Mechanisms of Resistance to RAF Inhibition in Melanomas Harboring a BRAF Mutation

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OVERVIEW

Treatment of V600E/K BRAF-mutated melanomas with RAF inhibitors (either vemurafenib or dabrafenib) results in rapid and dramatic responses in most patients – results that are associated with improved progression-free survival (PFS) and in the case of vemurafenib, overall survival (OS). However, resistance develops at a median time of approximately 6 months. Understanding the mechanisms of resistance is critical to develop strategies to prolong PFS and OS. Negative feedback mechanisms inherent in the MAPK pathway serve to modulate responses to these drugs. However, genetic changes develop within the tumor, which lead to reactivation of the MAPK and resistance to these drugs. The mechanisms that have been demonstrated in many patients by multiple investigators are (1) development of an activating mutation in NRAS, and (2) appearance of a BRAFV600E splice variant that encourages RAF dimerization. Several other mechanisms of resistance have also been described in individual patients or in preclinical models of resistance. In addition, there is evidence that activation of parallel pathways, such as the PI3K/AKT pathway, may represent another mechanism of resistance. Understanding the various mechanisms of resistance will inform our attempts to prevent resistance to RAF inhibitors.

Forty percent to 60% of melanomas harbor a driver mutation in BRAF, most commonly V600E or K. This leads to constitutive activation of the MAPK pathway and increased activation of ERK, which drives proliferation of the melanoma. Inhibitors of RAF kinases, such as vemurafenib or dabrafenib, effectively shut down ERK activation and lead to rapid tumor shrinkage in the majority of cases. Randomized trials have shown that both of these drugs improve PFS compared with dacarbazine chemotherapy; vemurafenib has also been shown to improve OS. Both of these drugs show remarkably similar response rates and improvements in PFS. New RAF inhibitors are in development, such as LGX818, and would be expected to have similar clinical efficacy.

Although 80% of patients with BRAF-mutated melanoma show some degree of tumor shrinkage when treated with a RAF inhibitor, and approximately 50% of patients achieve a formal partial response, clinical trials with both vemurafenib and dabrafenib show that most tumors develop resistance within 6 to 7 months, and approximately 10% are primarily refractory. Therefore, it is critical to understand the mechanisms of resistance to RAF inhibitors. I will review the current state of understanding of these mechanisms. It should be noted that many investigators are working on this problem, and the state of knowledge is currently in flux. Currently, there are some mechanisms that have been confirmed by multiple laboratories, some mechanisms that have been reported by individual investigators but not yet confirmed by others, and some mechanisms that are speculative.

MECHANISMS THAT REACTIVATE THE MAPK PATHWAY

Confirmed Mechanisms of Resistance

Almost all the mechanisms described so far lead to the reactivation of the MAPK pathway. One mechanism that is active in all tumors and which leads to modulation of the antitumor effect by RAF inhibitors is the inherent negative feedback mechanisms of the MAPK pathway. Inhibition by vemurafenib leads to decreased activation of ERK. This decreases the level of negative regulators such as Sprouty proteins and relieves the suppressive effects on RAS activation. With increased RAS activation, RAF kinases dimerize which allows vemurafenib to induce trans-activation of RAF. This leads to reactivation of MEK and then ERK. The final result is that, after a period of maximal suppression of ERK, the pathway regulates itself to maintain a low level of ERK activation. This can be seen most readily in vitro experiments. This observation probably explains why most clinical responses are partial responses.

Several other resistance mechanisms have been described that result from genetic changes in the melanoma (Table 1).

NRAS mutation. It is clear from the observations of several investigators that melanomas treated with vemurafenib can
acquire an activating mutation in NRAS. This leads to activation of the pathway. It would also be expected that this would lead to enhanced dimerization of RAF and further ERK activation. Whether the NRAS mutation preexists in the tumor or arises under the pressure of vemurafenib treatment remains unclear.

**BRAFV600E splice variant.** Another resistance mechanism is the development of a splice variant of the mutated BRAF mRNA. This splice variant results in a truncated form of the mutated BRAF kinase in which interaction with RAS is enhanced. This leads to dimer formation between the truncated, activated BRAF kinase and wild type RAF kinases. Once dimerized, vemurafenib induces trans-activation and subsequent reactivation of the MAPK pathway.

**Other Resistance Mechanisms of Uncertain Frequency**

There are a variety of other resistance mechanisms that have been identified in individual patients. There are others that have been described using in vitro and mouse models. The frequency by which these mechanisms cause resistance in patients remains uncertain.

