The linkage between WT GIST and KIT or PDGFRA mutant GIST remains unclear and could represent a very different variety of an otherwise similar appearing tumor. Given that GIST appears to be evolving new mutations to maintain strong KIT signaling in the TKI-resistant setting, approaches that act to inhibit GIST independent of the specific *KIT* mutation are appealing approaches to consider as future therapeutics. Key topics for further research in the immediate future will include identifying new agents with activity in the KIT signaling pathway and attempts to use existing agents better, for example in determining the role of TKI level testing in patient management.

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References

1. Rubin BP, Singer S, Tsao C, Duensing A, Lux ML, Ruiz R et al (2001) KIT activation is a ubiquitous feature of gastrointestinal stromal tumors. Cancer Res 61(22):8118–8121
2. Duensing A, Heinrich MC, Fletcher CD, Fletcher JA (2004) Biology of gastrointestinal stromal tumors: KIT mutations and beyond. Cancer Invest 22(1):106–116
3. Kindblom LG, Remotti HE, Aldenborg F, Meis-Kindblom JM (1998) Gastrointestinal pacemaker cell tumor (GIPACT): gastrointestinal stromal tumors show phenotypic characteristics of the interstitial cells of Cajal. Am J Pathol 152(5):1259–1269
4. Huizinga JD, Thuneberg L, Kluppel M, Malysz J, Mikkelsen HB, Bernstein A (1995) W/kit gene required for interstitial cells of Cajal and for intestinal pacemaker activity. Nature 373(6512):347–349
5. Nocka K, Majumder S, Chabot B, Ray P, Cervone M, Bernstein A et al (1989) Expression of c-kit gene products in known cellular targets of W mutations in normal and W mutant mice–evidence for an impaired c-kit kinase in mutant mice. Genes Dev 3(6):816–826
6. Besmer P, Murphy JE, George PC, Qiu FH, Bergold PJ, Lederman L et al (1986) A new acute transforming feline retrovirus and relationship of its oncogene v-kit with the protein kinase gene family. Nature 320(6061):415–421
7. Yarden Y, Kuang WJ, Yang-Feng T, Coussens L, Munemitsu S, Dull TJ et al (1987) Human proto-oncogene c-kit: a new cell surface receptor tyrosine kinase for an unidentified ligand. EMBO J 6(11):3341–3351
8. Miettinen M, Sobin LH, Sarlomo-Rikala M (2000) Immunohistochemical spectrum of GIST at different sites and their differential diagnosis with a reference to CD117 (KIT). Mod Pathol 13(10):1134–1142
9. Isozaki K, Hirota S, Miyagawa J, Taniguchi M, Shinomura Y, Matsuzawa Y (1997) Deficiency of c-kit + cells in patients with a myopathic form of chronic idiopathic intestinal pseudo-obstruction. Am J Gastroenterol 92(2):332–334
10. Isozaki K, Hirota S, Nakama A, Miyagawa J, Shinomura Y, Xu Z et al (1995) Disturbed intestinal movement, bile reflux to the stomach, and deficiency of c-kit-expressing cells in Ws/Ws mutant rats. Gastroenterology 109(2):456–464
11. Medeiros F, Corless CL, Duensing A, Hornick JL, Oliveira AM, Heinrich MC et al (2004) KIT-negative gastrointestinal stromal tumors: proof of concept and therapeutic implications. Am J Surg Pathol 28(7):889–894
12. Hirota S, Isozaki K, Moriyama Y, Hashimoto K, Nishida T, Ishiguro S et al (1998) Gain-of-function mutations of c-kit in human gastrointestinal stromal tumors. Science 279(5350):577–580
13. Kitayama H, Kanakura Y, Furitsu T, Tsujimura T, Oritani K, Ikeda H et al (1995) Constitutively activating mutations of c-kit receptor tyrosine kinase confer factor-independent growth and tumorigenicity of factor-dependent hematopoietic cell lines. Blood 85(3):790–798

14. Bergmann F, Gunawan B, Hermanns B, Hoer J, Schumpelick V, Fuzesi L (1998) Cytogenetic and morphologic characteristics of gastrointestinal stromal tumors. Recurrent rearrangement of chromosome 1 and losses of chromosomes 14 and 22 as common anomalies. Verh Dtsch Ges Pathol 82:275–278
