Mutations in the serine–threonine kinase BRAF at amino acid position V600 occur in 8% to 15% of colorectal cancers. BRAF<sup>V600</sup> mutations also occur in other tumor types, such as melanoma, non–small cell lung cancer (NSCLC), and hairy cell leukemia (HCL), suggesting that mutant BRAF could be broadly targeted regardless of histology. Unfortunately, response rates to mutant-specific BRAF inhibitors, such as vemurafenib and dabrafenib, vary by tumor type. Although BRAF inhibitors have achieved a mere 5% response rate in BRAF<sup>V600</sup>-mutant colorectal cancer, response rates in melanoma, NSCLC, and HCL are approximately 52%, 32%, and 96%, respectively (1–3). Thus, at least in solid tumors, the presence of a BRAF mutation is not a “slam dunk” for single-agent BRAF inhibition.

Mechanisms of resistance to single-agent BRAF inhibition also differ by histologic subtype. In BRAF<sup>V600</sup>-mutant colorectal cancer cells treated with vemurafenib, MAPK signaling is reactivated by the feedback activation of EGFR (Fig. 1A; ref. 3). Amplification of mutant BRAF has also been implicated in preclinical resistance to BRAF inhibitors in this context (4). Consistent with these findings, combined BRAF/MEK and BRAF/EGFR inhibition more effectively inhibits growth of BRAF<sup>V600</sup>-mutant colorectal cancer cells than BRAF inhibition alone (3). Other studies have shown that PIK3CA mutations and PTEN loss may also contribute to vemurafenib resistance in BRAF<sup>V600</sup>-mutant colorectal cancer cell lines (5). Additional molecular alterations across different tumor types (mostly melanoma) that modulate response to BRAF inhibition in BRAF<sup>V600</sup>-mutant tumors include <i>RAS</i> mutations, loss of the RAS negative regulator NF1, BRAF splice variants, increased expression of CRAF and COT, <i>MEK1</i> mutations, RB1 inactivation, and activation of MET, IGF1R, and HER3 receptor tyrosine kinases, among others (3). Clearly, reactivation of MAPK signaling is a recurring theme (3, 5).

Based on the identification that EGFR bypass signaling can mediate resistance to vemurafenib in BRAF<sup>V600</sup>-mutant colorectal cancer, clinical trials testing combinations of BRAF/MEK and BRAF/EGFR inhibitors in these patients are under way. Such combinations have demonstrated somewhat improved outcomes relative to BRAF inhibition alone, but acquired resistance still emerges (6, 7). In this issue of Cancer Discovery, Ahronian and colleagues (4) provide the first description of mechanisms of acquired resistance to combined BRAF/MEK and combined BRAF/EGFR inhibition in BRAF<sup>V600</sup>-mutant colorectal cancer. By utilizing both in vitro cell line models and clinical samples, they describe multiple distinct mechanisms of resistance to these BRAF inhibitor combinations, all of which reactivate the MAPK signaling pathway.

In vitro modeling of acquired resistance to combined BRAF/EGFR inhibition (vemurafenib/cetuximab) and combined BRAF/MEK inhibition (vemurafenib/selumetinib) resulted in acquired <i>KRAS</i><sup>G12D</sup> and <i>KRAS</i><sup>G13D</sup> substitution mutations, respectively (Fig. 1B). The authors further showed that these activating <i>KRAS</i> mutations increased phosphorylation of CRAF, MEK, ERK, and RSK, suggesting that mutant <i>KRAS</i>-mediated constitutive activation of the MAPK signaling pathway is responsible for resistance in this setting. Importantly, the cell line models of KRAS-mediated acquired resistance to combined BRAF/EGFR inhibition displayed cross-resistance to combined BRAF/MEK inhibition, and vice versa.

Ahronian and colleagues (4) also performed whole-exome and/or RNA sequencing of paired pretreatment and postresistance biopsies from 3 separate patients with BRAF<sup>V600</sup>-mutant colorectal cancer who progressed on either BRAF/EGFR or BRAF/MEK combination therapy. In the two described cases of acquired resistance to BRAF/EGFR combination therapy, the authors found amplification of BRAF (dabrafenib/panitumumab resistance) and <i>KRAS</i> (encorafenib/cetuximab resistance) to be unique to the resistant tumor (Fig. 1B). Of note, the <i>KRAS</i> amplification was identified in a lesion that had progressed through BRAF/MEK (dabrafenib/trametinib) combination therapy and continued to progress after the patient...
Figure 1. Schematic representation of mechanisms of resistance to BRAF/EGFR or BRAF/MEK inhibitor combinations in BRAF<sup>V600</sup>-mutant colorectal cancer (CRC). **A**, comparison of response of BRAF<sup>V600</sup>-mutant melanoma versus colorectal cancer to single-agent BRAF inhibition. Left, BRAF inhibitors (BRAFi) effectively inhibit MAPK signaling and induce tumor regression in over half of BRAF<sup>V600</sup>-mutant melanomas, when used as monotherapy. Mechanisms of resistance in nonresponsive tumors are not depicted. Right, BRAF inhibitors result in reactivation of EGFR-mediated MAPK signaling in BRAF<sup>V600</sup>-mutant colorectal cancer, contributing to the low overall response rate to single-agent BRAF inhibition. **B**, mechanisms of resistance to various combinations of BRAF/EGFR or BRAF/MEK inhibitors were identified by Ahronian and colleagues via *in vitro* modeling of acquired resistance or analysis of tumor samples after progression on combination therapy. KRAS<sup>G12D</sup> or KRAS<sup>G13D</sup> mutations and amplification of wild-type or mutant BRAF were found to confer resistance to dual inhibition of BRAF/EGFR and BRAF/MEK. A MEK<sup>F53L</sup> substitution mutation was found to confer resistance to dual BRAF/MEK inhibition. All models of resistance to combination BRAF/EGFR and BRAF/MEK inhibition retained sensitivity to ERK inhibition.

was switched to the BRAF/EGFR (encorafenib/cetuximab) regimen. This finding further validates the authors’ *in vitro* result that KRAS-mediated activation of MAPK signaling can confer cross-resistance to both BRAF/MEK and BRAF/EGFR inhibition.

