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Brief Communication

BRIEF COMMUNICATION

Dramatic Response of BRAF V600E Mutant Papillary Craniopharyngioma to Targeted Therapy

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Abstract

We recently reported that BRAF V600E is the principal oncogenic driver of papillary craniopharyngioma, a highly morbid intracranial tumor commonly refractory to treatment. Here, we describe our treatment of a man age 39 years with multiply recurrent BRAF V600E craniopharyngioma using dabrafenib (150 mg, orally twice daily) and trametinib (2 mg, orally twice daily). After 35 days of treatment, tumor volume was reduced by 85%. Mutations that commonly mediate resistance to MAPK pathway inhibition were not detected in a post-treatment sample by whole exome sequencing. A blood-based BRAF V600E assay detected circulating BRAF V600E in the patient's blood. Re-evaluation of the existing management paradigms for craniopharyngioma is warranted, as patient morbidity might be reduced by noninvasive mutation testing and neoadjuvant-targeted treatment.

Craniopharyngiomas are locally aggressive suprasellar tumors that arise adjacent to the optic nerves, pituitary gland, hypothalamus, and brainstem (1). Craniopharyngiomas compress and infiltrate these critical structures, causing profound neurological deficits. Standard treatment, including surgical resection and radiation therapy (2), may achieve local tumor control. Unfortunately, poor quality of life often follows aggressive local treatment because of permanent neurological and endocrine deficits. No standard chemotherapy exists, and when tumors recur after surgery and radiation, treatment is usually unsuccessful.

Craniopharyngiomas occur in two histological subtypes, adamantinomatous and papillary, with similar presentations and response to standard treatments (3). We recently reported that nearly all papillary craniopharyngiomas harbor the well-studied BRAFV600E alteration (4). In other BRAFV600E-mutated cancers, V600E mutation–specific BRAF inhibitors have profound antitumor effects (5,6). Adding the MEK inhibitor trametinib to BRAF inhibition reduces development of secondary squamous cell skin carcinomas and emergence of tumor resistance, further improving survival in patients with melanoma (7).

A man age 39 years who had undergone right craniotomy for a brain tumor elsewhere seven months earlier presented emergently to our hospital with confusion, visual deficits, severe headaches, and vomiting. The patient provided

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written informed consent for research studies, which were performed in accordance with protocols approved by the institutional review board at Dana-Farber/Harvard Cancer Center for dabrafenib and trametinib therapy and for genetic analysis. Detailed materials and methods for cranial computed tomography, immunohistochemistry, detection of BRAF V600E mutations in peripheral blood, and whole exome sequencing can be found in the Supplementary Materials (available online).

A cranial computed tomography exam showed a 4 cm suprasellar cystic tumor displacing the midbrain and causing obstructive hydrocephalus (Figure 1, A and B). Emergent tumor and cyst decompression was performed by craniotomy, with significant clinical improvement. Microscopic examination of the resected tissue showed only dense connective tissue with clusters of atypical squamous cells. Postoperative endocrinopathies included hypothyroidism, secondary adrenal insufficiency, and diabetes insipidus. Six weeks later, he became obtunded and imaging demonstrated a cystic recurrence of the suprasellar mass. He again underwent craniotomy for emergent decompression. Histology of the resected tumor secured the diagnosis of papillary craniopharyngioma (Supplementary Figure 1A, available online). Immunohistochemistry (IHC) using VE1 antibody, specific to the mutated BRAF V600E protein, revealed uniform staining of the neoplastic epithelium (Figure 1C); the V600E mutation was confirmed by allele-specific genetic testing. IHC for beta-catenin showed an absence of nuclear staining (Supplementary Figure 1B and Supplementary Methods, available online).

Two weeks later, after surgery and prior to his planned radiotherapy, the patient again experienced rapid neurological decline to a minimally responsive state, with radiographic recurrence of the cystic lesion; he required a fourth emergent craniotomy with cyst decompression, again with resolution of symptoms. In a staged procedure, the residual tumor was debulked through an endoscopic transsphenoidal approach, and the cyst wall was fenestrated into the third ventricle. Seven weeks later, the patient was readmitted for progressive bitemporal vision loss. MRI demonstrated expansion of the solid enhancing suprasellar tumor with a smaller cystic component (Figure 1D).

