KIT Oncogenic Mutations: Biologic Insights, Therapeutic Advances, and Future Directions

Jonathan A. Fletcher

See related article by Rubin et al., Cancer Res 2001;61:8118–21.

This commentary offers a historical perspective on a report in Cancer Research that showed that most gastrointestinal stromal tumors (GIST) have gain-of-function mutations in the KIT receptor tyrosine kinase (1). These gain-of-function mutations occurred in the extracellular dimerization domain or in three cytoplasmic domains of KIT, causing constitutive activation. As they occurred at early stages of GIST development, the findings suggested that KIT activation is pivotal in most GISTs and likely an initiating tumorigenic event. Furthermore, they predicted that KIT was a nearly universal therapeutic target in GIST, setting the stage for characterizing mechanisms of response and resistance to inhibitors of KIT signaling in GIST patients.

KIT Mutations Establish GIST as a Unique Biological and Clinicopathologic Entity

Three years prior to this report, Hirota and colleagues published a landmark study (2), showing KIT expression in 94% of GISTS and noting KIT exon 11 oncogenic mutations in 5 of the 6 human GIST samples sequenced. These findings implicated KIT activation as a fundamental oncogenic mechanism in GISTS. The findings also established expression of the KIT oncoprotein as a diagnostic biomarker for these theretofore poorly understood tumors and underscored biological relationships between GIST and interstitial cells of Cajal, the pacemaker cells of the gut that coordinate peristaltic activity throughout the gastrointestinal tract. These insights were transformative for the sarcoma field. Previously, “GIST” had not been a widely used acronym. Indeed, the unique biological and histologic features of GIST were so poorly understood that most pathologists and clinicians classified them as other types of sarcoma, typically as “leiomyosarcoma” if the GIST exhibited histopathologic features of malignancy. This was because GISTS have histologic resemblance to benign smooth muscle tumors, even if their underlying biology is substantially different (smooth muscle neoplasms neither express KIT nor have KIT genomic mutations). After the pivotal study by Hirota and colleagues, several groups confirmed KIT exon 11 mutations, which were found in approximately half of malignant cases but rarely in benign GISTS. Furthermore, GISTS with KIT exon 11 mutations seemed to be larger, higher grade, and associated with increased recurrence rate and lower survival, compared with those without demonstrable KIT mutations. However, our report showed that these prognostic correlates were spurious, stemming from technical difficulty detecting the KIT mutations in low-grade/early-stage GISTs, which often contain more nonneoplastic cells than high-grade GISTS.

KIT Mutations Are Not Restricted to Exon 11 or to Advanced GISTS

In the study by Rubin and colleagues (1), we were fortunate to use snap-frozen specimens, thereby permitting convenient cDNA sequencing of the entire 21-exon KIT coding sequence. This approach was more sensitive than genomic KIT exon 11 Sanger sequencing, because KIT transcripts are expressed primarily in the neoplastic cells from the GIST specimens. Therefore, the assays were not confounded by nonneoplastic cells, which comprise a substantial part of the overall cellularity, particularly in early GISTS. We found KIT oncogenic mutations in 44 of 48 GISTS, including each of 10 benign GISTS, 8 of 10 borderline GISTS (having histologic features intermediate between benign and malignant), and 26 of 28 malignant GISTS. This showed that KIT activation is not only an overarching event in GIST but an early and perhaps initiating event, in keeping with descriptions of germline KIT oncogenic mutations in GIST kindreds. We found KIT mutation hotspots not only in exon 11, but also in exon 9 (n = 6), resulting in tandem duplication of amino acids 502–503 in an extracellular dimerization region, exon 13 (n = 2; K642E substitutions in the kinase ATP-binding pocket), and exon 17 (n = 2; N822 substitutions in the kinase activation loop). Furthermore, by identifying a GIST subset that is truly KIT wild type, these studies led to intensive sequencing and proteomic queries that subsequently revealed the first examples of oncogenic PDGFRA mutations (3). KIT and PDGFRA are structurally similar receptor tyrosine kinases, and GISTS with mutations in either of these kinases have overlapping biological similarities (3). However, the kinase mutation type proved relevant in that GISTS with KIT exon 11 mutations are either gastric or small-bowel tumors (the two most common primary sites for GISTS) and can be either low grade or high grade, whereas those with KIT exon 9 mutations are more often small-bowel tumors and high grade. Those with PDGFRA mutations are often gastric, clinically indolent when localized, and with epitheloid histologically (contrasting with the majority of KIT-mutant GISTS, which have prominent spindle-cell morphology). Additional molecular subtypes of GISTS were identified subsequently, including those associated with neurofibromatosis type 1 or succinate dehydrogenase deficiency, the latter, as the Carney–Stratakis dyad of paraganglioma and GIST, often resulting from germline SDH subunit gene inactivating mutations (4).

