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CLINICAL STUDY

Molecular characteristics and clinical features of multifocal glioblastoma

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Abstract

Introduction Glioblastomas (GBMs) usually occur as a solitary lesion; however, about 0.5–35% present with multiple lesions (M-GBM). The genetic landscape of GBMs have been thoroughly investigated; nevertheless, differences between M-GBM and single-foci GBM (S-GBM) remains unclear. The present study aimed to determine differences in clinical and molecular characteristics between M-GBM and S-GBM.

Methods A retrospective review of multifocal/multicentric infiltrative gliomas (M-IG) from our institutional database was performed. Demographics, clinical, radiological, and genetic features were obtained and compared between M-GBM IDH-wild type (IDH-WT) vs 193 S-GBM IDH-WT. Mutations were examined by a targeted next-generation sequencing assay interrogating 315 genes.

Results 33M-IG were identified from which 94% were diagnosed as M-GBM IDH-WT, the remaining 6% were diagnosed as astrocytomas IDH-mutant. M-GBM and S-GBM comparison revealed that *EGFR* alterations were more frequent in M-GBM (65% vs 42% p = 0.019). Furthermore, concomitant *EGFR*/*PTEN* alterations were more common in M-GBM vs. S-GBM (36% vs 19%) as well as compared to TCGA (21%). No statistically significant differences in overall survival were observed between M-GBM and S-GBM; however, within the M-GBM cohort, patients harboring *KDR* alterations had a worse survival (*KDR*-altered 6.7 vs *KDR*-WT 16.6 months, $p = 0.038$).

Conclusions The results of the present study demonstrate that M-GBM genetically resembles S-GBM, however, M-GBM harbor higher frequency of *EGFR* alterations and co-occurrence of *EGFR*/*PTEN* alterations, which may account for their highly malignant and invasive phenotype. Further study of genetic alterations including differences between multifocal and multicentric GBMs are warranted, which may identify potential targets for this aggressive tumor.

Keywords Multifocal glioblastoma · Multicentric glioblastoma · *EGFR* · *KDR*

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Introduction

Glioblastoma (GBM) is the most common primary malignant tumor of the central nervous system (CNS) and is associated with poor prognosis despite current therapies [1–3]. GBMs usually occur as a solitary lesion; however, about 0.5 to 35% of all GBMs present with multiple lesions (M-GBM) [4–8]. Based on radiographic features, M-GBMs can be further divided into multifocal and multicentric GBMs. Multifocal GBMs have a clear contiguity pathway of spread between foci, which can be demonstrated by contiguous areas of T2-weighted signal on magnetic resonance imaging (MRI) of the brain. Meanwhile, multicentric GBMs are widely separated lesions that cannot be attributed to contiguous pathways [9]. The prognosis of multifocal and multicentric GBMs (M-GBM) have been historically reported to be more dismal than single foci GBM (S-GBM) [6–8].

The genetic landscape of GBMs has been thoroughly investigated revealing important genetic pathways that influence its clinicopathologic characteristics and outcomes [10, 11]. The exact mechanism of multifocality/multicentricity is still not fully comprehended, as studies defining genomic alterations of these tumors are scarce. The molecular characteristics of M-GBM might explain its inherent ability to migrate and invade, resulting in the poor outcome observed in these patients. It has been described that M-GBM lacks *IDH1*, *ATRX*, and *PDGFRA* mutations. Nevertheless, given the small sample sizes of prior studies, the true genomic differences between S-GBM and M-GBM remain unclear [8].

The goal of the present study is to determine from our institutional experience the molecular characteristics and clinical features between M-GBM and S-GBM, to allow a better understanding of genetic alterations in M-GBM that could explain their biology and behavior.

Material and methods

Patients and tumor samples

We performed a retrospective review of patients diagnosed with infiltrative glioma between January 2004 and December 2019. Patients were included if they had (1) histologic diagnosis of diffuse glioma; (2) multifocal or multicentric tumor; (3) mutation analysis by next-generation sequencing. Patients with multifocal tumors were defined as those having at least two distinct tumor foci, separated by at least 1 cm. Multifocal and multicentric GBM (M-GBM) were analyzed together and individually for a subanalysis. A cohort including 193 S-GBM, IDH-WT was used for comparison of demographic, clinical, and molecular characteristics (Online Resource 1). Patients diagnosed with diffuse astrocytic gliomas, IDH-WT, with molecular features of GBM, WHO grade IV were considered as GBM, IDH-WT according to the 3rd cIMPACT-NOW update [12].

