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Title Page

Bevacizumab Enhances Overall Survival in Newly Diagnosed Glioblastoma Patients with High COX-2 Expression

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Abstract

Bevacizumab (BEV) is known to improve progression-free survival (PFS) but not overall survival (OS) for newly diagnosed glioblastoma (ndGBM). Here, we evaluated the survival outcomes between temozolomide (TMZ)-only and TMZ+BEV treatments stratified based on the cyclooxygenase-2 (COX-2) expression, a rate-limiting enzyme involved in the cancer development. Fifty IDH-wildtype ndGBM patients treated between 2012 and 2023 were enrolled in this study. Pretreatment levels of COX-2 protein and mRNA expression were quantified, and survival analyses were performed based on the immunoreactivity score (IRS). Patients with high COX-2 expression (IRS ≥ 3, determined by its median) also exhibited higher COX-2 mRNA levels (ΔCt 6.43±1.64 vs. 7.67±0.81; $p = 0.020$). In patients with high COX-2 expression, TMZ+BEV had significantly longer median PFS and OS than those receiving TMZ-only (PFS: 22 vs. 8 months, $p < 0.001$; OS: 25 vs. 18 months, $p = 0.009$). In contrast, these benefits were not found in patients with low COX-2 expression (PFS: 12 vs. 15 months, $p = 0.875$; OS: 24 vs. 26 months, $p = 0.775$). Altogether, this study suggests that patients with high, but not low, COX-2 expression demonstrate survival benefits from the addition of BEV in ndGBM.

Keywords:

COX-2, Bevacizumab, Survival, Newly Diagnosed Glioblastoma

Introduction

Glioblastoma (GBM) is the most common type of glioma, accounting for approximately 50% of all cases, and is considered one of the most aggressive and devastating forms of malignant primary brain tumors due to its extremely poor prognosis [1]. The current standard therapy, introduced in 2005 and known as the Stupp protocol, combines surgical resection, radiotherapy, and chemotherapy with temozolomide (TMZ) and provides an overall survival (OS) of only 15–18 months [2,3]. Bevacizumab (BEV), a monoclonal antibody targeting vascular endothelial growth

factor A (VEGF-A), has subsequently been shown to reduce tumor size, alleviate glucocorticoid-related edema, and extend progression-free survival (PFS) [4,5]. This evidence led to the accelerated approval of BEV by the Food and Drug Administration as a second-line treatment for recurrent GBM in 2009 [6,7].

The phase III randomized trials, AVAglio and RTOG 0825, conducted between 2009 and 2011 to assess BEV in newly diagnosed glioblastoma (ndGBM), showed that BEV only improved PFS but not OS [8,9]. These subsequently resulted in the approval of BEV in Japan as an insurance-covered first-line treatment for newly diagnosed high-grade glioma in 2013, but not in other countries to date. Further analysis of the RTOG 0825 trial revealed that patients with high pretreatment relative cerebral blood volume (rCBV) had significantly improved OS, suggesting that those with a high angiogenic profile may benefit from BEV treatment [10]. Unfortunately, reliable angiogenic markers to predict a favorable response to BEV in ndGBM are currently lacking.

A preclinical study suggested that stratifying patients based on high Cyclooxygenase-2 (COX-2) expression may help identify individuals who are more likely to benefit from VEGF inhibitor therapy [11]. VEGF expression has been reported as a predictive biomarker for BEV response in biliary tract cancer [12]. However, its assessment in GBM remains challenging due to the inherently high baseline expression of VEGF in these tumors [13]. We hypothesized that COX-2 may represent an alternative angiogenic phenotype in GBM, as COX-2 functions as a rate-limiting enzyme in prostaglandin synthesis and promotes angiogenesis by activating prostaglandin E2 (PGE2) receptors and upregulating VEGF expression via the p38 and JNK kinase signaling pathways [14,15]. Moreover, COX-2 is a well-established biomarker for poor prognosis in GBM [16-18]. In this study, we evaluate the potential of COX-2 as a differentiating factor for identifying ndGBM patients who may benefit from BEV treatment.

Results

Treatment details of enrolled patients

A total of fifty patients with ndGBM were enrolled in this study, including 18 patients in the TMZ-only group and 32 patients in the TMZ + BEV group. Most patients were elderly, with a male predominance. Preoperative Karnofsky performance scale (KPS) was similar between groups, with comparable proportions of patients demonstrating moderate and good performance status. Most patients received carmustine (BCNU) wafer implantation. The majority were O6-methylguanine-DNA methyltransferase (MGMT)-negative (≤10% of cells) in the TMZ-only group. The TMZ+BEV group exhibited a similar distribution of MGMT status. Thirteen of the 18 patients in the TMZ-only group subsequently received BEV at the time of first recurrence. Detailed patient characteristics by treatment group are summarized in Table 1.

COX-2 protein levels and mRNA levels

Patients with an immunoreactivity score (IRS) of ≥ 3, based on the median IRS value (Fig. 1a), were classified as having high COX-2 protein expression. Accordingly, these patients exhibited higher levels of mRNA compared to those with low COX-2 protein expression, as indicated by lower ΔCt values (7.67 ± 0.81 vs. 6.43 ± 1.64, p = 0.020, Fig. 1b). Intratumoral COX-2 mRNA and protein expression in these patients were significantly correlated (r = -0.543, p = 0.007). The ICC for the IRS was 0.892, indicating good agreement between the two examiners.

A total of 23 samples with low COX-2 expression (15 from the TMZ + BEV group and 8 from the TMZ-only group) and 27 samples with high COX-2 expression (17 from the TMZ + BEV group and 10 from the TMZ-only group) were analyzed in this study. Notably, most patients with high COX-2 expression had a moderate KPS score before surgery. Patients' clinical characteristics based on COX-2 expression are presented in Supplementary Table 1. The representative IHC results for low and high COX-2 expression are depicted in Figures 1c and 1d.