15. Debiec-Rychter M, Lasota J, Sarlomo-Rikala M, Kordek R, Miettinen M (2001) Chromosomal aberrations in malignant gastrointestinal stromal tumors: correlation with c-KIT gene mutation. Cancer Genet Cytogenet 128(1):24–30
16. Heinrich MC, Rubin BP, Longley BJ, Fletcher JA (2002) Biology and genetic aspects of gastrointestinal stromal tumors: KIT activation and cytogenetic alterations. Hum Pathol 33(5): 484–495
17. Heinrich MC, Corless CL, Duensing A, McGreevey L, Chen CJ, Joseph N et al (2003) PDGFRA activating mutations in gastrointestinal stromal tumors. Science 299(5607):708–710
18. Corless CL, Schroeder A, Griffith D, Town A, McGreevey L, Harrell P et al (2005) PDGFRA mutations in gastrointestinal stromal tumors: frequency, spectrum and in vitro sensitivity to imatinib. J Clin Oncol 23(23):5357–5364
19. Manning G, Whyte DB, Martinez R, Hunter T, Sudarsanam S (2002) The protein kinase complement of the human genome. Science 298(5600):1912–1934
20. Kitamura Y, Hirotab S (2004) Kit as a human oncogenic tyrosine kinase. Cell Mol Life Sci 61(23):2924–2931
21. Nocka K, Buck J, Levi E, Besmer P (1990) Candidate ligand for the c-kit transmembrane kinase receptor: KL, a fibroblast derived growth factor stimulates mast cells and erythroid progenitors. EMBO J 9(10):3287–3294
22. Mol CD, Dougan DR, Schneider TR, Skene RJ, Kraus ML, Scheibe DN et al (2004) Structural basis for the autoinhibition and STI-571 inhibition of c-Kit tyrosine kinase. J Biol Chem 279(30):31655–31663
23. Mol CD, Lim KB, Sridhar V, Zou H, Chien EY, Sang BC et al (2003) Structure of a c-kit product complex reveals the basis for kinase transactivation. J Biol Chem 278(34):31461–31464
24. Duensing A, Medeiros F, McConarty B, Joseph NE, Panigrahy D, Singer S et al (2004) Mechanisms of oncogenic KIT signal transduction in primary gastrointestinal stromal tumors (GIST). Oncogene 23(22):3999–4006
25. Taylor ML, Metcalfe DD (2000) Kit signal transduction. Hematol Oncol Clin North Am 14(3):517–535
26. Zhang Z, Zhang R, Joachimiak A, Schlessinger J, Kong XP (2000) Crystal structure of human stem cell factor: implication for stem cell factor receptor dimerization and activation. Proc Natl Acad Sci USA 97(14):7732–7737
27. Gramza AW, Corless CL, Heinrich MC (2009) Resistance to Tyrosine Kinase Inhibitors in Gastrointestinal Stromal Tumors. Clin Cancer Res 15(24):7510–7518
28. Buchdunger E, Cioffi CL, Law N, Stover D, Ohno-Jones S, Druker BJ et al (2000) 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 295(1):139–145
29. Druker BJ, Tamura S, Buchdunger E, Ohno S, Segal GM, Fanning S et al (1996) Effects of a selective inhibitor of the Abl tyrosine kinase on the growth of Bcr-Abl positive cells. Nat Med 2(5):561–566
30. Tuveson DA, Willis NA, Jacks T, Griffin JD, Singer S, Fletcher CD et al (2001) STI571 inactivation of the gastrointestinal stromal tumor c-KIT oncoprotein: biological and clinical implications. Oncogene 20(36):5054–5058
31. Roskoski R Jr (2005) Structure and regulation of Kit protein-tyrosine kinase–the stem cell factor receptor. Biochem Biophys Res Commun 338(3):1307–1315
32. Fantl WJ, Johnson DE, Williams LT (1993) Signalling by receptor tyrosine kinases. Annu Rev Biochem 62:453–481
33. Hanks SK, Quinn AM, Hunter T (1988) The protein kinase family: conserved features and deduced phylogeny of the catalytic domains. Science 241(4861):42–52
34. Mol CD, Fabbro D, Hosfield DJ (2004) Structural insights into the conformational selectivity of STI-571 and related kinase inhibitors. Curr Opin Drug Discov Devel 7(5):639–648
