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Clinical, radiological and genomic features and targeted therapy in *BRAF* V600E mutant adult glioblastoma

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Abstract

Purpose—Although uncommon, detection of *BRAF*V600E mutations in adult patients with glioblastoma has become increasingly relevant given the widespread application of molecular diagnostics and encouraging therapeutic activity of BRAF/MEK inhibitors.

Methods—We performed a retrospective study of adult glioblastoma patients treated at Dana-Farber Cancer Institute/Brigham and Women's Hospital or Massachusetts General Hospital from January 2011 to July 2019 with an identified *BRAF*V600E mutation by either immunohistochemistry or molecular testing. Patient characteristics, molecular genomics, and preoperative MRI were analyzed.

Results—Nineteen glioblastoma patients were included, with median age at diagnosis of 41-years-old (range 22–69). Only 1/18 was *IDH1/2*-mutant; 10/17 had *MGMT* unmethylated tumors. The most common additional molecular alterations were *CDKN2A/2B* biallelic loss/loss-of-function (10/13, 76.9%), polysomy 7 (8/12, 66.7%), monosomy 10 (5/12, 41.7%), *PTEN* biallelic loss/loss-of-function (5/13, 38.5%) and *TERT* promoter mutations (5/15, 33.3%). Most tumors were well-circumscribed (11/14) and all were contrast-enhancing on MRI. Twelve patients

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Ethical approval This study was conducted under a Dana-Farber/Harvard Cancer Center Institutional Review Board protocol. A waiver was granted for informed consent due to its retrospective nature.

eventually developed subependymal or leptomeningeal dissemination. Six patients were treated with BRAF/MEK inhibition following disease progression after standard of care therapy, with 4/6 patients showing partial response or stable disease as best response. Median time to progression after BRAF/MEK inhibition was 6.0 months (95% CI 1.2–11.8). Grade 1 skin rash was present in 2 patients, but no other adverse events were reported. Median OS for the entire cohort was 24.1 months (95% CI 15.7–38.9).

Conclusion—Understanding the natural history and features of *BRAF*V600E glioblastoma may help better identify patients for BRAF/MEK inhibition and select therapeutic strategies.

Keywords

Glioblastoma; BRAF; Molecular targeted therapy; MEK inhibitor; BRAF inhibitor

Introduction

Despite advances in our understanding of glioma biology, patients with glioblastoma continue to have dismal outcomes with 5-year survival rates of about 6% [1] and median survival of between 14 to 19 months [2, 3]. There has been increased implementation of molecular diagnostics in clinical care for identification of molecular markers, which in turn can clarify diagnosis and prognosis in CNS tumors. In addition, there is a high level of interest in the detection of potentially targetable oncogenic mutations in glioma.

Activating mutations in *BRAF*(v-raf murine viral oncogene homolog B1), resulting in the substitution of glutamic acid for valine at position 600 (V600E mutation), are commonly present in a number of cancers, in particular in melanoma and colorectal cancer. Among primary CNS tumors, *BRAF*V600 driver events have been frequently identified in pleomorphic xanthoastrocytoma (PXA), gangliogliomas, as well as in ~ 1.7% of adult glioblastoma [4], where they are particularly enriched in the epithelioid variant of glioblastoma [5].

Detection of *BRAF*V600E mutations in adults with glioblastoma, through increased availability of molecular diagnostics, could have potentially life-extending effects due to the encouraging therapeutic activity of BRAF and mitogen-activated protein kinase (MEK) inhibitors, their relatively high tolerability and encouraging safety profiles in melanoma [6]. There is therefore considerable interest in using BRAF/MEK inhibitors in patients with *BRAF*V600E mutant GBM. Several case studies have described success with combination BRAF/MEK inhibitors in treatment of high-grade gliomas [7, 8].

As the incidence of this mutation in GBM is low, the natural history of these tumors is not well described. While uncommon, there might be a higher representation of this subtype of GBM in young adults [9]. Survival characteristics are also lacking and recurrence of *BRAF* V600E-mutant tumors has previously been documented with leptomeningeal involvement, a relatively rare event in GBM (estimated 2–14%) [10, 11].

