CANCER

# Resistance to MEK Inhibitors: Should We Co-Target Upstream?

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**Aberrant activation of the ERK pathway is common in human tumors. This pathway consists of a three-tiered kinase module [comprising the kinases RAF, mitogen-activated protein kinase (MAPK) kinase (MEK), and extracellular signal–regulated kinase (ERK)] which functions as a negative feedback amplifier to confer robustness and stabilization of pathway output. Because this pathway is frequently dysregulated in human cancers, intense efforts are under way to develop selective inhibitors of the ERK pathway as anticancer drugs. Although promising results have been reported in early trials for inhibitors of RAF or MEK, resistance invariably occurs. Amplification of the upstream oncogenic driver of ERK signaling has been identified as a mechanism for MEK inhibitor resistance in cells with mutant BRAF or KRAS. Increased abundance of the oncogenic driver (either KRAS or BRAF in the appropriate cellular context) in response to prolonged drug treatment results in increased flux through the ERK pathway and restoration of ERK activity above the threshold required for cell growth. For patients with BRAF mutant tumors, the results suggest that the addition of a RAF inhibitor to a MEK inhibitor may delay or overcome drug resistance. The data thus provide a mechanistic basis for ongoing trials testing concurrent treatment with RAF and MEK inhibitors.**

The ERK signaling pathway—which consists of the three-kinase cascade of RAF, MEK, and ERK—is frequently dysregulated in human cancer, often as a result of activating mutations in the *BRAF* and *RAS* genes (*KRAS*, *NRAS*, and *HRAS*). Given the high prevalence of ERK signaling aberrations and the dependence of RAS and BRAF mutant tumors on these oncogenic drivers, intense efforts are under way to identify inhibitors of this pathway for use as anticancer therapies. The RAF inhibitors PLX4032 and GSK2118436 have demonstrated remarkable clinical activity in individuals with melanomas harboring the BRAF(V600E) mutation (*1, 2*). Selective inhibitors of MEK have also shown promise, though with response rates lower than those observed with inhibitors of RAF (*3*). This difference in efficacy has been attributed to the broader therapeutic index of RAF inhibitors that affect ERK pathway activity in a mutant-specific manner (*4*). Nevertheless, as has been the pattern with inhibitors of other oncogenic kinases such as epidermal growth factor receptor and ABL, the clinical benefit of these therapies is limited by the emergence of drug resistance.

Using AZD6244, a selective, non–adenosine 5′-triphosphate competitive inhibitor of MEK1 and MEK2, Little *et al*. now identify amplification of the oncogenic driver of ERK signaling (BRAF or KRAS) as a mechanism of acquired resistance to MEK inhibitors (*5*). The approach taken was to generate AZD6244-resistant subclones of colo205 cells, a cell line with a gain-of-function mutation in BRAF (V600E), and HCT-116 cells, a cell line with a gain-of-function mutation in KRAS (G13D), through continuous culture in increasing concentrations of drug. Comparison of the resistant clones and the parental clones in the absence of drug showed increased MEK and ERK activity in the resistant cells as compared with the parental counterparts. Drug treatment of the resistant sublines was effective in reducing ERK activity (as assessed by phosphorylated ERK). However, given the higher basal activity of the pathway in the resistant cells, an increased concentration of drug was required to inhibit pathway activity sufficiently to induce growth arrest. The results suggest that the resistant cells had adapted to drug exposure by increasing flux through the ERK signaling pathway in order to maintain ERK activity above the threshold required for cell proliferation. This result is notable because Bollag and colleagues showed that induction of tumor regression by the RAF inhibitor PLX4032 required almost complete suppression of ERK signaling by the drug (*6*). One implication of this work is that perturbations that even modestly diminish the ability of a drug to inhibit ERK signaling may result in clinical resistance by allowing residual ERK activity to rise above the threshold required to maintain cell growth.

In both the mutant BRAF and KRAS cell lines, the mechanistic basis for the increase in basal MEK and ERK activity and thus drug resistance proved to be analogous: amplification of the oncogenic driver of pathway activity (Fig. 1). In the case of the drug-resistant colo205 clones, increased abundance of BRAF was the result of *BRAF* amplification. Similarly, in the drug-resistant HCT-116 clones, elevated KRAS abundance was due to *KRAS* amplification. These results are consistent with those of Corcoran *et al*., who also identified increased activation of MEK and ERK resulting from BRAF amplification as the basis for AZD6244 resistance in two additional colorectal cancer cell lines with the V600E mutant form of BRAF (*7*). Similarly, increased abundance of activated KRAS confers resistance to the MEK inhibitor CI-1040 in C26 mouse colon cancer cells, which harbor a gain-of-function mutation in KRAS (G12V) (*8*).

