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CLINICAL STUDY

Additional genetic alterations in *BRAF*-mutant gliomas correlate with histologic diagnoses

Antonio Dono[1,2] · Jennifer Vu[1] · Molly Anapolsky[2] · Gabriella Hines[1] · Takeshi Takayasu[1] · Yuanqing Yan[2] · Nitin Tandon[2,3] · Jay-Jiguang Zhu[2,3] · Meenakshi B. Bhattacharjee[1,3] · Yoshua Esquenazi[2,3,4,5] · Leomar Y. Ballester[1,2,3,6]

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Abstract

Introduction Recently, the term "Diffuse glioma, *BRAF* V600E-mutant" has been recommended for IDH-wildtype gliomas with *BRAF* p.V600E mutation and without *CDKN2A/B* deletion. However, additional alterations in gliomas that coexist with *BRAF*-mutations are poorly defined.

Methods We analyzed next-generation sequencing results in 315 cancer-associated genes for 372 gliomas from our institution (2010 to 2017). In addition, we reviewed IDH-WT gliomas with mutation and copy-number alterations available in cBioPortal, to further characterize *BRAF*-mutant gliomas.

Results Seventeen (4.6%) showed *BRAF* mutations. Tumor types included 8 glioblastomas, 2 epithelioid glioblastomas (E-GBM), 2 pleomorphic xanthoastrocytomas (PXA), 1 anaplastic oligodendroglioma, 1 diffuse astrocytoma, and 3 pilocytic astrocytomas. Fifty-three percent (53%) of cases exhibited *BRAF*-alterations other than p.V600E. The majority of the tumors were localized in the temporal lobe (52.9%). In addition to *BRAF* mutations, glioblastomas showed concomitant mutations in *TP53* (3/8), *CDKN2A/B*-loss (6/8), *TERT*-promoter (6/8), and/or *PTEN* (5/8). Both E-GBMs and PXAs showed *CDKN2A/B*-loss and *BRAF* p.V600E with absence of *TERTp, TP53,* and *PTEN* mutations. Similar findings were observed in *BRAF*-mutant infiltrating gliomas from cBioPortal.

Conclusions Knowledge of additional alterations that co-occur with *BRAF*-mutations in gliomas may improve diagnosis and help identify patients that could benefit from targeted therapies. Furthermore, we provide examples of two patients whose tumors responded to *BRAF* pathway inhibitors, arguing in favor of these therapies in patients with *BRAF*-mutant gliomas.

Keywords *BRAF* mutant glioma · *BRAF* p.V600E · Cobimetinib · PXA · Epithelioid glioblastoma

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✉ Yoshua Esquenazi
Yoshua.EsquenaziLevy@uth.tmc.edu

✉ Leomar Y. Ballester
Leomar.Y.Ballester@uth.tmc.edu

[1] Department of Pathology and Laboratory Medicine, McGovern Medical School, The University of Texas Health Science Center at Houston, Houston, TX, USA

[2] Vivian L. Smith Department of Neurosurgery, McGovern Medical School, The University of Texas Health Science Center at Houston, Houston, TX, USA

[3] Memorial Hermann Hospital-TMC, Houston, TX, USA

[4] Center for Precision Health, School of Biomedical Informatics, The University of Texas Health Science Center at Houston, Houston, TX, USA

[5] Department of Pathology and Laboratory Medicine, Department of Neurosurgery, McGovern Medical School, UT Neuroscience, University of Texas Health Science Center at Houston, 6431 Fannin St., MSB 2.136, Houston, TX 77030, USA

[6] Vivian L. Smith Department of Neurosurgery and Center for Precision Health, UT-Neuroscience, McGovern Medical School, The University of Texas Health Science Center at Houston, 6400 Fannin Street, Suite # 2800, Houston, TX 77030, USA

Introduction

Gliomas account for over 75% of all malignant primary central nervous system (CNS) tumors and have a variety of genomic alterations that may result in dysregulation of growth-factor signaling pathways and contribute to tumorigenesis. Glioblastoma (GBM) is the most aggressive and prevalent type of adult glioma [1]. The median overall survival (OS) is < 2 years despite maximal surgical resection and chemoradiation [2]. However, GBMs are a genetically heterogeneous group of tumors and understanding their molecular drivers is critical for accurate diagnosis, prognosis, and incorporation of targeted therapies.

The RAS–RAF–MEK extracellular signal-regulated kinase (*ERK*) signaling cascade is frequently mutated in human cancers. It transduces a growth signal from the cell membrane to the nucleus via a chain of protein kinases and is responsible for cellular proliferation and survival. A commonly altered gene in this pathway is v-raf murine sarcoma viral oncogene homolog B1 (*BRAF*), a RAF family serine/threonine kinase protein that is part of the RAS–RAF–MEK–ERK pathway [3]. *BRAF* alterations are common in several tumor types including melanoma, carcinomas from colorectal, thyroid and ovarian origins, and primary brain tumors [4]. *BRAF* alterations are most commonly found in circumscribed (or non-diffuse) gliomas like pilocytic astrocytoma (PA), ganglioglioma, pleomorphic xanthoastrocytoma (PXA), and anaplastic PXA, but have also been described in epithelioid glioblastoma (E-GBM) [5–10].

Interestingly, common clinical, histologic, immunohistochemical, and molecular features have been described for E-GBM and anaplastic PXAs, including the *BRAF* p.V600E mutation [11]. Moreover, methylation studies show that the majority of cases with a histologic diagnosis of E-GBM cluster with PXAs, obscuring the divide between these 2 apparently distinct entities [12]. While the genetic landscape of anaplastic PXAs shows recurring concomitant *BRAF* and *CDKN2A/B* alterations and frequent *TERTp* mutations [13], the additional genetic alterations that coexist with *BRAF* mutations in gliomas remain poorly understood. A better understanding of these additional alterations could improve the molecular classification of *BRAF*-mutant gliomas.