35. Joensuu H, Roberts PJ, Sarlomo-Rikala M, Andersson LC, Tervahartiala P, Tuveson D et al (2001) Effect of the tyrosine kinase inhibitor STI571 in a patient with a metastatic gastrointestinal stromal tumor. N Engl J Med 344(14):1052–1056
36. van Oosterom AT, Judson I, Verweij J, Stroobants S, Donato di Paola E, Dimitrijevic S et al (2001) Safety and efficacy of imatinib (STI571) in metastatic gastrointestinal stromal tumours: a phase I study. Lancet 358(9291):1421–1423
37. van Oosterom AT, Judson IR, Verweij J, Stroobants S, Dumez H, di Paola ED et al (2002) 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 38(Suppl 5):S83–S87
38. Demetri GD, von Mehren M, Blanke CD, Van den Abbeele AD, Eisenberg B, Roberts PJ et al (2002) Efficacy and safety of imatinib mesylate in advanced gastrointestinal stromal tumors. N Engl J Med 347(7):472–480
39. Blanke CD, Demetri GD, von Mehren M, Heinrich MC, Eisenberg B, Fletcher JA et al (2008) 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 26(4):620–625
40. Dagher R, Cohen M, Williams G, Rothmann M, Gobburu J, Robbie G et al (2002) Approval summary: imatinib mesylate in the treatment of metastatic and/or unresectable malignant gastrointestinal stromal tumors. Clin Cancer Res 8(10):3034–3038
41. Blanke CD, Rankin C, Demetri GD, Ryan CW, von Mehren M, Benjamin RS et al (2008) 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 26(4):626–632
42. Verweij J, Casali PG, Zalcberg J, LeCesne A, Reichardt P, Blay JY et al (2004) Progression-free survival in gastrointestinal stromal tumours with high-dose imatinib: randomised trial. Lancet 364(9440):1127–1134
43. Zalcberg JR, Verweij J, Casali PG, Le Cesne A, Reichardt P, Blay JY et al (2005) Outcome of patients with advanced gastrointestinal stromal tumours crossing over to a daily imatinib dose of 800 mg after progression on 400 mg. Eur J Cancer 41(12): 1751–1757
44. Heinrich MC, Corless CL, Demetri GD, Blanke CD, von Mehren M, Joensuu H et al (2003) Kinase mutations and imatinib response in patients with metastatic gastrointestinal stromal tumor. J Clin Oncol 21(23):4342–4349
45. Debiec-Rychter M, Sciot R, Le Cesne A, Schlemmer M, Hohenberger P, van Oosterom AT et al (2006) KIT mutations and dose selection for imatinib in patients with advanced gastrointestinal stromal tumours. Eur J Cancer 42(8):1093–1103
46. Sciot R, Debiec-Rychter M, Daugaard S, Fisher C, Collin F, van Glabbeke M et al (2008) Distribution and prognostic value of histopathologic data and immunohistochemical markers in gastrointestinal stromal tumours (GIST): An analysis of the EORTC phase III trial of treatment of metastatic GIST with imatinib mesylate. Eur J Cancer 44(13):1855–1860
47. Heinrich MC, Owzar K, Corless CL, Hollis D, Borden EC, Fletcher CD et al (2008) 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 26(33):5360–5367
48. Gastrointestinal Stromal Tumor Meta-Analysis Group (Meta-GIST) (2010) 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 28(7): 1247–1253
49. Van Glabbeke M, Verweij J, Casali PG, Le Cesne A, Hohenberger P, Ray-Coquard I et al (2005) Initial and late resistance to imatinib in advanced gastrointestinal stromal tumors are predicted by different prognostic factors: a European Organisation for Research and Treatment of Cancer-Italian Sarcoma Group-Australasian Gastrointestinal Trials Group study. J Clin Oncol 23(24):5795–5804
50. Sleijfer S, Wiemer E, Seynaeve C, Verweij J (2007) Improved insight into resistance mechanisms to imatinib in gastrointestinal stromal tumors: a basis for novel approaches and individualization of treatment. Oncologist 12(6):719–726
