Curr Oncol Rep (2013) 15:386–395
DOI 10.1007/s11912-013-0316-6

SARCOMAS (SR PATEL, SECTION EDITOR)

Exploring Novel Therapeutic Targets in GIST: Focus on the PI3K/Akt/mTOR Pathway

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Published online: 20 April 2013

Abstract Gastrointestinal stromal tumors (GISTs) are the most common soft tissue sarcoma, and most feature abnormalities in two genes encoding the receptor tyrosine kinases (RTKs), *KIT*, and *PDGFRA*. The RTK inhibitor imatinib revolutionized treatment in GIST; however, drug resistance remains a challenge. Constitutive autophosphorylation of RTKs is linked to phosphatidylinositol 3-kinase (PI3K)/Akt/mammalian target of rapamycin (mTOR) pathway hyperactivation, which is central to oncogenic signaling, and known to be dysregulated in GIST. Preclinical experiments have confirmed that inhibiting the PI3K/Akt/mTOR pathway is a rational target for therapy. Early studies using mTOR inhibitors have shown limited success, which may be due to the activation of Akt that occurs following mTORC1 inhibition. Therefore, targeting PI3K or Akt, which lie upstream of mTORC1, may translate into more complete pathway inhibition. Several treatment strategies are currently being developed in phase 1 and 2 clinical trials. Compounds currently in development include pan-Class I PI3K inhibitors, dual PI3K/mTOR inhibitors, and Akt inhibitors. The aim of this review is to highlight the evidence for targeting PI3K/Akt/mTOR-dependent mechanisms in GIST and to evaluate the existing preclinical and clinical data supporting this strategy.

Keywords Akt · GIST · Imatinib · Inhibitor · KIT · mTOR · PDGFRA · PI3K · RTK · Targeted therapy

Introduction

Gastrointestinal stromal tumors (GISTs) represent about 18 % of all sarcomas [1], and are the most common mesenchymal tumors of the GI tract [2]. Nevertheless, GISTs are rare, only representing approximately 1 % of all GI tract malignancies, or approximately 10–20 cases per million individuals [3, 4]. GISTs are thought to originate from the interstitial cells of Cajal (or corresponding precursor cells), which play a role in the regulation of peristalsis [5]. GISTs originate predominantly in the stomach (70 %) and small intestine (10–25 %) [6], but can occur throughout the GI tract, including in the rectum and rarely in the esophagus or colon [7].

The characteristic ongogenic mechanism leading to GIST development is a gain-of-function mutation in one of two genes, *KIT* or *PDGFRA*, located on chromosome 4q12. These encode two type III receptor tyrosine kinases (RTKs), c-KIT (CD117) and platelet-derived growth factor receptor alpha (PDGFRA) [8, 9]. Natural/physiologic ligands for these receptors include stem cell factor (SCF) and PDGF, respectively. Early *in vivo* studies confirmed that gene-activating *KIT* mutations led to the development of GISTs in transgenic mice [10]. In humans, most GISTs (85 %) have a mutation in the *KIT* gene and, of the patients without a *KIT* mutation, one-third (5–7 % of all patients with GIST) have a mutation in the gene encoding PDGFRA [11]. Mutations in these genetic loci lead to ligand-independent autostimulation of the receptors, causing activation of pathways that inhibit apoptosis and promote cell proliferation and cell cycle activation, thus facilitating subsequent oncogenesis.

Advances in the understanding of GIST biology led to the development of treatments targeting known genetic abnormalities in *KIT* and *PDGFRA*. Originally developed to target the Bcr–Abl fusion protein in chronic myeloid leukemia, imatinib mesylate (imatinib; Gleevec®/Glivec®) was introduced as a novel treatment for GISTs in 2001 and has revolutionized therapeutic strategies for this disease. Imatinib targets multiple RTKs and binds the adenosine triphosphate (ATP)-docking region of c-KIT and PDGFRA, thus preventing the transfer of phosphate groups from ATP to the receptors themselves, thereby hindering the activation of their intracellular substrates.

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In 2008, the US Food and Drug Administration (FDA) converted a prior accelerated approval of imatinib to full (regular) approval for the treatment of patients with c-KIT-positive unresectable and/or metastatic malignant GISTs [12].

Prior to the introduction of imatinib, there were few options for patients with inoperable or recurrent GIST following surgery, since radiotherapy and conventional chemotherapy have generally proven ineffective at tackling these types of sarcoma [13, 14]. However, even with the advent of imatinib, median progression-free survival (PFS) remains at approximately 2 years, and secondary resistance to imatinib after showing some initial benefit is seen in 40–50 % of patients within 2 years of therapy [15]. Patients who are resistant or intolerant to first-line imatinib treatment may be treated with the multikinase inhibitor sunitinib malate (sunitinib; Sutent®), an FDA-approved second-line therapy for GIST [16]. In a phase 3 study, the clinical benefit rate of sunitinib was found to be 65 % (7 % partial response, 58 % stable disease) [17]. However, the duration of benefit was limited and resistance was fairly universal. Specifically, sunitinib has been reported to show limited activity against *KIT* exon 17 and *PDGFRA* exon 18 mutant phenotypes [18].