The majority of *BRAF* mutations in cancer affect the kinase domain at the 600th codon, where valine is substituted for glutamate (V600E). *BRAF* p.V600E confers approximately a tenfold greater kinase activity than wild-type (WT) *BRAF* [4]. While this mutation is often associated with poor prognosis in some gliomas [7, 14], it has also been shown to correlate with better prognosis in cases of isocitrate dehydrogenase wildtype glioblastomas (GBM IDH-WT) [15]. Immunohistochemistry with a BRAF p.V600E specific antibody is a useful tool for the detection of this mutation in gliomas. However, this approach is limited because it can only identify tumors with the V600E alteration. Targeted therapies, such as *BRAF* p.V600E (dabrafenib, vemurafenib) and *MEK* (trametinib, cobimetinib) inhibitors, have proven to delay progression in melanoma patients with *BRAF* p.V600 mutations [16]. Moreover, these drugs have been recently used in the treatment of *BRAF*-mutant high-grade gliomas and have shown responses, highlighting the importance of recognizing *BRAF*-altered gliomas in clinical practice [17–20].

The goal of this study is to evaluate additional genetic alterations in *BRAF*-mutant gliomas in a large glioma institutional registry (n = 372) and in cBioPortal. This study will allow a better understanding of additional genetic alterations that frequently coexist with *BRAF* mutations in gliomas and their diagnostic implications.

Methods

Patients and samples

372 consecutive glioma patients undergoing biopsy or surgical resection and whose tissue was evaluated by NGS (2010 to 2017) were included in this study, after approval by the institutional review board of the University of Texas Health Science Center at Houston (UTHealth) and Memorial Hermann Hospital, Houston, TX. Clinical data (age, gender, histologic diagnosis, imaging studies, recurrence, and survival) were collected using medical records and compiled using a REDCap database. Diagnosis were performed following the 2016 WHO Classification of Tumors of the CNS by board-certified neuropathologists.

Next-generation sequencing

Tumors were analyzed for genetic alterations by a targeted next-generation sequencing assay (NGS) interrogating 315 genes (FoundationOne®, Foundation Medicine Inc., Cambridge, MA, USA). The FoundationOne® assay was performed in a clinical laboratory improvement amendments (CLIA)-certified laboratory, as previously described [21, 22]. Tumor mutation burden (TMB) was calculated based on the number of somatic mutations in sequenced genes and extrapolating the value to the genome as a whole using a validated algorithm [23, 24]. TMB was reported as a number of mutations per megabase (mb) of genome.

cBioPortal for cancer genomics

We searched for *BRAF*-mutant gliomas with mutation and copy-number alterations available in cBioPortal (https://www.cbioportal.org/) (data accessed on August 2020) [25, 26]. We included cases from the following cBioPortal datasets: Brain Tumor PDXs (Mayo Clinic, 2019), Brain Lower Grade Glioma (TCGA, PanCancer Atlas), Glioblastoma Multiforme (TCGA, PanCancer Atlas), Memorial-Sloan Kettering Cancer Center (MSKCC, 2019), LGG University of California San Francisco (UCSF 2014), and GBM Columbia (Columbia, 2019) [27–30]. The cBioPortal Oncoprint tool was utilized to perform the oncoplots of both UTHealth and cBioPortal datasets [25, 26].

Results

Characteristics of the study cohort

The institutional database (UTHealth cohort) included 372 patients with a mean age of 57 years (range 2–87 years). There were 219 (58.9%) males, 263 (70.7%) Caucasians, 52 (14.0%) Hispanic, 36 (9.7%) African-American, 15 (4.0%) Asians, and 6 (1.6%) of other racial backgrounds. Histologic diagnoses in descending order of frequency include: GBM IDH-WT (249/372, 66.9%), GBM IDH-mutant (24/372, 6.5%), oligodendroglioma IDH-mutant and 1p/19q co-deleted (OD) (22/372, 5.9%), anaplastic astrocytoma (AA) IDH-mutant (16/372, 4.3%), diffuse astrocytoma (DA) IDH-mutant 15 (4.0%), AA, IDH-WT (13/372, 3.5%), anaplastic oligodendroglioma, IDH-mutant and 1p/19q codeleted (AO) (12/372, 3.2%), DA IDH-WT (9/372, 2.4%), pilocytic astrocytoma (PA) (6/372, 1.6%), PXA (3/372, 0.8%), E-GBM (2/372, 0.6%), and subependymal giant cell astrocytoma (1/372, 0.3%).

