Targeting BRAF Mutations in High-Grade Neuroendocrine Carcinoma of the Colon

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Abstract
Mutations in the RAS/RAF/MEK/ERK pathway leading to constitutive activation and uncontrolled cellular growth have been identified in various human malignancies, making this pathway a target for potential therapeutics. The activating BRAFV600E mutation is one well-characterized oncogenic mutation that has been described and targeted with clinical success in various malignancies, including melanoma and hairy cell leukemia. Although BRAF-directed treatments have yielded clinical benefit in a subset of tumor types, such as melanoma, thyroid cancer, and lung cancer, BRAF inhibition fails to confer a clinical benefit in colon cancer. Identification of patients for whom BRAF inhibition may produce clinically meaningful outcomes is imperative. The incidence of BRAF mutations in neuroendocrine carcinoma (NEC) is estimated to be 5% to 10%. A recent case series demonstrated benefit in targeting the BRAFV600E mutation in metastatic high-grade rectal NECs. Combination BRAF and MEK inhibition is known to yield improved outcomes compared with BRAF inhibition alone in melanoma. This report presents 2 patients with high-grade colorectal NECs who had different responses to treatment with combined BRAF/MEK inhibition after experiencing disease progression through first-line platinum-based chemotherapy. One patient experienced an excellent initial response to therapy before ultimately experiencing progression, and in the other patient initially had stable disease before eventually experiencing progression. These cases highlight the complicated role BRAF mutations play in gastrointestinal NECs, and the need for further research to identify not only patients who may benefit from BRAF-directed therapies but also strategies to avoid development of resistance.

Neuroendocrine tumors (NETs) of the gastrointestinal tract occur at an approximate incidence of 6.2 per 100,000 worldwide, and are the second most prevalent gastrointestinal tract tumor behind colorectal cancer (CRC). Many factors, including site of origin, stage, and biological features, influence treatment decisions. Metastatic, high-grade, poorly differentiated neuroendocrine carcinomas (NECs) are typically aggressive tumors characterized by a Ki-67 level of >20% and a mitotic index of >20 mitoses per 10 high-powered field, and are generally treated with chemotherapy regimens used for small cell lung cancer. Despite treatment, prognosis for high-grade NEC remains poor, with a median survival of <1 year, underlining the need to develop effective targeted therapies that can produce durable responses. Targetable genetic features of high-grade NECs have not been comprehensively described. In a recent case series, Klempner et al reported BRAF mutations in 9% of 108 total cases of colorectal NEC, and described 2 cases of

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treatment-refractory high-grade rectal NECs harboring \( \text{BRAF}^{\text{V600E}} \) mutations. \( \text{BRAF}^{\text{V600E}} \) mutation has been identified in both solid and hematologic malignancies, including adenocarcinoma of the colon and lung, papillary thyroid cancer, melanoma, and hairy cell leukemia. In \( \text{BRAF}^{\text{V600E}} \)-mutated melanoma, combined BRAF and MEK inhibition has shown superiority to BRAF monotherapy and has proven to be an effective treatment for metastatic disease. In the Klempner et al study, 2 patients with metastatic high-grade rectal NEC with \( \text{BRAF}^{\text{V600E}} \) mutations were treated with combined BRAF/MEK inhibition after experiencing disease progression through treatment with platinum-based regimens. Both patients had excellent and durable partial responses to therapy of 7 and 9 months, respectively, at the time of writing. However, the use of combination BRAF/MEK inhibition in patients with colonic or small bowel NEC has not been described. This report presents 2 cases of NEC, which demonstrate both the promises and limitations of BRAF inhibition in gastrointestinal NEC, and highlight the need for ongoing preclinical and clinical research to optimize patient selection for these treatments.

