1. Introduction

The receptor tyrosine kinase (RTK), c-KIT, and other type III RTKs, PDGF receptor (PDGFR), Colony-stimulating factor-1 receptor (c-FMS) and FLT3 (Fms-like tyrosine kinase receptor-3) play important roles in cancer. KIT was first identified as a retroviral oncogene, as the product of the "white spotting" (W) locus in mice, and at the protein level, as a marker of human acute myeloid leukaemia (AML) and normal haemopoietic progenitor cells (reviewed [1]). The phenotype of the W mouse (characterised by white spotting of the coat, anaemia, lack of mast cells and infertility) and many subsequent studies on human tissues demonstrated the key functional role of KIT and its ligand, Stem Cell Factor (SCF) in haemopoiesis, melanogenesis and fertility (especially spermatogenesis). More recently KIT was shown to be necessary for the development and function of gut pacemaker cells, the Interstitial Cells of Cajal (ICC). This pattern of c-KIT expression and function has predicted the cancers in which KIT abnormalities, typically mutations leading to constitutive activation of the intrinsic kinase, are crucially involved (reviewed [2]).

Typical of the Type III RTK family, the KIT protein consists of an extracellular region made up of five immunoglobulin (Ig)-like domains, a single transmembrane domain, and an intracellular region including a split kinase domain and the "kinase insert domain" (KID) which binds to SCF. The KID contains additional activating phosphorylation sites and is a target for some inhibitors (reviewed [3]).

2. Mechanism of oncogenic KIT activation

Mutations in KIT are typically gain-of-function activating mutations, resulting in constitutive activation of the kinase and enhanced cell proliferation. KIT activation can also occur via other mechanisms, such as binding of the ligand, SCF, which is constitutively expressed in the gut, and other receptors and ligands, such as PDGF and c-FMS. In addition, mutations in other genes, such as PI3K and RAS, can also activate KIT downstream pathways (reviewed [4]).

3. Signalling pathways downstream of KIT

The activation of KIT results in a cascade of signal transduction pathways, including the Ras/PI3K/Akt and MAPK pathways, which are involved in cell proliferation, survival, invasion, and angiogenesis. The Ras/PI3K/Akt pathway is involved in cell proliferation, survival, invasion, and angiogenesis. The MAPK pathway is involved in cell proliferation, differentiation, and survival. The activation of these pathways results in the transcription of target genes, which can lead to tumour growth and progression.

4. KIT kinase inhibitors and primary resistance

KIT inhibitors, such as imatinib, sunitinib, and dasatinib, have been shown to be effective in treating cancers driven by KIT mutations. However, primary resistance can occur due to the presence of resistant mutations in the kinase domain of KIT, such as T670I and D816V. These mutations can prevent the binding of KIT inhibitors, resulting in primary resistance.

5. Secondary resistance to kinase inhibitory drugs

Secondary resistance to KIT inhibitors can occur due to the development of mutations in downstream pathways, such as PDGF or SCF, which can activate KIT. This can result in the development of acquired resistance to KIT inhibitors. In addition, other pathways, such as PI3K and AKT, can also become activated, leading to the development of resistance.

6. Clinical trials of KIT inhibitors

Clinical trials have shown that KIT inhibitors are effective in treating KIT-driven cancers, with imatinib being the first KIT inhibitor to be approved for the treatment of gastrointestinal stromal tumours (GIST). However, the development of resistance remains a major challenge in the treatment of KIT-driven cancers. Therefore, new strategies are needed to overcome resistance and improve patient outcomes.

7. New approaches to treatment of cancers driven by mutant KIT

New approaches to the treatment of cancers driven by mutant KIT include the use of combination therapies, such as KIT inhibitors in combination with other drugs, and the use of different KIT inhibitors with different mechanisms of action. In addition, new strategies, such as the use of immunotherapies, may also be effective in treating KIT-driven cancers.

8. Conclusion

In conclusion, KIT is an important target for the treatment of cancers driven by mutant KIT. However, the development of resistance remains a major challenge. Therefore, new strategies are needed to overcome resistance and improve patient outcomes.
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This box summarises key points contained in the article.

2. Mechanism of oncogenic KIT activation

2.1 Overview

The domain structure of KIT and the location of some common mutations are illustrated in Figure 1. In the normal course of events ligand binding triggers receptor dimerisation, relief of auto-inhibitory interactions and trans-phosphorylation within dimer pairs followed by recruitment, phosphorylation and activation of downstream signalling proteins. There are multiple sites of KIT mutation in cancers, with some “hot-spots” corresponding to the intracellular and extracellular juxtamembrane domains (exons 8, 9 and 11) and the activation loop of the kinase domain (exon 17) which lead to disruption of auto-inhibitory mechanisms. It is noteworthy that the common sites of KIT mutation differ markedly between cancers. This may reflect differential effects of the various mutations on downstream signalling pathways (Section 3).