51. Heinrich MC, Corless CL, Blanke CD, Demetri GD, Joensuu H, Roberts PJ et al (2006) Molecular correlates of imatinib resistance in gastrointestinal stromal tumors. J Clin Oncol 24(29): 4764–4774
52. Agaram NP, Wong GC, Guo T, Maki RG, Singer S, Dematteo RP et al (2008) Novel V600E BRAF mutations in imatinib-naive and imatinib-resistant gastrointestinal stromal tumors. Genes Chromosomes Cancer 47(10):853–859
53. Demetri GD, Wang Y, Wehrle E, Racine A, Nikolova Z, Blanke CD et al (2009) Imatinib plasma levels are correlated with clinical benefit in patients with unresectable/metastatic gastrointestinal stromal tumors. J Clin Oncol 27(19):3141–3147
54. Antonescu CR, Besmer P, Guo T, Arkun K, Hom G, Koryotowski B et al (2005) Acquired resistance to imatinib in gastrointestinal stromal tumor occurs through secondary gene mutation. Clin Cancer Res 11(11):4182–4190
55. Heinrich MC, Maki RG, Corless CL, Antonescu CR, Harlow A, Griffith D et al (2008) Primary and secondary kinase genotypes correlate with the biological and clinical activity of sunitinib in imatinib-resistant gastrointestinal stromal tumor. J Clin Oncol 26(33):5352–5359
56. Liegl B, Kepten I, Le C, Zhu M, Demetri GD, Heinrich MC et al (2008) Heterogeneity of kinase inhibitor resistance mechanisms in GIST. J Pathol 216(1):64–74
57. Wardelmann E, Merkelbach-Bruse S, Pauls K, Thomas N, Schildhaus HU, Heinicke T et al (2006) Polyclonal evolution of multiple secondary KIT mutations in gastrointestinal stromal tumors under treatment with imatinib mesylate. Clin Cancer Res 12(6):1743–1749
58. Conca E, Negri T, Gronchi A, Fumagalli E, Tamborini E, Pavan GM et al (2009) Activate and resist: L576P-KIT in GIST. Mol Cancer Ther 8(9):2491–2495
59. Blay JY (2010) Pharmacological management of gastrointestinal stromal tumours: an update on the role of sunitinib. Ann Oncol 21(2):208–215
60. O'Farrell AM, Abrams TJ, Yuen HA, Ngai TJ, Louie SG, Yee KW et al (2003) SU11248 is a novel FLT3 tyrosine kinase inhibitor with potent activity in vitro and in vivo. Blood 101(9): 3597–3605
61. Mendel DB, Laird AD, Xin X, Louie SG, Christensen JG, Li G et al (2003) In vivo antitumor activity of SU11248, a novel tyrosine kinase inhibitor targeting vascular endothelial growth factor and platelet-derived growth factor receptors: determination of a pharmacokinetic/pharmacodynamic relationship. Clin Cancer Res 9(1):327–337
62. Prenen H, Cools J, Mentens N, Folens C, Sciot R, Schoffski P et al (2006) Efficacy of the kinase inhibitor SU11248 against gastrointestinal stromal tumor mutants refractory to imatinib mesylate. Clin Cancer Res 12(8):2622–2627
63. Faivre S, Delbaldo C, Vera K, Robert C, Lozahic S, Lassau N et al (2006) Safety, pharmacokinetic, and antitumor activity of SU11248, a novel oral multitarget tyrosine kinase inhibitor, in patients with cancer. J Clin Oncol 24(1):25–35
64. Demetri GD DJ, Fletcher JA, Morgan JA, Fletcher CD, Kazanovicz A, Van Den Abbeele A, Baum C, Maki R, Heinrich MC (2004) SU11248, a multi-targeted tyrosine kinase inhibitor, can overcome imatinib (IM) resistance caused by diverse genomic mechanisms in patients (pts) with metastatic gastrointestinal stromal tumor (GIST). J Clin Oncol. 2004 ASCO Annual Meeting Proceedings (Post-Meeting Edition), vol 22, No 14S (July 15 Supplement) 3001
65. Deprimo SE, Huang X, Blackstein ME, Garrett CR, Harmon CS, Schoffski P et al (2009) Circulating levels of soluble KIT serve as a biomarker for clinical outcome in gastrointestinal stromal tumor patients receiving sunitinib following imatinib failure. Clin Cancer Res 15(18):5869–5877
66. George S, Blay JY, Casali PG, Le Cesne A, Stephenson P, Deprimo SE et al (2009) Clinical evaluation of continuous daily dosing of sunitinib malate in patients with advanced gastrointestinal stromal tumour after imatinib failure. Eur J Cancer 45(11):1959–1968
