

IN FOCUS

# Towards a Unified Model of RAF Inhibitor Resistance

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**Summary:** ATP-competitive RAF inhibitors elicit profound but often temporary antitumor responses in patients with *BRAF*-mutant melanoma. Analysis of tumor samples collected at the time of disease progression indicates that alterations within the extracellular signal-regulated kinase (ERK) pathway that result in reactivation of ERK signaling are present in most patients. Mutations in the phosphoinositide 3-kinase/AKT pathway that enhance the adaptive response to RAF inhibitors also contribute to RAF inhibitor resistance in a subset of patients. *Cancer Discov*; 4(1); 27-30. ©2014 AACR.

See related articles by Wagle et al., p. 61 (13), Shi et al., p. 69 (18), Shi et al., p. 80 (7), and Van Allen et al., p. 94 (11).

RAF inhibitors (vemurafenib and dabrafenib) have profound clinical activity in patients with *BRAF*-mutant melanoma, but their therapeutic effects are limited by the emergence of drug resistance. Initial responses to RAF inhibitor therapy are also highly variable and complete remissions are rare. This has spurred extensive laboratory and clinical research efforts to understand the molecular basis of acquisition of resistance to RAF inhibitors. The goals of this work include the development of therapeutic strategies that are useful for treating resistant tumors or, preferably, that delay or prevent the emergence of drug-resistant clones.

The unique feature of selective, ATP-competitive RAF inhibitors is that they inhibit extracellular signal-regulated kinase (ERK) signaling and thus cellular proliferation only in cells that harbor *BRAF* mutations. In other tumor cells, particularly those with RAS activation, these drugs cause a paradoxical activation of ERK signaling that can be associated with accelerated cellular proliferation and cancer progression. Physiologic activation of RAS causes the dimerization and activation of RAF family protein kinases. Binding of the RAF inhibitor to one member of the RAF dimer causes the allosteric transactivation of the other, unbound RAF protein (1). In contrast, *BRAF*<sup>V600E</sup> signals as a monomer that is inhibited by drug binding (2).

The mechanism of action of RAF inhibitors suggests that molecular lesions that cause *BRAF*<sup>V600E</sup> to dimerize in tumor cells would cause resistance to vemurafenib. Laboratory and clinical studies now support this model. Expression of an alternatively spliced variant of *BRAF*<sup>V600E</sup> that constitutively dimerizes causes resistance to RAF inhibitors in a model system and has been shown to be expressed in a significant fraction of melanomas from patients with acquired resistance (2). Moreover, mutations in *NRAS*, loss of *NF1* function, and activation of upstream receptor tyrosine kinases, lesions expected to induce RAS-dependent RAF dimerization, have

all been shown to confer acquired resistance to RAF inhibitors in model systems, patients, or both (2-4). Additional molecular events, particularly amplification of *BRAF*<sup>V600E</sup> and mutations in the *MAP2K1* gene, which encodes MEK1, have also been shown to occur in tumors with acquired resistance and functionally validated as causal (5, 6).

The common feature of each of these mechanisms of resistance is that they result in activation of ERK signaling that is insensitive to the RAF inhibitor. Thus, RAF inhibitor resistance is often associated with maintenance of activation of the oncogene-driven pathway. Previously only a small number of samples had been analyzed for each of the above resistance lesions, and thus their prevalence and whether they coexist with other lesions that affect the phenotype was unknown. Two studies in the current issue of *Cancer Discovery* provide further data as to the prevalence of RAF inhibitor resistance mechanisms and identify novel resistance mechanisms, several of which are consistent with prior laboratory studies.

In the study by Shi and colleagues (7), the investigators analyzed 100 tumor samples collected from 44 patients using targeted methods to identify known mechanisms of RAF inhibitor resistance and, in parallel, unbiased whole exome sequencing (WES), to attempt to identify novel resistance mechanisms. In their cohort, approximately 60% of disease progression biopsies harbored mutations within the mitogen-activated protein kinase (MAPK) pathway identified in prior work as responsible for RAF inhibitor resistance. These included *NRAS* mutations (18%), *BRAF* amplification (19%), *BRAF* splice variants (13%), and *MAP2K1* mutations (3%). *CDKN2A* deletion was observed in 7% of biopsies, and was also grouped by the investigators with the ERK pathway alterations, as cyclin D expression is dependent on the ERK pathway in *BRAF*-mutant cells (8). However, it is not clear that loss of the *CDKN2A* product p16<sup>INK4a</sup> is sufficient to cause resistance, as, in such tumors, cyclin D1 is still ERK dependent and *CDKN2A*-null models often retain sensitivity to RAF and MAP-ERK kinase (MEK) inhibitors (8, 9).