Study data were collected from the electronic medical record of Memorial Hermann Hospital and managed using REDCap electronic data capture tools hosted at the University of Texas Health Science Center at Houston (UT Houston) [13, 14]. These included age, gender, Karnofsky performance status (KPS), histologic diagnosis, tumor location, radiographic extent of resection, treatment strategy, recurrence, and survival. Tumors were classified by a Board-certified neuropathologist following the 2016 WHO Classification of Tumors of the Central Nervous System [15]. This study was approved by the Institutional Review Board of the UT Houston and Memorial Hermann Hospital, Houston, TX.

Targeted sequencing

Tumor samples were analyzed for genomic alterations by a targeted next-generation sequencing assay (NGS) interrogating 315 genes and 28 gene rearrangements (FoundationOne CDx®, Foundation Medicine, Inc., Cambridge, MA, USA). The FoundationOne® assay was performed in a clinical laboratory improvement amendments certified laboratory, as previously described [16–18]. Telomerase reverse transcriptase promoter (*TERTp*) status was not available for four patients.

Co-occurrence of most common mutations

To evaluate the co-occurrence of *EGFR*/*PTEN*, *EGFR*/*KDR*, and *EGFR*/*PTEN*/*TERTp* alterations, we compared M-GBM vs S-GBM. In addition, to further investigate these differences we compared our results to the known incidence of these mutations in The Cancer Genome Atlas (TCGA) PanCancer Atlas for *EGFR*/*PTEN* and *EGFR*/*KDR*, and to a published study evaluating co-occurrence of *EGFR*/*PTEN*/*TERTp* alterations, as TCGA lacks *TERTp* information [19–22].

Statistical analyses

Clinical, demographic, and frequency of genomic alterations were evaluated by Fisher's exact test or Mann–Whitney U test for categorical and continuous variables, respectively. Overall survival was calculated as the time in months from diagnosis to death or date of last available follow-up. Kaplan–Meier method was used to plot survival curves and statistical significance was examined by the univariable

log-rank test. Cox proportional hazard regression models were utilized for univariable and multivariable analysis to calculate the hazard ratio (HR) estimates with 95% confidence interval (CI). Multivariable Cox proportional hazard regression model analysis was adjusted for the following set of covariates: age, male gender, KPS, Stupp protocol, salvage bevacizumab, multifocal vs multicentric, and *KDR* mutant gene, as these are factors well-known to affect survival [23], *KDR* was selected as it was found to be significant in univariable analysis. A p-value ≤ 0.05 was considered statistically significant. Statistical analyses were performed in EZR (1.40) [24] and Prism v.8.2.1 (GraphPad, La Jolla, California, USA).

Results

Clinico-demographic characteristics of multifocal infiltrative gliomas

A total of 564 patients with infiltrative gliomas (IG) were identified, from which 41 (7.3%) had a multifocal/multicentric profile (M-IG). There were 39/395 (9.9%) M-GBM IDH-WT patients, 2/69 (2.9%) astrocytomas IDH-mutant (IDH-MT) presented multiple lesions at the time of initial diagnosis. From the 41 patients, 33 met the inclusion criteria and were further analyzed (Online Resource 1).

The 33M-IG patients had a median age of 60 years (range 26–77). There were 17 males and 16 females, 24.2% had KPS scores ≥ 80, and 29/33 (88%) underwent resection, meanwhile, the remaining 12% underwent biopsy. Temozolomide was prescribed to 31 (93.9%) patients with concomitant radiotherapy to 30 (90.9%) according to the Stupp protocol [25]. Moreover, some patients were treated with 1st line bevacizumab (7/33, 21.2%), irinotecan (5/33, 15.2%), and tumor-treating fields (5/33, 15.2%). Histologically, 31 patients (94%) were diagnosed as GBM, IDH-WT, 1 (3%) GBM IDH-mutant (IDH-MT), and 1 (3%) diffuse astrocytoma (DA), IDH-MT. Furthermore, 22/33 (66.7%) patients could be further classified as multicentric and 30 (90.9%) patients had enhancing tumors (Fig. 1). The demographic and clinical characteristics are depicted in Table 1.