Given the rapid regrowth of this tumor and the presence of BRAFV600E, we initiated the oral RAF inhibitor, dabrafenib (Day 0) (150 mg, orally twice daily). MRI after four days of dabrafenib treatment revealed a 23% decrease in tumor enhancing volume (7.32 cc) and a 32% decrease in the associated cyst volume (0.39 cc) (Supplementary Methods, available online). On dabrafenib Day 17, another MRI showed continued tumor response: 52% enhancing volume decrease and 70% cyst volume reduction (Figure 1E). Because MEK inhibition can enhance the effects of BRAF inhibition, we added the MEK inhibitor trametinib (2 mg, orally twice daily) to the regimen (7) on Day 21. After an additional 14 days of dual inhibitor therapy, the enhancing tumor volume had been reduced by 85% and tumor-associated cyst had been reduced by 81% (Figure 1, F and G). The patient remained clinically stable; toxicity was limited to one day of low-grade fever.

On treatment Day 38, the patient underwent repeat endoscopic transsphenoidal surgery for consolidation tumor resection. Three weeks after surgery and one week after dabrafenib and trametinib were stopped, he had definitive radiation treatment to the tumor (50.4 Gy in 28 fractions), which he tolerated well. Seven months later, the patient remains free of new symptoms.

The histology showed substantial treatment effect with a brisk infiltrate of foamy macrophages and fibrosis (Figure 2A). Expression of BRAFV600E mutant protein was retained by immunohistochemistry (Figure 2A). While B-cells were scarce, (Supplementary Figure 1, C and D, available online) treatment led to a sharp increase in CD8-positive T-lymphocytes (Figure 2B). Notably, IHC for MIB-1 showed that the proliferation index before treatment was 22.1% but that it was sharply reduced after treatment (<0.5%) (Figure 2B).

By analogy with work in melanoma and thyroid cancer (8–10), we tested blood collected during the treatment course for the presence of circulating BRAFV600E DNA (8–10) (Supplementary Methods, available online). We detected the mutation circulating in the patient's blood on multiple occasions (Figure 2C). The mutant DNA was absent in the blood of five nonrelated individuals without craniopharyngioma or other malignancies. We performed whole exome sequencing on the pre- and post-treatment tissue resection samples (Supplementary Methods, available online). We found that BRAFV600E was clonal in both tissue samples by analyzing the variant allelic frequency (Figure 2D) (4,11), consistent with the uniform staining seen with the VE1 BRAF antibody (Figure 2A). As we observed previously in papillary craniopharyngiomas (4), the tumor's pre- and post-treatment mutation rates were low, with only 1.02 and 0.55 mutations/MB, respectively. The BRAFV600E mutation was the only alteration seen in this tumor previously detected in other papillary craniopharyngiomas (4) (Supplementary Table 2, available online). Importantly, no mutations known to mediate melanoma resistance to BRAF inhibition emerged in the post-treatment tumor sample (Supplementary Table 2, available online).

We report herein the first exceptional therapeutic response to combined BRAF and MEK-targeted therapy in a multiply recurrent papillary craniopharyngioma with genetically confirmed BRAFV600E mutation. Both the solid and cystic portions of the tumor shrank rapidly. The course of dabrafenib/trametinib therapy had low toxicity. Genomic studies confirmed BRAFV600E to be present in all tumor cells both before and after treatment, with the mutation also detectable in peripheral blood during treatment.

Craniopharyngiomas are locally aggressive suprasellar tumors that often reach a large size before symptomatic presentation. Most patients never return to premorbid functional levels or to good quality of life even after multimodality treatment (12). Much of this morbidity results from surgery and radiation treatment immediately adjacent to sensitive visual, endocrine, and neurological structures. A neoadjuvant or entirely nonoperative treatment strategy for craniopharyngiomas, such as those used for prolactinomas or suprasellar germinomas, would be attractive.