Characteristics of BRAF-mutant gliomas

BRAF mutations were identified in 17/372 (4.6%) cases: 1/9 (11.1%) DA IDH-WT, 8/249 (3.2%) GBM IDH-WT, 2/2 (100%) E-GBMs, 1/12 (8.3%) AO, 3/6 (50%) PA, and 2/3 (66.7%) PXAs (Fig. 1). There were 8 females and 9 males with a median age of 50 years (range 2–83 years). The median age by diagnosis was: GBM IDH-WT (51.5; range 23–71 years), E-GBM (23.5; range 23–24 years), PXA (24; range 21–27 years), and PA (15; range from 2 to 26 years). Seizures were a presenting symptom in 5/17 (29.4%) patients. The anatomic distribution of *BRAF*-altered gliomas is shown in Fig. 2a. Lesions occurred in the temporal lobe in 9/17 (53%) cases; 4/8 GBM IDH-WT, 1/2 E-GBMs, 2/2 PXAs, 1/1 DA IDH-WT, 2/3 PAs. Two *BRAF*-altered GBM IDH-WT occurred in a midline location (#4 with brainstem involvement and #8 involving thalamus). The majority of patients with GBM IDH-WT (including both E-GBM cases) underwent resection followed by TMZ (10/10) and radiotherapy (RT) (9/10) according to the Stupp protocol [31], Online Resource 1. The median OS of patients with GBM IDH-WT with a *BRAF* alteration was 12.8-months and 7/8 (87.5%) patients are now deceased. All patients with PAs, PXAs, and E-GBM are alive at the moment of this report with an average OS of 52.4, 145.6, and 12.1 months, respectively. The DA IDH-WT and AO patients with a *BRAF* alteration are deceased with an OS of 16.1 and 22.7 months, respectively.

Additional genomic alterations in BRAF-mutant gliomas

NGS identified 179 genomic alterations involving 99 genes. The median number of mutations per patient was 10 (range 7–17). Most alterations were reported as variants of unknown significance (VUS) (105/173 or 60.7%). As demonstrated in Fig. 2b, 9/17 (53%) patients had the *BRAF* p.V600E mutation and 9/17 (53%) had *BRAF* mutations distinct from p.V600E (one patient had two *BRAF* alterations, p.V600E and a non-V600E mutation). Other *BRAF* alterations included: *BRAF-KIAA1549* fusion in PA, *BRAF* amplification, p.K483E, p.D594N, and p.N5811 in GBM IDH-WT, and BRAF p.L597Q in a patient with DA IDH-WT.

Genomic alterations for the 17 patients are reported in Fig. 1 and Online Resource 1. All cases except patient 14 (AO) were IDH-WT. In addition to *BRAF* alterations, *BRAF*-mutant gliomas showed mutations in *TP53*, *TERTp*, *CDKN2A/B,* and *PTEN,* among others. Online Resource 2 shows the mutations observed in these genes and their predicted effects on protein function (gain/loss of function).

Patients with *BRAF*-mutant GBM IDH-WT (n = 8) frequently showed additional alterations in *PTEN* (n = 5), *CDKN2A/B* (n = 6), and *TERTp* mutations (n = 6). In contrast, E-GBMs and PXAs exhibited *BRAF* p.V600E and *CDKN2A/B* mutations without *TERTp, PTEN*, or *TP53* mutations. Additionally, *MTAP* was mutated in two patients, 1/2 E-GBM and 1/2 PXA. We compared the frequency of *CDKN2A/B*, *TERTp, PTEN*, and *TP53* mutations between E-GBM/PXA (n = 4) and GBM/DA IDH-WT (n = 9). GBM/DA IDH-WT patients had increased frequency of concomitant *CDKN2A/B* and *TERTp* mutations compared to E-GBM/PXA (77.8% vs. 0%). Also, *TP53* or *PTEN* mutations were more commonly present in GBM/DA compared to E-GBM/PXA (77.8% vs. 0%).

BRAF-mutant gliomas in cBioPortal datasets

In cBioPortal, 82/2185 (3.8%) gliomas with a *BRAF* mutation were identified. Fifty-five out of the 82 patients were

