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What lessons can be learned from the GIST paradigm that can be applied to other kinase-driven cancers

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Abstract

Gastrointestinal stromal tumor (GIST) is the most common sarcoma of the intestinal tract, known to be notoriously refractory to conventional chemotherapy or radiation. It is an ideal solid tumor model to apply our understanding from aberrant signal transduction to drug development, since nearly all tumors have a mutation in the *KIT* or, less often, *PDGFRA* or *BRAF* gene. The constitutively activated *KIT* and *PDGFRA* oncoproteins serve as crucial diagnostic and therapeutic targets. The discovery of oncogenic *KIT* activation as a central mechanism of GIST pathogenesis, suggested that inhibiting or blocking *KIT* signaling might be the milestone in the targeted therapy of GISTs. Indeed, imatinib mesylate inhibits *KIT* kinase activity and represents the front line drug for the treatment of unresectable and advanced GISTs, achieving a partial response or stable disease in about 80% of patients with metastatic GIST. *KIT* mutation status has a significant impact on treatment response, emerging in recent years as a leading paradigm for genotype-driven targeted therapy. During this review parallels with other models in oncology which share their addiction to a particular mutationally activated kinase will be contrasted. A better understanding of oncogene addiction as a common theme across tumors of diverse histologies underlies the clinical success of targeting such kinases with several selective kinase inhibitors. Remarkable as well is the similarity displayed in the mechanisms of drug failure, after a successful but temporary clinical response to kinase inhibition. Reactivation of the same oncogenic kinase by often acquisition of second site mutations is another emerging paradigm of secondary resistance in these tumor models. The complexity of polyclonal resistance in imatinib-resistant patients argues that single next-generation kinase inhibitors will not be beneficial in all mutant clones. Other broad therapeutic strategies could include combination of kinase inhibitors with targeting *KIT* downstream targets, such as PI3-K or MAPK/MEK inhibitors.

Keywords

GIST; *KIT*; imatinib; TKI; resistance

KIT Biology and KIT-Mediated Oncogenesis

KIT is a member of the type III transmembrane receptor tyrosine kinase (RTK) family, which are comprised of five extracellular immunoglobulin domains, a single transmembrane

region, an inhibitory cytoplasmic juxtamembrane domain, and a split cytoplasmic kinase domain separated by a kinase insert segment (fig. 1) [1]. Under physiologic conditions, binding of the KIT ligand, stem cell factor (SCF), to the extracellular domain of the receptor results in receptor dimerization, activation of the intracellular tyrosine kinase domain through autophosphorylation of specific tyrosine residues, and receptor activation [2]. The downstream signal transduction pathways includes the mitogen activated protein kinase- (MAPK), phosphatidylinositol 3'-kinase-(PI3K), and Janus kinase/signal transducers and activators of transcription- (JAK/STAT) pathways. The intracellular signalling through KIT plays a critical role in the development of several mammalian cell types, including melanocytes, hematopoietic progenitor cells, mast cells, primordial germ cells, and interstitial cells of Cajal [3–4]. As a consequence, loss-of-function mutations at either KIT or KIT Ligand murine loci generate deficiencies in all these major cell systems. In contrast, the presence of KIT receptor activating mutations have been implicated in the pathogenesis of several human tumors, including seminomas [5], mastocytosis [6], acute myelogenous leukemias [7], and more recently in melanomas [8], suggesting a central role for KIT in oncogenesis.

KIT oncogenic activation is the dominant pathogenetic mechanism in GIST

The majority of *KIT* mutations in GIST are somatic, although a few families with germline mutations have been identified [9]. The frequency of *KIT* mutation in GIST ranges between 80% and 85%. KIT mutations are found predominantly in the juxtamembrane domain of KIT receptor, which have been shown to promote KIT dimerization in the absence of SCF and release the receptor from its auto-inhibited conformation, resulting in constitutive activation. The most common site of *KIT* mutations is in the 5' end of exon 11. The types of mutations occurring in this hot-spot are quite heterogeneous, including in-frame deletions of variable sizes, point mutations, or deletions preceded by substitutions. Although mutations at this site are not associated with a specific clinicopathologic phenotype, the presence of deletions rather than substitutions predicts a more aggressive behavior [10]. A less common hot-spot is located at the 3' end of exon 11, which includes mainly internal tandem duplications mutations (ITDs) [11]. GIST patients harboring ITD-type mutations follow a more indolent clinical course and their tumors are with predilection located in the stomach [11]. The 2nd most common site of *KIT* mutations, accounting for 10–15% of GIST patients, is located in exon 9, coding for the extracellular domain. Most *KIT* exon 9 mutations represent an insertion of two amino acids, AY 502–503. GISTs harboring *KIT* exon 9 mutations are characterized by small bowel location and aggressive clinical behavior [11].

