Case Series

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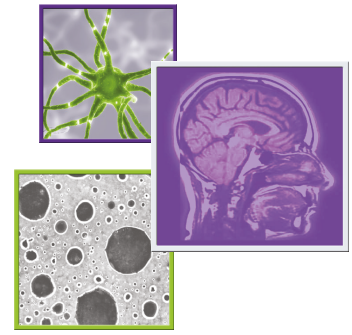

Challenges of targeting *BRAF* V600E mutations in adult primary brain tumor patients: a report of two cases

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Aim: Therapeutic targeting of *BRAF* alterations in primary brain tumor patients has demonstrated clinical activity in case reports and early trials; however, there is limited high-level evidence of the efficacy. **Patients & results:** Targeting *BRAF* V600E mutations with concurrent dabrafenib and trametinib in anaplastic pleomorphic xanthoastrocytoma resulted in a transient radiographic and clinical response and no therapeutic benefit in a patient with an epithelioid glioblastoma. **Conclusion:** *BRAF*/*MEK* inhibition did not produce a durable treatment effect in glioblastoma or pleomorphic xanthoastrocytoma with *BRAF V600E* alterations. Heterogenicity of related cases in the literature makes an evaluation of efficacy *BRAF* targeting therapies in gliomas difficult and requires additional investigation.

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Next-generation sequencing of cancers has become commonly used to identify potentially targetable alterations [1]. *BRAF* is a gene that has roles in cellular proliferation, apoptosis, angiogenesis, migration and differentiation via the RAS/RAF/MEK/ERK kinase pathway. When mutated in cancers, it acts as an oncogene, leading to uncontrolled proliferation [2]. Targeting this pathway with *BRAF* and *MEK* inhibitors has been a successful approach to treat some cancers, including *BRAF* V600E mutant anaplastic thyroid cancer [3], metastatic non-small-cell lung cancer [4] and melanoma [5]. Among primary brain tumor subtypes, *BRAF* V600E mutations are found in up to two-thirds of pleomorphic xanthoastrocytomas, up to a half of gangliogliomas and in nearly a tenth of pilocytic astrocytomas [6]. The fusion oncogene *KIAA1549-BRAF* has also been identified in up to 66% of pilocytic astrocytomas and pediatric low-grade diffuse astrocytomas but at lower rates in higher grade gliomas [7–9]. There have not been any randomized trials in adults using *BRAF*-targeting therapies in gliomas and the literature is limited to one early open basket trial and approximately 28 case reports [10,11]. However, these case reports are likely biased toward unusually positive results. Here, we describe cases of anaplastic pleomorphic xanthoastrocytoma (PXA) and epithelioid glioblastoma with *BRAF* V600E mutations treated with trametinib and dabrafenib with variable outcomes.

Case series

Case 1

A 23-year-old woman presented with headaches and, after a near-total resection, was found to have a right temporal-parietal lobe mass diagnosed initially as a glioblastoma, IDH-wild type. She received standard therapy of concurrent radiation and temozolomide. Next-generation sequencing using DNA isolated from formalin-fixed paraffin-embedded tumor tissue identified a *BRAF* V600E alteration (with calculated mean allele frequency of 71%) and homozygous deletion of *CDKN2A/B*. Re-evaluation of the tissue slides revised the diagnosis to an anaplastic