The extracellular juxtamembrane domain consisting of the fourth and fifth Ig-like loops is involved in correctly orienting receptor monomers and stabilising dimers induced by binding of dimeric SCF [17]. Specifically, this region, encoded by exons 8 and 9, directly mediates dimer interactions. Exon 8 is an important mutation site in AML and results in small deletions/substitution usually involving D419 [18]. Exon 9 mutations, most commonly an AY insertion [19], occur in about 10% of GIST cases and may act by a similar mechanism. The mechanisms of KIT activation by exon 11 and 17 mutations of paediatric cases had KIT mutations [10,11]. A higher proportion of non-CBF AML cases (~ 30% of all AML) display activating mutations in the closely related RTK, FLT3.

As predicted, activating KIT mutations have also been observed in germ cell cancers. In testicular seminomas frequencies of up to 26% have been reported [12]. KIT mutations also occur in about 30% of cases of unilateral ovarian dysgerminomas but not other histological types [13].

Activating KIT mutations and amplifications have been demonstrated in melanoma at relatively low frequency (approximately 5%) and mostly in those occurring at accral, mucosal or chronic sun damaged sites [14]. A much larger proportion of melanomas have mutations in B-Raf, which is a downstream effector of KIT and would provide a similar proliferative stimulus [15]. Interestingly, earlier studies indicated that, in the majority of melanomas, KIT expression is lost on progression [16] indicating distinct roles for KIT in different types of melanoma.

While KIT is now known to also be expressed in other tissues, for example, vascular endothelial cells, astrocytes, renal tubules, breast glandular epithelial cells and sweat glands, recurrent mutations have not been described in corresponding cancers and early studies have generally failed to show efficacy of KIT inhibitors in these cancers. This is in accord with the concept that the target must be a “driver” of the cancer (e.g., as evidenced by mutation or over-expression) to be a useful therapeutic target.

auto-regulatory domains. Activating mutation of KIT was first reported in the human mast cell leukaemia line, HMC-1 [9]. Two mutations in the same allele, leading to amino acid substitutions V560G and D816V in the juxta-membrane auto-regulatory domain and the kinase domain, respectively, were reported. Each mutation was capable of causing ligand-independent KIT activation as demonstrated by tyrosine auto-phosphorylation and promotion of factor-independent growth of murine Ba/F3 cells. Activating mutations of KIT are now known to occur in almost all cases of systemic mastocytosis (SM) and are often present in other haemopoietic lineages in these patients indicating that the target may be in the stem cell compartment [4].

The most striking involvement of KIT is in gastrointestinal stromal tumours (GIST) which are derived from the ICC. Activating mutations of KIT in GIST were first reported by Hirota and co-workers [5]. It is now known that more than 80% of cases display KIT mutations resulting in constitutive activation, while a smaller proportion (~ 6%) of cases have mutations in the closely related PDGFR [6]. Use of KIT kinase inhibitors has provided a paradigm shift in treatment of these cancers.

In AML KIT mutation is relatively uncommon and is confined to Core Binding Factor (CBF) leukaemias which comprise around 17% of AML and are characterised by t(8;21) or inv(16) [7]. These translocations/inversions result in fusion genes such AML-ETO and CBFB-MYHII, respectively, leading to impaired differentiation, while subsequent KIT mutation provides a proliferative and survival advantage [8,9]. Since KIT expression is down-regulated during normal myelopoiesis, any disease resulting from activating mutation of KIT might be self-limiting in the absence of a differentiation block. This is in contrast with the mast cell lineage where KIT continues to be highly expressed in mature cells. Recent large studies in CBF AML indicate that around 37% of adult cases and 19% of pediatric cases had KIT mutations [10,11]. A higher proportion of non-CBF AML cases (~ 30% of all AML) display activating mutations in the closely related RTK, FLT3.

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are discussed in Sections 2.2 and 2.4 in the context of GIST and haematological malignancies, respectively.