In about 10% of GIST patients no mutations in either *KIT* or *PDGFRA* have been identified, although uncontrolled KIT kinase activation has been noted even in the absence of mutation [12]. However, despite the KIT signaling pathway being activated the response to imatinib in the wild-type GIST patients has been disappointing. Among adult patients, the wild-type GIST subset represents a heterogeneous group of with no particular association with anatomic location or clinical outcome. In contrast, GISTs occurring in children or in type 1 neurofibromatosis are nearly always wild-type [13–14]. In fact, pediatric GISTs represent a distinct clinico-pathologic and molecular subset, with predilection for females, multifocal gastric tumors, and wild-type genotype [13].

The prognostic significance of *KIT* and *PDGFRA* mutations has been examined from the pre-imatinib era. In a large series of patients who underwent surgical resection for primary localized GIST, specific *KIT* mutations had prognostic significance according to univariate but not multivariate analysis [15]. In particular patients with exon 11 point mutations or insertions had a favorable prognosis, whereas those with *KIT* exon 11 DEL557-8 and/or *KIT* exon 9 mutations had a poor prognosis.

KIT as a therapeutic target in melanoma and other cancers

Recently, activating mutations and/or gene amplification of *KIT* have been found in 39% of mucosal, 36% of acral, and 28% of melanomas that arise in chronically sun-damaged skin (defined by the presence of solar elastosis on pathology review) [8]. The clinical importance of *KIT* mutations in melanoma relates to the fact that approved drugs are available for inhibition of its kinase activity and are already used successfully in other cancers. The spectrum of *KIT* mutations seen in melanoma partially overlaps with that seen in GIST, where most mutations also occur in exon 11. The common hot spot in melanoma is the *L576P* substitution, comprising 35% of all *KIT* mutations [16]. In contrast with GIST, mutations in the first and second kinase domains of *KIT* are much more frequent, with a combined frequency of 25% [16]. Although limited in numbers thus far, clinical experiences confirm *KIT* as a melanoma therapeutic target, with patients experiencing dramatic and durable responses to treatment, which in some cases may be dose-dependent [17–18]. The importance of proper patient selection is highlighted by the negative experience with imatinib in prior trials with unselected melanoma patients. Three phase II trials of imatinib in metastatic melanomas were carried out before the discovery of *KIT* mutations in melanoma and have proven mostly disappointing [19–20] [21]. In retrospect, one of the trials found a near complete response of a patient with metastatic acral melanoma 12 weeks of treatment [20], consistent with the finding of genetic alterations of *KIT* in acral melanomas. Together, these results have renewed the enthusiasm for *KIT* inhibitors and indicate the promise of treatments targeted against specific genetic alterations in melanoma. The results to date are particularly encouraging, as they occur in acral and mucosal melanomas, which are characterized by a high degree of chromosomal instability with numerous amplifications [22]. The fact that *KIT* amplifications have been found either isolated or in conjunction with *KIT* mutations [8, 23] has raised concern that tumors with amplification might be more prone to resistance, as therapy could select tumor cells with higher copy number as an escape mechanisms. The observation that responses seem to occur despite the presence of numerous other genetic alterations are indicative that melanomas with *KIT* mutations are “addicted” to *KIT* activation.

Mutations of *KIT* are overall infrequent in adult AML (2%–8%) and tend to cluster within the activation loop (exon 17) and a region of the extracellular domain integral to receptor dimerization (exon 8). Their prevalence is much higher, however, in adult patients with core binding factor (CBF) AML (6%–48%) and may be associated with worse clinical outcome in this patient population [7, 24–25]. There is little data with regard to imatinib therapy in AML patients with CBF *KIT* mutations. In a series of three patients, imatinib had transient activity in a patient with exon 8 in-frame deletion, but none in the two patients carrying D816Y and D816V mutations [26]. A recent report identifies the presence of a V530I *KIT*

exon 10 mutation in a patient with unresectable aggressive extra-abdominal fibromatosis who had a dramatic response to imatinib therapy[27]. This substitution affecting the transmembrane domain of KIT has been previously reported in the CBF AML [7, 24]. Similar with GIST patients harboring mutations in the 2nd kinase domain of KIT, most patients with systemic mastocytosis, expressing the oncogenic KIT mutation D816V, are resistant to imatinib therapy.