Given these promising therapeutic avenues, further recognition and characterization of this subtype of GBM, through clinical, imaging and other pathological features would

be important. Of equal value would be the understanding of the natural history and prognosis of *BRAFV600E* mutant GBM. We investigated the demographics, clinical course, radiologic characteristics, and genetic mutations in an adult cohort of *BRAFV600E* mutant glioblastoma, which has not previously been well described.

Methods

Cohort selection

This retrospective study included adults (≥ 18-years-old) with a pathological diagnosis of newly diagnosed glioblastoma WHO grade IV, treated at Dana-Farber Cancer Institute/Brigham and Women's Hospital or Massachusetts General Hospital from January 1, 2011 to July 31, 2019. Patients with *BRAFV600E* mutation on immunohistochemistry staining or molecular diagnostics were included after a review of an institutional pathology database.

Data analysis

All charts were reviewed and data were collected retrospectively for each patient, including: (1) demographic data; (2) clinical data (including all tumor-directed therapy); (3) radiological data; and (4) pathological and molecular information.

Imaging features

Qualitative MRI data was reviewed by three investigators (ML, KS, EG) using Visually Accessible Rembrandt Images (VASARI) feature set at the time of diagnosis. Leptomeningeal or subependymal dissemination was determined by imaging review or positive CSF cytology. For patients started on BRAF/MEK inhibition, response to treatment was assessed using the Response assessment in Neuro-oncology (RANO) criteria [2].

Pathological and molecular features

Histologic diagnosis was performed in accordance with the 2016 WHO classification [2]. Molecular features were evaluated, including *IDH1/2* and *BRAFV600E* mutations, by IHC and/or NGS, and O6-methylguanine-DNA methyltransferase (*MGMT*) promoter methylation status. Targeted NGS sequencing was conducted using validated institutional platforms [12, 13] and analyzed for single nucleotide variants, indels, copy number variants, and structural variants. Copy number variants were further evaluated using micro-array-based comparative genomic hybridization.

Statistical analysis

Statistical analyses were performed using STATA version 16.0 software. Time-to-event analyses for overall survival (OS) were calculated using the Kaplan–Meier method from time of glioblastoma diagnosis to date of death. Progression-free survival (PFS) was calculated as treatment start date to date of progression or death. Difference in survival was compared between groups using the log-rank test. Patients lost to follow up were censored at the date of last contact.

Results

Patient characteristics

BRAFV600E mutations were identified in 19 patients with newly-diagnosed GBM. Sixteen (84.2%) patients were identified based on NGS and 3 (15.8%) were identified based on immunohistochemistry alone (NGS not performed). Median age at diagnosis was 41 years (range 22–69) and 68.4% (13/19) were female (Table 1).

Pathological and molecular characteristics

The tumors displayed a variety of histomorphological appearances, including: 5 that displayed prominent or focal epithelioid features (one of which additionally displayed areas of neuronal differentiation that were synaptophysin positive); two with prominent giant cell components; one gliosarcoma and one GBM with spindled fascicular pattern; one with regional rhabdoid cytomorphology; and a sparsely sampled case with histology reminiscent of ependymoma. In all cases the final integrated histomolecular neuropathological diagnosis was glioblastoma, WHO grade IV, and included consensus diagnosis for cases with non-canonical glioblastoma histological appearances. Only 1/18 (5.6%) was *IDH1 (R132S)* mutant, which would now be reclassified as a Grade IV *IDH*-mutant astrocytoma [14], and 10/17 (58.8%) were *MGMT* promoter unmethylated. *BRAFV600E* IHC had been performed in addition to NGS on 7 patients, with 6 (71.4%) interpreted by neuropathology as showing positive staining (Supplemental Fig. 1) and the 7th patient's initial non-diagnostic pathology consisted only of fragments of focally mildly hypercellular brain tissue with rare atypical cells, in which the *BRAFV600E* IHC was negative and the positive molecular testing was conducted on the subsequent diagnostic resection specimen. An additional 3 cases were diagnosed by positive *BRAFV600E* IHC alone. The intensity and quality of *BRAFV600E* immunopositivity was variable across cases, and on molecular testing, although the *BRAFV600E* variant was commonly present at an allelic frequency (median 34.7%, interquartile range 20.1–47.8) that was suggestive of a clonal mutation, the variant allelic frequency was as low as 8%. On molecular analysis, the most common alterations were *CDKN2A/2B* biallelic loss/loss-of-function (76.9%, 10/13), polysomy 7 (66.7%, 8/12), monosomy 10 (41.7%, 5/12), *PTEN* biallelic loss/loss-of-function (38.5%, 5/13) and *TERT* promoter mutations (33.3%, 5/15). No *EGFR* amplification, *EGFRvIII* variants, or *H3F3A* mutations were identified (Fig. 1).