Disease progression in patients with GISTs treated with imatinib and sunitinib is usually due to a variety of resistance mechanisms. Primary resistance, defined as progression within the first 3–6 months, occurs in approximately 10–15 % of patients treated with imatinib [19, 20]. This is mainly due to the presence of pre-existing factors, including mutational status, and is associated with persistent c-KIT phosphorylation and activation of downstream mediators such as Akt [21]. Primary resistance is most often associated with *KIT* exon 9 mutations and *KIT* wild-type GISTs, which happen to be less sensitive to imatinib treatment. Similarly, the most frequent *PDGFRA* mutation in GISTs, D842V on exon 18, also exhibits primary resistance to imatinib [21, 22••]. On the other hand, secondary (acquired) imatinib resistance has been detected in up to 70 % of patients and is most commonly the result of additional mutations acquired in the KIT or PDGFRA kinase domain after the induction of therapy [23–26]. Other potential mechanisms of resistance to RTK inhibitors include downregulation or loss of c-KIT and protein kinase C theta (PKCθ) activation [27], and overexpression of insulin-like growth factor receptor 1 (IGFR1), which is most frequently seen in wild-type GIST [28].

Although imatinib has clearly extended the survival of patients with metastastic GIST, median overall survival is only 5 years [29]. Therefore, despite the benefit of existing targeted therapies for many patients with GIST, there remains an unmet medical need for more effective treatments with longer-term benefit and, preferably, a curative potential. Considering the complexity of primary and acquired genomic mutations that drive drug resistance, novel compounds that target RTK receptors alone may not be capable of overcoming all of the different resistance mechanisms to current therapies, thus a different strategy is warranted.

RTKs such as c-KIT and PDGFRA control a diverse array of intracellular mediators. In particular, some signaling pathways activated downstream of c-KIT have been the subject of intense investigation to identify new targets for drug development, especially for GISTs that are resistant to RTK inhibitors. These include the phosphatidylinositol 3-kinase (PI3K)/Akt/mammalian target of rapamycin (mTOR), Ras/RAF/ERK, JAK/STAT, and Src kinase pathways. Despite their diverse regulatory capacity, these pathways are central to oncogenic signaling since they are capable of promoting cell proliferation and dampening the apoptotic process.

Of particular interest is the PI3K/Akt/mTOR pathway, which is frequently activated in GIST. This pathway is thought to be linked with imatinib resistance [21], and has been shown to be involved in oncogenesis and tumor progression at various disease stages [30]. Constitutive activation of c-KIT through autophosphorylation has been linked to dysregulated PI3K/Akt/mTOR pathway signaling [30]; therefore, the PI3K/Akt/mTOR pathway represents an attractive target in cancer therapy for drugs used in combination with upstream RTK inhibitors such as imatinib. There are several classes of drugs in phase 1–2 trials that target elements of this pathway. Compounds currently in development include pan-Class I PI3K inhibitors, dual PI3K/mTOR inhibitors, and Akt inhibitors.

The aim of this review is to highlight the evidence for PI3K/Akt/mTOR-dependent mechanisms in the pathophysiology of GIST and describe how this pathway links to disease-specific biomarkers, in particular the c-KIT and PDGFRA signaling cascades. The review will address the rationale for therapeutic targeting of the PI3K/Akt/mTOR pathway in GIST, and evaluate the existing preclinical and clinical data supporting this strategy.

PI3K/Akt/mTOR Signaling Pathway in GIST

The PI3Ks are a family of lipid kinases involved in the early stages of a signaling cascade intimately linked to processes that govern cell survival, proliferation, and differentiation [31]. The PI3K family consists of three classes, characterized according to their structural homology and substrate specificity [32, 33]. Class I PI3Ks are further categorized into two groups, Class IA and IB, with Class IA being the most frequently implicated in cancer [34]. Class I PI3Ks are heterodimers that comprise separate catalytic (p110) and regulatory (p85) subunits. A set of regulatory monomers are encoded by the PI3K regulatory subunit genes *PIK3R1*, *PIK3R2*, and *PIK3R3*, giving rise to five p85 isoforms: p85α (including splice variants p55α and p50α), p85β, and p55γ. The Class I

PI3K catalytic subunit is made up of three p110 isoforms including p110α, p110β, and p110δ, which are derived from *PIK3CA*, *PIK3CB*, and *PIK3CD*, respectively [35].