Targeting PDGFRA in GIST and beyond

PDGFRA is also a member of the type III receptor tyrosine kinase, which is activated by mutations or small deletions in a subset of GIST [28] and childhood acute myeloid leukemia's [29], as well as fusion with FIP1L1 in hypereosinophilic syndrome [30] and systemic mastocytosis associated with eosinophilia [31]. In GIST, mutations in PDGFRA and KIT are mutually exclusive and about one-third of GISTs lacking *KIT* mutations harbor a mutation in *PDGFRA*, within exons 12, 14, or 18 [28, 32]. Most GISTs with mutated *PDGFRA* have a distinct phenotype, including gastric location, epithelioid morphology, variable/absent KIT expression by immunohistochemistry, and an indolent clinical course [32]. The most common hot spot involves the 2nd kinase domain, exon 18 *D842V* substitution (which corresponds to exon 17 of KIT), which is associated with insensitivity to imatinib therapy. More recently *PDGFRA* mutations have been identified in the vast majority of inflammatory fibroid polyps, which are benign mesenchymal lesions associated with a rich eosinophilic infiltrate, often occurring in the stomach [33].

BRAF V600E mutated family of tumors – welcoming its newest member: GIST!

BRAF mutations are identified in 7% of all cancers. *BRAF* mutations are detected in more than half of melanoma cases and their incidence is dependent to ultraviolet light exposure, being most common in melanomas arising in skin intermittently exposed to the sun [22]. Apart from melanoma, *BRAF* mutations are also implicated in the pathogenesis of certain epithelial malignancies, such as papillary thyroid carcinoma, colorectal carcinoma, as well as in some benign / pre-neoplastic lesions, such as melanocytic nevi and serrated colonic polyps [34–36]. In a recent study, we have identified a primary *BRAF* mutation in 7% of adult GIST patients, lacking *KIT/PDGFRA* mutations [13]. The *BRAF*-mutated GISTs show predilection for small bowel location and high risk of malignancy. KIT protein is consistently overexpressed in these cases and there are no distinctive features microscopically that would differentiate them from *KIT*-mutated GISTs [13]. The mechanism through which *BRAF* activating mutations in GISTs may affect KIT signaling remains unclear. Similar to other tumor types where *BRAF* mutations are more commonly observed, the mutations seen in GIST are also located within the exon 15 V600E hot spot. No other mutations were identified in the *BRAF* exon 11 or *NRAS* exons 2 and 3. At least one patient carrying a *BRAF* mutation who was treated at our institution for metastatic GIST showed insensitivity for both imatinib and sunitinib therapy. In keeping with this observation, untreated tumors from a *KIT*^{V558} /+ GIST mouse model showed consistent activation of ERK1/2 phosphorylation, while imatinib treatment had no effect on ERK activation [37]. These findings delineate a new molecular group of patients who may benefit

from selective BRAF inhibitors, as an alternative therapeutic option to imatinib. The emerging remarkable success of RAF inhibitor (PLX4032, PLEXXIKON) against the V600E-mutated melanoma, suggests that similar responses may be seen with other tumor types sharing its dependence to oncogenic RAF. An identical *V600E BRAF* mutation was also identified in one of the 28 patients who developed acquired resistance to imatinib, lacking a defined mechanism of drug resistance [13]. This patient was a 66-year old man with a primary high risk gastric GIST which displayed a heterozygous *PDGFRA* exon 18 deletion. After 20 month of imatinib treatment, the resected resistant areas showed a divergent differentiation into a KIT-negative rhabdomyosarcoma phenotype. Thus secondary *BRAF* mutations could represent an alternative mechanism of imatinib resistance, albeit rare, in GIST or other kinase-mutated neoplasms.

Alternative activated signaling pathways in wild-type and syndromic GIST

Another interesting line of evidence is the transcriptional upregulation and protein overexpression of IGF1R in pediatric GIST [13, 38]. This finding could indicate drug sensitivity to IGF1R-directed agents, such as the IGF1R antibodies, currently being tested in phase II clinical trials. Furthermore, inhibition of IGF1R activity by NVP-AEW541 (a small molecule IGF1R inhibitor, Novartis) was demonstrated in GIST cell lines, leading to cytotoxicity and apoptosis via AKT and MAPK signaling [39]. However the mechanisms of IGF1R upregulation is still under investigation, since no gene amplification or activating mutations have been so far detected [38 and Cristina Antonescu, unpublished data].