Patients’ survival based on COX-2 expression and treatment groups

The TMZ + BEV group demonstrated a significant improvement in PFS (18 vs 9 months, respectively, p = 0.031) compared to the TMZ-only group, but no significant difference in OS (25 vs 22 months, respectively, p = 0.098) (Figure 2a and 2b). In the TMZ-only group, high COX-2 expression was associated with a significant reduction in OS but not in PFS compared to low COX-2 expression (OS: 18 vs. 26 months, respectively, p = 0.018; PFS: 8 vs 15 months, respectively, p = 0.123) (Fig. 2c and 2d). A multivariate regression model revealed that high COX-2 expression significantly increased the risk of events related to OS in the TMZ-only group (hazard ratio [HR]: 3.78, 95% confidence interval [CI]: 1.13–12.64, p = 0.03) (Supplementary Figure 1). In contrast, the TMZ + BEV group exhibited no significant differences in both OS and PFS between patients with high versus low COX-2 expression (OS: 25 vs. 24 months, respectively, p = 0.525; PFS: 22 vs. 12 months, respectively, p = 0.693) (Fig. 2e and 2f).

In patients with high COX-2 expression, the TMZ + BEV group demonstrated a significant improvement in both PFS and OS compared to the TMZ-only group (PFS: 22 vs. 8 months, respectively, p < 0.001; OS: 25 vs. 18 months, respectively, p = 0.009) (Fig. 3a and 3b). A multivariate regression model revealed that TMZ + BEV treatment significantly reduced the risk of events related to both PFS (HR PFS: 0.18, 95% CI: 0.06–0.56, p = 0.003; Table 2) and OS (HR OS: 0.29, 95% CI: 0.11–0.79, p = 0.016; Table 3) in patients with high COX-2 expression. In contrast, in the low COX-2 expression group, the TMZ + BEV treatment showed no significant improvement in either PFS or OS compared to the TMZ-only group (PFS: 12 vs. 15 months, respectively, p = 0.875; OS: 24 vs. 26 months, respectively, p = 0.775) (Fig. 3c and 3d).

Discussion

GBM is characterized by high vascularity and overexpression of proangiogenic cytokines, including VEGF-A and COX-2 [19,20]. This has led to the hypothesis that anti-angiogenic therapies may be effective for patients with this condition [21]. BEV, an angiogenesis inhibitor, has been used as a second-line chemotherapy for recurrent GBM in various countries [7]. In Japan, this therapy is uniquely approved as a first-line chemotherapy in conjunction with standard treatment for newly diagnosed high-grade

glioma. However, BEV has been reported as a frequently cost-ineffective treatment in Japan, as it only improves PFS without affecting OS [22]. These unsatisfactory treatment outcomes may be attributed to the heterogeneous nature of GBM [21]. In this study, we investigated whether participants with a high angiogenic profile prior to treatment, as indicated by high COX-2 expression, might derive greater benefit from BEV treatment. Our study demonstrated that patients with high COX-2 expression receiving TMZ + BEV experienced improved PFS and OS compared to those receiving TMZ-only. Consistent with this, our multivariate analysis adjusting for age, KPS score, extent of resection, MGMT status, and treatment group, demonstrated that TMZ + BEV treatment was an independent predictor of improved PFS and OS in patients with high COX-2 expression. However, this improvement was not observed in patients with low COX-2 expression. Altogether, these results suggest that adding BEV to standard treatment in ndGBM specifically benefits patients with high pretreatment COX-2 expression.

It is important to acknowledge that high COX-2 expression is widely recognized as a poor prognostic factor in glioma patients. Zhang et al. reported that high COX-2 expression was negatively associated with OS in glioma patients, with a median OS of 18 months for those with high COX-2 expression compared to 25 months for those with low COX-2 expression ($p < 0.05$) [17]. Wang et al. also reported that high COX-2 expression correlated with shorter OS compared to low COX-2 expression in GBM patients (10.5 months vs. 24 months, $p = 0.008$) [18]. Consistent with previous studies, our findings indicated that in the TMZ-only group, high COX-2 expression is associated with poorer OS outcomes, with a median OS of 18 months for patients with high COX-2 expression compared to 26 months for those with low COX-2 expression ($p = 0.018$). However, this difference was no longer observed when BEV was added to TMZ chemotherapy (OS: 25 vs. 24 months, respectively, $p = 0.525$). This suggests that the addition of BEV to standard treatment enhances OS in patients with high COX-2 expression, achieving a duration comparable to that of patients with low COX-2 expression.

COX-2 exhibits pleiotropic effects that are advantageous for cancer development, including promoting angiogenesis, metastasis, tumor proliferation, infiltration, and

resistance to anticancer drugs. Consequently, its overexpression is primarily associated with tumors that exhibit aggressive behavior [18,23-25]. Previous study has reported that high-grade gliomas are more likely to exhibit high COX-2 expression compared to low-grade gliomas [17]. In a study specifically examining ndGBM, high COX-2 expression was observed in 52.6% of patients [18]. This finding aligns with our study, in which we identified high COX-2 expression in 54% of the GBM patient population, suggesting that more than half of these patients could be candidates for personalized chemotherapy. In contrast, high VEGF expression has been associated with a greater benefit from BEV treatment in biliary tract cancer [12]. However, stratifying GBM patients based on VEGF expression remains challenging, as over 85% of these tumors exhibit high VEGF expression, limiting its utility as a discriminatory biomarker [13]. Consequently, COX-2 expression may serve as a more practical biomarker for stratifying ndGBM patients who could benefit from BEV chemotherapy.

