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Rona Yaeger, MD, is an Assistant Attending at Memorial Sloan-Kettering Cancer Center. She is also a member of the laboratory of Neal Rosen, MD, PhD. Her research studies the role of mitogenic and survival signaling pathways in colorectal cancer, focusing on the effects of selective therapeutics against nodes in these pathways. She is interested in the dysregulation of mitogen-activated protein kinase (MAPK) signaling that commonly occurs in colorectal cancer, resulting from epidermal growth factor receptor (EGFR) activation or activating mutations in RAS or RAF, and the consequences of this dysregulation on parallel and downstream signaling. Through her research, she recently identified the emergence of KRAS mutations with acquired resistance to EGFR targeting agents. Dr. Yaeger graduated from New York University School of Medicine and completed her internal medicine residency training at The New York Presbyterian Hospital-Columbia University Medical Center and her oncology fellowship at Memorial Sloan-Kettering Cancer Center.

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BRAF Mutations in Colorectal Cancer: Clinical Relevance and Role in Targeted Therapy

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Experts have long appreciated that the clinical entity we call colorectal cancer is a phenotype made up of numerous different genotypes, accounting for the wide variation in clinical course and responses to therapies experienced by different individuals. Observations reported by Khambata-Ford et al¹ and subsequently confirmed by Amado et al² have made the practicing community aware that activating mutations in exon 2 of the gene encoding for KRAS, a key signal transduction protein, result in primary resistance to anti-epidermal growth factor receptor (EGFR) agents by leading to EGFR-independent activation of mitogen-activated protein kinase (MAPK) signaling.

BRAF is a signal transduction protein that is downstream of KRAS in the MAPK pathway. Approximately 5% to 10% of colorectal cancers harbor mutations in BRAF; the most common is the V600E mutation. The occurrence of these mutations in colorectal cancers raises 2 important questions: what are the clinical characteristics of a colorectal cancer with a V600E BRAF mutation, and can that mutation be targeted for therapeutic advantage?

BRAF as a Prognostic and Predictive Marker

From a prognostic perspective, it is clear that BRAF mutations confer a particularly poor prognosis, regardless of the therapeutic intervention used. In a combined analysis of the CRYSTAL and OPUS trials, which explored the addition of cetuximab to front-line chemotherapy for metastatic colorectal cancer, a substantially diminished overall survival was seen for patients with BRAF-mutated versus BRAF wild-type disease.³

The question of BRAF as a predictive marker of resistance to anti-EGFR monoclonal antibodies has been addressed; the results, however, are complicated. In the chemotherapy-refractory setting, responses to cetuximab or panitumumab in BRAF-mutated tumors are extremely rare. Di Nicolantonio et al⁴ reported that, in the chemotherapy-refractory setting, BRAF mutation confers resistance to cetuximab or panitumumab. No responses were seen in the 11 patients included.⁴ An additional study reported that none of the 5 patients with BRAF-mutated tumors showed a response, and found that progression-free survival was significantly shorter than that seen in BRAF wild-type tumors.⁵ De Roock et al⁶ reported a response rate of 8.3% (2 of 24) in patients whose tumors had BRAF mutations versus 38.0% in patients with BRAF wild-type tumors (124 of 326; $P=.0012$). Combining the results of these 3 studies shows response for 2 of 40 BRAF-mutated tumors. This suggests that the salvage activity of anti-EGFR agents in V600E BRAF-mutated tumors is similar to that seen in KRAS-mutated tumors.

More recent data from the combined analysis of the CRYSTAL and OPUS trials have raised questions about the potential for activity of anti-EGFR agents in conjunction with active chemotherapy in the front-line setting in BRAF-mutated tumors.³ We should note that these data are derived from a nonpreplanned analysis of a nonpreplanned combination of these 2 trials, each of which used a different chemotherapy backbone (FOLFIRI vs. FOLFOX) and each of which has a different prespecified primary end point (overall vs. progression-free survival). Therefore, the statistical validity of the observations is less than optimal. Nevertheless, although

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BRAF was a consistently poor prognostic marker, patients who received cetuximab appeared to fare better in terms of overall (14.1 vs. 9.9 months) and progression-free survival (7.1 vs. 3.7 months) than those who received chemotherapy alone. As would be expected from the small numbers of patients involved, these differences do not reach statistical significance. This observation will require confirmation in prospective randomized trials; however, the possibility of some clinically meaningful degree of activity from the first-line addition of cetuximab or panitumumab to initial chemotherapy in patients with BRAF-mutated colorectal cancer cannot be excluded.