Although most commonly occurring in sporadic settings, GIST has been described as part of certain syndromes, such as familial GIST, Carney's triad, Carney-Stratakis syndrome and neurofibromatosis. The association of multifocal gastric GIST with paragangliomas and pulmonary chondromas affecting mostly females is diagnostic of Carney's triad. More recently it was recognized that the autosomal dominant inheritance of the dyad "paraganglioma and gastric GIST", or the "Carney-Stratakis syndrome" (CSS), represents a separate condition which affects both males and females and lacks the association with pulmonary chondromas. Mutations of genes coding for succinate dehydrogenase (SDH) subunits, typically associated with familial paragangliomas, are also identified in CSS [40]. In contrast the pathogenesis of Carney's triad tumors, including GIST, remains undefined, with a recent comprehensive genetic analysis of 41 tumors from 37 patients failed to identify any activating mutations in the coding region of *KIT*, *PDGFRA*, and *SDH A-D* [41]. GIST occurring in the setting of neurofibromatosis type 1 is secondary to a somatic inactivation of the wild-type NF1 allele in the tumor [42]. Only infrequently, *KIT* or *PDGFRA* mutations have been documented in these tumors [14].

Imatinib treatment in GIST: a leading paradigm for genotype-driven targeted therapy

GIST patients harbor different oncogenic mutations in *KIT* and *PDGFRA*, which have distinct responses to imatinib. Imatinib mesylate (STI571, Gleevec™, Novartis Pharmaceuticals, Basel, Switzerland) is a selective tyrosine kinase inhibitor whose targets include ABL, BCR-ABL, KIT, and PDGFR. Imatinib is a 2 phenyl-amino-pyrimidine

derivative, which specifically binds to the inactive conformation of the ABL kinase or the inactive form of KIT. Imatinib was first applied in 1998 to refractory CML and subsequently to advanced GIST patients in 2000 [43–44]. Imatinib achieves partial responses or stable disease in nearly 80% of GIST patients, and remarkably the 2 year survival in advanced GIST is now 75–80% [45]. The long-term outcome of imatinib treatment for metastatic GIST has emerged from several large trials. Approximately 45% of patients with metastatic GIST have a measurable response after administration of imatinib, while about 30% will have at least stable disease [45].

However, responses to imatinib depend on the functional domain affected [46]. Patients with *KIT* exon 11 mutations have a partial response rate of 84% compared with a 0% partial response rate among patients without *KIT* mutations [46]. By analogy with other receptor tyrosine kinases, the juxtamembrane domain may function as a negative regulator of the KIT kinase and disruption of the conformational integrity of this domain may impair its negative regulatory function. Deletion of tyr-567 and val-568 in the normal *KIT* gene have been shown to enhance agonist induced KIT signaling by altering the specificity of docking sites for cytoplasmic signaling molecules in the activated KIT receptor. By contrast, mutations in exon 9 of KIT, which encodes the fifth extracellular immunoglobulin-like loop, are less responsive, albeit this limitation can be partially overcome by a high-dose regimen (400 mg twice daily) [47]. Activating oncogenic mutations within the ectodomain of KIT map to the D5-D5 interface, which presumably stabilize receptor dimers and thus activate the kinase in the absence of ligand [48]. In spite of an activated KIT pathway, the imatinib response in GIST patients with a wild-type genotype for *KIT/PDGFR α /BRAF* has been disappointing. Furthermore, tumors with activation loop mutations particularly show the least response to imatinib inhibition.

Similar with the GIST paradigm, the different types of *EGFR* mutations detected lung cancer have different prognostic values. Although *EGFR* mutations in exons 19 or 21 are correlated with clinical factors predictive of response to gefitinib and erlotinib, *EGFR* exon 19 deletion mutations had a longer median survival than patients with *EGFR* L858R point mutation [49–50].

Tyrosine kinase inhibitors (TKI) therapy is standard of care for patients with metastatic/advanced GIST. And although the optimal duration of treatment is not known, it is recommended to continue therapy indefinitely or as long as they are experiencing a clinical benefit. Imatinib interruption in patients with advanced GIST controlled with imatinib is often associated with a high risk of disease progression within 1 year. In most patients, however, the disease is controlled with imatinib re-challenge, with no statistically significant differences in imatinib-refractory progression free survival between interruption and continuation [51–52]. No apparent difference was seen in sunitinib activity between the intermittent and continuous daily dosing schedules.

Evaluating Tumor Response to TKI Therapy: Radiology and Pathology Perspective. Learning from the Imatinib-Treated GIST lesson

