Maximum Resection and Immunotherapy Improve Glioblastoma Patient Survival: A Single-institution Prognostic Analysis

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Abstract

Purpose. Glioblastoma (GBM) is a refractory disease with a poor prognosis and various methods, including maximum resection and immunotherapy, have been tested to improve outcomes. This retrospective study analyzed the prognostic factors of initially diagnosed glioblastoma patients at our institution to analyze the effect of these methods on prognosis.

Methods. Two hundred seventy-seven patients with initially diagnosed glioblastoma who were treated in our institution from 2009 to 2020. Various data, including extent of removal (EOR) and type of adjuvant therapy, were examined and prognostic relationships were analyzed.

Results. The median OS of the entire 277-case cohort, 200 non-biopsy cases, and 77 biopsy cases were 16.6 months, 19.7 months, and 9.7 months, respectively. Gross total removal (GTR; 100% of EOR) was achieved in 32.9% of the cases. Univariate analysis revealed younger age, right side, higher Karnofsky performance status, GTR, intraoperative MRI use for removal, proton therapy, combination immunotherapy, and discharge to home as good prognostic factors. Intraoperative MRI use and EOR were closely related. In the multivariate analysis, GTR, proton therapy, and combination of immunotherapies including autologous formalin-fixed tumor vaccine were the significant prognostic factors. A multivariate analysis of 91 GTR cases showed that immunotherapy contributed to prognostic improvements. The median OS and 5-year OS% values were 36.9 months and 43.3% in GTR cases receiving immunotherapy.

Conclusion. GTR, proton therapy, and immunotherapy were good prognostic factors in single-center GBM cases. Tumor vaccine therapy for GTR cases achieved a notably high median survival time and long-term survival ratio, indicating its usefulness in GTR cases.

Introduction

Glioblastoma (GBM) is generally a refractory disease with a poor prognosis for the majority of patients (1). Past papers have shown prognosis-related factors in glioblastoma patients receiving standard radio-chemotherapy, such as extent of removal (2-4), patient age, isocitrate dehydrogenase (IDH) mutations, and methylation status **of O6-methylguanine-DNA methyl-transferase (MGMT)**promotor among others (5-7). In addition, multiple immunotherapies for GBM patients have been conducted in an attempt to improve prognoses (8,9). Since January 2013, we have utilized intraoperative magnetic resonance imaging (MRI) to improve the extent of removal (10, 11), in addition to advanced treatments, including immunotherapy and proton therapy (12-15). In March 2013, we started a double-blinded, randomized phase IIb/III trial of autologous formalin-fixed tumor vaccine (AFTV) with standard chemotherapy and temozolomide (TMZ) for newly diagnosed GBM to evaluate the efficacy of AFTV for prevention of recurrence and/or cure of residual tumor burden (UMIN000010602) (13), and since October, 2020, GBM patients who had gross-total removal (GTR), resulting in no residual gadolinium enhancement, or subtotal removal (STR) are enrolled in a double-blinded, randomized phase III trial of AFTV. Similarly, in a double-blinded, randomized phase II trial of dendritic cell vaccine ICT-107, GBM patients who had undergone GTR, resulting in no residual gadolinium enhancement, or STR were enrolled. (16) In that study, progression-free survival (PFS) in the

intent-to-treat (ITT) population was significantly increased in the ICT-107 cohort by 2.2 months. In a phase 3 trial of an autologous tumor, featuring lysate-pulsed, dendritic cell vaccine (DCVax®-L) with standard therapy for newly diagnosed GBM, 63% of enrolled cases were GTR cases (17) and the median overall survival (mOS) of the overall ITT population (according to blinded interim data) was 23.1 months from the time of surgery. Moreover, various clinical methodologies, such combining immune checkpoint inhibitors to overcome relapse-mediating immunosuppressive mechanisms in the GBM microenvironment after monotherapy, are being tested (8, 18). However, no currently published paper has examined the superiority of immunotherapies in a phase III clinical study. The purpose of this study is thus to analyze the prognostic factors in consecutive cases of initially diagnosed glioblastoma in our institution for the past 11 years during the TMZ era and evaluate both the extent of removal and effect of these advanced treatments on GBM cases.