Class IA PI3Ks are activated by RTKs, G-protein-coupled receptors, and a number of oncogenes such as Ras (Fig. 1). Following RTK phosphorylation, PI3K is recruited to the internal leaflet of the plasma membrane, anchoring to the tyrosine phosphate residues of RTKs via its p85 regulatory subunit [36]. Here the PI3K catalytic subunit is disinhibited allowing PI3K to, in turn, phosphorylate phosphatidylinositol 3,4,5-bisphosphate (PIP_2) to yield phosphatidylinositol 3,4,5-trisphosphate (PIP_3). The tumor suppressor phosphatase and tensin homolog (PTEN) acts to negatively regulate the pathway by dephosphorylating PIP_3 to form PIP_2 [37]. Prior generation of PIP_3 by PI3K, however, results in translocation of the serine–threonine protein kinase Akt to the plasma membrane. Akt indirectly activates mTOR, which alongside the regulator-associated protein of mTOR (raptor) and other complementary proteins form mTOR complex 1 (mTORC1). mTORC1 activation is initiated through the Akt-mediated inhibition of tuberous sclerosis 2 (TSC2), allowing Ras homolog enriched in brain (Rheb) accumulation and mTORC1 functional activation [38]. mTORC1 has been shown to subsequently activate downstream S6 kinase 1 (S6K1) and S6K2, leading to increased protein synthesis, cell survival, and growth signals [39–41], which are key steps required for sustained tumor growth. In addition, S6K plays an important role in the inhibition of the PI3K/Akt/mTOR pathway, and is implicated in a negative feedback mechanism to regulate PI3K activation and block mTORC2. mTORC2, a regulatory protein complex that principally comprises mTOR and the rapamycin-insensitive companion of mTOR (rictor), facilitates the activation of Akt [42]. Thus, blocking mTORC2 allows the control of a critical arm of the pathway aimed at preventing Akt

