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KRAS and *KIT* Gatekeeper Mutations Confer Polyclonal Primary Imatinib Resistance in GI Stromal Tumors: Relevance of Concomitant Phosphatidylinositol 3-Kinase/AKT Dysregulation

Introduction

KIT juxtamembrane oncogenic mutations (encoded by *KIT* exon 11) are found in 67% of GI stromal tumors (GISTs) and are inhibited potently by imatinib. Virtually all patients with these mutations therefore achieve clinical benefit when treated with imatinib. Imatinib resistance in *KIT* exon 11–mutant GISTs typically occurs after 18 to 24 months of response or disease stabilization, most often resulting from expansion of multiple tumor clones harboring secondary *KIT* kinase domain mutations.[1] However, approximately 10% of patients with GISTs have primary imatinib resistance, defined by clinical progression within 3 to 6 months after initiating therapy. Such GISTs typically lack *KIT* and platelet-derived growth factor receptor alpha (*PDGFRA*) mutations, or contain particular mutations, such as *PDGFRA* D842V, that are intrinsically imatinib resistant.[2] To our knowledge, this is the first report of polyclonal heterogeneity—including *KRAS* mutation— as a mechanism of primary imatinib resistance in a patient with GIST.

Case Report

A 61-year-old man presented to an outpatient clinic in October 2003 with an 8-week history of progressive left shoulder pain, nausea, and fatigue. A left upper quadrant mass was palpable on physical examination. Laboratory data were normal, other than a hematocrit level of 28.3% and a platelet count of 536,000/uL. An abdominal computed tomography (CT) scan revealed a 19.7 × 13.1-cm mass arising from the anterior wall of the stomach, accompanied by five liver metastases, all less than 1 cm in maximal diameter. Endoscopic biopsy demonstrated a spindle cell GIST (Fig 1A) with 20 mitoses per 50 high-power fields (hpf), diffuse cytoplasmic KIT expression (Fig 1B), CD34 expression, and no expression of smooth muscle actin and cytokeratins.

The patient received imatinib 400 mg per day and experienced symptomatic improvement within 1 month, including resolution of shoulder pain, softening of the palpable mass, and normalization of the blood counts. A follow-up CT scan after 6 weeks of treatment with imatinib showed that the gastric mass (22.7 × 13.1 cm) had typical post-therapy changes, including hypodensity and a decrease in wall thickness (Fig 2A). The liver metastases were unchanged. A CT scan at week 16 of imatinib treatment showed reduction of the hypodense overall gastric residual mass to 15.4 × 11.6 cm; however, a new, hyperdense 2.7 × 2.0-cm nodule was present at the caudal aspect of the mass (Fig 2B). The patient continued to receive imatinib, and a follow-up CT scan 2 months later showed progression of the hyperdense nodule to 4.9 × 5.6 cm, now accompanied by additional progressing nodules in the bed of the gastric primary (Fig 2C). An upper GI bleed prompted resection of the gastric mass, which was performed 24 hours after the last imatinib dose. During this surgery, subcentimeter peritoneal implants were observed but not removed. Histologically, the gastric mass was spindle cell–type GIST. Genomic analyses by Sanger sequencing, Ion Torrent (Life Technologies, Carlsbad, CA), and Sequenom MassArray System (Sequenom, San Diego, CA) were performed in clinically responding (region No. 1) versus clinically progressing (regions No. 2 and No. 3) aspects of the mass. Region No. 1 was hypocellular, nonmitotic, and therefore consistent with stable/responding disease, whereas regions No. 2 and No. 3 had 60 and 55 mitoses per 50 hpf, respectively, and were therefore consistent with progressing, imatinib-resistant disease. Each of these three regions expressed KIT strongly and had a homozygous *KIT* exon 11 E554_V559del mutation (Fig 3A) and a homozygous *PTEN* missense mutation, C124S (Fig 3B), which is known to abrogate PTEN lipid- and protein-phosphatase activity and PTEN-mediated phospholipase

