Abstract

**Purpose:** Many patients with \( \text{BRAF}^{V600E} \) mutant melanoma treated with BRAF inhibitors experience a rapid response, but ultimately develop resistance. Insight into the mechanism of resistance is critical for development of more effective treatment strategies.

**Experimental Design:** Comprehensive genomic profiling of serial biopsies was performed in a patient with a \( \text{BRAF}^{V600E} \) mutant metastatic melanoma who developed resistance to vemurafenib. An \( \text{AGAP3}^{-}\text{BRAF} \) fusion gene, identified in the vemurafenib-resistant tumor, was expressed in \( \text{BRAF}^{V600E} \) melanoma cell lines, and its effect on drug sensitivity was evaluated.

**Results:** Clinical resistance to vemurafenib in a melanoma harboring a \( \text{BRAF}^{V600E} \) mutation was associated with acquisition of an \( \text{AGAP3}^{-}\text{BRAF} \) fusion gene. Expression of the \( \text{AGAP3}^{-}\text{BRAF} \) fusion in \( \text{BRAF}^{V600E} \) mutant melanoma cells induced vemurafenib resistance; however, these cells remained relatively sensitive to MEK inhibitors. The patient experienced clinical benefit following treatment with the combination of a BRAF and a MEK inhibitor. Rebiopsy of the tumor at a later time point, after BRAF and MEK inhibitors had been discontinued, showed loss of the \( \text{AGAP3}^{-}\text{BRAF} \) fusion gene. Mixing experiments suggest that cells harboring both \( \text{BRAF}^{V600E} \) and \( \text{AGAP3}^{-}\text{BRAF} \) only have a fitness advantage over parental \( \text{BRAF}^{V600E} \) cells during active treatment with a BRAF inhibitor.

**Conclusions:** We report acquisition of a BRAF fusion as a novel mechanism of acquired resistance to vemurafenib in a patient with melanoma harboring a \( \text{BRAF}^{V600E} \) mutation. The acquisition and regression of clones harboring this fusion during the presence and absence of a BRAF inhibitor are consistent with rapidly evolving clonal dynamics in melanoma.

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**Introduction**

\( \text{BRAF}^{V600E/K} \) mutations are found in approximately half of malignant melanomas (1–3). These activating mutations in the MAPK/ERK pathway drive tumor progression, survival, and metastasis by promoting cell-cycle progression, facilitating escape from apoptosis, and abrogating immune destruction (4). The development of selective BRAF inhibitors, including vemurafenib and dabrafenib, was a landmark in the treatment of melanoma and has led to significant improvements in clinical response rate (RR), progression-free survival (PFS), and overall survival (OS), compared with chemotherapy for patients with \( \text{BRAF} \)-mutant melanoma (5, 6). Although an initial reduction in tumor volume is observed in the majority of patients with \( \text{BRAF}^{V600E} \) mutant melanoma treated with BRAF inhibitors, clinical resistance associated with progression of disease develops in most patients and limits the long-term utility of these agents. This has led to the clinical investigation and approval of combination BRAF and MEK inhibitor therapy (dabrafenib plus trametinib; vemurafenib plus combretastatin) that is associated with superior RR, PFS, and OS compared with BRAF inhibitor monotherapy (7–9).

Several mechanisms of acquired resistance to vemurafenib have been identified and these include secondary \( \text{NRAS} \) mutations (10), mutations in \( \text{ARAF} \) and \( \text{CRAF} \) (11), amplification of \( \text{BRAF} \) (12), activation of other prosurvival signaling pathways, including the phosphoinositide-3-kinase (PI3K) pathway (11), and amplification of upstream receptor tyrosine kinases such as MET (13). To date, over a dozen acquired mechanisms of resistance are described and, often, multiple mechanisms of
BRAF (16) and recently have been found in some melanomas that lack MAPK signaling. Sequencing, although there often is evidence of reactivation of malignant melanoma in which comprehensive genomic profile analysis must be used to identify the presence of a BRAF-BRAF fusion in a melanoma with a known BRAF V600E mutation at the time of progression on vemurafenib therapy. This finding suggests that BRAF rearrangements may be a novel mechanism of acquired resistance to BRAF inhibitors.