Consistent with prior preclinical studies, mutations in the phosphoinositide 3-kinase (PI3K)-PTEN-AKT pathway were also identified by WES as potential mediators of RAF inhibitor resistance (9). Mutations in *AKT1/AKT3* (Q79K and E17K) and putative functional alterations in positive regulators of the pathway (*PIK3CA*, *PIK3CG*) and in negative regulatory genes

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(*PIK3R2*, *PTEN*, and *PHLPP1*) were detected in 22% of resistant samples. The functional consequences of these latter lesions and their role in conferring resistance have in most cases not yet been fully defined and require further laboratory study.

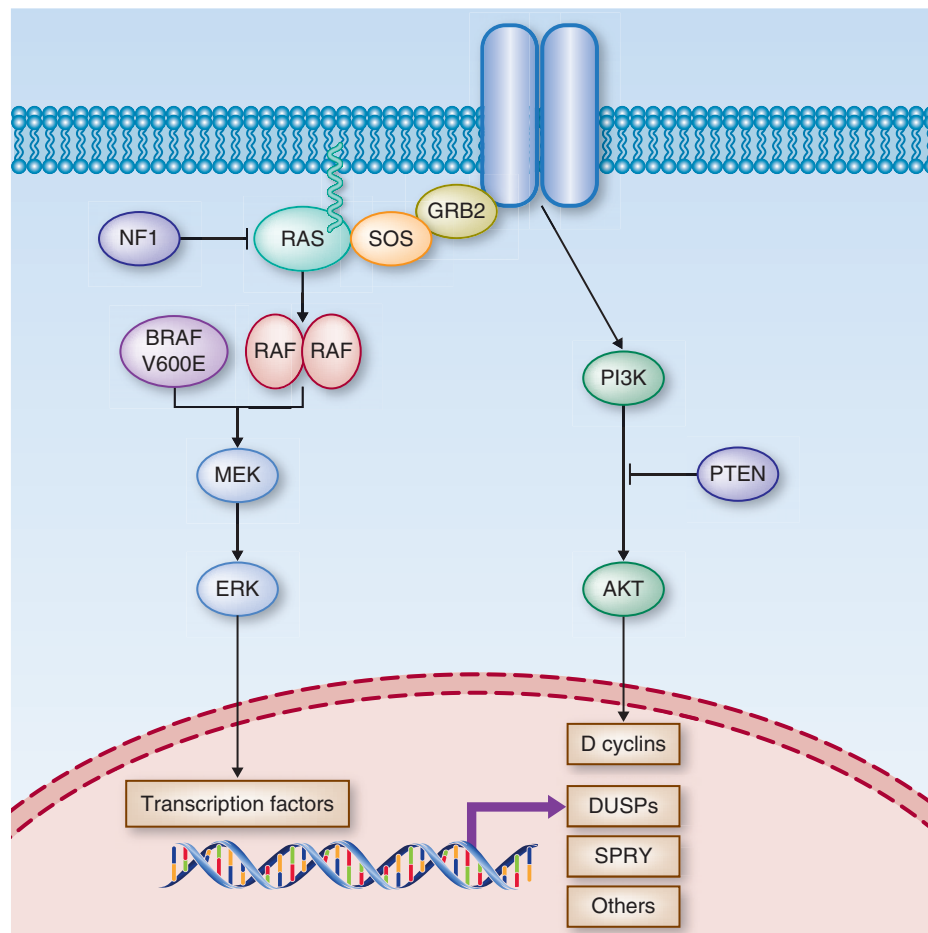
In the study by Shi and colleagues (7), multiple biopsies were obtained at different times or from disparate locations from several patients, and more than a single lesion in the ERK pathway was identified in multiple patients typically within different tumor biopsies. A detailed phylogenetic analysis of multiple progressive lesions from a subset of these patients suggested branching evolution of tumors in which the development of genetic diversity was not linearly associated with time. In one patient, for whom nine samples from progressing tumors were obtained over 726 days of BRAF inhibitor therapy, five distinct drivers of acquired BRAF inhibitor resistance were detected, including *KRAS* mutation, *BRAF* alternative splicing, and *BRAF* amplification. These results are consistent with a prior case report, which suggested that distinct mechanisms of BRAF inhibitor resistance were present in two different progressing lesions from a single patient (10). This finding of heterogeneity of resistance drivers within individual patients suggests that biopsy of individual lesions may have limited utility in guiding subsequent therapy selection. Novel methods for the analysis of circulating tumor cells or tumor-derived DNA in plasma may provide a better picture of the spectrum of resistance mechanisms within individual patients and may allow for the detection of resistant clones and therapy modification before evidence of clinical progression.