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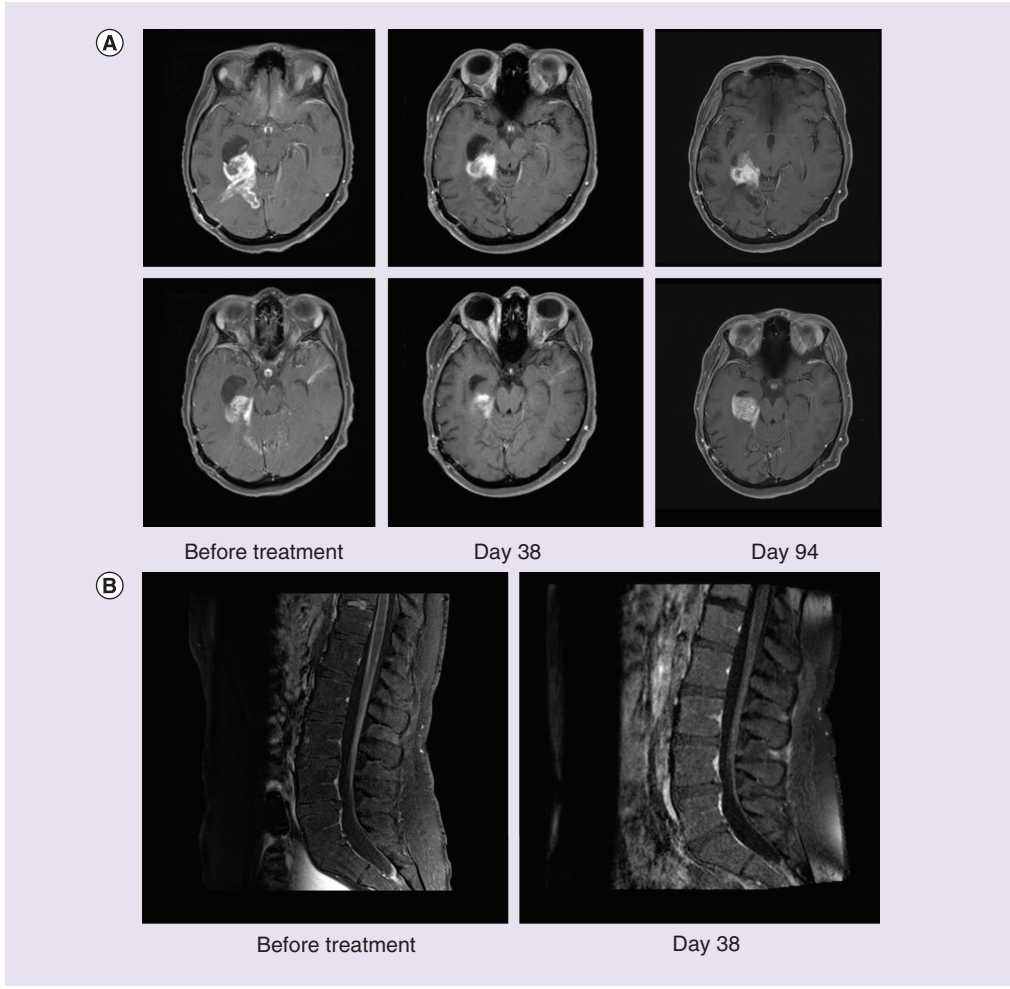

Figure 1. Gadolinium-enhanced T1 sequences magnetic resonance imaging from case 2. (A) Right temporal anaplastic pleomorphic xanthoastrocytoma with *BRAF* V600E mutation before, 38 and 94 days after initiating dabrafenib at 150 mg twice a day and trametinib 2 mg daily. Partial treatment response is seen at day 38 of treatment followed by reoccurrence at day 94 of treatment. **(B)** Diffuse enhancement of the cauda equina before (left) and radiographic improvement (right) 38 days after concurrent treatment with dabrafenib and trametinib. Recurrence in the spine occurred on day 94 (not shown).

PXA. A total of 161 days after diagnosis, she was admitted to the hospital for a clinical decline and was found to have radiographic progression, including a mass in the right temporal horn of the lateral ventricle and marked diffuse enhancement of cauda equina and distal spinal cord meninges consistent with leptomeningeal metastasis. She was started on dabrafenib 150 mg twice daily and trametinib 2 mg by mouth daily, which she tolerated well. Follow-up imaging 38 days later showed a decreased size of the right temporal lesion from 5.2 × 3 cm to 2.3 × 2.2 cm and markedly decreased enhancement of the cauda equina and conus (Figure 1). Clinically, she had lessened back pain and improved energy levels. Durable response to targeted therapy was not seen and, 94 days after starting targeting therapy, she had radiographic and clinical decline. She was transitioned to monotherapy with bevacizumab dosed at 10 mg per kg every 2 weeks intravenously and transitioned to hospice. Her overall survival from tissue diagnosis was 292 days and from the start of concurrent dabrafenib and trametinib was 131 days.