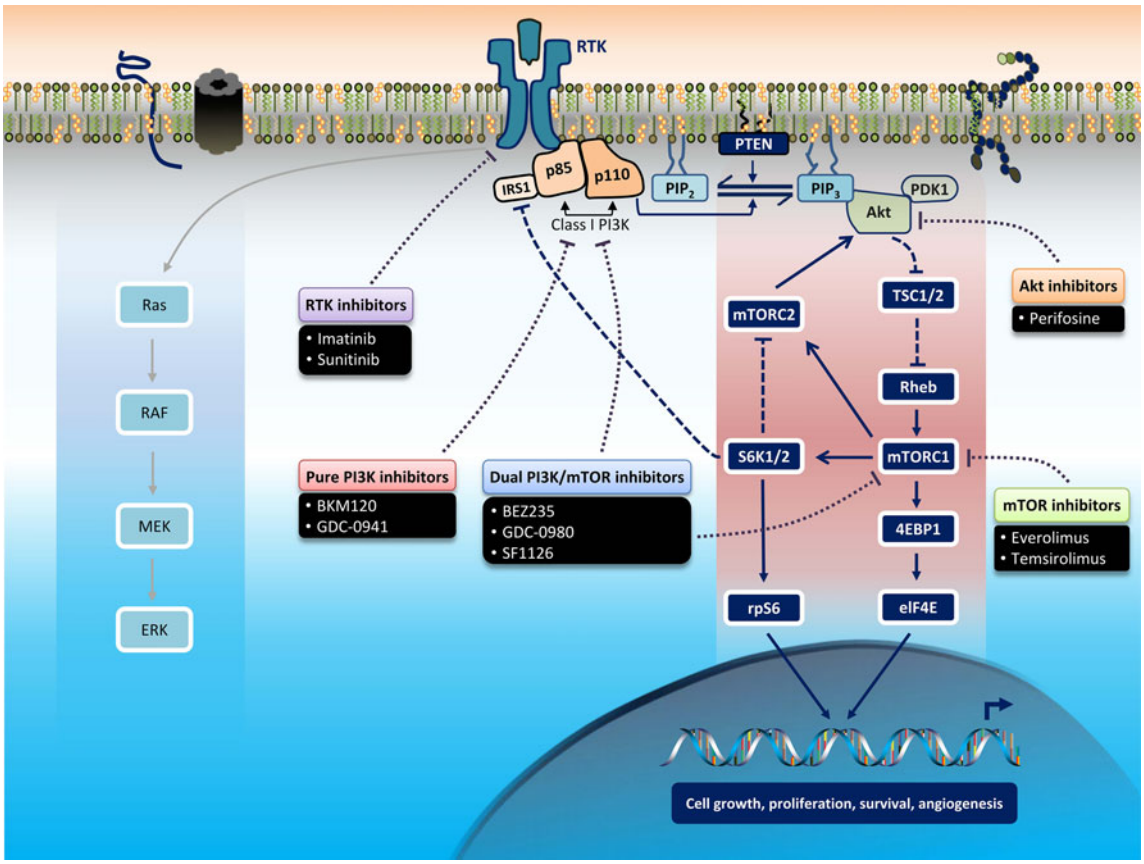

Fig. 1 The PI3K/Akt/mTOR pathway and inhibitors in clinical development. List of inhibitors of the PI3K/Akt/mTOR pathway in clinical trials used for treatment of gastrointestinal stromal tumors. Solid lines represent activating actions, hyphen-dashed lines represent inhibitory actions by natural molecules, and dotted lines represent inhibitory actions exerted by pharmaceutical agents. *4EBP1* factor 4E binding protein 1; *Akt* also protein kinase B (PKB); *ERK* extracellular signal-related kinase; *IRS1* insulin receptor substrate 1; *MEK* mitogen-activated protein/ERK kinase; *mTOR* mammalian target of rapamycin; *mTORC* mTOR complex; *PDK1* pyruvate dehydrogenase lipoamide kinase isozyme 1; *PI3K* phosphatidylinositol 3-kinase; *PIP_2* phosphatidylinositol 4,5-bisphosphate; *PIP_3* phosphatidylinositol 3,4,5-trisphosphate; *PTEN* phosphatase and tensin homolog; *RAF* RAF proto-oncogene serine/threonine protein kinase; *Ras* rat sarcoma oncogene; *Rheb* Ras homolog enriched in brain; *rpS6* ribosomal protein S6; *RTK* receptor tyrosine kinase; *S6K* ribosomal S6 kinase; *TSC1/2* tuberous sclerosis protein

signaling. These steps alongside Ras/RAF/MEK/ERK signaling are vital processes in oncogenesis and present a potentially fruitful area of investigation in GIST therapy.

Genetic Alterations of the PI3K/Akt/mTOR Pathway

In GIST, the core mechanism of PI3K/Akt/mTOR pathway activation is through mutation-induced stimulation of the upstream RTKs c-KIT and PDGFRA [43••]. In a recent study of 108 patients with GIST, downstream mTOR activation, detected by phosphorylation of S6 and factor 4E binding protein 1 (4EBP1), was present in 38 % and 83 % of *KIT*- and *PDGFRA*-mutated tumor samples, respectively [44]. Interestingly, pathway activation was also identified in 74 % of *KIT* and *PDGFRA* wild-type tumor samples [44], suggesting that hyperactivation of the PI3K/Akt/mTOR pathway also plays a frequent role in GIST cells that arise through alternate molecular aberrations, and that inhibition of the PI3K pathway may be therapeutically useful in tumors with primary resistance.

Genetic alterations in the PI3K/Akt/mTOR pathway, including *PI3KCA*-activating mutations, amplification of *PIK3CA*, and mutations of *PTEN* or loss of PTEN protein expression have been well documented in some cancers [38, 45, 46]. By contrast, relatively little is known about PI3K/Akt/mTOR pathway alterations in GIST, especially in those tumors lacking *KIT* or *PDGFRA* mutations [47]. In a small study of 65 GIST samples, mono-allelic loss of *PTEN* was frequently observed, but mutation or epigenetic silencing of *PTEN* was not evident [48]. Recently, in an effort to identify novel pathogenic mutations in GIST, Daniels *et al.* identified a *PIK3CA* mutation at H1047L, which coincided with a 15-bp deletion in *KIT* exon 11 [47]. This was the first *PIK3CA* mutation identified in GIST and this discovery supports the need for further research into the frequency of aberrations in PI3K pathway elements in this disease.

Involvement of the PI3K/Akt/mTOR Pathway in Advanced and Resistant GIST

The PI3K/Akt/mTOR pathway is highly active in imatinib-resistant GISTs, usually as a result of secondary mutations in the *KIT* or the *PDGFRA* kinase domains [49••]. In an analysis of tumor samples from 32 patients with GISTs, expression of phosphorylated KIT (p-KIT), phosphorylated Akt (p-Akt), proliferating cell nuclear antigen (PCNA), and B-cell lymphoma 2 was shown to be elevated in imatinib-resistant GISTs compared with samples from imatinib-responsive patients [49••]. By contrast, expression of total c-KIT protein, mitogen-activated protein kinase (MAPK), p-MAPK, and p-mTOR was comparable in all GIST samples, suggesting that the PI3K/Akt pathway may be a more relevant therapeutic target than the MEK/MAPK pathway in imatinib-resistant GIST patients with a secondary *KIT* mutation [49••].

In a preclinical investigation, Western immunoblotting and protein activation assays were used to evaluate the effects of inhibiting pathways downstream of c-KIT in imatinib-sensitive, imatinib-resistant/*KIT* mutant, and wild-type GIST cell lines [30]. The authors observed a complete loss of 4EBP1 phosphorylation following PI3K inhibition (using LY294002) in all cell lines evaluated, whereas blockade of c-KIT and mTOR by imatinib and everolimus, respectively, only led to a partial (0–50 %) decrease in 4EBP1 phosphorylation, demonstrating incomplete suppression of mTOR signaling [30]. Additional cell viability studies confirmed this, as PI3K inhibition induced cell growth arrest and apoptosis in all cell lines, including those that were imatinib resistant. This was in contrast to inhibition of mTOR or MEK, which only resulted in minor anti-proliferative effects, and inhibition of STAT or phospholipase C gamma (PLCγ), with no effects on cell proliferation [30]. The PI3K/Akt/mTOR pathway was, therefore, considered to be of greater importance in resistant GIST than other pathways downstream of c-KIT or PDGFRA [30, 49••].