Our study focused exclusively on COX-2 expression in newly diagnosed cases, which may limit its applicability to recurrent cases. It is well-established that recurrent cases can exhibit an altered angiogenic profile due to prior chemotherapy regimens. This is evidenced by changes in rCBV in patients following BEV treatment and the upregulation of COX-2 expression in GBM cell lines after TMZ treatment [10,26-28]. Nevertheless, previous studies have shown that recurrent GBM patients with a high angiogenic profile, as determined by MRI perfusion and VEGF-A expression, also derive greater benefit from BEV treatment [29-35]. These results suggest that angiogenic markers, including COX-2, could aid in identifying recurrent cases that may respond well to BEV. Therefore, evaluating high COX-2 expression as a predictor of treatment efficacy in recurrent cases warrants further investigation.

Studies confirming patient stratification through biomarkers to enhance BEV efficacy in ndGBM are currently lacking, particularly since BEV is approved for this indication only in Japan. In this study, we demonstrated that only patients with high COX-2 expression, who mostly presented with moderate preoperative KPS, derived a survival benefit from the addition of BEV to standard treatment. While BEV is primarily reported to alleviate symptoms such as cerebral edema, our findings highlight its

potential to improve survival in this molecularly defined subgroup, underscoring COX-2 as a predictive biomarker in ndGBM. Despite this novel finding and efforts to ensure the robustness of the analysis, the retrospective design, small sample size, and potential subjectivity of IHC scoring are notable limitations of our study. While we complemented IHC with COX-2 mRNA expression analysis using qRT-PCR, broader RNA-based methods such as RNA sequencing or targeted transcriptomic panels may provide higher molecular accuracy and warrant evaluation in future studies.

Overall, our study highlights the importance of stratifying GBM patients before initiating BEV in newly diagnosed cases, utilizing COX-2 expression as a predictive biomarker. While high COX-2 expression is well known to be associated with poor survival outcomes, the addition of BEV could be a potential option to improve survival outcomes in these patients. This study also suggests the potential for expanding BEV indications as a first-line personalized chemotherapy option for high COX-2 expression patients in other countries and potentially restricting its use in patients with low COX-2 expression to minimize treatment costs in Japan. Future studies should confirm these results in a larger patient population.

Methods

Study Design and data collection

We conducted a retrospective study in our institute between 2012 and 2023. This study was carried out in accordance with the Declaration of Helsinki. The study was approved, and the requirement for informed consent was waived by the ethics committee of Tottori University, Faculty of Medicine (No. 23A149). Data were retrospectively collected from electronic medical records, including variables such as age, sex, KPS, date of surgery, tumor location, tumor resection status, pathological diagnosis, MGMT status, Mindbomb E3 Ubiquitin Protein Ligase-1 (MIB-1) index, initial treatment plan, recurrence, treatment plan after recurrence, and mortality.

Patient selection and treatment

The inclusion criteria for patients in our study were as follows: 1) age ≥ 18 years; 2) newly diagnosed isocitrate dehydrogenase wild-type GBM confirmed by pathological diagnosis; 3) availability of formalin-fixed paraffin-embedded (FFPE) or frozen tissue from tumor resection prior to chemotherapy; 4) routine follow-up MRI after the initial surgery to monitor recurrence and survival outcomes.

After surgery, all patients received radiotherapy in conjunction with concomitant TMZ at a dose of 75 mg/m^2 per day, administered seven days a week from the first to the last day of radiotherapy. This was followed by adjuvant TMZ therapy at a dose of 150–200 mg/m^2 per day for five days during each 28-day cycle. In the TMZ + BEV group, BEV therapy was initiated concurrently with radiotherapy and TMZ. BEV was typically administered as an intravenous infusion at a dose of 10 mg/kg every two weeks. However, treatment interruptions of up to four weeks and dose reductions to a minimum of 7 mg/kg were allowed in cases of adverse events, such as proteinuria or hypertension. The addition of BEV therapy was chosen for ndGBM patients, particularly those who underwent partial resection or who presented with significant peritumoral edema. The extent of resection (EOR) was classified based on the estimated percentage of tumor removed: biopsy (<10%), partial resection (10–89%), subtotal resection (90–95%), and gross total resection (>95%). The treatment plan was established by two senior neurosurgeons (A.K. and T.H.).

COX-2 Immunohistochemistry (IHC) and immunoreactivity score (IRS)

All available FFPE samples (n = 50) were selected for IHC staining to assess COX-2 protein expression using a COX-2 antibody (1:2000) (ab179800, Abcam, UK). The FFPE samples were deparaffinized with xylene and rehydrated in ethanol. Antigen retrieval was performed using citrate buffer (H-3300-250, Vector Laboratories, US) in a microwave at 500W for 10 minutes, followed by cooling at room temperature for 20 minutes. The slides were washed twice with Tris Buffer Saline with Tween®20 (TBS-T) (T9142, Takara Bio, Japan) for 5 minutes each. Staining was performed using an avidin-biotin complex kit (Streptavidin/Biotin Blocking Kit, SP-2002, Vector Laboratories, US). After washing with TBS-T, the slides were incubated with BLOXALL

Blocking solution for 10 minutes (VECTASTAIN® Elite® ABC Universal PLUS kit, Peroxidase, Horse Anti-Mouse/Rabbit IgG, PK-8200, Vector Laboratories, US) to quench endogenous peroxidase activity. Nonspecific binding was blocked using 2.5% Normal Horse Serum (PK-8200, Vector Laboratories, US) for 20 minutes. Sections were then incubated with the primary antibody (ab179800, Abcam, UK), diluted in SignalStain® Ab diluent (1:2000) (Cell Signaling Technology, US), overnight at 4°C. After washing with TBS-T, the slides were incubated with a secondary antibody VECTASTAIN biotinylated horse anti-rabbit/mouse (PK-8200, Vector Laboratories, US). Following additional TBS-T washes, sections were incubated in the VECTASTAIN Elite ABC reagent (PK-8200, Vector Laboratories, US). The antibody binding was visualized by incubating the sections in 3,3′-diaminobenzidine (DAB) (Vectorlabs DAB substrate kit, no nickel) for 1 minute. The sections were then washed in distilled water and counterstained with hematoxylin (Muto Pure Chemicals, Japan) to visualize nuclei. Alcohol and xylene were used to dehydrate and clear the sections before mounting them under micro cover glass (Muto Pure Chemicals, Japan).

