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REVIEW ARTICLE

BRAF Mutations in CNS Tumors—Prognostic Markers and Therapeutic Targets

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Abstract

Gliomas are a heterogeneous group of brain tumors with limited therapeutic options. However, identification of *BRAF* V600E mutations in a subset of gliomas has provided a genomic-targeted approach for management of these diseases. In this review, we aimed to review the role of *BRAF* V600E in gliomagenesis, to characterize concurrent genomic alterations and their potential prognostic implications, and to review comprehensively the efficacy data of *BRAF* inhibitors (combined or not with *MEK* inhibitors) for the treatment of low- and high-grade gliomas. We also provide a summary of the toxicity of these agents and describe resistance mechanisms that may be circumvented by alternative genomic approaches. Although the efficacy of targeted therapy for management of *BRAF* V600E-mutant gliomas has mostly been assessed in small retrospective and phase 2 studies with heterogeneous populations, the data generated so far are a proof of concept that genomic-directed therapies improve outcomes of patients with refractory/relapsed glioma and underpin the need of comprehensive genomic assessments for these difficult-to-treat diseases. In the future, the role of targeted therapy in the first-line setting and of genomic-directed therapies to overcome resistance mechanisms should be assessed in well-designed clinical trials.

Key Points

Targeted therapy with *BRAF* and *MEK* inhibitors improves outcomes of patients with *BRAF* V600E-mutant gliomas refractory to standard treatments.

The efficacy of targeted therapy in patients with *BRAF* V600E-mutant glioma underscores the importance of comprehensive genomic assessments for patients with central nervous system tumors.

Genomic-directed strategies to overcome resistance mechanisms should be assessed in clinical trials.

1 Introduction

Gliomas are a heterogeneous group of primary neoplasms of the central nervous system (CNS) that differ in clinical presentation, molecular characteristics, and prognosis. The 2021 World Health Organization (WHO) classification of CNS tumors has acknowledged the evolving understanding of the molecular characteristics of gliomas and has introduced new subtypes based on specific molecular alterations [1, 2]. However, management of patients with glioma has not changed substantially in the last two decades, as treatment continues to be based on gross tumor resection that can be followed by either radiation therapy, chemotherapy, or a combination of both [3, 4]. This is particularly concerning for patients with high-grade and refractory gliomas, for whom therapeutic options are limited and survival is dismal [5–8].

Identification of driver alterations has led to the development of targeted therapies that have resulted in improvements in recurrence-free survival, progression-free survival, and overall survival across several solid malignancies [9–13]. One such alteration occurs in the v-raf murine viral oncogene homolog B1 (*BRAF*). In physiological conditions, *BRAF* regulates the mitogen-activated protein kinase (MAPK) pathway, which is involved in the expression of genes related to cellular proliferation and survival. *BRAF* is

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also implicated in the development of adult tissues, including the CNS [14]. The most frequent alteration in *BRAF* is a point mutation characterized by substitution of valine for glutamic acid in codon 600 (V600E), which causes constitutive *BRAF* activation, independent of upstream *RAS* signaling [15]. *BRAF* mutations are frequent in melanomas [16] and also occur in colorectal cancers [17], non-small cell lung cancer [18], and thyroid carcinomas [19]. In gliomas, *BRAF* mutations have been identified across several histological subtypes and are more frequent in low-grade tumors [20]. However, the prognostic significance of *BRAF* mutations in gliomas is still debatable in the literature [21–23].

Dual inhibition of *BRAF* and mitogen-activated protein kinase kinase (*MEK*) is a standard of care for patients with *BRAF* V600-mutant melanomas, non-small cell lung cancers, and thyroid carcinomas [9–11, 13, 24, 25]. Targeted therapies, either with *BRAF* inhibitor monotherapy or in combination with a *MEK* inhibitor, have also been evaluated in patients with *BRAF* V600E-mutant recurrent or refractory gliomas in small retrospective series and prospective studies, with some patients benefiting from durable responses and extended survival [26–31]. However, there is no consensus on the best timing of initiation of these targeted therapies in patients with gliomas, and there is a concern regarding mechanisms of resistance and long-term toxicities, especially in patients with low-grade tumors [4, 32, 33].

In this review, we aim to discuss the role of *BRAF* in gliomagenesis and the prognostic implications of concurrent genomic alterations, as well as to describe the current landscape of the management of patients with *BRAF* V600E-mutant gliomas. We also discuss toxicities related to *BRAF* and *MEK* inhibition, and we summarize the evidence on mechanisms of resistance and possible approaches to overcome them.

2 *BRAF* V600 Mutations in Gliomas: Gliomagenesis, Concurrent Genomic Alterations, and Prognostic Implications

BRAF mutations are grouped in three classes according to their kinase activity. Class I *BRAF* mutations are characterized by strong activation and increased kinase activity of the MAPK pathway (approximately 500–700 fold as compared with wild-type *BRAF*). Class I *BRAF* mutations generate abnormal proteins that are constitutively activated without the need of dimerization and are sensitive to *BRAF* and *MEK* dual inhibition [34]. *BRAF* V600E is the most frequent class I mutation found in gliomas [35]. Class II mutations have lower kinase activity as compared with class I mutations, usually occur in the activation segment, and signal as *RAS*-independent dimers [34]. *KIAA1549*:*BRAF*, a *BRAF* fusion frequently found in pilocytic astrocytoma, functions as a class II *BRAF* mutation [36]. Class III *BRAF* mutations have lower kinase activity as compared to wild-type *BRAF* but may be implicated in tumorigenesis when they dimerize in the context of *RAS* upstream activation [34]. Class III mutations represent only 10% of *BRAF* mutations in gliomas, and are associated with *NF1* loss-of-function mutations or *EGFR* amplification [35].