Although resistance to the MEK inhibitor was due to amplification of the oncogenic driver in each case, resistance to AZD6244 emerged more rapidly in KRAS-mutant HCT-116 cells than in BRAF-mutant colo205 cells. This is not surprising because KRAS mutant cells vary in their dependence on MEK, whereas BRAF mutant tumors exhibit almost uniform sensitivity to MEK inhibition (*9*). A potential explanation for this finding is that KRAS mediates transformation through a number of downstream effectors in addition to ERK. HCT-116 cells also harbor a second activating mutation in *PIK3CA*, the gene encoding the catalytic subunit of phosphoinositide-3-kinase α, and are intrinsically more resistant to MEK inhibition than are KRAS mutant cells with wild-type *PIK3CA* (*10*).

It should be highlighted that the mechanisms reported in these papers as mediating resistance to MEK inhibitors are distinct from those proposed as the basis for

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resistance to the RAF inhibitor PLX4032 (*11*–*13*). This is not totally unexpected because—in contrast to MEK inhibitors, which suppress ERK activity in all cells—RAF inhibitors affect ERK pathway activity in a mutant-specific manner (*14*–*17*). In tumor or normal cells with wild-type BRAF, RAF inhibitors induce ERK signaling by transactivating RAS-dependent RAF dimers (*17*). In cells with mutant BRAF, RAS activity is too low to support adequate RAF dimer formation, and thus RAF inhibitors potently suppress ERK signaling. This mutant-selective inhibition of ERK signaling in BRAF mutant cells by PLX4032 is probably the basis for the broader therapeutic index and thus greater clinical efficacy of RAF inhibitors as compared with MEK inhibitors, which inhibit ERK activity in both tumor and normal cells. Probably because of this property of RAF kinases, the common theme in studies of RAF inhibitor resistance is the identification of perturbations that facilitate the formation of RAF dimers. These include increased abundance of CRAF or RAS activation due to either activating mutation in NRAS (Q61K) or alterations leading to upstream activation of receptor tyrosine kinases, such as PDGFR-β (platelet-derived growth factor receptor–β) and IGF-1R (insulin-like growth factor 1 receptor) (*11*, *12*). These latter perturbations not only abrogate the ability of RAF inhibitors to suppress ERK signaling but also activate parallel signaling pathways that likely diminish the dependence of the tumor cell on MEK and ERK activity.

One motivation for studying mechanisms of resistance is that such studies may suggest rational therapeutic strategies for patients whose tumors exhibit intrinsic or acquired resistance. In the case of amplification of BRAF(V600E) shown by Little *et al*. and Corcoran *et al*., Little *et al*. postulate that concurrent inhibition of RAF may restore sensitivity to the MEK inhibitor by reducing pathway flux to amounts comparable with the drug-sensitive parental cells. Consistent with this hypothesis, partial small interfering RNA–mediated knockdown of BRAF or KRAS in the appropriate resistant cell lines restored sensitivity of the resistant cells to the degree exhibited by the parental cell lines. Furthermore, the combination of inhibitors of RAF and MEK was more effective than either agent alone.

Consistent with these results, new evidence suggests that targeting multiple nodes within the same signaling cascade (for example, RAF and MEK) may not be redundant from a "signaling network" point of view. Work by Sturm *et al*. revealed that three-tiered kinase modules, such as the RAF-MEK-ERK cascade, function as negative feedback amplifiers that confer robustness and stabilize pathway output, properties that can attenuate the effects of selective inhibitors of pathway intermediaries (*18*). The data provide a mathematical basis for the observation that inhibitors of multiple nodes within such feedback loops may cooperate to durably suppress pathway output when used in combination. These results and those of other groups (*19*, *20*), along with the observation by Bollag *at al.* that tumor regression required near complete inhibition of ERK activity (*6*), suggest that combining inhibitors of MEK and RAF may prove to be a more efficacious clinical strategy than the use of either treatment alone. Improved efficacy with the combination may manifest as either an increase in the frequency of complete responses or as a delay in the emergence of drug resistance. Because clones with *BRAF* amplification may preexist at low frequency in a subset of patients, their prospective identification, if clinically feasible, may also be useful in guiding the initial choice of therapy (*21*).