The IRS is a semi-quantitative scoring system employed to assess COX-2 protein levels.[17] The IRS consists of two parameters: staining intensity and cell positivity rate. Staining intensity was assessed under 100x magnification using a light microscope and graded as follows: 0, none; 1, weak; 2, moderate; and 3, strong. The cell positivity rate was assessed under 200x magnification in hotspot areas of tumor cells and graded as follows: 0, no positive cells; 1, positive cell rate ≤10%; 2, positive cell rate between 10% and 50%; 3, positive cell rate between 50% and 80%; and 4, positive cell rate between 80% and 100%. Positive COX-2 expression in perinecrotic areas was excluded. The IRS was calculated by multiplying the scores from the two parameters. Two independent observers (I.K. and H.K.) evaluated all the stained samples, and the level of agreement was assessed using the intraclass correlation coefficient (ICC). An ICC value greater than 0.75 was considered indicative of high reliability. Any discrepancies in the IRS between the two observers were resolved through mutual agreement.

COX-2 mRNA levels using Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR)

qRT-PCR was employed to quantify the mRNA expression levels of COX-2. RNA extraction was conducted on all available frozen tissue samples (n = 23) using RNeasy Kits for RNA Purification (Qiagen, Germany), following the manufacturer's instructions. One-step qPCR was performed using the SuperScript™ III Platinum™ SYBR™ Green One-Step qRT-PCR Kit (Thermo Fisher Scientific, USA), in accordance with the manufacturer's protocol. The following COX-2 primer sequences were used: forward primer, 5'-CCTGTGCCTGATGATTGC-3'; reverse primer, 5'-CTGATGCGTGAAGTGCTG-3' [36]. GAPDH was employed as an endogenous control gene, with the following primer sequences: forward primer, 5'-GTCTCCTCTGACTTCAACAGCG-3'; reverse primer, 5'-ACCACCCTGTTGCTGTAGCCAA-3' [37]. All reactions were conducted using the ViiA 7 Real-Time PCR System (Applied Biosystems, USA). ΔCt values were calculated by subtracting the Ct value of the endogenous control from the Ct value of the target gene, and these values were used to determine mRNA levels.

Statistical analysis

The median IRS was utilized as the cut-off value to categorize COX-2 protein expression into low and high groups. We then compared the mean ΔCt values of COX-2 between the low and high protein expression groups using an independent samples t-test. Additionally, the correlation between the IRS and the ΔCt values of COX-2 was assessed using Pearson's correlation test. Survival analysis was performed using Kaplan-Meier curves to evaluate PFS and OS, with differences assessed using the log-rank test. PFS was defined as the time from surgery to tumor recurrence according to the Response Assessment in Neuro-Oncology criteria, while OS was defined as the duration from diagnosis until death [38]. Hazard ratios were analyzed using a Cox regression model employing a backward elimination method. We included the covariates of age (<60 and ≥60 years), KPS [moderate (50–70) and good (80–100)], extent of resection (<90% and ≥90%), and MGMT status (positive staining >10%) due to their significant clinical impact on OS. Additionally, covariates with significant

univariate p-values were selected from variables including sex, tumor location [eloquent areas (motor, sensory, language, visual field) and non-eloquent areas], adjuvant treatments [carmustine (BCNU) wafers, tumor treating fields (TTF)], treatment after recurrence in the TMZ-only group (with or without BEV), and the MIB-1 index. A p-value <0.05 was considered statistically significant. All statistical analyses were performed using SPSS version 29.

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Statements & Declarations

Conflicts of Interest

The authors have no relevant financial or non-financial interests to disclose.

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Ethics approval

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the ethics committee of Tottori University, Faculty of Medicine (No. 23A149). As this was a retrospective study, the requirement for informed consent was waived by the ethics committee.

Author Contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by A.K., I.K., and H.K. The first draft of the manuscript was written by I.K. and A.K. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Human ethics and consent to participate

Not applicable.

Consent to publish

Not applicable.

Availability of data and material

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request

Clinical trial number

Not applicable.

Figure Captions

Fig. 1 (a) IRS of COX-2 expression: The median IRS value across all groups was 3, with IRS ≥ 3 classified as high COX-2 expression. (b) ΔCt analysis of COX-2 expression revealed significantly lower mean ΔCt values in the high-expression group, indicating higher mRNA levels compared to the low-expression group. Representative immunohistochemistry (IHC) staining images show (c) low COX-2 expression with an

IRS of 1, derived from an intensity score of 1 and a positivity cell rate of 1, and (d) high COX-2 expression with an IRS of 9, derived from an intensity score of 3 and a positivity cell rate of 3

Fig. 2 Kaplan-Meier survival analysis of PFS (a) and OS (b) comparing the TMZ-only and TMZ+BEV groups. The TMZ+BEV group showed a significant improvement in PFS but no significant difference in OS. Kaplan-Meier survival analysis stratified by COX-2 expression in the TMZ-only group (c, d) and the TMZ+BEV group (e, f) revealed that, in the TMZ-only group, high COX-2 expression was associated with no significant difference in PFS (c) but significantly reduced OS (d). In contrast, the TMZ+BEV group showed no significant differences in either PFS (e) or OS (f) between low and high COX-2 expression