The frequency of *BRAF* mutations in gliomas varies according to histological subtypes and age of presentation. In the pediatric population, *BRAF* V600E mutation occurs in 7% of all glioma cases [37], but it can occur in 20% of pediatric low-grade gliomas (LGGs) [38]. In adults, *BRAF* V600E mutation occurs in only 4.6% of cases (mostly young adults), more frequently in epithelioid glioblastoma, pleomorphic xanthoastrocytoma, and anaplastic pleomorphic xanthoastrocytoma [37, 39]. However, it is possible that the frequency of *BRAF* V600E mutation in adult patients with glioma is underestimated, since *BRAF* testing is not routinely performed in all institutions. This is suggested by a single-center study in Japan in which all patients had access to comprehensive genomic profiling testing. In that cohort, 8% of patients with glioma had a *BRAF* V600E mutation [40]. In a study with 1320 central nervous system tumor samples, 96 *BRAF* mutations were detected (93 *BRAF* V600E), which were more frequently found in pleomorphic xanthoastrocytoma (63% of adult cases, 69% of pediatric cases), anaplastic pleomorphic xanthoastrocytoma (38% of adult cases, 100% of pediatric cases), and ganglioglioma (21% of adult cases, 18% of pediatric cases) [20]. Upon recurrence, *BRAF* V600E-mutant pediatric LGGs treated with either surgery, radiation, or chemotherapy continue to present *BRAF* V600E mutation in 98% of cases [41]. However, *BRAF* V600E-mutant glioblastoma subclones may not expand after exposure to radiation and alkylating chemotherapy, notwithstanding their theoretical proliferative advantage, and these tumors will not present *BRAF* V600E mutation at the time of recurrence [42]. Despite sharing the same point mutation, the prognosis of patients with *BRAF* V600E-mutant gliomas varies widely [39, 43], which implies that *BRAF* V600E is not the sole driver to influence tumorigenesis and clinical outcomes.

Preclinical data have demonstrated that *BRAF* V600E alone is insufficient to cause gliomagenesis. In a pilocytic astrocytoma model, neurospheres derived from human fetal cerebral cortex were infected with a lentivirus containing *BRAF* V600E. Although there was evidence of MAPK pathway activation in the infected cells, this did not result in significantly increased proliferation as compared to controls. These cells eventually stopped proliferating and demonstrated evidence of oncogene-induced senescence [44]. However, loss of function of p16Ink4a and p14Arf (products coded by *CDKN2A*) in association with *BRAF* V600E is sufficient to induce tumorigenesis, which implies that

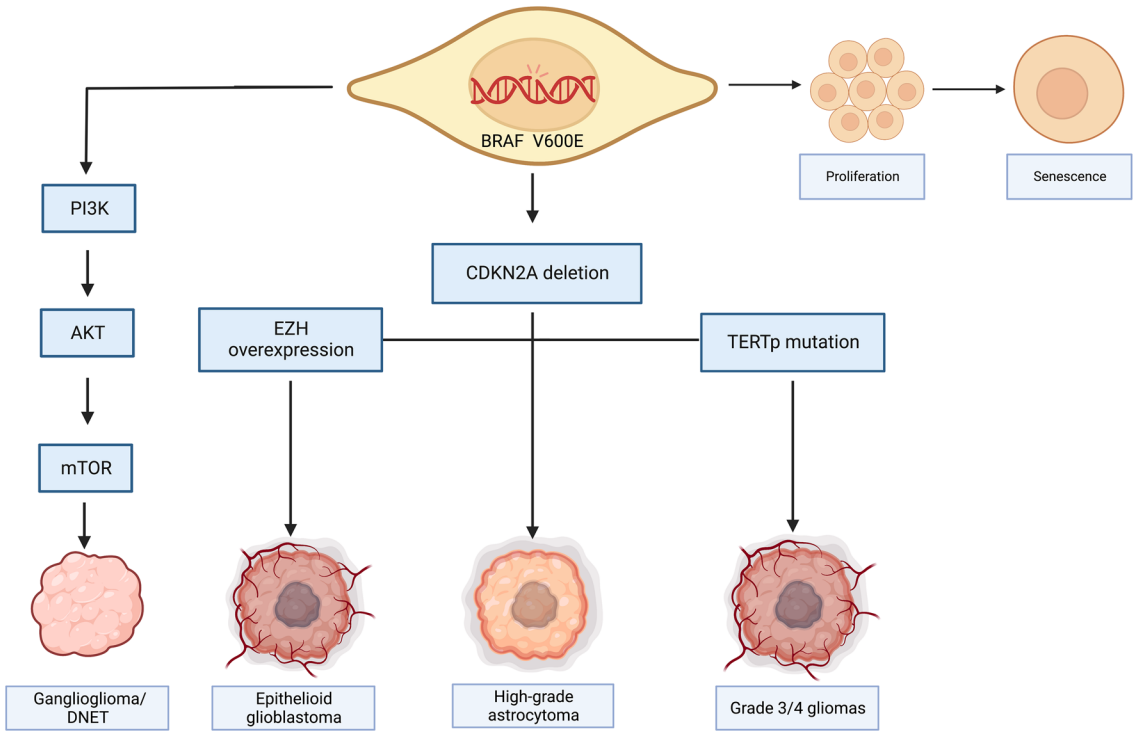

Fig. 1 Evolutionary pathways of a progenitor neural cell with *BRAF* V600E mutation and concurrent genomic alterations that lead to development of different gliomas. DNET: Dysembryoplastic neuroepithelial tumor; EZH: Enhancer of zeste homolog 2; TERTp: TERT promoter. Created with BioRender.com