2.2 GIST

The major region of KIT mutation in GIST is within exon 11 occurring in about 65% of patients [19]. This exon encodes the intracellular juxtamembrane domain (JMD), a key autoregulatory domain of RTKs which stabilises the inactive conformation of the kinase domain in which the “activation loop” is incorrectly positioned for catalysis [20]. Crystal structure analysis of KIT has shown that in the absence of ligand the JMD folds back into the active site of the kinase (Figure 2) [21]. Several active and inactive conformations of KIT, like other RTKs, are believed to exist in equilibrium such that in the absence of ligand the inactive conformations are predominant and the receptor has very low basal activity. Receptor dimerisation following ligand binding allows this basal activity to bring about transphosphorylation of Y568 and Y570 residues in the JMD releasing its interaction with the kinase domain and promoting the fully active conformation [20,22]. Disruption of the JMD by mutation is believed to lead to activation of the kinase by removal of this autoinhibition. In GIST there are many different exon 11 mutations including point mutations, tandem duplications, deletions and insertions and, while the precise mechanisms may differ, these are all believed to act by preventing the interaction of the JMD with the active site cleft [6]. As an example, from the crystal structure of autoinhibited KIT (PDB 1T45; [21]) it can be seen that the V560G substitution [23,24] would result in the loss of multiple hydrophobic interactions (e.g., with N787, I789 and F848) required to stabilise binding of the JMD to the kinase domain.

2.3 Melanoma

The most common KIT mutations found in melanoma are L576P and K642E, encoded within exons 11 and 13, respectively [14]. These substitutions have also been reported in GIST. From the crystal structure of autoinhibited KIT [21],
it is apparent that the side chain of residue K642 forms an H-bond with the backbone of T574 which stabilises the autoinhibited structure. L576 stabilises a very short helix in the JMD, which would be disrupted upon mutation to a proline. Additionally, the L576 sidechain makes hydrophobic contacts with the αC helix (V643, Y646). As above, mutation of either residue is expected to favour kinase activation.

2.4 Haematological malignancies
In haemopoietic malignancies, as well as germ cell tumours, the main region of mutation is exon 17 which codes for the activation loop of the kinase domain. This region undergoes a major conformational shift on kinase activation (Figure 2).

In the inactive state the activation loop obstructs access of substrates to the active site and the DFG sequence at its N-terminal end is incorrectly aligned for its role in catalysis. Relief of JMD autoinhibition results in kinase activation by greatly favouring conformations in which the activation loop is shifted away from the active site and the DFG motif adopts a position enabling correct alignment of ATP and catalysis [25]. The active conformation may be stabilized by phosphorylation of Y823 [22,26]. In systemic mastocytosis (SM) the predominant activation loop mutation results in substitution of D816 usually by V but sometimes by H or Y. In CBF AML, D816 and N822 substitutions are common activating mutations [27]. The D residue corresponding to position 816 in KIT is highly conserved in RTKs indicating a key functional role and cell line studies have indicated that the loss of D was important, rather than the residue that replaced it. Early molecular modelling work indicated that substitution of D816 in KIT destabilised intramolecular interactions in the inactive conformation of the kinase domain and markedly favoured the active conformation [28]. More recently kinetic studies demonstrated that kinase domain activation is strikingly enhanced in D816H mutant KIT compared to WT [29]. This group showed that the D816H substitution also destabilises the interaction of the JMD with the kinase domain.

3. Signalling pathways downstream of KIT

3.1 Signalling by WT KIT
Once activated, KIT autophosphorylates on multiple Y residues which serve as docking sites for downstream effectors.
Several pathways act downstream of KIT to affect cell survival and proliferation [30]. Src family kinases (SFK), the p85 subunit of phosphatidylinositol 3-kinase (PI3K), phospholipase C-gamma and adapters that lead to activation of MAP kinase pathways are directly recruited and activated by binding to phospho-Y residues on the receptor.

### 3.2 Signalling by mutant KIT

The different types of KIT mutation associated with particular cancers may be a consequence of their differential effects on signalling pathways downstream of KIT- and/or tissue-specific requirements for particular pathways. Mouse knock-in experiments, in which the wild type Kit alleles were replaced by alleles coding for receptor lacking recruitment sites for particular downstream effectors, resulted in tissue-selective consequences (e.g., [31]). It has also been shown that various mutations have differential effects on the activation of signalling pathways downstream of KIT. For example, the MAPK pathway is activated by WT KIT following SCF stimulation, and by mutant KIT in primary GISTs and cell lines [32], but not in AML cells or immortalised early myeloid cells expressing D816 mutant KIT where PI3K and/or STAT3 activation appear to be key effectors [33,34]. In contrast, STAT3 is not activated by WT KIT signalling [34]. Whereas WT KIT is mostly located on the cell surface, the D816 mutant is largely in intracellular compartments [35] which may account for its ability to activate STAT3. Others have shown that STAT3 activation by another RTK, c-Met, depends on trafficking to an endosomal compartment [36]. Recruitment and activation of SFK are of central importance in KIT signalling, but this requirement is overcome by the D816V mutation which confers a Src-like substrate specificity on KIT itself [37]. Alteration of substrate specificity by the corresponding mutation in murine Kit, D814V, was also reported [38].

