Neuro-Oncology Advances
3(1), 1–8, 2021 | https://doi.org/10.1093/noajnl/vdab127 | Advance Access date 1 October 2021

Prognostic implications of epidermal and platelet-derived growth factor receptor alterations in 2 cohorts of IDH*wt* glioblastoma

Iyad Alnahhas, Appaji Rayi, Maria del Pilar Guillermo Prieto Eibl, Shirley Ong, Pierre Giglio, and Vinay Puduvalli

Division of Neuro-Oncology, Department of Neurology, the Ohio State University Wexner Medical Center, Columbus, Ohio, USA (I.A., A.R., M.P.G.P.E., S.O., P.G., V.P.); Present address: Thomas Jefferson University, Philadelphia, Pennsylvania, USA (I.A.); Present address: Charleston Area Medical Center, Charleston, West Virginia, USA (A.R.); Present address: University of Miami, Miami, Florida, USA (M.P.G.P.E.); Present address: MD Anderson Cancer Center, Houston, Texas, USA (V.P.)

Corresponding Author: Iyad Alnahhas, MD, MSc, Division of Neuro-oncology, Department of Neurology, Thomas Jefferson University, 910 Walnut St, Room 310G, Philadelphia, PA 19107, USA (iyad.alnahhas@jefferson.edu).

Abstract

Background. Glioblastoma remains a deadly brain cancer with dismal prognosis. Genetic alterations, including *IDH* mutations, 1p19q co-deletion status and *MGMT* promoter methylation have been proven to be prognostic and predictive to response to treatment in gliomas. In this manuscript, we aimed to correlate other mutations and genetic alterations with various clinical endpoints in patients with IDH-wild-type (IDHwt) glioblastoma.

Methods. We compiled a comprehensive clinically annotated database of IDHwt GBM patients treated at the Ohio State University Wexner Medical Center for whom we had mutational data through a CLIA-certified genomic laboratory. We then added data that is publicly available from Memorial Sloan Kettering Cancer Center through cBioPortal. Each of the genetic alterations (mutations, deletions, and amplifications) served as a variable in univariate and multivariate Cox proportional hazard models.

Results. A total of 175 IDHwt GBM patients with available *MGMT* promoter methylation data from both cohorts were included in the analysis. As expected, *MGMT* promoter methylation was significantly associated with improved overall survival (OS). Median OS for MGMT promoter methylated and unmethylated GBM was 26.5 and 18 months, respectively (HR 0.45; P = .003). Moreover, *EGFR/ERBB* alterations were associated with favorable outcome (HR of 0.37 (P = .003), but only in *MGMT* promoter unmethylated GBM. We further found that patients with *EGFR/ERBB* alterations who also harbored *PDGFRA* amplification had a significantly worse outcome (HR 7.89; P = .025).

Conclusions. Our data provide further insight into the impact of genetic alterations on various clinical outcomes in IDHwt GBM in 2 cohorts of patients with detailed clinical information and inspire new therapeutic strategies for IDHwt GBM.

Key Points

- In our analysis of 2 cohorts of IDHwt GBM, EGFR/ERBB alterations were associated with favorable outcome in *MGMT* promoter unmethylated GBM.
- Patients with EGFR/ERBB alterations who also harbored PDGFRA amplification had significantly worse outcome.

Importance of the Study

In the era of personalized medicine, it has become routine practice to sequence tumors to aide in decisions making regarding treatment options, either for the sake of clinical trials inclusion, or for salvage treatment for patients who have exhausted standard-of-care options. In this paper, we report our experience with CLIA-certified molecular sequencing tests for patients with IDH-wild-type glioblastoma at the Ohio State University. We correlate mutations and genetic alterations with various clinical endpoints in patients from our cohort as well as a publically available cohort from Memorial-Sloan Kettering Cancer Center.

Gliomas are tumors that arise from the glial cells in the central nervous system. Glioblastoma (GBM) is the most common malignant glioma and represents astrocytoma grade IV.[1] Despite our deeper understanding of the genomic alterations that precipitate gliomagenesis, only a few genetic and epigenetic modifications have been identified to be meaningful in clinical practice: At the chromosomal level, simultaneous copy number losses of chromosomes 1p and 19q—based on the World Health Organization 2016 classification of brain tumors—define oligodendrogliomas.[2] Oligodendrogliomas have better survival outcomes compared to astrocytomas.[3,4] Similarly, it is well established that isocitrate dehydrogenase (*IDH*) 1 and 2 mutations carry favorable outcomes in patients with astrocytomas and secondary GBM.[5] Finally, the promoter methylation status of the gene encoding for the repair enzyme O6-methylguanine-DNA methyltransferase (MGMT) predicts response to the standard-of-care alkylating chemotherapy agent used in glioma: temozolomide.[6]