Identification of BRAFV600E, a key activator of ERK/MAPK signaling, and the development of inhibitors specific to this mutated oncoprotein have substantially improved outcomes in melanoma (7). However, thyroid cancers, colorectal carcinomas, and lung carcinomas with BRAFV600E mutation are often insensitive to BRAF inhibition (13). Others, like hairy cell leukemia (6) and Erdheim-Chester disease (14), show durable responses. Like these, craniopharyngiomas have a low frequency of somatic mutations and are not genomically complex (4).

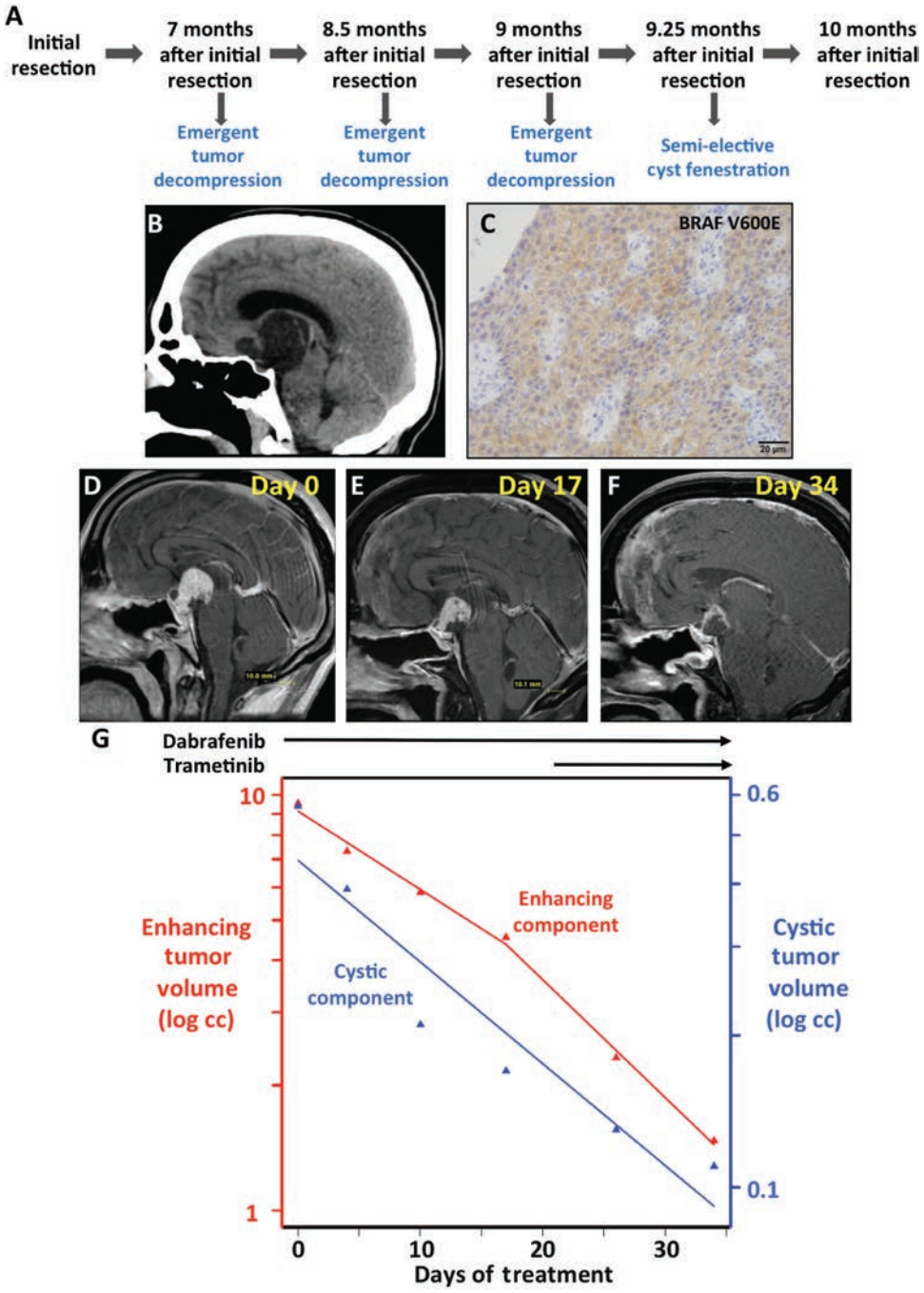

Figure 1. Targeted inhibition of BRAF/MEK pathway in a patient with a multiply recurrent papillary craniopharyngioma. **A)** Demonstration of the patient represented to our hospital with obtundation three times within a span of eight weeks with CT imaging revealing re-accumulation of a suprasellar cyst. **B)** The MRI on initial presentation to our institution seven months following his initial resection at an outside institution. **C)** The specimen obtained at time of the second neurosurgical decompression at our institution, which revealed diffuse positivity for BRAF V600E by IHC (VE1 antibody). We have validated the specificity of the antibody in prior work (4,16). **D)** The brain MRI following five craniotomies when the patient was re-admitted 10 months following the initial resection at the outside hospital for bitemporal vision loss. The brain MRI showed recurrence of solid enhancing tumor. **E)** The MRI scan after the patient received dabrafenib alone for 17 days with 52% reduction in enhancing tumor volume. **F)** The MRI scan after an additional 14 days of dabrafenib and trametinib treatment; the final volumetric reduction in the enhancing mass was 85%. **G)** The tumor volume for both the enhancing (**red**) and cyst (**blue**) components. Note logarithmic axes.

Further experience is needed to define the frequency, durability, and extent of BRAF treatment response in papillary craniopharyngiomas.

Neoadjuvant treatment requires minimally invasive or noninvasive tumor diagnosis. While MRI characteristics that suggest the diagnosis of papillary craniopharyngioma have been described (15), currently definitive diagnosis requires a tissue biopsy using transcranial stereotactic or transsphenoidal endoscopic techniques. Our finding of detectable BRAFV600E in peripheral blood was unexpected; we know

