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

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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.

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 (IDHwt) GBM from Memorial Sloan Kettering Cancer Center (MSKCC) through cBioPortal. 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. 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
Results

Patient Characteristics

Of the 73 patients with IDHwt 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; \( P = .464 \)).
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; \( P = .009 \)). 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/ERBBwt 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).

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

| OSU+MSK with MGMT (N = 175) | Univariate Analysis | Multivariate Model (0.001) |
|-----------------------------|---------------------|-----------------------------|
|                             | \( P \) value (HR)  | \( P \) 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                   | .974                |                             |
| PDGFR/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)         |                             |

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 who 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
who were positive for \textit{EGFR/ERBB} alterations and \textit{PDGFRA} amplification was 18.44 m compared to 34.06 m for patients positive for \textit{EGFR/ERBB} alterations without \textit{PDGFRA} amplification.

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

### 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. \textit{CDKN2A/B, EGFR, TP53,} and \textit{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 \((P = .023)\), \textit{MGMT} \((HR 0.54; P = .086)\), and \textit{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 \((P = .022)\), \textit{MGMT} promoter methylation \((HR 0.29; P < .001)\), \textit{EGFR/ERBB} alterations \((HR 0.40; P = .011)\), \textit{CDKN2A/B} loss \((HR 0.39; P = .012)\), and \textit{PIK3R1} mutations \((HR 0.04; P = .003)\) as significant...
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 \( (P = .007) \).

**Leptomeningeal spread**

Five out of 65 (77%) 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 \((N = 1)\), PDGFR amplification \((N = 1)\), PDGFR Y849K subclonal mutation \((N = 1)\), CDKN2A/B loss \((N = 2)\), TP53 mutations \((N = 3)\), and TERT promoter mutations \((N = 3)\). No case had EGRF amplification or EGFRVIII 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).

![Figure 4](image.png)

_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._

**Discussion**

We compiled a comprehensive clinically annotated database of with detailed demographic and clinical data of 73 patients with IDHwt GBM. We then added data that is publicly available from MSKCC through cBioPortal. This cohort included 204 patients with IDHwt 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. 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 PDGFR. More specifically, patients with an EGFR/ERBB alteration who also exhibited PDGFR 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 PDGFA and PDGFRB, respectively) are both expressed as transmembrane receptors on GBM cell surface and are drivers of glioma growth.
PDGFRα amplification is the more common alteration and occurs in 13.1% of GBM. While concurrent amplification of EGFR and PDGFRα has been reported in up to 5% of GBM, EGFR and PDGFRα co-expression at the mRNA level is seen in 37% of GBM sphere lines as reported by Chakravarty et al. The paper also showed functional transactivation of PDGFRc by EGFR: EGF stimulation in this setting can result both in EGFR-EGFR homodimerization as well as EGFR-PDGFRc heterodimerization which can drive proliferation. 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. We believe that the interaction between EGFR and PDGFR 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, 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. Likewise, EGFR amplification has been previously reported to be lost in 16–27% of recurrent samples. We illustrate how CDKN2A/B, EGFR, TP53, 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, 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. 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/ERBBwt tumors. Moreover, there appears to be a significant interaction between EGFR and PDGFR. 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

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### 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.

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