Besides tumor-treating fields, little progress has been made in the management of GBM over the past 2 decades despite enormous research efforts. In fact, GBM was the first cancer type to be analyzed by The Cancer Genome Atlas (TCGA) project which revealed several genomic subtypes of the tumor.[7] Genomic alterations—including copy number variations (CNV) and mutations—lead to activation of oncogenes and inactivation of tumor suppressor genes. Hence, we now better understand the various cellular mechanisms and pathways utilized by gliomas for growth and survival. The most common altered pathways in GBM include: receptor tyrosine kinase (RTK/RAS) pathway (eg, via amplification of epidermal growth factor receptor [*EGFR*] and platelet-derived growth factor receptor [*PDGFR*]), phosphatidylinositol 3-kinase (*PI3K*) pathway (eg, via deletion of the tumor suppressor [*PTEN*]), cell cycle pathway (eg, via mutations in *CDKN2A/B* and *RB1*), *P53* pathway (eg, via mutations in *P53* and *MDM2*), and telomere length maintaining pathways (eg, *TERT* promoter mutations).[8]

Targeted therapies emerged to tackle specific pathways utilized by cancer cells. While these treatments have had successes in cancers such as melanoma and non-small-cell lung cancer, clinical trials of small molecule inhibitors, antibodies, vaccines, and kinase inhibitors targeting these pathways have not improved overall survival (OS) in patients with GBM. A key reason for failure of target inhibition in gliomas appears to be tumor heterogeneity and cancer cell plasticity leading to redundant inputs that maintain the downstream signaling pathways allowing the cancer cells to survive even if one upstream signaling receptor is blocked.[9]

In this study, we aimed to correlate genomic alterations with clinical patient outcomes in 2 cohorts of IDH*wt* GBM and to assess interactions among the various alterations.

Methods

Under an IRB-approved protocol, we compiled a comprehensive clinically annotated database of adult patients with GBM at The Ohio State University (OSU) for whom we had next-generation sequencing (NGS) data through CLIA-certified commercial platforms (Foundation One and Tempus). The database included information detailing the pathologic diagnosis, age, race, gender, performance status, tumor location, treatments utilized, occurrence of complications (radiation necrosis, leptomeningeal spread, or thromboembolic disease defined as deep vein thrombosis and/or pulmonary embolism at any time during the disease course), molecular classifications (*IDH* mutations, *MGMT* promoter methylation, 1p19q co-deletion), programmed death ligand-1 (PD-L1) protein expression on immunohistochemistry, and survival data, in addition to NGS data.

Furthermore, we added publicly available data from a cohort of *IDH*-wild-type *(IDH*wt) GBM from Memorial Sloan Kettering Cancer Center (MSKCC)[10] through cBioPortal.[11,12] We also acquired *MGMT* promoter methylation data for this cohort from MSKCC as this data was not available through cBioPortal. The aim was to correlate mutational data with clinical outcomes, namely OS. Each of the mutations/alterations served as a variable in univariate and multivariate analyses.

We grouped certain alterations under one variable based on the core signaling pathway altering functions.[8] The molecular variables included are listed in Table 1.

Statistical Analysis

A Cox's proportional hazards model was used for univariate and multivariate survival analyses. Backward stepwise regression models were used when looking at subgroups to decrease the number of variables in smaller cohorts. Chi-square tests were used for categorical variables. A nonparametric test was used to compare the median

Table 1. Variables Included in the Univariate and Multivariate Survival Models Grouped Based on Function in the Core Signaling Pathways

Variable	Mutations/Alterations
MDM/TP53	*MDM2*, *MDM4* or *TP53* mutations
CDKN2A/B	*CDKN2A/B/C* deletions or mutations (rare)
CDK/CCND	*CDK4*/*CDK6* amplifications, or *CCND1*/*CCND2* amplifications
RB1	*RB1* mutations/deletions
EGFR/ERBB	*EGFR* amplifications/mutations or *ERBB* (2-4) mutations.
FGFR	*FGFR* mutations/amplifications
PDGFRA/KIT	*PDGFRA* mutations/amplifications
NF1	*NF1* mutations
PTEN	*PTEN* mutations/deletions
PIK3R1	*PIK3R1* mutations/deletions
PI3K gain	PIK3CA mutations, *mTOR* mutations or *AKT* amplifications
MYC	*MYC*: *MYC*/*MYCN* amplifications
TERT	*TERT* promoter mutations

age for the 2 cohorts as age was not normally distributed *P* values less than .05 were considered significant. IBM SPSS Statistics 26 was used.