A small body of evidence suggests that PTEN loss may play a role in GIST progression and resistance to therapy. In an early study of PTEN expression in a small cohort of 21 patients with aggressive GIST, multivariate analysis revealed that underexpression or low immunoreactivity of PTEN by immunohistochemistry in these samples was a prognostic factor for malignancy [50]. Here, the authors showed that an increase of 25 % or more in PTEN low/negative cells was associated with aggressive disease [50]. Furthermore, in the aforementioned study of 65 GIST samples, PTEN loss was identified at a much higher frequency in metastatic or imatinib-resistant samples (16 % and 36 %, respectively) than in primary samples (9 %) [48]. In preclinical experiments, imatinib- and sunitinib-resistant cell lines arising from long-term exposure to sunitinib were found to harbor epigenetic silencing of *PTEN*, with consequent PI3K/AKT pathway overactivation [51]. This suggests that in addition to *PTEN* deletion, *PTEN* silencing could also represent a potential mechanism for the development of resistance to RTK inhibition.

Targeting the PI3K/AKT/mTOR Pathway in GIST

There are several strategies under investigation focusing on PI3K/Akt/mTOR components in cancer (Fig. 1), with a number of inhibitors targeting different nodes of the pathway currently in clinical development for GIST (Table 1). First to be developed were the synthetic rapamycin derivatives such as everolimus, temsirolimus, and ridaforolimus, which allosterically inhibit mTORC1 (but not mTORC2) by binding to FKBP12, which lies adjacent to the catalytic site

Table 1 Clinical trials in GIST using drugs targeting elements of the PI3K/Akt/mTOR pathway

Intervention	Company	Trial phase	Trial number	Target	Status	Condition
BKM120 + imatinib	Novartis Pharmaceuticals	Phase 1b	NCT01468688	c-KIT + PI3K Class I	Recruiting	Third-line GIST
Everolimus + imatinib	Novartis Pharmaceuticals	Phase 2	NCT00510354	c-KIT + mTOR	Ongoing	Progressive GIST
Everolimus + imatinib	Novartis Pharmaceuticals	Phase 1–3	NCT01275222	c-KIT + mTOR	Completed	Resistant GIST
Temsirolimus	Wyeth/Pfizer	Phase 2	NCT00087074	mTOR	Completed	Soft tissue sarcoma or GIST
Perifosine + imatinib	Æterna Zetaris	Phase 2	NCT00455559	c-KIT + Akt	Completed	Resistant GIST

A list of clinical trials using inhibitors of the PI3K/Akt/mTOR pathway in solid tumors, including GISTs

Akt also known as protein kinase B (PKB); *c-KIT* tyrosine protein kinase KIT (CD117); *GIST* gastrointestinal stromal tumor; *mTOR* mammalian target of rapamycin; *PI3K*, phosphatidylinositol 3-kinase

of the complex [52, 53]. Dual PI3K/mTOR inhibitors such as BEZ235, GDC-0980, and SF1126 followed, and were developed to target the catalytic sites of both PI3K and mTOR, which share structural similarity [52]. Since dual PI3K/mTOR inhibitors directly bind the catalytic sites of their targets, they inhibit all Class IA PI3Ks and both mTORC1 and mTORC2 [54•]. Subsequent advances in drug design led to the development of "pure" PI3K inhibitors or "pan-Class I" PI3K inhibitors, such as BKM120 and GDC-0941, which show selectivity for the catalytic sites of Class I PI3K only, and not for mTOR [52]. Finally, in addition to inhibitors of PI3K and/or mTOR, agents that target Akt have also been developed, such as the oral alkylphosphocholine compound perifosine [55, 56]. In the following sections, the preclinical and clinical evidence supporting the use of these agents in GIST are described.

Rapamycin Analogs: Inhibitors of mTOR

Imatinib and everolimus have demonstrated enhanced, synergistic tumor responses in murine GIST xenografts [57]. When measuring fluorodeoxyglucose (FDG) uptake by FDG positron emission tomography (PET), combination treatment revealed superior antitumor effects when both drugs were used together compared with either drug given as a single agent; however, no evidence was available to assess PI3K or Akt activation levels [57]. Despite this demonstration of synergy, other preclinical experiments suggest that it remains unclear whether the addition of everolimus is capable of fully overcoming resistance to imatinib. For example, preclinical studies using GIST cell lines showed that the combination of imatinib and everolimus had additive effects in imatinib-sensitive cell lines, but not in imatinib-resistant lines [30].

