Multiple Gastrointestinal Polyps in Patients Treated with BRAF Inhibitors

Ravi K. Amaravadi1,2, Kathryn E. Hamilton1, Xiaohong Ma1, Shengfu Piao1, Armando Del Portillo3, Katherine L. Nathanson1,2, Matteo S. Carlino4,5, Georgina V. Long5, Igor Puzanov6, Xiaowei Xu7, Jennifer J.D. Morrissette7, Kenneth Y. Tsai8, Keith T. Flaherty9, Jeffrey A. Sosman6, Grant R. Goodman10, Grant A. McArthur11, Anil K. Rustgi1,2, David C. Metz1,2, Lynn M. Schuchter1,2, Paul B. Chapman1,2, and Antonia R. Sepulveda3

Abstract

Purpose: BRAF inhibitors (BRAFi) extend survival in BRAF-mutant melanoma but can promote the growth of Ras-mutant neoplasms. This study determined if gastrointestinal polyps found in BRAFi-treated patients harbored Ras mutations.

Experimental Design: Colon and gastric polyps were identified and resected from BRAFi-treated melanoma patients. Next-generation sequencing (NGS) was performed on polyps. The ability of BRAFi to promote polyp formation was functionally characterized in ApcMin+/− mice. MAPK and β-catenin pathway activity was assessed by immunohistochemistry in mouse and human polyps.

Results: Fourteen patients treated with BRAFi underwent endoscopy to assess for polyps. Seven out of 7 patients >40 years of age and treated for >2 years were found to have colonic tubular adenomas with 4 out of the 7 patients having 5 or more polyps. One patient presented with bleeding from hyperplastic gastric polyps that recurred 6 months after BRAFi rechallenge. NGS performed on polyps found no mutations in MAPK pathway genes, but found APC mutations in all tubular adenomas. A significant increase in the number of polyps was observed in BRAFi-treated compared with control-treated ApcMin+/− mice (20.8 ± 9.2 vs 12.8 ± 0.1; P = 0.016). No polyps were observed in BRAFi-treated wild-type mice.

Conclusions: BRAFi may increase the risk of developing hyperplastic gastric polyps and colonic adenomatous polyps. Due to the risk of gastrointestinal bleeding and the possibility of malignant transformation, further studies are needed to determine whether or not endoscopic surveillance should be recommended for patients treated with BRAFi.

Introduction

BRAF inhibitors (BRAFi), including vemurafenib and dabrafenib, extend survival in stage IV BRAF(V600E)-mutant melanoma patients (1, 2), and produce a 45% 2-year survival rate (3, 4). Although the median progression-free survival is approximately 7 months (1, 4, 5), in some cases patients have been treated for 3 to 5 years continuously with BRAFi. Early in the development of BRAFi, treatment-associated cutaneous squamous cell carcinoma (SCC) raised concerns regarding oncogenic risks. In phase II trials of BRAFi, 10% to 26% of patients developed cutaneous SCC or keratoacanthoma (4, 5). Molecular characterization of these SCCs found that some tumors harbored HRAS mutations (6, 7). BRAFi inhibition in HRAS mutant/BRAFi wild-type cutaneous SCC cells leads to paradoxical increase in mitogen active protein kinase (MAPK) signaling (8). Given the potential paradoxical activation of MAPK signaling especially in the presence of RAS mutations, there is concern that accelerated growth of other more life-threatening neoplasms is possible in patients treated with BRAFi. Reports of the progression of a preexisting NRAS-mutant chronic myelomonocytic leukemia in a melanoma patient treated with vemurafenib (9), of the progression of a KRAS-mutant colon cancer (10), and the development of a KRAS-mutant pancreatic cancer (11) in two separate patients treated with combined BRAF and MEK inhibition (dabrafenib and trametinib), underscore this possibility. Furthermore, the concern over BRAFi-associated neoplasms is increased given the adjuvant studies of vemurafenib or dabrafenib in resected stage II and III melanoma.