Traditional tumor response criteria such as Response Evaluation Criteria in Solid Tumors (RECIST) are based on unidimensional tumor size and do not take into considerations changes in tumor metabolism, tumor density, and decrease in the number of intra-tumoral vessels. All of these changes indicate response to TKI therapy in patients with GIST. Hence, response assessment according to RECIST is known to be insensitive in evaluating response to TKI therapy [53]. Decreased density on contrast-enhanced CT indicates response to therapy and correlates with tumor necrosis or myxoid degeneration. The CT response criteria proposed by Choi et al. use both tumor density and size to assess response to TKI in GIST [54–55]. These criteria correlate well with PET in predicting response compared to RECIST, however, they are not universally accepted or applied at ease outside specialized centers. Although the utility of PET scan was emphasized in the initial years of TKI treatment, refined radiologic criteria (such as the Choi criteria) have surpassed the need for PET scan. Thus CT scans with intra-venous contrast are the preferred routine imaging modality for GIST patients undergoing TKI therapy [54]. When a GIST responds to imatinib it generally becomes homogeneous and hypodense (fig. 2A), while the tumor vessels and solid enhancements disappear. These changes can be seen within 1 to 2 months in most clinically responding GISTs and have shown to have a prognostic value, even in the absence of size shrinkage of the tumor bulk. Recognizing the response pattern is critical in selecting patients who may benefit from surgical debulking or who may need switching to a second line inhibitor instead. Conversely, when imatinib-resistance occurs, the reverse in tumor density and sometimes nodule within nodule translate to tumor progression (fig. 2B). Refining evaluation criteria of TKI response has not only been a remarkable success in the clinical management of GIST patients, but also underscores a significant departure from assessing clinical responses to conventional chemotherapy using conventional imaging methods.

As a subset of GIST patients showing either stable disease or partial response to imatinib therapy become candidates for surgical debulking, the availability of large volume tumor specimens has been a great resource for pathologists to investigate histologic responses to TKI. This opportunity has been left unparalleled in other solid tumor models, where either the response to TKI is short-lived or the surgical debulking is not a feasible alternative option. The histologic response to imatinib therapy in GIST is heterogeneous, varying from nodule to nodule within the same resection, as well as within the same lesion [56]. Some tumors may show only gross tumor necrosis, with large, central areas of cystification and hemorrhage, whereas the remaining solid areas are viable microscopically. In other examples, extensive gelatinous and myxoid degeneration is appreciated grossly, often seen in liver metastases (Fig. 3). Dense hyalinization with complete loss of tumor cells is rare (Fig. 4). Even in tumors with a very good histologic response, microscopic foci of viable and KIT positive cells are often present (Fig. 4C). The assessment of tumor proliferation (Ki67 index) in these viable areas may serve as an indicator of the indolent nature of the residual viable tumor. A negative or low Ki67 index in the cellular areas correlates more often with tumor response (i.e. foci of quiescent tumor, Fig. 4D). Conversely, the degree of

tumor necrosis alone may not be as informative to assess tumor response versus resistance, microscopically. A subset of responsive GIST tumors shows weak or even negative KIT immunostaining, compared to the pre-imatinib tumor sample (Fig. 4C). Furthermore, a small group of imatinib responsive tumors acquire smooth muscle features, suggesting that imatinib may induce a trans-differentiation towards a smooth muscle phenotype, through chronic inactivation of KIT signaling [56]. Second-site *KIT* mutations are quite rare in GISTs responsive to imatinib compared with imatinib-resistant tumors [56]. This is in contrast with CML, where *BCR-ABL* kinase domain mutations have been detected more often in chronic phase patients who had stable disease on imatinib and predicted subsequent clinical relapse [57].

How are the surgical approaches combined with molecular targeted therapy?

There are several reasons to consider surgical resection in patients with metastatic GIST who are being treated with molecular therapy. While TKI induce marked tumor regression in the majority of patients, complete responses are rarely achieved. One possible hypothesis is that the chance of resistance is proportional to the amount of residual viable GIST following therapy with TKI. While it has been shown that tumor load can continue to decrease even after a year of imatinib therapy, the median time to best response is approximately 3.5 months and there is little incremental shrinkage after 9 months [58]. Thus, patients with advanced GIST who have stable or responsive disease on imatinib may benefit from elective surgical resection. The optimal timing of surgery in relation to imatinib therapy for patients with advanced GIST is still debatable, but our experience in responsive disease suggests that surgical resection after 3–6 months with TKI is beneficial if their disease appears completely resectable [59]. However, it is critical to continue tyrosine kinase inhibition postoperatively to delay, or possibly even prevent, subsequent progression. Patients with metastatic GIST who were randomized to stop imatinib after 1 year of therapy subsequently developed progressive disease [60]. The optimal duration of tyrosine kinase inhibitor therapy after resection of metastatic GIST is unknown at this time. Once resistance to imatinib develops, there is currently only a small chance of rescuing the patient and surgical debulking has not shown benefit in patients who develop imatinib resistance following a delay in surgical resection [59].

Interestingly, co-administration of targeted agents with conventional chemotherapy appears to be contra-productive in lung cancers with EGFR mutations and can even defeat the effects of targeted agent [61]. This is because chemotherapeutic agents may promote cell cycle arrest and therefore suppress apoptosis triggered by acute inactivation of an oncogenic kinase achieved through targeted therapy. This mechanism has potentially contributed to the disappointing results observed when EGFR kinase inhibitors were administered together with conventional chemotherapy drugs in non-small-cell lung cancer [61].

