Published in final edited form as:

Target Oncol. 2013 September ; 8(3): 183–188. doi:10.1007/s11523-013-0266-9.

Bevacizumab-Based Treatment in Colorectal Cancer with a *NRAS* Q61K Mutation

Filip Janku^{1,*}, Jennifer J. Wheler^{1,*}, David S. Hong¹, and Razelle Kurzrock²

¹Department of Investigational Cancer Therapeutics (Phase I Clinical Trials Program), The University of Texas MD Anderson Cancer Center, 1515 Holcombe Blvd., Houston, TX 77030

²Moore's Cancer Center, The University of California San Diego, 3855 Health Sciences Drive, La Jolla, CA 92093

Abstract

Despite development of new therapies, metastatic colorectal cancer (mCRC) largely remains an incurable disease. Approximately 2–6% of colorectal cancers harbor *NRAS* mutations. The anti-VEGF antibody bevacizumab is a backbone of most therapeutic regimens; however, biomarkers predicting its activity are not known. We report two cases of mCRC with a Q61K *NRAS* mutation that had a favorable response to bevacizumab and the histone deacetylase inhibitor valproic acid. In contrast, none of 10 patients with wild-type *NRAS* or unknown *NRAS* status and mutated *KRAS* (*NRAS* and *KRAS* mutations are mutually exclusive) responded to the same regimen. These results suggest that *NRAS* mutation merits further investigation as a potential biomarker predicting the efficacy of bevacizumab-based treatment.

Keywords

NRAS mutation; Colorectal cancer; Bevacizumab; Q61K

Introduction

Colorectal cancer is the third most frequent cause of death from cancer in men and women in the United States, and it is estimated that more than 49,000 Americans died of this disease in 2011.[1] Metastatic colorectal cancer is not curable, except for a small proportion of patients with isolated liver metastasis, and the median overall survival usually does not exceed 2 years.[2; 3] Standard treatment options include oxaliplatin or irinotecan in combination with 5-fluorouracil/leucovorin or capecitabine. These cytotoxic drugs may be combined with targeted agents such as monoclonal antibodies cetuximab or panitumumab, which target epidermal growth factor receptor (EGFR), or the monoclonal antibody bevacizumab, which targets vascular endothelial growth factor (VEGF).[2; 3; 4] While patients with a mutated *KRAS* oncogene are known not to derive benefit from anti-EGFR antibodies, there is no marker predicting response to bevacizumab. As a result, bevacizumab is widely used even though only a subset of patients derive benefit from it.

Address for correspondence: Filip Janku, MD, Department of Investigational Cancer Therapeutics; The University of Texas MD Anderson Cancer Center; 1515 Holcombe Blvd., FC8.2057, Box 0455; Houston, TX 77030, fjanku@mdanderson.org; Phone 713-794-1226; Fax 713-563-0566.

*Both authors contributed equally to this manuscript

Conflicts of interest: The authors have declared that no competing interests exist.

Ethical statement: All treatment and analysis were approved by MD Anderson Institutional Review Board and were conducted according to the principles expressed in the Declaration of Helsinki.

Case reports

Patient 1 was a 55-year-old man who was diagnosed with rectal adenocarcinoma in April 2004. In May 2004, he underwent a low anterior resection. The final pathology reading demonstrated moderate to poorly differentiated adenocarcinoma invading through the muscularis propria with one out of two lymph nodes infiltrated by carcinoma. On imaging (computed tomography [CT] of chest, abdomen, and pelvis), there was no evidence of distant metastasis (pT2pN1M0). The patient received adjuvant chemotherapy with oxaliplatin, 5-fluorouracil, leucovorin (FOLFOX) and adjuvant external chemoradiation with concurrent continuous infusion of 5-fluorouracil. He was disease-free until May 2006, when he was diagnosed with a left hepatic lobe and small pulmonary metastases. He received palliative chemotherapy with FOLFOX in combination with bevacizumab from July to September 2006, with a partial response. In October 2006, he underwent left hepatic metastasectomy, which removed a solitary liver metastasis (moderately differentiated adenocarcinoma with clear margins) and then continued on chemotherapy with FOLFOX in combination with bevacizumab from December 2006 to March 2007. He remained progression-free until September 2007, when he was found to have enlarging pulmonary metastases. Then in October 2007, chemotherapy was initiated with irinotecan, 5-fluorouracil, leucovorin (FOLFIRI) and bevacizumab, resulting in stable disease. Because of poor tolerance his treatment was changed to capecitabine and bevacizumab in March 2008, which continued until progression of pulmonary metastases in March 2009.