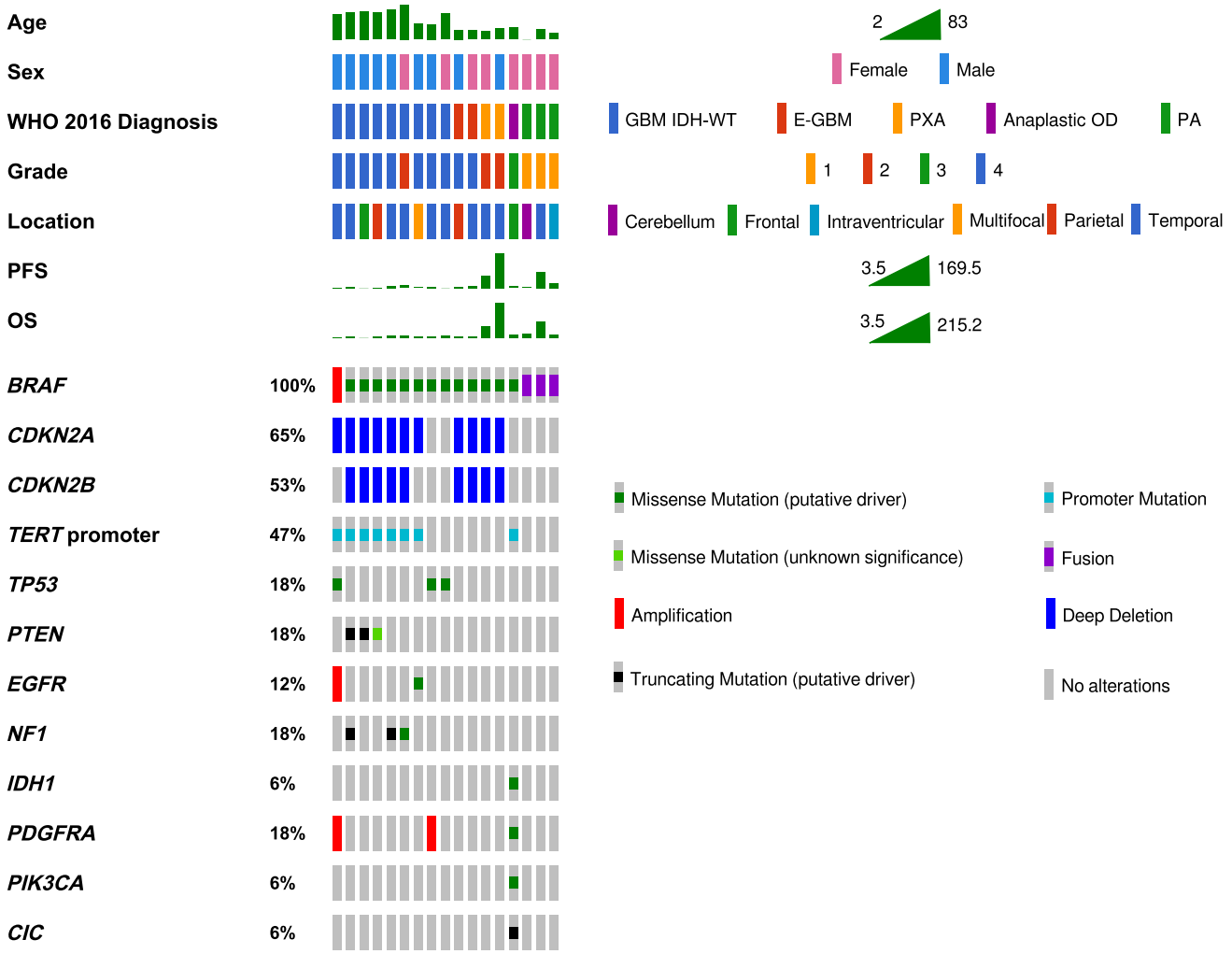

Fig. 1 Mutations in cancer-related genes in 17 patients with *BRAF*-mutant gliomas. *BRAF*-mutant GBMs frequently showed *TERTp, CDKN2A/B, PTEN,* and/or *TP53* mutations. E-GBMs and PXAs showed similar genomic alterations and similar age at diagnosis. PAs showed *BRAF*-KIAA1549 fusion even when located in the temporal lobe. *WHO* World Health Organization, *PFS* progression-free survival, *OS* overall survival, *GBM IDH-WT* glioblastoma IDH-wildtype, *E-GBM* epithelioid glioblastoma, *PXA* pleomorphic xanthoastrocytoma, *OD* oligodendroglioma, *PA* pilocytic astrocytoma. The GBM group includes one case with a histologic diagnosis of diffuse astrocytoma IDH-WT that showed molecular features of GBM WHO grade 4, according to cIMPACT-NOW Update 3 [46]

included in the study, as some patients were incompletely characterized, had unclear histologic diagnosis, or *BRAF* gene deletion, truncation, or variant of unknown significance (Online Resource 3). Fifty-six *BRAF* mutations were identified in the 55 patients (1 patient had two non-V600E point mutations). *BRAF* alterations included p.V600E (23/82, 41%), amplification (14/82, 25%), non p.V600E point mutations (11/82, 20%) and fusions (8/82, 14%) including *KLHL7-BRAF*, *BRAF-TPR, BRAF-ATF7, FAM131B-BRAF, BRAF-UBE2H, BRAF-KIAA1549,* and intragenic fusions (Fig. 2c). Most cases were classified as GBM, IDH-WT (33/55, 60%), while the remaining cases included 7 (12.8%) DA IDH-Mutant, 3 (5.6%) PA, 2 (3.6%) GBM IDH-Mutant, 2 (3.6%) AA IDH-Mutant, 2 (3.6%) DA IDH-WT, 2 (3.6%) ganglioma, 1 (1.8%) AA IDH-WT, 1 (1.8%) OD, 1 (1.8%) anaplastic PXA, and 1 (1.8%) PXA. The most common additional mutations in *BRAF*-mutant gliomas in cBioPortal were *CDKN2A/B, TERTp, TP53, PTEN, TP53, IDH1/IDH2,* and *ATRX* (Fig. 3). Considering the subgroup of patients with available *TERTp* status, all *TERTp*-mutant cases were diagnosed as GBM IDH-WT or OD. Interestingly, *BRAF*-amplification was the most common *BRAF* alteration in IDH-Mutant astrocytomas (7/11 63.7%) and PA showed *BRAF* fusions (*BRAF-KIAA1549 or FAM131B-BRAF).*