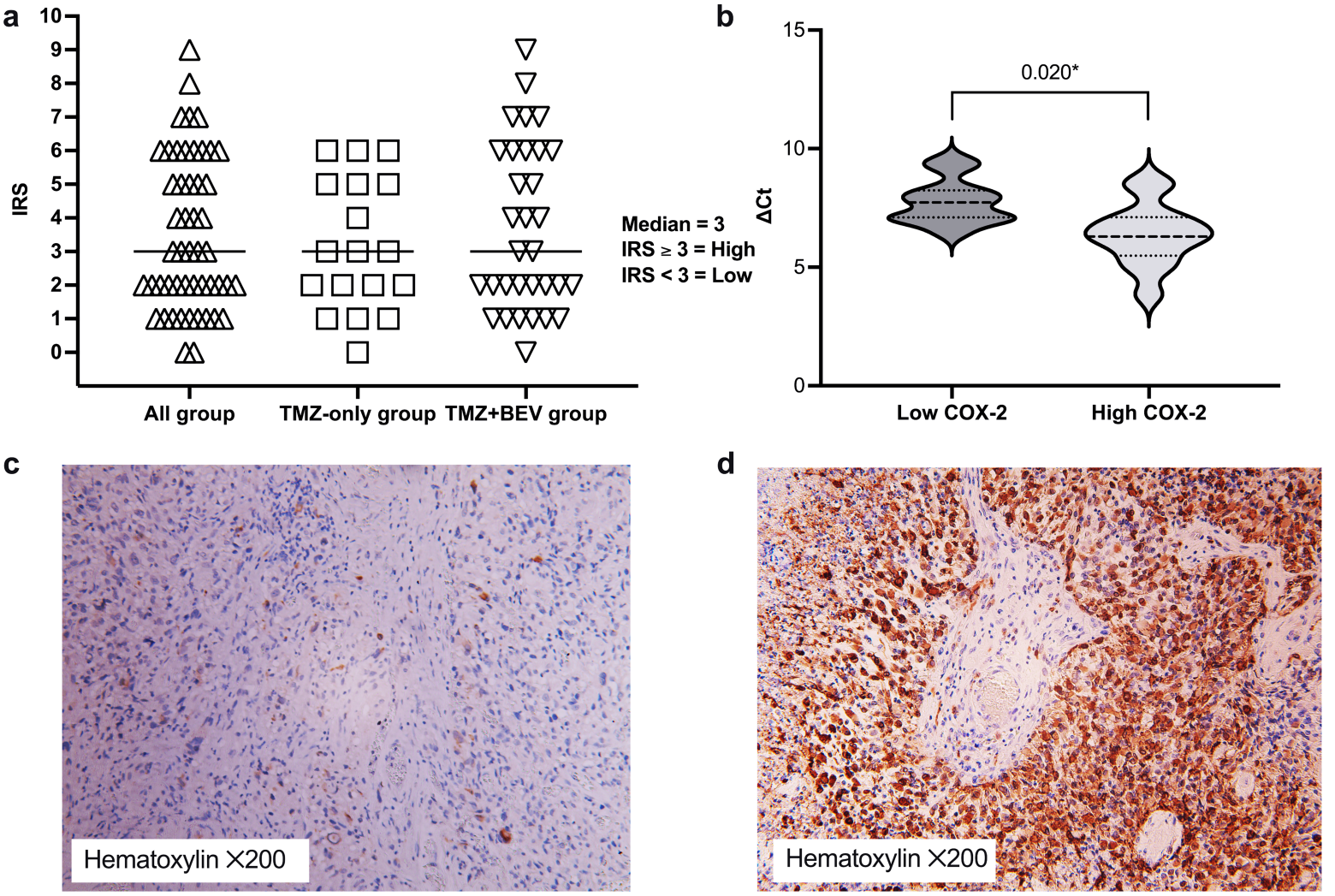
Fig. 3 Exploratory data analysis stratified by high COX-2 (a, b) and low COX-2 (c, d) expression. High COX-2 expression was associated with significantly improved PFS (a) and OS (b) in the TMZ+BEV group compared to the TMZ-only group. In contrast, low COX-2 expression showed no significant improvement in either PFS (c) or OS (d) between the TMZ+BEV and TMZ-only groups