Radiological features and leptomeningeal metastasis

Fourteen patients were included for analysis of presurgical tumor imaging characteristics, summarized in Table 1. Most tumors were well circumscribed (78.6%, 11/14) and all were contrast-enhancing on MRI. While only 2 patients (14.3%) had leptomeningeal or ependymal involvement at diagnosis, 12/19 (63.2%) or 10 additional patients, developed subependymal or leptomeningeal dissemination at progression, including distal leptomeningeal metastasis to the spinal cord (n = 2) and the posterior fossa (n = 4). Two patients underwent a lumbar puncture for suspected leptomeningeal metastasis, and both had malignant cells present on cytology. Median number of months to leptomeningeal metastasis was 15.9 months (95% CI 3.13–35.20) and median OS after leptomeningeal metastasis was 4.7 months (95% CI 0.4–8.8). There was no significant difference in overall survival in the

patients who developed leptomeningeal spread vs those who did not (24.1 months vs 21.1 months, $p = 0.51$) (Table 2).

BRAF/MEK inhibition

Six patients were treated with BRAF/MEK inhibition following progression after standard-of-care therapy. None received upfront treatment with BRAF or BRAF/MEK inhibition. Of these, 4 (66.7%) were treated on an “off-label” compassionate use basis, and 2 (33.3%) were enrolled in a clinical trial. One patient was on vemurafenib alone and 5 were on a combination of dabrafenib and trametinib. Half of the patients treated with BRAF/MEK inhibitor therapy (3/6) demonstrated a partial response (as illustrated in Fig. 2) and 1 experienced stable disease as their best response. Median time to progression after BRAF/MEK inhibitor therapy was 6.0 months (95% CI 1.3–11.8). There were no reported significant adverse events (AE) except for mild rash (CTCAE Grade 1) in 2 patients.

Survival outcomes

Median OS for the entire cohort was 24.1 months (95% CI 15.7–38.9). Formal evaluation of the effect of BRAF/MEK inhibitor therapy on OS was limited by our small sample size but a non-statistically significant trend favoring longer OS was observed with BRAF/MEK inhibitor therapy; specifically, median OS for patients on BRAF/MEK inhibitor therapy was 35.6 months (95% CI 24.1–not estimable) compared to 17.0 months (95% CI 5.7–38.9) among patients who did not receive BRAF/MEK inhibitor therapy ($p = 0.42$) (Fig. 3).

Discussion

Compared to the general glioblastoma patient population, which has a median age at diagnosis of 65 years and a median OS of 14.6 months [2], our cohort had a median age at diagnosis of 41 years and a median OS of 24.1 months. *BRAFV600E* mutations have been described in younger patients with glioma, although the majority are low grade, *IDHwt* gliomas [9, 15, 16]. *BRAFV600E* mutations are also particularly enriched (50–67%) in epithelioid GBM [5], which tend to affect younger patients with a reported median age of 25–30 [17, 18].

A study of pediatric patients with secondary *BRAFV600E* mutant high grade gliomas suggest that these tumors initially had increased time to malignant transformation to high grade glioma and improved survival [19]. Although our cohort included only newly diagnosed glioblastoma, *BRAFV600E* mutation status may be a better marker of prognosis in the younger adult *IDHwt* GBM population compared to histological grading. Indeed, historic cohorts have shown that *IDHwt* diffuse gliomas in pediatric and young adult cohorts with a *BRAFV600E* mutation, *FGFR* alterations or *MYB* or *MYBL1* rearrangement tend to have a better prognosis and good overall survival, prompting unique considerations and characterization highlighted by the cIMPACT-NOW update 4 [20]. This prognostic difference highlights the importance of *BRAFV600E* mutation testing in young adults with *IDHwt* high grade gliomas. In terms of histopathology, although a substantial plurality of our cases exhibited epithelioid morphology, we found that *BRAFV600E* mutations were present in a wide variety of histological variants of GBM. Only one patient had an *IDH*