Methods

Patient enrollment:

A total of 277 patients with initially diagnosed glioblastoma who were treated in our institution from June 2009 to March 2020, including previous clinical trials at our institution, were registered in this retrospective study. Although TMZ was approved in Japan in September 2006, nimustine (ACNU) was widely used until May 2008 at our institution while many patients had unknown prognoses up through May 2009. Therefore, all patients until May 2009 at the same time of both ACNU and unknown prognosis patients were excluded. The study protocol was approved by the ethics committee of our institution (number R01-202). Various data, including patient age, sex, main location of the tumor, tumor side, **Karnofsky Performance**Status (KPS) of patients, extent of removal (EOR), interactive photodynamic diagnostic diagnosis (PDD) using 5-aminolevulinic acid (5-ALA), use of intraoperative MRI, IDH status, p53 status, MGMT status, type of radiotherapy, type of adjuvant therapy, and patient return at discharge were examined for relationships to the prognoses. As for EOR evaluated with T1weighted-images after gadolinium administration on MRI within 3 days after surgery, GTR for complete disappearance of the contrast area (100% of EOR), STR for tumor volume reduction of 90% or more or tumor residuals with a maximum diameter of 10 mm or less, partial removal (PR) for 5% or more and less than 90%, and biopsy for less than 5% were set as fixed definitions. Information on the PDD fluorescence intensity was collected from surgical records and classified as strong, vague, or negative and those cases with positive fluorescence intensity but without any detailed descriptions were classified as positive.

Intraoperative MRI system for tumor removal or biopsy and postoperative treatments

Our intraoperative MRI system, the VISIUS® Surgical Theatre (IMRIS, Minnetonka, MN, USA), features both high-field (1.5T) ceiling-mounted and movable magnets, (10) to confirm the presence or absence of residual tumors for cases with maximum removal or to confirm accurate positioning for biopsy. Our institution uses the ceiling-mounted magnet to check for removal and the movable magnet for biopsy. For postoperative radiotherapy (RT), extended local radiation using 60 Gy (30 fraction) total irradiation dose or similar protocols were classified as ‘conventional RT’, RT using 45 Gy (15 fraction) or similar protocols

were ‘hypo-fractionated RT’, and RT using proton beams or combined with proton beams were classified as ‘proton therapy’. The critical inclusion criteria for this therapy were as follows: predicted radiation necrosis was unlikely to be fatal, the potential resectability of a lesion when brain necrosis was found in the 96.6 Gy irradiation range (15), and patients who accepted the advanced therapy after careful informed consent that fully explained possible complications. In addition, rare cases of whole brain radiotherapy (WBRT) were grouped separately. For postoperative adjuvant therapy, cases treated according to the modified Stupp regimen (RT concomitant with TMZ followed by TMZ maintenance therapy until recurrence or for 12 to 24 times) were classified into the ‘TMZ’ group. Cases using immunotherapy in addition to the modified Stupp regimen were classified into the ‘immunotherapy’ group. Of the 39 cases in the immunotherapy group at our institution, 31 cases received autologous formalin-fixed tumor vaccine (AFTV), the manufacturing method of which is described in the past paper of ours (12), 2 cases received interferon beta, and 6 cases received other drugs. Three of these cases with AFTV also received proton therapy. The critical inclusion criteria for the AFTV therapy were as follows: patients who underwent maximum possible resection (at least non-biopsy surgery) of the tumor, at least 1.5 g of neoplastic tissue for AFTV preparation was available (12), and patients who accepted the advanced therapy after careful informed consent that fully explained possible complications. Patients who started bevacizumab (BEV) before or concomitant with RT were classified into the ‘bevacizumab’ group. Our institution’s clinical protocol indicates that BEV treatment be used for low KPS patients receiving biopsy or PR surgery and most BEV cases also used TMZ.

Detection of p53, MGMT and IDH statuses,

For immuno-histochemistry (IHC) surveys of p53 and MGMT statuses, the corresponding staining indices were calculated as the average number of positive cells in the best-stained tumor areas with a total number of cells not less than 1000, as described previously (12). For category analyses, cases with 10% or more positive cells were rated as positive while cases with fewer than 10% positive cells were rated as negative for both MGMT and p53 (12). For p53 status, positive status, as determined by IHC in our institute, and mutation results from Sanger sequencing technique performed by Kansai Molecular Diagnosis Network for CNS tumors (KNBTG) in recent cases were grouped for analysis. In recent cases, MGMT status was also determined by methylation-specific polymerase chain reaction (PCR) or pyrosequencing in our institute, or quantitative methylation-specific PCR following the bisulfite modification of tumor genomic DNA performed by KNBTG. For IDH status, data results from Sanger sequencing by KNBTG for most of recent cases and IHC in our institute, using IDHR132H antibody, for the other cases ≥**55** years old were grouped for analysis since the 2016 World Health Organization classification of brain tumors proscribes sequencing for IDH in GBM patients ≥**55**years old.