Clinical Relevance of KIT Mutations

Our studies showed that most GISTS express KIT and contain KIT oncogenic mutations, whereas GIST morphologic mimics do not. With improved diagnostics, particularly incorporating IHC as a standard aspect of the work-up, it became clear that GIST, a previously nebulous entity, was surprisingly the most common...
subtype of all sarcomas. Whereas GIST had formerly been among the most chemoresistant sarcomas, the advent of imatinib and other tyrosine kinase inhibitor drugs transformed the disease into a poster child for success in targeted therapeutics. Most patients with metastatic GISTs have at least 2 years of excellent disease control with first-line imatinib therapy, and, remarkably, more than 25% of these patients remain on imatinib after 5 years of therapy (5), with more than 15% still alive after 15 years from initial imatinib. These remarkable clinical advances underscored the need to estimate prognosis and treatment benefit for individual patients with early-stage disease prior to the appearance of clinical metastases, leading to the development of practical and useful risk-classification schemes, in which the terms “low risk” versus “high risk” embody much of what was previously referred to as “benign” versus “malignant.” Aversion to calling GISTs “benign” stemmed from the recognition that even the smallest and most low-grade GIST could occasionally metastasize, but this tendency can lead to exaggeration, sending the incorrect message that even harmless appearing GISTs (convincingly benign) have some finite and definable risk of metastasis.

Our preclinical demonstration of imatinib response in KIT-mutant GISTs led Demetri and colleagues to rapidly evaluate the therapeutic strategy in patients with metastatic GIST, most of whom had major responses (6). The initial clinical studies led to larger multicenter collaborative efforts and served as the basis for worldwide regulatory approval of imatinib as an effective therapy. These and other studies showed that GISTs with the common KIT exon 11 mutations respond particularly well to imatinib (5), whereas those with KIT exon 9 mutations are less potently inhibited and therefore benefit from imatinib dose escalation. Several primary mutations, such as PDGFRA D842V, are imatinib resistant but relatively rare in advanced GISTs. Even patients with near-complete imatinib responses invariably have residual surviving GIST cells, including resistant subclones, which sow the seeds for eventual clinical progression (8). Demetri and colleagues therefore evaluated other KIT/PDGFRα inhibitor drugs with different target inhibition profiles. These studies demonstrated that sunitinib, which inhibits certain KIT structural variants more potently than imatinib, can induce clinical disease control and prolong survival when given after failure of imatinib. This work served as the basis for worldwide regulatory approval of sunitinib for second-line treatment of advanced GIST. Unfortunately, response duration to sunitinib in an unselected population of GIST patients is relatively brief (6–9 months) because many patients have polyclonal imatinib resistance, in which some cells have acquired secondary KIT exon 13 mutations (sensitive to sunitinib), whereas others have secondary KIT exon 17 mutations (resistant to sunitinib as well as imatinib). These KIT exon 13 and 17 mutations account for 80% to 90% of acquired imatinib resistance and are on the same allele (in “cis”) with the primary KIT mutations. Therefore, the primary and secondary mutations collaborate to determine the imatinib-resistant KIT oncoprotein structure, and sunitinib has differential binding interactions with these various structural forms. Regorafenib, which was approved as a third-line therapy for GIST, is similarly effective against some but not all imatinib-resistant mutations in KIT. Fortunately, because KIT biology is essential in most GISTs, the tumors remain addicted to the unique constellation of KIT-dependent signaling pathways and cannot readily develop drug resistance by supplanting KIT with other oncogenic pathways. Exceptions include acquisition of secondary NF1/RAS and PI3K/mTOR pathway-activating mutations, but these mutations activate pathways immediately downstream of KIT/PDGFRA, rather than engaging unrelated oncogenic pathways.