67. Guo T, Hajdu M, Agaram NP, Shinoda H, Veach D, Clarkson BD et al (2009) Mechanisms of sunitinib resistance in gastrointestinal stromal tumors harboring KITAY502-3ins mutation: an in vitro mutagenesis screen for drug resistance. Clin Cancer Res 15(22):6862–6870
68. Weisberg E, Manley PW, Breitenstein W, Bruggen J, Cowan-Jacob SW, Ray A et al (2005) Characterization of AMN107, a selective inhibitor of native and mutant Bcr-Abl. Cancer Cell 7(2):129–141
69. Prenen H, Guetens G, de Boeck G, Debiec-Rychter M, Manley P, Schoffski P et al (2006) Cellular uptake of the tyrosine kinase inhibitors imatinib and AMN107 in gastrointestinal stromal tumor cell lines. Pharmacology 77(1):11–16
70. Von Mehren MRP, Casali PG, Blay J, Debiec-Rychter M, Dumez M, Cheung W, Feifel B, Veronese M and Demetri GD (2007) 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. Abstract: 10023. In: ASCO Annual Meeting 2007
71. Sevinc A (2009) Activity of nilotinib (AMN-107) alone in advanced gastrointestinal stromal tumors progressing on imatinib and sunitinib. Case report. Chemotherapy 55(2):132–136
72. Reichardt P CP, Blay J, Von Mehren M, Schoffski P, Hosseinzadeh S, Tanaka C, Gsponer T, Veronese ML, Demetri GD (2006) A phase I study of AMN107 alone and in combination with imatinib in patients (pts) with imatinib-resistant gastrointestinal stromal tumors (GIST). J Clin Oncol. 2006 ASCO Annual Meeting Proceedings Part I, vol 24, No 18S (June 20 Supplement), 9545 2006 ASCO Annual Meeting. Abstract 9545
73. Thomas SM, Brugge JS (1997) Cellular functions regulated by Src family kinases. Annu Rev Cell Dev Biol 13:513–609
74. Schittenhelm MM, Shiraga S, Schroeder A, Corbin AS, Griffith D, Lee FY et al (2006) Dasatinib (BMS-354825), a dual SRC/ABL kinase inhibitor, inhibits the kinase activity of wild-type, juxtamembrane, and activation loop mutant KIT isoforms associated with human malignancies. Cancer Res 66(1):473–481
75. Demetri GD, Lo Russo P, MacPherson IR, Wang D, Morgan JA, Brunton VG et al (2009) Phase I dose-escalation and pharmacokinetic study of dasatinib in patients with advanced solid tumors. Clin Cancer Res 15(19):6232–6240

76. Lyons JF, Wilhelm S, Hibner B, Bollag G (2001) Discovery of a novel Raf kinase inhibitor. Endocr Relat Cancer 8(3):219–225
77. Wilhelm S, Chien DS (2002) BAY 43–9006: preclinical data. Curr Pharm Des 8(25):2255–2257
78. Guida T, Anaganti S, Provitera L, Gedrich R, Sullivan E, Wilhelm SM et al (2007) Sorafenib inhibits imatinib-resistant KIT and platelet-derived growth factor receptor beta gatekeeper mutants. Clin Cancer Res 13(11):3363–3369
79. Guo T, Agaram NP, Wong GC, Hom G, D'Adamo D, Maki RG et al (2007) Sorafenib inhibits the imatinib-resistant KITT670I gatekeeper mutation in gastrointestinal stromal tumor. Clin Cancer Res 13(16):4874–4881
80. Wiebe L, Kasza K, Maki RG (2008) Sorafenib is active in patients with imatinib and sunitinib-resistant gastrointestinal stromal tumors (GIST): a phase II trial of the University of Chicago Phase II Consortium. J Clin Oncol 26, 553 s Abstract 10502
81. Maki RG, D'Adamo DR, Keohan ML, Saulle M, Schuetze SM, Undevia SD et al (2009) Phase II study of sorafenib in patients with metastatic or recurrent sarcomas. J Clin Oncol 27(19): 3133–3140
82. Reichardt P, Montemurro M, Gelderblom H, Blay J et al (2010) Comparison of two doses of imatinib for the treatment of unresectable or metastatic gastrointestinal stromal tumors: a meta-analysis of 1,640 patients. Gastrointestinal Stromal Tumor Meta-Analysis Group (MetaGIST). J Clin Oncol 28(7):1247–1253