deletion of *CDKN2A* supresses oncogene-induced senescence in neural progenitors [45]. Similarly, activation of the PI3K/Akt/mTOR pathway may also overcome senescence, as evidenced in *BRAF* V600E-mutant gangliogliomas and dysembryoplastic neuroepithelial tumors [46].

BRAF V600E mutation and *CDKN2A* homozygous deletion may be the initiating genomic events of a spectrum of gliomas with different histological characteristics and prognosis (Fig. 1). For example, in cases of epithelioid glioblastoma derived from anaplastic pleomorphic xanthoastrocytoma, the presence of *BRAF* V600E mutation and *CDKN2A* homozygous deletion has been evidenced in both low-grade and high-grade areas; however, differentiation to a more aggressive phenotype seems to be related to the overexpression of enhancer of zeste homolog 2 (EZH2), one of the proteins of the polycomb repressive complex 2, which catalyzes the trimethylation of H3K27. Overexpression of EZH2 has been observed in high-grade areas of epithelioid glioblastomas and of epithelioid differentiation in anaplastic pleomorphic xanthoastrocytomas carrying both *BRAF* V600E mutation and *CDKN2A* homozygous deletion, and it has been associated with worse survival [47]. Moreover, downregulation of both *CDKN2A* and *MTAP* (both localized at chromosome 9p21) has been observed in *BRAF* V600E-mutant pediatric high-grade gliomas (HGGs) [48].

The occurrence of *TERT* promoter mutation is associated with a higher histologic grade in *BRAF* V600E-mutant gliomas. Gabler et al [49]. developed a panel of glioma-derived cell lines to analyze the interplay between *BRAF* V600E and *TERT* promoter mutations. They observed that only cell lines with both *BRAF* V600E and *TERT* promoter mutation expressed *TERT* mRNA, and this finding was significantly more frequent in grades 3 and 4 gliomas as compared to LGGs (28% versus 3%, $p = 0.003$). Additionally, only *BRAF* V600E-mutant tumor cells with loss of *CDKN2A* and *TERT* promoter mutation developed stable and immortalized cell lines. These findings suggest that *TERT* promoter mutation and *CDKN2A* homozygous deletion have synergistic effects that lead to loss of oncogene-induced senescence caused by *BRAF* V600E mutation, and this results in a more aggressive phenotype.

The prognosis of patients with *BRAF* V600E-mutant gliomas is the result of a combination of histological grade and concurrent genomic alterations, as suggested by clinical observations. In a meta-analysis with survival data from 1308 patients with glioma, the presence of *BRAF* V600E mutation was associated with improved survival, but this benefit was restricted to young patients (< 35 years old) and with LGGs [21]. It is possible that some of these patients may have "molecularly defined glioblastoma," i.e.,

a histologically low-grade tumor with the molecular characteristics of a glioblastoma (e.g., *IDH* wild-type, gain of chromosome 7/loss of chromosome 10); nonetheless, these patients still had good outcomes [50]. In contrast, the presence of *BRAF* V600E mutation in gangliogliomas, a WHO grade 1 tumor more frequent in children, adolescents, and young adults, is associated with shorter recurrence-free survival [23]. Parenthetically, the co-occurrence of *H3K27M* mutation with *BRAF* V600E mutation in patients with grade 1 gangliogliomas does not result in the adverse prognosis seen in patients with *H3K27M*-mutant diffuse midline gliomas [51]. In adults, *BRAF* V600E-mutant gliomas are less likely to have co-occurring *IDH1/2*, *ATRX*, and *TP53* mutations, but homozygous deletion of *CDKN2A* or *CDKN2B* is a frequent co-occurring genomic event [22], and is also a marker of high-grade malignant astrocytomas in children [52]. Indeed, in patients with *BRAF* V600E-mutant pediatric LGGs, progression-free survival and overall survival were worse as compared with their *BRAF* wild-type counterparts (10 year progression-free survival for *BRAF* V600E-mutant versus *BRAF* wild type: 27% versus 60.2%; 10 year overall survival: 83.9% versus 92.1%), and patients with *CDKN2A* homozygous deletion and *BRAF* V600E mutation had worse outcomes than patients with *CDKN2A* homozygous deletion alone (10 year progression-free survival: 0% versus 45.9%, respectively) [53]. Therefore, a comprehensive genomic evaluation of *BRAF* V600E-mutant gliomas is necessary to prognosticate adequately and treat these tumors. This may be difficult in the context of lesions that are not amenable to surgical resection, but liquid biopsy may be an alternative to molecularly characterize these tumors [54].

