Successful Imatinib Therapy for Neuroendocrine Carcinoma With Activating *KIT* Mutation: A Case Study

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Abstract

Neuroendocrine tumors (NET) and gastrointestinal stromal tumors (GIST) are believed to originate from the cells of Cajal that are randomly dispersed along the aerodigestive tract. Despite their distinct morphologic appearance, NET and GIST may share oncogenic mechanisms. Often presenting in the metastatic setting, treatment options for patients with NET are limited. This case report presents a patient with refractory metastatic NET that did not respond to conventional chemotherapy. The patient was treated with a KIF11 inhibitor in a phase I clinical trial and experienced a prolonged and clinically meaningful partial response. On progression at 20 months, the patient's tumor was sequenced to reveal a *KIT* exon 11 mutation. Institution of imatinib therapy achieved a rapid and sustained antitumor effect with profound clinical benefit. Despite previously reported *KIT* expression in NET, this is the first documented case of an activating *KIT* mutation in NET and of successful treatment with both a KIF11 inhibitor and imatinib, each of which was elucidated through molecular profiling of the patient's tumor. Imatinib may be a valuable therapy in NET harboring activating *KIT* mutations. (*J Natl Compr Canc Netw* 2014;12:847–852)

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Learning Objectives

Upon completion of this activity, participants will be able to:

- Summarize the criteria on which the classification of NET is based and list some markers for NET
- Describe the rationale for the use of imatinib in the treatment of patients with NET with activating *KIT* mutations
- Evaluate the data supporting the hypothesis that NET and GIST arise from a common precursor

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Neuroendocrine tumors (NET) and gastrointestinal stromal tumors (GIST) are believed to originate from the cells of Cajal¹⁻³ that are randomly dispersed along the aerodigestive tract. Despite their distinct morphologic appearance, NET and GIST may share oncogenic mechanisms. Often presenting in the metastatic setting, patients with NET have limited treatment options. This case report presents a patient with refractory metastatic NET that did not respond to conventional chemotherapy. The patient was treated with a kinesin 11 (KIF11) inhibitor in a phase I clinical trial and experienced a prolonged and clinically meaningful partial response. On progression at 20 months, the patient's tumor was sequenced to reveal a *KIT* exon 11 mutation. Institution of imatinib therapy achieved a rapid and sustained antitumor effect with profound clinical benefit. Despite previously reported *KIT* expression in NET, this is the first documented case of an activating *KIT* mutation in NET and of successful treatment with both a KIF11 inhibitor and imatinib, each of which was elucidated through molecular profiling of the patient's tumor. Imatinib may be a valuable therapy in NET harboring activating *KIT* mutations.

Case Report

A 48-year-old man with a past medical history of hypertension and testicular cancer in 1988, which was successfully treated with radiation and surgery, presented in August 2009 to a local emergency department with severe right upper-quadrant pain exacerbated with cough and inspiration without flushing or diarrhea. A CT scan revealed an enlarged liver extending to the pelvis with numerous hypodense metastatic foci without evidence of a primary tumor (Figure 1A). Fine-needle aspiration (FNA) biopsy of the liver demonstrated neuroendocrine carcinoma

Figure 1 CT images of the abdomen after oral and intravenous contrast administration at baseline (A), after 3 months of KIF11 inhibitor therapy (B), and after 4 months of imatinib therapy (C).

with rare mitoses. The amount of biopsy material was insufficient to obtain Ki-67 or any other testing. He underwent initial treatment with 4 cycles of cisplatin and etoposide, which produced minimal to no improvement. Four months later he presented to Fox Chase Cancer Center to review further treatment options. The patient received 8 months of 5-fluorouracil and streptozocin-based chemotherapy without significant improvement.⁴ His physical examination at that time was remarkable for painful hepatomegaly, with a liver edge palpable below the umbilicus as measured at the mid-sternal line. Laboratory tests revealed an elevated chromogranin A level at 40.0 ng/mL (normal range, 1.9–15.0 ng/mL; Figure 2).

In 2010, sunitinib and everolimus were not available for treatment outside of clinical trials. Additionally, the patient had no identifiable pancreatic mass to suggest a pancreatic origin of the extensive metastatic process involving the liver. Therefore, in an effort to identify putative drug targets for this patient, the authors conducted *in silico* comparisons of global mRNA profiles of NET and colon adenocarcinoma using publicly available Gene Expression Omnibus data sets (GSE2109). This showed enrichment of NET samples for several transcripts known to be bona fide markers of neuroendocrine cells: SV2C (synaptic vesicle glycoprotein 2C), CHGA, CHGB (chromogranin A and B), and TPH1 (tryptophan hydroxylase), supporting the overall validity of the approach. Another family of molecules highly expressed in NET compared with adenocarcinoma (false discovery rate <20%) were kinesin family proteins 1A, 3A, and 5C. Based on this information, the patient was enrolled in a phase I clinical trial using an inhibitor of KIF11.