mutant tumor, with a non-canonical *IDH* mutation, *IDH1* R132S, which has been recurrently reported as a pathogenic variant in infiltrative gliomas (COSMIC). Patients with *BRAF* V600E mutations represent a distinct biological cohort from those harboring canonical *IDH* mutation in pediatric low grade gliomas, as these represent different oncogenic pathways [21]. A review of the available cohorts on cBio-Portal regrouping the TCGA and MSKCC glioma datasets also confirms that these driver events appear to be mutually exclusive in high grade glioma, although non-canonical *IDH* mutations are occasionally associated with *BRAF*V600E mutations. Compared to the pediatric patients where this appears to be more distinct, the presence of several driver events may be present in older patients with gliomas. The true significance of this co-mutation in our cohort remains unclear given the small sample size and our selection of only Grade IV gliomas.

In our study, seven patients (40%) had *BRAF*V600E IHC in addition to molecular testing, in which all diagnostic cases exhibited concordance between IHC and NGS panel testing. In one case, the IHC on the initial non-diagnostic tissue was negative, whereas the molecular testing of the diagnostic resection specimen was positive. The intensity and quality of staining varied across cases, and may reflect the *BRAF*V600E variant allele frequency. Taken together, the findings from our cohort and others' studies support that *BRAF*V600E testing be considered for *IDH*wt GBM patients that are younger or exhibit epithelioid histology. For most institutions that do not routinely conduct targeted NGS panel testing of brain tumors, *BRAF*V600E IHC can be an inexpensive and widely-available screening tool, which if equivocal or negative, should prompt for molecular testing. The latter should preferably include targeted NGS panel testing, so as to also identify any additional potential therapeutic targets in young GBM patients or epithelioid GBMs.

Overall, the radiographic appearance of *BRAF*V600E mutations at diagnosis tended to be heterogeneous, although the majority were (1) well circumscribed, (2) heterogeneously enhancing and (3) had a cystic component. While none of these baseline features necessarily distinguish the *BRAF*V600E mutant tumors from other *IDH*wt GBMs, we observed that the majority of these patients had imaging evidence of leptomeningeal spread and in some, distant metastasis.

Leptomeningeal dissemination is a relatively rare phenomenon in glioblastoma with estimated rates of less than 5% [10]. In our cohort, 63% of patients exhibited evidence of subependymal or leptomeningeal dissemination on imaging, most frequently at the time of recurrence, suggesting a particular propensity for such phenomena among *BRAF*V600E mutant glioblastomas. Four patients in our cohort harbored co-existing *TERT* promoter and *CDKN2A/CDKN2B* mutations—genetic features commonly associated with *BRAF*V600E mutant gliomas laying on the spectrum between PXA and epithelioid glioblastoma [17], which have often been associated with leptomeningeal dissemination [22]. While early studies suggested that leptomeningeal dissemination might be a feature of disease duration, this is unlikely to be the only factor as the time to antemortem diagnosis of leptomeningeal spread in GBM is quite wide [23]. Intrinsic tumor characteristics rather than disease duration may also predict the propensity for leptomeningeal dissemination. Our study suggests that *BRAF*V600E may be a predisposing genetic factor. In this context, while whole spine imaging and CSF analyses for malignant cells are not usually part of routine screening in

GBM patients, the threshold for doing so should be lower in patients harboring a *BRAF* V600E mutation.

Our cohort had an objective response rate of 50% to BRAF/MEK inhibition, all of which were partial responses. Two patients had recurrent disease with leptomeningeal spread at the time of initiation and both demonstrated a partial response to therapy. A prior case series had also described dramatic responses to dabrafenib in 3 patients with *BRAF*V600E recurrent gliomas and leptomeningeal disease [24]. BRAF/MEK inhibitors are widely available therapies, having demonstrated substantial effectiveness for patients with *BRAF*V600 mutant metastatic melanoma, including those with brain involvement [25]. Combined BRAF/MEK inhibition has been showed to improve both PFS and OS in metastatic melanoma [26]. This combination is thought to overcome the development of resistance through reactivation of the MAPK pathway. Notably, among 11 patients with malignant diffuse glioma enrolled in a basket trial of vemurafenib alone, one demonstrated a partial response and 5 patients experienced stable disease [27]. Additionally, some case series have observed similarly encouraging responses to BRAF/MEK targeted therapy in recurrent *BRAF*V600E mutant adult glioma [8], including a case of prolonged complete response with combination therapy after initial failure with BRAF-inhibition alone [28]. Encouragingly, preliminary results from an open-label basket trial of combination therapy with dabrafenib and trametinib (NCT02034110), which enrolled 37 patients with *BRAF* V600E mutant HGG, demonstrated an overall response rate of 26% including 1 complete response, and most responders displayed prolonged durations of response [29]. BRAF/MEK inhibition was safely tolerated, with less than a 10% rate of serious adverse events (i.e. CTCAE Grade 3 or more). A number of trials are ongoing both in adult and pediatric patients with newly diagnosed and recurrent glioma (e.g. NCT03919071, NCT03973918, NCT02684058) to further evaluate the impact of BRAF/MEK inhibitor therapy among patients with *BRAF*V600E mutant gliomas.