BRAF as a Therapeutic Target

The selective BRAF inhibitor vemurafenib (formerly known as PLX-4032) has achieved high response rates and increased overall survival in patients with V600E BRAF-mutated melanoma.⁷ The experience in colorectal tumors with V600E BRAF mutations has revealed only minimal activity for this agent, however. Kopetz et al⁸ treated 21 patients with V600E BRAF mutations with single-agent vemurafenib; only one patient experienced an objective response.

Preclinical studies are underway to investigate the reasons why these agents lack activity in metastatic colorectal cancer. Pharmacodynamic studies in tumor samples from patients with melanoma in a phase I trial of vemurafenib indicate that near-complete inhibition of pathway signaling is necessary to effectively inhibit tumor growth.⁹ Two recent reports suggest that vemurafenib treatment fails to sufficiently inhibit MAPK in colorectal cancer because of reactivation of EGFR signaling.^{10,11} In a process of “adaptive resistance,”¹² when vemurafenib inhibits BRAF, it activates growth factor receptors previously suppressed by negative feedback signals resulting from the high MAPK activity in these tumors. Activation of growth factor receptors in turn activate RAS and CRAF and overcomes the inhibitory effect of vemurafenib (Figure 1). Colorectal tumors have relatively high EGFR expression and ligand production, and therefore are primed for continued growth despite treatment with vemurafenib. Thus, adaptive resistance develops in colorectal cancer much more rapidly than in melanoma.

Further, the adaptive resistance effect is probably magnified by the selective efficacy of current BRAF inhibitors such as vemurafenib. Vemurafenib inhibits mutated BRAF only, and mutated BRAF signals as a monomer. Paradoxically, vemurafenib has been shown to activate MAPK signaling in tumors with wild-type BRAF, in which



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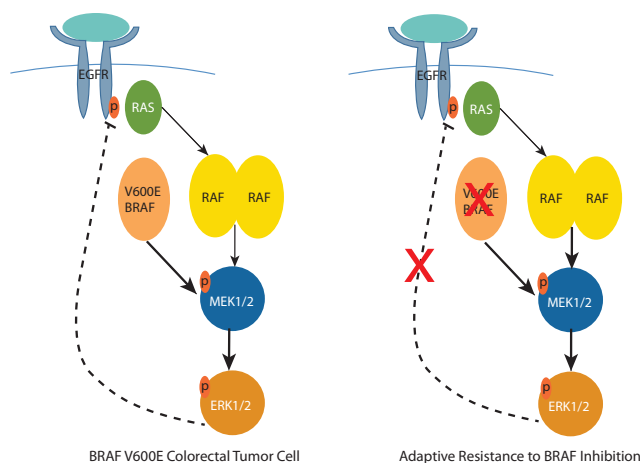


Figure 1 Adaptive resistance.

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RAF signals as a dimer.¹³ As EGFR signals through RAS, feedback reactivation of EGFR with vemurafenib will lead to RAS activation and the formation of dimers of the RAF protein, against which vemurafenib is ineffective. Based on these data, we and others propose new studies to test the clinical efficacy of combining EGFR and BRAF inhibitors in patients with BRAF-mutant colorectal cancer.

Conclusions

Our understanding of the prevalence and relevance of BRAF mutations in colorectal cancer continues to evolve. Available evidence strongly suggests that BRAF-mutant colorectal cancers carry a poor prognosis, and that these tumors are insensitive to EGFR inhibition in the chemotherapy-refractory setting to a similar degree as tumors with exon 2 KRAS mutations. Intriguing but suboptimal data raise the possibility of some degree of activity of EGFR agents in conjunction with active chemotherapy in the front-line management of metastatic BRAF-mutated colorectal cancer. Vemurafenib, the selective inhibitor of mutated BRAF that has shown important single-agent activity in V600E BRAF-mutated melanoma, is virtually inactive as a single agent in colorectal cancers harboring that same mutation. The reasons for this, and strategies to overcome that resistance, are the subject of ongoing investigations.

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