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BRAF internal deletions and resistance to BRAF/MEK inhibitor therapy

Douglas B. Johnson[1], **Merrida A. Childress**[1,2], **Zachary R. Chalmers**[3], **Garrett M. Frampton**[3], **Siraj M. Ali**[3], **Samuel M. Rubinstein**[1], **David Fabrizio**[3], **Jeffrey S. Ross**[3], **Sohail Balasubramanian**[3], **Vincent A. Miller**[3], **Philip J. Stephens**[3], **Jeffrey A. Sosman**[4,*], and **Christine M. Lovly**[1,2,*]

[1]Department of Medicine, Vanderbilt University Medical Center and Vanderbilt Ingram Cancer Center, Nashville TN

[2]Department of Cancer Biology, Vanderbilt University Medical Center and Vanderbilt Ingram Cancer Center, Nashville TN

[3]Foundation Medicine Inc., Cambridge MA

[4]Northwestern University Medical Center and Robert H. Lurie Comprehensive Cancer Center, Chicago IL

Summary

BRAF and MEK inhibitors have improved clinical outcomes in advanced, *BRAF*^{V600}-mutated melanomas. Acquired resistance occurs in most patients, with numerous and diverse drivers. We obtained pre-treatment and progression biopsies from a patient who progressed on dabrafenib and trametinib. In addition to a preserved *BRAF*^{V600E} mutation, an internal deletion (rearrangement) of *BRAF* was observed in the progression sample. This deletion involved exons 2-8, which includes the Ras-binding domain, and is analogous to previously documented *BRAF* fusions and splice variants known to reactivate RAS-RAF-MEK-ERK signaling. In a large cohort of melanomas, 10 additional internal deletions were identified (0.4% of all melanomas; 9 of which had concurrent *BRAF* mutations), as well as sporadically in other tumor types. Thus, we describe a novel mechanism of resistance to BRAF and MEK inhibition.

Keywords

BRAF; internal deletion; resistance; rearrangement; dabrafenib; trametinib; vemurafenib

The combination of BRAF and MEK inhibitors produce potent responses and improved overall survival in patients with advanced melanoma harboring *BRAF*^{V600} mutations (Long et al., 2016). Acquired resistance limits the clinical benefit of these agents in most patients,

Contact Information: Address: 777 PRB, 2220 Pierce Ave, Nashville, TN 37232. Douglas.b.johnson@vanderbilt.edu.
*Contributed equally as senior authors

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and arises at a median of 9-11 months on therapy (Flaherty et al., 2012; Robert et al., 2014). Mechanisms of acquired resistance are numerous and diverse, and include alterations activating the RAS-RAF-MEK-ERK pathway, and those promoting alternative signaling networks (Rizos et al., 2014; Shi et al., 2014; Van Allen et al., 2014). Genomic and transcriptomic alterations driving resistance that affect *BRAF* specifically include gene amplification and alternate splicing. The latter alteration removes the inhibitory RAS-binding domain (RBD), thus promoting constitutive signaling (Poulikakos et al., 2011).

Other genomic alterations in *BRAF* have been described across the spectrum of human tumors. These include atypical (non-V600) *BRAF* mutations, *BRAF* fusions, and *BRAF* kinase domain duplications, that each promote RAS-RAF-MEK-ERK signaling, and are variably sensitive to RAF or MEK inhibitors (Botton et al., 2013; Dahlman et al., 2012; Hutchinson et al., 2013; Klempner et al., 2016). Short, oncogenic internal deletions in *BRAF* have also been described in pancreatic cancer, which confer resistance to BRAF inhibitors (Chen et al., 2016).

Large internal deletions (also termed rearrangements) in *BRAF* that delete the RBD would be predicted to confer similar functional consequences as *BRAF* fusions or alternatively-spliced *BRAF*. These alterations, however, have not been described in human cancers to our knowledge. Here, we describe a large *BRAF* internal deletion in a melanoma sample with acquired resistance to BRAF and MEK inhibitors, and evaluate the incidence of *BRAF* internal deletions across human cancer types.