Our findings suggest that *BRAF*V600E screening should be considered for *IDH*wt GBM patients who are young adults, have leptomeningeal dissemination, or display epithelioid histomorphology, in order to potentially identify patients for targeted *BRAF*V600E therapy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Funding

No funding was received in support of this study.

Conflict of interest

M.J. Lim-Fat; K.W. Song; B.M. Andersen; D.A. Forst; E.R. Gerstner and I. Arrillaga-Romany report no disclosures relevant to the manuscript; J.B. Iorgulescu received support from the NIH (NCI K12CA090354) and Conquer Cancer Foundation/Sontag Foundation Young Investigator Award; J.T. Jordan received honoraria from Elsevier and Consulting for Navio Theragnostics, Recursion Pharmaceuticals, CereXis, Inc, and Health2047, Inc; D.A. Reardon received Research support (paid to DFCI) from Acerta Pharmaceuticals; Agenus; Celldex; EMD Serono; Incyte; Inovio; Midatech; OmniOX; Tragara, advisory/consultation (paid to Dr. Reardon): Abbvie; Advantagene; Agenus; Amgen; Bayer; Bristol-Myers Squibb; Celldex; Del-Mar; EMD Serono; Genentech/Roche; Invax; Inovio; Merck; Merck KGaA; Monteris; Novocure; Oncorus; Oxigene; Regeneron; Stemline; Taiho Oncology, Inc., Honoraria

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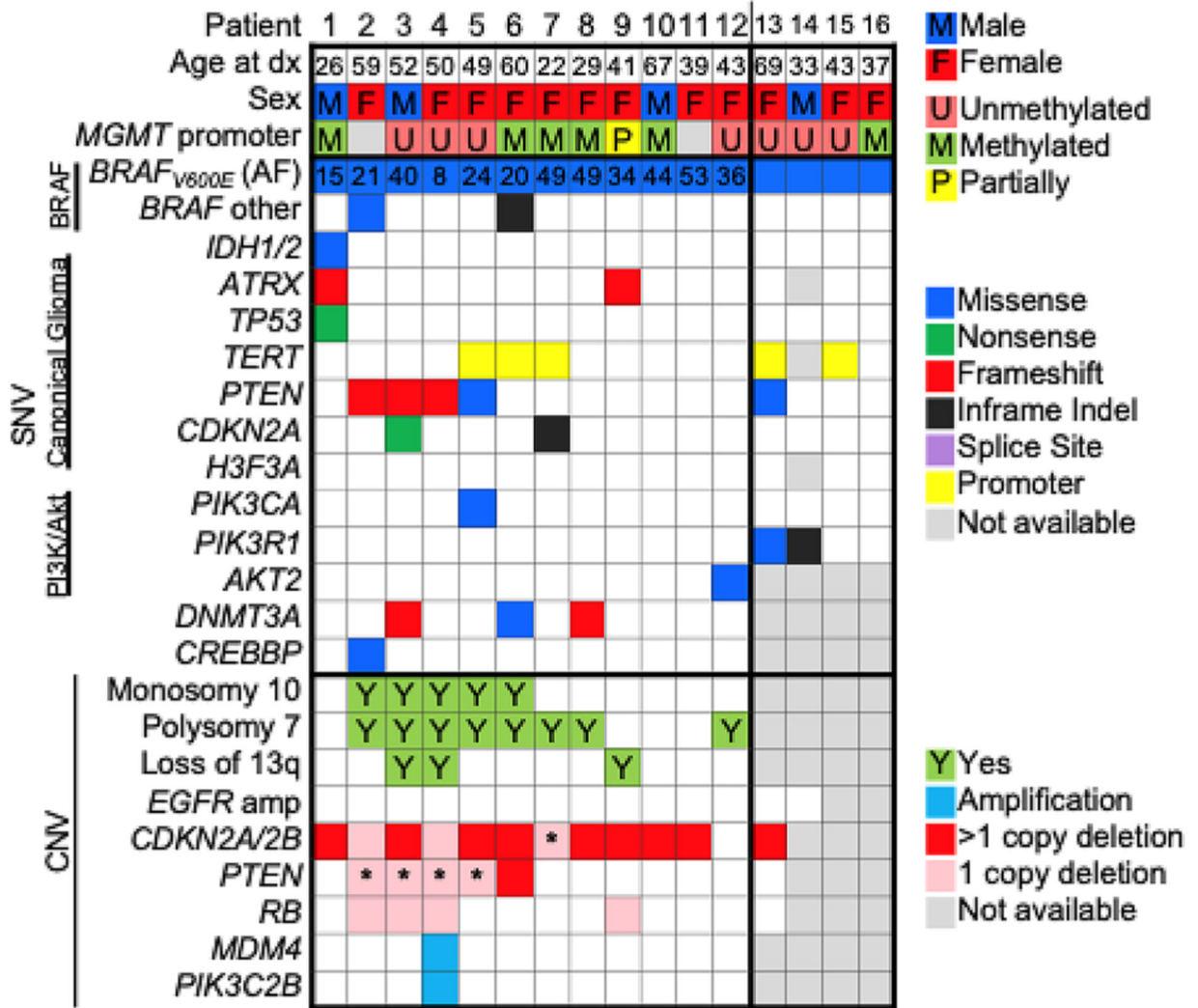