Here, we report several patients with advanced BRAF(V600E)-mutant melanoma who were treated for a long term with BRAFi, and were found to have intestinal polyps. Genetic characterization of these intestinal polyps revealed no mutations in MAPK pathway genes; however, mutations in the APC (adenomatous
Translational Relevance

BRAF inhibitors (BRAFi) can promote the growth of cutaneous squamous cell carcinoma, and possibly other Ras-driven neoplasms. We describe a high incidence of multiple gastrointestinal polyps in patients treated with BRAFi for ≥2 years. Next-generation sequencing determined that these colon polyps harbored only adenomatous polyposis coli (APC) mutations. BRAFi inhibition significantly increased the number of polyps in APC Min+/− mice but not wild-type mice, providing further evidence that BRAFi may promote the progression of existing intestinal polyps.

Patients and Methods

Patients and lesion samples

Patients participated in the phase I trial of vemurafenib (NCT00405587), the phase II study of vemurafenib (NCT00949702), the vemurafenib expanded access protocol (NCT01248936), the phase I trial of dabrafenib (NCT00880321) or received the commercial drug. All patients had BRAFV600E metastatic melanoma and received either 720 or 960 mg of vemurafenib or 150 mg dabrafenib twice daily. Patients provided written informed consent for the molecular analysis of lesions obtained during treatment. Esophagogastroduodenoscopy (EGD) and colonoscopy were performed in the standard manner under conscious sedation.

Molecular analysis of tumor specimens

DNA was extracted from formalin-fixed and paraffin-embedded (FFPE) tissue sections of polyps and was sequenced by next-generation sequencing (NGS) on the Ion Torrent (AmpliSeq Cancer Hotspot panel v.2; Life Technologies), and MiSeq (Illumina TruSeq Cancer Hotspot panel) platforms. Library preparation for Ion Torrent sequencing of 50 genes was performed on a 318 chip using 10 to 15 nanograms of DNA and the Ion PGM sequencer (Life Technologies). Library preparation for MiSeq sequencing of 47 genes (MiSeq reagent kit v 2; Illumina) using 250 nanograms of genomic DNA, on the MiSeq platform. Ion Torrent data were analyzed with the Ion Torrent Suite v.3.4 (Life Technologies). MiSeq sequencing data were analyzed using an in-house analysis pipeline (12).

For more information, including genes sequenced and methods to detect amplifications, see Supplementary Methods.

Studies in mice

Animal procedures were performed in accordance with guidelines from the local animal ethics committee. Two cohorts with 15 C57Bl/6-APCmin+/− mice (The Jackson Laboratory) each were fed control chow or PLX4720 (the laboratory tool compound for vemurafenib)-infused (high dose) chow (13) for 28 days. Upon sacrifice, small intestine and colon were cut open length-wise, and polyp number and size were evaluated in a blinded fashion for proximal small intestine (PSI), distal small intestine (DSI), and colon using a Nikon SMZ645 microscope. Wild-type C57Bl6 mice were treated with control and PLX chow for 6 months and sacrificed. All gastrointestinal tracts were formalin fixed, and paraffin embedded. Immunohistochemistry for phospho-ERK, and β-catenin, was performed as previously described (14) using the following antibodies: Phospho-ERK (Cell Signaling 4370) and β-catenin, was performed as previously described (14) using the following antibodies: Phospho-ERK (Cell Signaling 4370) and β-catenin (Cell Signaling 9562). Stained slides were evaluated for histologic features of mouse intestinal polyps, the number of polyps, and nuclear phospho-ERK and β-catenin were scored in a blinded fashion. For the latter, a polyp with a well-oriented nucleus containing at least 10 immunoreactive cells was scored as positive for phospho-ERK and β-catenin.