3 Targeted Therapy in *BRAF* V600E-Mutant Gliomas: the Evidence

The efficacy of targeted therapies in other *BRAF* V600-mutant malignancies [9–13, 24, 25], including in patients with melanoma brain metastases [55], has led to the investigation of these agents for management of gliomas. In the preclinical setting, the *BRAF* inhibitor PLX-4720 resulted in increased survival in a murine astrocytoma model, and this was augmented when combined with palbociclib, a CDK4/6 inhibitor. Similar results were obtained in a human xenograft astrocytoma model with *BRAF* V600E mutation and *CDKN2A* deletion [45]. In a *BRAF* V600E-mutant pediatric glioma cell model, activity of the MAPK pathway was reduced by 59–63% with vemurafenib and by 72–74% with trametinib [56]. However, combined inhibition of *BRAF* and *MEK* results in more pronounced and prolonged inhibition of the MAPK pathway in glioma cell models than either agent alone, and this translated into more potent tumor growth inhibition in xenograft models while preventing *ERK* paradoxical reactivation and reducing the risk of development of cutaneous squamous cell carcinoma related to *BRAF* inhibitor monotherapy [57, 58].

In the clinical setting, activity of targeted therapy in patients with *BRAF* V600E-mutant gliomas has been demonstrated in several case reports [59–64], as well as in retrospective series. In a retrospective study with nine patients with *BRAF* V600E-mutant pediatric LGGs, the objective response rate (ORR) with dabrafenib monotherapy was 41.7%, disease control rate (DCR) was 100%, and progression-free survival was 26.1 months [32]. In another study, vemurafenib monotherapy led to treatment response in 57% (4/7) of patients with pediatric LGGs [65]. In a multiinstitutional retrospective series comprising 56 patients with pediatric LGGs (mostly pilocytic astrocytomas and gangliogliomas), dabrafenib monotherapy resulted in ≥ 25% tumor reduction in 80% of patients. Tumor responses were achieved at a median of 4 months and were sustained with a median time on treatment of 17.4 months. The presence of *CDKN2A* homozygous deletion did not seem to affect treatment response. Progressive disease was observed in eight patients, five of which achieved tumor control after adding a *MEK* inhibitor to dabrafenib therapy [30].

These results were replicated in a phase 1/2a clinical trial in pediatric patients with refractory *BRAF* V600E-mutant LGGs. In 32 evaluable patients, ORR with dabrafenib monotherapy was 44%, median duration of response was 26 months, and disease control rate was 78% [66]. In another phase 1 study with 19 patients with pediatric LGGs, the ORR with vemurafenib monotherapy was 32% and some responses were maintained with 40 months of follow-up [67]. A phase 1 trial of trametinib alone or with dabrafenib in pediatric patients with refractory LGGs demonstrated higher ORR in patients receiving combination therapy (25% versus 15%), and progression-free survival was longer in patients receiving dual inhibition (36.9 versus 16.4 months) [68]. Finally, a randomized phase 2 study of dabrafenib and trametinib versus carboplatin and vincristine in patients with relapsed pediatric LGGs demonstrated superiority of targeted therapy with higher ORR (47% versus 11%) and superior median progression-free survival (20.1 versus 7.4 months) [69]. These results underpin the clinical efficacy of targeted therapy in patients with refractory pediatric LGGs.

Evidence for first-line treatment with targeted therapy in patients with pediatric LGG is scarce, with one retrospective study (including patients with different MAPK pathway alterations) demonstrating an ORR of 75% and DCR of 100% [70]. In another retrospective study with 19 patients with pediatric HGG treated with upfront targeted therapy (11 with *BRAF* and *MEK* inhibitor combination, and 8 with *BRAF* inhibitor monotherapy), the response rate (in 14 evaluable patients) was 57%. The estimated progression-free survival at 3 and 5 years was 65% and 44%, respectively, and

overall survival at 3 and 5 years was 82%. These results compare favorably to historical controls of patients with *BRAF* V600E-mutant pediatric HGG treated with radiotherapy and conventional chemotherapy [71]. These studies suggest there is a survival advantage for using targeted therapy in the first-line setting, but confirmation of this hypothesis depends on results of ongoing prospective clinical trials.

In patients with relapsed or refractory pediatric HGGs, the combination of dabrafenib and trametinib was assessed in a single-arm phase 2 trial. Among 41 patients enrolled, ORR was 56.1%. Median progression-free survival was 9 months, median duration of response was 22.2 months, and median overall survival 32.8 months [72]. These results compare favorably to a retrospective cohort of patients with pediatric HGGs treated with dabrafenib alone (11 patients, ORR 36%, and median progression-free survival 10 months) [30].

In contrast to the pediatric population, data on the efficacy of targeted therapy in adults with *BRAF* V600E-mutant gliomas were very limited until recently. In a retrospective study of 28 adult patients with refractory or disseminated *BRAF* V600E-mutant gliomas with high-grade features, 13 patients were treated with *BRAF* inhibitor monotherapy, and 15 patients received a combination of *BRAF* and *MEK* inhibitors. In the whole cohort, tumor responses were achieved in 11 (39%) patients, and responding patients had a median reduction of tumor burden of 78%. The probability of response in patients treated with dual inhibition was not statistically different when compared with patients treated with *BRAF* inhibitor alone (27% versus 54%, $p = 0.25$). The authors observed that, despite being treated in the recurrent setting, responding patients achieved a median progression-free survival that was longer than achieved with standard first-line treatment (18 versus 7 months, $p = 0.047$). Additionally, tumor response was associated with improvement in performance status [31].