**Case 1**

A 66-year-old Ashkenazi Jewish woman with a past medical history of early-stage invasive ductal carcinoma of the left breast, treated definitively with surgery and adjuvant tamoxifen therapy, presented after a routine colonoscopy revealed a 1.6-cm cecal polyp with pathologic findings consistent with a poorly differentiated, high-grade (Ki-67, 50%) NEC involving the submucosa and mucosa of the cecum associated with a sessile serrated adenoma (SSA). Triple-phase CT of the abdomen and pelvis revealed a large hemangioma but no other suspicious lesions (Figure 1A). MRI of the abdomen and pelvis confirmed imaging evidence of a 9.4 x 8.4-cm hemangioma, but also showed 2 small lesions in the inferior right lobe of the liver with ring enhancement concerning for metastatic deposits (Figure 1B, C). A subsequent 18F-FDG PET/CT scan revealed multiple hypermetabolic hepatic lesions as well as nodularity adjacent to the right psoas muscle, with intense FDG avidity consistent with metastatic disease (Figure 2A, B). A 68-Gallium DOTATATE PET revealed no abnormal radiotracer uptake. Ultrasound-guided liver biopsy confirmed metastatic, poorly differentiated NEC. The patient was started on intravenous cisplatin (75 mg/m² on day 1) and etoposide (100 mg/m² per dose on days 1–3), and restaging 18F-FDG PET/CT performed after 2 cycles of chemotherapy showed progression of hepatic metastatic disease with evidence of multiple new hypermetabolic hepatic lesions (Figure 2C, D). 18F-FDG PET/CT was used for restaging because many hepatic lesions were not visible on either CT or MRI.

Next-generation sequencing of the liver biopsy specimen performed by Foundation Medicine demonstrated intact DNA mismatch repair (MMR) function within the tumor. Additional testing revealed genomic alterations in \( \text{BRAF}^{\text{V600E}} \) and TP53, as well as amplifica-
tion in FLT3 and CDK8. Based on data from Klempner et al, the patient was initiated on oral treatment with off-label dabrafenib, 150 mg twice daily, and trametinib, 2 mg daily. Restaging scans performed 8 weeks after initiation of dabrafenib/trametinib combination therapy demonstrated significant interval decrease in number, size, and FDG-avidity of multiple hypermetabolic foci throughout the liver, with only a few residual small foci, in addition to interval resolution of the previously noted hypermetabolic soft tissue densities within the pericecal/pericolic region (Figure 2E, F). This was accompanied by significant symptomatic improvement, including increased appetite and weight gain.

The patient tolerated combination dabrafenib and trametinib well for 5 months at full doses, with a mild self-limited acneiform rash the only toxicity. Unfortunately, her disease progressed after 5 months of treatment, with development of diffuse hepatic metastases and abdominal lymphadenopathy. Biopsy of a liver lesion following progression revealed ARID1B Q123* that was not reported on the pretreatment biopsy, in addition to persistence of BRAF V600E and TP53 H214R mutations.

With regard to germline genetic testing, the patient underwent complete sequencing of BRCA1 and BRCA2 at the time of her breast cancer diagnosis in 2014, with no detectable mutations in these genes. After her diagnosis of metastatic poorly differentiated NEC, she declined additional genetic testing when the likelihood of a hereditary cancer predisposition syndrome was determined to be low.

Case 2

A 51-year-old man underwent his first screening colonoscopy and was found to have an ulcerated lesion at the hepatic flexure, with pathologic findings consistent with a hyperplastic polyp. However, given the suspicious macroscopic appearance of the lesion during colonoscopy, this was followed by a laparoscopic partial right colectomy with ileocolic anastomosis. Pathologic findings revealed a 3-cm ulcerated, poorly differentiated carcinoma with neuroendocrine and squamoid differentiation arising in an SSA with cytologic low- and high-grade dysplasia, positive for CK7 and synaptophysin on immunohistochemistry. Metastatic carcinoma was seen in 3 of 32 lymph nodes, and all margins were uninvolved. Ki-67 was focally >80% by semi-manual quantitation. Additional testing revealed intact DNA MMR function within the tumor. His postoperative course was complicated by the development of an incisional abscess. Triple-phase CT of the abdomen/pelvis showed no evidence of metastatic disease.