### Additional Biological Perturbations in KIT-Mutant GISTs

The initiating events in virtually all GISTs are gain-of-function mutations in KIT or PDGFRA or loss-of-function mutations in NF1 or SDH family genes. Notably, KIT/PDGFRA activation is both essential to the development of most GISTs and has crucial ongoing roles in tumor maintenance. For this reason, sequential biological perturbations contributing to GIST progression generally build upon a backdrop of KIT/PDGFRA oncogenic activation, either refining and redirecting the KIT oncogenic signal or leveraging KIT/PDGFRα activation through dysregulation of unrelated pathways. KIT oncoproteins in GIST transmit oncogenic signals via the PI3K and RAS/RAF/MEK pathways, and the ETV1 transcription factor is a key effector of MEK/MAPK, activating many genes that induce GIST proliferation (9). ETV1 expression in GIST requires both KIT/PDGFRA and MEK activation, underscoring therapeutic opportunities for RAS pathway inhibition. Other observations highlighting the oncogenic importance of RAS/RAF/MEK are acquisition of classic RAS or BRAF oncogenic functions in a subset of KIT-mutant GISTs, endowing these GISTs with imatinib resistance.

Biological drivers of GIST progression include mutations regulating the cell-cycle inhibitors CDKN2A, RB1, and TP53. These mutations are rarely described in early “low-risk” GISTs and probably contribute to transition from low-risk to more mitotically active high-risk disease. More than 75% of metastatic KIT/PDGFRA-mutant GISTs have genomically mediated loss of dystrophin expression (10), corresponding to acquisition of clinically aggressive behavior. In these tumors, dystrophin down-regulation is often caused by intragenic deletions within the dystrophin gene, DMD, destroying the DMD open reading frame. Restoration of dystrophin expression inhibits invasion, migration, and anchorage-independent growth. These observations show that genomically mediated dystrophin inactivation is a frequent event during GIST progression and consistent with a critical contributory role for dystrophin inactivation in enabling GIST metastasis.

### Future Directions

Although second-line sunitinib is a multikinase inhibitor with anti-VEGFR activity, there is no evidence that activity beyond the KIT/PDGFRα targets is a crucial determinant of GIST benefit in patients. Rather, all evidence suggests that patients with imatinib-resistant GISTs only respond when the progressing metastases contain sunitinib-responsive KIT mutations in exons 13 or 14, typically V654A or the “gatekeeper” T670I substitutions in the KIT ATP-binding pocket. Similarly, there are no data to suggest that third-line regorafenib activity against imatinib-resistant GISTs is due to inhibition of targets beyond KIT/PDGFRα. These observations highlight ongoing opportunities to develop more selective and potent drugs effective against KIT primary exon 9 and 11 mutations, in isolation and in “cis” with V654A, T670I, or activation loop (exons 17 and 18) mutations. Such strategies could be administered at higher effective doses (with fewer nonspecific

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toxicities than sunitinib or regorafenib), thereby maximizing KIT/PDGFRα inhibition. Therapeutic progress will also result from more effective targeting of downstream pathways that convey KIT/PDGFRα oncogenic signals, of which RAS/RAF/MEK and PI3K/AKT remain promising. Biological and therapeutic advances in GISTs should be translatable to other cancers with the same KIT oncogenic mutations, including subsets of acute myeloid leukemia, mast cell disease, germ cell tumor, and melanoma. In addition, GIST remains a paradigm example for all kinase-driven cancers, from which much has been learned with relevance to lung cancers, melanomas, and many other forms of human neoplasia.

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No potential conflicts of interest were disclosed.

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