Table 1. Characteristics of patients treated with BRAFi who underwent GI endoscopy

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age, y</th>
<th>BRAFi</th>
<th>Time on BRAFi, y</th>
<th>Family history of colon cancer</th>
<th>Prior colonoscopy</th>
<th>Number of colonic polyps</th>
<th>Number of gastric polyps</th>
</tr>
</thead>
<tbody>
<tr>
<td>VEN63</td>
<td>52</td>
<td>VEM</td>
<td>0.63</td>
<td>None</td>
<td>None</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>VEN65</td>
<td>54</td>
<td>VEM</td>
<td>0.32</td>
<td>None</td>
<td>None</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>VEN50</td>
<td>69</td>
<td>VEM</td>
<td>0.67</td>
<td>Father</td>
<td>Yes, 1 adenoma</td>
<td>1 T A</td>
<td>0</td>
</tr>
<tr>
<td>VEN52</td>
<td>52</td>
<td>VEM</td>
<td>0.76</td>
<td>None</td>
<td>None</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>VEN53</td>
<td>53</td>
<td>VEM</td>
<td>0.83</td>
<td>None</td>
<td>None</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>VEN61</td>
<td>59</td>
<td>VEM</td>
<td>0.88</td>
<td>None</td>
<td>None</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>VEN62</td>
<td>64</td>
<td>VEM</td>
<td>2.05</td>
<td>Mother</td>
<td>None</td>
<td>1 T A</td>
<td>0</td>
</tr>
<tr>
<td>VEN63</td>
<td>48</td>
<td>VEM</td>
<td>2.75</td>
<td>Mother</td>
<td>None</td>
<td>1 T A</td>
<td>0</td>
</tr>
<tr>
<td>WMD1</td>
<td>61</td>
<td>DAB</td>
<td>5</td>
<td>Brother</td>
<td>None</td>
<td>8 T A</td>
<td>NA</td>
</tr>
</tbody>
</table>

Abbreviations: DAB, dabrafenib; GM, grandmother; HP, hyperplastic polyp; MSKCC, Memorial Sloan Kettering Cancer Center; NA, not available; PMAC, Peter Macallum Cancer Center; TA, tubular adenoma; UPENN, University of Pennsylvania; VAN, Vanderbilt-Ingram Cancer Center; VEM, vemurafenib; WMD1, Westmead Hospital.
Results

Characteristics of melanoma patients who developed polyps

In the phase I trial of vemurafenib, 48 patients were treated with therapeutic doses of vemurafenib (15). Four of the 8 patients treated continuously for more than 2 years underwent EGD and colonoscopy (Table 1). The first patient (UPENN1) presented with hemodynamically unstable gastrointestinal bleeding 2.5 years after starting vemurafenib. He had a negative EGD and colonoscopy immediately prior to starting therapy. EGD demonstrated six gastric polyps (Fig. 1A) and a duodenal ulcer (not shown). The polyps were resected and histologic analysis demonstrated hyperplastic gastric polyps. Colonoscopy demonstrated five polyps. Histologic examination demonstrated typical adenomatous polyps with features of tubular adenomas (Fig. 1B). The patient developed anemia and fatigue 6 months after rechallenge with vemurafenib. Repeat EGD, colonoscopy, and histologic analysis demonstrated recurrence of six bleeding hyperplastic gastric polyps (Fig. 1C) and no recurrence of colonic polyps. Patient UPENN2 was asymptomatic when she underwent endoscopy and was found to have seven colonic polyps (6 tubular adenomas and 1 hyperplastic polyp) after 2.25 years of vemurafenib (Supplementary Fig. S1). She resumed vemurafenib treatment after resection of polyps and had repeat EGD and colonoscopies after 1 year, which showed no recurrent or new polyps. Patient MSKCC1 underwent colonoscopy due to persistent diarrhea after 2.76 years of vemurafenib and was found to have 10 colonic polyps (7 tubular adenomas and 3 hyperplastic polyps). Like patient UPENN1, this patient had a negative colonoscopy prior to enrollment on the phase I study, increasing the likelihood that the 10 polyps were associated with drug therapy. Patient PMAC1, who unlike the other patients was younger than 40, was found to have no polyps despite 3.5 years of vemurafenib therapy.