Shortly after the initiation of the clinical trial treatment, a rapid improvement in symptoms was documented: pain decreased markedly, obviating the need for narcotics, and the patient's body weight increased back to baseline secondary to improvements in appetite. Consistent with the observed clinical benefit, blood chromogranin A levels precipitously declined (Figure 2). Physical examination confirmed decreased pain and a progressive reduction of the liver vertical size on palpation beginning from week 4 of therapy. On repeat imaging, a partial response was confirmed after 3 months of KIF11 inhibitor therapy by RECIST criteria (Figure 1B).⁵ Overall, the patient gained 20 pounds and his abdominal pain

Imatinib Sensitivity in Neuroendocrine Carcinoma

Figure 2 Serum chromogranin A measurements (reference range, 1.9–15.0 ng/mL).

subsided to 3 on a scale of 10. This response lasted for 20 months, followed by slow tumor progression as manifested by recurrence of abdominal pain, rising chromogranin A levels (Figure 2), and more than a 25% increase in target lesion size.

Because of a shortage of cellular material in the initial FNA, a second core needle biopsy of the liver was obtained in June 2012 and a comprehensive analysis of putative tumor biomarkers (Caris molecular profiling assay, Caris Life Sciences, Inc., Irving, TX) was ordered. The second biopsy revealed an intermediate-grade neuroendocrine carcinoma (Figure 3), staining positive for chromogranin and synaptophysin, with 20% of tumor cells positive for Ki-67. Targeted sequencing of the tumor DNA revealed an activating mutation in exon 11 (V560del, c.1679_1681delTTG) of the *KIT* receptor tyrosine kinase.⁶ This mutation has been previously associated with efficacy of imatinib in patients with GIST.⁷ A splice variant of *BAP1* (2056+1C>T), a gene associated with familial cancer risk,⁸ and amplification of *MCL1* were detected in the tumor DNA. Immunohistochemical evaluation of *KIT* expression in the tumor revealed strongly (3+) positive staining on 100% of cells (Figure 3), reinforcing the idea that, in this case, *KIT* overexpression and the presence of an activating mutation represented the central mechanism of this patient's carcinoma. Based on this information, therapy was initiated with imatinib at a standard dose of 400 mg/d. After 2 weeks of imatinib

therapy, the patient's pain began to subside and the chromogranin A declined from 44 to 4 ng/mL. On physical examination after 2 months of imatinib, the patient's recurrent hepatomegaly resolved. A CT scan of the liver confirmed a marked reduction of the hepatic lesions and normalization of the organ size (Figure 1A). At the time of writing, 46 months after his initial diagnosis, the patient had been continuing on imatinib at 400 mg/d for 12 months with good tolerability. Chromogranin A level at 12 months remains low at 4 ng/mL.

Discussion

The number of reported cases of NET in the United States from 1975 to 2004 is more than 100,000, which is more than that of pancreatic cancer and stomach cancer combined. Since 1973, the incidence of neuroendocrine cancer has increased more than 5-fold. Most NET occur in the gastrointestinal tract, with nearly 42% occurring in the small bowel.⁹ These tumors are classified based on site of origin and grade, as assessed by mitotic count and Ki-67 score. Well-differentiated tumors (low- and intermediate-grade, corresponding to a Ki-67 index of <3% and 3%–20%, respectively) behave and are treated markedly differently from poorly differentiated NET. A recent analysis of SEER data shows the median survival of well-differentiated NETs with distant metastases to be 33 months. This decreases to only 5 months for tumors that are poorly differentiated with distant metastases. Although increasing grade portends a worse prognosis and ultimately dictates a differing treatment approach, great heterogeneity remains even among well-differentiated NET. Finding therapeutic vulnerabilities in NET is of high clinical importance. For example, a subset of NET (mostly of the foregut origin) is characterized by low expression of O-6-methylguanine-DNA methyltransferase (MGMT), which portends greater sensitivity to alkylating agents such as temozolomide and streptozocin.¹⁰

This patient presented with a large hepatic tumor burden, making aggressive treatment via surgical debulking of the liver a poor option. Not surprisingly, treatment with aggressive chemotherapeutic regimens resulted in modest success consistent with the limited studies of chemotherapy in metastatic carcinoid tumors.¹¹ However, the subsequent response and clinical benefit that the patient experienced sec-

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Figure 3 Hematoxylin-eosin (A) and immunohistochemical (B) evaluation of KIT expression in a core biopsy of a hepatic metastasis.

ondary to treatment with the KIF11 inhibitor was both surprising and remarkable.

KIF11 is a homotetrameric protein that attaches to antiparallel microtubules, causing formation of a bipolar spindle allowing complete separation of chromosomes during mitosis. Inhibition of KIF11 results in a monopolar spindle apparatus causing incomplete separation of chromosomes and apoptosis.¹² The present case is the first documentation in the literature of a kinesin-5 inhibitor providing clinical benefit to a patient with a metastatic NET. Overexpression of KIF11 and its subsequent use as a therapeutic target in bladder cancer has been documented, but this strategy is not considered to be a standard treatment option.¹³ The mechanism through which KIF11 inhibition produced such a dramatic response in the present patient, whose tumor was refractory to chemotherapy, remains to be elucidated.