Results

Patient Characteristics

Of the 73 patients with IDH*wt* GBM from the OSU cohort included in the study, *MGMT* promoter methylation data was available on 68/73 patients and these patients were included in the survival analysis. Similarly, we were able to obtain *MGMT* promoter methylation data on 107/204 patients from the MSKCC cohort. Only patients with known *MGMT* promoter methylation status were included in this analysis as we figured that any model that does not include MGMT would be inaccurate. Figure 1 shows a flow diagram of the analyses performed on each or both cohorts and number of patients included in each analysis.

The median age for the OSU cohort was 60 years (20–78) and the median age for the MSKCC cohort was 61 years (22–91; P = .649). The median OS for the OSU cohort of 20.88 months (95% CI, 15.88–25.87) was similar to that for the MSKCC cohort of 18.77 months (95% CI, 16.46–21.08; P = .464).

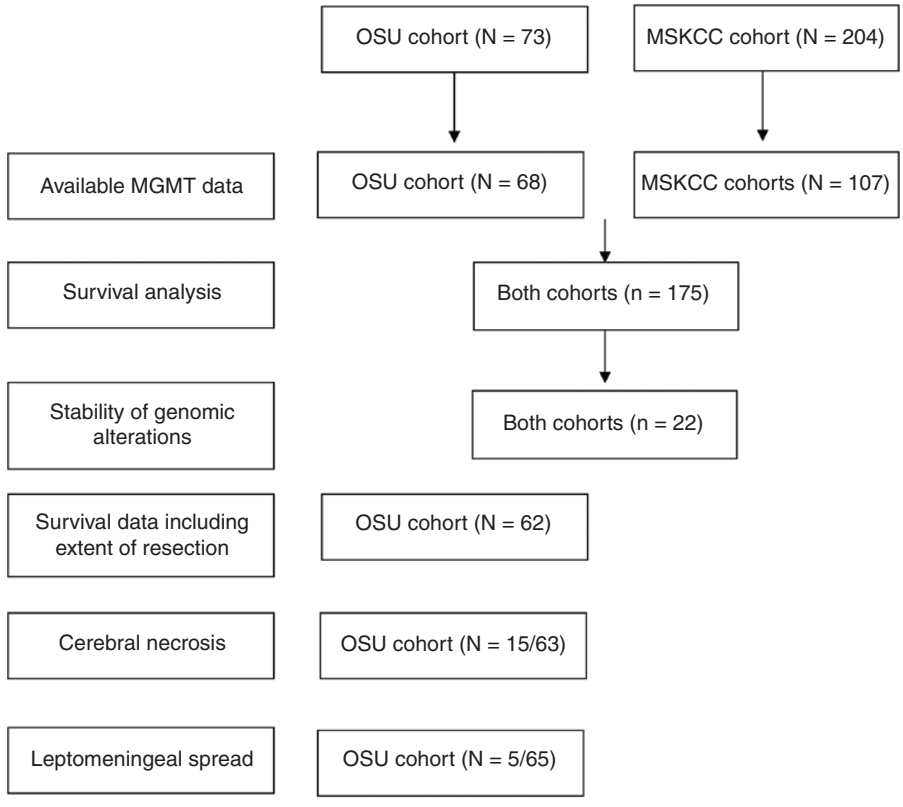

Figure 1. A flow diagram showing the analyses performed on the OSU and MSKCC cohorts and number of patients included in each analysis.

Genomic Alterations and Overall Survival (both cohorts)

A total of 175 patients were included in this analysis. Results of the univariate and multivariate analyses are shown in Table 2. As expected, *MGMT* promoter methylation was significantly associated with improved OS. Median OS for methylated and unmethylated *MGMT* promoter GBM were 26.5 and 18 months, respectively (multivariable HR 0.45; $P = .003$). Unexpectedly, *EGFR/ERBB* alterations were also associated with significantly improved OS. OS for *EGFR/ERBB* altered GBM and *EGFR/ERBBwt* GBM were 24.95 and 16.86 months, respectively (multivariable HR 0.31; $P < .001$). *EGFR* amplification, specifically, was also associated with improved OS (multivariable HR 0.41; $P = .009$).