Materials and Methods
Study approval
Patient case 1 described in this report was enrolled in an Institutional Review Board (IRB)-approved investigational trial for tumor sequencing (protocol2012002075) conducted at the Rutgers Cancer Institute (CINI) in New Brunswick, New Jersey, in accordance with the Belmont Report. This patient provided written informed consent prior to study enrollment. Approval for use of de-identified data for patient case 2, including a waiver of informed consent and a Health Insurance Portability and Accountability Act (HIPAA) waiver of authorization, was obtained from the Western IRB (protocol20152817), in accordance with the U.S. Common Rule, by Foundation Medicine, Inc.

Tumor sequencing
Formalin-fixed tumor samples, and DNA extracted from peripheral blood specimens, were analyzed by a comprehensive genomic profiling assay performed on indexed, adaptor-ligated, hybridization captured libraries targeting all exons of 315 cancer-related genes (FoundationOne) at a commercial CLIA-certified laboratory, Foundation Medicine.

Construct generation
The cDNA encoding AGAP3–BRAF fusion gene was synthesized (Life Technologies) and placed in Gateway entry vector. A V5-tagged expression clone (pCDNA3.2/V5-DEST) was then created by performing an LR recombination reaction using gateway LR clonase II enzyme mix (Invitrogen Life technologies, cat. No.11791-020) according to the manufacturer's protocol. The expression of protein was verified by transient transfection of plasmid in 293T cells and Western blot analysis probing with antibody against V5.

Creation of stable cell line and colony formation assay
UACC903 melanoma cells with original BRAF V600E mutations were transfected with either empty vector or the V5-tagged AGAP3–BRAF fusion vector. Cells were then selected on Genticin (G418)-containing RPMI media. Expression of fusion protein was verified by Western blot. The colony-forming ability of these cells expressing either the empty vector or the AGAP3–BRAF fusion vector was evaluated by plating 300 cells/well in a 6-well plate. The cells were allowed to grow for 15 days. The colonies were then stained with crystal violet. To evaluate the effect of a BRAF inhibitor (PLX4720) or a MEK inhibitor (AZD6244) on the colony-forming ability of cells expressing the AGAP3–BRAF fusion protein, cells were plated in a 6-well plate as previously described. Cells were treated with different concentrations of drugs (0.25, 50, 75, 100, and 200 nmol/L) and allowed to grow for 15 days supplemented with fresh media every 3 days. The number of colonies was counted after staining with crystal violet. The percentage of survival was plotted compared with untreated cells from the data collected from three independent experiments.

Cell proliferation assay
To evaluate the effect of a BRAF inhibitor (vemurafenib) and a MEK inhibitor (trametinib) on the proliferation of UACC903 cells harboring either a BRAF V600E mutation or the AGAP3–BRAF fusion gene along with the BRAF V600E mutation, cells were seeded into 96-well plates (4,000 cells/well). Cells were treated with the indicated concentrations of BRAF inhibitor (vemurafenib) or MEK inhibitor (trametinib) and grown for 6 days. The proliferation of cells was measured by incubating them with CellTititer 96 Aqueous One Solution reagent (Promega) for 3 hours and measuring the absorbance at 490 nm. The values were plotted as an average of three independent experiments.

Western blot analysis
UACC903 cells expressing either empty vector or cells expressing the V5-tagged AGAP3–BRAF fusion protein were treated overnight with BRAF inhibitor (1 μmol/L), MEK inhibitor (500 nmol/L), or the combination of both drugs (500 nmol/L each). Protein extracts were prepared using NTEN buffer (Tris pH 8.0, NaCl 150 mmol/L, EDTA 1 mmol/L, NP40 0.5% and protease and phosphatase inhibitors) and resolved by SDS-PAGE. Blots were probed with antibodies against V5 (Bethyl Laboratories), phosphorylated-MEK (pMEK) and total MEK, phosphorylated-ERK (pERK) and total ERK, and BRAF (Cell Signaling Technology).

