Patterns of Resistance in Gastrointestinal Stromal Tumor (GIST):
Implications for Genetic Testing and Therapeutics.

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The discovery of constitutive KIT activation as the 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 metastatic GISTs. In spite of a high rate of response in patients with KIT exon 11 mutated GISTs, the failure rate is significantly higher in patients with a wild type genotype, suggesting an alternative activated pathway not targeted by imatinib therapy. The most common mechanism of resistance is through polyclonal acquisition of second site mutations in the kinase domain, which highlight the future therapeutic challenges in salvaging these patients after failing kinase inhibitors monotherapies. During this talk will take the opportunity to summarize the recent knowledge accumulated on mechanisms of failure to targeted therapy in GIST, including second line therapies available. In addition will provide an updated discussion on diagnostic pitfalls, including changes secondary to imatinib resistance.

Pathologic and Molecular Heterogeneity of Acquired Imatinib-Resistance: Future Challenges for Targeted Monotherapy. It has become clear that most patients who initially respond to tyrosine kinase inhibitors 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 \(^1\). 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 \(^1\). The predominant mechanism of acquired resistance to imatinib is via additional mutations in the KIT kinase domain, which are found in 46-67% of patients \(^1-3\). 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)\(^1\). 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) most often, but they also occur in the ATP-binding domain (exons 13 and 14) \(^1\). The first and the second mutation are consistently located on the same allele of the KIT gene (cis-location). 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 \(^1\). 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.
Increased mitotic index is consistently seen in biopsies or surgical specimens of clinically resistant GIST patients. The tumors share similar histologic features, such as marked increased cellularity, increased nuclear pleomorphism as compared to pre-imatinib biopsies, as well as a significantly higher mitotic activity. Both primary and secondary imatinib resistant tumors show strong and diffuse KIT immunopositivity, as well as high levels of phosphorylated KIT by Western Blotting. The degree of KIT phosphorylation appears significantly higher than in the non-treated, imatinib-naive GISTs, however, within the resistant subset, the degree of KIT activation is consistently high, regardless of the type of primary KIT mutation, the status of secondary KIT mutations, or the type of clinical resistance (primary versus secondary). Only rarely, loss of KIT expression is noted in imatinib-resistant tumors, suggesting activation of novel KIT-independent oncogenic pathways.

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’ 3, 4. 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 the 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. In a recent study, Liegl et al 5 demonstrated extensive intra- and inter-lesional heterogeneity of resistance mutations in patients with clinically progressing
GIST. Of the 53 metastases studied from 14 patients, 6 patients had 2-5 secondary mutations in separate metastases, and 3 patients with two secondary mutations within same metastasis. However 2\textsuperscript{nd} site mutations were not found in wild-type GISTs or in KIT-mutant GISTs showing unusual morphology and/or loss of KIT expression by IHC, indicating a distinct mechanism of resistance in these patients.

Crystallographic studies of the KIT-imatinib complex reveal that, similar to BCR-ABL, imatinib binds the inactive conformation of the kinase. There are two possible mechanisms of how resistance to imatinib therapy may develop. First, second site mutations may stabilize the active conformation of the KIT kinase which prevents imatinib binding. Alternatively, 2\textsuperscript{nd} site mutations may specifically interfere with imatinib binding without affecting the overall KIT kinase conformation. Other mechanisms may also be involved. However, in half of imatinib-resistant GIST cases there are no identifiable 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 \textsuperscript{6,7}.

In a recent study we have identified the presence of a \textit{V600E BRAF} exon 15 mutation in one of 28 imatinib resistant GIST, lacking a defined mechanism of drug resistance. Our patient had a high risk gastric GIST, expressing both KIT and PDGFRA proteins in the primary tumor and direct sequencing demonstrated a primary \textit{PDGFRA} exon 18 deletion in the absence of \textit{BRAF} mutations. The imatinib resistant tumor, resected after 20-months of imatinib therapy, showed not only loss of KIT and PDGFRA protein expression, but
trans-differentiation into a rhabdomyosarcoma phenotype. Thus secondary \textit{V600E BRAF} mutations could represent an alternative mechanism of imatinib resistance. This result is intriguing and suggests a possible role of \textit{BRAF} mutations in triggering imatinib-resistance as well as inducing a KIT or PDGFRA negative phenotype in the resistant tumor \textsuperscript{8}. Kinase inhibitors targeting BRAF may be effective therapeutic options in this molecular GIST subset. The mechanism of primary resistance remains unclear.