A woman in her 30s with advanced melanoma with axillary lymph node, liver, thoracic spine, and subcutaneous disease involvement presented to our clinic. She was highly symptomatic from an extensive disease burden with pain and fatigue. Laboratory testing showed elevated lactate dehydrogenase; molecular testing revealed a $BRAF^{V600E}$ mutation. She was initiated on dabrafenib and trametinib and experienced rapid clinical improvement (Figure 1). A CT scan performed two months following start of therapy demonstrated partial response in nearly all visible tumors (-32% decrease in measurable tumor diameters). Four months following treatment initiation, she experienced increasing size of a subcutaneous back lesion, and rise in LDH. She was continued on dabrafenib and ipilimumab was added in hopes of inducing a durable response; trametinib was discontinued for safety reasons (given prior reports of colon perforation with the triple combination) (Minor et al., 2015). She experienced further progressive disease in numerous lesions approximately 2 months later. Based on the rapid painful tumor growth, she underwent palliative resection of the subcutaneous lesion on her back. Thereafter, she was treated with pembrolizumab without response and ultimately died of progressive disease.

The subcutaneous tumor on the patient's back was biopsied one week prior to initiation of dabrafenib and trametinib, and the same lesion was resected following disease progression (Figure 1). Both samples were evaluated by an extensively validated hybrid-capture based next generation sequencing platform that sequences exons from 315 genes and introns from 28 genes (Frampton et al., 2013). Sequencing from both the pre-treatment and disease-progression samples identified $BRAF^{V600E}$, $PTEN^{G129E}$, and *TERT* promoter mutations, as well as *CDKN2A/B* loss. Minimal change in $BRAF^{V600E}$ mutant allele frequency was noted

between pre-treatment (42%) and progression (41%) tumors. The sample obtained at disease progression was also found to have a *BRAF* internal deletion (rearrangement). Breakpoints in introns 1 and 8 were identified, thus resulting in the loss of exons 2-8, which includes the inhibitory RAS-binding domain (Figure 2A). The breakpoints resulted in an internal, in-frame deletion rather than a fusion product, which was confirmed by RNA sequencing (Figure 2B; Supplemental Figure 2). *IGF1R* amplification was also identified in the progression sample only, consistent with prior data that resistance to BRAF + MEK combination therapy is usually mediated by multiple resistance mechanisms functioning in concert (Long et al., 2014; Moriceau et al., 2015; Wagle et al., 2014).

We then surveyed the spectrum of tumors sequenced by Foundation Medicine, to assess for additional *BRAF* internal deletions that remove the RBD, while sparing the kinase domain. Among 2439 melanomas, 10 additional *BRAF* internal deletions were identified (0.4% of all melanomas). Of these 10 samples, 9 had concurrent *BRAF* mutations, including $BRAF^{V600}$ mutations in 6 and atypical, non-V600 *BRAF* mutations in 3 (Table 1). No *NRAS* or *KRAS* mutations were observed in these samples. *BRAF* internal deletions involving the RBD and sparing the kinase domain were also identified in a diverse set of 16 other samples, including in multiple myeloma, glioblastoma, breast cancer, non-small cell lung cancer, and others (Table 1; Supplemental Figure 1). A surprisingly high number of these (7 of 16; 44%) were found to have concurrent *BRAF* mutations.

Comprehensive genomic profiling of paired pre-treatment and disease progression biopsies, has uncovered numerous mechanisms of resistance to targeted therapies in diverse tumor types. Using this approach, we identified a novel *BRAF* internal deletion mimicking previously reported alternative splicing events that confers resistance to BRAF and MEK inhibition. While other alterations in *BRAF* arise in the context of BRAF inhibitor therapy (amplifications and alternative splicing) or in $BRAF^{V600}$ wild type samples (fusions, kinase duplications, and short internal deletions), this is the first report of a large *BRAF* internal deletion involving exons encoding the RAS-binding domain. Interestingly, in melanoma, all but one of these tumors harbored concurrent *BRAF* mutations. Taken with the results of our paired biopsies, we would hypothesize that these internal deletions may serve as weak oncogenes on their own, but enhance mutant BRAF signaling. An unexpectedly high number of patients with other cancers also harbored concurrent *BRAF* point mutations, providing further evidence for this hypothesis.