Table 2. Univariate and Multivariate Analyses Correlating the Various Genomic Alterations With Overall Survival

OSU+MSK with *MGMT* ($N = 175$)	Univariate Analysis *P* value (HR)	Multivariate Model (0.001)- *P* value (HR)
Age	.619	.793 (1.003)
MGMT	.008 (0.53)	.003 (0.45)
MDM/P53	.507	.023 (0.49)
CDKN2A/B	.765	.388
CDK/CCND	.706	.985
RB1	.145	.06 (0.367)
EGFR/ERBB	.003 (0.49)	.0002 (0.31)
FGFR	.568	.378
PDGFRA/KIT	.941	.974
NF1	.208	.641
PTEN	.026 (1.66)	.513
PIK3R1	.936	.067 (0.38)
PI3K gain	.386	.281
MYC	.988	.183
TERT promoter	.043 (1.93)	.074 (2.07)

Favorable survival related to *EGFR/ERBB* alterations appeared to hold true only when *MGMT* promoter was unmethylated: in a backward stepwise regression model ($P = .003$) of the 112 unmethylated *MGMT* promoter cohort, *EGFR/ERBB* alterations were associated with favorable HR of 0.37 ($P = .003$; Figure 2). Median OS for *MGMT* unmethylated, *EGFR/ERBB* altered GBM was 25.05 months compared to 15.12 months for *MGMT* unmethylated, *EGFR/ERBB*wt GBM. In patients with unmethylated *MGMT* promoter GBM, *MDM/P53* alterations were also associated with favorable outcome (HR 0.51; $P = .027$). On the other hand, when *MGMT* promoter was methylated ($N = 63$), a backward stepwise model ($P = .001$) revealed *FGFR* and *PTEN* as markers of worse survival (HR 10.23; $P = .001$ and HR 3.02; $P = .012$, respectively).

Association Between *EGFR/ERBB* and *PDGFRA* Amplification

The multivariable analysis hinted at an association between *EGFR/ERBB* and *PDGFRA*. We therefore performed separate analyses for patients who were positive for *EGFR/ERBB* alterations ($N = 78$; Table 3) and those were not. In patients with *EGFR/ERBB* alterations (or those who specifically had EGFR amplification), univariate and multivariate analyses showed that those who also had *PDGFRA* ($N = 5$) amplification had significantly worse survival (multivariate HR 7.89; $P = .025$; Figure 3). Median OS for patients

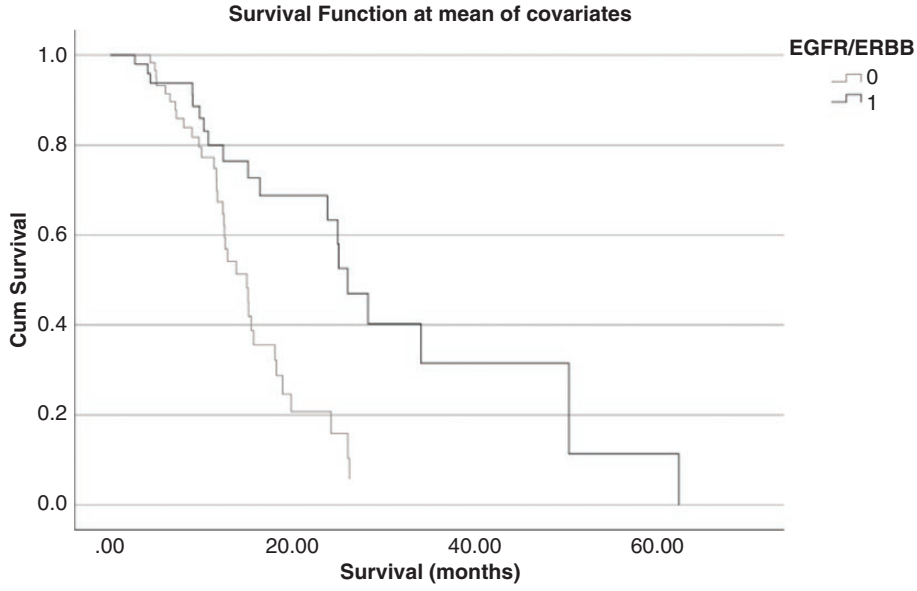

Figure 2. The presence of EGFR/ERBB alterations was associated with favorable outcome in a cohort of 112 patients with unmethylated MGMT promoter GBM.

who were positive for *EGFR/ERBB* alterations and *PDGFR*A amplification was 18.44 m compared to 34.06 m for patients positive for *EGFR/ERBB* alterations without *PDGFR*A amplification.

In patients without *EGFR/ERBB* alterations, a backward stepwise model ($P = .008$), revealed only *MGMT* promoter methylation as a favorable marker (HR 0.45; $P = .023$). Age was associated with worse outcome (HR 1.02; $P = .047$).