In addition, the authors identified co-occurring acquired missense mutations in MEK1 and ARAF in a BRAF<sup>V600</sup>-mutant colorectal cancer sample with acquired resistance to BRAF/MEK inhibition (dabrafenib/trametinib). Follow-up *in vitro* analysis revealed that the MEK1<sup>F53L</sup> mutation, but not the ARAF<sup>Q489L</sup>
mutation, was sufficient to confer resistance to dabrafenib/trametinib in vitro, although the two mutations were not tested for cooperativity within the same cell (Fig. 1B). As the authors point out, this result highlights a separate important principle regarding the significance of findings revealed by comprehensive mutational profiling, i.e., that thorough and efficient biologic validation of novel mutations is critical to distinguish passenger versus driver mutations, especially when two obvious signaling molecules in the same pathway harbor mutations.

Taken together, the translational data from Ahronian and colleagues (4) have multifaceted implications for our ongoing understanding of the underlying biology and clinical treatment of \(BRAF^{V600}\)-mutant tumors. Context-dependent differences in sensitivity to single-agent RAF inhibition reveal varying levels of “addiction to mutant BRAF signaling” across histologic subtypes. These differences appear to extend to the setting of combination therapies as well. Although combined BRAF/EGFR and combined BRAF/MEK inhibition thus far demonstrate improved clinical outcomes in \(BRAF\)-mutant colorectal cancer compared with RAF inhibition alone, response rates to combination therapy still range from 12% to 29% only. By comparison, in \(BRAF\)-mutant melanoma, combined BRAF/MEK inhibition has demonstrated a response rate of 64% (8). Biologic mechanisms underling these phenomena are not yet fully understood but could be explained by differences in factors such as cell lineage, epigenetics, and microenvironment of the tumor. The 12% to 29% response rate indicates that it will take more than dual combination therapy to target \(BRAF^{V600}\)-mutant colorectal cancer to the same extent as \(BRAF^{V600}\)-mutant melanoma; however, it is also evident that even in \(BRAF^{V600}\)-mutant melanoma, dual BRAF/MEK inhibition is not curative.

Finally, the authors of this study leave us with the hope that newly developed ERK inhibitors may successfully overcome MAPK-driven resistance to combined BRAF/EGFR or BRAF/MEK inhibition in \(BRAF^{V600}\)-mutant colorectal cancer. Recent preclinical studies have also demonstrated that ERK inhibitors may be effective at overcoming acquired resistance to BRAF/MEK inhibition in \(BRAF^{V600}\)-mutant melanoma (9). It remains to be seen whether ERK inhibitors will be effective in overcoming all mechanisms of resistance to BRAF/MEK/EGFR-targeted therapies, as well as whether ERK inhibitors will be useful as first-line therapies across different histologic groups of \(BRAF\)-mutant tumors. Factors mediating sensitivity to ERK inhibition will probably vary according to cellular context, as we have seen with BRAF inhibitors. Biomarkers of sensitivity to ERK inhibition will be important as clinicians are faced with questions about how best to administer these therapies to provide maximal benefit to patients.

These studies spark a broader discussion regarding the utility of monotherapy versus combination therapy in personalized cancer medicine. Maximizing combination therapy in the first-line setting makes intuitive sense, with the goal being to limit any residual resistant disease that will ultimately result in tumor progression or recurrence. However, the current reality in metastatic solid tumors is that resistance almost invariably develops for the targeted agents in clinical use. It remains to be seen whether the addition of new “downstream” ERK inhibitors will result in longer time to resistance. It is also unknown whether resistance to such newer therapies will result in more aggressive tumors that are unresponsive to other targeted agents (10).

In the case of \(BRAF^{V600}\)-mutant colorectal cancer, for example, the addition of an ERK inhibitor to the BRAF/MEK or BRAF/EGFR combination could significantly extend progression-free survival. However, if acquired resistance after the addition of an ERK inhibitor occurs within a similar time frame as seen with BRAF/MEK and BRAF/EGFR inhibitor combinations, a patient could potentially benefit from receiving the dual inhibitor combination first, followed by an ERK inhibitor once the tumor has progressed. The question of which strategy will provide the best outcome for patients can only be answered clinically. In the meantime, studies such as those performed by Ahronian and colleagues (4) are critical to pave the road ahead.

**Disclosure of Potential Conflicts of Interest**

W. Pao is Global Head, Oncology Discovery and Translational Area, at Roche; reports having received commercial research grants from AstraZeneca, Bristol-Myers Squibb, and Symphogen; and has been a consultant/advisory board member for AstraZeneca, Bristol-Myers Squibb, and Exelixis. No potential conflicts of interest were disclosed by the other author.

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**REFERENCES**

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