Our case suggests that *BRAF* internal deletions likely contribute to acquired resistance to BRAF +/- MEK inhibition in concert with other resistance mechanisms. One could also speculate that these alterations drive primary resistance to BRAF inhibitors if present in the pre-treatment setting. We elected not to model these alterations *in vitro*, since our groups and others have extensively shown that removal of the RAS-binding domain through fusions or alternate splicing activates RAS-RAF-MEK-ERK signaling and mediates resistance to BRAF inhibitors (Hutchinson et al., 2013; Poulikakos et al., 2010; Shi et al., 2014). Characterizing effects of particular *BRAF* internal deletions is an important future direction. Further, evaluating the effect of tumor heterogeneity and outgrowth of resistant clones is another critical question (Shi et al., 2014). It also remains unclear how the addition of a

MEK inhibitor contributes to the development of *BRAF* internal deletions, as compared with patients treated with BRAF inhibitor monotherapy.

Other potential resistance mechanisms were identified in this patient as well, including *PTEN* mutations. Pre-existing *PTEN* alterations decrease the duration of, but do not preclude therapeutic responses, whereas acquired mutations/loss appear to mediate (or contribute to) therapeutic resistance (Van Allen et al., 2014). Thus, *PTEN* mutations may have contributed to the short duration of response in this case (4 months). *IGF1R* genomic amplification may have also played a role, as overexpression at the protein level has been described as a mechanism of acquired resistance to BRAF inhibitors (Nazarian et al., 2010). These intrinsic and acquired genomic alterations likely mediated resistance to BRAF/MEK inhibition synergistically. Several elegant studies have demonstrated that resistance to combination therapy is driven by multiple mechanisms functioning in concert, supporting this notion (Long et al., 2014; Moriceau et al., 2015; Villanueva et al., 2013; Wagle et al., 2014).

We and others have also demonstrated that MEK or unselective RAF inhibitors (e.g. sorafenib) may be effective in patients with *BRAF* fusions (Botton et al., 2013; Hutchinson et al., 2013; Menzies et al., 2015). In the context of BRAF or BRAF + MEK inhibitor resistant melanoma, these agents are unlikely to provide a significant clinical benefit as monotherapy. However, in tumors harboring *BRAF* internal deletions without concurrent $BRAF^{V600}$ mutations (including those with concurrent non-V600 mutations), MEK or RAF inhibition should be investigated. In conclusion, this is the first report of a *BRAF* internal deletion to our knowledge and defines an uncommon but recurrent genetic subset of melanomas and other cancers.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Significance

This is the first report of a *BRAF* internal deletion affecting the RAS-binding domain in a similar fashion to fusions or splice variants in *BRAF. BRAF* internal deletions were identified in melanomas and other tumor types, and thus comprise a recurrent, albeit uncommon genetic subset of cancer. Finally, the identification of this alteration in a progression sample (but not pre-treatment) suggests *BRAF* internal deletions mediate resistance to BRAF/MEK inhibition.