He was then referred to the Clinical Center for Targeted Therapy. The molecular profile of the tumor sample from the left hepatic metastasectomy showed wt *KRAS*, wt *BRAF*, wt *EGFR*, wt *KIT*, wt *PIK3CA*, intact PTEN expression and a Q61K *NRAS* mutation (181C>A). In April 2009, he initiated an investigational therapy with carboplatin and a nucleoside antimetabolite. His tumors were slowly growing until he was found to have unequivocal disease progression in the lungs and liver in September 2009. Then he initiated a clinical trial with the anti-VEGF monoclonal antibody bevacizumab (11 mg/kg IV on days 1 and 15 of 28 days) in combination with the histone deacetylase (HDAC) inhibitor valproic acid (5.3 mg/kg PO daily). The first restaging with CT of chest, abdomen, pelvis showed about 11% improvement per Response Evaluation Criteria in Solid Tumors (RECIST) and this was maintained for 13.5 months until November 2010 (Figure 1).^[5] Western blotting of peripheral mononuclear blood cells obtained before the first study drug administration and on day 15 of cycle 1 confirmed increased histone acetylation (data not shown).

Patient 2 was a 35-year-old man who was diagnosed with sigmoid adenocarcinoma in August 2005; he then underwent sigmoid resection, which revealed moderately differentiated adenocarcinoma infiltrating through the bowel, and six out of thirteen lymph nodes were infiltrated. A CT of chest, abdomen, and pelvis showed no evidence of distant metastasis (pT3pN2M0). He received 6 months of adjuvant chemotherapy with FOLFOX. He was disease-free until April 2008, when he was diagnosed with left hepatic lobe metastasis. He received palliative chemotherapy with FOLFOX in combination with bevacizumab from April to October 2008, with response shown by attenuated FDG uptake on positron emission tomography (PET). The patient then underwent regional treatment for liver metastasis with a Cyberknife. Eventually, he presented with recurrent liver metastases and resumed chemotherapy with FOLFOX in combination with bevacizumab from July to October 2009, with a response noted in a follow up FDG-PET. In November 2009, he underwent an explorative laparotomy with the intent to remove liver metastasis, but this was aborted due to a perioperative finding of peritoneal carcinomatosis. In December 2009, he initiated chemotherapy with FOLFIRI and bevacizumab, but because of poor tolerance this treatment was changed after 7 cycles to irinotecan, capecitabine (XELIRI) and cetuximab, which continued until disease progression in November 2010. Starting in January 2011, he

initiated an experimental Phase I therapy with a recombinant IgG1 antibody product consisting of two antibodies against EGFR. At the time of disease progression in March 2011, he initiated treatment with a p38 mitogen-activated-protein kinase (MAPK) inhibitor and progressed on that in May 2011. Then he was referred to the Clinical Center for Targeted Therapy at MD Anderson Cancer Center. The molecular profile of the tumor sample from the primary tumor showed wt *KRAS*, and additional analysis from the omental metastasis revealed a Q61K *NRAS* mutation and V272M + R273C *TP53* mutations. The tumor showed wt *BRAF*, wt *EGFR*, wt *KIT*, wt *PIK3CA*, and no aberrations in the additional 35 oncogenes tested in the panel.

In July 2011, he initiated a clinical trial with the anti-VEGF monoclonal antibody bevacizumab (11 mg/kg IV on days 1 and 15 of 28 days) in combination with the HDAC inhibitor valproic acid (5.3 mg/kg PO daily). The first restaging CT of chest, abdomen, and pelvis after 8 weeks of therapy showed a 27% improvement, and the second restaging after 16 weeks of therapy showed a 35% improvement per RECIST compared to baseline (Figure 2). He continues on therapy for 8+ months.