### 4. KIT kinase inhibitors and primary resistance

#### 4.1 Overview - imatinib resistance

Inhibition of the tyrosine kinase activity of the BCR/ABL oncoprotein by imatinib (Gleevec®) in chronic myeloid leukaemia (CML) has revolutionised the treatment of this disease [39]. Although imatinib is highly selective compared with other tyrosine kinase inhibitors, it also potently blocks the activity of KIT and PDGFR, suggesting a potential role in treatment of cancers driven by mutant forms of these receptors. At an early stage of evaluation it was noted that clinically relevant KIT mutants differed greatly in their sensitivity to imatinib. Specifically, substitutions at position D816 in the activation loop rendered the kinase almost completely resistant to the drug at clinically achievable doses [23,40]. This likely reflects the mutation strongly favouring the active conformation of the KIT kinase domain to which, as in the case of BCR/ABL, imatinib cannot bind [21,41]. In contrast, an exon 11 mutation resulting in the substitution V560G enhanced sensitivity to imatinib by more than 10-fold [23]. Similar results were obtained with another JMD mutant [42]. These observations have striking implications for the application of imatinib in GIST and SM in particular.

#### 4.2 Imatinib in treatment of GIST

The action of imatinib in the treatment of GIST has been extensively evaluated and it is currently the first line treatment for metastatic disease and in an adjuvant setting for prevention of relapse of poor prognosis GIST following surgical resection (reviewed [43,44]). As outlined above, more than 80% of GISTs have activating mutations in KIT and a further ~5% have mutations in PDGFR. When patients with metastatic disease were treated with imatinib (400 mg/day) striking responses were observed with increased relapse-free or progression-free survival (discussed further in Section 6.1). An interesting difference was noted between patients with KIT mutations in exon 11 (~65%) and those with mutations in exon 9 (~10%), with the former group having stronger, more durable responses [19]. Responses of the exon 9 mutant group could be improved by increasing the imatinib dose to 800 mg/day [45]. These results are likely to reflect the difference in kinase activation mechanisms between the two classes of mutant described above. Exon 9 mutations affect an early stage of receptor activation and probably act by mimicking the action of the ligand. Thus they would be expected to respond to imatinib in a similar way to wild-type (WT) KIT. In contrast, exon 11 mutants probably act, in general, by releasing the kinase domain from auto-inhibition by the JMD. Importantly, this autoinhibitory mechanism also interferes with imatinib binding [21]. Hence JMD mutations such as V560G enhance imatinib inhibition and clinical responses. Similar enhancement of kinase inhibition is seen in cells expressing V560G KIT with the imatinib-related second generation inhibitor, nilotinib [24].

#### 4.3 Imatinib in treatment of SM and AML

Imatinib has also been evaluated for treatment of SM which is typically characterised by activation loop mutations in KIT, particularly the D816V substitution [46]. As stated above, this mutant form of KIT is highly resistant to the drug and most cases have proved refractory. Imatinib has also been tested without success in AML. In some instances, this was probably due to the use of unselected cases since most would not have had KIT mutations. CBF leukaemias frequently display KIT mutations in exon 8, usually involving D419, or in exon 17, most commonly affecting D816 or N822, but not in exon 11 [47]. None of these mutant forms display the enhanced imatinib sensitivity of exon 11 mutant KIT, however the N822K mutant and those involving D419 have similar sensitivity to WT KIT [18,27], suggesting that detection of CBF AML patients with these mutations may select a subgroup of patients likely to respond.
4.4 Newer KIT inhibitors

To approach the primary imatinib resistance of D816 mutant KIT that commonly occurs in SM and also in AML, melanoma and germ cell tumours, newer drugs have been evaluated. These include multi-targeted inhibitors, dasatinib and PKC412, both of which can bind to the active conformation of the kinase domain favoured by D816 mutants. Dasatinib is also a potent inhibitor of SFKs which are important mediators of KIT actions, thus this inhibitor potentially has dual targets of KIT kinase activity per se and SFK-mediated responses [48]. However, although dasatinib strongly inhibits D816 mutant KIT in cell line models, its activity on mutant relative to WT KIT still depends on the particular amino acid substitution (Y>>F>V) [49] and varies between reports [50,51]. Unfortunately, studies using dasatinib in SM patients have yielded disappointing results [52].