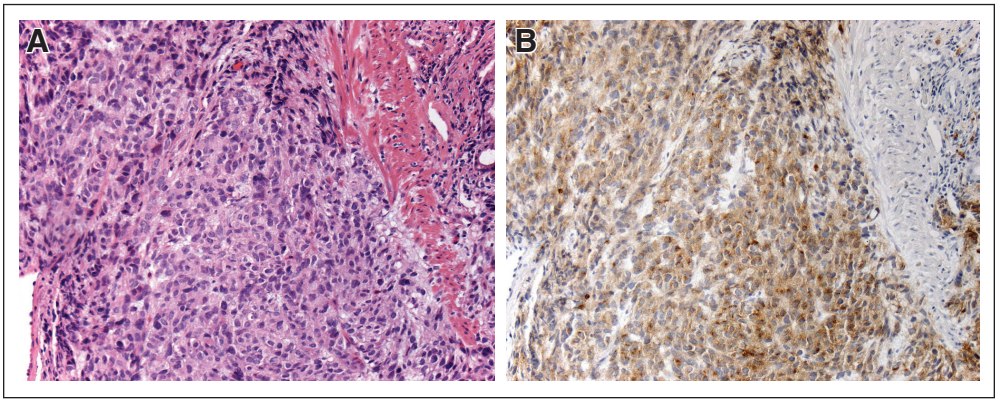

Fig 1.

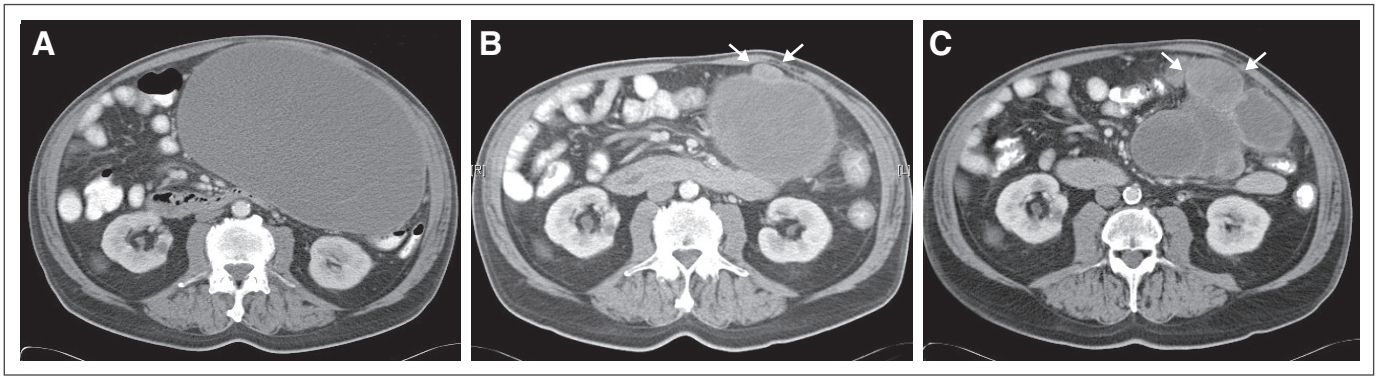

Fig 2.

regulation.[3] The imatinib-responsive region No. 1 had no additional mutations, whereas imatinib-resistant region No. 2 had a *KRAS* G12R mutation by Sequenom analysis, which was corroborated by *KRAS* genomic sequencing and transcript allelic subcloning and sequencing (Fig 3C). Imatinib-resistant region No. 3 had a *KIT* gatekeeper T670I mutation (Fig 3D), which is known to confer imatinib resistance.[4] Immunoblotting evaluations confirmed strong KIT expression in both imatinib-responsive and -resistant regions (Fig 4); however, KIT was activated, as assessed by phosphoKIT Y721 expression, only in region No. 3 with the *KIT* T670I mutation, mitogen-activated protein kinase was hyperactivated only in region No. 2 with *KRAS* G12R, whereas AKT was hyperactivated in both of these regions (Fig 4).

Imatinib was resumed, but the patient manifested further progression of intra-abdominal disease 4 weeks postoperatively and died 5 months later while receiving high-dose imatinib (800 mg per day). He was unable to receive second-line therapy because progression occurred during the window between completion of a phase III trial and US Food and Drug Administration approval of sunitinib for imatinib-resistant GIST.