The NET in this patient also displayed high expression of KIT via immunohistochemistry. Express-

sion of KIT has been variable, as shown in the few preclinical studies performed of NET. KIT expression by immunohistochemistry in carcinoid tumors ranges from 0% to 38%.^{14,15} Based on these data and the high expression of platelet-derived growth factor receptors (PDGFR) α and β in carcinoid tumors, a phase II trial of imatinib in an unselected population of patients with these tumors was previously conducted. Imatinib is a small molecule inhibitor of multiple tyrosine kinases, including KIT, PDGFR, and ABL. In this trial, a partial response was seen in just 1 patient (4%). Although this response was durable, it was not associated with KIT overexpression on immunohistochemistry analysis. However, an additional 63% of patients in this trial experienced stable disease, with a median progression-free survival (PFS) of 5.9 months, and 7 patients (26%) experienced a biochemical response, resulting in a significantly improved PFS compared with those without a biochemical response (115 vs 24 weeks; $P=.003$).¹⁴ In an unselected population, some signal of efficacy was thus seen, but this treatment confers only limited benefit to this patient cohort as a whole. Imatinib has not been further pursued in this disease. Furthermore, the present patient's tumor was found to have a "classical" exon 11-activating mutation of KIT (Figure 4). Notably, KIT mutations are seen rarely, but randomly, in a variety of human cancers other than GIST in which the therapeutic significance of these mutations remains unclear. In the

Figure 4 (A) Schema of KIT protein domains (green) and gene exons (light blue) and (B) the prevalence of mutations in KIT relative to exons. (C) Frequency of KIT gene mutations in different tumor types.

Abbreviations: CNS, central nervous system; GIST, gastrointestinal stromal tumors; NET, neuroendocrine tumors.

(B, C) Data from the COSMIC database. Available at: <http://cancer.sanger.ac.uk/cancergenome/projects/cosmic/>. Accessed May 5, 2014.

present patient, the use of imatinib led to impressive symptomatic and radiographic improvements during his current 9-month treatment.

The interstitial cells of Cajal, the “pacemaker cells” of the gastrointestinal tract, likely give rise to GIST.¹⁶ Huizinga et al¹⁷ showed that knockout of *KIT* in a mouse model results in loss of cells of Cajal. Three years after this discovery, Hirota et al¹⁸ published their findings of *KIT* mutations in GIST. Interestingly, case reports have described the simultaneous occurrence of mixed GIST and neuroendocrine carcinomas in the same tumor, suggesting the existence of a shared precursor giving rise to both cancers.^{3,19,20} A subset of GIST arises from autonomous ganglia (gastrointestinal autonomic nerve tumors [GANT]²¹) of proximal bowel (stomach, small intestine, and mesentery). Some GANT and GIST may present with epithelioid microscopic features, but nearly uniformly demonstrate gastrointestinal tract involvement and are almost uniformly synaptophysin and chromogranin-negative,²² in contrast to the presence of both neuroendocrine biomarkers expressed in the present case. This may give merit to the idea that GIST and NET tumors divert from a common precursor, and in so doing, may prompt clinicians to search for *KIT* mutations in NET.

Conclusions

This report describes the treatment benefit afforded to a patient with a metastatic NET carcinoma through the use of 2 novel therapeutic agents. Available mRNA profiling data demonstrated a rationale for inhibition of kinesin family proteins in carcinoid tumors. A phase I trial involving the use of an inhibitor of kinesin-5 produced clinically meaningful symptomatic improvement and a partial response with a 20-month durability. Subsequently, at the time of progression, molecular analysis revealed an unexpected finding: *KIT* overexpression associated with a *KIT* exon 11 mutation. The present patient is currently continuing on treatment with imatinib, with evidence of a second clinical benefit and a corresponding biochemical response. Although the durability of his benefit remains to be seen, the authors feel that these data further support efforts to pursue detailed molecular characterization of individual tumors in efforts to improve cancer treatment. This may serve not only to identify new therapeutic tar-

gets but also to tease out the subset of patients who truly derive benefit from largely discarded therapeutic avenues.

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Posttest Questions

1. NET are classified by which of the following?
 - a. Ki-67
 - b. Chromogranin level
 - c. Kinesin level
 - d. Tumor cell anaplasia
2. Patients with metastatic poorly differentiated NET of the gastrointestinal tract are typically considered for initial treatment via a platinum doublet (carboplatin or cisplatin plus etoposide), with high-grade pathology determined by the presence of a mitotic rate of >20 mitoses per high-pow-

ered field or a Ki-67 greater than:

- a. 10%
 - b. 15%
 - c. 20%
3. Which of the following drugs is NOT used to treat cancer positive for KIT-activating mutations:
 - a. Everolimus
 - b. Sunitinib
 - c. Imatinib
 - d. Dasatinib