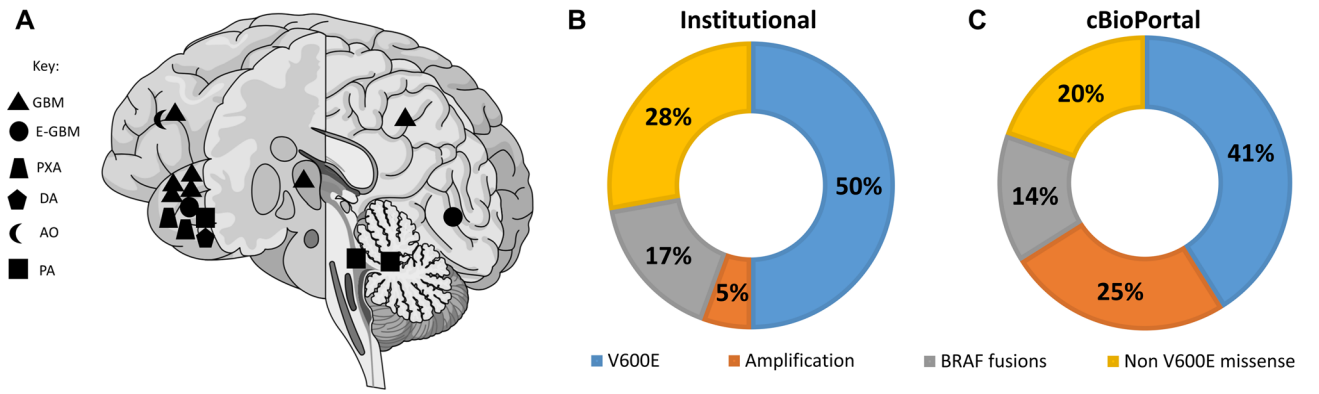

Fig. 2 **a** Anatomic location for the 17 *BRAF*-mutant gliomas demonstrating that 52% (9/17) of the *BRAF*-mutated gliomas occurred in the temporal lobe. One *BRAF*-mutant glioma occurred in a midline location (thalamus) and one tumor showed frontal and brainstem involvement. **b** *BRAF*-mutant gliomas from the institutional database showing that 53% of patients have the V600E mutation. **c** *BRAF*-mutant gliomas from cBioPortal showing that 44% of cases have the V600E mutation. Other alterations included *BRAF* amplification, non V600E missense mutation, loss, and *KLH7-BRAF* fusion

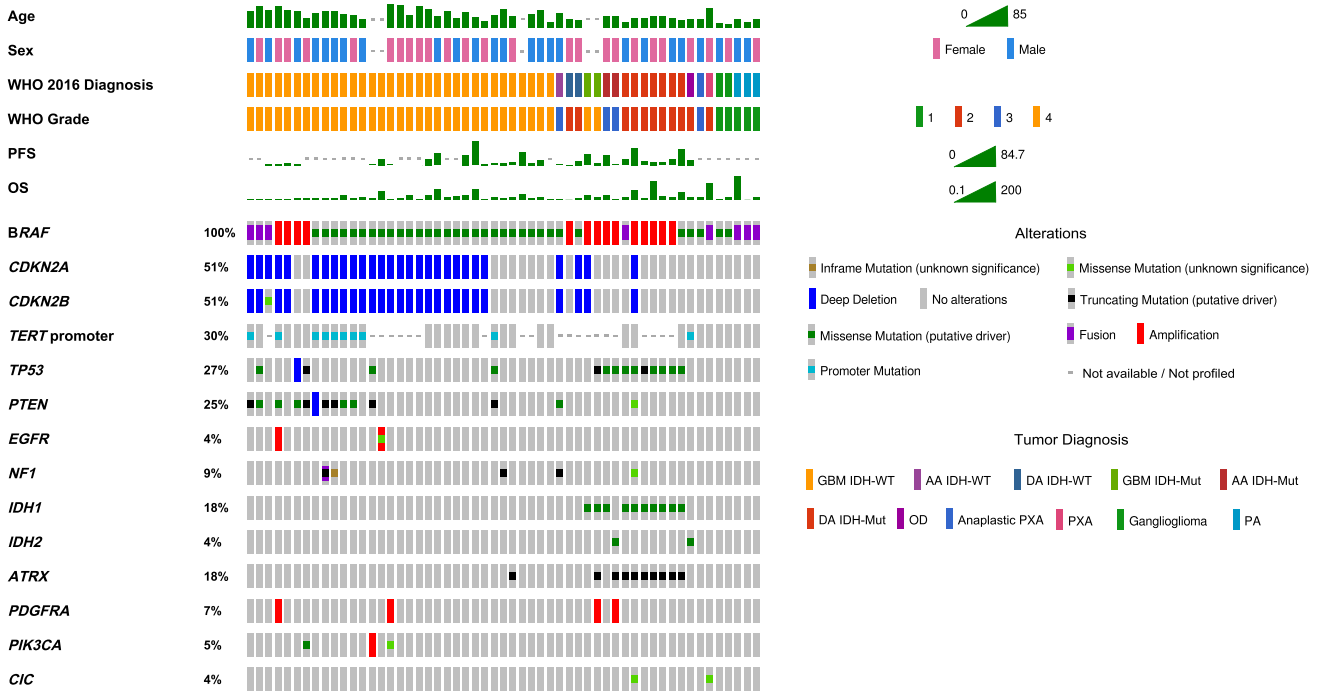

Fig. 3 *BRAF*-mutant, IDH-WT, gliomas in cBioPortal. Fifty-five (55/2185, 2.5%) gliomas were identified to harbored *BRAF* activating mutations in six datasets available at cBioPortal. *WHO* World Health Organization, *PFS* progression-free survival, *OS* overall survival, *WT* wildtype, *Mut* mutant, *GBM* glioblastoma, *AA* anaplastic astrocytoma, *DA* diffuse astrocytoma, *PXA* pleomorphic xanthoastrocytoma, *OD* oligodendroglioma, *PA* pilocytic astrocytoma

Clinical use of BRAF inhibitors

Only two patients in our study (7 and 12) received targeted therapies with *BRAF* pathway inhibitors (cobimetinib, vemurafenib).

Case 7 is a 63-year-old female with an infiltrating *BRAF*-mutant (p.N581I—*BRAF* class 3 mutation), *TP53*-mutant (p.L257Q) glioma, diagnosed as GBM IDH-WT. After initial therapy and multiple therapeutic regimens for progressive disease, the patient's MRI demonstrated worsening tumor burden (Fig. 4a, b). Therefore cobimetinib (MEK inhibitor) was initiated, as preclinical data suggest that class 3 *BRAF* mutations are resistant to RAF inhibitors like vemurafenib [32]. MRI 6-weeks after cobimetinib demonstrated