Wild-type GISTs lacking *KIT* and *PDGFRA* mutations frequently show primary imatinib resistance, and although some of these are succinate dehydrogenase-deficient because of *SDHA*, *SDHB*, or *SDHC* mutations,[5,6] others have no known genetic mutations. To test the hypothesis that such GISTs might contain *RAS* mutations or other *KIT* downstream mutations, we used a Sequenom panel to screen for *RAS*, *BRAF*, and *PI3KCA* mutations in *KIT/PDGFRA* wild-type GISTs from 27 patients. Only one of these 27 GISTs contained demonstrable mutation(s): this was a high-risk GIST (8-cm gastric primary with 62 mitoses per 50 hpf) that contained both *HRAS* G12V and *PIK3CA* H1047R mutations. *PIK3CA* H1047R is a gain-of-function mutation that accounts for approximately 20% of *PIK3CA* mutations in advanced human cancers[7] and is associated with response to phosphatidylinositol 3-kinase (PI3K)/AKT/mammalian target of rapamycin pathway inhibitors.[8]

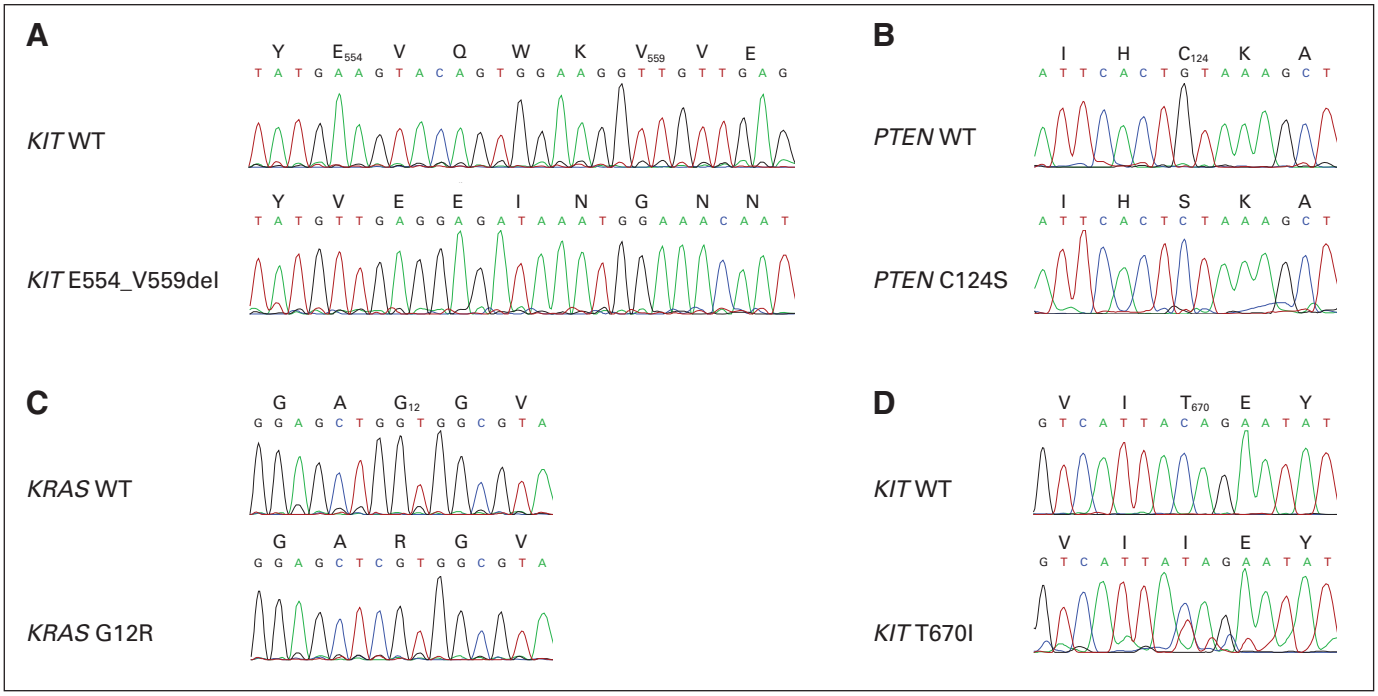

Fig 3.