Cell-mixing studies
To test if the UACC903 cells expressing the AGAP3–BRAF fusion protein have a survival advantage over the parental UACC903 cells harboring the BRAF V600E mutation alone when treated with the BRAF inhibitor, vemurafenib, the UACC903 parental cells harboring BRAF V600E alone were transfected with pCDNA3-GFP empty vector. Green fluorescence protein (GFP)-positive cells were sorted by fluorescence-activated cell sorting (FACS; BD Biosciences Influx high-speed cell sorter) and grown in RPMI medium. UACC903 parental cells (GFP) and cells
expressing the AGAP3-BRAF fusion gene were mixed together in a 10:1 ratio (2000:200) well in a 12-well plate. The mixed population of cells was either untreated or treated with 500 nmol/L of BRAF inhibitor (vemurafenib) for 7 days. The effect of BRAF inhibition on cell growth was then evaluated by imaging for GFP-positive UACC903 parental cells and cells expressing the fusion protein (by differential interference contrast imaging [DIC]) at 10× magnification using a Zeiss Axiovert 200M inverted fluorescence microscope. Twenty images were taken for each treatment. The percentage of GFP-positive (UACC903 parental cells) and non-GFP cells expressing the AGAP3-BRAF fusion protein (by DIC) were quantified before and after treatment with vemurafenib and plotted.

**Results**

**Patient case 1**

A 53-year-old man was initially diagnosed 3 years previously with a 0.7-mm-thick melanoma on the anterior chest wall that was treated with wide surgical excision and clear margins. The patient did well for 3 years and then presented with an enlarging left axillary mass. A PET-CT scan showed the presence of a PET-avid 4.4 cm left axillary mass, as well as PET-avid lesions at T4 and right ilium, consistent with bony metastatic disease. A fine-needle aspiration biopsy of the T4 lesion was performed with diagnostic analysis. This specimen was sent for comprehensive genomic profiling (FoundationOne, Foundation Medicine), which identified both a BRAF\(^{V600E}\) mutation and a novel AGAP3-BRAF fusion gene involving the joining of exons 1 to 9 of AGAP3 in frame to exons 9 to 18 of BRAF. Also present were a VHL\(^{1527}\) nonsense mutation, CDKN2A/B loss, an alteration in STK11 (STK11\(^{+}\) splice site 464+1G>C), an ARID1A truncating mutation (ARID1A\(^{A1576G}\)), and a subclonal mutation in DNMT3A (DNMT3A\(^{R736H}\), Table 1). To determine if the AGAP3-BRAF fusion was detectable prior to treatment with vemurafenib, a pretreatment biopsy specimen was also sent for sequencing using the same assay. The pretreatment specimen had the BRAF\(^{V600E}\) mutation but no BRAF fusion. Identical alterations in VHL, and CDKN2A loss, were also present in the pretreatment biopsy specimen. Intriguingly, the pretreatment biopsy had focal deletion of STK11 but no evidence of the splice-site mutation. The DNMT3A\(^{R736H}\) mutation was also present subclonally in the pretreatment specimen (Table 1).

These results were presented at the CINI Molecular Tumor Board. Following panel discussion, it was recommended that the patient be treated with combination BRAF inhibition and MEK inhibition, based on prior disease progression on ipilimumab therapy and the fact that he was not a candidate for any U.S.-based trials of nivolumab or pembrolizumab available at that time due to his history of brain metastases. The patient was treated with the combination of dabrafenib and trametinib and initially experienced marked clinical improvement with significant reduction of right upper quadrant pain and overall improvement in quality of life. However, the patient experienced increasing right-upper quadrant pain accompanied by an increase in the left axillary mass within 2 months, and evidence of disease progression was observed on imaging.