In the second study by Van Allen and colleagues (11), WES was performed on paired pretreatment and progression samples collected from 45 patients, of whom 14 developed resistance soon after initiation of therapy (within 12 weeks). They also detected several resistance mechanisms that had been previously identified to confer RAF inhibitor resistance, including mutations in *NRAS*, *MAP2K1*, and *NF1* and *BRAF* amplification. They did not seek to assess the expression of alternatively spliced *BRAF*<sup>V600E</sup> isoforms. Mutations in the *MAP2K2* gene (which encodes the MEK2 kinase) were also identified in four patients, and focal amplification of *MITF*, a master lineage transcription factor, was identified in one. *In vitro* studies confirmed that the resistance mutations in *MAP2K1* and *MAP2K2* detected exclusively in the resistant samples were associated with resistance to both RAF and MEK inhibitors, albeit to varying degrees. Expression of these resistance alleles did not, however, confer resistance to a selective ERK inhibitor. In contrast, forced overexpression of *MITF* was sufficient to induce resistance to the BRAF, MEK, and ERK inhibitors studied. Furthermore, consistent with the results of Shi and colleagues (7), multiple resistance alterations within the MAPK pathway were found to co-occur in several patients for whom multiple disease progression samples were available.

In sum, the data support prior studies of BRAF inhibitor resistance in which alterations that cause reactivation of ERK signaling were found to occur in most patients. These studies provide support for the testing of RAF and MEK inhibitors in combination in patients with *BRAF*-mutant melanoma. Early results from a randomized phase II trial comparing combined RAF and MEK inhibition to RAF inhibitor monotherapy suggest a delay in median time to progression with the com-

bination (9.4 months with the combination of RAF and MEK inhibitors versus 5.8 months with RAF monotherapy; ref. 12). In contrast, MEK inhibitors have shown little activity if initiated following disease progression on RAF inhibitors. A third study by Wagle and colleagues (13) in this issue of *Cancer Discovery* reports on the first in depth study of tumor samples collected from patients treated with the RAF and MEK inhibitor combination. Consistent with the preclinical studies highlighted above demonstrating that *MEK1* and *MEK2* mutations can confer RAF and MEK inhibitor resistance, a *MEK2*<sup>Q60P</sup> mutation was identified in 1 of 5 patients studied. Of greater surprise to the investigators, one patient had a *BRAF* splice variant lacking exons 2-10 and a second patient had *BRAF* amplification. It should be noted that amplification of *BRAF* has previously been identified as a mechanism of MEK inhibitor resistance in cells with mutant BRAF (14). One hypothesis to explain this result is that increased abundance of the oncogenic driver (in this case BRAF) in response to prolonged drug treatment results in increased flux through the ERK pathway and restoration of ERK activity above the threshold required for inhibition of cell proliferation. As the antitumor activity of RAF inhibitors requires near-complete inhibition of ERK activation, minimal reactivation of ERK signaling may be sufficient for clinical progression (15). A similar mechanism of increased basal ERK activation in cells expressing RAF splice variants may also explain the selection for this alteration in patients treated with the RAF and MEK inhibitor combination. These early results from Wagle and colleagues (13) suggest that clinical strategies that achieve more maximal inhibition of ERK signaling may have greater clinical efficacy. These include novel RAF and MEK inhibitors with alternative mechanisms of kinase inhibition or greater potency and the use of intermittent treatment schedules (16). Alternatively, combined RAF and ERK inhibitor therapy may result in more durable treatment responses or may simply result in the selection of alterations downstream of ERK such as RB1 loss with similar kinetics. Finally, it should be noted that early trials of combined inhibition of RAF and MEK did not escalate the doses of each drug beyond their recommended single-agent doses. As the combination of a RAF inhibitor (which activates ERK in normal cells) and a MEK inhibitor (which inhibits ERK in normal cells) has less ERK-dependent toxicities than the use of either drug alone, further dose escalation of these agents may be achievable and may result in greater antitumor effects by inducing more maximal ERK pathway inhibition in *BRAF*-mutant tumors.