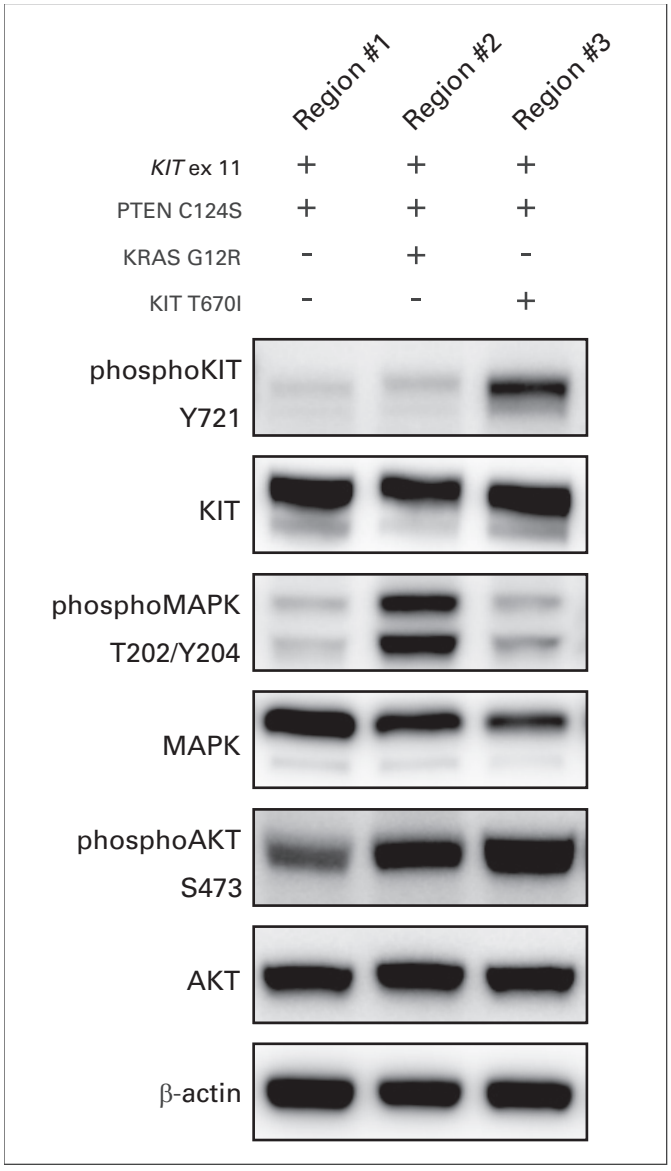

Fig 4.

Discussion

Although pretreatment genomic intratumoral heterogeneity has been implicated as a cause of treatment failure in several solid tumors,[2] this report provides the first evidence, to our knowledge, of primary imatinib resistance resulting from intratumoral genomic heterogeneity. This resistance, manifested already at 16 weeks of treatment, was related (in separate lesions) to *KRAS* mutation and the *KIT* gatekeeper mutation T670I. Recently, Miranda et al[9] reported in vitro evidence that *KRAS* mutations might bestow imatinib resistance in GIST, and our case report corroborates that the *KRAS* gain-of-function mutation is a contributor to clinical imatinib resistance, even in the face of therapeutic KIT oncoprotein inhibition. Notably, Diaz et al[10] predicted by mathematical modeling that *KRAS*-mutant subclones are present before initiation of anti–epidermal growth factor receptor treatment in some colorectal cancers. Similarly, it is conceivable that *KRAS* mutations are present as minor subclones in more untreated GISTs than previously appreciated, and are then enriched for by KIT/PDGFRA-inhibitor therapies.

Most GISTs with RAS pathway dysregulation by BRAF or NF1 alterations have been low-grade and low-risk tumors,[11-15] whereas the two *RAS*-mutant cases reported here—both containing concurrent *PI3K-PTEN* mutations— are decidedly high grade. Notably, our case report relates to a *KIT* exon 11–mutant GIST in which the *KRAS* mutation was restricted to a subregion of the tumor and was therefore acquired after the *KIT* exon 11 mutation. However, our mutation screens in 27 *KIT/PDGFRA* wild-type GISTs demonstrated a case with concomitant *PIK3CA* and *HRAS* mutations, suggesting that PI3K and RAS pathway genetic coactivation provides a transforming equivalent to KIT activation in some GISTs lacking *KIT* mutations. In this sense, we propose that KIT/PDGFRA oncogenesis in high-grade GISTs is most effectively supplanted when the PI3K/AKT and RAS/RAF/MEK pathways are both constitutively activated by independent mutations. This hypothesis is appealing in that GIST *KIT/PDGFRA* mutations are known to coactivate both PI3K and RAS downstream pathways.[16] The hypothesis warrants evaluation in a larger group of patients with GISTs, but if true, adds a level of complexity to KIT/PDGFRA downstream resistance mechanisms and accounts for why such mechanisms, potentially requiring mutational hits to genes in two pathways, infrequently cause imatinib resistance. In keeping with this hypothesis, we note that the *PTEN* C124S mutation reported here was demonstrated along with *KIT* exon 11 mutation in both imatinib-sensitive and imatinib-resistant aspects of the GIST. Therefore, the *PTEN* mutation was not directly responsible for imatinib resistance, but likely created a biologic state that was permissive for KRAS G12R–transforming activity, with KRAS G12R being a known imatinib-resistance mechanism.[9]