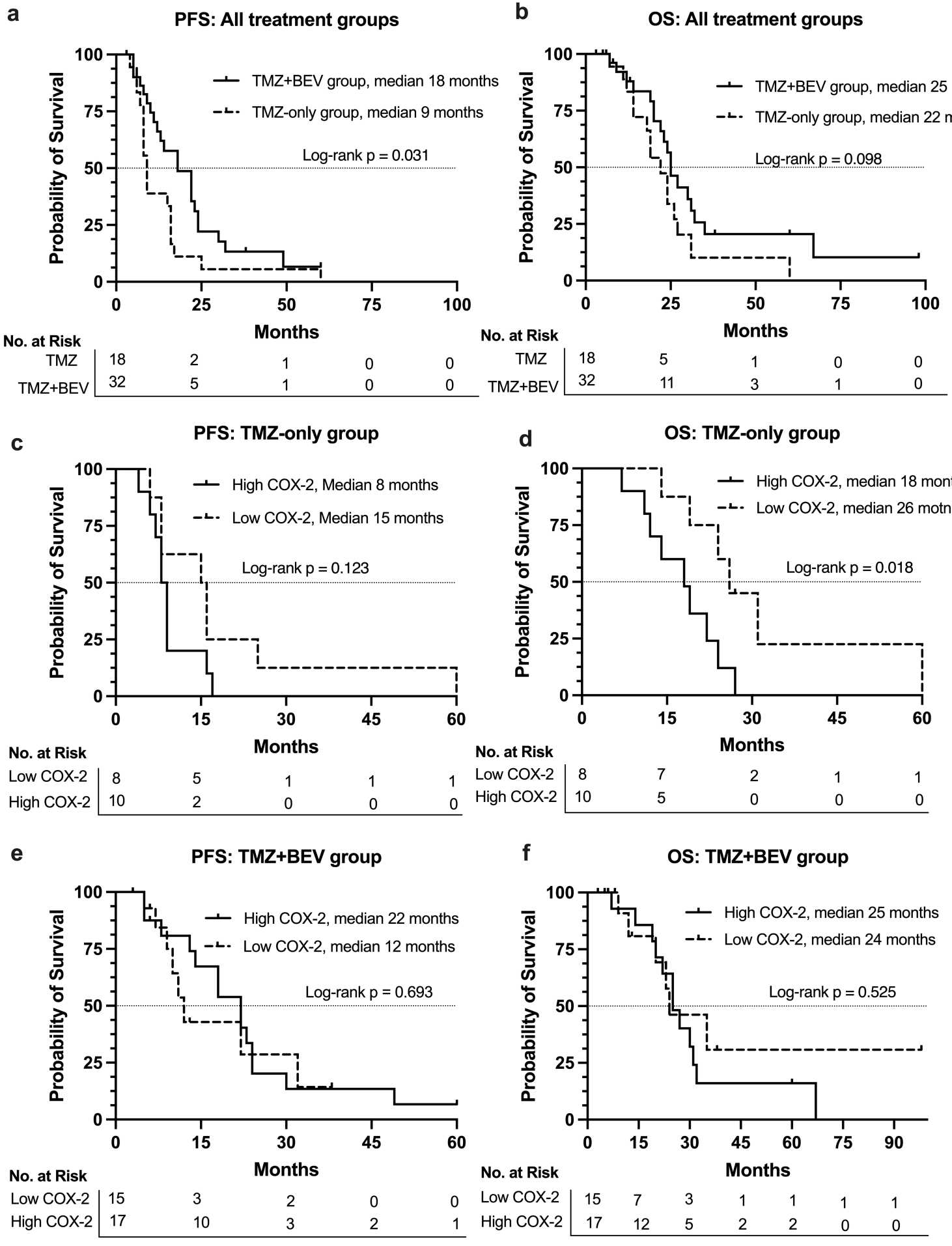
Tables

Table 1. Patient baseline characteristics

Table 2. Univariate and multivariate analyses of covariates associated with PFS in high COX-2 group

Table 3. Univariate and multivariate analyses of covariates associated with OS in high COX-2 group


a
IRS
10
9
8
7
6
5
4
3
2
1
0
All group
TMZ-only group
TMZ+BEV group
Median = 3
IRS ≥ 3 = High
IRS < 3 = Low
b
ΔCt
15
10
5
0
0.020*
Low COX-2
High COX-2
c
Hematoxylin ×200
d
Hematoxylin ×200


a
PFS: All treatment groups
Probability of Survival
TMZ+BEV group, median 18 months
TMZ-only group, median 9 months
Log-rank p = 0.031
Months
No. at Risk
TMZ 18 2 1 0 0
TMZ+BEV 32 5 1 0 0
b
OS: All treatment groups
Probability of Survival
TMZ+BEV group, median 25 months
TMZ-only group, median 22 months
Log-rank p = 0.098
Months
No. at Risk
TMZ 18 5 1 0 0
TMZ+BEV 32 11 3 1 0
c
PFS: TMZ-only group
Probability of Survival
High COX-2, Median 8 months
Low COX-2, Median 15 months
Log-rank p = 0.123
Months
No. at Risk
Low COX-2 8 5 1 1 1
High COX-2 10 2 0 0 0
d
OS: TMZ-only group
Probability of Survival
High COX-2, median 18 months
Low COX-2, median 26 motnhs
Log-rank p = 0.018
Months
No. at Risk
Low COX-2 8 7 2 1 1
High COX-2 10 5 0 0 0
e
PFS: TMZ+BEV group
Probability of Survival
High COX-2, median 22 months
Low COX-2, median 12 months
Log-rank p = 0.693
Months
No. at Risk
Low COX-2 15 3 2 0 0
High COX-2 17 10 3 2 1
f
OS: TMZ+BEV group
Probability of Survival
High COX-2, median 25 months
Low COX-2, median 24 months
Log-rank p = 0.525
Months
No. at Risk
Low COX-2 15 7 3 1 1 1 1
High COX-2 17 12 5 2 2 0 0