Clinical results with rapamycin analogs in imatinib-refractory GIST have been modest. A nonrandomized, open-label, phase 1/2 study evaluated the feasibility of everolimus plus imatinib as combined therapy for patients with advanced GIST who were refractory to imatinib [2]. In the arm consisting of first-line imatinib nonresponders who were treated with everolimus plus imatinib, 17 % of patients were progression-free after 4 months, and in those who had failed both imatinib and sunitinib or other RTK treatments, 37 % were deemed progression-free after 4 months [2]. In the intention-to-treat population, median PFS was 1.9 and 3.5 months and median overall survival was 14.9 and 10.7 months for the first- and second-line failures, respectively. The most common adverse effects in the phase 1 study included nausea, diarrhea, fatigue, and anemia, whereas in the phase 2 study, these included hypokalemia, anemia, lymphopenia, fatigue, and vomiting [2]. In a multicenter, single-arm phase 2 trial of everolimus and imatinib in imatinib-resistant GIST, the best response at 16 weeks was stable disease, which was observed in nine of 27 patients (33 %), including one patient with a *KIT* exon 12 mutation who was classified as progression-free for >33 months [58]. In another phase 2 study, a subgroup of patients with imatinib- and sunitinib-resistant GISTs treated with everolimus in combination with imatinib exhibited a disease control rate (complete response, partial response, or stable disease) of 27 % (n=15), again indicating that this combination may have moderate potential in this type of heavily-pretreated patient population [59].

The observation that rapamycin analogs showed limited activity in imatinib-resistant GIST models *in vitro* [30], and modest activity in the clinical trials described above could be attributed to paradoxical increases in Akt signaling, which occur following mTORC1 inhibition and the resulting abrogation of the S6K negative feedback loop (Fig. 1). Inhibition of mTORC1 prevents activation of the mTORC1 substrate S6K, stopping it from negatively regulating PI3K and Akt signaling via insulin receptor substrate 1 (IRS-1) and mTORC2, respectively [60]. Inhibiting mTORC1 and not mTORC2 may, therefore, lead to PI3K and Akt activation, promoting resistance to the effects of rapamycin analogs. In

preclinical experiments with GIST cell lines, blocking PI3K activity using LY294002 led to complete inhibition of 4EBP1 phosphorylation, whereas the combination of c-KIT and mTOR inhibitors (imatinib/everolimus) exhibited a 0–50 % reduction only [30]. Furthermore, PI3K inhibition (but not mTOR inhibition) led to apoptosis in imatinib-resistant cell lines, which also featured decreased Akt, S6, and 4EBP1 phosphorylation. Therefore, PI3K inhibition might be an effective strategy to overcome the challenge of pathway re-activation that follows mTORC1 inhibition. These compounds target events upstream of mTOR activation, by inhibiting the early step in PIP_3 generation at the plasma membrane, prior to Akt recruitment, phosphorylation, and downstream mTORC1 activation.

Pan-Class I PI3K Inhibitors

Combining imatinib with PI3K inhibitors facilitates the blockade of cell-surface c-KIT signaling and PI3K activation, the primary intracellular step in the pro-growth PI3K/Akt/mTOR pathway. This approach avoids the inhibition of the S6K negative feedback loop that occurs when directly inhibiting mTORC1 with rapalogs. Concomitant RTK/PI3K inhibition also ensures the prevention of PI3K-induced activation of Akt itself, which is often unaffected by treatment with rapamycin analogs. During *in vitro* studies, the pan-Class I PI3K inhibitor BKM120 combined with imatinib demonstrated antiproliferative effects in a panel of GIST cell lines including imatinib-resistant (GIST430) and imatinib-sensitive (GIST882) cell cultures [61]. Here, inhibition of cell growth was greater when the two compounds were used in combination compared with either drug used alone.

GDC-0941, another pan-Class I PI3K inhibitor, demonstrated pro-apoptotic effects *in vivo* when combined with imatinib in GIST xenograft models [62]. GDC-0941 alone showed minimal activity on cancer cell apoptosis and tumor volume, decreasing mitotic activity two-fold compared with untreated control. By contrast, the combination of GDC-0941 plus imatinib led to a 95 % decrease of tumor volume from baseline compared with imatinib alone (85 %). Furthermore, the combination of imatinib and GDC-0941 led to a 15-fold increase in apoptosis compared with untreated control, whereas single-agent imatinib only instigated a three-fold increase in apoptosis [62]. Interestingly, cell signaling studies showed that GDC-0941 and imatinib monotherapies only partially inhibited Akt activation, which may explain the comparably lower efficacy of the two agents when used alone. By contrast, GDC-0941 and imatinib in combination successfully abolished PI3K/Akt/mTOR pathway activation. Tumor growth recommenced rapidly following discontinuation of imatinib treatment alone, while growth control was greater in the dual-agent treatment group after cessation of treatment [62]. Collectively, these observations suggest that targeting c-KIT and PI3K together could potentially be a useful approach to therapy in GIST.