PKC412 is a multi-targeted staurosporin analogue which has been evaluated as a FLT3 inhibitor in AML [53]. It is proposed to bind to the “hinge region” of the KIT active site which is much less influenced by conformational changes associated with activation. In the absence of crystal structures of PKC412 in complex with KIT or related kinases, docking into a homology model of the active FMS kinase has been used to predict the mode of drug binding. It was shown that PKC412 could be docked into the conformation of the active FMS kinase (based on PDB 1PKG; [26]), however, it could not be docked into an inactive conformation crystal structure of FMS) [54]. The situation is completely analogous for KIT (authors’ unpublished data), where we have not been able to dock PKC412 into either of the two inactive conformation crystal structures, whereas it can be docked into the active conformation of KIT (PDB 1PKG). PKC412 is an effective inhibitor of D816V mutant KIT [24,55] and has shown promise in treatment of SM [56].

5. Secondary resistance to kinase inhibitory drugs

5.1 Resistance due to secondary KIT mutation in drug-binding residues

Similar to the case of CML, resistance to imatinib arises in initially responsive GIST patients treated with the drug, and as with CML, this is almost always due to a secondary mutation in the same KIT allele as the original mutation [57]. Secondary mutations identified in this way involve a limited number of sites. The most common are point mutations in exons 13 and 14 (encoding the N-terminal lobe of the kinase domain) resulting in amino acid substitutions V654A or T670I, respectively, which confer imatinib resistance by interfering with drug binding [58,59]. Residue T670, analogous to T315 in BCR/ABL, is the “gatekeeper” residue located at the entrance to the hinge region of the ATP binding cleft. Imatinib forms an H-bond with T670 and this is lost on mutation. Furthermore, the presence of a bulky substituent, either naturally as in the case of FLT3, or through mutation as in T670I KIT, obstructs binding of imatinib and related drugs such as nilotinib, and also dasatinib conferring resistance. Residue V654 is directly involved in imatinib binding and replacement with the smaller A residue results in loss of this hydrophilic interaction [59]. To counter imatinib insensitivity due to the “gatekeeper” mutations, the multi-targeted kinase inhibitor sunitinib was developed. This compound, which does not extend beyond the gatekeeper into the catalytic region (Figure 3) is a potent inhibitor of the T670I and V654A KIT mutants. Sunitinib is approved for treatment of GIST following relapse on imatinib [60].
5.2 Resistance due to secondary activation loop mutations

Neither T670I nor V654A is an activating mutation. In contrast, other mutations conferring secondary resistance affect the activation loop of the kinase domain in particular residue D816, D820, N822 or Y823. D816V and N822K are primary activating mutations which may confer secondary imatinib resistance by mechanisms similar to those responsible for primary resistance as described above. While the D816V mutation confers resistance by strongly favouring the active conformation of the kinase domain, the precise mechanism in the case of N822K is less certain. Although it is clearly an activating mutation, in the primary context N822K remains similar in imatinib sensitivity to WT KIT [27]. When combined with a JMD (exon 11) mutation, the resultant double mutant also displays similar imatinib sensitivity to WT KIT, however substantially less than the JMD mutant alone (author’s unpublished data). Sunitinib, like imatinib, selectively binds to the inactive conformation of the kinase domain and fails to inhibit D816 mutant forms [29,61].

6. Clinical trials of KIT inhibitors

6.1 GIST

Prior to the discovery of frequent KIT mutation in GIST [5] and the advent of targeted kinase inhibitors, metastatic GIST was refractory to existing therapy and median survival was around 18 months. Early clinical trials of imatinib for treatment of patients with metastatic GIST showed remarkable success. Partial responses or disease stabilisation were achieved in around 80% of patients with 2 year survival of 75 – 80% [62]. This study provided the basis for FDA approval of imatinib for treatment of metastatic GIST. Ten percent of patients displayed primary resistance (defined as progression within the first 6 months of treatment) the frequency of which was related to KIT mutational status (WT>exon 9>exon 11) [19]. It was subsequently shown that patients with exon 9 mutation responded better to an escalated dose of imatinib [45]. Recent data indicate a median survival of GIST patients with advanced disease of 5 years with 34% of patients alive for > 9 years [63]. At this stage it is unclear what duration of imatinib treatment is necessary, but one study has shown that discontinuation in responding patients after 3 years was associated with rapid progression [64].

Following the success in treating patients with advanced disease, imatinib has been trialled in an adjuvant context in GIST patients with primary disease following potentially curative surgery. A definitive Phase III trial reported by Dematteo and co-workers [65] demonstrated significant benefit of one year of imatinib therapy post-surgery. This led to FDA approval for adjuvant use of imatinib for patients with high risk of recurrence (based on tumour size, location, mitotic index, bleeding or rupture). Several other trials are currently underway (reviewed [63]). Imatinib has also been evaluated in a neoadjuvant setting in which the drug is given prior to, as well as post- surgery [66]. It has been proposed that this approach could lead to tumour de-bulking in advanced disease allowing subsequent successful surgery.