The comparison between S-GBM and M-GBM revealed that the latter was less likely to have gross total resection (GTR, 12.9% vs 33.5%, $p=0.02$) but was treated more frequently with 1st line irinotecan (1.5% vs 12.9%, $p=0.007$) and bevacizumab (9.3% vs 19.4%, $p=0.11$). Re-resection was less likely to occur in M-GBM patients

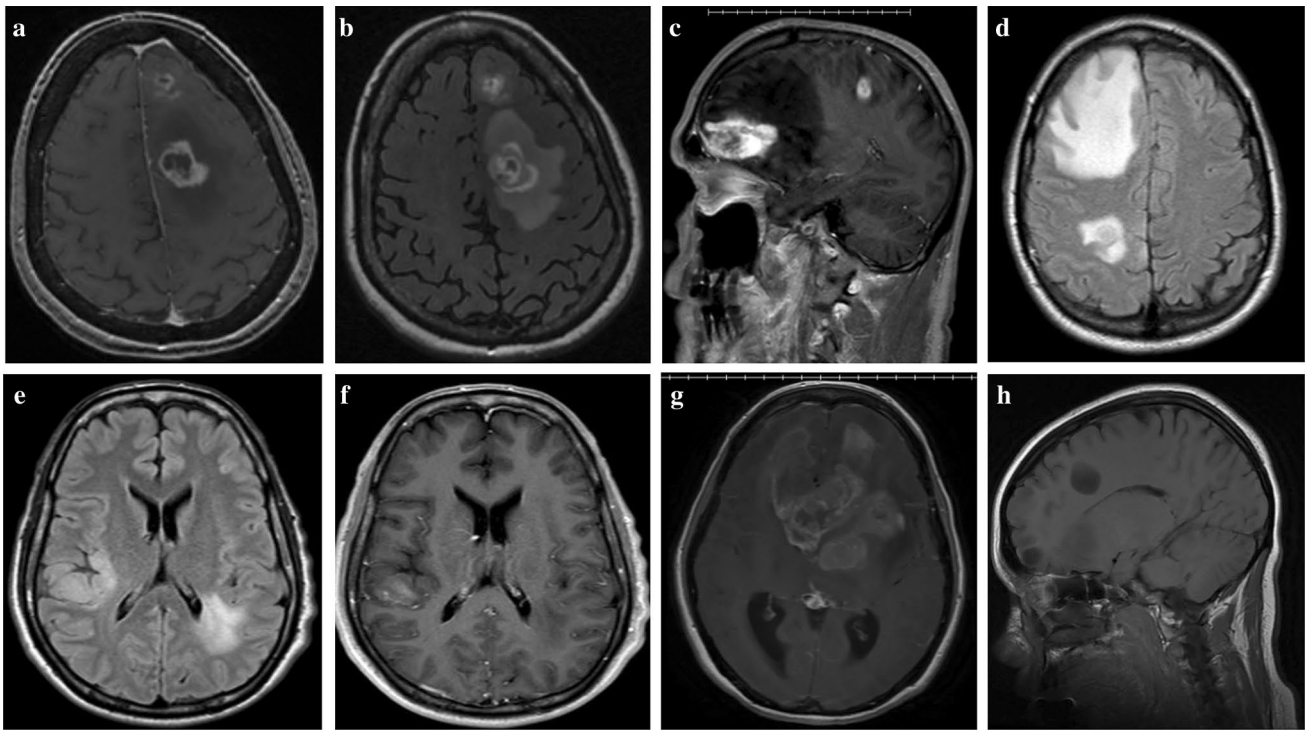

Fig. 1 M-GBM MRI imaging. Patient 1, axial T1 post contrast (**a**) and axial T2 flair (**b**) demonstrating a multifocal GBM IDH-WT. Patient 27, sagittal T1 post contrast (**c**) and axial T2 Flair (**d**) demonstrating a multicentric GBM IDH-WT. Patient 20, axial T2 Flair (**e**), and axial T1 post contrast (**f**) demonstrating a multicentric partially enhancing GBM IDH-WT. Patient 32, axial T1 post contrast (**g**) demonstrating a multifocal GBM IDH-MT. Patient 33, sagittal (**h**) MRI demonstrating a multicentric diffuse astrocytoma IDH-MT

Table 1 Demographics, clinical characteristics and treatments of patients with GBM IDH-WT