Dabrafenib and trametinib were then discontinued. The patient was subsequently treated with anti-programmed death-1 (PD-1) antibody therapy (pembrolizumab) when it became available as part of an expanded access protocol. However, he again had worsening disease in the same left axillary tumor, which became painful within several months of initiating pembrolizumab therapy. The patient underwent palliative resection of this tumor and continued on anti-PD-1 therapy. Nevertheless, he soon exhibited clear evidence of disease progression at multiple tumor sites and anti-PD-1 therapy was discontinued. Comprehensive genomic profiling was again performed on the secondary lesion in the right chest wall, which revealed a combination of several alterations that had been present in the pretreatment biopsy.

![Figure 1](Image)

**Figure 1.** Representative CT images showing left axillary metastasis and liver metastases. **A**, Prior to vemurafenib treatment; **B**, after 2 months of vemurafenib therapy; and **C**, following development of disease progression in the left axilla on vemurafenib.
resected left axillary lymph node tumor which had progressed in the absence of BRAF- or MEK-targeted therapy. Biopsy results from the resected tumor specimen revealed the presence of the BRAF\textsuperscript{V600E} mutation, but no evidence of the AGAP3–BRAF rearrangement (Table 1). Of note, because the region of intron 8 of BRAF harboring the breakpoint of the gene rearrangement was sequenced to >125× depth in all samples (Table 1), the presence of the fusion should have been detected even if present at low frequencies. CDKN2A loss and the VHL\textsuperscript{E52} splice-site mutations seen in prior axillary lymph node biopsy specimens were also present.

Following review of these results at the CINJ Molecular Tumor Board, reinitiation of the combination of dabrafenib and trametinib was recommended. Unfortunately, the patient Developing resistance to treatment with a BRAF inhibitor, ALK244, for 6 hours was able to significantly reduce MEK and ERK phosphorylation in both parental and UACC903 cells expressing the AGAP3–BRAF fusion gene (Fig. 2C). Colony formation assays demonstrated that cells harboring the AGAP3–BRAF fusion, unlike parental UACC903 cells harboring the BRAF\textsuperscript{V600E} mutation alone, were relatively resistant to treatment with a BRAF inhibitor. However, the UACC903 parental cells harboring the BRAF\textsuperscript{V600E} mutation alone, and the cells also expressing the AGAP3–BRAF fusion were similarly sensitive to treatment with a MEK inhibitor (Fig. 2D).

To complement the colony formation assay, a cell proliferation assay was also performed, in this case using the clinically relevant MEK inhibitor, trametinib. In this assay, UACC903 cells harboring both the BRAF\textsuperscript{V600E} mutation and the AGAP3–BRAF fusion gene were significantly less sensitive to BRAF inhibition than parental UACC903 cells (Fig. 2E). Both the UACC903 parental as well as cells expressing the fusion gene were sensitive to MEK inhibitor (trametinib), however, at higher concentrations (500 and 1,000 nmol/L) the cells harboring both the BRAF\textsuperscript{V600E} mutation and the AGAP3–BRAF fusion gene were less sensitive to MEK inhibition compared with parental cells (Fig. 2E). Although this relative resistance was seen only at high trametinib concentrations, it suggests that the presence of the BRAF fusion may also induce some partial resistance to MEK inhibition.

Cells expressing the AGAP3–BRAF fusion gene have a growth advantage in the presence of BRAF inhibitor Mixing studies to evaluate the relative growth advantage that the AGAP3–BRAF fusion may impart to melanoma cells already harboring a BRAF\textsuperscript{V600E} mutation showed that, in the setting of treatment with vehicle alone, around 90% of the cells were the