We treated an additional 10 patients, who had metastatic colorectal cancer with wt *NRAS* (n=3) or unknown *NRAS* status and mutated *KRAS* (n=7, *KRAS* and *NRAS* mutations are mutually exclusive), and none of the patients experienced any tumor shrinkage per RECIST. Their median progression-free survival was 1.8 months (range, 0.3–3.9 months).

Discussion

We report two cases of heavily pretreated patients with metastatic colorectal cancer and a *NRAS* Q61K mutation treated with anti-VEGF monoclonal antibody bevacizumab and the HDAC inhibitor valproic acid. Both patients were heavily pretreated with standard therapies including bevacizumab. Both patients demonstrated tumor shrinkage per RECIST at the time of first restaging, which lasted for more than 13 months in patient 1. Patient 2 has achieved a partial response and continued on therapy at the time of this report for 8+ months. None of the 10 patients with metastatic colorectal cancer and wt *NRAS* or mutated *KRAS* (*KRAS* mutations do not coexist with *NRAS* mutations) treated on the same regimen showed prolonged stable disease or tumor regression.

NRAS mutations occur in subsets of diverse solid tumors, with the highest prevalence (14–30%) in malignant melanoma.[6; 7; 8] *NRAS* mutations have been reported in 2–6% of patients with colorectal cancers and in a retrospective analysis were associated with low responses to cetuximab and chemotherapy.[6; 7; 9] *NRAS* belongs to a family of small G proteins with three homologues (*HRAS*, *NRAS*, *KRAS*) coded by different *RAS* gene loci. *RAS* activates mitogen-activated-protein kinase (MAPK) and PI3K/AKT/mTOR signaling, which are two major pathways involved in the progression of multiple cancers.[10] *In vitro* studies link *NRAS* function to antiapoptotic signaling.[11] Preclinical models in a genetically engineered mouse model demonstrated that *KRAS* mutations in colonic epithelium promote hyperproliferation via MEK signaling, whereas epithelial cells with *NRAS* mutations suppressed apoptosis.[12] In addition, preclinical models using cell line-derived xenografts with knocked out MEK expression demonstrated decreased angiogenesis and tumor growth compared to xenografts with intact MEK.[13] Therefore, it is conceivable that aberrations activating the MAPK pathway can promote angiogenesis. However, 8 of 10 patients with metastatic colorectal cancer with wt *NRAS* (n=3) or unknown *NRAS* status (n=7) had *KRAS* mutations in codon 12 or 13 and their outcomes on bevacizumab and valproic acid were dismal (of note, 5 of these patients also tested negative for *BRAF* mutations).

The role of *NRAS* mutations in selecting therapeutic regimens for cancer is currently unknown. Preclinical models with cell lines from different human cancers with *NRAS* mutations showed inhibition when treated with the MEK inhibitor CI-1040, and human colon cancer xenografts with *NRAS* mutations were sensitive to the MEK inhibitor AZD6244.[14; 15] In addition, preclinical data on melanoma cell lines and early clinical observations suggest that melanomas with *NRAS* mutations are sensitive to HSP90 inhibitors.[16; 17]

Bevacizumab is an anti-VEGF monoclonal antibody, which in most cancers has only modest activity when given as a single agent but prolongs survival in combination with chemotherapy in several metastatic cancers.[4; 18; 19] Survival prolongation is approximately 2 months and it is plausible that a small subset of patients benefit more substantially while most patients do not. Valproic acid, widely used for treating seizures, is also a HDAC inhibitor. Inhibiting histone deacetylase activity results in histone acetylation, which is associated with up-regulated gene transcription.[20] By promoting gene transcription, valproic acid induces differentiation, growth inhibition, and apoptosis.[21] Combinations of antiangiogenic agents and HDAC inhibitors are expected to have synergistic antiangiogenic activity.[22; 23; 24]