Case 2

A 47-year-old man presented with auditory and visual hallucinations, followed by a generalized seizure. After gross-total resection, he was found to have a right temporal lobe mass diagnosed as anaplastic astrocytoma, IDH-wild type, with molecular features of glioblastoma grade IV based on EGFR amplification, O-6-methylguanine-DNA methyltransferase (MGMT) promotor non-methylated. He received concurrent radiation and temozolomide

without adjuvant temozolomide. He had reoccurrence 2 years later and was treated with two additional cycles of temozolomide before stopping due to continued progression. He was placed on infusions of bevacizumab dosed at 10 mg per kg every 2 weeks intravenously for 8 months until radiographic progression, including a new extracranial extension of tumor in the right parotid gland and lymph nodes. Subtotal resection of the tumor was consistent with epithelioid glioblastoma. Next-generation sequencing using DNA isolated from formalin-fixed paraffin-embedded tumor tissue demonstrated mutations in *BRAF* V600E (with calculated mean allele frequency of 57%), *EGFR* T263P, amplification of *CDK6*, point mutation of *TERT* (promoter-146C>T), androgen receptor (G461_G473del) and homozygous deletion of *CDKN2A/B*. The patient underwent a further 2 months of treatment with bevacizumab and additional radiation to his neck, parotid gland and intracranial mass for a total of 6000 cGy in 30 fractions. Further progression was seen 3.5 months later and included a new lesion in the left lateral ventricle. He was started on oral dabrafenib 150 mg twice daily and trametinib 2 mg daily, which he tolerated well. Only 43 days later, he demonstrated further clinical and radiographic progression and was transitioned to hospice care. His overall survival from initial tissue diagnosis was 3.3 years and from his second surgery was 240 days.

Discussion

Both cases had *BRAF* V600E alterations targeted by dabrafenib and trametinib without durable response. Patient one, with an anaplastic PXA, had a transient response followed by rapid progression. The patient with epithelioid glioblastoma in case 2 had no benefit. Notably, the patient with epithelioid glioblastoma also had a gain of function mutation of *EGFR* (T263P). Colon cancers frequently have high expression of *EGFR* and further activation of *EGFR* has been seen in *BRAF* V600E mutant colon cancers treated with *BRAF* inhibitors and subsequent treatment resistance [12]. Conversely, *EGFR* expression in melanoma tends to be low and has been suggested to be a contributing reason why there has been an 80% response rate to BRAF inhibition in melanoma versus 5% in colon cancer [13,14]. This observation has also been seen in preclinical studies, showing that *EGFR* amplification in glioma cell lines leads to resistance to *BRAF* inhibition and that blocking *EGFR* may be a means to overcome resistance [15]. This resistance could be mediated in part to adaptive feedback reactivation of MAPK signaling, often mediated by *EGFR* which can, in turn, lead to activation of other *RAF* kinases, such as *CRAF*, which are resistant to *BRAF* inhibitors (Figure 2) [16,17]. MEK inhibitors are also known to enhance epidermal growth factor-induced *AKT* activation [18,19]. Studies in prostate cancer have shown that androgen receptor signaling also activates the RAS/MAPK pathway [20]. However, it is unclear to what degree the androgen receptor alteration in case 2 contributed to resistance to *BRAK/MEK* inhibition and there is insufficient data to support using androgen deprivation in primary brain tumor patients. Even without *EGFR* amplification, *BRAF/MEK* inhibition results in a multitude of poorly understood resistance mechanisms, including negative regulation between *RAS-ERK* and *PI3K-AKT* (Figure 2) [16,21].

Almost all reported cases of adult brain tumor patients with *BRAF* alteration have been treated with targeting therapy after progression on temozolomide and some also after bevacizumab [10]. Including the patients presented here, many cases in the literature have concurrent treatments including temozolomide, carmustine, tumor treating fields and surgically placed carmustine wafers, making assessments of efficacy difficult [22–24]. Further complicating the issue, of the 28 or more adult *BRAF* V600E mutant glioma case reports described in the literature, there is a mixed use of dabrafenib versus vemurafenib, with or without trametinib [10]. Dabrafenib is believed to have superior enhanced blood–brain barrier penetration compared with vemurafenib, as evidenced by increased response rates (16 vs 31%) in intracranial melanoma and, similarly, concurrent MEK with *BRAF* inhibition may delay resistance to therapy [10,25]. There are currently no trials to support which combination of these therapies are superior in primary brain tumors. Targeting *BRAF* alterations in glioma patients may yield different responses across histologies, similar to our experience and what has been observed in a Phase II basket study of a variety of malignancies [26]. A recent open-label, nonrandomized, multicohort trial evaluated 24 patients with *BRAF* V600E mutant brain tumors, which included PXAs, gangliogliomas and high-grade diffuse astrocytomas treated with vemurafenib. In contrast to the positive single case reports and, similar to our presented cases, an overall response rate of 25% with mixed results was seen in each group [11]. Limitations to targeting therapies in neuro-oncology patients include overcoming the blood–brain barrier [27] and intratumor heterogeneity [28,29].