Fig. 1. *BRAF V600E* glioblastoma patient demographics and molecular alterations. A computation plot displaying the demographic and molecular characteristics of the 16 patients with *BRAF V600E* mutant glioblastoma that underwent next generation sequencing. The *BRAF V600E* variant was frequently present at a clonal allele frequency (AF; median 34.7%, interquartile range 20.1–47.8). No *EGFR* amplification, *EGFRvIII* variants, or *H3F3A* mutations were identified. *Single copy deletion concurrently with a loss-of-function single nucleotide variant is suggestive of biallelic inactivation

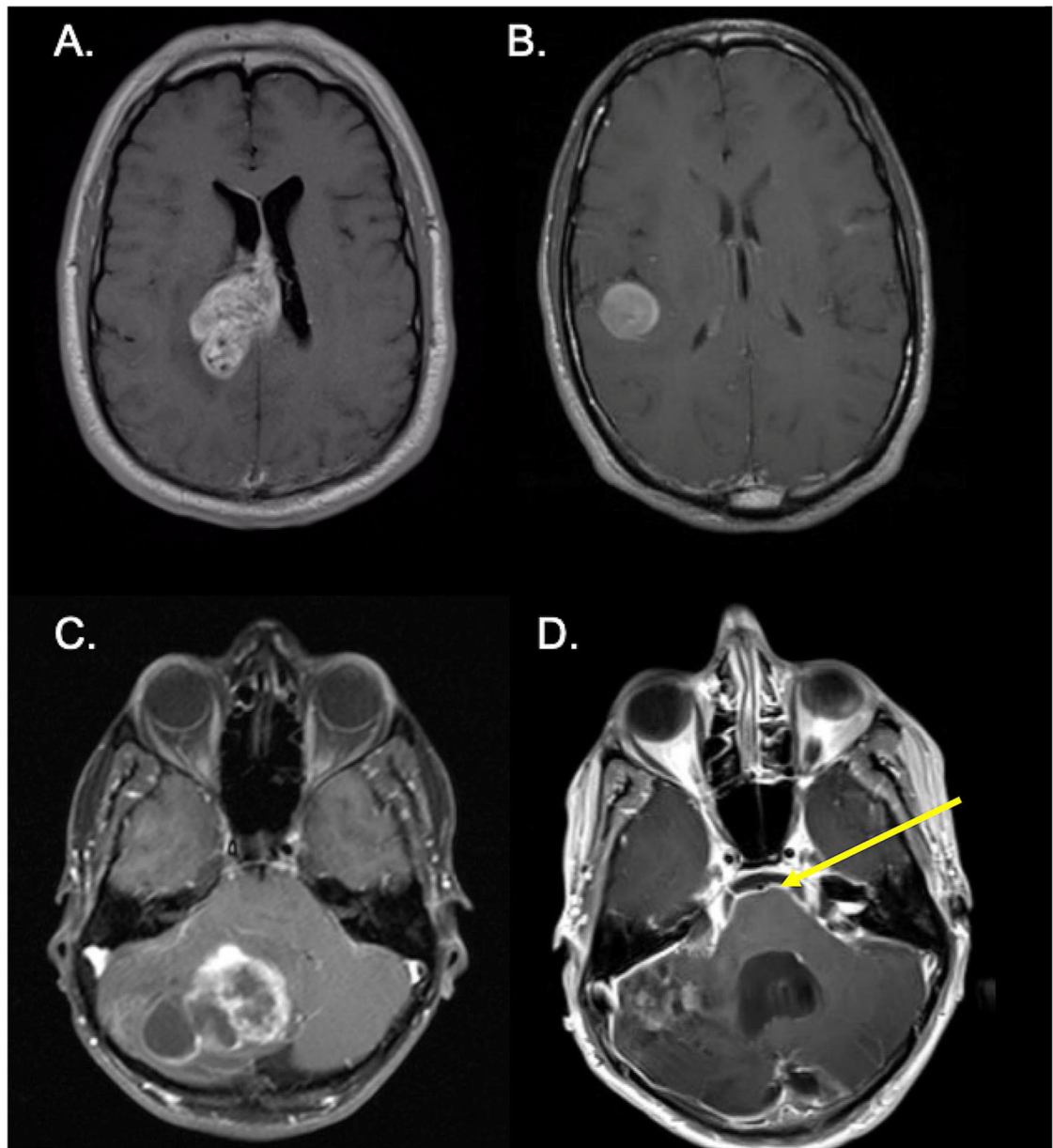