Adult patients with *BRAF* V600E-mutant gliomas have been evaluated in a few basket trials. The NCI-MATCH trial assessed the efficacy of dabrafenib and trametinib in patients with *BRAF* V600E-mutant solid tumors and included five patients with CNS tumors (one each of epithelioid glioblastoma, pilocytic astrocytoma, anaplastic astroblastoma, pleomorphic xanthoastrocytoma, and histiocytic sarcoma). Three of these patients were evaluable for response, and a partial response was seen in two of them, while the other patient had stable disease. One patient sustained response for approximately 15 months [29].

The activity of single-agent vemurafenib in patients with recurrent *BRAF* V600E-mutant gliomas was assessed in the VE-BASKET study, a multicohort, nonrandomized trial. In total, 24 patients with glioma were enrolled, of which 11 had malignant diffuse glioma (5 with anaplastic astrocytoma and 6 with glioblastoma) and 7 had pleomorphic xanthoastrocytoma. In the whole cohort, confirmed ORR was 25% (one partial response was observed in the malignant diffuse glioma subcohort, and one complete and two partial responses were observed in the pleomorphic xanthoastrocytoma subcohort). Despite utilizing RECIST criteria for assessing response, the responses rates reported in the VE-BASKET study would be similar if RANO criteria were applied, as all but two responders (both in the pleomorphic xanthoastrocytoma subcohort) had at least 50% reduction in the sum of the largest diameters of the target lesions. Median progression-free survival was 5.5 months, and median overall survival was 28.2 months. The later result was influenced by the outcomes of patients with pleomorphic xanthoastrocytoma (median overall survival not reached); patients with malignant diffuse glioma had a median overall survival of 11.9 months [26].

A small phase 2 trial assessed encorafenib and binimetinib in patients with recurrent *BRAF* V600E-mutant HGG. This trial enrolled only five patients, and it was closed because of slow accrual. Three patients achieved a radiological response (two complete responses) and another had stable disease [73]. The ROAR study prospectively evaluated the combination of dabrafenib and trametinib in patients with *BRAF* V600E-mutant tumors. This study enrolled 58 patients with recurrent glioma: 45 had HGGs (31 with glioblastoma), and 13 had LGGs. In the HGG cohort, ORR by RANO criteria was 33%, and median duration of response was 31.2 months. Median progression-free survival was 5.5 months and median overall survival was 17.6 months. In contrast, the ORR in the LGG cohort was 54%, and median duration of response, median progression-free survival, and median overall survival were not reached [27, 28].

It should be emphasized that patients with HGG treated with targeted therapy within the VE-BASKET and ROAR trials had been previously treated with multiple lines of therapy, and the results obtained in these studies compare favorably with lomustine and bevacizumab, an approved combination for treating patients with recurrent glioblastomas and grade 4 astrocytomas (median progression-free survival: 4.2 months; median overall survival: 9.1 months) [8, 26–28].

Table 1 summarizes the efficacy results of targeted therapy for patients with *BRAF* V600E-mutant gliomas across several studies.

Although with small numbers and with a heterogeneous population, results of both VE-BASKET and ROAR trials reveal the different biological behaviors of LGGs and HGGs when treated with targeted therapies. Similar to what is seen in the pediatric population [30], in whom *BRAF* V600E-mutant LGGs are more frequent, adult patients with low-grade *BRAF* V600E-mutant gliomas may achieve long-term control of disease with *BRAF* inhibitor alone; however, patients with HGG seem to require a more aggressive

Table 1: Efficacy of targeted therapy in BRAF V600E-mutant glioma across multiple studies

Low-grade glioma								
Author [ref]	Most frequent histology	Population	Type of study	Drugs tested	N	ORR	PFS[a]	OS[a]
Pérez et al. [32]	PA	Pediatric	Retrospective	Dabrafenib	9	41.7%	26.1 months	Not provided
Nobre et al. [30]	PA	Pediatric	Retrospective	Dabrafenib	56	80%	~3 years	Not provided
Del Bufalo et al. [65]	GG	Pediatric	Retrospective	Vemurafenib	7	57%	Not provided	Not provided
Hargrave et al. [66]	PA	Pediatric	Phase 1/2a	Dabrafenib	32	45%	35 months	Not reached
Nicolaides et al. [67]	PA	Pediatric	Phase 1	Vemurafenib	19	31.5%	Not reached	Not provided
Bouffet et al. [68]	Not specified	Pediatric	Phase 1/2	Dabrafenib + trametinib	36	52.8%	Not reached	Not provided
				Trametinib	13	38.5%	26.9 months	Not provided
Bouffet et al. [69]	PA	Pediatric	Phase 2	Dabrafenib + trametinib	73	47%	20.1 months	Not reached
Kaley et al. [26]	PXA	Adult	Basket trial	Vemurafenib	12	41.6%	5.7 months[b]	Not reached[b]
Subbiah et al. [28]	GG	Adult	Phase 2	Dabrafenib + trametinib	13	54%	Not reached	Not reached
High-grade glioma								
Author [ref]	Most frequent histology	Population	Type of Study	Drugs tested	N	ORR	PFS[a]	OS[a]
Berzero et al. [31]	GBM	Adult	Retrospective	Vemurafenib	11	55%	7 months (whole cohort)	39% at 2 years (whole cohort)
				Vemurafenib + cobimetinib	5	20%		
				Dabrafenib	2	50%		
				Dabrafenib + tramentinib	10	30%		
Nobre et al. [30]	GBM	Pediatric	Retrospective	Dabrafenib	11	36%	10 months	Not provided
Kaley et al. [26]	GBM	Adult	Basket trial	Vemurafenib	12	8.3%	5.3 months	11.9 months
Schreck et al. [73]	GBM and aPXA	Adult	Phase 2	Encorafenib + binimetinb	5	60%	Not provided	Not provided
Hargrave et al. [72]	GBM	Pediatric	Phase 2	Dabrafenib + trametinib	41	56.1%	9 months	32.8 months
Subbiah et al. [28]	GBM	Adult	Phase 2	Dabrafenib + trametinib	45	33%	5.5 months	17.6 months

aPXA: anaplastic pleomorphic xanthoastrocytoma; GBM: glioblastoma; GG: ganglioglioma; *N*: number of patients included; ORR: objective response rate; OS: overall survival; PA: pilocytic astrocytoma; PFS: progression-free survival; PXA: pleomorphic xanthoastrocytoma