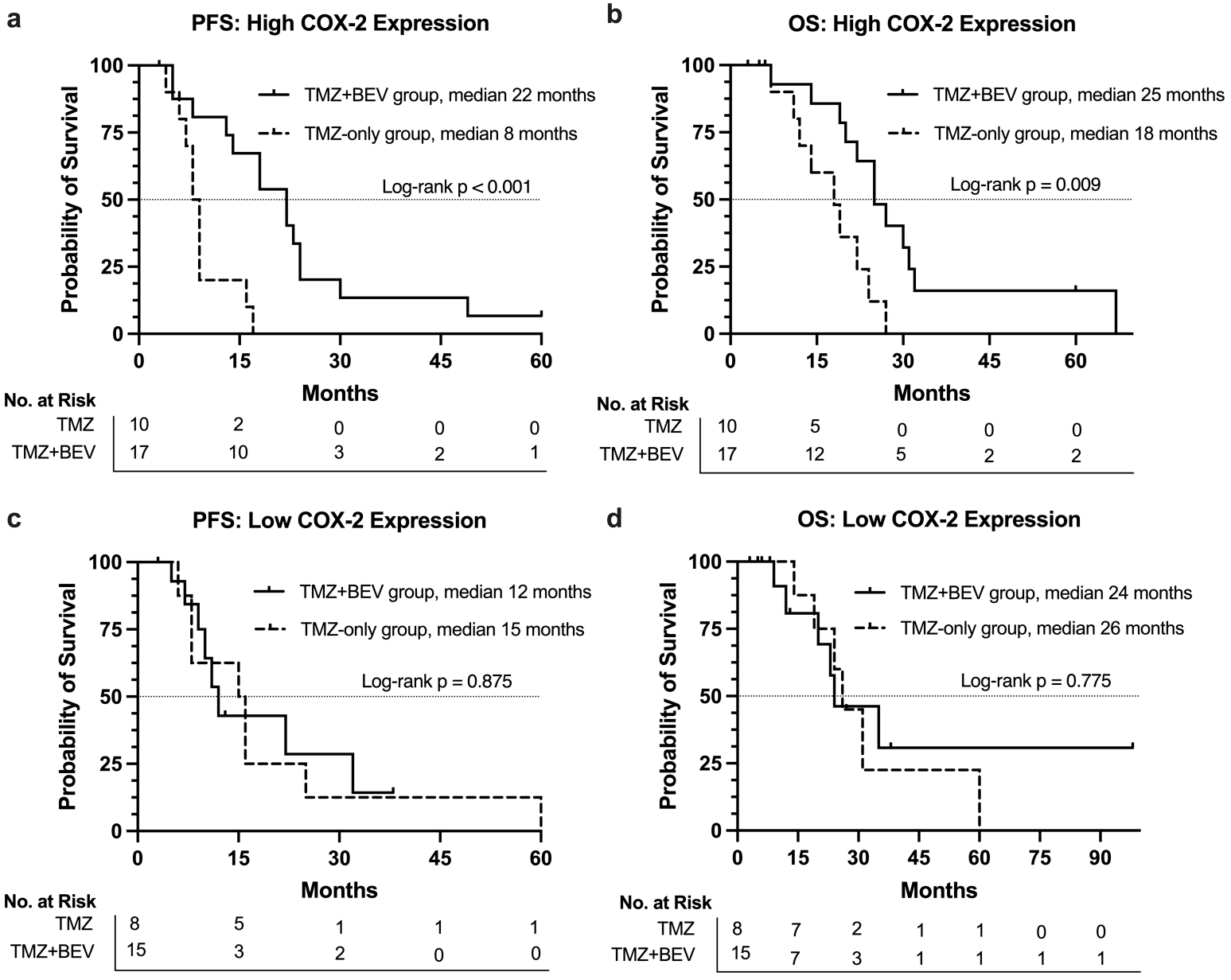
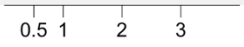


Table 2. Univariate and multivariate analyses of covariates associated with PFS in high COX-2 group

Subgroup	n	Median PFS months	95% CI	Univariate P-Value	HR (95% CI)	Multivariate P-Value
Age, years				**0.21**	**1.66 (0.46 to 5.90)**	**0.44**
<60	4	13	0.00 to 29.66			
≥60	23	14	2.66 to 25.34			
Sex				**0.4**	**NA**	**NA**
Male	15	14	8.50 to 19.50			
Female	12	9	0.00 to 23.14			
KPS Score				**0.71**	**1.12 (0.42 to 2.93)**	**0.83**
Moderate (50-70)	17	16	8.16 to 23.84			
Good (≥ 80)	12	9	7.55 to 10.46			
Tumor location				**0.29**	**NA**	**NA**
Non-eloquent area	16	18	1.81 to 34.19			
Eloquent area	11	13	6.53 to 19.47			
EOR				**0.88**	**0.89 (0.33 to 2.41)**	**0.83**
<90	10	16	0.00 to 39.38			
≥90	17	14	4.31 to 23.69			
BCNU Wafer				**0.01**	**0.72 (0.19 to 2.70)**	**0.62**
No	6	6	2.39 to 9.60			
Yes	21	18	10.98 to 25.02			
TTF				**0.45**	**NA**	**NA**
No	24	14	3.41 to 24.59			
Yes	3	18	0.39 to 35.60			
MGMT Status				**0.98**	**1.24 (0.63 to 2.43)**	**0.54**
Negative (≤ 10%)	13	17	4.95 to 13.05			
Positive (> 10%)	12	9	2.11 to 31.89			
Missing	2	NA*				
MIB-1 Index				**0.38**	**NA**	**NA**
Low (≤ 40%)	14	14	8.36 to 19.64			
High (>40%)	13	17	2.24 to 31.76			
Treatment				**< 0.001**	**0.18 (0.06 to 0.56)**	**0.003**
TMZ-only	10	8	6.76 to 9.24			
TMZ+BEV	17	22	14.61 to 29.39			



PFS = Progression-free survival; COX-2 = Cyclooxygenase-2; CI = Confidence interval; HR = Hazard ratio; NA = Not applicable; KPS = Karnofsky performance scale; EOR = Extent of resection; BCNU = 1,3-Bis(2-chloroethyl)-1-nitrosourea; TTF = Tumor Treating Field; MGMT = O6-methylguanine-DNA methyltransferase; IHC = Immunohistochemistry; MIB-1 = Mindbomb E3 Ubiquitin Protein Ligase-1; TMZ = Temozolomide; BEV = Bevacizumab, * = Missing data on MGMT patients were censored for analysis