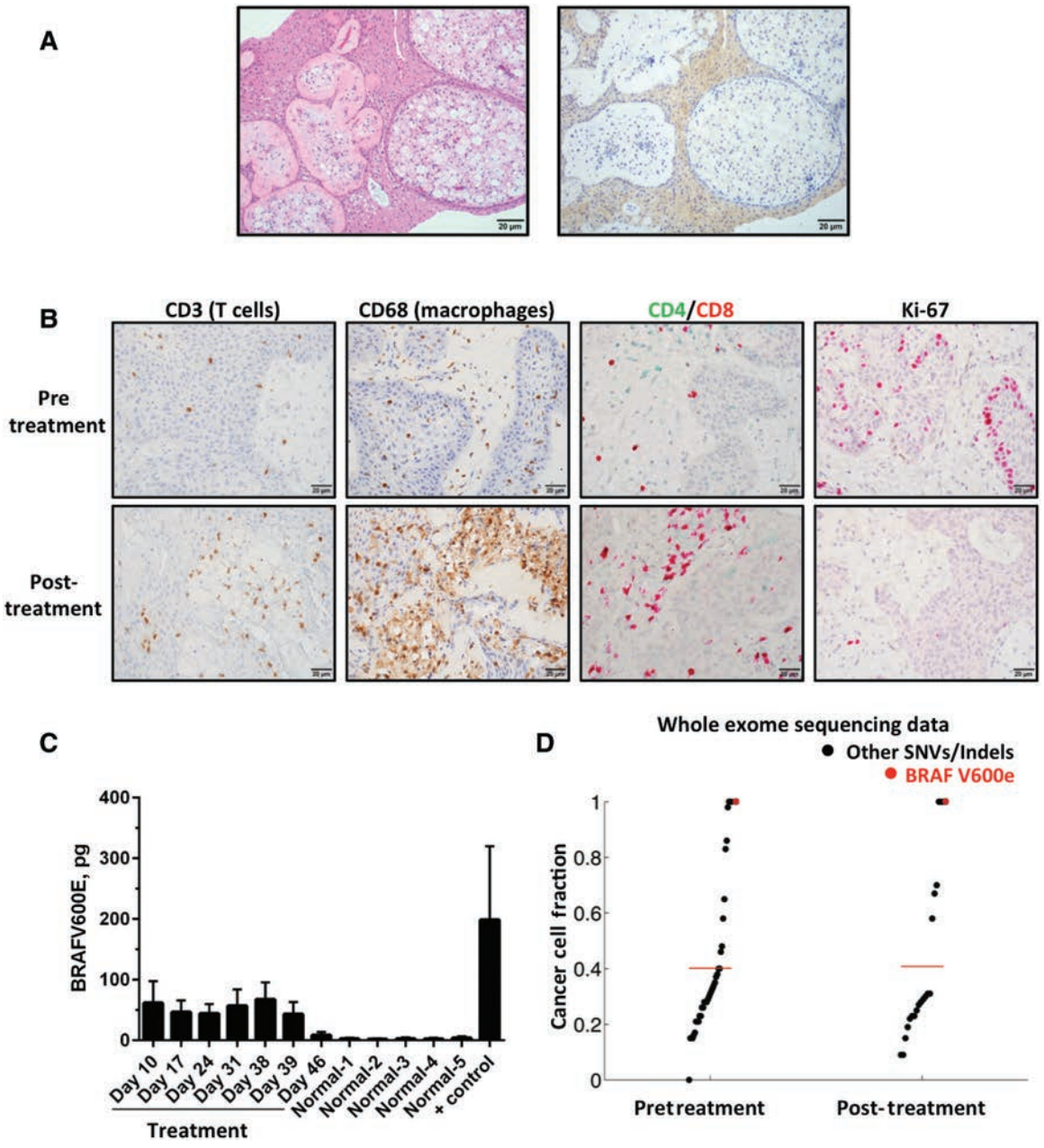

Figure 2. Characterization of dabrafenib/trametinib effects in a craniopharyngioma using pre- and on-treatment samples. **A)** Hematoxylin and eosin (H&E)–stained section (**left**) of the resected residual papillary craniopharyngioma tissue post-treatment that shows that some of the fibrovascular cores are engorged with foamy macrophages, while others are densely sclerotic. **A)** The immunohistochemistry (IHC) for BRAF V600E (VE1 antibody) on the post-treatment resection specimen. **B)** IHC for immune markers on pre- and post-treatment samples, including CD3 (T cells), CD4 (**green**)/CD8 (**red**), CD68 (macrophages). B) IHC for Ki-67 (Mib1) (**right panels**) to monitor proliferative index pre- and post-treatment. **C)** Detection of circulating BRAF V600E RNA in the blood of a patient with papillary craniopharyngioma using a polymerase chain reaction–based assay. Samples: blood samples were available starting from the 10th day after dabrafenib treatment was started and were taken weekly thereafter. Trametinib was initiated on Day 20. Mean values and standard deviation **error bars** are plotted. Surgery was performed on Day 38; Normal 1–5 are controls from individuals with no history of papillary craniopharyngioma or other BRAF V600E mutant tumor. D) The cancer cell fraction (CCF) distribution plot of single nucleotide variations and small insertions/deletions (Indels) from whole exome sequencing data from the pre- and post-treatment resection samples. Indicated in **red** is BRAF V600E, which has a cancer cell fraction of 1 indicating clonal status. All other events are in **black**. The **red line** indicates the mean CCF. SNV = single nucleotide variation.

of no previous example of circulating tumor cells or cell-free DNA in intracranial benign tumors. A possible caveat is that the DNA may have been released as a consequence of the multiple surgical procedures, the concurrent drug treatment, or both. If the presence of BRAFV600E can be detected before any treatment, this would offer the opportunity to test prospectively for V600E in the blood of craniopharyngioma patients and thereby facilitate neoadjuvant-targeted therapy. Shrinking tumors before definitive treatment with surgery/radiation could improve the safety and efficacy of initial treatment, potentially reducing the disabling morbidities that often follow current treatment.

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