The potential use of a PI3K inhibitor plus imatinib will be explored in a multi-arm, open-label phase 1b study using the PI3K inhibitor BKM120 in patients with GIST who are refractory to imatinib and sunitinib (NCT01468688). This nonrandomized safety study will determine the maximum tolerated dose and/or recommended phase 2 dose of combined imatinib and BKM120 as third-line treatment for patients with GIST.

Dual PI3K/mTOR Inhibitors

A particular challenge with mTORC1 inhibitors involves the S6K and mTORC2 feedback loops that regulate the activity of PI3K and Akt, respectively. Dual PI3K/mTOR inhibitors could, therefore, offer a theoretical advantage over single PI3K and mTOR inhibitors as they may offer more complete blockade of the PI3K/AKT/mTOR pathway. In preclinical *in vitro* experiments, the dual PI3K/mTOR inhibitor BEZ235 exhibited single-agent antiproliferative effects in imatinib-sensitive cell lines with and without biallelic PTEN loss (GIST882LY and GIST882 cell lines, respectively) and in a cell line with the imatinib-resistant D820A kit mutation (GIST48) [48]. BEZ235 plus imatinib demonstrated synergistic effects in all three of these cell lines; however, the combination did not inhibit imatinib-resistant cells with c-KIT loss (GIST48B), which may suggest activation of alternate pathways in some resistant cell lines. The observation that BEZ235 was able to partially reduce Akt and S6 phosphorylation in GIST cells with either small-interfering RNA (siRNA) silencing of PTEN or biallelic loss of the corresponding gene suggests that PI3K inhibition may be a relevant strategy in GIST malignancies that exhibit these alterations.

A recent investigation employed bilateral c-KIT-characterized GIST xenografts in mice, which included independent, imatinib-sensitive (exon 11) and -resistant (exon 9) mutations [63]. Combination treatment with BEZ235 and imatinib led to complete inhibition of PI3K signaling in imatinib-resistant and also imatinib-sensitive GIST xenografts. BEZ235 and imatinib acted synergistically to increase apoptosis in imatinib-sensitive cells when compared with either compound alone. When used independently, BEZ235 and imatinib were able to stabilize tumor growth in both xenograft models, while their combination caused a 66 % reduction of tumor growth in imatinib-sensitive xenografts. Furthermore, continued growth inhibition was observed in imatinib-sensitive mice treated with imatinib and BEZ235, taking place even after treatment withdrawal [63].

SF1126, a novel compound that comprises LY294002 (an inhibitor of PI3K superfamily members, including PI3K and mTOR) linked to an RGD tripeptide, is able to deliver targeted therapy to tumor endothelia [64]. Early reports from a

phase 1 trial of 28 patients with advanced solid tumors treated with SF1126 showed it to be well tolerated, with only one dose-limiting toxicity observed (grade 3 diarrhea). Stable disease lasting ≥8 weeks was observed in 11 of 28 patients, and included three patients with GIST who had stable disease lasting 11–20 weeks [64]. Update reports should provide more conclusive data on all the patient subgroups included. More recently, a phase 1 dose-escalation study of the dual PI3K/mTOR inhibitor GDC-0980 in patients with various advanced solid tumors including breast, sarcoma, and GIST, reported three patients with GIST who showed ≥25 % decrease in FDG uptake as assessed by FDG-PET [65]. This also included a patient who exhibited a 58 % decrease in FDG uptake and who continued on the study for >6 months [65].

Akt Inhibitors

Akt is a key element of the PI3K pathway and central to many intracellular signaling cascades. To date, there is limited evidence, both preclinical and clinical, of Akt inhibitors in GIST; however, one phase 2 study investigated the Akt inhibitor perifosine in combination with imatinib in patients with imatinib-resistant disease [55]. Perifosine demonstrated good efficacy in wild-type c-KIT GIST with four of five patients demonstrating stable disease as per Response Evaluation Criteria In Solid Tumors [55]. Overall, however, the study treatment showed limited clinical activity, with stable disease identified as best response in 16 of 36 evaluable patients [55]. At the time of writing, it appears that the further pursuit of Akt inhibition as therapy for GIST is unlikely, given that no clinical trials using this intervention are underway.

Future Perspectives

Targeted therapy can potentially benefit 85 % of patients with GIST; however, resistance remains a problem for those treated with current first- and second-line therapies. The initial introduction of imatinib into the therapeutic armamentarium used for GIST treatment led to significant improvements in clinical benefit, but most patients still progress eventually. Multiple analyses of tumor samples from imatinib and sunitinib-resistant patients using molecular, fluorescence *in situ* hybridization, and histological methods have highlighted the variation of drug resistance mechanisms in GIST. Therapeutic decision making is, therefore, significantly complicated due to the presence of intra- and interlesional heterogeneity in resistance mechanisms [66].