Table 3. Univariate and multivariate analyses of covariates associated with OS in high COX-2 group

Subgroup	n	Median OS months	95% CI	Univariate P-Value	HR (95% CI)	Multivariate P-Value
Age, years				**0.14**	**2.24 (0.49 to 10.22)**	**0.29**
< 60	4	31	13.39 to 48.60			
≥ 60	23	22	15.46 to 28.45			
Sex				**0.45**	**NA**	**NA**
Male	15	22	14.15 to 29.85			
Female	12	22	12.70 to 31.29			
KPS Score				**0.83**	**0.56 (0.19 to 1.62)**	**0.28**
Moderate (50-70)	17	22	14.43 to 29.57			
Good (≥ 80)	12	24	18.05 to 29.95			
Tumor location				**0.54**	**NA**	**NA**
Non-eloquent area	16	22	12.69 to 31.30			
Eloquent area	11	22	15.80 to 28.19			
EOR				**0.67**	**0.76 (0.25 to 2.31)**	**0.62**
<90	10	16.5	3.76 to 34.25			
≥90	17	22	17.29 to 26.71			
BCNU Wafer				**0.045**	**1.15 (0.23 to 5.71)**	**0.86**
No	6	14	9.71 to 18.29			
Yes	21	22	20.25 to 27.75			
TTF				**0.45**	**NA**	**NA**
No	24	22	17.56 to 26.45			
Yes	3	25	NA			
MGMT Status				**0.85**	**1.37 (0.64 to 2.94)**	**0.42**
Negative (≤ 10%)	13	22	15.35 to 24.65			
Positive (> 10%)	12	20	15.72 to 28.28			
Missing	2	NA*				
MIB-1 Index				**0.38**	**NA**	**NA**
Low (≤ 40%)	14	24	15.80 to 32.19			
High (> 40%)	13	22	16.97 to 27.04			
Treatment				**0.009**	**0.29 (0.11 to 0.79)**	**0.016**
TMZ-only	10	18	11.31 to 24.69			
TMZ+BEV	17	25	19.41 to 30.59			

KPS = Karnofsky performance scale; MGMT = O6-methylguanine-DNA methyltransferase; MIB-1 = Mindbomb E3 Ubiquitin Protein Ligase-1; BCNU = 1,3-Bis(2-chloroethyl)-1-nitrosourea; OS = Overall Survival; TMZ = Temozolomide; BEV = Bevacizumab; COX-2 = Cyclooxygenase-2; EOR = Extend of resection; HR = Hazard ratio; CI = Confidence interval; M: Mann-Whitney U test; NA = Not applicable, * = Missing data on MGMT patients were censored for analysis

Table 1. Patient baseline characteristics

Parameters	All patients (n=50)	TMZ-only (n=18)	TMZ+BEV (n=32)
Age, Median (Range), years	72 (36-84)	74.5 (55-82)	68 (36-84)
< 60, n (%)	10 (20)	1 (5.6)	9 (28.1)
≥ 60, n (%)	40 (80)	17 (94.4)	23 (71.9)
Sex, n (%)			
Male	30 (60)	11 (61.1)	19 (59.4)
Female	20 (40)	7 (38.9)	13 (40.6)
KPS score, Median (Range)	70 (50-100)	80 (50-90)	70 (60-100)
Moderate (50-70), n (%)	26 (52)	8 (44.4)	18 (56.3)
Good (≥ 80), n (%)	24 (48)	10 (55.6)	14 (43.8)
Tumor location, n (%)			
Non-eloquent area	32 (64)	12 (66.7)	20 (62.5)
Eloquent area	18 (36)	6 (33.3)	12 (37.5)
EOR, (% tumor removal)			
Biopsy (< 10%), n (%)	6 (12)	0 (0)	6 (18.8)
Partial resection (10-89%), n (%)	15 (30)	4 (22.4)	11 (34.4)
Subtotal resection (90-95%), n (%)	15 (30)	9 (50)	6 (18.8)
Gross total resection (> 95%), n (%)	14 (28)	5 (27.8)	9 (28.1)
BCNU Wafer, n (%)			
Yes	34 (68)	9 (50)	25 (78.1)
No	16 (32)	9 (50)	7 (21.9)
TTF, n (%)			
Yes	10 (20)	2 (11.1)	8 (25)
No	40 (80)	16 (88.9)	24 (75)
MGMT, n (%)			
Positive (> 10%)	20 (40)	5 (27.8)	15 (46.9)
Negative (≤ 10%)	27 (54)	13 (72.2)	14 (43.8)
Data missing	3 (6)	0 (0)	3 (9.4)
MIB-1 index (Mean±SD, %)	45.92±17.08	43.61±18.45	47.27±16.30
Details of BEV Treatment			
Use of BEV, n (%)	45 (90)	[a]13 (72.2)	32 (100)

BEV cycle, Median (Range)	21 (1-113)	[a]15 (1-41)	23.5 (1-113)
Prognosis			
PFS, median (range) months	10.5 (3-60)	9 (4-60)	19 (7-60)
OS, median (range) months	20 (3-98)	12.5 (3-60)	21 (3-98)

TMZ = Temozolomide; BEV = Bevacizumab; KPS = Karnofsky performance scale; EOR = Extent of resection; BCNU = 1,3-Bis(2-chloroethyl)-1-nitrosourea; TTF = Tumor Treating Field; MGMT = O6-methylguanine-DNA methyltransferase; IHC = Immunohistochemistry; MIB-1 = Mindbomb E3 Ubiquitin Protein Ligase-1; PFS = Progression-free survival; OS = Overall Survival

[a]BEV was administrated to the patients in the TMZ-only group at the time of their first recurrence.