The mechanism of action explaining the salutary effect of bevacizumab in these two patients with colorectal cancer and a Q61K *NRAS* mutation remains unclear. First, the effect may be due to bevacizumab. Indeed these patients were previously treated on bevacizumab-containing regimens for approximately 22 and 13 months, respectively, before showing progression. However, with their prior therapy, the role of bevacizumab versus combination chemotherapy such as FOLFOX or FOLFIRI is unclear. Alternatively, it is possible that the addition of valproic acid contributed to therapeutic benefit in these cases since bevacizumab has only modest activity as single agent.[18] In theory, epigenetic modulation with valproic acid, which promotes transcription of tumor suppressor genes, may contradict the antiapoptotic effect of *NRAS* mutations, and in combination with bevacizumab, lead to enhanced antiangiogenesis. Alternatively, bevacizumab and valproic acid may induce autophagy to the point of autophagic cell death. Finally, bevacizumab and valproic acid may inhibit a critical, as yet unknown, pathway activated by *NRAS*. It is however conceivable that the therapeutic effect seen is due to bevacizumab, and the fact that single agent activity occurs in only a minority of patients with colorectal cancer is consistent with this notion, especially if *NRAS* mutation is a biomarker of response (since *NRAS* mutations occur in a minority of patients with colorectal cancer). *NRAS* mutations may serve as potential biomarkers to predict the anticancer effect of bevacizumab and valproic acid. Nevertheless, our observations should be interpreted with caution. Even though it is possible that *NRAS* mutations can lead to increased angiogenesis, they are not necessarily predictive for response to bevacizumab.

Bevacizumab is an essential component of nearly all standard-of-care regimens for metastatic colorectal cancer.[2; 4] Despite being a targeted agent, which apparently is effective in only a small subset of patients, the relevant target or biomarker predicting its activity has not been identified. To understand the underlying molecular basis of efficacy, a bedside to bench foray has to be applied and preclinical experiments in *NRAS* mutant colorectal cancer models need to be designed. Further, preclinical and clinical studies are warranted to investigate the role of *NRAS* mutation in bevacizumab-based therapies in colorectal cancer.

Acknowledgments

Financial support: Supported by Grant Number RR024148 from the National Center for Research Resources, a component of the NIH Roadmap for Medical Research (<http://nihroadmap.nih.gov/clinicalresearch/overview-translational.asp>). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

All treatment and analysis were performed in accordance with MD Anderson Institutional Review Board guidelines. We thank Ms. Joann Aaron for scientific review and editing of this article.