Table 3. Univariate and Multivariate Analyses Correlating the Various Genomic Alterations With Overall Survival Within *EGFR/ERBB* Altered Tumors

OSU+MSK with *MGMT* (*EGFR/ERBB* altered) ($N = 78$)	Univariate Analysis *P* value (HR)	Multivariate Model (0.023)- *P* value (HR)
Age	.261	.120
MGMT	.224	.241
MDM/P53	.520	.317
CDKN2A/B	.434	.505
CDK/CCND	0.742	.976
RB1	.686	.878
FGFR	.495	.981
*PDGFR*A/KIT	.034 (3.86)	.025 (7.89)
NF1	.361	.877
PTEN	.01 (2.60)	.454
PI3K gain	.112	.175
MYC	.196	.721
TERT promoter	.031 (5.17)	.139

Stability of Genomic Alterations Across Recurrent Samples

Four patients from the OSU cohort and 22 patients from the MSKCC cohort had DNA sequencing performed on recurrent samples. *CDKN2A/B*, *EGFR*, *TP53*, and *PTEN* alterations were most consistent between the primary and recurrent samples (Figure 4).

Genomic Alterations and Clinical Outcomes (OSU cohort)

Overall survival

Looking at the OSU cohort alone, we were able to incorporate extent of resection into the model ($N = 62$). A strongly significant backward stepwise regression model ($P < .001$) revealed that extent of resection ($P = .023$), *MGMT* (HR 0.54; $P = .086$), and *EGFR/ERBB* alterations (HR 0.31; $P = .003$) correlate with significant favorable outcomes. Biopsy only compared to gross total resection carried significantly worse outcome (HR 4.97; $P = .006$). Subtotal was not significantly different from gross total resection in the multivariate model. Also, of note, presence of thromboembolic disease was also associated with worse OS in the OSU cohort in univariate but not multivariate analysis (univariate HR 2.26; $P = .009$).

Progression-free survival

A strongly significant backward stepwise model ($P < .001$) revealed extent of resection ($P = .022$), *MGMT* promoter methylation (HR 0.29; $P < .001$), *EGFR/ERBB* alterations (HR 0.40; $P = .011$), *CDKN2A/B* loss (HR 0.39; $P = .012$), and *PIK3R1* mutations (HR 0.04; $P = .003$) as significant

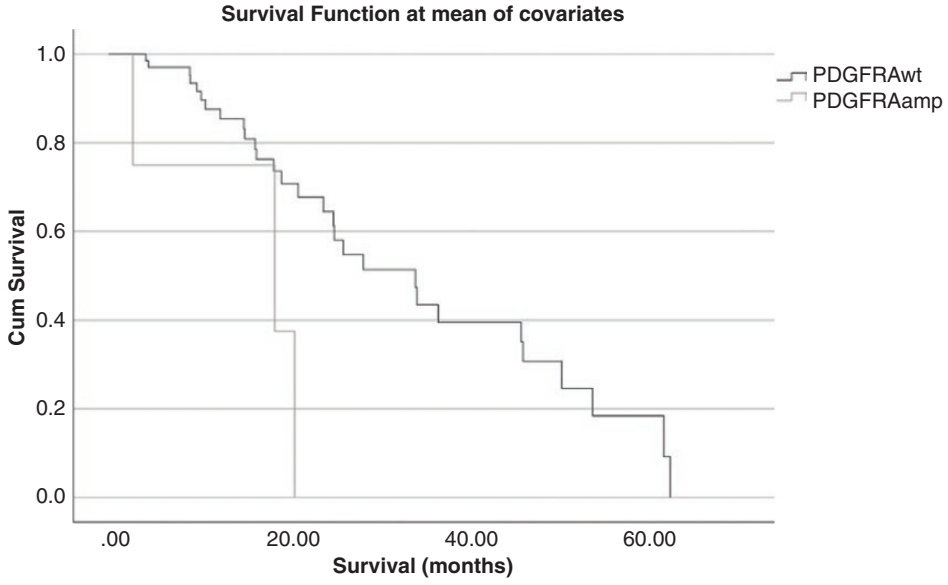

Figure 3. The presence of PDGFRA amplification in EGFR/ERBB altered GBM was associated with worse survival in a cohort of 78 patients.