Adjuvant Targeted Therapy in GIST. Can we extrapolate this experience to other cancers?

In a recent randomized phase III, double-blind, placebo-controlled, multicenter trial, following the resection of a primary GIST, adjuvant therapy with imatinib was safe and significantly prolonged recurrence-free survival compared to placebo treatment [62]. Eligible patients had complete gross resection of a primary GIST at least 3 cm in size and positive for the KIT protein by immuno-histochemistry. Patients were randomly assigned to imatinib 400 mg or to placebo daily for 1 year after surgical resection. Patients assigned to placebo were eligible to crossover to imatinib treatment in the event of tumor recurrence. The primary endpoint was recurrence-free survival. Accrual was stopped early because the trial results crossed the interim analysis efficacy boundary for recurrence-free survival. However there was no difference observed in the overall survival in the short term follow-up available. These findings impacted on the management of patients with primary GIST and have prompted the FDA approval of imatinib in the adjuvant setting. However, optimum duration of postoperative treatment has not yet been determined. Adjuvant imatinib after complete resection for primary GIST is recommended for at least 12 months in intermediate- to high risk patients. Higher risk patients may require longer treatment.

In other cancers however the clinical benefit of adjuvant TKI remains still under investigation. In lung cancer, patients with *EGFR* exon 19 or 21 mutations in their resected adenocarcinoma are being offered at MSKCC enrollment in an on-going single-arm phase 2 clinical trial of erlotinib as adjuvant therapy, based on the prospective phase 2 data in metastatic NSCLC documenting very high radiologic response rates in this molecular subgroup. The efficacy results will be then compared to the treatment arm of the on-going multicenter, placebo controlled phase 3 clinical trial of adjuvant erlotinib in patients with resected NSCLC which expresses EGFR by IHC, or demonstrates EGFR amplification by FISH, a.k.a. RADIANT study (Randomized Double-Blind Trial In Adjuvant NSCLC with Tarceva) [63–64].

Mechanisms of Acquired Resistance to TKI therapy –An Emerging Common Theme of Drug Failure. Future Challenges for Targeted Monotherapy

It has become clear that most patients who initially respond to TKI eventually acquire resistance. Although the 2-year survival of patients with metastatic GIST treated with imatinib approximates 72%, half of the patients develop disease progression by 2 years [65]. In only a minority of cases, patients are insensitive to the drug, so-called primary resistance. In most imatinib resistant GISTs, KIT is reactivated and the downstream signaling pathways remain KIT dependent [65]. The acquired resistance to imatinib typically occurs through second site mutations in KIT, detected in 46–67% of patients, which bypass the inhibitory effects of the drug by two possible mechanisms [65–66]. First, second site mutations may specifically interfere with imatinib binding without affecting the overall KIT kinase conformation. Alternatively, these mutations may stabilize the active conformation of the KIT kinase which prevents imatinib binding. Other mechanisms may also be involved.

However, one third of imatinib-resistant GIST lack secondary mutations, suggesting that additional mechanisms of resistance might be responsible, such as KIT genomic amplification and activation of an alternative receptor tyrosine kinase protein in the absence of KIT expression. The mechanism of primary resistance remains unclear.

The fact that resistance occurs at the level of KIT and not by additional mutations in downstream components or other signaling pathways are the most stunning illustration of the specificity of oncogene addiction and underscore the unique role of KIT as a therapeutic target in these tumors. In GIST, the second site mutations occur without exception on the same allele as the primary mutation and affect either the first or the second kinase domains, leading to an imatinib-resistant KIT oncoprotein [65, 67]. The mechanism for the development of second site *KIT* mutations remains unclear, but resistant patients with identifiable secondary mutations have been treated with imatinib longer than resistant patients lacking secondary mutations (median 27 versus 14.5 months) [65]. These findings suggest that clonal selection of existing mutations prior to imatinib therapy is unlikely to explain acquired resistance. Secondary mutations are typically not seen in the pre-imatinib or primary resistant tumors. These secondary mutations tend to be single amino acid substitutions in the catalytic domain (exon 17) or in the ATP-binding domain (exons 13 and 14) [65]. The frequency of secondary mutations is also determined by the location of the primary *KIT* mutations, with GISTs harboring *KIT* exon 11 mutations more commonly becoming imatinib-resistant due to acquisition of secondary mutations, as compared to *KIT* exon 9 mutated GIST [65]. This observation further supports that the probability of developing a secondary mutation increases with duration of imatinib treatment, which often is longer in GISTs harboring exon 11 mutation than in those with exon 9 mutation or wild-type GISTs.