Characteristic	All GBM IDH-WT n = 224	S-GBM n = 193	M-GBM n = 31	*p*-value	Multifocal GBM n = 10	Multicentric GBM n = 21	*p*-value
Age at diagnosis, median [IQR]	61 [54–68]	61 [54–69]	61 [54–67]	0.827	59.5 [55–68]	61 [54–66]	1.00
Male (%)	60	61	55	0.599	30	67	0.120
Non-Hispanic White (%)	69	68	74	0.676	60	81	0.380
Karnofsky performance status 80–100 (%)	34	35	26	0.414	40	19	0.381
1st Line therapy (%)							
Stupp protocol	90	89	94	0.749	100	86	0.553
Bevacizumab	11	9	19	0.114	20	19	1.00
Irinotecan	3	1.5	15	**0.008**	10	14	1.00
Tumor-treating fields	13	13	16	0.577	10	19	1.00
Surgical resection (%)				**0.0009**			0.097
Gross-total resection	32	35	13		30	5	
Near-total resection	16	18	3		0	5	
Subtotal resection	41	37	71		50	80	
Biopsy	11	10	13		20	10	
Salvage therapy[a] (%)							
Re-operation	36	39	19	0.072	44	6	**0.035**
Temozolomide	44	44	38	0.669	33	41	1.00
Bevacizumab	63	63	65	0.828	89	53	0.098
Irinotecan	32	31	35	0.818	44	29	0.667
Tumor-treating fields	31	30	38	0.486	44	35	0.692
Radiotherapy	20	20	19	1.00	22	17	0.588
Stereotactic radiosurgery	34	34	31	0.822	44	35	0.382
Genetic alterations (%)							
CDK4-MT	11.2	10.9	12.9	0.759	40	0	**0.007**
CDKN2A/B-MT	71	70	77	0.523	50	91	**0.022**
EGFR-MT	45	42	65	**0.019**	70	62	1.00
KDR-MT	9	8	13	0.31	10	14	1.00
KIT-MT	9	8	13	0.31	10	14	1.00
NF1-MT	16	18	7	0.185	0	10	1.00
PIK3CA-MT	12	12	7	0.545	0	10	1.00
PTEN-MT	50	48	58	0.338	90	43	**0.020**
TERTp-MT[b]	81	81	79	0.799	89	74	0.63
TP53-MT	29	30	23	0.523	50	10	**0.022**

GBM glioblastoma, *WT* wild type, *MT* mutant, *IQR* interquartile range, *S-GBM* single foci glioblastoma, *M-GBM* multifocal/multicentric glioblastoma

[a]Not all patients had a recurrence, for the salvage therapies a total of 154 GBM IDH-WT were taken into account [128 S-GBM and 26M-GBM (9 multifocal and 17 multicentric)]

[b]Tested in 221 patients as *TERTp* status was not available for 3 patients. Fisher's exact test or Mann–Whitney U test was performed for categorical and continuous variables respectively. *P*-values were two-sided and a $p \leq 0.05$ was considered as statistically significant and are represented in bold