References

1. Siegel R, Ward E, Brawley O, Jemal A. Cancer statistics, 2011: the impact of eliminating socioeconomic and racial disparities on premature cancer deaths. *CA Cancer J Clin.* 2011; 61:212–236. [PubMed: 21685461]
2. Hurwitz H, Fehrenbacher L, Novotny W, Cartwright T, Hainsworth J, Heim W, Berlin J, Baron A, Griffing S, Holmgren E, Ferrara N, Fyfe G, Rogers B, Ross R, Kabbinavar F. Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. *N Engl J Med.* 2004; 350:2335–2342. [PubMed: 15175435]
3. Van Cutsem E, Kohne CH, Hitre E, Zaluski J, Chang Chien CR, Makhson A, D’Haens G, Pinter T, Lim R, Bodoky G, Roh JK, Folprecht G, Ruff P, Stroh C, Tejpar S, Schlichting M, Nippgen J, Rougier P. Cetuximab and chemotherapy as initial treatment for metastatic colorectal cancer. *N Engl J Med.* 2009; 360:1408–1417. [PubMed: 19339720]
4. Saltz LB, Clarke S, Diaz-Rubio E, Scheithauer W, Figer A, Wong R, Koski S, Lichinitser M, Yang TS, Rivera F, Couture F, Sirzen F, Cassidy J. Bevacizumab in combination with oxaliplatin-based chemotherapy as first-line therapy in metastatic colorectal cancer: a randomized phase III study. *J Clin Oncol.* 2008; 26:2013–2019. [PubMed: 18421054]
5. Therasse P, Arbuck SG, Eisenhauer EA, Wanders J, Kaplan RS, Rubinstein L, Verweij J, Van Glabbeke M, van Oosterom AT, Christian MC, Gwyther SG. New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. *J Natl Cancer Inst.* 2000; 92:205–216. [PubMed: 10655437]
6. Forbes SA, Bindal N, Bamford S, Cole C, Kok CY, Beare D, Jia M, Shepherd R, Leung K, Menzies A, Teague JW, Campbell PJ, Stratton MR, Futreal PA. COSMIC: mining complete cancer genomes in the Catalogue of Somatic Mutations in Cancer. *Nucleic Acids Res.* 2010; 39:D945–950. [PubMed: 20952405]
7. Janku F, Lee JJ, Tsimberidou AM, Hong DS, Naing A, Falchook GS, Fu S, Luthra R, Garrido-Laguna I, Kurzrock R. PIK3CA mutations frequently coexist with RAS and BRAF mutations in patients with advanced cancers. *PLoS One.* 2011; 6:e22769. [PubMed: 21829508]
8. Ellerhorst JA, Greene VR, Ekmekcioglu S, Warneke CL, Johnson MM, Cooke CP, Wang LE, Prieto VG, Gershenwald JE, Wei Q, Grimm EA. Clinical correlates of NRAS and BRAF mutations in primary human melanoma. *Clin Cancer Res.* 2011; 17:229–235. [PubMed: 20975100]
9. De Roock W, Claes B, Bernasconi D, De Schutter J, Biesmans B, Fountzilias G, Kalogeras KT, Kotoula V, Papamichael D, Laurent-Puig P, Penault-Llorca F, Rougier P, Vincenzi B, Santini D, Tonini G, Cappuzzo F, Frattini M, Molinari F, Saletti P, De Dosso S, Martini M, Bardelli A, Siena S, Sartore-Bianchi A, Tabernero J, Macarulla T, Di Fiore F, Gangloff AO, Ciardiello F, Pfeiffer P, Qvortrup C, Hansen TP, Van Cutsem E, Piessevaux H, Lambrechts D, Delorenzi M, Tejpar S. Effects of KRAS, BRAF, NRAS, and PIK3CA mutations on the efficacy of cetuximab plus chemotherapy in chemotherapy-refractory metastatic colorectal cancer: a retrospective consortium analysis. *Lancet Oncol.* 2010; 11:753–762. [PubMed: 20619739]
10. Malumbres M, Barbacid M. RAS oncogenes: the first 30 years. *Nat Rev Cancer.* 2003; 3:459–465. [PubMed: 12778136]
11. Wolfman JC, Palmby T, Der CJ, Wolfman A. Cellular N-Ras promotes cell survival by downregulation of Jun N-terminal protein kinase and p38. *Mol Cell Biol.* 2002; 22:1589–1606. [PubMed: 11839824]