Microscopically, the clinically resistant tumors if resected show marked increased cellularity and a significantly higher mitotic activity compared to pre-imatinib biopsies (Fig. 5). Both primary and secondary imatinib resistant tumors show strong and diffuse KIT immunopositivity (Fig. 5B). The degree of KIT phosphorylation appears significantly higher than in the non-treated, imatinib-naïve GISTs, however, within the resistant subset, the degree of KIT activation is consistently high, regardless of the status of secondary *KIT* mutations or the type of clinical resistance (primary versus secondary) [65]. Only rarely, loss of KIT expression is noted in imatinib-resistant tumors, suggesting activation of KIT-independent oncogenic pathways. One example is the ‘*dedifferentiated GIST*’, defined as tumor progression from a conventional KIT positive GIST to an anaplastic KIT negative tumor. Changing phenotype after chronic exposure to imatinib includes not only the loss of KIT reactivity, but also the aberrant expression of epithelial and muscle markers [68]. More recently Liegl et al. reported five cases of progressing metastatic GIST with heterologous rhabdomyoblastic differentiation after imatinib treatment. Primary *KIT* mutations were detected in both the conventional GIST and rhabdomyoblastic components, but no secondary mutations of the type associated with TKI resistance were identified in the dedifferentiated or rhabdomyoblastic areas [69].

Another level of complexity relies on the fact that long-term imatinib therapy leads to clonal selection of distinct resistant tumor subclones, the so-called ‘*polyclonal acquired resistance*’

[70–71]. Thus, each tumor nodule under progression may undergo individual clonal evolution, resulting in multiple secondary mutations developed at different metastatic sites within the same patient. As such, designing salvage strategies in these imatinib-resistant settings should address inhibition of all known genomic activating mutations in the oncoprotein. The complexity of secondary mutations in imatinib-resistant patients argues that single next-generation kinase inhibitor will not be beneficial in all mutant clones.

Confronted with such a wide range of mutations one would like to predict the spectrum and frequency of vulnerable sites for acquired mutations under the selective pressure of individual TKI in an *in vitro* model. Learning from the CML experience, fast-forward mutagenesis on *BCR-ABL* transformed Ba/F3 cells has shown the following: 1) a remarkable overlap with the pattern of mutations seen in imatinib-resistant CML patients; 2) although ENU-induced mutagenesis is expected to induce mutations in multiple proteins, resistant clones were almost exclusively seen in the *BCR-ABL* kinase domain (T315I) at relevant concentrations; 3) decrease of resistant clones outgrowth with drug combination, at lower concentrations compared to single agents; 4) combining drugs with different mechanisms of inhibition (i.e. allosteric with ATP-binding-site inhibitors) suppress completely the emergence of resistance mutations [72, 73–75]. Following this example, we were able to determine the pattern and spectrum of second site mutations using an *in vitro* mutagenesis screen in KIT [76]. Imatinib selection of Ba/F3 *KIT* exon 11 mutants identified a 90% mutation rate, with D655, T670, D816 and N822 as resistant hot spots. In contrast imatinib selection of the Ba/F3 *KIT* exon 9 mutants showed a significantly lower rate of secondary mutations, with T670, D816 and D820 as hot spots. Overall the *in vitro* resistance screen showed a good correlation with the genotypes observed in imatinib-resistant patients. However, the fast-forward mutagenesis results suggest that the location to either ectodomain or juxtamembrane domain of the primary *KIT* mutation may trigger different mechanisms of drug resistance, with a higher rate of resistant mutations seen with primary *KIT* exon 11 mutants [76].

Once resistance to imatinib develops, there is currently only a small chance of rescuing the patient. Dose escalation of imatinib in 133 patients who progressed at 400 mg per day resulted in a median time to progression of only 81 days, and only 18% were progression-free at 1 year [77]. In the setting of active disease progression on TKI therapy, discontinuation therapy may lead to accelerated tumor growth by withdrawing control of sensitive clones of the disease (even if limited disease sites have been shown to exhibit resistance to therapy and hence to progress more rapidly). Therefore in the absence of a clinical trial testing of a different hypothesis continuing TKI therapy should be an essential component of best supportive care for patients with progressive disease. Supporting these observations, Fumagalli et al showed that rechallenging patients with imatinib after standard and investigational therapeutic options fail resulted in disease activity and prolonged survival [78]. In the context of limited progression, patients who are no longer experiencing benefit from current TKI therapy should be given another trial of previously tolerated and effective therapy.