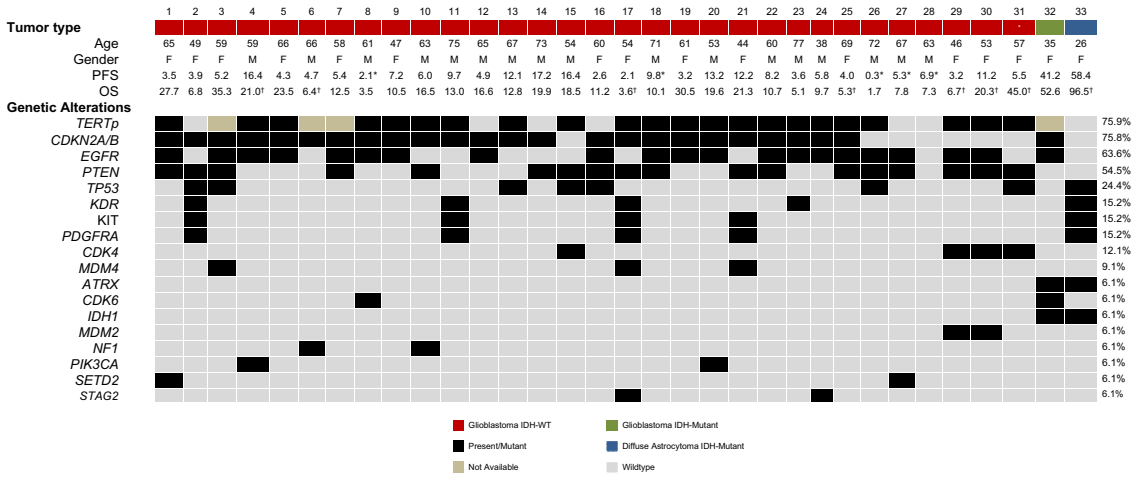

Fig. 2 Mutations in cancer-related genes of 33 multicentric/multifocal infiltrative glioma patients. *PFS* progression-free survival (months), *OS* overall survival (months), *WT* wild type, *Mut* mutant, *M* male, *F* female. *Anaplastic astrocytoma IDH-WT with molecular features of GBM WHO grade IV according to cIMPACT-NOW Update 3

(39.8% vs 19.2%, $p = 0.07$). Furthermore, the analysis between multifocal (n = 10) and multicentric (n = 21) GBM IDH-WT showed that within this group, multifocal patients were more likely to undergo re-resection and salvage therapies at recurrence (Table 1).

Genetic alterations

NGS analysis revealed that *TERTp* was mutated in 22/29 (75.9%) patients. *CDKN2A/B* loss was observed in 25/33 (75.7%), while *EGFR* alterations in 21/33 (63.6%) patients. *PTEN* was mutated in 18/33 (54.5%) patients. The other frequently altered genes were *TP53* in 8/33 (24.4%), *KDR*, *KIT*, and *PDGFRA*, each mutated in 5/33 (15.2%) patients (Fig. 2; Table 1).

The comparison between S-GBM and M-GBM revealed that *EGFR* alterations were more frequently present in M-GBM (65% vs 42% $p = 0.019$, Table 1 and Online Resource 2). Moreover, we observed that concomitant mutations in *EGFR* and *PTEN* genes were more frequently observed in M-GBM (M-GBM 36% vs S-GBM 19%, $p = 0.05$). Also, the percentage of patients harboring the aforementioned mutation concomitantly with *TERTp* mutations was higher in M-GBM (M-GBM 25% vs S-GBM 18%, $p = 0.43$). Analysis of TCGA data revealed a co-occurrence of *EGFR*/*PTEN* alterations in 81/378 (21.4%) GBM IDH-WT cases. This was similar to the S-GBM group (19%) but lower than M-GBM patients in our study [19, 20, 22]. The co-occurrence of *EGFR*, *PTEN*, and *TERTp* also appeared to be higher in M-GBM (25%) compared to prior reports (5.6%) [21]. Also, we evaluated the co-occurrence of *EGFR*/*KDR* alterations in the entire cohort (n = 224) and M-GBM (n = 31), in which there was not an association between these genes ($p = 0.34$ and 0.12, respectively). These results were further validated by the lack of association in the TCGA database between *EGFR*/*KDR* alterations ($p = 0.49$) and *EGFR* amplification and *KDR* alterations ($p = 0.17$) [19, 20, 22].

Prognosis and survival

Univariable analysis of GBM IDH-WT patients (n = 224) demonstrated that patients with age < 55 years, KPS 80 or higher, and 1st line Stupp protocol had improved OS. However, only age and 1st line Stupp protocol were independent predictors of poor survival after multivariable analysis. Importantly, we observed a trend towards shorter survival (13.0 vs 17.9 months, $p = 0.31$) in M-GBM patients (Fig. 3a, Online Resource 3).

No statistically significant differences were observed between multifocal and multicentric GBM. However, multifocal GBM showed a trend towards better outcomes (Fig. 3b). In addition, M-GBM patients with *KDR* alterations had a worse survival than *KDR*-WT patients in univariable analysis (6.7 vs 16.6 months, $p = 0.038$) and multivariable analysis (HR 9.3 [1.17–73.8], $p = 0.035$, Fig. 3c; Table 2). The effect of *KDR* alterations in the outcome of S-GBM was observed in the univariable log-rank test, in which *KDR* altered S-GBM had a worse survival than their *KDR*-WT counterparts (11.4 vs 18.5 months, $p = 0.015$). However, this difference was not observed after multivariable analysis (*KDR* altered HR 1.78 [0.74–4.31], $p = 0.20$). Survival differences