**Activation through RTKS.** Melanoma is known to express a large variety of RTKs that signal through the MAPK pathway.

**MECHANISMS OF RESISTANCE TO RAF INHIBITORS**

**TABLE 1. Mechanisms of Resistance to RAF Inhibition in V600 BRAF-Mutated Melanoma**

<table>
<thead>
<tr>
<th>Mechanism</th>
<th>Clinical Evidence</th>
<th>Other Evidence</th>
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<tr>
<td>NRAS mutation</td>
<td>Observed in resistant melanomas</td>
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<tr>
<td>Splice variant of BRAFV600E mRNA</td>
<td>Observed in resistant melanomas</td>
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<td>Activation of RTKs</td>
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<td>MET</td>
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<tr>
<td>IGF-1R</td>
<td>↑ expression has been seen in resistant tumors</td>
<td>In vitro models</td>
</tr>
<tr>
<td>PDGFRβ</td>
<td></td>
<td>In vitro models</td>
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<tr>
<td>NF1 loss</td>
<td>May be associated with short PFS</td>
<td>In vitro models</td>
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<tr>
<td>↑ Transcription of BRAFV600E mRNA</td>
<td>↑ copy number of BRAFV600E allele observed in some resistant tumors</td>
<td>In vitro models</td>
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<tr>
<td>↑ COT expression</td>
<td>↑ COT transcription observed in two cases of resistant melanomas</td>
<td>In vitro models</td>
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<tr>
<td>MEK1 mutation</td>
<td>Observed in one case of resistant melanoma</td>
<td>In vitro models</td>
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</tbody>
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**KEY POINTS**

- Most BRAF-mutated melanomas respond to vemurafenib but develop resistance.
- Feedback mechanisms in the MAPK pathway make complete responses unusual.
- There are multiple possible mechanisms of resistance, all of which so far involve reactivation of the MAPK pathway.
- NRAS mutations and splice variants of BRAFV600E mRNA are the two most common mechanisms identified so far.
- Concomitant strategies to inhibit the PI3K pathway may be required to prevent resistance.

Activation of these would be expected to lead to resistance. Several investigators have conducted kinase screens that point to MET activation as a potential resistance mechanism. Activation of other RTKs have been proposed as potential resistance mechanisms, including IGF-1R and PDGFRβ. Supporting data from clinical samples are not yet available.

**Increased expression of mutated BRAF kinase.** By transferring human melanomas to immunosuppressed mice and treating with vemurafenib, investigators developed resistant melanoma clones with enhanced transcription and translation of the mutated BRAF kinase. These melanoma cells appeared to be addicted to the BRAF mutation in that withdrawing vemurafenib led to decreased tumor growth. In four of 20 vemurafenib-resistant BRAFV600E-mutated melanomas, Lo et al. found an increased copy number of the BRAFV600E allele.

**Increased expression of COT.** COT (encoded by the MAP3K8 gene) activates ERK through a MEK-dependent mechanism. In a screen that overexpressed a library of kinase overexpression in a BRAFV600E melanoma, investigators found that overexpression of COT led to vemurafenib resistance. It has been difficult to determine how frequently this mechanism occurs in patients treated with vemurafenib, although the investigators were able to show that in two of three resistant tumors tested, increased transcription of MAP3K8 developed in vemurafenib-resistant melanomas.

**MAP2K1 mutations.** Mutations in MAP2K1 (encoding MEK1) are detected in some BRAF-mutated melanomas and would be expected to lead to vemurafenib resistance. An in vitro mutagenesis screen demonstrated that P124L and Q56P mutations in MAP2K1 led to resistance to an analog of vemurafenib. Clinically, a patient has been described in which a MAP2K1 mutation was identified in a resistant metastasis. This was an activating mutation and was associated with resistance to vemurafenib.

**NF1 loss.** NF1 inhibits RAS activation. Loss of NF1 would be predicted to result in resistance to vemurafenib. A recent
analysis of melanoma exon sequencing showed 16/121 melanomas harbored a \textit{NF1} missense or nonsense mutation.\textsuperscript{11} This analysis, and another recent data set,\textsuperscript{14} showed that \textit{NF1} mutations often occur in \textit{BRAF}-mutated melanomas. Although one might expect \textit{NF1} loss to be a mechanism of resistance to vemurafenib, this has not yet been clearly demonstrated clinically. In one patient, a \textit{NF1} nonsense mutation found in the initial tumor was associated with a short PFS.\textsuperscript{15}

**PARALLEL PATHWAYS LEADING TO RESISTANCE**

Given that RAS activates both the MAPK and the PI3K/akt pathway, the latter has received special attention as a potential parallel pathway. Indeed, it has been long known that PTEN is frequently deleted in \textit{BRAF}-mutated melanomas\textsuperscript{16} and recently, PTEN deletion was shown to be required for the malignant phenotype in a \textit{BRAFV600E} mouse model.\textsuperscript{17} In human \textit{BRAF}-mutated melanoma tumors, 44% also contained a mutation or deletion of PTEN.\textsuperscript{11} In \textit{BRAFV600E} cell lines treated with RAF inhibitor, upregulation of certain RTKs, such as MET\textsuperscript{5} or IGF-1R,\textsuperscript{6} leads to AKT activation suggesting the possibility that the PI3K/akt pathway can serve as a rescue pathway for RAF inhibition. These observations suggest strongly that a subset of melanomas rely on activation of the PI3K/akt pathway and that successful therapy will require inhibition of this pathway as well as the MAPK pathway.

Other pathways have received less attention but could play a role in vemurafenib resistance.

**CONCLUSION**

The challenge going forward is to obtain tumor biopsies pretreatment and at the time of tumor progression so that we can determine the mechanism of resistance in the individual patient and to catalog these mechanisms. The range and frequency of these mechanisms of resistance will guide future strategies to prevent and overcome resistance to these drugs.

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**Disclosures of Potential Conflicts of Interest**

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**References**