Fig. 2. Radiological appearance of *BRAFV600E* mutant glioblastoma. MRI imaging of the brain showing subependymal (a) and subcortical (b) involvement of glioblastoma. Progression of initial cerebellar tumor (c) with leptomeningeal metastasis (yellow arrow; d)

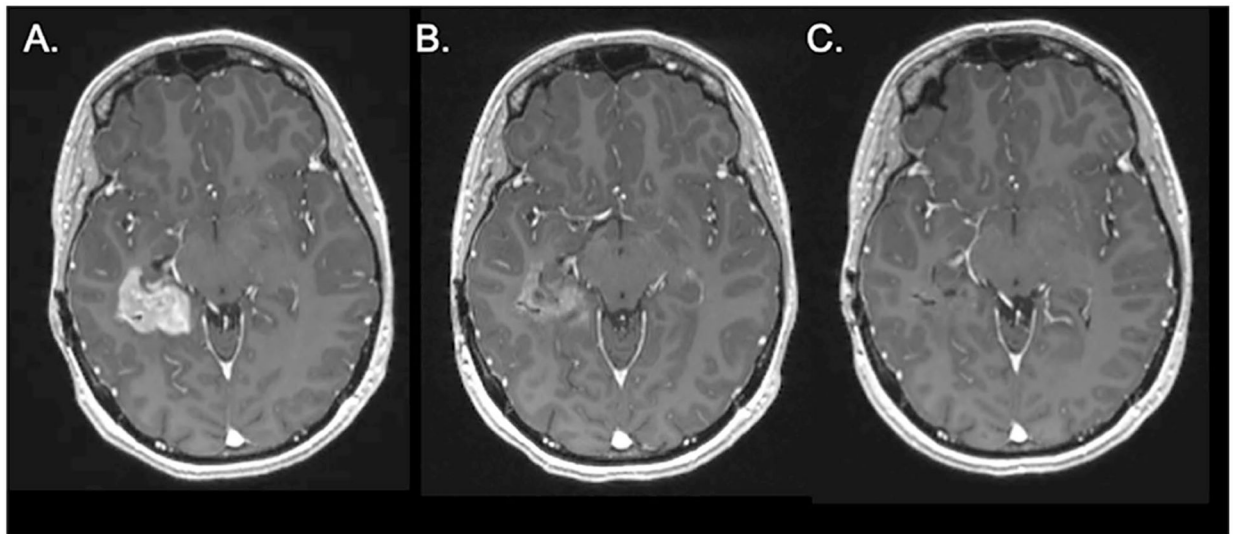


Fig. 3. Radiographic responses to BRAF/MEK inhibitors for *BRAFV600E* mutant glioblastomas. An axial MRI post-contrast T1 sequence of a patient with *BRAFV600E* mutant glioblastoma at initiation of dabrafenib/trametinib (A). Follow-up scan shows partial response at 1 month (B) and near complete response at 3 months (C)