Experience with BRAF targeting therapies in pediatric brain tumors is generally limited to case reports and series. This includes a complete response to vemurafenib in a 9-year-old boy with glioblastoma with a *BRAF* V600E mutation with no description of EGFR or loss of *CDKN2A* [30]. Another case series of four children with recurrent PXAs with *BRAF* V600E mutations were treated with vemurafenib and found progressive disease in one patient,

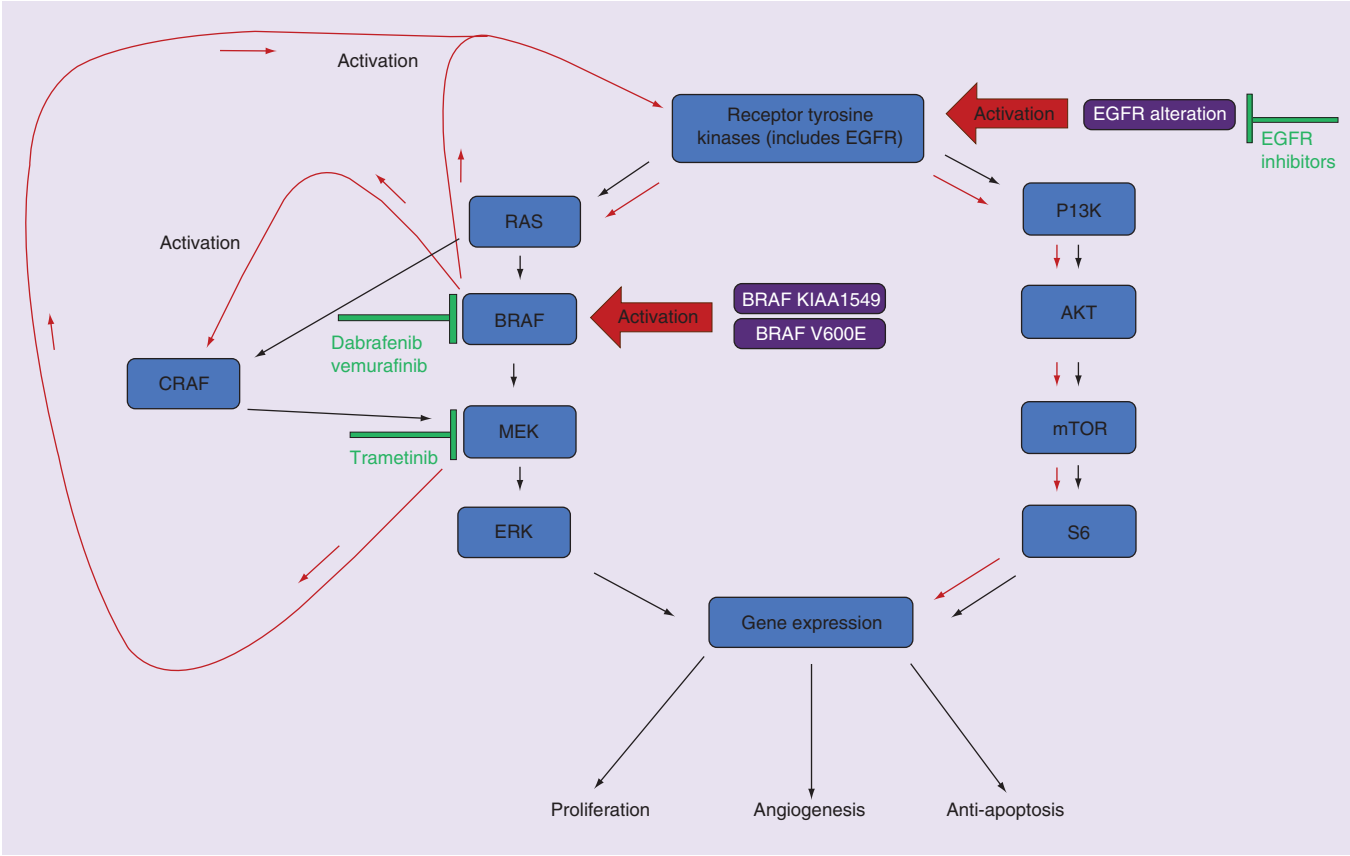

Figure 2. RTK/MAPK feedback pathways of RAS/P13K following *BRAF* and *MEK* inhibition. Inhibition of *BRAF* leads to reduced *ERK*-dependent feedback and increase activation of RTK and activation of alternative RAF, such as *CRAF* which stimulates *MEK* resulting in reactivation of the MAPK pathway. Inhibiting *MEK* concurrently with *BRAF* helps prevent reactivated MAPK pathway but results in increased activation of RTK and increased P13K/AKT pathway activation, which can result in treatment resistance. Alterations in *EGFR* that result in amplification may result in resistance to *BRAF* inhibition and are a possible concurrent therapeutic target. Red arrows indicate an increase of activation of pathways.
AKT: Protein kinase B; BRAF: Proto-oncogene B-Raf; CRAF: Proto-oncogene c-RAF; mTOR: Mammalian target of rapamycin; P13K: Phosphoinositide 3-kinase; RTK: Receptor tyrosine kinase; S6: p70 ribosomal protein S6 kinase.

stable in two and a partial response in another, with a median progression free survival of 5 months and overall survival of 8 months [31]. Recently, there was a preliminary analysis of a Phase I/II trial (NCT01677741) using monotherapy with dabrafenib in 32 recurrent pediatric patient brain tumor patients (of which 41% were pilocytic astrocytomas and 22% were gangliogliomas) with *BRAF* V600E mutations had an overall response rate of 44% [32]. In pediatric gliomas, *BRAF* V600E and *CDKN2A* mutations have a tendency to undergo malignant transformation and have poorer outcomes. Additionally, loss of *CDKN2A* has been identified to contribute independently to poor outcomes in *BRAF* V600E mutant pediatric low-grade glioma [33,34]. Based on this observation, it is possible that the loss of *CDKN2A* in the patients presented could also play a role in resistance to targeted therapy. However, this is contradicted by the findings in a boy with low-grade astrocytoma with a *BRAF* V600E mutation treated with dabrafenib