[a]Numbers refer to median, unless otherwise indicated. [b]Results refer only to PXA cohort

inhibition of the MAPK pathway, which is achieved by the combination of *BRAF* and *MEK* inhibitors [57]. Given the lack of randomized data, the decision of whether treating a patient with *BRAF* inhibitor alone or combined with a *MEK* inhibitor may rely on tumor grade, toxicity, and possible mechanisms of resistance (discussed in detail below).

4 *BRAF* and *MEK* Inhibition-Related Toxicity

Data on short- and long-term toxicity of *BRAF* and *MEK* inhibitors are furnished largely from clinical trials in melanoma, in which these drugs were evaluated in phase 3 trials with long follow-up. Currently, there are three *BRAF* and *MEK* inhibitor combinations approved for metastatic melanoma based on randomized trials combining *BRAF* and *MEK* inhibitors in comparison to *BRAF* inhibitor monotherapy: dabrafenib and trametinib, encorafenib and binimetinib, and vemurafenib

Table 2 Adverse events more frequently associated with *BRAF* or *BRAF* and *MEK* inhibition

BRAF inhibitor monotherapy	*BRAF* + *MEK* inhibitor
Hyperkeratosis	AST/ALT increase
Skin papilloma	Decreased left ventricular ejection fraction
Keratoacanthoma	QT interval prolongation
Cutaneous squamous cell carcinoma	Hypertension
Alopecia	Serous retinopathy

and cobimetinib [10, 11, 74, 75]. These trials reveal there are class effect adverse events (i.e., that are common to any *BRAF* inhibitor or to any combination of *BRAF* and *MEK* inhibitors) and drug-specific adverse events (Tables 2 and 3).

Monotherapy with a *BRAF* inhibitor is associated with increased rates of cutaneous toxicities as compared with dual *BRAF* and *MEK* inhibition, especially hyperkeratosis (6–40%) and cutaneous squamous cell carcinoma/keratoacanthoma (8–29%) [10, 11, 13, 74]. Dual inhibition significantly reduces the incidence of cutaneous toxicities, but it is more likely to cause increase of AST (13–22%) and ALT (13–23%) [10, 11, 13, 75]. Additionally, the combination of *BRAF* and *MEK* inhibitors increases the rate of visual adverse events, such as blurred vision (16%), serous retinopathy (20%), and retinal detachment (8%) [10, 11, 13, 74, 76]. Fortunately, most of these events are mild and are primarily managed by close monitoring or temporarily withholding the drugs. The discontinuation rate of *BRAF* and *MEK* inhibitors due to adverse events varies between 10% and 16%, similar to the discontinuation rates of *BRAF* inhibitor monotherapy [10, 11, 13, 74, 75].

Cardiac adverse events, such as decreased left ventricular ejection fraction, cardiac failure, and QT interval prolongation, have been closely monitored in clinical trials on patients with melanoma and, despite being more frequent with *BRAF* and *MEK* dual inhibition, occur at a low incidence and are mostly mild (e.g., the incidence of grade ≥ 3 decreased left ventricular ejection fraction varies between 1% and 2%) [10, 11, 74]. In comparison with *BRAF* inhibitor monotherapy, the combination of *BRAF* and *MEK* inhibitors is associated with increased risk of pulmonary embolism (2.2% versus 0.4%, RR 4.36, 95% CI 1.23–15.44, $p = 0.02$), hypertension (19.5% versus 14%, RR 1.49, 95% CI 1.12–1.48, $p = 0.005$), and decreased left ventricular ejection fraction (8.1% versus 2%, RR 3.72, 95% CI 1.74–7.95, $p < 0.001$), particularly in patients younger than 55 years (RR 26.50, 95% CI 3.58–196.10, $p = 0.001$) [77]. Currently, there are no specific recommendations on how to monitor and treat patients with decreased left ventricular ejection fraction related to *BRAF* and *MEK* inhibitors, but holding the drugs and consultation with cardiology is recommended [78].

Dabrafenib and trametinib is the most frequently studied combination in the treatment of *BRAF* V600E-mutant gliomas [27–29, 69, 72]. Pyrexia is a frequent adverse event of this combination (mostly associated with dabrafenib), and it occurs in 59% of patients with melanoma [10]. In patients with LGG treated within the ROAR study, pyrexia occurred in 62% of patients; however, in the HGG cohort, the frequency of pyrexia dropped to 24% [27]. This difference is likely due to more frequent use of steroids in patients with HGG. Classically, pyrexia is managed by holding dabrafenib, prescribing antipyretics, continuing trametinib, and resuming dabrafenib after resolution of pyrexia. However, in an adjuvant trial of dabrafenib and trametinib for fully resected stage III melanoma, pyrexia was managed by immediately holding both drugs and by resuming them after the patient remained asymptomatic for at least 24 hours. This approach reduced the rate of grade ≥ 3 pyrexia and resulted in comparable rates of recurrence-free survival as holding dabrafenib alone [79]. The rates of fatigue and nausea are comparable between the melanoma and glioma trials [10, 27].