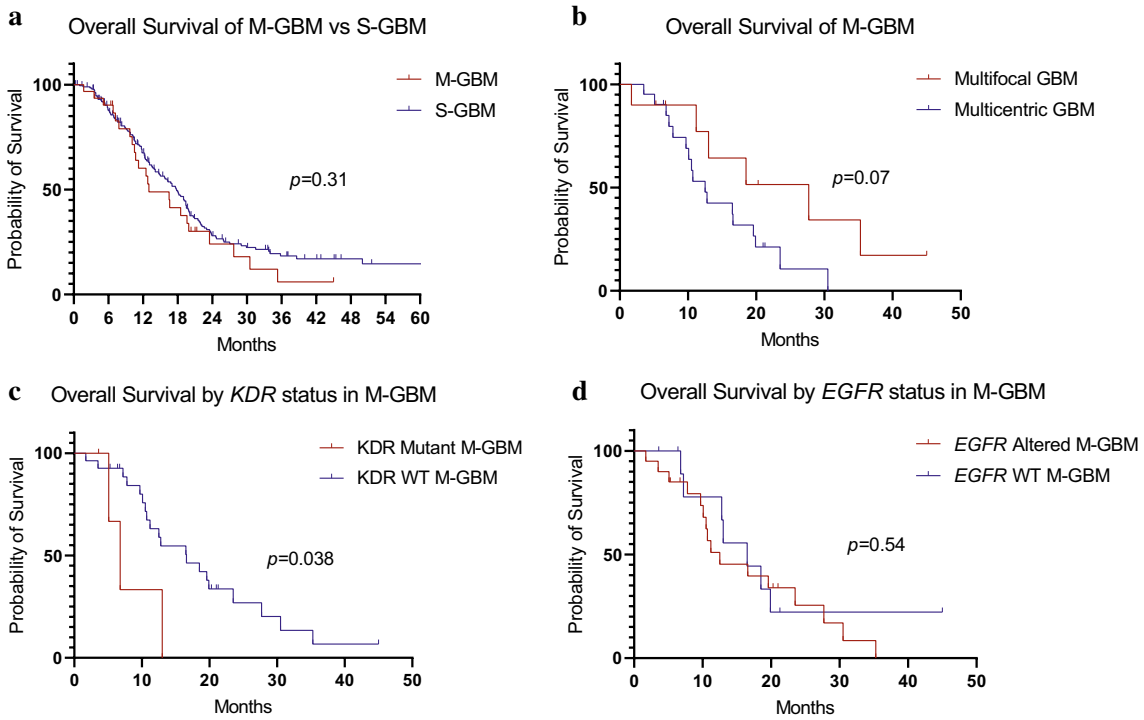

Fig. 3 Overall survival in GBM IDH-WT. **a** Overall survival of GBM IDH-WT (n = 224) by multifocality, in which there was not statistically significant difference between the survival of M-GBM (n = 31) and S-GBM (n = 193, 13.0 vs 17.9 months, $p = 0.31$). **b** Overall survival within M-GBM (n = 31). There was not statistically significant difference between multifocal (n = 10) and multicentric (n = 21) patients (27.7 vs 12.5 months, $p = 0.07$). **c** Overall survival of M-GBM (n = 31) by KDR gene status, in which KDR mutant patients (n = 4) had worse survival compared to KDR WT patients (n = 27, 6.7 vs 16.6 months, $p = 0.038$). **d** Overall survival of M-GBM by EGFR gene status, in which there was not statistically significant difference between EGFR altered (n = 20) and EGFR WT (n = 10) patients (12.5 vs 16.5 months, $p = 0.54$). *GBM* glioblastoma, *M-GBM* multifocal/multicentric GBM, *S-GBM* single foci GBM, *WT* wild type. Kaplan–Meier curves were examined by the log-rank test, a $p \leq 0.05$ was considered as statistically significant

Table 2 Multivariable Cox proportional hazard regression model of overall survival in patients with M-GBM (n = 31)

