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Making Sense of *MEK1* Mutations in Intrinsic and Acquired BRAF Inhibitor Resistance

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Summary

In this issue of *Cancer Discovery*, Shi and colleagues add further insight into the role of exon 3 *MEK1* mutations in BRAF inhibitor resistance by demonstrating the presence of ^{P124S}*MEK1* and ^{I111S}*MEK1* mutations concurrently with $^{V600E/K}$*BRAF* mutations at baseline in 16% of melanoma specimens. Although the presence of ^{P124S}*MEK1* or ^{I111S}*MEK1* mutations did not predict for resistance, and these alleles were not selected for upon BRAF inhibition, other exon 3 *MEK1* mutations, such as C121S, did convey resistance, suggesting a role for defined exon 3 *MEK1* mutations in acquired BRAF inhibitor resistance.

The discovery that 50% of all cutaneous melanomas harbor activating mutations in the serine/threonine kinase *BRAF* has revolutionized expectations for melanoma therapy. In the recent phase III randomized clinical trial, 53% of patients selected on the basis of their melanomas harboring a position-600 mutation showed good levels of response to the small-molecule BRAF inhibitor vemurafenib [Zelboraf, PLX4032; Genentech (1)]. Despite these promising results, and the unprecedented hope this offers to patients with melanoma, significant hurdles remain. We do not yet fully understand why nearly one half of all patients with *BRAF*-mutant melanoma do not show a response to BRAF inhibitors, as measured by Response Evaluation Criteria in Solid Tumors (RECIST) criteria. Among the patients with *BRAF*-mutant melanoma whose tumors do respond well to treatment initially, most responses tend to be short-lived, with resistance emerging in nearly every case. Updated data from the phase II clinical trial of vemurafenib shows an average progression-free survival of

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No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: K.S.M. Smalley

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7 months, with an overall survival of 16 months. It is clear that both intrinsic and acquired resistance to BRAF inhibitors is common and efforts are underway to identify these resistance mechanisms and to develop mitigation strategies.

A large number of potential BRAF inhibitor resistance mechanisms have now been described. The identified mechanisms differ from those implicated in the acquired resistance to other targeted therapy agents, such as imatinib in chronic myeloid leukemia and erlotinib in non–small cell lung cancer, in that they do not involve secondary kinase mutations at the "gatekeeper" site. Instead, a diverse array of acquired resistance mechanisms have been reported, including mutations in *NRAS*, upregulated expression of a number of receptor tyrosine kinases (IGF1R, PDGFR-β), truncations of *BRAF*, amplification of *BRAF*, and increased expression of kinases known to activate the mitogen-activated protein kinase (MAPK) signaling pathway, particularly COT and CRAF [Fig. 1 (2)].

Melanomas are well known to be "addicted" to signaling through the Ras/Raf/MEK/ERK MAPK pathway, with the majority of melanoma driver oncogenes reported thus far known to activate this signal transduction cascade. Even among those melanomas lacking the obvious MAPK pathway activators such as oncogenic *BRAF* and *NRAS*, constitutive levels of MEK/ERK signaling are still required. It therefore comes as little surprise that melanomas possess multiple means to reactivate MAPK signaling and that many of the acquired BRAF inhibitor resistance mechanisms reported so far, including most of those that have been convincingly validated in clinical specimens, are MAPK dependent. The fact that near total MAPK pathway blockade is required for BRAF inhibitor efficacy in patients with melanoma suggests that even modest increases in the level of signaling through the Ras/Raf/MEK/ERK pathway are sufficient to convey drug resistance, and there is now experimental evidence to support this contention (3).