Importantly, the rate of neurotoxicity in patients with glioma was not increased while on treatment with dabrafenib and trametinib. Intracranial hemorrhage was not reported in the ROAR study, and seizures occurred in four (8.9%) patients in the HGG glioma cohort [27, 28]. This is consistent with the rates of tumor-related epilepsy in patients with HGG [80]. Headaches were observed in 45% of patients treated with dabrafenib and trametinib, which is in line with what was reported in patients with metastatic melanoma [10, 27, 55].

Vemurafenib causes skin rashes in 67.5% and photosensitivity reactions in 37.8% of patients when given as monotherapy; when combined with cobimetinib, the rate of skin

Table 3 Adverse events more frequently associated to each of the *BRAF* and *MEK* inhibitor combinations

Dabrafenib + trametinib	Encorafenib + binimetinib	Vemurafenib + cobimetinib
Pyrexia	CPK increased	CPK increased
Chills	Constipation	Photosensitivity
Peripheral edema	Abdominal pain	Diarrhea
	Dizziness	

rashes increases to 72.5% and photosensitivity reactions to 47.8% [81]. In the glioma cohort of the VE-BASKET study, the frequency of photosensitivity with single-agent vemurafenib was 38% (nine patients) and of skin rashes was 29% (seven patients). There were no grade ≥ 3 photosensitivity reaction or skin rashes [26]. Most of the adverse events occurring with vemurafenib and cobimetinib are managed with dose reduction or interruption, and supportive care [81].

Encorafenib and binimetinib cause increase of creatine phosphokinase in 26% of patients. The same adverse event is observed in 35.2% of patients receiving vemurafenib and cobimetinib. Constipation, abdominal pain, and dizziness are also more frequent with encorafenib and binimetinib [74].

The data presented above were generated by studies in adult populations with melanoma or gliomas. In pediatric patients with LGG, the rate of pyrexia with single agent dabrafenib is 28% [66]. In a phase 1 trial, the rate of pyrexia in patients with pediatric LGGs receiving dabrafenib and trametinib was 50% [68]. In another phase 1 study investigating vemurafenib in 19 patients with pediatric LGGs, 10 patients developed grade 3 maculopapular rash; however, photosensitivity was observed in only one patient. Another patient developed cutaneous squamous cell carcinoma after four cycles of vemurafenib [67]. Decreased left ventricular ejection fraction with *BRAF* and *MEK* inhibition seems to occur at similar rates as seen in adult populations [68]. Taken together, the pattern of adverse events observed in the pediatric population seems to be consistent with that observed in adults, but larger trials with long-term follow-up are needed to adequately assess the safety of *BRAF* and *MEK* inhibitors in children.

5 Mechanisms of Resistance to *BRAF* and *MEK* Inhibition in Gliomas and Possible Solutions

Development of resistance to targeted therapy is a concern when treating patients with *BRAF* V600E-mutant gliomas. In patients treated with *BRAF* inhibitor monotherapy, reactivation of the MAPK pathway through *ERK* paradoxical activation is a well-described mechanism of resistance and can be prevented by concurrent *MEK* inhibition [58]. Data from clinical trials in patients with *BRAF* V600-mutant melanoma demonstrate that upfront dual inhibition of *BRAF* and *MEK* not only delays progression (10.5 versus 5.6 months) but also improves overall survival when compared with *BRAF* inhibitor alone (1 year overall survival: 79% versus 70%), and the overall survival benefit is superior with upfront combination despite introduction of *MEK* inhibitor following progression while on *BRAF* inhibitor monotherapy [82]. However, patients with *BRAF* V600E-mutant pediatric LGGs have prolonged control of disease with *BRAF* inhibition alone, and most patients who present disease progression can have their disease successfully controlled with addition of a *MEK* inhibitor [30, 83]. This suggests that gliomas have distinct mechanisms of resistance to *BRAF* inhibition when compared with melanomas.

Putative mechanisms of resistance to *BRAF* inhibition in LGG and HGG include alterations in genes that modulate receptor tyrosine kinase activity, such as *CBL* (an E3 ubiquitin-ligase) and *ERFFI1* (ERBB receptor feedback inhibitor 1), *NF1* loss-of-function missense mutations, activating mutations in *MAP2K1*, emergent mutations in *PTEN* and *PIK3C2G*, alterations in cell cycle regulators (such as *BAP1* and *ANKHD1*) and *TET2* alterations (a gene involved in epigenetic modulation of DNA). Additionally, resistance to *BRAF* inhibitors may emerge from a switch from *BRAF* to *CRAF*-mediated *ERK* activation, making it independent from *BRAF* V600E. Resistance may also emerge through changes in gene expression, as demonstrated by an RNA sequencing analysis that revealed enrichment of mesenchymal (*TGFB1*-high) and proneural (*TGFB1*-low) genotypes with different expressions of *EGFR*, *YAP1* and *KRAS*. Those changes may occur at low variant allele frequencies, but this may be clinically significant in the context of low drug penetration through the blood–brain barrier [84]. Other mechanisms of resistance to *BRAF* inhibition include increased expression of *AXL*, a gene that activates the JAK/STAT, MAPK/ERK and PI3K/AKT pathways, increased *EGFR* expression, and elevated Wnt signaling [85]. The occurrence of *in cis BRAF* L514V mutation, which allows dimerization with *BRAF* V600E and results in decreased sensitivity to *BRAF* inhibitor monotherapy, has also been described as a resistance mechanism in *BRAF* V600E-mutant glioma [86], and this could potentially be counteracted by pan-*RAF* inhibition [87], although this has not been prospectively evaluated.