Statistical analysis

For the analysis, $\chi 2$ test, Fisher’s exact test and logrank tests were used for univariate analyses and the Cox proportional hazard model was used for multivariate analysis with $p < 0.05$ considered as significant. Statistical calculations were performed with IBP SPSS Statistics V25.0 software.

Results

Analysis for all 277 initially diagnosedGBM cases

Table 1 and Table 2 show various data for all 277 cases, including age; sex; location of main lesion; side of the lesion (right, left, other side [median, bilateral, multicentric]); KPS; EOR of the lesion; use of intraoperative PDD and fluorescence intensity; use of intraoperative MRI (for removal or for biopsy); IDH, p53, and MGMT promotor statuses of the lesion; type of RT; type of combination therapy; outcome at discharge (discharge to home, transfer to other facilities or dead during hospitalization); and their relationship to patient prognoses. The median OS of the entire 277-case cohort, the 200 non-biopsy cases, and the 77 biopsy cases were 16.6 months, 19.7 months, and 9.7 months, respectively with GTR (100% of EOR) achieved in 32.9% of the cases. Univariate analysis revealed that younger age, right side, higher KPS, GTR, intraoperative MRI use for removal, p53 status, proton therapy, combination immunotherapy, and discharge to home were good prognostic factors. Cases with IDH mutant status had longer OS (median OS was 28 months) than those with IDH wildtype (median OS was 15 months) and cases with MGMT methylated/negative status had longer OS (median OS was 21 months) than those with unmethylated/positive (median OS was 18 months), although no significant differences were seen.

Table 3 shows the results of a multivariate analysis regarding relation among OS and 7 factors (65-year-old or less, right side, KPS 70 or more, GTR, p53 negative/wild status, proton therapy, and immunotherapy groups). Intraoperative MRI use was excluded from multivariate analyses as a relating factor of EOR since they were closely related as shown in Fig1 indicates that only 40 (33.6%) out of 119 non-biopsy cases without intraoperative MRI resulted in GTR while 51 (63.0%) out of 81 non-biopsy cases with intraoperative MRI for removal were GTR (p=0.000, $\chi 2$ test). In addition, return trips after discharge were also excluded as a confounding factor with patient's prognosis itself and the type of radiotherapy (Conventional/Proton versus Others, p=0.000, Fisher's exact test). In the multivariate analysis, GTR, proton therapy, and immunotherapy were the significant prognostic factors (Table3). Immunotherapy was more often selected for GTR cases than non-GTR cases (p=0.00, chi-square test) and any interactions between RT and EOR types (p>0.1, chi-square test) were not found. Figure 2 shows the survival curves comparing each favorable prognostic factor group with the other groups, visually confirming that cases in the GTR and immunotherapy groups show a tailed plateau curve, indicative of many long-term survivors. In the multivariate analysis regarding PFS, GTR (p=0.000, Exp=1.76) and immunotherapy (p=0.015, Exp=1.68) were the significant prognostic factors (detailed data not shown).

Sub-analysis regarding GTR and non-GTR cases

Table 4 shows the results of a multivariate analysis regarding relationship among OS and candidate factors of 91 GTR cases in order to extract those factors that contributed to the best prognosis in the GTR group. In this analysis, immunotherapy contributed to improvements in prognoses, while proton therapy did not contribute, despite no interaction between RT and EOR types (p>0.1, chi-square test). The PFS and OS curves for these factors in the GTR group are shown in Fig. 3. As shown in Fig. 3-B, the median OS was 36.9 months and the 5-year OS% was 43.3% in patients who underwent surgery resulting in GTR and

immunotherapy. Table 4 shows the results of a multivariate analysis regarding relationships between OS and candidate factors in 186 non-GTR cases. In this analysis, preoperative KPS and proton therapy, but not immunotherapy, contributed to improvements in prognoses.

Sub-analysis regarding AFTV cases

Table 5 shows the results of a multivariate analysis regarding relationship among OS and candidate factors search for good prognostic factors in 31 AFTV cases, which were the majority (79.5%) of the immunotherapy group. Here, only 3 cases (11%) out of 27 confirmed IDH statuses were IDH wildtype. The median OS of these 3 IDH mutant cases was 29.5 months. As for MGMT status, only 7 cases (28%) out of 25 confirmed status were methylated/negative status, others were unmethylated/positive status. Twenty cases resulted in GTR after surgery, 7 cases were STR, and 4 cases were PR. In this analysis, GTR was a good prognostic factor, in line with results seen in the analysis of the entire immunotherapy cohort. Figure 4 shows the PFS and OS curves for these factors in the AFTV group. Patients with AFTV had a median OS of 26.5 months and the GTR group had a notably good prognosis of 34.4 months for median OS and 40% for 5-year OS%.