In this issue of *Cancer Discovery,* Shi and colleagues (4) present new data exploring the potential role of exon 3 *MEK1* mutations in intrinsic and acquired BRAF inhibitor resistance. This work builds on a previous study in which investigators implicate the role of an acquired exon 3 ^{C121S}*MEK1* mutation in one patient with melanoma who did not respond to vemurafenib therapy (5). Using a series of matched (pre- and posttreatment) samples from 31 patients treated with either vemurafenib or dabrafenib (GSK2118436), the authors performed whole-exome sequencing and demonstrated that 5 of 31 specimens harbored either ^{P124S}*MEK1* or ^{I111S}*MEK1* mutations concurrently with $^{V600E/K}$*BRAF* mutations. These mutations were somatic, and no equivalent mutations in MEK2 were noted in the pretreatment samples. Unexpectedly, failure of BRAF inhibitor therapy did not select for exon 3 *MEK1* mutations, and the only patients with *MEK1* mutations in their melanomas upon disease progression already harbored these at baseline.

Of clinical significance, the presence of either the ^{P124S}*MEK1* or ^{I111S}*MEK1* mutations was not predictive of up-front resistance to BRAF inhibitor therapy, with 4 of the 5 patients with concurrent *BRAF* and *MEK1* mutations displaying objective responses in the tumors biopsied and 3 of these patients ultimately achieving an overall partial response (as measured by RECIST criteria). Because these exon 3 *MEK1* mutations did not appear to predict for intrinsic BRAF inhibitor resistance, the authors next performed *in vitro* studies

and observed that lentiviral-mediated introduction of either wild-type *MEK1*, ^{P124S}*MEK1*, or ^{I111S}*MEK1* did not increase activation of extracellular signal-regulated kinase (ERK) in melanoma cells that were $^{V600E/K}$*BRAF* mutant. In contrast, introduction of the ^{P124S}*MEK1* mutant into human cells (HEK293) lacking oncogenic *BRAF* increased phospho-ERK signaling, suggesting that the oncogenic *BRAF* in melanoma cells was dominant over the 2 exon 3 *MEK1* mutants with regard to ERK activation. Although it is not yet clear why mutant *BRAF* would be more effective at activating ERK than ^{P124S}*MEK1* or ^{I111S}*MEK1*, melanoma cells are known to have impaired feedback inhibition in the Ras/Raf/MEK/ERK signaling pathway, suggesting that inhibition/activation at different levels of the signaling cascade may not be equivalent (6).

Consistent with the identified *MEK1* mutants having little influence upon phospho-ERK levels, further studies by Shi and colleagues (4) showed neither of the *MEK1* mutants to convey vemurafenib resistance in either growth or clonogenic assays. Not all exon 3 *MEK1* mutants identified from patients with melanoma were similarly inactive with regards to vemurafenib resistance. In agreement with previously published work, Shi and colleagues (4) provided *in vitro* data demonstrating the ability of the ^{C121S}*MEK1* mutation to restore levels of phospho-ERK signaling in the face of BRAF inhibition leading to a decrease in the sensitivity to vemurafenib (5). It therefore seems that although some mutations in *MEK1* may play a role in BRAF inhibitor resistance, the baseline presence of a MEK1 mutation is not necessarily predictive of a diminished drug response. Further study of a larger series of pre- and posttreatment biopsies is therefore required to determine the spectrum of *MEK1* mutations that have utility as predictive biomarkers.

The evolving experience of targeted therapy in melanoma and other tumors shows single-agent inhibitor strategies to be largely ineffective at delivering durable antitumor responses. Although combination therapy strategies may yet prove to be curative in genetically defined cases, much is still to be done in terms of developing combination therapies and matching these with tumor genotypes. In line with the observation that restoration of MAPK signaling mediates resistance to vemurafenib, Shi and colleagues (4) demonstrated that “vertical inhibition” of the MAPK pathway through combined vemurafenib and MEK inhibitor (AZD6244) treatment led to a strong degree of synergy in melanoma cell lines with ^{V600E}*BRAF* mutations. This observation, which corroborates earlier studies in which the authors demonstrated that combined BRAF and MEK inhibition delays the onset of BRAF inhibitor resistance, is currently the subject of an eagerly awaited phase II clinical trial [dabrafenib plus trametinib (GSK1120212); ref.¤7, 8]. The combination of a BRAF plus a MEK inhibitor is likely to prove particularly beneficial in preventing the paradoxical MAPK signaling-driven development of squamous tumors that often occur in patients with melanoma receiving BRAF inhibitor therapy (9).