Second Line Therapy Paradigm – similarities with first line TKI regarding genotype-dependent response and selection of double KIT-mutant resistant clones

Sunitinib malate (Pfizer) is the only FDA approved second-line therapy for patients with imatinib-resistant or imatinib-intolerant GIST. The clinical benefit of sunitinib is genotype dependent in regards to both primary and secondary mutations, with GIST harboring KIT ectodomain mutations being the most sensitive. Because sunitinib activity encompasses a broader spectrum of targeted kinases compared with imatinib, including anti-vascular endothelial growth factor receptor activity, it is possible that additional mechanisms play a role in the acquisition of resistance. However, data obtained from clinical samples as well as from *in vitro* mutagenesis suggest that sunitinib resistance shares similar pathogenetic mechanisms seen in imatinib failure, with acquisition of secondary mutations in the activation loop conferring resistance to both drugs [76]. The three patients from our Institution who developed sunitinib resistance after at least one year of clinical benefit had a similar primary mutation in *KIT* exon 9 and second site mutations in the *KIT* kinase activation domain (N822K, D820Y and D820E) [76]. In keeping with the clinical observations, the *KIT* mutations observed by the *in vitro* mutagenesis recapitulated the type and location of second-site *KIT* mutations observed in both imatinib- and sunitinib-resistant patients. Thus all sunitinib-resistant secondary mutations identified in the Ba/F3 *KIT*^{502-3AYins} cells were similarly point mutations in the KIT kinase activation loop [76].

Analogous Emerging Paradigms in Sarcomas – the Battle for Defining Additional Targetable Oncogenic Kinases

A number of other activated kinases have been identified, some of them by mining the global transcriptional output of sarcoma types. The oncogenic up-regulation of these receptor tyrosine kinases was secondary either to activating mutations (angiosarcoma) or to a gene fusion leading to constitutive expression of the ligand (and its receptor, presumably through an autocrine or paracrine loop, in dermatofibrosarcoma protuberans). Thus, in a recent expression profiling study, angiosarcomas (AS) were characterized by up-regulation of vascular specific receptor tyrosine kinases, including *TIE1*, *KDR* (*VEGFR2*), *TEK* (*TIE2*), and *FLT1* (*VEGFR1*) [79]. Full sequencing of these candidate genes identified mutations in *KDR* in 10% of AS patients. A *KDR*-positive genotype was typically associated with strong *KDR* protein expression. *KDR*-mutated positive tumors were limited to the breast anatomic location, with or without history of prior radiation. Transient transfection of *KDR* mutants into COS-7 cells showed ligand-independent activation of the kinase, which was inhibited with specific *KDR* inhibitors [79]. These results provide basis for the activity of VEGFR-directed therapy in the treatment of AS.

The COL1A1-PDGFB fusion product in dermatofibrosarcoma protuberans (DFSP) signals through the PDGF receptor in an autocrine loop [80], which can be blocked using tyrosine kinase inhibitors acting at PDGFR, such as imatinib. A number of clinical studies have shown a high response rate to imatinib therapy in both locally advanced and metastatic DFSP [81–83]. As imatinib blocks PDGFRB signaling, these results support the concept that

DFSP cells are dependent on aberrant activation of PDGFRB for cellular proliferation and survival.

Conclusion

The era of oncogene-directed therapy in sarcoma has begun, most convincingly following the GIST paradigm and taking full advantage of the kinases selectively targeted by imatinib and other more broad-based TKIs being tested in clinical trials. The topics discussed in this review provide a strong support for shifting our understanding toward an oncogene-defined cancer subclassification and promise that other sarcoma types can be susceptible to a similar strategy, as global methodology of screening for candidate targets is more widely applied to an increasingly number of tumors.

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Fig 1.

Genomic structure of *KIT* gene in relation to the functional domains of KIT protein.

Fig 2.

Evaluating imatinib response and progression in GIST by CT. A. 54-year old male with metastatic GIST showing stable disease after 14 month of therapy, with a homogeneous and hypodense tumor, with decrease in the number of intra-tumoral vessels and solid enhancements; B. focal tumor progression, with the typical 'nodule within nodule' appearance (long arrows), detected by an increase in tumor density.

Fig 3.
Gross appearance of a liver metastasis from an imatinib-responsive GIST patient, showing a distinctive gelatinous, myxoid cut surface.

Fig 4. Microscopic appearance of the liver metastasis from Fig 2 showing a low cellularity spindle cell GIST, with increased fibrous stroma and no mitoses (A,B). The KIT expression was weak and focal (C), while the Ki67 proliferation index confirmed the lack of mitotic activity (D).

Fig 5. Microscopic appearance of an imatinib-resistant GIST, showing high cellularity and mitotic activity (A); KIT expression was diffuse and strong (B), while Ki67 showed a high proliferation index (C).