As mechanisms of resistance are identified, potential therapies to overcome resistance are being explored in the preclinical and clinical settings. Glioma cell lines treated with the *BRAF* inhibitor PLX-4720 present decreased expression of the protein tyrosine phosphatase *PTPN9*, a negative regulator of *EGFR*. As a result, those cell lines present hyperexpression of *EGFR*, making them resistant to *BRAF* inhibition. The combination of PLX-4720 and neratinib, an *EGFR* inhibitor, has been demonstrated to reduce tumor growth in *BRAF* V600E inhibitor-resistant xenograft models [88].

Inhibition of autophagy has been identified as a possible therapeutic approach to overcome different resistance mechanisms, including *KRAS* and *NRAS* activation, and *EGFR* hyperexpression [89]. In a preclinical model, the combination of vemurafenib and chloroquine (which functions as an autophagy inhibitor) resensitized resistant *BRAF*

V600E-mutant glioma cells to *BRAF* inhibition [90]. In the clinical setting, this combination has led to extended tumor control in a patient with *BRAF* V600E-mutant pleomorphic xanthoastrocytoma who had disease progression on dabrafenib and trametinib [91]. A clinical trial combining dabrafenib, trametinib, and hydroxychloroquine for patients with recurrent *BRAF*-altered gliomas previously exposed to *BRAF* and *MEK* inhibitors is actively recruiting patients (NCT04201457).

Activation of mTOR pathway is a mechanism of resistance to *MEK* inhibitors, and combination of trametinib with the mTORC1/2 inhibitor sapanisertib has been investigated in glioma xenograft models. This combination effectively inhibited downstream signaling of both MAPK and mTOR pathways, as well as cell cycle regulator proteins such as CDK1, CDK2, CDK4, CDK5, and CDK6. Decrease of VEGF expression was also noted with trametinib and sapanisertib combination. However, upregulation of *EGFR* and class 1 histone deacetylase proteins has been identified as potential resistance mechanisms to this combination [92]. Similarly, the combination of dabrafenib, trametinib, and an HSP90 inhibitor resulted in inhibition of both MAPK and mTOR pathways. This combination resulted in cytotoxic effects in *BRAF* and *MEK* inhibitor-resistant glioma cells, and this effect was observed both in vitro and in vivo [93]. In another study with glioma xenograft models, *MEK1* inhibition with selumetinib caused *STAT3* activation, and combined inhibition with *LLL12* (a *STAT3* inhibitor) induced tumor complete responses[94].

Although multiple mechanisms of resistance have been described and possible therapeutic approaches to overcome resistance have been identified, these findings have not yet been translated to clinical practice. This is due to the rarity of *BRAF* V600E-mutant gliomas, and to the difficulty in acquiring tissue samples at the time of relapse. Additionally, trials with targeted therapy have only been conducted after relapse to standard first-line treatment, and it is not known what the impact of front-line targeted therapy in patients with *BRAF* V600E-mutant gliomas would be. A phase 2 trial (NCT03919071) is assessing the event-free survival in patients with newly diagnosed *BRAF* V600E-mutant glioma treated with dabrafenib and trametinib maintenance following radiation therapy, but this study is not expected to be completed until 2027.

6 Conclusions

The identification of *BRAF* V600E mutations in gliomas has provided new therapeutic approaches for patients who otherwise would have limited treatment options. Results from retrospective and prospective studies provide evidence that targeted therapy can lead to tumor shrinkage, extended disease control, and prolonged survival in patients with gliomas refractory to standard first-line therapies. The results of these trials also evidence that genomic-directed therapies may be effective for patients with glioma. However, there are questions that still need to be answered in well-conducted clinical trials. For example, it is unknown whether patients with LGG are best treated with a *BRAF* inhibitor alone or in combination with a *MEK* inhibitor upfront. Additionally, for this population with a better prognosis, long-term follow-up is necessary to assess potential late toxicities of targeted therapy. For patients with HGG, results of first-line targeted therapy are eagerly waited. Whenever possible, patients who develop progressive disease should have genomic studies to assess mechanisms of resistance that can be targeted by alternative genomic approaches that should be evaluated in prospective clinical trials. Finally, the effectiveness of targeted therapy in patients with *BRAF* V600E-mutant gliomas highlights the importance of a comprehensive genomic evaluation for patients affected with primary brain malignancies.

Data availability Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

Declarations

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Conflict of Interest Thiago P. Muniz is a prior recipient of the Novartis Oncology Young Canadian Investigator Award. Warren P. Mason has received honoraria from Bayer, Viatris, Inc., and GlaxoSmithKline, and is part of the advisory board for Novocure.

Ethics Approval Not applicable.

Consent to Participate Not applicable.

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Author Contributions Thiago P. Muniz: conceptualization, methodology, data curation, writing—original draft, writing—review and editing. Warren P. Mason: conceptualization, writing—review and editing, supervision.

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