Finally, recent laboratory studies have shown that in *BRAF*-mutant tumors, high levels of ERK-dependent negative feedback potently suppress ligand-dependent mitogenic signaling (17). In the context of *BRAF*-mutant cells, relief of this ERK-dependent feedback results in the rapid adaptation of the cancer cell that attenuates the antitumor effects of the RAF inhibitor. One consequence of this adaptive response is RAS activation, the formation of RAF dimers, and a partial rebound in ERK activation (Fig. 1). The term “adaptive resistance” has been proposed to describe this blunting of the therapeutic response caused by rapid, adaptive changes to the signaling network following treatment with selective inhibitors of oncoprotein-activated pathways. In a fourth study by Shi and colleagues (18) in this issue of *Cancer Discovery*, the investigators demonstrate



**Figure 1.** Mechanisms of resistance to RAF inhibitors. Before exposure to RAF inhibitors, *BRAF*<sup>V600E</sup>-mutant tumors have hyperactivated ERK signaling. This results in negative-feedback suppression of upstream receptor tyrosine kinase signaling. In this state, expression of active RAS-GTP is low and *BRAF*<sup>V600E</sup> signals as a monomer, which is sensitive to RAF inhibitors. Upon treatment with vemurafenib, ERK-dependent negative feedback is rapidly downregulated, resulting in the restoration of receptor signaling, RAS activation, induction of RAF dimers, and activation of parallel signaling pathways, including the PI3K-AKT pathway. Activation of PI3K/AKT, which coregulates the expression of some downstream effectors of ERK transformation including D cyclins, results in diminished dependence of RAF signaling. This rapid adaptive response to RAF inhibition attenuates the antitumor effects of RAF inhibitors and thus may promote the selection of subclones harboring coalterations that confer higher levels of RAF inhibitor resistance. These molecular alterations include *NRAS* and *KRAS* mutations, loss of *NF1* function, aberrantly spliced *BRAF* variants that lack the RAS binding domain, *BRAF* amplification, and mutations in *MEK1* and *MEK2*, among others.

that *BRAF* (and *BRAF* + *MEK*) inhibitor therapy results in an increase in *PI3K/AKT* signaling that is associated with enhanced clonogenic growth dependence on *PI3K* and *AKT*. This adaptive response was attenuated by *PTEN* expression but could be rescued by the introduction of a mutant *AKT*, in this case *AKT1*<sup>Q79K</sup>, a resistance allele identified in their study of disease progression samples (7). Taken in sum, the results suggest that the early adaptive response of *BRAF*-mutant cells to *ERK* pathway inhibition may promote the selection of resistant clones that harbor additional genomic events that confer higher levels of *RAF* inhibitor resistance. The data also support combinatorial approaches that attenuate the adaptive response, including the addition of a *PI3K* or *AKT* inhibitor to the *RAF* and *MEK* (or *ERK*) inhibitor combination. As previous attempts to combine *MAPK* and *PI3K* pathway inhibitors have been limited by overlapping toxicities, upfront testing of intermittent treatment schedules should be considered. As cyclin D1 expression is coregulated by both *ERK* and *PI3K/AKT*

activation, evaluation of a *CDK4* inhibitor in combination with the *RAF/MEK* inhibitor combination should also be pursued. In summary, the four studies reviewed above add significant new insights to our understanding of resistance to *RAF* inhibitor therapy and provide support for rational combinatorial treatment approaches that delay or prevent the emergence of drug-resistant clones.

**Disclosure of Potential Conflicts of Interest**

D.B. Solit is a consultant/advisory board member of Pfizer. No potential conflicts of interest were disclosed by the other author.

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**REFERENCES**

1. Poulidakos PI, Zhang C, Bollag G, Shokat KM, Rosen N. *RAF* inhibitors transactivate *RAF* dimers and *ERK* signalling in cells with wild-type *BRAF*. *Nature* 2010;464:427-30.