The presence of primary mutations and the development of secondary mutations in *KIT* give rise to differential drug sensitivity in patients with GIST. Drug sensitivity due to these mutations varies between the drugs that are either currently available (imatinib, sunitinib) or are in advanced stages of development (sorafenib, nilotinib) [22••, 67]. Primary mutations tend to occur in loci encoding juxtamembrane regions of c-KIT, most frequently due to abnormalities in exons 9 and 11. On the other hand, secondary mutations are associated with the intracellular kinase domain and are present in exons 13, 14, 17, and 18. It is believed that different mutations result in conformational changes in the c-KIT protein thus affecting drug binding, and limiting the effectiveness of RTK inhibitors [67]. For example, sunitinib was shown to bind to the unactivated conformation of the c-KIT ATP-binding domain, thereby preventing c-KIT autoactivation [67]. One of the major limitations of targeting c-KIT is, therefore, its susceptibility to a diverse number of mutations, thus preventing c-KIT inhibitors from demonstrating prolonged antitumor effects. A strategy focusing on downstream direct PI3K pathway inhibition could potentially lead to a more comprehensive blockade of RTK signals compared with treatments that specifically target RTKs themselves.

As resistance to RTK inhibitors often stems from *KIT* and *PDGFRA* abnormalities in GIST, increased understanding of the signaling pathways downstream of mutant *KIT* and *PDGFRA* tyrosine kinases will enable more targeted treatment regimens to be developed. In the future, to maximize clinical efficacy and benefit, novel therapeutic strategies could be tailored according to appropriate biomarkers and patient-specific mutation profiles when these become available [68]. This has been particularly problematic in GIST as relatively little is known about mutations in wild-type and resistant GIST, and what is known is too complex and heterogeneous for any realistic personalization of therapy. Without a good understanding of the genetic abnormalities in GIST, it could be difficult to characterize novel biomarkers for future targeted treatments, such as PI3K inhibitors. The PI3K/Akt/mTOR pathway has been identified as an important mediator of GIST tumorigenesis, particularly in metastatic and resistant tumors, and components of the PI3K/Akt/mTOR pathway are, therefore, attractive therapeutic targets for clinical investigation. However, more work is required to establish whether specific molecular alterations (both in the PI3K pathway and in upstream RTKs) will predict a beneficial response to PI3K inhibitor-based therapy, not just in GIST, but in other tumor types as well [68].

Despite promising preclinical data, early studies of mTOR inhibitors in combination with imatinib in imatinib-resistant patients were disappointing, perhaps due to feedback activation of the pathway or a reliance on mTOR-independent pathways downstream of PI3K. Therefore, targeting the messengers upstream of mTOR may be a more effective approach in the future. More recent investigations have, therefore, focused on the potential of PI3K and Akt inhibitors in combination with imatinib, and are currently being investigated in early-stage clinical studies. In addition to PI3K inhibitors, agents that target other signaling pathways

downstream of c-KIT and PDGFRA, such as MEK or IGFR1 [28], could also prove useful surrogate targets. This could offset the current challenges of multiple drug resistance observed in GIST treatment, as tumor cells try to negate proliferative responses around the boundaries of current drug strategies.

Conclusions

The introduction of imatinib led to a significant improvement in the management of GIST. Despite this, resistance to RTK inhibitor treatment remains a problem with the majority of patients seeing disease progression as a result of a diversity of secondary mutations in *KIT* and *PDGFRA*. The PI3K/Akt/mTOR pathway plays a crucial role in driving the oncogenic disease process by facilitating tumor cell growth and proliferation. Components of this pathway are attractive targets for novel drug development. Several approaches are being developed to target the PI3K/Akt/mTOR pathway with inhibitors of PI3K itself, dual PI3K/mTOR inhibitors, and Akt inhibitors under investigation. These drugs may potentially offer more broad inhibition of pro-growth cellular mechanisms than single-agent RTK inhibitors. Early studies using mTOR inhibitors have shown limited success, which may be due to the reactivation of the PI3K pathway that occurs following mTORC1 inhibition. Therefore, targeting PI3K or Akt (which lie upstream of mTORC1), may translate into more complete pathway inhibition, and potentially, a greater anti-tumor effect. The evidence in this review highlights the need for further studies in this expanding area of research, warranting continued focus on PI3K/Akt/mTOR signaling and its potential as a therapeutic target in GIST.

Acknowledgments Financial support for medical editorial assistance was provided by Novartis Pharmaceuticals. Allan P. Kiprianos, PhD provided medical editorial assistance with this manuscript.

Conflict of Interest Shreyaskumar Patel has been a consultant for Novartis, Merck, GSK, and Johnson and Johnson and has received grant/research support from Infinity, Johnson and Johnson, PharmaMar, and Eisai.

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- • Of importance
- •• Of major importance

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