### Table 1. Next-generation DNA sequencing results on serial biopsy specimens

<table>
<thead>
<tr>
<th>Test</th>
<th>Premutation biopsy (bone)</th>
<th>Axillary lymph node biopsy (progressing on vemurafenib)</th>
<th>Axillary lymph node biopsy (progressing on immunotherapy, off kinase inhibitors for &gt;3 months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRAF\textsuperscript{V600E} MAF</td>
<td>30% (685)</td>
<td>54% (879)</td>
<td>51% (208)</td>
</tr>
<tr>
<td>VHL\textsuperscript{E52} MAF</td>
<td>53% (557)</td>
<td>22% (567)</td>
<td>15% (123)</td>
</tr>
<tr>
<td>ARID1A Y560 (depth)</td>
<td>0% (670)</td>
<td>8% (676)</td>
<td>0% (197)</td>
</tr>
<tr>
<td>DNMT1SR736H (depth)</td>
<td>1% (567)</td>
<td>2% (642)</td>
<td>0% (132)</td>
</tr>
<tr>
<td>STK11 splice site 464–1G–C (depth)</td>
<td>0% (229)</td>
<td>45% (348)</td>
<td>36% (78)</td>
</tr>
<tr>
<td>STK11 copy-number alteration</td>
<td>Loss</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>CDKN2A/B copy-number alteration</td>
<td>Loss</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>AGAP3–BRAF fusion</td>
<td>Absent (0 supporting reads in manual confirmation)</td>
<td>Present with 23 supporting chimeric read pairs</td>
<td>Absent (0 supporting reads in manual confirmation)</td>
</tr>
<tr>
<td>BRAF intron 8 average coverage</td>
<td>546.57</td>
<td>568.21</td>
<td>125.45</td>
</tr>
</tbody>
</table>

NOTE: Asterisk denotes stop codon. Abbreviation: MAF, mutant allele frequency.
parental UACC903 cells, retaining the original ratio of mixed cells (Fig. 3A). However, when cells were treated with vemurafenib for 7 days, the percentage of GFP-positive cells decreased dramatically, to <20% of cells. This result demonstrates that, in the absence of a BRAF inhibitor, cells harboring both BRAFV600E and the AGAP3–BRAF fusion gene do not have a significant growth advantage. However, treatment with a BRAF inhibitor resulted in a relative dominance of cells harboring the AGAP3–BRAF fusion. These data suggest that cells harboring both BRAFV600E mutation and the AGAP3–BRAF fusion gene are only more fit than parental cells harboring the BRAFV600E mutation alone when in the presence of BRAF inhibitor treatment.

Patient case 2
In addition to the index case, we report on an additional patient case demonstrating the co-occurrence of a BRAF rearrangement with a BRAFV600E mutation in the setting of acquired resistance to vemurafenib and/or MEK inhibitors. Patient case 2 is a 48-year-old man with metastatic melanoma harboring a BRAFV600E mutation who developed progressive disease following treatment with the combination of dabrafenib and trametinib. DNA sequencing of a biopsy of a progressive lesion showed the resistant tumor to harbor both a BRAFV600E mutation and a CSTF3–BRAF fusion gene that retained the full kinase domain of BRAF.

Figure 2.
Structural and functional characterization of the AGAP3–BRAF fusion. A, Illustration of AGAP3–BRAF rearrangement event resulting from an inversion in chromosome 7. B, Representation of BRAF and AGAP3 genes and key domains. RBD, Ras binding domain; CR, Cysteine–rich domain; Miro, Mitochondrial Rho GTPase domain; PH, Pleckstrin homology domain. C, UACC903 cells harboring BRAFV600E expressing either empty vector or V5-tagged AGAP3–BRAF fusion were either untreated or treated with PLX4720 (BRAF inhibitor), AZD6244 (MEK inhibitor), or both agents. Cell extracts were processed for Western blotting and probed with the indicated antibodies. D, Parental UACC903 cells and UACC903 cells with stable expression of V5-AGAP3–BRAF were treated with increasing concentrations of BRAF inhibitor (PLX4720) left panel or MEK inhibitor (AZD6244), right panel and colony formation is plotted. E, Parental UACC903 cells and UACC903 cells with stable expression of V5-AGAP3–BRAF were treated with increasing concentrations of a BRAF inhibitor (vemurafenib, left) or a MEK inhibitor (trametinib, right), for 4 days and the cell proliferation was measured with Celltiter 96 Aqueous One Solution reagent (Promega), and percent cell proliferation is plotted as an average of three independent experiments.
Discussion