In summary, our findings demonstrate *KRAS* mutation and polyclonal heterogeneity as mechanisms of primary imatinib resistance in GIST, show that both KRAS and HRAS isoforms can contribute to GIST oncogenesis, and highlight the conjoined nature of the PI3K/AKT and RAS/RAF signaling pathways in GIST tumorigenesis. These findings validate the PI3K/AKT/ mammalian target of rapamycin pathway and RAS/RAF/MEK pathways as concurrently relevant in GIST oncogenic signaling.

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REFERENCES

1. Liegl B, Kepten I, Le C, et al: Heterogeneity of kinase inhibitor resistance mechanisms in GIST. J Pathol 216:64-74, 2008
2. Gramza AW, Corless CL, Heinrich MC: Resistance to tyrosine kinase inhibitors in gastrointestinal stromal tumors. Clin Cancer Res 15:7510-7518, 2009
3. Alvarez-Breckenridge CA, Waite KA, Eng C: PTEN regulates phospholipase D and phospholipase C. Hum Mol Genet 16:1157-1163, 2007
4. Heinrich MC, Corless CL, Blanke CD, et al: Molecular correlates of imatinib resistance in gastrointestinal stromal tumors. J Clin Oncol 24:4764-4774, 2006
5. Janeway KA, Kim SY, Lodish M, et al: Defects in succinate dehydrogenase in gastrointestinal stromal tumors lacking KIT and PDGFRA mutations. Proc Natl Acad Sci U S A 108:314-318, 2011
6. Miettinen M, Wang ZF, Sarlomo-Rikala M, et al: Succinate dehydrogenase-deficient GISTs: A clinicopathologic, immunohistochemical, and molecular genetic study of 66 gastric GISTs with predilection to young age. Am J Surg Pathol 35:1712-1721, 2011
7. Janku F, Wheler JJ, Naing A, et al: PIK3CA mutations in advanced cancers: Characteristics and outcomes. Oncotarget 3:1566-1575, 2012
8. Janku F, Wheler JJ, Naing A, et al: PIK3CA mutation H1047R is associated with response to PI3K/AKT/mTOR signaling pathway inhibitors in early-phase clinical trials. Cancer Res 73:276-284, 2013
9. Miranda C, Nucifora M, Molinari F, et al: KRAS and BRAF mutations predict primary resistance to imatinib in gastrointestinal stromal tumors. Clin Cancer Res 18:1769-1776, 2012
10. Diaz LA Jr, Williams RT, Wu J, et al: The molecular evolution of acquired resistance to targeted EGFR blockade in colorectal cancers. Nature 486:537-540, 2012
11. Agaram NP, Wong GC, Guo T, et al: Novel V600E BRAF mutations in imatinib-naive and imatinib-resistant gastrointestinal stromal tumors. Genes Chromosomes Cancer 47:853-859, 2008
12. Hostein I, Faur N, Primois C, et al: BRAF mutation status in gastrointestinal stromal tumors. Am J Clin Pathol 133:141-148, 2010
13. Agaimy A, Terracciano LM, Dirnhofer S, et al: V600E BRAF mutations are alternative early molecular events in a subset of KIT/PDGFRA wild-type gastrointestinal stromal tumours. J Clin Pathol 62:613-616, 2009
14. Daniels M, Lurkin I, Pauli R, et al: Spectrum of KIT/PDGFRA/BRAF mutations and phosphatidylinositol-3-kinase pathway gene alterations in gastrointestinal stromal tumors (GIST). Cancer Lett 312:43-54, 2011
15. Miettinen M, Fetsch JF, Sobin LH, et al: Gastrointestinal stromal tumors in patients with neurofibromatosis 1: A clinicopathologic and molecular genetic study of 45 cases. Am J Surg Pathol 30:90-96, 2006
16. Bauer S, Duensing A, Demetri GD, et al: KIT oncogenic signaling mechanisms in imatinib-resistant gastrointestinal stromal tumor: PI3-kinase/AKT is a crucial survival pathway. Oncogene 26:7560-7568, 2007

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