EGD and colonoscopy findings that were available for additional patients that were treated with BRAFi are presented in Table 1. The histology for nearly every colonic polyp was tubular adenoma. In most cases, patients underwent endoscopy for either symptoms of dyspepsia, colitis, or for routine colon cancer screening. Patient WMD1 from Australia was treated with dabrafenib for 5 years, and on surveillance 18-fluorodeoxy (FDG) positron emission tomography/computed tomography (PET/CT) scan (Fig. 2A), an FDG avid lesion in the transverse colon was found and interpreted as a potential melanoma metastases. Colonoscopy revealed eight colonic polyps that were in some cases >10 mm in size, including a 15-mm transverse colon polyp, which corresponded to the FDG avid lesion on the PET/CT scan (Fig. 2B). In this small cohort of patients, those who were >40 years of age and who had been treated with BRAFi for >2 years all were found to have colonic polyps. Once polyps reach larger proportions (>10 mm) they may be detected radiographically and may be mistaken for melanoma progression. However, a possible limiting factor for this observation is that 7 of 10 patients did not have a baseline colonoscopy prior to the start of BRAFi therapy and in 2 of the remaining 3 patients, the prior colonoscopy was more than 1 year prior to the start of BRAFi therapy. Additional confounding factors such as family history and advanced age may have played a significant role in these observations.
Genetic characterization of polyps. A stepwise accumulation of somatic mutations in the APC, KRAS, DCC, SMAD4, and TP53 genes has been described to explain the transformation of normal colonic epithelium to adenomatous polyps and adenocarcinoma (16). APC mutations are found in early adenomas, KRAS mutations in late adenomas, and TP53 mutations in adenocarcinoma. There is very little known about the pathogenesis of hyperplastic gastric polyps, but TP53 and PIK3CA mutations may be present in dysplastic hyperplastic polyps (17, 18). We performed NGS on patient polyps using both the AmpliSeq and TruSeq cancer panels on the Ion Torrent (n = 12), and Illumina (n = 7) sequencing platforms, respectively (Table 2), expecting to see somatic mutations in RAS genes. To increase yield in individual patients, DNA extracted from small polyps was combined for sequencing assays. In general, there was agreement between the two platforms as far as mutated genes. In colonic polyps, no mutations in MAPK pathway genes (e.g., RAS, RAF) were found, but all adenomas tested had APC mutations, with the most common mutation being truncation mutations in the β-catenin binding domain (R1450X). We found two of eight tubular adenomas harbored previously described mutations in the GSK3B (glycogen synthase kinase-3B) phosphorylation site of β-catenin (19). One patient’s adenoma had a low allelic frequency of two pathogenic TP53 mutations more typical of adenocarcinomas rather than adenomas. In this sample, there was no histologic evidence of adenocarcinoma. No somatic mutations were found in gastric hyperplastic polyps, except a KRAS mutation, which given the low allele frequency may be a spurious result.

BRAF inhibition increases the number of polyps in Apc Min+/− mice. C57BL/6J-Apc Min+/− mice harbor a truncation in the APC gene in the β-catenin binding motif, similar to the adenomas we found in melanoma patients treated with BRAFi. These mice develop adenomatous polyps that are localized to the small intestine rather than the colon, in contrast with humans where the APC-mutant polyps are typically found in the colon. This genotype produces 100% penetrance leading to uniform anemia and death by 3 months (20). Examination of the GI tract of Apc Min+/− mice (N = 15 each) fed control or PLX4720 (BRAFi) chow for 28 days found no polyps in the stomach, few in the colon, with the majority residing in the small intestine (Fig. 3A). The mean number of visible polyps in the small intestine was significantly increased in PLX4720 fed mice compared with control mice, in the proximal small intestine and in the total intestine (Fig. 3B). There was a nonsignificant but consistent increase in polyps in the distal small intestine as well. No significant difference in the incidence of colon polyps was observed. Histologic examination of polyps (Fig. 3C) found the number of polyps was increased (Fig. 3D) in mice fed PLX4720 chow compared with control chow in the proximal (mean ± SEM 9.2 ± 0.5 vs. 5.8 ± 0.2 polyps; P = 0.004) and total small intestine (20.8 ± 1.9 vs. 12.8 ± 0.1; P = 0.016). Invasive adenocarcinoma of the colon was found in one of 15 mice fed PLX4720 chow and zero of 15 mice treated with control chow. It is known that Apc min+/− polyps occasionally progress to invasive adenocarcinoma without any other intervention in this model. To determine if PLX4720 can induce polyp formation in the absence of Apc mutations in mice, cohorts of C57Bl6 mice (WT mice) were fed control (N = 3) or PLX4720 (N = 4) chow for 6 months. No polyps were found in the intestines of mice fed PLX or control chow, suggesting that that BRAF inhibition interacts specifically with the APC mutation to accelerate polyposis. Immunohistochemistry against phospho-ERK and β-catenin in Apc Min+/− mouse polyps demonstrated nuclear phospho-ERK and β-catenin that localized to the epithelial cells in the most proliferative segments of adenomatous tissue. No significant difference in the percentage of adenomatous epithelium with nuclear localization of phospho-ERK or β-catenin in Apc Min+/− mice fed control chow versus PLX4720 chow were found (Fig. 4A and B). Unlike the mouse tissue, human polyps obtained from BRAF-mutant melanoma patients treated with BRAFi had almost no nuclear β-catenin, and nuclear phospho-ERK was present in a patchy distribution (Fig. 4C and D). Taken together, these data indicate that APC mutation can confer sensitivity to BRAF-mediated acceleration of polyposis in the absence of RAS mutation.