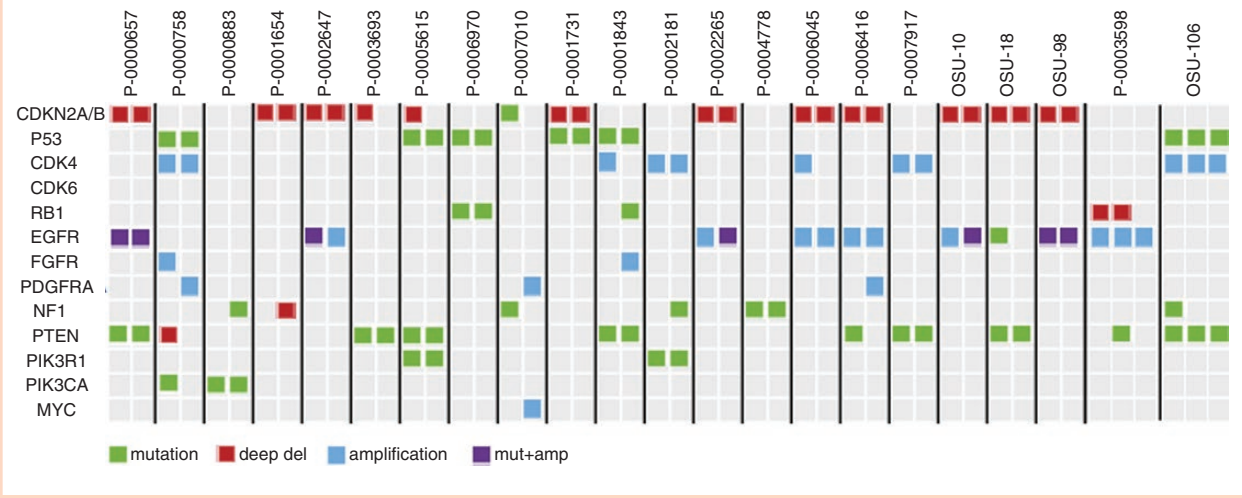

Figure 4. Mutations and copy number variations in primary and recurrent samples for each patient are shown in pairs. MSKCC patient IDs are as identified in cBioPortal.

favorable outcomes. Biopsy only as opposed to gross total resection carried significantly worse risk of progression (HR 3.627; $P = .007$).

Cerebral necrosis

Treatment-related cerebral necrosis is a potential complication in patients with gliomas. Information about cerebral necrosis was available on 63 patients. Nine out of 24 (37.5%) of patients with methylated *MGMT* promoter GBM developed RN versus 6/39 (15.4%) in the unmethylated group. Chi-square test ($P = .045$).

Leptomeningeal spread

Five out of 65 (7.7%) patients (with available data) developed leptomeningeal disease (LMD). All tumors appeared to have extended into the ventricles on brain imaging by the time of development of LMD. Four tumors had evidence of subependymal spread prior (range 45–93 days) to development of LMD. Four patients had available CSF studies, 2 of whom had evidence of atypical or malignant cells. CSF protein was elevated in all 4 samples (range 113–492 mg/dL). CSF glucose was low in all 4 samples (range < 10–46 mg/dL). *MGMT* promoter was methylated in 2 tumors and unmethylated in 2 and unknown in 1. The genomic alterations varied among samples and included *EGFR* V765M mutation ($N = 1$), *PDGFRA* amplification ($N = 1$), *PDGFRA* Y849C subclonal mutation ($N = 1$), *CDKN2A/B* loss ($N = 2$), T*P53* mutations ($N = 3$), and *TERT* promoter mutations ($N = 3$). No case had *EGFR* amplification or *EGFR*vIII mutation. Other alterations observed included *DNMT3A* mutation, *KIT*, *MYC*, and *MDM2* amplifications and *RB1* losses. PD-L1 expression in tumor samples ranged from 5% to 40%. One intracranial sample had a 20% PD-L1 expression with LMD from this tumor exhibiting 40% PD-L1 expression. Median OS was 11.67 months (range 6.05–18.31). Median OS after LMD diagnosis was 2.76 months (range 1.91–7.39).

Discussion

We compiled a comprehensive clinically annotated database of with detailed demographic and clinical data of 73 patients with IDH*wt* GBM. We then added data that is publicly available from MSKCC through cBioPortal. This cohort included 204 patients with IDH*wt* GBM. The aim was to correlate different mutational data with clinical outcomes, namely survival. Each of the mutations/alterations served as a variable in univariate and multivariate analyses. Survival analyses were performed on 175 patients for whom *MGMT* promoter methylation status is known.