Despite the success of imatinib therapy, approximately half of the patients with metastatic disease develop resistance within 2 years, and as in the case of BCR/ABL in CML, this is almost always due to a second mutation in the same allele of KIT as the primary mutation [57]. The nature of these mutations (which prevent imatinib binding either directly or by influencing the conformation of the binding site) and second-generation inhibitors designed to overcome this resistance are discussed in the previous section. From a clinical perspective, an important observation is that different lesions, and sometimes different regions of the same lesion, in imatinib-resistant patients harbour different secondary mutations [67,68]. Since no second generation drug is active on all of these mutants, this raises a possible need for use of drug combinations with potential complications due to toxicity and drug interactions. Sunitinib was developed as an inhibitor of mutants with imatinib-resistance due to secondary mutation in the drug binding residues, T670I and V654A of KIT. It has proved successful in treating many cases of GIST following relapse on imatinib and has received FDA approval for use in this context [60]. However, activation loop mutants are insensitive to sunitinib [29,69]. Relapse of a patient with a tertiary mutation in the activation loop of KIT was reported during sunitinib treatment following relapse on imatinib due to V654A mutation [70]. Other kinase inhibitors, mostly broad spectrum, are currently being evaluated in clinical trials in GIST (reviewed [44]).

6.2 SM and AML

In haemopoietic malignancies mutant forms of KIT have been observed in CBF AML and SM. While the majority of AML patients achieve remission with aggressive chemotherapy, relatively few survive long-term, so that new treatments are urgently needed. Early trials of imatinib in unselected cases of AML were unsuccessful (e.g., [71]), probably due to the relatively low rate of KIT mutations (around 7% overall) and the frequency of resistant D816 mutations. A small study examined imatinib responses in three CBF AML cases with KIT mutations; two patients with D816 mutation failed to respond while the third with an exon 8 mutation showed clinical benefit [72]. Because of its low toxicity, imatinib warrants further investigation in patients with KIT exon 8 or N822K mutations which are relatively common in CBF AML and sensitive to the drug [18,27]. Dasatinib, which has higher activity on D816 mutant KIT, is currently being trialled in AML (http://clinicaltrials.gov), for example in combination with induction chemotherapy in AML (NCT01238211) and in maintenance therapy in CBF AML (NCT00850382). PKC412 (midostaurin), which is currently under evaluation for treatment of FLT3 mutant AML [53], is also a very good inhibitor of D816V mutant KIT and has potential for treatment of cases of AML with this mutant as driver.
In SM, KIT mutation (usually D816V) has been observed in both indolent (ISM) and aggressive (ASM) forms of the disease. ISM is a chronic disease which is treated for symptomatic relief, whereas ASM is a debilitating and incurable disease with shortened life expectancy [46,52] and is a candidate for therapy with KIT inhibitors. Treatment with imatinib has generally failed to deliver benefit [73,74], likely reflecting its lack of activity on D816 mutant KIT. While dasatinib has reasonable activity on this mutant in cell lines, results in clinical studies have been disappointing with limited responses [75,76]. PKC412 is a good inhibitor of D816 mutant KIT and was shown to have activity in one SM patient [56]. In a subsequent Phase II trial conducted by this group, it showed considerable efficacy, but at the expense of substantial toxicity. This and other ongoing studies have been recently reviewed [52,77].

6.3 Melanoma
Melanomas commonly express KIT and, since treatments of metastatic melanoma prior to the advent of inhibitors of mutant B-Raf had very poor response rates, early studies were conducted as to the effect of imatinib in this disease. These studies on unselected patients failed to demonstrate significant benefit. Subsequently a subset of melanoma cases, mostly those with accral or mucosal phenotype, and lacking B-Raf or K-Ras mutations, have been shown to have activating KIT mutations, most commonly L576P (exon 11) or K642E (exon 13) (e.g., [78]). Two Phase II trials of imatinib in metastatic melanoma with activating KIT mutations were reported [78,79] recently, both showing significant benefit from drug treatment. In one study, responses occurred across the spectrum of mutations [79], while in the other responses were restricted to patients with L576P or K642E mutations [78]. The results for cases with L576P mutation were surprising since in vitro studies indicated that this mutant has low sensitivity to imatinib [80,81]. Two patients with melanoma harbouring L576-mutated KIT were successfully treated with dasatinib, which may have been due to its activity on SFK as well as or instead of KIT [81]. In a Phase II study, dasatinib was shown to have minor benefit for treatment of metastatic melanoma and to cause substantial toxicity [82]. It is possible that imatinib or other KIT inhibitors may have a role in treatment of melanomas driven by KIT mutations, but a much fuller evaluation is required.