Figure 2. (A, B) 18F-FDG PET/CT scan completed in case 1 before therapy showing multiple hypermetabolic hepatic lesions (arrows), maximal standard uptake value (SUV) of 11.9, confirmed as metastatic disease on biopsy. (C, D) 18F-FDG PET/CT scan completed after 2 cycles of chemotherapy showing progression of hepatic metastatic disease with evidence of multiple new hypermetabolic hepatic lesions (arrows). (E, F) 18F-FDG PET/CT scan completed 8 weeks after initiation of combination therapy with dabrafenib/trametinib showing significant interval decrease in number, size, and FDG avidity of multiple hypermetabolic foci throughout the liver, with only a few residual small foci remaining; maximal SUV of 5.0.
The patient received 3 of 4 planned cycles of adjuvant intravenous chemotherapy with cisplatin (75 mg/m\(^2\) per dose on day 1 of a 21-day cycle) and etoposide (100 mg/m\(^2\) per dose on days 1–3 of a 21-day cycle). Cycle 4 was withheld due to worsening tinnitus and impaired hearing. Surveillance imaging at 2 and 4 months after surgery showed no evidence of metastatic disease; however, restaging 6 months after surgery revealed tumor recurrence at the site of resection (Figure 3A). Biopsy revealed a \textit{BRAF}\textsuperscript{V600E} mutation, a deleterious \textit{TP53} splice site mutation, and several other nonactionable mutations on a targeted HaloPlex panel (Agilent Technologies).\(^\text{10}\) He was enrolled on a clinical trial (ClinicalTrials.gov identifier: NCT01713972) studying dabrafenib (50 mg orally twice daily) and the vascular endothelial growth factor receptor (VEGFR) tyrosine kinase inhibitor (TKI) pazopanib (600 mg orally daily) in patients with \textit{BRAF}-mutated advanced malignant tumors. He received 1 month of treatment before developing acute onset abdominal pain and fevers, and imaging results were concerning for abscess formation at the anastomotic site. He underwent exploratory laparotomy, ileocolostomy resection, and abdominal washout with wide drainage for ileocolostomy perforation. Pathology revealed omentum with acute inflammation and abscess formation, with no evidence of malignancy in 13 lymph nodes. He recovered from surgery and was discontinued from the trial, but continued on single-agent dabrafenib due to the \textit{BRAF}\textsuperscript{V600E}-mutated tumor.

He tolerated treatment well, with initial restaging imaging consistent with SD, but rapid disease progression was noted after 6 months of treatment, most notably in a peripancreatic/periduodenal mass that had substantially increased from 1.7 x 1.9 cm to 5.2 x 8.4 cm (Figure 3B). He went on to receive an MEK inhibitor (binimetinib, 45 mg orally twice daily) as part of a clinical trial (ClinicalTrials.gov identifier: NCT01885195), but restaging studies after 2 cycles of therapy showed significant disease progression, and he was transitioned to best supportive care and died 6 weeks later.