Discussion

In this study, younger age, right side, higher KPS, GTR, intraoperative MRI use for removal, p53 status, proton therapy, combination immunotherapy, and discharge to home were good prognostic factors in the univariate analysis. Among these, GTR, proton therapy, and immunotherapy were extracted as good prognostic factors, while the use of intraoperative MRI was closely related to EOR. In previous reports, intraoperative MRI was observed to improve the removal rate (19, 20) and our current study is consistent with these reports. As for the right side resulting in a good prognosis in the univariate analysis, the requirement of complex informed consent for advanced therapies, including proton therapy and immunotherapy, may be involved. Informed consent that contained explanations of possible complications tended to include more non-aphasic patients (the majority of whom had right-side lesions) in these advanced therapies. For instance, 20 (61%) out of 33 patients who underwent proton therapy had right-sided lesions as did 24 (61%) out of 39 patients who received immunotherapy (detailed data not-shown).

In the sub-analysis, immunotherapy was a good prognostic factor in the GTR group while GTR was also a good prognostic factor in the AFTV group that happens to represent the majority of immunotherapy given in our institution. Our previous, prospective clinical studies have also shown that high EOR prolongs PFS and OS, although significant differences were only obtained in univariate analyses using the small number of patients that were enrolled in the study (12, 18). In the present study, however, the significance of this result seems to be high because of the large, albeit retrospective, amount of patient data (277 cases). Moreover, sub-analysis using GTR patient data (Fig3) shows immunotherapy can produce long survival (for up to 5 years) in approximately 40% of patients if the tumor lesion is surgically removed without any

residual bulk. To the best of our knowledge, no previous study has clarified this phenomenon. A recent meta-analysis using 9 total studies, representing 806 GBM patients, showed that half of GBM patients have PD-L1 overexpression and this expression in tumor tissues is significantly related to a poor OS (HR = 1.63, $P = 0.003$) with heterogeneity ($I^2 = 51\%$) (21). This result indicates that tumor bulk in most GBM cases engenders resistance to cytotoxic T cell lymphocytes (CTLs), an idea bolstered by our previous studies that showed immunosuppressive PD-1-positive cells and M2 macrophages colocalized to GBM tissue in early relapse cases after AFTV (22, 23). We therefore speculate that vaccine therapies, including AFTV combined with immune checkpoint inhibitors, M2 macrophage inhibitors, or local therapy that provokes a local immune response, will prolong OS for both GTR and non-GTR cases. Combinations of immunotherapy based on this concept are expected to become the next generation of immunotherapy. (18)

In this study, IDH status was not a statistically significant prognostic factor throughout the entire analysis (Table1) or in the 31 AFTV cases (detailed data not shown). The mOS values of IDH mutant GBM patients were fairly high (28.0 months in the entire analysis and 29.5 months in a sub-analysis using AFTV cases) and we speculate that this is due to the low number of IDH mutant cases. In a recent meta-analysis of GBM, 67 (36.61%) of 183 *IDH1* wild-type GBM cases were PD-L1-positive, while only one (3.85%) of 26 *IDH1* mutant GBM cases were PD-L1-positive. (21) The pooled OR indicates that PD-L1 positivity was closely related to *IDH1* status (OR = 9.92, $P = 0.007$),(21) revealing that CTLs in the GBM microenvironment are more effective in IDH1 mutant GBM. Future studies will accumulate the data to confirm this theory. In our study, p53-negative/wild type was a good prognostic factor in the univariate analysis of the entire dataset as shown in Table1 (mOS values were 19.1, 16.4, and 15.7 months in p53 negative/wildtype, p53 positive/mutant, and unexamined cases, respectively, p=0.047 by the logrank test). In this regard, p53 status had no effect on prognosis in the univariate analysis of 238 cases (excluding immunotherapy cases; median OS values were 15.9, 13.7, and 14.7 months in p53 negative/wildtype, p53 positive/mutant, and unexamined cases, respectively, p=0.162 by the logrank test) and we assumed that the immunotherapies improved the prognoses of p53 negative/wildtype GBM patients. Our previous studies also suggest that this type is a good prognostic factor in GBM patients who receive AFTV but future prospective studies are needed to verify this.

Limitations of this study must be acknowledged and, to address them, a multicenter, randomized Phase III trial on AFTV was begun to clarify immunotherapy benefits in GTR patients. Detailed information about MRI data including existence of contrast enhancement (CE) in the lesion, volume of CE area and volume of fluid-attenuated inversion recovery high-intensity area were not included in the analysis. Heterogeneity in type of testing for IDH, p53, and MGMT statuses might make the results inaccurate and our insufficient analysis of molecular markers of intratumoral tissues related to prognosis in the immunotherapy group, outside of these 3 markers, should be rectified by additional studies. In addition, as only 3 patients received immunotherapy combined with proton therapy, the effect of this combination cannot be clearly stated in this study.