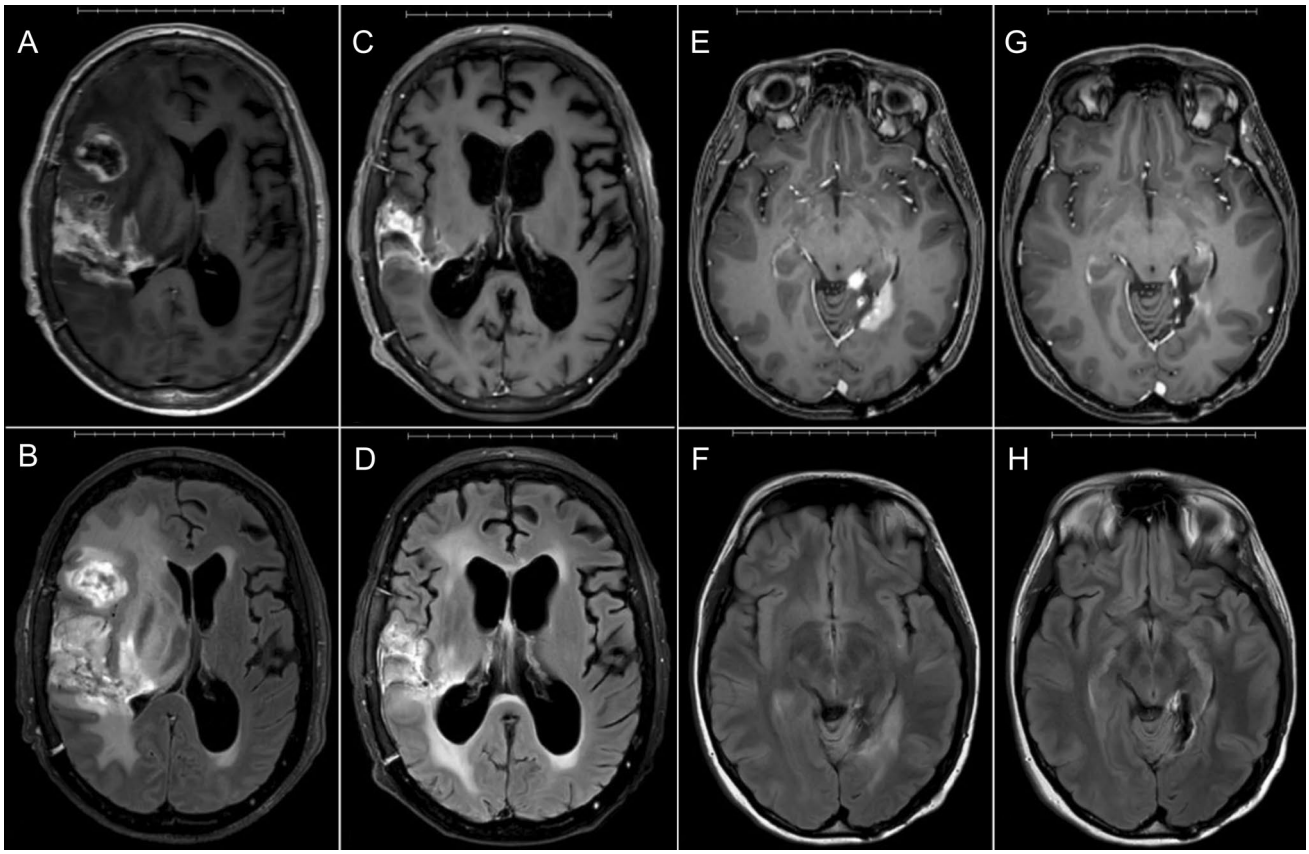

Fig. 4 T1 post-contrast magnetic resonance imaging (T1c-MRI) and T2 Flair-MRI in a GBM patient with a *BRAF* p.N581I mutation (patient 7) before (**a**, **b**) and after (**c**, **d**) 6-weeks of treatment with cobimetinib. T1 post-contrast magnetic resonance imaging (T1c-MRI) and T2 Flair-MRI in a patient with PXA with *BRAF* p.V600E mutation (patient 12) before (**e**, **f**) and after (**g**, **h**) 10-weeks of cobimetinib and vemurafenib treatment. The post-treatment images exhibited a significant decrease in enhancement and vasogenic edema

a significant decrease in the enhancing tumor and vasogenic edema consisting with tumor response (Fig. 4c, d). The patient was deceased 5-months after initiating cobimetinib.

Case 12 is a 21-year-old female who underwent resection of a relatively circumscribed, *BRAF*-mutant (p.V600E) and *CDKN2A/B*-mutant glioma, diagnosed as PXA, WHO grade II. After initial therapy and multiple therapeutic regimens for progressive disease, the patient's MRI demonstrated tumor progression (Fig. 4e, f). Subsequently, cobimetinib and vemurafenib treatment (MEK and *BRAF* inhibitors) were added to the treatment regimen. MRI 10-weeks after cobimetinib/vemurafenib showed an excellent response (Fig. 4g, h). At the moment of this report, 10-months since the last recurrence, the patient is alive and continuing oral therapy with cobimetinib and vemurafenib with no disease progression.

Discussion

A growing body of genomic information has provided the opportunity to include genotype in the diagnosis and treatment of cancer patients [33]. Although previous studies have focused on *BRAF* p.V600E mutations in gliomas [3, 6, 9], the association of *BRAF* mutations with other genomic alterations in gliomas remains to be further refined.