83. Dubreuil P, Letard S, Ciufolini M, Gros L, Humbert M, Casteran N et al (2009) Masitinib (AB1010), a potent and selective tyrosine kinase inhibitor targeting KIT. PLoS One 4(9):e7258
84. Soria JC, Massard C, Magne N, Bader T, Mansfield CD, Blay JY et al (2009) Phase 1 dose-escalation study of oral tyrosine kinase inhibitor masitinib in advanced and/or metastatic solid cancers. Eur J Cancer 45(13):2333–2341
85. Le Cesne A, Blay JY, Bui BN, Bouche O, Adenis A, Domont J et al (2010) Phase II study of oral masitinib mesilate in imatinib-naive patients with locally advanced or metastatic gastro-intestinal stromal tumour (GIST). Eur J Cancer 46(8):1344–1351
86. Drevs J, Hofmann I, Hugenschmidt H, Wittig C, Madjar H, Muller M et al (2000) Effects of PTK787/ZK 222584, a specific inhibitor of vascular endothelial growth factor receptor tyrosine kinases, on primary tumor, metastasis, vessel density, and blood flow in a murine renal cell carcinoma model. Cancer Res 60(17):4819–4824
87. Thomas AL, Morgan B, Horsfield MA, Higginson A, Kay A, Lee L et al (2005) Phase I study of the safety, tolerability, pharmacokinetics, and pharmacodynamics of PTK787/ZK 222584 administered twice daily in patients with advanced cancer. J Clin Oncol 23(18):4162–4171
88. Mross K, Drevs J, Muller M, Medinger M, Marme D, Hennig J et al (2005) Phase I clinical and pharmacokinetic study of PTK/ZK, a multiple VEGF receptor inhibitor, in patients with liver metastases from solid tumours. Eur J Cancer 41(9):1291–1299
89. Joensuu H, De Braud F, Coco P, De Pas T, Putzu C, Spreafico C et al (2008) Phase II, open-label study of PTK787/ZK222584 for the treatment of metastatic gastrointestinal stromal tumors resistant to imatinib mesylate. Ann Oncol 19(1):173–177
90. Mahalingam D, Swords R, Carew JS, Nawrocki ST, Bhalla K, Giles FJ (2009) Targeting HSP90 for cancer therapy. Br J Cancer 100(10):1523–1529
91. Nakatani H, Kobayashi M, Jin T, Taguchi T, Sugimoto T, Nakano T et al (2005) STI571 (Glivec) inhibits the interaction between c-KIT and heat shock protein 90 of the gastrointestinal stromal tumor cell line, GIST-T1. Cancer Sci 96(2):116–119
92. Bauer S, Yu LK, Demetri GD, Fletcher JA (2006) Heat shock protein 90 inhibition in imatinib-resistant gastrointestinal stromal tumor. Cancer Res 66(18):9153–9161
93. Li CF, Huang WW, Wu JM, Yu SC, Hu TH, Uen YH et al (2008) Heat shock protein 90 overexpression independently predicts inferior disease-free survival with differential expression of the alpha and beta isoforms in gastrointestinal stromal tumors. Clin Cancer Res 14(23):7822–7831
94. Wagner AJ, Morgan JA, Chugh R (2008) Results from phase 1 trial of IPI-504, a novel HSP90 inhibitor, in tyrosine kinase inhibitor-resistant GIST and other sarcomas. Journal of Clinical Oncology, 26, 553 s Abstract 10503
95. Lane AA, Chabner BA (2009) Histone deacetylase inhibitors in cancer therapy. J Clin Oncol 27(32):5459–5468
96. Kovacs JJ, Murphy PJ, Gaillard S, Zhao X, Wu JT, Nicchitta CV et al (2005) HDAC6 regulates Hsp90 acetylation and chaperone-dependent activation of glucocorticoid receptor. Mol Cell 18(5): 601–607
97. Liu Y, Tseng M, Perdreau SA, Rossi F, Antonescu C, Besmer P et al (2007) Histone H2AX is a mediator of gastrointestinal stromal tumor cell apoptosis following treatment with imatinib mesylate. Cancer Res 67(6):2685–2692
98. Muhlenberg T, Zhang Y, Wagner AJ, Grabellus F, Bradner J, Taeger G et al (2009) Inhibitors of deacetylases suppress oncogenic KIT signaling, acetylate HSP90, and induce apoptosis in gastrointestinal stromal tumors. Cancer Res 69(17):6941–6950
99. Floris G, Debiec-Rychter M, Sciot R, Stefan C, Fieuws S, Machiels K et al (2009) High efficacy of panobinostat towards human gastrointestinal stromal tumors in a xenograft mouse model. Clin Cancer Res 15(12):4066–4076