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2. Poulidakos PI, Persaud Y, Janakiraman M, Kong X, Ng C, Moriceau G, et al. RAF inhibitor resistance is mediated by dimerization of aberrantly spliced BRAF(V600E). *Nature* 2011;480:387–90.
3. Nazarian R, Shi H, Wang Q, Kong X, Koya RC, Lee H, et al. Melanomas acquire resistance to B-RAF(V600E) inhibition by RTK or N-RAS upregulation. *Nature* 2010;468:973–7.
4. Whittaker SR, Theurillat JP, Van Allen E, Wagle N, Hsiao J, Cowley GS, et al. A genome-scale RNA interference screen implicates NF1 loss in resistance to RAF inhibition. *Cancer Discov* 2013;3:350–62.
5. Shi H, Moriceau G, Kong X, Lee MK, Lee H, Koya RC, et al. Melanoma whole-exome sequencing identifies (V600E)B-RAF amplification-mediated acquired B-RAF inhibitor resistance. *Nat Commun* 2012;3:724.
6. Wagle N, Emery C, Berger MF, Davis MJ, Sawyer A, Pochanard P, et al. Dissecting therapeutic resistance to RAF inhibition in melanoma by tumor genomic profiling. *J Clin Oncol* 2011;29:3085–96.
7. Shi H, Hugo W, Kong X, Hong A, Koya RC, Moriceau G, et al. Acquired resistance and clonal evolution in melanoma during BRAF inhibitor therapy. *Cancer Discov* 2014;4:80–93.
8. Solit DB, Garraway LA, Pratilas CA, Sawai A, Getz G, Basso A, et al. BRAF mutation predicts sensitivity to MEK inhibition. *Nature* 2006;439:358–62.
9. Xing F, Persaud Y, Pratilas CA, Taylor BS, Janakiraman M, She QB, et al. Concurrent loss of the PTEN and RB1 tumor suppressors attenuates RAF dependence in melanomas harboring (V600E)BRAF. *Oncogene* 2012;31:446–57.
10. Romano E, Pradervand S, Paillusson A, Weber J, Harshman K, Muehlethaler K, et al. Identification of multiple mechanisms of resistance to vemurafenib in a patient with BRAFV600E-mutated cutaneous melanoma successfully rechallenged after progression. *Clin Cancer Res* 2013;19:5749–57.
11. Van Allen EM, Wagle N, Sucker A, Treacy DJ, Johannessen CM, Goetz EM, et al. The genetic landscape of clinical resistance to RAF inhibition in metastatic melanoma. *Cancer Discov* 2014;4:94–109.
12. Flaherty KT, Infante JR, Daud A, Gonzalez R, Kefford RF, Sosman J, et al. Combined BRAF and MEK inhibition in melanoma with BRAF V600 mutations. *N Engl J Med* 2012;367:1694–703.
13. Wagle N, Van Allen EM, Treacy DJ, Frederick DT, Cooper ZA, Taylor-Weiner A, et al. MAP kinase pathway alterations in BRAF-mutant melanoma patients with acquired resistance to combined RAF/MEK inhibition. *Cancer Discov* 2014;4:61–8.
14. Corcoran RB, Dias-Santagata D, Bergethon K, Iafrate AJ, Settleman J, Engelman JA. BRAF gene amplification can promote acquired resistance to MEK inhibitors in cancer cells harboring the BRAF V600E mutation. *Sci Signal* 2010;3:ra84.
15. Bollag G, Hirth P, Tsai J, Zhang J, Ibrahim PN, Cho H, et al. Clinical efficacy of a RAF inhibitor needs broad target blockade in BRAF-mutant melanoma. *Nature* 2010;467:596–9.
16. Das Thakur M, Salangsang F, Landman AS, Sellers WR, Pryer NK, Levesque MP, et al. Modelling vemurafenib resistance in melanoma reveals a strategy to forestall drug resistance. *Nature* 2013;494:251–5.
17. Lito P, Pratilas CA, Joseph EW, Tadi M, Halilovic E, Zubrowski M, et al. Relief of profound feedback inhibition of mitogenic signaling by RAF inhibitors attenuates their activity in BRAFV600E melanomas. *Cancer Cell* 2012;22:668–82.
18. Shi H, Hong A, Kong X, Koya RC, Song C, Moriceau G, et al. A Novel AKT1 mutant amplifies an adaptive melanoma response to BRAF inhibition. *Cancer Discov* 2014;4:69–79.