7. New approaches to treatment of cancers driven by mutant KIT

7.1 Cancer stem cells and resistance to inhibitor therapy
Imatinib has profoundly improved the outlook for GIST patients, but the question remains as to whether this cancer can ever be eradicated with kinase inhibitors alone. Live tumour cells persist in patients treated with imatinib, and patients rapidly relapse on drug withdrawal [64]. A similar situation occurs with targeting BCR/ABL in CML where complete elimination of the disease may fail due to the insensitivity of the leukaemic stem cell pool to kinase inhibitors [59] and much can be learned from that experience. A study using a K641E knock-in mouse model system indicated that normal ICC progenitors express low levels of c-Kit. Neither they nor their spontaneously transformed variants were sensitive to imatinib [83]. The authors suggest that cancer stem cell drugs, possibly combined with imatinib, might target these cells.

7.2 Targeting synergising oncogenes
From the above and other evidence, it follows that development of overt malignancy requires additional “hits” as well as mutant KIT and targeting these together with KIT is likely to be a successful therapeutic approach. Occult GIST “tumorlets” are frequently found in the stomachs of older individuals at autopsy. These have typical KIT mutations seen in GIST, but have benign characteristics [84,85]. These observations indicate that KIT mutation is an early but insufficient event in GIST development, consistent with observations of familial GIST. Recently a key role for the ETS family transcription factor ETV-1 acting in synergy with mutant KIT was reported in GIST [86]. Similarly, in CBF AML mutant KIT requires the co-expression of transcription factor fusion proteins that promote survival and block differentiation [8,9]. In SM co-operating oncogenic stimuli also appear to be necessary for transformation. The presence of cells expressing the D816V KIT mutant in the blood of healthy individuals and in patients with indolent SM has been reported [46,87].

Gleixner and co-workers [88] recently reported that KIT-independent activation of SFKs, Btk and Lyn, synergises with D816V KIT in HMC-1 leukaemic mast cells. Growth of HMC-1 cells could be blocked by the synergistic action of dasatinib, which inhibits both SFK and D816V KIT, and bosutinib which inhibits SFK but not KIT. Similarly, inhibition of Btk and Lyn with low-dose dasatinib (suboptimal for D816V KIT inhibition) combined with PKC412 (which effectively inhibits D816V), or with bosutinib and PKC412, blocked HMC-1 cell growth [50,88]. Targeting synergising oncogenes with small molecule inhibitors appears to be a promising approach although some of these inhibitors have a broad activity spectrum and combinations may cause unacceptable toxicity.

Studies of GIST cell lines indicate that many cells become quiescent rather than undergoing apoptosis when treated with imatinib and can resume proliferation when the drug is withdrawn [89]. Tumour cells commonly have upregulated survival mechanisms which are required for the cells to cope with the stress induced by oncogenic disruptions to cell signalling. Targeting mediators of cell survival is being explored as a therapeutic approach. In GIST cell lines apoptosis could be induced with antagonists to the upregulated BCL2 anti-apoptotic protein [90], while reversing down-regulation of...
pro-apoptotic BIM could bring about cell death [91]. Autophagy was shown to be another mechanism by which GIST cell lines avoid death on treatment with KIT inhibitors [89]. Treatment with lysosome-targeting agents such as antimalarials chloroquine or quinacrine synergised with imatinib in blocking survival and growth of GIST cells in vitro.

7.3 New approaches to KIT inhibition
A major problem with the use of KIT inhibitors is either primary or secondary resistance due to D816 mutations and the lack of good drugs to target them. The success of imatinib and sunitinib in GIST is largely due to the scarcity of these mutants, in contrast to SM in particular. To inhibit D816 mutants, a drug needs to bind to the active conformation of the kinase which is more structurally constrained than the inactive conformation to which drugs like imatinib and sunitinib bind. Drugs that bind to the active conformation, such as dasatinib and PKC412 are, in general, broad spectrum kinase inhibitors with correspondingly greater toxicity. While a large number of new small molecule kinase inhibitors are under evaluation for KIT inhibition (reviewed [44]), experience with dasatinib and PKC412 suggests that they will not be effective as single agents in patients whose cancers have D816 KIT mutations. An alternate or additional approach may be to target the stability of the mutant KIT protein. Fumo et al. [92] showed that the chaperone protein, Hsp90 binds to mutant forms of KIT (including D816V) in HMC-1 mast cell leukaemia lines. Treatment of the cells with the small molecule Hsp90 inhibitor 17-AAG led to degradation of KIT, inhibition of its signalling and promoted cell death. More recently, IPI-504, a 17-AAG derivative with greater solubility and improved pharmacological characteristics, has been tested in xenograft models of GIST with exon 11 or 13 KIT mutations [93]. The drug was shown to cause KIT degradation and block cell proliferation in the tumours, as well as inducing tumour necrosis and shrinkage. Furthermore, it acted synergistically with imatinib or sunitinib. However, higher doses, especially in combination with the kinase inhibitor, caused liver toxicity. It remains to be seen whether this will prove to be limiting in humans. Hsp90 inhibitors are currently being evaluated in Phase II clinical trials in GIST [44].