As expected, *MGMT* promoter methylation was significantly associated with better outcome HR 0.45; $P = .003$. Furthermore, GBM with *EGFR*/*ERBB* alterations (and specifically *EGFR* amplification) had better outcome compared to *EGFR*/*ERBBwt* GBM. However, this appeared to be true only in *MGMT* promoter unmethylated GBM. Previous literature is unclear in prognostic role of *EGFR* in GBM, as some studies suggested favorable outcome and others suggested worse outcome.[13,14] However, to our knowledge this has not been looked at previously in the setting of multivariable analysis and specifically *MGMT* promoter methylation. This finding will need to be validated in bigger datasets.

In the OSU cohort alone, more aggressive surgical resection, *MGMT* promoter methylation and *EGFR*/*ERBB* alterations yielded favorable OS and PFS outcomes. Moreover, focusing on patients with *EGFR*/*ERBB* alterations, in both cohorts, univariate and multivariate analysis showed an association between *EGFR* and *PDGFRA*. More specifically, patients with an *EGFR*/*ERBB* alteration who also exhibited *PDGFRA* amplification had significantly worse survival (HR 7.89; $P = .025$) hinting to a potential interaction between the 2 receptors that needs further evaluation.

PDGFRα and PDGFRβ (encoded by *PDGFRA* and *PDGFRB,* respectively) are both expressed as transmembrane receptors on GBM cell surface and are drivers of glioma growth.

PDGFRA amplification is the more common alteration and occurs in 13.1% of GBM.[7] While concurrent amplification of *EGFR* and *PDGFRA* has been reported in up to 5% of GBM,[7] EGFR and PDGFRA co-expression at the mRNA level is seen in 37% of GBM sphere lines as reported by Chakravarty et al.[15] The paper also showed functional transactivation of PDGFRα by EGFR: EGF stimulation in this setting can result both in EGFR-EGFR homodimerization as well as EGFR-PDGFRα heterodimerization which can drive proliferation.[15] Further supporting this interaction, Hegi et al. observed that the expression of p-EGFR correlated with p-PDGFRβ in a window of opportunity study of 22 patients with recurrent GBM who were treated with at least 5 days of gefitinib (an *EGFR* inhibitor) prior to re-resection.[16] We believe that the interaction between *EGFR* and *PDGFRA* alterations may have therapeutic implications and escaping to PDGFR signaling may in part explain the failure of EGFR targeted therapy in GBM.

Tumor heterogeneity and multiple RTK pathway activation have rendered GBM highly resistant to targeted treatment,[17] particularly in the recurrent setting. GBM has been reported to change methylation subclass upon recurrence, which may further point to emergence of adaptive resistance mechanisms to therapy.[18] Likewise, *EGFR* amplification has been previously reported to be lost in 16–27% of recurrent samples.[18,19] We illustrate how *CDKN2A/B*, *EGFR*, T*P53*, and *PTEN* alterations were most consistent between the primary and recurrent samples, however, none of the mutations/copy number variations appear to be invariably consistent between the primary and recurrent samples.

As previously described,[20] *MGMT* promoter methylation was associated with increased risk of treatment related cerebral necrosis: (37.5%) versus (15.4%) in the methylated and unmethylated groups, respectively, in the OSU cohort. Furthermore, GBM patients who developed leptomeningeal disease had no one consistent genetic alteration. Rather, in all instances, the lesions had extended toward the ventricles prior to development of LMD.

In summary, NGS provides valuable information when caring for patients with GBM. The study is limited by the small sample size, especially for the analyses performed on the OSU cohort alone, leading to low power to make definitive statistical conclusions. The study is also limited by the fact that glioblastoma was diagnosed solely based on pathologic evaluations; new diagnostic entities have been established based on methylation testing.[21] The study is rather hypothesis generating and our findings will need validation from other bigger cohorts. We find that *MGMT* unmethylated GBM that harbors *EGFR/ERBB* alterations appear to have better prognosis in comparison to *MGMT* unmethylated *EGFR/ERBB*wt tumors. Moreover, there appears to be a significant interaction between *EGFR* and *PDGFRA*. This may in part explain EGFR inhibition resistance in GBM and highlights the plasticity of GBM cells. Combining targeted treatments against these 2 receptors may be an attractive therapeutic target.

Keywords

EGFR | GBM | IDH | MGMT | next-generation sequencing | PDGFR

Acknowledgment

We thank Dr. Ingo Mellinghoff and the MSKCC team for providing *MGMT* methylation data for the cBioPortal dataset.

Funding

This project did not receive any funding support.

Conflict of interest statement. The authors have no conflicts of interest to disclose.

Authorship Statement. I.A., A.R., M.G.P.: Project design, data collection, data analysis, manuscript writing. S.O., P.G., V.P.: Project design, data analysis, manuscript writing.