\textit{Imatinib-Resistance Triggered Phenotype Change}. Dedifferentiation, defined as tumor progression from a conventional KIT positive GIST to an anaplastic/pleomorphic KIT negative tumor has been previously described after chronic exposure to imatinib and can represent a diagnostic pitfall. Changing phenotype in these cases includes not only complete loss of KIT reactivity but also acquiring aberrant expression for epithelial and muscle markers \textsuperscript{9}. More recently Liegl et al reported five cases of progressing metastatic GIST with heterologous rhabdomyoblastic differentiation after imatinib treatment. The rhabdomyoblastic components showed positivity for desmin and myogenin, while were negative for KIT expression. Primary \textit{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 rhabdomyoblastic areas \textsuperscript{10}.

\textit{Second-line Targeted Therapy in Imatinib-Resistant GIST patients}. It is now apparent that during the chronic course of imatinib treatment a significant subset of GIST patients may develop resistance. 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\textsuperscript{11,12}. 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\textsuperscript{13}. A considerable effort is focused into the development of novel tyrosine kinase inhibitors. Sunitinib malate (Sutent, Pfizer, New York, NY), a multitargeted TKI with potent anti-angiogenic and direct anti-tumor activities, is the only FDA approved 2\textsuperscript{nd} line therapy for patients with imatinib-resistant or -intolerant GIST (February 2006). Sunitinib is a highly multi-targeted tyrosine kinase inhibitor that potently inhibits FLT3, PDGFR\textsubscript{A}, PDGFR\textsubscript{B}, VEGFR\textsubscript{1}, VEGFR\textsubscript{2}, and KIT\textsuperscript{14}. Clinical benefit of sunitinib is genotype-dependent of both the primary and secondary mutations in KIT. It has been shown to give at least short term clinical benefit in about 65\% of GIST patients that are refractory to imatinib. Particularly, it shows superior efficacy in GIST patients hosting KIT exon 9 mutations\textsuperscript{15}. However, only about one-quarter of patients who are switched to sunitinib will continue to have responsive disease a year later\textsuperscript{16}. Other dual KIT-VEGFR inhibitors presently tested in clinical trials of imatinib/sunitinib-resistant GIST include a number of second-generation small molecule inhibitors, such as nilotinib, dasatinib and sorafenib. According to in vitro data these inhibitors appear to have higher efficacy and potency \textit{in vitro} in the imatinib-resistant mutations\textsuperscript{17}.

\textbf{Alternative Therapeutic Options.} Due to the proliferation advantage and apoptosis resistance conferred by oncogenic KIT signaling, GISTs are quite resistant to conventional modalities of chemo- and radiotherapy. Alternative salvage options other than inhibiting KIT signaling pathway are presently being explored. These strategies promise a therapeutic solution to the challenge of heterogeneous imatinib resistance.
Bauer et al.\textsuperscript{18} investigated the activity of heat shock protein 90 (HSP90) inhibitor 17-allylamino-18-demethoxy-geldanamycin (17-AAG) in enhancing cellular degradation of constitutively activated KIT oncoprotein. Thus, 17-AAG was effective in inhibiting KIT-dependent imatinib sensitive and imatinib-resistant GIST cell lines, but not KIT-independent GIST cells, suggesting that its effects result mainly from inactivation of KIT oncoprotein. Taking a different approach, Sambol et al.\textsuperscript{19} showed the efficacy of flavopiridol, a cyclin-dependent kinase inhibitor, on a primary GIST cell line, by induction of apoptosis and transcriptional down-regulation of KIT. A possible combination of targeted kinase inhibitors with drugs such as flavopiridol or 17-AAG may be more effective in the setting of polyclonal acquired resistance. Other broad therapeutic strategies include targeting KIT downstream targets, such as PI3-K or MAPK/MEK inhibitors\textsuperscript{20}. 
References