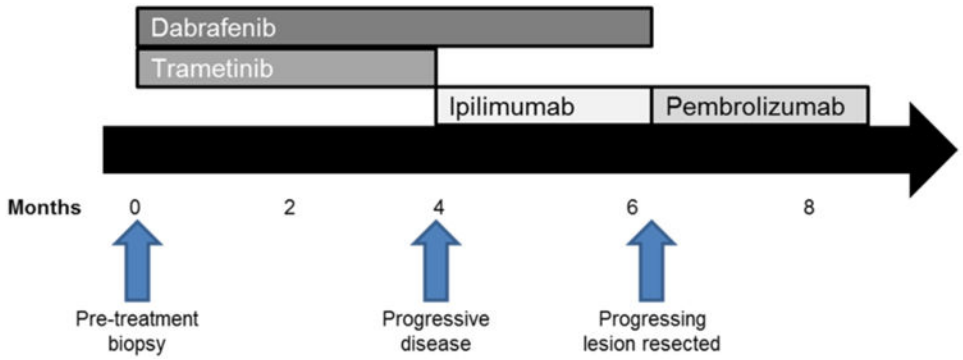

Figure 1. Timecourse of treatment and biopsies

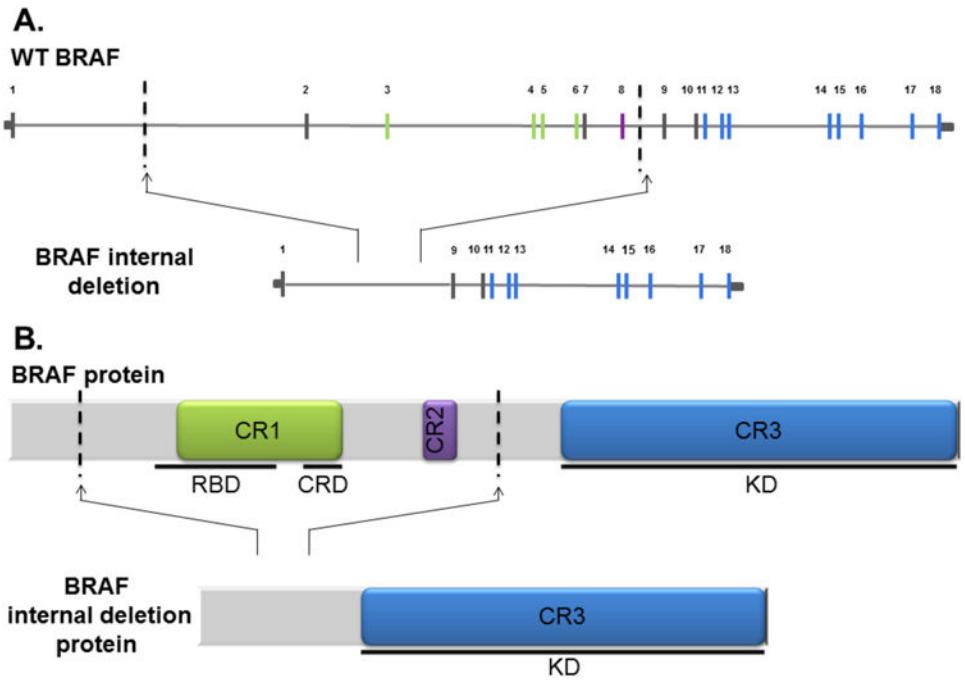

Figure 2.

(**A**) Scaled genomic representation of BRAF (top) and BRAF internal deletion observed in our case (bottom). Exons are numerically labeled. Exons encoding highly conserved protein domains are depicted in green (CR1), purple (CR2), and blue (CR3). Dashed lines indicate the breakpoints found in the patient case report that resulted in the B-Raf internal deletion variant depicting the loss of exons 2-8. (**B**) Protein schematic of full length B-Raf (top). Highly conserved protein domains are depicted in green, purple, and blue: CR1 including the inhibitory Ras binding domain (RBD) as well as the cysteine rich domain (CRD) required for Ras binding; CR2; and CR3 which contains the entire kinase domain. Dashed lines indicate genomic breakpoint locations in our observed case.

Table 1
Genomic and pathologic details of tumors harboring *BRAF* internal deletions

Disease Ontology	N-Ras Mutations	K-Ras Mutations	B-Raf Mutations	BRAF Exons Deleted
unknown primary melanoma	ND	ND	V600 K601>E	2-8
unknown primary melanoma	ND	ND	V600E	4-8
unknown primary melanoma	ND	ND	V600K	2-10
unknown primary melanoma	ND	ND	ND	2-10
unknown primary melanoma	ND	ND	V600E	3-10
skin melanoma	ND	ND	V600E	2-8
skin melanoma	ND	ND	T599I	3-8
skin melanoma	ND	ND	V600E	2-8
head and neck melanoma	ND	ND	L597S	2-8
vulva melanoma	ND	ND	D594G	3-8
bladder urothelial (transitional cell) carcinoma	ND	ND	D587H	3-8
bone marrow multiple myeloma	ND	ND	V600E	2-8
bone marrow multiple myeloma	ND	ND	V600E	2-8
bone marrow multiple myeloma	ND	ND	ND	2-10
brain glioblastoma	ND	ND	ND	3-8
breast carcinoma (nos)	ND	ND	ND	4-7
breast invasive ductal carcinoma	ND	ND	ND	5-9
lung adenocarcinoma	ND	G12C	ND	2-9
lung non-small cell lung carcinoma	ND	ND	D594G	4-8
lung non-small cell lung carcinoma	ND	ND	G464A	4-8
nasopharynx and paranasal sinuses carcinoma	ND	ND	L485F	3-8
pancreas ductal adenocarcinoma	ND	ND	S467L	2-10
prostate acinar adenocarcinoma	ND	ND	ND	4-8
thymus thymoma	ND	ND	ND	3-10
unknown primary adenocarcinoma	ND	ND	ND	2-7
unknown primary germ cell tumor	ND	amplification	ND	3-8

ND: Not detected. NOS: Not otherwise specified