References

1. Ostrom QT, Cioffi G, Gittleman H, et al. CBTRUS Statistical Report: primary brain and other central nervous system tumors diagnosed in the United States in 2012-2016. *Neuro Oncol.* 2019;21(Suppl 5):v1–v100.
2. Louis DN, Perry A, Reifenberger G, et al. The 2016 World Health Organization Classification of Tumors of the Central Nervous System: a summary. *Acta Neuropathol.* 2016;131(6):803–820.
3. Cairncross G, Wang M, Shaw E, et al. Phase III trial of chemoradiotherapy for anaplastic oligodendroglioma: long-term results of RTOG 9402. *J Clin Oncol.* 2013;31(3):337–343.
4. Erdem-Eraslan L, Gravendeel LA, de Rooi J, et al. Intrinsic molecular subtypes of glioma are prognostic and predict benefit from adjuvant procarbazine, lomustine, and vincristine chemotherapy in combination with other prognostic factors in anaplastic oligodendroglial brain tumors: a report from EORTC study 26951. *J Clin Oncol.* 2013;31(3):328–336.
5. Yan H, Parsons DW, Jin G, et al. IDH1 and IDH2 mutations in gliomas. *N Eng J Med.* 2009;360(8):765–773.
6. Hegi ME, Diserens AC, Gorlia T, et al. MGMT gene silencing and benefit from temozolomide in glioblastoma. *N Engl J Med.* 2005;352(10):997–1003.
7. Brennan CW, Verhaak RG, McKenna A, et al.; TCGA Research Network. The somatic genomic landscape of glioblastoma. *Cell.* 2013;155(2):462–477.
8. Cancer Genome Atlas Research N. Comprehensive genomic characterization defines human glioblastoma genes and core pathways. *Nature.* 2008;455(7216):1061–1068.
9. Touat M, Idbaih A, Sanson M, Ligon KL. Glioblastoma targeted therapy: updated approaches from recent biological insights. *Ann Oncol.* 2017;28(7):1457–1472.
10. Zehir A, Benayed R, Shah RH, et al. Mutational landscape of metastatic cancer revealed from prospective clinical sequencing of 10,000 patients. *Nat Med.* 2017;23(6):703–713.

11. Cerami E, Gao J, Dogrusoz U, et al. The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. *Cancer Discov.* 2012;2(5):401–404.
12. Gao J, Aksoy BA, Dogrusoz U, et al. Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. *Sci Signal.* 2013;6(269):pl1.
13. Hobbs J, Nikiforova MN, Fardo DW, et al. Paradoxical relationship between the degree of EGFR amplification and outcome in glioblastomas. *Am J Surg Pathol.* 2012;36(8):1186–1193.
14. Li J, Liang R, Song C, Xiang Y, Liu Y. Prognostic significance of epidermal growth factor receptor expression in glioma patients. *Onco Targets Ther.* 2018;11:731–742.
15. Chakravarty D, Pedraza AM, Cotari J, et al. EGFR and PDGFRA co-expression and heterodimerization in glioblastoma tumor sphere lines. *Sci Rep.* 2017;7(1):9043.
16. Hegi ME, Diserens A-C, Bady P, et al. Pathway analysis of glioblastoma tissue after preoperative treatment with the EGFR tyrosine kinase inhibitor gefitinib--a phase II trial. *Mol Cancer Ther.* 2011;10(6):1102–1112.
17. Patel AP, Tirosh I, Trombetta JJ, et al. Single-cell RNA-seq highlights intratumoral heterogeneity in primary glioblastoma. *Science.* 2014;344(6190):1396–1401.
18. Körber V, Yang J, Barah P, et al. Evolutionary trajectories of IDHWT glioblastomas reveal a common path of early tumorigenesis instigated years ahead of initial diagnosis. *Cancer Cell.* 2019;35(4):692-704. e612.
19. van den Bent MJ, Gao Y, Kerkhof M, et al. Changes in the EGFR amplification and EGFRvIII expression between paired primary and recurrent glioblastomas. *Neuro Oncol.* 2015;17(7):935–941.
20. Brandes AA, Franceschi E, Tosoni A, et al. MGMT promoter methylation status can predict the incidence and outcome of pseudoprogression after concomitant radiochemotherapy in newly diagnosed glioblastoma patients. *J Clin Oncol.* 2008;26(13):2192–2197.
21. Bender K, Perez E, Chirica M, et al. High-grade astrocytoma with piloid features (HGAP): the Charité experience with a new central nervous system tumor entity. *J Neurooncol.* 2021;153(1):109–120.