Table 2. Disease-associated somatic mutations in intestinal polyps from patients on BRAFi

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sample</th>
<th>Gene</th>
<th>Mutation (AA change)</th>
<th>Allele frequency (%)</th>
<th>Total coverage (fold)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colonic polyps</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MSKCC1</td>
<td>Pooled TA</td>
<td>APC</td>
<td>PI441A</td>
<td>15</td>
<td>473</td>
</tr>
<tr>
<td></td>
<td></td>
<td>APC</td>
<td>TI448S</td>
<td>30</td>
<td>518</td>
</tr>
<tr>
<td>UPENN1</td>
<td>Pooled TA</td>
<td>CTNB1</td>
<td>S45F</td>
<td>49</td>
<td>6118</td>
</tr>
<tr>
<td></td>
<td></td>
<td>APC</td>
<td>G106X</td>
<td>8</td>
<td>3905</td>
</tr>
<tr>
<td>UPENN1</td>
<td>Large TA</td>
<td>APC</td>
<td>G106X</td>
<td>27</td>
<td>4379</td>
</tr>
<tr>
<td></td>
<td>Pooled TA</td>
<td>APC</td>
<td>Q138X</td>
<td>29</td>
<td>23882</td>
</tr>
<tr>
<td></td>
<td>Pooled TA</td>
<td>TP53</td>
<td>R181H</td>
<td>5</td>
<td>1166</td>
</tr>
<tr>
<td></td>
<td>Pooled TA</td>
<td>TP53</td>
<td>Y234C</td>
<td>4</td>
<td>4463</td>
</tr>
<tr>
<td></td>
<td>Pooled TA</td>
<td>TP53</td>
<td>R175H</td>
<td>5</td>
<td>4523</td>
</tr>
<tr>
<td></td>
<td>Pooled TA</td>
<td>APC</td>
<td>1450X</td>
<td>30</td>
<td>2485</td>
</tr>
<tr>
<td></td>
<td>Pooled TA</td>
<td>APC</td>
<td>1450X</td>
<td>41</td>
<td>2275</td>
</tr>
<tr>
<td></td>
<td>Pooled TA</td>
<td>CTNB1</td>
<td>S45F</td>
<td>54</td>
<td>1529</td>
</tr>
<tr>
<td></td>
<td>Pooled TA</td>
<td>APC</td>
<td>PI441A</td>
<td>9</td>
<td>679</td>
</tr>
<tr>
<td></td>
<td>Pooled TA</td>
<td>APC</td>
<td>PI422T</td>
<td>25</td>
<td>673</td>
</tr>
<tr>
<td></td>
<td>Pooled TA</td>
<td>APC</td>
<td>PI450X</td>
<td>16</td>
<td>11170</td>
</tr>
<tr>
<td></td>
<td>Pooled TA</td>
<td>APC</td>
<td>Q1477X</td>
<td>10</td>
<td>14079</td>
</tr>
<tr>
<td>Gastric polyps</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UPENN1</td>
<td>Gastric polyp</td>
<td>None</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UPENN1</td>
<td>Gastric polyp</td>
<td>None</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UPENN1</td>
<td>Gastric polyp</td>
<td>None</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UPENN1</td>
<td>Gastric polyp</td>
<td>None</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: AA, amino acid; MSKCC, Memorial Sloan Kettering Cancer Center; TA, tubular adenoma; UPENN, University of Pennsylvania; VAN, Vanderbilt-Ingram Cancer Center.
Discussion