The frequency of *BRAF* p.V600E in GBM IDH-WT, E-GBMs, DA IDH-WT, and PXAs in our study are similar to those previously described [6, 8, 34]. Consistent with previous studies [13, 34, 35], the mean age of diagnosis for *BRAF*-mutant PA and PXAs were 15 and 24 years, respectively. The median age of diagnosis for *BRAF*-mutant GBM IDH-WT was 51.5 years. This age is younger than the reported mean age for GBM IDH-WT (~65 years) [1, 8]. Unlike IDH mutations that are associated with infiltrating gliomas occurring at a younger age (<55 years), *BRAF* mutations were associated with gliomas occurring at a wide range of ages (range 23–71 years) [36]. The median OS of the *BRAF*-mutant GBM IDH-WT gliomas was

12.8 months, lower than the median OS of 19.1 months for the remaining 241 GBM, IDH-WT (*BRAF*-wildtype) cases in our database. The average survival of patients with PA (52.4 months) and PXA (145.2 months) was consistent with the expected long survival for patients with these tumor types [13, 37, 38].

Epithelioid GBM vs. PXA

The age and the mutations in the two E-GBMs in our database strongly resembled those of the two PXAs. The four patients were diagnosed in early adulthood (21–27 years) and exhibited *BRAF* p.V600E and *CDKN2A/B* mutations, and lacked *TERTp, PTEN*, or *TP53* mutations. In cBioPortal, there is 1 PXA with a *BRAF-TPR* fusion and 1 anaplastic PXA with BRAF p.V600E and a CIC mutation. Interestingly, a recent study of whole-genome methylation analysis of 64 E-GBMs concluded that the histopathologically defined E-GBM does not represent a single diagnostic entity. E-GBM cases could be classified into one of three molecularly and biologically distinct categories based on the genome methylation signature; (i) PXA with favorable prognosis, predominantly in children and young adults (38/64, 59.4%), (ii) GBM, IDH-WT with poor prognosis, mainly occurring in older adults, albeit with more frequent *BRAF* mutations (17/64, 26.6%), and (iii) pediatric GBM *RTK1* of intermediate prognosis in children and young adults, associated with chromothripsis and frequent *PDGFRA* amplifications (9/64, 14%) [12]. Therefore, the two cases diagnosed as E-GBMs on histologic grounds alone (given the presence of necrosis) in our study might be better classified as PXAs, based on the current understanding of molecular alterations of these tumors [11, 12]. Although the follow up is limited, the average OS of the E-GBM patients was 12.1 months. At the time of this report, both E-GBM patients are alive and without recurrence, which would be uncommon for patients with GBM [31].

Other alterations in BRAF-mutant gliomas

In GBM IDH-WT, *BRAF* mutations are strongly associated with *TERTp* and *PTEN* mutations and mutations in either *TP53* or *CDKN2A/B*. *TP53* mutations and *CDKN2A/B* loss appear to be mutually exclusive in cases with *BRAF* missense mutations, as previously reported [39]. In agreement with this observation, in the cBioPortal dataset, *TP53* mutation and *CDKN2A/B* loss are mutually exclusive events ($p \leq 0.001$, n = 885) [25, 26, 40]. Our results show an association between coexisting BRAF p.V600E, *CDKN2A/B* loss, and *TERTp* mutations and a diagnosis of GBM, IDH-WT. In contrast, PXA/E-GBMs showed BRAF p.V600E and *CDKN2A/B* loss without *TERTp* mutations. These data suggest that *TERTp* mutations can help distinguish *BRAF*-mutant GBM from PXA/E-GBM.

We identified 2 oligodendrogliomas, IDH-mutant and 1p/19q codeleted with a *BRAF* mutation and 11 IDH-mutant astrocytomas with *BRAF* alterations. While *BRAF* alterations have been rarely detected in IDH-mutant tumors, the significance of this finding remains to be determined [41].

BRAF-mutant gliomas and the temporal lobe

The frequencies of PXAs and E-GBMs localizing to the temporal lobe in previous studies were similar to our cohort (3/4, 75%) [8, 35]. However, the previously reported frequency of GBMs occurring in the temporal lobe (28%) is lower than that of the *BRAF*-mutant GBM, IDH-WT cases in our study (4/8, 50%). This supports the observation that *BRAF*-mutant GBMs have a predilection for the temporal lobe [42, 43]. Our data did not show an association between PXA/E-GBM histology or *CDKN2A/B* loss and localization to the temporal lobe. However, the number of PXA/E-GBMs in our study is small.

BRAF p.V600E immunohistochemistry and targeting BRAF in gliomas

Two patients in our study (#7-GBM and #12-PXA) were treated with cobimetinib and vemurafenib and both responded favorably. Patient 7 had a non-V600E *BRAF* mutation, suggesting that MAPK pathway inhibitors could be incorporated in the treatment of gliomas with *BRAF* mutations other than the p.V600E. Importantly, in the present study, taking into account cases in our cohort and the cBioPortal dataset, 57% of *BRAF*-mutations were non-V600E. Recognizing *BRAF*-mutations opens the possibility of incorporating targeted therapy (*BRAF* and MEK inhibitors) in the treatment plan of glioma patients, which could be missed if *BRAF* status is only evaluated with the BRAF p.V600E specific antibody. Moreover, identifying the particular *BRAF* mutations might be crucial, as preclinical data has shown that some might not respond to *BRAF* inhibitors (class 3 mutations) and should be better treated with MEK inhibitors, as shown in case 7 [32].