7.4 Targeting KIT downstream signalling
Finally, it may be possible to target downstream signalling pathways that are required for KIT-dependent growth. For example, in haemopoietic cells transformed by D816V mutant KIT, P13K is constitutively and potently activated. Pharmacological inhibition of P13K caused death of these cells in vitro and mutation of the major KIT recruitment site for P13K (Y721F) blocked tumorigenicity in syngeneic mice in vivo [33]. Similarly this pathway appears to be critical in survival of GIST [94]. Several agents targeting P13K or its downstream effectors AKT and mTOR are currently under evaluation [95]. In haemopoietic cell line models and CBF AML patients the serine/threonine protein phosphatase PP2A, a known tumour suppressor, is strikingly down-regulated [96]. This phosphatase is a key regulator of multiple KIT signalling pathways. Pharmacological reactivation of PP2A with FTY720 (a nontoxic drug recently FDA-approved for treatment of multiple sclerosis because of its immunosuppressive action) reduced proliferation and induced apoptosis of murine early myeloid cells expressing D816V KIT in vitro and retarded their growth in syngeneic mice [96]. FTY720 also induced apoptosis in AML patient blasts harbouring D816V/Y KIT [96,97]. This approach offers particular promise in cancers involving D816 mutations which are highly resistant to commonly used KIT kinase inhibitors.

8. Conclusion
Mutant forms of KIT are drivers of several cancers including GIST, SM and subsets of AML, melanoma and testicular seminomas. Multiple different mutations are responsible for constitutive activity of KIT in a tumour-selective fashion. The small molecule inhibitor, imatinib, has had great benefit in treatment of GIST, but so far kinase inhibitors have made little impact in SM or other malignancies. This is due, in part, to the lack of well-validated inhibitors of forms of KIT with certain activation loop mutations. In GIST, treatment with imatinib results in disease control but not eradication and drug resistance frequently develops due to secondary mutation in KIT resulting in loss of drug binding. While new drugs such as sunitinib are effective on the most common of these, this drug is not effective on activation loop mutations. In relapsing GIST patients, different tumour foci frequently contain different secondary mutations such that no single drug is likely to be effective. At present there is no known inhibitor that is active on all observed KIT secondary mutants. Meanwhile, considerable progress is being made on identifying pathways that act synergistically with KIT to transform cells. Co-targeting these pathways may lead to tumour control (or even eradication) while being well-tolerated by normal cells, a concept known as "synthetic lethality" [98]. A particular challenge is to target tumour stem cells which seem to be especially refractory to KIT inhibition.

9. Expert opinion
The observation that different oncogenic mutant forms of KIT vary greatly in sensitivity to imatinib illustrated the need for mutation analysis in individual patients in order to determine which patients would benefit from treatment with the drug as well as the appropriate dose to use. This is particularly true in the case of GIST and CBF AML which are heterogeneous with respect to the presence and type of KIT mutations with different inhibitor sensitivity. Mutation analysis of key exons of KIT is now technically straightforward, relatively inexpensive and is economically justified in
view of the high cost of the drugs as well as patient benefit. The development of secondary and tertiary resistance mutations in GIST has required development of new second-line drugs, but currently there remains a lack of good inhibitors for some mutant forms of KIT. Even where effective inhibition of KIT is achieved in GIST, disease eradication does not occur. There are several exciting candidates which could be targeted together with KIT, especially in the context of more rapid and extensive reduction of tumour burden. These include modifiers of mutant KIT degradation, cell death or autophagy, and of signalling pathways required for KIT-mediated transformation, for example, the PI3K pathway and PP2A. Application of combination therapies during initial disease treatment should lead to optimum tumour clearance and correspondingly reduce the problem of drug-resistant relapse. However, for disease cure, eradication of tumour stem cells is necessary and is not achieved by current therapies. To meet this objective, it is likely that co-operating oncopgenes will need to be targeted together with KIT. Important candidates have been identified in GIST and CBF AML.

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Declaration of interest

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