Table 1Patient characteristics for 19 patients with glioblastoma harboring *BRAF*V600E mutation

	Total, n = 19 (%)
Median age at diagnosis, year (range)	41.0 (22–69)
Female sex	13 (68.4%)
Race	
Caucasian	14 (73.6%)
African-American	2 (10.5%)
Other	3 (15.8%)
KPS at baseline 80 (n = 18)	14 (77.8%)
IDH 1/2wildtype ^a (n = 18)	17 (94.4%)
MGMT promoter(n = 17)	
Methylated	6 (35.3%)
Partially methylated	1 (5.9%)
Unmethylated	10 (58.8%)
Anatomical location	
Supratentorial	18 (94.7%)
Midline	2 (22.2%)
Multifocal	1 (5.3%)
Extent of resection (n = 17)	
Gross total resection	10 (58.8%)
Subtotal resection	5 (29.4%)
Biopsy	2 (11.8%)
First-line Standard-of-care chemoradiation and adjuvant TMZ	16 (84.2%)
BRAF/MEK inhibition as salvage treatment (n = 6)	
BRAF inhibition alone	1 (16.7%)
BRAF/MEK inhibition	5 (83.3%)
Radiological features at diagnosis (n = 14)	
Circumscribed	11 (78.6%)
Superficial location	6 (42.9%)
Cortical involvement	8 (57.1%)
Ependymal/leptomeningeal extension	2 (14.3%)
Heterogeneous enhancement	13 (92.9%)
Cystic appearance	10 (71.4%)
Hemorrhagic appearance	4 (28.6%)

^aIDH mutation was assessed by next generation sequencing and/or immunohistochemistry

KPS Karnofsky Performance Status; *IDH* Isocitrate dehydrogenase; *MGMT* O⁶-methylguanine-DNA methyltransferase; *TMZ* temozolomide; *BRAF* B-Raf Proto-Oncogene; *MEK* mitogen-activated protein kinase

Table 2

Summary of treatment and objective response of BRAF/MEK inhibition in 6 patients

Study ID number	BRAF/MEK inhibitor	Treatment Prior to BRAF/MEKi	Measurable tumor at initiation	Best response	Time to progression on inhibitor (months)	Overall survival (months)
9	Dabrafenib 150 mg BID, then 100 mg BID after 2 months/trametinib 2 mg QD, then 1.5 mg QD after 2 months	Concurrent RT/TMZ, 6 cycles of adjuvant TMZ	Yes	Stable disease	4.7	26.7
10	Vemurafenib 960 mg BID every other week, then 960 mg BID after 2 months	Concurrent RT/TMZ, 12 cycles of adjuvant TMZ	Yes	Stable disease	1.7	N/A
12	Dabrafenib 150mg BID/trametinib 2 mg QD	Concurrent RT/TMZ, 5 cycles of adjuvant TMZ	Yes	Partial response	6.6	N/A
13	Dabrafenib 150 mg BID / trametinib 2 mg QD	Concurrent RT/TMZ, 6 cycles of adjuvant TMZ	Yes	Partial response	10.9 (intermittently adherent)	35.6
15	Dabrafenib 150 mg BID/trametinib 2 mg QD (received for 13 days at 4 mg QD, then on hold due to decreased LVEF)	Concurrent RT/TMZ, 6 cycles of adjuvant TMZ, Avastin, Carboplatin, repeat RT	No	Progressive disease	1.3	24.1
16	Dabrafenib 150 mg BID/trametinib 2 mg QD	Concurrent RT/TMZ, 6 cycles of adjuvant TMZ	Yes	Partial response	11.9 (intermittently adherent)	39.7

BID twice daily, *QD* once daily, *RT* radiation therapy, *TMZ* temozolomide