BRAF mutant adults and pediatric gliomas

Our cohort includes mostly adult patients, which differs from most prior studies that include *BRAF*-mutant gliomas in the pediatric population [44]. This difference is driven by the clinical practice at our institution and it allows us to explore *BRAF*-mutant gliomas in an adult cohort. A recent study determined that nearly all pediatric low-grade gliomas

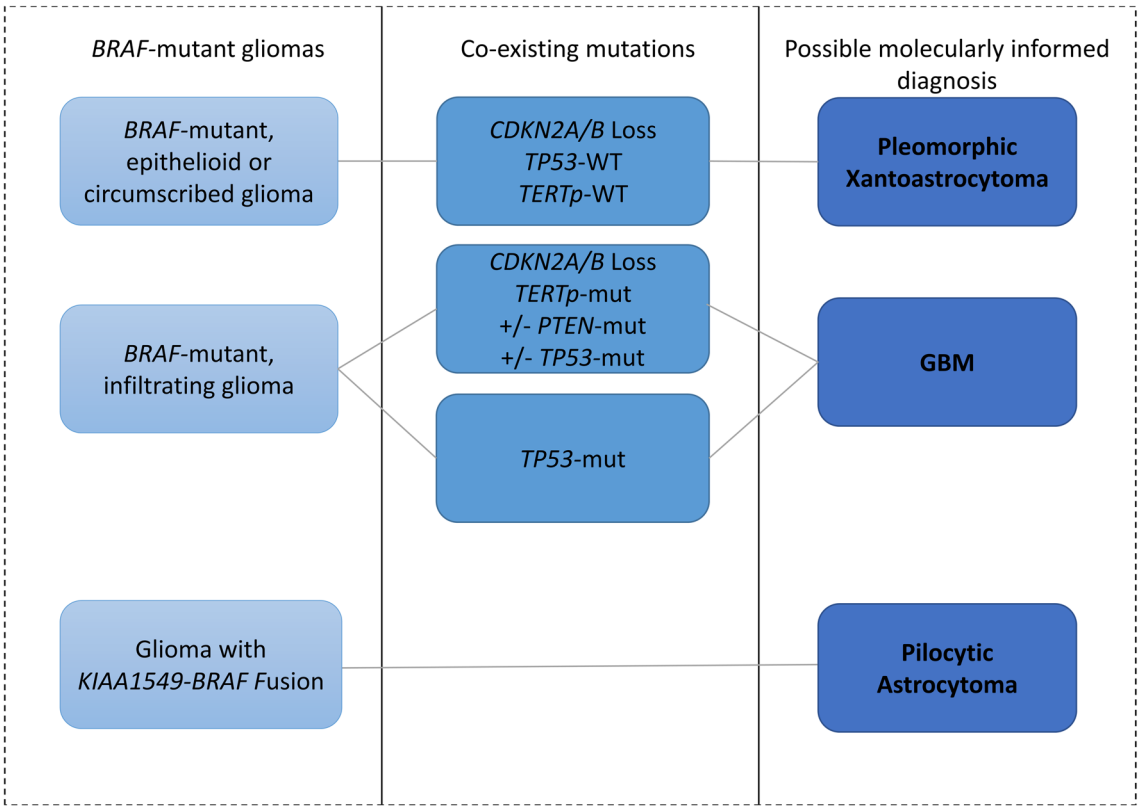

Fig. 5 Possible molecularly informed diagnosis of *BRAF*-mutant gliomas. Incorporation of additional alterations that co-occur in *BRAF*-mutant gliomas may improve their molecularly informed diagnosis. Circumscribed and epithelioid *BRAF*-mutant gliomas with *CDKN2A/B* loss and intact *TP53* and *TERTp* are most likely Pleomorphic xanthoastrocytomas. In contrast, *BRAF* mutant infiltrating gliomas with *CDKN2A/B* loss, *TERTp* mutations, and with or without *PTEN or TP53* are most likely GBM. Similarly, *BRAF*-mutant infiltrating gliomas with *TP53* mutation are most likely GBMs. Circumscribed gliomas with the *KIAA1549-BRAF* fusion are most likely pilocytic astrocytomas

harbored RAS/MAPK activation pathway alterations, with *KIAA1549-BRAF* fusion, *BRAF* p.V600E, or *NF1* mutations accounting for 66% of cases [45]. Pediatric patients in our study included 1 PA with *KIAA1549-BRAF* and 2 gangliogliomas (p.V600E-mutant). In contrast to pediatric gliomas, *BRAF* alterations are infrequent in adults' gliomas but can be detected in ~ 2–5% of patients with p.V600E being the most common.

Summary

In this study, we defined additional alterations in *BRAF*-mutant gliomas and their potential diagnostic implications. Our results demonstrate that gliomas with *BRAF* mutations frequently exhibit additional alterations in *TP53, TERTp, CDKN2A/B*, and *PTEN* and show a favorable response to *BRAF* and/or MEK inhibitors. Knowledge of alterations that co-occur with *BRAF* mutations in gliomas, in particular, alterations involving *CDKN2A/B, TERTp, TP53*, and *PTEN* may improve glioma diagnosis (Fig. 5). For example, *BRAF*-mutant gliomas with co-existing *CDKN2A/B, TERTp, TP53,* or *PTEN* mutations may be considered as GBM, while *BRAF*-mutant tumors with only *CDKN2A/B* alterations, but no mutations in *TERTp, TP53,* or *PTEN* appear to be better classified as PXAs. However, we recognize that our conclusions are limited by a small sample size (due to the rarity of *BRAF*-mutant infiltrating gliomas) and the absence of *TERTp* information in cBioPortal. Nonetheless, the additional genetic alterations that coexist with *BRAF* mutations can inform the diagnosis of gliomas including PXA, E-GBM, and "*BRAF*-mutant GBM IDH-WT". Accurate diagnosis of these tumors is critical as their prognosis and treatment differs.

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Data availability The datasets generated during and/or analyzed during the current study are available upon reasonable request to the corresponding authors.

Compliance with ethical standards

Conflict of interest The authors declared no conflict of interest.

Ethical approval This retrospective study was approved by the institutional review board of The University of Texas Health Science Center at Houston and Memorial Hermann Hospital, Houston, TX, following the 1964 Helsinki Declaration and its later amendments.

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