Here we describe the first report of a MAPK-targeted therapy potentially accelerating neoplastic growth in benign gastric epithelium, and in APC-mutant colonic tissue lacking MAPK mutations. One patient had a low frequency TP53 mutation present in her polyps, suggesting that the eventual malignant transformation of these adenomas is possible. Because BRAF-associated colonic adenomas undetectable by CT scanning could potentially progress to frank colon adenocarcinoma if left untreated, we view this finding as a significant and unanticipated adverse event. It is important to note that we did not find any histology consistent with adenocarcinoma in any of the cases described in this report and clinical trials of single-agent BRAFi have not reported any cases of adenocarcinoma during the specified follow-up period. The high number of polyps and the recurrence of the gastric polyps in 1 patient that was rechallenged with vemurafenib provided some clinical evidence of a potential association. Despite this observation, colonic adenomas are common in patients >50 years of age. One study encompassing 4 million patients found that detection rates of adenomas especially nonadvanced adenomas (<5 mm) have increased significantly over the years 2003 to 2012 to >30% in men and >20% in women in 2012 (21). The projected annual transition rates from advanced adenomas to colorectal cancer strongly increase with age from 2.6% in 55 to 65 year old and 5.1% to 5.6% in >80 year old men or women (22). Other studies, which include both advanced and nonadvanced adenomas, have estimated 5% of adenomas progress to colon cancer over 7 to 10 years (23). While multiple adenomas are common in the general population, the incidence of ≥5 adenomas was estimated in one study to be 0.4%, and the risk of progression to colon cancer if more than five polyps were observed was 24% in this study (24). While this is an older study, in a more modern series of 889 patients, 5 or more polyps were found in 4% of patients, and multiple adenomatous polyps, or a polyp >10 mm significantly increased the odds of finding concurrent high-grade dysplasia or adenocarcinoma (25). Due to the limitation of follow-up times in any endoscopy study, the precise risk and the rate of transformation from colonic adenoma to adenocarcinoma is difficult to ascertain. In our study, 4 of 14 patients treated with BRAFi had five or more colonic polyps, further supporting the potential association between BRAFi and polyp development. It is clear that the patients described here are a highly selected population and the rate of multiple polyps could be much lower in a larger population.

Gastric polyps are found in only 6% of all endoscopies. Multiple gastric polyps are found in 1.5% of all endoscopies (26). While PPI therapy is a risk factor for developing gastric polyps, 7% of PPI patients develop gastric polyps after an average of 32 months, and 90% of PPI-associated polyps are fundic gland polyps not hyperplastic polyps like those observed here (27).
While BRAFi associated gastric polyps may have a low malignant potential, our experience indicates they can be associated with clinically significant gastrointestinal bleeding. In practice, common hyperplastic gastric polyps often do not spontaneously bleed. In addition, those that are found are often left in place. The added burden and risk of screening for and resecting these lesions in patients treated with BRAFi with no evidence of bleeding needs to be considered carefully against the prognosis of their malignancy.

The NGS platforms used in this study were able to rule out most deleterious mutations in MAPK genes such as BRAF, CRAF, and NRAS, but this platform is not designed to definitively detect functional amplifications of MAPK genes that may in fact interact with APC mutations. Nevertheless, the finding of APC mutations in the absence of RAS or other MAPK mutations provided the opportunity to test the causative association between BRAFi and polyp formation using wild-type mice and the Apc Min +/− mouse model. The lack of polyp formation in wild-type mice treated for 6 months with BRAFi suggests that BRAFi do not initiate polyp development but could increase the rate of polyp growth in epithelium that has lost APC function. Although a high percentage of neoplastic cells with nuclear localization of phospho-ERK was not observed in adenomas from either patients or mice treated with BRAFi, it is possible that paradoxical MAPK activation took place at earlier time points during therapy. The lack of a high percentage of nuclear β-catenin is consistent with a previous report that indicates that APC loss by itself is insufficient for β-catenin nuclear localization, which requires concurrent MAPK activation (28). Additional laboratory studies in genetically engineered cell lines will be necessary to define the mechanistic interaction between MAPK and β-catenin signaling in this context.