Reactivation of MAPK signaling is not the only mechanism used by melanoma cells to escape from BRAF inhibition, and it has been suggested that resistance may also be managed through the dual targeting of mutant *BRAF* and components of the PI3K/AKT/mTOR pathway (2, 3). At this juncture, we do not have good biomarkers for segregating ^{V600E}*BRAF* mutant melanomas into those that would benefit from the BRAF and MEK inhibitor combination versus those that would respond better to the BRAF and

PI3K/AKT inhibitor combination. Ultimately, however, these issues may be difficult to resolve. There is a growing realization that tumors are much more heterogeneous than previously suspected, and it is expected that this will complicate our efforts to develop patient-specific combination therapies. A recent whole-exome sequencing analysis of multiple specimens taken from the same patient with renal cell carcinoma highlighted the nature of intratumoral diversity and showed that two thirds of the mutations found in one specimen were not detected in other tumor biopsies from the same patient (10). Most remarkably of all, a favorable prognosis gene signature and an unfavorable prognosis gene signature were found in different regions of the same tumor nodule (10). With this in mind, it is likely that multiple BRAF inhibitor resistance mechanisms could coexist within the same patient's melanoma and require different management strategies. There is already evidence from the whole-genome sequencing of cell lines that melanoma is a tumor with a very high mutational load (11). This mutational complexity is likely to drive the high levels of intratumoral heterogeneity already known to exist in melanoma and lead to the development of evolutionarily diverse tumors within the same patient. Understanding this heterogeneity and developing strategies to manage multiple coexistent therapeutic escape routes will prove critical in our efforts to develop combination therapies that deliver durable responses to patients with disseminated melanoma.

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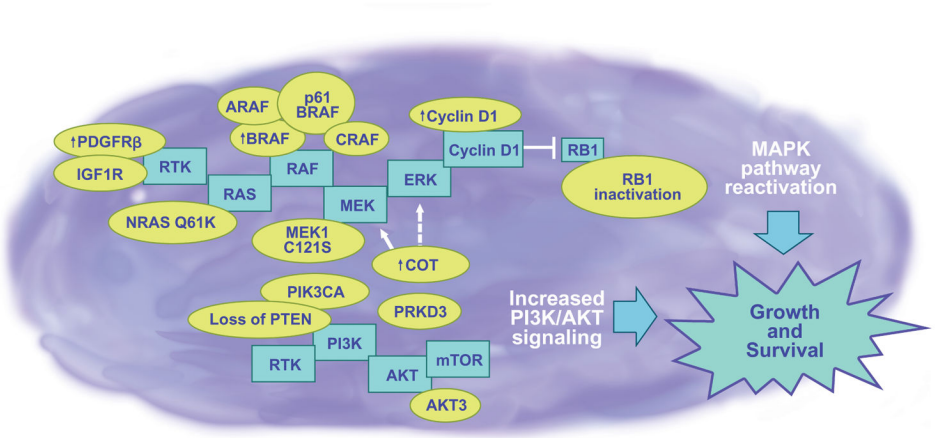

Figure 1.

Various mechanisms (shown in yellow balloons) have been defined that can lead to BRAF inhibitor resistance in baseline and disease-progressed BRAF-mutated melanomas and include PDGFRβ and IGF1R receptor tyrosine kinase signaling, secondary *NRAS* mutations, ^{V600E}*BRAF* amplification, ^{V600E}*BRAF* p61 splice variant, RAF isoform signal switching, ^{C121S}*MEK1* mutation, COT amplification, increased AKT activity, loss of PTEN, PRKD3, amplified cyclin D1, and RB1 inactivation. The central theme of BRAF inhibitor escape is the reactivation of MAP kinase and increased PI3K/AKT/mTOR signaling (skeleton pathways shown in blue boxes), which leads to melanoma growth and survival. As a further complication to the incredibly diverse resistance landscape, it has been demonstrated that multiple mutations, and possibly others that have yet to be identified, can occur within the same melanoma tumor or cell line, resulting in intratumor heterogeneity. It is expected that the complexity of mutations that can occur within one tumor will ultimately redirect our strategies towards individualized therapy.