Variable	Multivariable HR [95% CI]	*p*-value
Age at diagnosis > 55 years	0.51 [−0.13 to 1.91]	0.315
Male	4.87 [0.90 to 26.3]	0.065
KPS ≥ 80	1.32 [0.36 to 4.80]	0.677
Stupp protocol	0.09 [0.006 to 1.39]	0.086
Salvage bevacizumab	0.55 [0.14 to 2.10]	0.382
Multifocal	0.38 [0.08 to 1.77]	0.219
KDR MT	9.30 [1.17 to 73.8]	**0.035**

P-values were two-sided and a $p < 0.05$ was considered as statistically significant and are represented in bold

M-GBM multifocal/multicentric glioblastoma, *KPS* Karnofsky performance status, *MT* mutant, *HR* hazard ratios, *CI* confidence interval

in M-GBM patients were not observed between *EGFR*-altered (12.5 months) and *EGFR*-WT (16.5 months) tumors (Fig. 3d; Table 2).

Discussion

Several studies on M-GBM have reported decreased survival when compared to S-GBM; however, studies focusing on the molecular characteristics of M-GBM are scarce. It is still unclear whether M-GBM represents a distinct biologic variant of GBM or if multifocal progression is part of the natural history of the disease [7, 8]. The current study represents, the largest M-GBM cohort with comprehensive genomic characterization, in which subtle molecular differences were observed. Our data suggest that M-GBM genetically resembles typical GBM.

In the present cohort, we identified that 9.9% of GBM IDH-WT had multiple lesions at diagnosis, which is comparable to recent studies that have used similar criteria to define M-GBM [5, 7, 8]. Interestingly, we identified 2 cases (2.9%) of multifocal astrocytomas IDH-MT. A recent study reported that multifocality is an independent predictor for *IDH1*-WT status, as only 1/102 (1%) patients had an *IDH1* mutation [26]. The multifocal tumor, in this case, was an oligodendroglioma, IDH-mutant and 1p/19q co-deleted. A prior study by Liu et al. [8] reported all multifocal patients

to be IDH-WT. In our study, both patients (32 and 33) were astrocytomas, IDH-mutant (1p/19q intact). Thus, to the best of our knowledge, these cases represent the first reports of multifocal IDH-MT astrocytomas (Figs. 1g, h, 2).

Previous studies have described the differential expression of MAPK, PTEN, MGMT, and EGFR between S-GBM and M-GBM; however, these studies are limited by their small sample size and lack of comprehensive molecular characterization [5, 7, 27]. A recent study on 18M-GBM patients with mutation analysis identified the absence of *IDH1*, *ATRX*, or *PDGFRA* mutations in all patients [8]. These results contrast with our findings in which in addition to the observed IDH1 p.R132H mutation in two patients, we also observed mutations in *ATRX* and *PDGFRA* amplification in 2.9% and 15.2% of patients, respectively. *ATRX* mutations are widely distributed across the gene and are mostly truncating (including frame shift and nonsense variants) as seen in the current study (patient 32 with an *ATRX* N111fs*16 and patient 33 with an *ATRX* E1541*) [28]. *ATRX* mutations occur in 78% and 63% of GBM, IDH-MT and astrocytoma IDH-MT, respectively, and only in 3% of GBM IDH-WT [29]. Moreover, a Japanese study identified loss of ATRX expression in 28.6% of 14 multicentric astrocytomas, IDH-WT [27]. Thus, it is not surprising that *ATRX* mutations were not observed in previous smaller cohorts. *PDGFRA* discrepancies between our study and previous studies could be also explained by sample size, as we observed that *PDGFRA* is amplified in 15.2% of M-IG and 12.9% of M-GBM. This represents roughly the same known frequency of *PDGFRA* amplifications in GBM, as 13.5% of S-GBM and 14.7% of GBMs in TCGA harbored *PDGFRA* amplification [19]. Moreover, *PDGFRA* amplification has been reported in 16.7% of a small M-GBM study [30].

EGFR alterations are found in 30–50% of all GBMs and are associated with tumor development and progression [19, 31]. Therefore, *EGFR* has been proposed as a potential therapeutic target for GBMs; however, clinical trials targeting this gene have been unsuccessful [32, 33]. A previous study with six M-GBM found aberrations in *EGFR* in all patients [30]. In our study, we also identified that *EGFR* alterations were more common in M-GBM than in S-GBM (65% vs 42%, $p = 0.019$).