Additional clinical studies are ongoing to determine the rate of polyp formation and the demographics of patients most likely to develop these polyps. As the combination of BRAF and MEK inhibition was found to be superior to BRAFi alone (29) and is becoming more widely adopted as a standard of care in melanoma, studies are needed to determine if dual inhibition of the MAPK pathway in this manner would have any effect on the likelihood of developing colonic and/or gastric polyps. Importantly, in preclinical models of HRAS-mutant BRAFWT cutaneous squamous carcinoma, BRAFi-associated paradoxical MAPK activation and growth of squamous tumors could be blunted by adding a MEK inhibitor to the BRAFi (7). This finding was recapitulated in the randomized phase III trial of dabrafenib and trametinib versus dabrafenib alone, where the incidence of squamous cell carcinoma was significantly decreased in the combination arm that included the MEK inhibitor (30). Before these studies are complete, it is premature to recommend widespread serial colonoscopy in patients treated with BRAFi, especially because no cases of frank adenocarcinoma were observed in this cohort of patients. However, given the risk of gastrointestinal bleeding, judicious surveillance in asymptomatic patients older than 40 with a family history of colon cancer, or a personal history of polyps and more than 2 years of BRAFi therapy, may be considered reasonable.

Disclosure of Potential Conflicts of Interest
R.K. Amaravadi reports receiving commercial research grants from Genentech. M.S. Carlino reports receiving speakers bureau honoraria from GlaxoSmithKline and Novartis. G.V. Long is a consultant/advisory board member for Amgen, Bristol-Myers Squibb, GlaxoSmithKline, Merck, Novartis, and Roche. J. Puzanov and K.T. Flaherty are consultants/advisory board members for Roche. G.R. Goodman has ownership interests (including patents) in Roche/Genentech. G.A. McArthur reports receiving commercial research grants from Celgene, Novartis, Pfizer, and Ventana, and is a consultant/advisory board member for Proverxus. P.B. Chapman reports receiving commercial research grants from Roche/Genentech, commercial research support from GlaxoSmithKline, and is a consultant/advisory board member for Bristol-Myers Squibb. No potential conflicts of interest were disclosed by the other authors.
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): R.K. Amaravadi, K.E. Hamilton, X. Ma, I. Puzanov, A.K. Rustgi

Study supervision: R.K. Amaravadi, A.K. Rustgi

Grant Support
Funding for this work was provided in part by Genentech through a sponsored research agreement with University of Pennsylvania (to R.K. Amaravadi). Additional resources used included the Molecular Pathology and Imaging, Molecular Biology and Gene Expression, and Transgenic and Chimeric Mouse Cores of the University of Pennsylvania Molecular Studies in Digestive and Liver Diseases Center (NIH/NIDDK P30 DK050306). DNA sequencing was performed using the Abramson Cancer Center Genomics Core services (P30 CA016520). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received February 25, 2015; revised June 8, 2015; accepted June 26, 2015; published OnlineFirst July 22, 2015.

www.aacrjournals.org Clin Cancer Res; 21(23) December 1, 2015

5221

BRAF Inhibitors and GI Polyps

References
Multiple Gastrointestinal Polyps in Patients Treated with BRAF Inhibitors

Ravi K. Amaravadi, Kathryn E. Hamilton, Xiaohong Ma, et al.


Updated version
Access the most recent version of this article at:

Supplementary Material
Access the most recent supplemental material at:
http://clincancerres.aacrjournals.org/content/suppl/2015/07/21/1078-0432.CCR-15-0469.DC1

Cited articles
This article cites 30 articles, 8 of which you can access for free at:
http://clincancerres.aacrjournals.org/content/21/23/5215.full#ref-list-1

Citing articles
This article has been cited by 1 HighWire-hosted articles. Access the articles at:
http://clincancerres.aacrjournals.org/content/21/23/5215.full#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.