Standard testing for BRAF mutations is usually undertaken by amplicon-based DNA sequencing to identify hotspot BRAF mutations (21). These assays, however, will not detect BRAF fusions, as the coding sequences of individual exons are not altered. Similarly, BRAF fusions are not detectable with standard whole-exome sequencing, as the fusion junctions are located in the introns. One approach to identifying rearrangements is through hybrid capture of “hotspot introns.” High-depth sequencing of select introns not only sensitively detects rearrangements but analysis of the junction sequence can identify the partner genes involved. As intron 8 of BRAF is captured in the FoundationOne assay, it can identify novel rearrangements of BRAF involving this region. Other targeted approaches to identify fusion proteins include target enrichment based RNA-seq analysis, where target enrichment can be accomplished by hybrid capture, single primer-extension technology, or the anchored multiplex polymerase chain reaction (PCR) approach (22, 23).

BRAF fusion proteins were initially reported and characterized in pediatric pilocytic astrocytomas (16). The majority of pilocytic astrocytomas harbor gene fusions that result in an in-frame fusion of the N-terminal exons of KIAA1549 to exons 9 to 18 of BRAF, leading to a fusion protein that contains the intact wild-type kinase domain of BRAF. The N-terminal region of KIAA1549 is thought to mediate constitutive dimerization of the fusion protein and thus activation of BRAF kinase activity. Introduction of a first-generation BRAF inhibitor, such as vemurafenib, causes paradoxical activation of this fusion protein and increased MEK phosphorylation (24, 25). Cells harboring BRAF fusions respond to vemurafenib in a manner similar to cells harboring RAS mutations, through downstream MAPK pathway activation. As such, vemurafenib should be avoided in these settings; however, either MEK inhibitors or second-generation “paradox-breaking” BRAF inhibitors may be effective in the setting of BRAF fusions.

BRAF fusions have been reported as primary driver mutations in a subset of melanomas without a BRAF<sup>V600E</sup> mutation (26) and have been shown to be associated with resistance to BRAF inhibitors (19, 26). Here, we report an AGAP3–BRAF fusion that was detected in a melanoma with a known BRAF<sup>V600E</sup> mutation at the time of development of resistance to vemurafenib. The presence of the AGAP3–BRAF fusion was not detected in pretreatment samples, consistent with this fusion protein representing the mechanism of resistance to vemurafenib. Of note, it was expected...
that the AGAP3–BRAF fusion harbors a wild-type kinase domain, as seen in other reported cases of BRAF fusion genes, including the prior report of an AGAP3–BRAF fusion. This was confirmed by laboratory studies that demonstrated that the AGAP3–BRAF fusion induces resistance to vemurafenib in cells with preexisting BRAF\(^{V600E}\) mutation, as compared with cells lines harboring a BRAF\(^{V600E}\) mutation. Cells harboring both BRAF\(^{V600E}\) and AGAP3–BRAF fusion remained sensitive to a MEK inhibitor, suggesting that MEK inhibition can overcome this mechanism of resistance (27, 28). Of note, cells harboring both the BRAF\(^{V600E}\) mutation and the AGAP3–BRAF fusion did show some partial resistance to trametinib at high doses in proliferation assays (Fig. 2E), suggesting that BRAF fusion genes may also impart some resistance to MEK inhibitors. The case showing development of a CSF3–BRAF fusion in the setting of acquired resistance to the combination of dabrafenib and trametinib also suggests that some BRAF fusion genes may impart clinical resistance to MEK inhibitors as well. The relatively transient response in the index case also supports this possibility.