12. Haigis KM, Kendall KR, Wang Y, Cheung A, Haigis MC, Glickman JN, Niwa-Kawakita M, Sweet-Cordero A, Sebolt-Leopold J, Shannon KM, Settleman J, Giovannini M, Jacks T. Differential effects of oncogenic K-Ras and N-Ras on proliferation, differentiation and tumor progression in the colon. *Nat Genet.* 2008; 40:600–608. [PubMed: 18372904]
13. Mavria G, Vercoulen Y, Yeo M, Paterson H, Karasarides M, Marais R, Bird D, Marshall CJ. ERK-MAPK signaling opposes Rho-kinase to promote endothelial cell survival and sprouting during angiogenesis. *Cancer Cell.* 2006; 9:33–44. [PubMed: 16413470]
14. Solit DB, Garraway LA, Pratilas CA, Sawai A, Getz G, Basso A, Ye Q, Lobo JM, She Y, Osman I, Golub TR, Sebolt-Leopold J, Sellers WR, Rosen N. BRAF mutation predicts sensitivity to MEK inhibition. *Nature.* 2006; 439:358–362. [PubMed: 16273091]
15. Davies BR, Logie A, McKay JS, Martin P, Steele S, Jenkins R, Cockerill M, Cartledge S, Smith PD. AZD6244 (ARRY-142886), a potent inhibitor of mitogen-activated protein kinase/extracellular signal-regulated kinase 1/2 kinases: mechanism of action in vivo, pharmacokinetic/pharmacodynamic relationship, and potential for combination in preclinical models. *Mol Cancer Ther.* 2007; 6:2209–2219. [PubMed: 17699718]
16. Grbovic OM, Basso AD, Sawai A, Ye Q, Friedlander P, Solit D, Rosen N. V600E B-Raf requires the Hsp90 chaperone for stability and is degraded in response to Hsp90 inhibitors. *Proc Natl Acad Sci U S A.* 2006; 103:57–62. [PubMed: 16371460]
17. Banerji U, Affolter A, Judson I, Marais R, Workman P. BRAF and NRAS mutations in melanoma: potential relationships to clinical response to HSP90 inhibitors. *Mol Cancer Ther.* 2008; 7:737–739. [PubMed: 18375819]
18. Margolin K, Gordon MS, Holmgren E, Gaudreault J, Novotny W, Fyfe G, Adelman D, Stalter S, Breed J. Phase Ib trial of intravenous recombinant humanized monoclonal antibody to vascular endothelial growth factor in combination with chemotherapy in patients with advanced cancer: pharmacologic and long-term safety data. *J Clin Oncol.* 2001; 19:851–856. [PubMed: 11157039]
19. Sandler A, Gray R, Perry MC, Brahmer J, Schiller JH, Dowlati A, Lilenbaum R, Johnson DH. Paclitaxel-carboplatin alone or with bevacizumab for non-small-cell lung cancer. *N Engl J Med.* 2006; 355:2542–2550. [PubMed: 17167137]
20. Marks P, Rifkind RA, Richon VM, Breslow R, Miller T, Kelly WK. Histone deacetylases and cancer: causes and therapies. *Nat Rev Cancer.* 2001; 1:194–202. [PubMed: 11902574]
21. Johannessen CU, Johannessen SI. Valproate: past, present, and future. *CNS Drug Rev.* 2003; 9:199–216. [PubMed: 12847559]
22. Qian DZ, Kachhap SK, Collis SJ, Verheul HM, Carducci MA, Atadja P, Pili R. Class II histone deacetylases are associated with VHL-independent regulation of hypoxia-inducible factor 1 alpha. *Cancer Res.* 2006; 66:8814–8821. [PubMed: 16951198]
23. Qian DZ, Wang X, Kachhap SK, Kato Y, Wei Y, Zhang L, Atadja P, Pili R. The histone deacetylase inhibitor NVP-LAQ824 inhibits angiogenesis and has a greater antitumor effect in combination with the vascular endothelial growth factor receptor tyrosine kinase inhibitor PTK787/ZK222584. *Cancer Res.* 2004; 64:6626–6634. [PubMed: 15374977]
24. Kim MS, Kwon HJ, Lee YM, Baek JH, Jang JE, Lee SW, Moon EJ, Kim HS, Lee SK, Chung HY, Kim CW, Kim KW. Histone deacetylases induce angiogenesis by negative regulation of tumor suppressor genes. *Nat Med.* 2001; 7:437–443. [PubMed: 11283670]

September 12, 2009

November 24, 2009

Figure 1. Imaging showing response to bevacizumab and vaproic acid in Patient 1

The baseline CT of chest, abdomen and pelvis shows lung and liver metastases. After 8 weeks of therapy there is improvement shown by reduced density of left lung metastasis in lung windows (top scan) and a reduction in the size of right lung and liver metastases in soft tissue windows (middle and bottom picture).

Figure 2. Imaging showing response to bevacizumab and vaproic acid in Patient 2

The baseline CT of chest, abdomen and pelvis of Patient 2 shows ascites, right lung pleural based, and peritoneal metastases. After 8 weeks of therapy there is improvement in size of all lesions with resolution of ascites.