100. Bauer S, Parry JA, Muhlenberg T, Brown MF, Seneviratne D, Chatterjee P et al (2010) Proapoptotic activity of bortezomib in gastrointestinal stromal tumor cells. Cancer Res 70(1):150–159
101. Van Oosterom AT DH, Desai J, Stroobants S, Van Den Abbeele AD, Clement P, Shand N, Kovarik J, Tsyrlova A, Demetri GD (2004) Combination signal transduction inhibition: a phase I/II trial of the oral mTOR-inhibitor everolimus (E, RAD001) and imatinib mesylate (IM) in patients (pts) with gastrointestinal stromal tumor (GIST) refractory to IM. J Clin Oncol, 2006 ASCO Annual Meeting Proceedings Part I, vol 24, No 18S (June 20 Supplement), 2006: 9545
102. Dumez H, Reichard P. Blay JY (2008) A phase I-II study of everolimus (RAD001) in combination with imatinib in patients (pts) with imatinib-resistant gastrointestinal stromal tumors (GIST). CRAD001C2206 Study Group. J Clin Oncol, 26, 557 s Abstract 10519
103. Fabbro D, Ruetz S, Bodis S, Pruschy M, Csermak K, Man A et al (2000) PKC412–a protein kinase inhibitor with a broad therapeutic potential. Anticancer Drug Des 15(1):17–28
104. Debiec-Rychter M, Cools J, Dumez H, Sciot R, Stul M, Mentens N et al (2005) Mechanisms of resistance to imatinib mesylate in gastrointestinal stromal tumors and activity of the PKC412 inhibitor against imatinib-resistant mutants. Gastroenterology 128(2):270–279
105. Reichardt P PD, Lindner T, Heinrich MC, Cohen PS, Wang Y, Yu R, Tsyrlova A, Dimitrijevic S, Blanke C (2005) 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. 2005 ASCO Annual Meeting Proceedings, vol 23, No 16S, Part I of II (June 1 Supplement), 3016
106. Palassini E FE, Coco P, Piovesan C, Dileo P, Bertulli R, Casali PG (2008) Combination of PKC412 and sirolimus in a metastatic patient with PDGFRA-D842 V gastrointestinal stromal tumor (GIST). J Clin Oncol 26 (May 20 suppl; abstr 21515)
107. Trent JC, Ramdas L, Dupart J, Hunt K, Macapinlac H, Taylor E et al (2006) Early effects of imatinib mesylate on the expression of insulin-like growth factor binding protein-3 and positron

emission tomography in patients with gastrointestinal stromal tumor. Cancer 107(8):1898–1908
108. Pantaleo MA, Astolfi A, Di Battista M, Heinrich MC, Paterini P, Scotlandi K et al (2009) Insulin-like growth factor 1 receptor expression in wild-type GIST: a potential novel therapeutic target. Int J Cancer 125(12):2991–2994
109. Braconi C, Bracci R, Bearzi I, Bianchi F, Sabato S, Mandolesi A et al (2008) Insulin-like growth factor (IGF) 1 and 2 help to predict disease outcome in GIST patients. Ann Oncol 19(7):1293–1298
110. Steigen SE, Schaeffer DF, West RB, Nielsen TO (2009) Expression of insulin-like growth factor 2 in mesenchymal neoplasms. Mod Pathol 22(7):914–921
111. Bolland M, Benn D, Croxson M, McCall J, Shaw JF, Baillie T et al (2006) Gastrointestinal stromal tumour in succinate dehydrogenase subunit B mutation-associated familial phaeochromocytoma/paraganglioma. ANZ J Surg 76(8):763–764
112. Neumann HP, Erlic Z (2008) Maternal transmission of symptomatic disease with SDHD mutation: fact or fiction? J Clin Endocrinol Metab 93(5):1573–1575
113. McWhinney SR, Pasini B, Stratakis CA (2007) Familial gastrointestinal stromal tumors and germ-line mutations. N Engl J Med 357(10):1054–1056
114. Pasini B, McWhinney SR, Bei T, Matyakhina L, Stergiopoulos S, Muchow M et al (2008) 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 16(1): 79–88
115. Janeway KA LB, Nose V, Hornick JL, Barretina J, Dahia PL, Fletcher JA (2009) Complete loss of succinate dehydrogenase b (SDHB) in pediatric gastrointestinal stromal tumors. Connective Tissue Oncology Society, abstract 39408