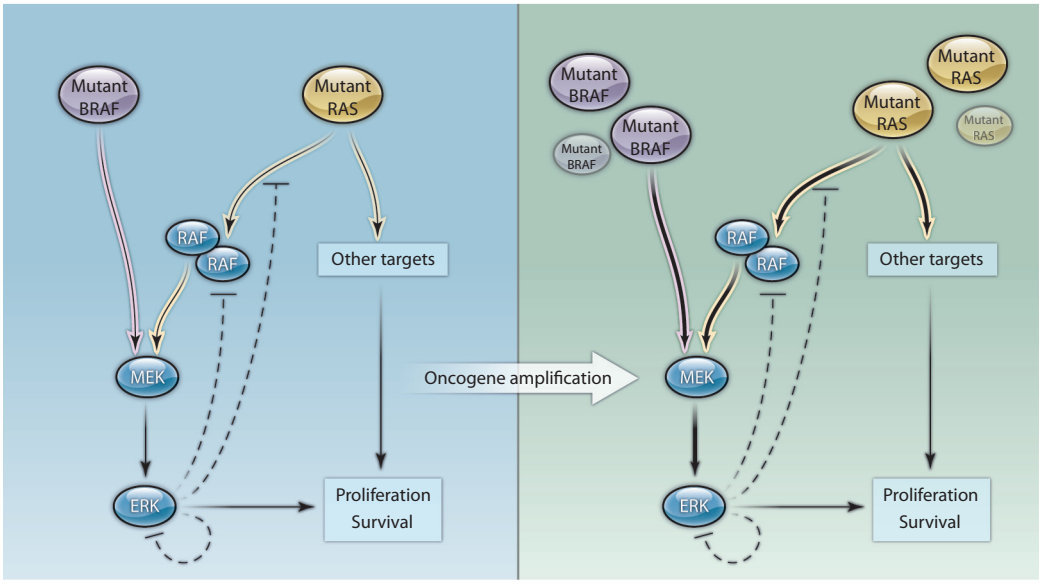


**Fig. 1.** Amplification of the oncogenic driver of ERK pathway activity confers resistance to MEK inhibitors. The RAF-MEK-ERK pathway is a three-tiered kinase cascade with a central role in regulating cell proliferation and survival. In cells with wild-type BRAF, RAS proteins activate the RAF kinases [ARAF, BRAF, and CRAF (RAF1)] in part by facilitating their dimerization. Activated RAF phosphorylates MEK1 and MEK2 (MEK), which in turn phosphorylate ERK1 and ERK2 (ERK). ERK pathway activation in BRAF(V600E) mutant cells is RAS-independent and does not require the formation of RAF dimers. The ERK pathway contains a classical feedback loop in which the abundance of feedback elements, such as Sprouty and dual-specificity phosphatase family proteins, is determined by ERK activity.

Because RAF inhibitors have the property of paradoxically activating ERK signaling in normal cells (all of which have wild-type BRAF), combining inhibitors of RAF and MEK may also have the added benefit of abrogating the normal tissue toxicities observed with the use of each drug alone. The most common toxicity associated with the use of MEK inhibitors is an acneiform rash resulting from inhibition of ERK activity in normal skin (*22*). In contrast, treatment with RAF inhibitors is often associated with the development of a hyperkeratotic skin rash and the induction of keratoacanthomas and squamous cell carcinomas, presumably as a result of increased ERK signaling in normal skin (*1*). Because MEK and RAF inhibitors would be predicted to have antagonistic effects on ERK output in normal tissues, their combined use may result in an attenuation of the toxicities observed with either drug alone.

In summary, Little *et al*. and Corcoran

*et al*. identify amplification of the upstream oncogenic driver of ERK signaling as a mechanism for MEK inhibitor resistance in BRAF or KRAS mutant cells. These studies were performed by using colorectal cancer models; it will thus be interesting to know whether such findings contribute to MEK-inhibitor resistance in metastatic melanoma or other cancer cell lineages. Furthermore, the clinical relevance of these findings in cell lines will require validation in tumor samples from patients treated with MEK inhibitors. For patients with BRAF mutant tumors, the results also provide a mechanistic basis for trials testing concurrent treatment with both RAF and MEK inhibitors. Such trials are currently accruing patients with BRAF mutant metastatic melanoma (clinicaltrials.gov identifiers NCT01072175 and NCT01231594), and the results are eagerly awaited.

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