Discussion

As additional information is learned regarding the molecular mutations that drive oncogenesis, targeted therapeutics have played an integral role in the treatment of malignancies across various tumor types. Aberrant signaling through the RAS/RAF/MEK/ERK pathway has been identified in many human malignancies, and as a result, this pathway has sparked interest for potential therapeutic targets.\(^\text{11,12}\) Activating \textit{BRAF} mutations are found in most cases at codon 600, and typically result from replacement of the amino acid valine by glutamic acid.\(^\text{12}\) \textit{BRAF}\textsuperscript{V600E} mutations, which have been found to occur frequently in SSAs,\(^\text{13,14}\) lead to constitutive activation of \textit{BRAF} kinase, resulting in activation of the MEK kinases and downstream targets involved in cellular proliferation. Although the success of \textit{BRAF}/MEK inhibition has been well documented in metastatic melanoma, targeting \textit{BRAF} mutations in CRC has led to mostly disappointing results,\(^\text{15,16}\) prompting further studies\(^\text{17}\) and alternative treatment strategies. In one recently reported phase II study, treatment with vemurafenib in combination with cetuximab and irinotecan in patients with metastatic \textit{BRAF}\textsuperscript{V600E}-mutated CRC led to improved progression-free survival (4.4 vs 2.0 months) and a higher disease con-
trol rate (67% vs 22%) compared with cetuximab and irinotecan alone. These data suggest that simultaneous EGFR and BRAF inhibition combined with chemotherapy may be an effective therapeutic option in metastatic BRAFV600E-mutated and RAS wild-type CRC. At the 2016 ESMO meeting, Corcoran et al reported that patients with BRAFV600E-mutated metastatic CRC treated with triplet dabrafenib/trametinib/panitumumab had improved median progression-free survival (not reached) compared with those treated with combination dabrafenib/panitumumab (3.4 months) or combination trametinib/panitumumab (2.8 months). In a similar recently reported phase III study, treatment with the BRAF inhibitor encorafenib in combination with the MEK inhibitor binimetinib and cetuximab showed promising results, with an overall response rate of 41% and nearly a third of patients demonstrating prolonged stable disease up to 9.3 months. As the frequency of genetic testing continues to increase, the prevalence of BRAF mutations and therapeutic benefit of various combination therapies remains to be seen.

Although combination platinum-based chemotherapy has long been the standard first-line treatment for metastatic high-grade NECs, responses typically lack durability, indicating an unmet clinical need for the development of more effective therapies. Genomic alterations in TP53 and Rb are commonly seen in high-grade NECs. In a recent case series, Klempner et al described 2 patients with treatment-refractory high-grade rectal NECs that were noted to harbor BRAFV600E mutations. These patients were treated with combined BRAF/MEK inhibition and had an excellent durable response to therapy. In this retrospective review of 109 cases of colorectal NEC (grade 1–3) that had previously undergone comprehensive genomic profiling, Klempner et al identified 10 samples (9%) with BRAF mutations. Of these, 8 harbored the BRAFV600E mutation and 2 had non-V600E alterations (G469A and R671Q), and 8 occurred in patients with high-grade (grade 3) tumors. Rates of BRAF mutations in CRC have varied from approximately 7% to 14% in prior large studies, whereas the reported rate in NETs has ranged from 7% to 17%. The role of BRAF-directed therapy in patients with NET and these mutations remains unclear, but the robust clinical responses seen in the 2 patients harboring the BRAFV600E mutation described by Klempner et al suggests that a subset of patients may derive meaningful clinical benefit.

The cases we present reflect 2 different responses to BRAF-directed therapies. One patient with metastatic poorly differentiated cecal NEC had a dramatic but brief clinical and radiographic response to combined BRAF/MEK inhibition. A second patient with NEC arising from the hepatic flexure of the colon had stable disease for approximately 6 months, including 1 month of combined BRAF/VEGFR TKI inhibition on clinical trial and 5 months of BRAF inhibition alone on clinical trial, before ultimately experiencing disease progression. This patient was subsequently treated with a MEK inhibitor as part of a subsequent trial, but ultimately his disease continued to progress rapidly. To date, a number of mechanisms of resistance to BRAF inhibition have been described, leading to reactivation of the MAP kinase pathway or activation of other proliferative pathways. Although the transient benefit seen in the patient with cecal NEC highlights the success of BRAF inhibition, the lack of a durable response in this patient and the absence of any significant response in the second patient with a colonic NET emphasize the need for further study of the mechanisms of resistance to BRAF-directed therapies. Both of our patients had NEC of midgut origin, as opposed to the Klempner et al case series, in which the 2 patients with durable responses to BRAF inhibition had hindgut NEC. Whether the embryonic origin of the primary tumor influences response to BRAF inhibitor therapy requires further study.

Conclusions

BRAF-targeted therapy is an exciting and potentially promising option for patients with BRAFV600E-mutated, high-grade NEC, with dramatic responses observed in some. However, patient selection will be critical, because for some patients, responses are less robust and duration of clinical benefit limited, as demonstrated in the 2 cases presented. How best to optimize patient selection for these therapies is an important area that warrants exploration in prospective clinical trials.

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