These findings suggest that cells harboring both the BRAF\(^{V600E}\) mutation and the AGAP3–BRAF fusion were present at a very low frequency in the pretreatment setting and could not be detected even at relatively high sequencing depth. Treatment with vemurafenib induced selection pressure that allowed preferential growth of cells harboring the BRAF fusion gene, making them the dominant clone and driving tumor resistance and progression. The resistant tumor also had a mutation in ARID1A that was not detected in the pretreatment biopsy; it is not clear if this mutation plays any role in vemurafenib resistance, especially as the mutant allele frequency was relatively low. Of note, the pretreatment biopsy had evidence of STK11 deletion; however, neither of the two later biopsies showed evidence of STK11 deletion, but instead had STK11 splice mutations that should lead to loss of function. This finding is consistent with clonal heterogeneity in this patient with a large tumor burden, with convergent evolution for STK11 loss in both clones. The presence of identical BRAF and VHL mutations and CDKN2A deletion in all samples is consistent with evolution from a common ancestral clone.

Interestingly, the same tumor site noted to have the AGAP3–BRAF fusion and BRAF\(^{V600E}\) mutation later progressed after 3 months of anti–PD-1 antibody therapy, during which there was no treatment with either a BRAF or a MEK inhibitor. When this site was excised for palliation, sequencing revealed only the presence of the BRAF\(^{V600E}\) mutation, and no evidence of AGAP3–BRAF fusion despite the fact that the region of intron 8 of BRAF that harbored the breakpoint was sequenced to greater than 125× depth. This finding suggests that the clone harboring both the BRAF\(^{V600E}\) mutation and the AGAP3–BRAF fusion may have been more fit than clones harboring the BRAF\(^{V600E}\) mutation alone only in the setting of ongoing treatment with vemurafenib. However, when selection pressure from BRAF and MEK inhibition was not present, clones harboring BRAF\(^{V600E}\) alone may have had a fitness advantage, thereby dominating the recurrent mass. This hypothesis is supported by the reported data from mixing studies showing relative outgrowth of cells harboring both BRAF\(^{V600E}\) and AGAP3–BRAF fusion only in setting of BRAF inhibitor treatment (Fig. 3A).

There may be very rapid clonal dynamics under selection for the BRAF inhibitors in malignant melanoma, and dominant clonal populations may rapidly shift during tumor progression when drugs are discontinued (Fig. 3B). Similar rapid clonal dynamics have been reported in colon cancer, with transient emergence of clones harboring KRAS mutations emerging during development of cetuximab resistance followed by their regression when cetuximab is discontinued (29). Rechallenge with cetuximab at the time point when KRAS-mutant clones were undetectable led to clinical response (29).

Due to the possibility of a mixed response, the biopsy showing the presence of the AGAP3–BRAF fusion was obtained when the patient was still receiving vemurafenib therapy. If vemurafenib had been completely discontinued before the biopsy was performed, this mechanism of resistance may not have been detected, as the clone with BRAF\(^{V600E}\) alone could have rebounded rapidly. Of note, this patient did experience some clinical benefit, although brief in duration, upon rechallenge with a BRAF inhibitor after progression on immunotherapy. This result suggests that rechallenge with BRAF/MEK inhibitors may be of benefit in the setting of interval progression of kinase inhibitor therapy, as has been reported by others (30–32).

This is the first report of a BRAF rearrangement as the mechanism of acquired resistance to vemurafenib in a melanoma with a preexisting BRAF\(^{V600E}\) mutation. It is possible that such BRAF rearrangements may represent an underappreciated mechanism for acquired resistance to BRAF inhibitors. It is hard to estimate the frequency of such events, as post-resistance biopsies using assays that can identify fusion genes are not routinely performed. Given the potential for rapid clonal dynamics, the timing of biopsy of progressive disease to look for mechanisms of resistance may be critical, as there may be rapid rebound of parental clones following discontinuation of kinase inhibitor therapy. Serial biopsies, or serial use of circulating cell-free tumor DNA assays, may identify clinical settings where rechallenge with BRAF inhibitors after progression off therapy may be effective.

Disclosure of Potential Conflicts of Interest
S.M. Ali has ownership interest (including patents) in Foundation Medicine. No potential conflicts of interest were disclosed by the other authors.

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BRAF Fusion as a Novel Mechanism of Acquired Resistance to Vemurafenib in BRAF^V600E^ Mutant Melanoma

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