Importantly, *EGFR* alterations have been associated with an increased infiltrative and invasive phenotype [30, 34], and therefore with a multifocal appearance as seen in our study. Notably, we observed that co-occurrence of *EGFR* and *PTEN* alterations, as well as, *EGFR*, *PTEN*, and *TERTp* alterations, was increased in M-GBM compared to both S-GBMs in our cohort and published literature [19–22]. These findings are in accordance with the results of a previous study that described 67% of M-GBM showed alterations in *EGFR*, *PTEN*, and *TERTp* [30]. Therefore M-GBM genetically resembles typical GBM with an increased incidence of *EGFR* mutations and co-occurrence of *EGFR*, *PTEN*, and *TERTp* alterations.

A previous study has also identified a higher expression of *CYB5R2* in M-GBM, which has been independently associated with worse OS and has been proposed as a potential prognostic and diagnostic marker [8]. Unfortunately, we did not examine the expression of this gene in our study.

Overall survival and multifocality

Multifocality has been consistently correlated with poor survival in GBM [4–8, 35, 36]. In our study, M-GBM trended towards a worse survival compared to S-GBM; however, it did not reach statistical significance. This could be explained due to the limited sample size, the relatively small percentage of biopsies performed in these cases (12.2%) compared to prior reports (28–100%) [5–7, 35–38], and the more aggressive adjuvant upfront approach demonstrated by increased utilization of chemotherapeutic agents in these patients (bevacizumab and irinotecan) compared to S-GBM (Online Resource 4). Prior centers have also described an aggressive approach for these cases, suggesting multiple craniotomies for maximal cytoreduction that could potentially improve survival [9]. Interestingly, we identified for the first time that alterations in *KDR* predicted worse survival within the M-GBM group. Even though our results should be taken with caution and validated in a larger cohort, *KDR*, also known as *VEGFR2*, is a tyrosine kinase receptor that plays an important role in GBM angiogenesis, aggressiveness, and progression [39–42]. Previous studies have demonstrated that the *KDR* CAGT haplotype increases GBM aggressiveness and that concomitant high mRNA expression of *KDR*, *FLT1*, and *VEGFA* has been associated with shorter survival [39, 40]. Bevacizumab, a monoclonal antibody towards VEGF, is one of the few drugs currently approved for GBM treatment. A recent study has demonstrated that *EGFR*-altered patients with recurrent GBM, have a significantly shorter time to progression when treated with bevacizumab [43]. Moreover, *KDR* activation through paracrine secretion of VEGF-C has been demonstrated to represent an escape mechanism employed by GBM to counteract bevacizumab therapy, which could potentially explain the worse survival seen in *KDR*-altered patients [44]. While *KDR* alterations have been demonstrated to predict bevacizumab response in other malignancies such as colon cancer [45], it is still unknown if alterations in *KDR* and its related genes *FLT1* and *VEGFA* could predict response to bevacizumab in GBM.

Multifocal vs multicentric GBM

Finally, we investigated the differences between multifocal and multicentric GBM. While no demographic differences were observed, our study revealed that multicentric GBM

was less likely to undergo re-resection, salvage therapies, and GTR. Prior studies have also reported worse outcomes in multicentric GBM compared to multifocal GBM (3 vs. 11 months) [6], which is in concordance with our results. Interestingly, various genetic differences were observed in the univariate analysis between these groups demonstrating differences in *CDKN2A/B*, *CDK4*, *PTEN*, and *TP53* genes that have not been previously described and may warrant further study (Table 1).

Despite several limitations, such as the retrospective nature, limited sample size, and lack of sequencing information from distinct tumor foci, the present study represents the largest cohort of multifocal/multicentric gliomas undergoing comprehensive genetic characterization and demonstrates the unique molecular features of this aggressive type of GBM.

Conclusions

The results of the present study demonstrate that M-GBM genetically resembles S-GBM, however, M-GBM have a higher frequency of *EGFR* alterations and co-occurrence of *EGFR*/*PTEN* alterations, which may account for their highly malignant and invasive phenotype.

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Author contributions Study design: AD, LYB, and YE. Data recollection: AD, EW, and AR Data analysis: AD and VLR. Manuscript writing: AD, EW, and YE. Manuscript revision and editing: AD, NT, LYB, and YE. Study supervision: LYB and YE. Approved final manuscript: all authors.

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Data availability The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.Code availability Not applicable.

Consent to participate Not applicable.

Consent for publication Not applicable.

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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