for 40 weeks before progression who was found to have acquired a *BRAF* L514V mutation, leading to resistance after genetic analysis of tissue before and after targeted therapy [35]. These studies, similar to those in adults, are difficult to interpret given the heterogeneity of histologies and treatments and should be interpreted with caution when extrapolating to adults and vice versa.

Notably, both presented cases represent treatment with BRAF/MEK inhibitors, without other concomitant therapy, after definitive radiographic progression outside of prior radiation fields. Combinatorial multiagent regimens may be favored in the future but remain to be seen.

Conclusion

In this case series, minimal clinical benefit was seen after targeting *BRAF* V600E in anaplastic PXA and glioblastoma with concurrent dabrafenib and trametinib. These cases suggest that targeting *BRAF* alterations through *BRAF*/*MEK* inhibition in the treatment of high-grade diffuse gliomas in adult patients will not be as successful as it is in other *BRAF* mutated cancers. Based on studies of different malignancies, *EGFR*-amplified gliomas with *BRAF* alterations may be resistant to *BRAF* inhibition. To avoid publication biases, there is a need to report poor responders in brain tumor patients treated with molecularly targeted therapies in *BRAF* and other alterations. Additional studies are needed to understand when these targeting agents could play a role in combination with other treatments to overcome redundant oncogenic molecular pathways.

Executive summary

- Targeting *BRAF* mutations has shown to be beneficial for a variety of cancers; however, there is limited high-level evidence of the efficacy in primary brain tumors.
- Concurrent inhibition of *BRAF* and *MEK* with dabrafenib and trametinib did not produce a durable treatment effect in pretreated adults with recurrent glioblastoma or anaplastic pleomorphic xanthoastrocytoma with mutations in *BRAF*V600E.
- Similar to other cancers, a gain of function mutations in *EGFR* could lead to resistance to *BRAF*-targeting therapies in primary brain tumors and may be a concurrent therapeutic target with *BRAF*-inhibiting treatments.
- Heterogenicity of *BRAF*-targeting therapy in primary brain tumors in the literature makes an evaluation of the efficacy of these therapies difficult and requires additional investigation.

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