In conclusion, GTR, proton therapy, and immunotherapy were good prognostic factors in the multivariate analysis of single-center GBM cases. Notably, tumor vaccine therapy for GTR cases achieved high median survival times and long-term survival ratios, revealing that vaccine therapy should be performed for GTR cases.

Declarations

Availability of data and material

The datasets generated during and/or analyzed during the current study are not publicly available since they include a few unpublished data from original clinical studies but are available from the corresponding author on reasonable request.

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Conflicts of Interest Disclosure

The authors declare that no conflicts of interest exist in this study. This study was supported by a Grant-in-Aid for Scientific Research in Japan (Grant number: 18K08962).

Author Contributions

All authors contributed to the study conception and design. Material preparation, data collection was performed by Dr. EI and Dr. NS and analysis was performed by Dr. EI. The first draft of the manuscript was written by EI and the second draft was modified by EI, NS and MM. all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Tables

Table 1. Background and diagnostic factors in all 277 cases and their relationship to patient prognoses.

Factors		Patient numbers		Median PFS (months)	P values	Median OS (months)	P values
All cases		277		8.7		16.6	
Age	Median (percentile)	66	(57-74)				
	66 or more	149	(53.8%)	8.2	0.334	13.8	0.006
	65 or less	128	(46.2%)	9.4		19.3	
Sex	Men (%)	165	(59.6%)	8.4	0.281	16.6	0.966
	Women	112	(40.4%)	8.7		15.7	
Main Location	Frontal	104	(37.5%)	8.6	0.395	16.2	0.179
	Temporal	93	(33.6%)	8.3		15.1	
	Parieto-Occipital	39	(14.1%)	9.2		24.8	
	Others	41	(14.8%)	9.8		15.7	
Side	Right	132	(47.7%)	9.8	0.030	19.1	0.013
	Left	118	(42.6%)	8.2		15.9	
	Others	27	(9.7%)	5.1		11.0	
KPS	Median (percentile)	70	(60-80)				
	70 or more	190	(68.6%)	9.2	0.190	19.7	0.006
	60 or less	87	(31.4%)	8.6		11.2	
EOR	Non-biopsy	200	(72.2%)	9.5		19.7	
	GTR (100% of EOR)	91	(32.9%)	11.3	0.000	26.5	0.000
	STR	58	(20.9%)	9.2		21.7	
	PR	51	(18.4%)	5.3		11.1	
	Biopsy	77	(27.8%)	7.1		9.7	
5-ALA	Strong	190	(68.6%)	9.1	0.096	16.8	0.300
	Vague	31	(11.2%)	6.7		14.4	
	Positive	11	(4.0%)	11.5		18.0	

	Negative	8	(2.9%)	10.3		13.7		
	Not used/Unknown	37	(13.4%)	4.9		16.7		
Intraoperative MRI	Yes (for removal)	82	(29.6%)	11.2	0.009	21.7	0.000	
	Yes (for biopsy)	12	(4.3%)	6.4		8.2		
	Not used	183	(66.1%)	8.1		14.7		
IDH	Mutant	17	(6.1%)	14.6	0.365	28.0	0.256	
	Wild	197	(71.1%)	8.6		14.5		
	Not examined	63	(22.7%)	8.4		21.6		
p53	Positive/Mutant	109	(39.4%)	8.3	0.307	16.4	0.047	
	Negative/Wild	129	(46.6%)	9.7		19.1		
	Not examined	39	(14.1%)	8.2		15.7		
MGMT	Negative/Methylated	44	(15.9%)	11.2	0.451	21.4	0.267	
	Positive/Unmethylated	117	(42.2%)	7.9		18.1		
	Not examined	116	(41.9%)	8.9		15.1		

Table 2 Radiation therapy, combination therapies, and discharge destination outcomes for all 277 cases and their relationship to patient prognoses.

Factors		Patient numbers		Median PFS (months)	P values	Median OS (months)	P values
All cases		277		8.7		16.6	
RT	Conventional	195	(70.4%)	9.0	0.000	17.7	0.000
	Hypofractionation	41	(14.8%)	7.3		8.6	
	WBRT	5	(1.8%)	5.5		8.1	
	Proton	33	(11.9%)	11.3		28.7	
	Not used	3	(1.1%)	2.8		2.8	
Combination therapies	TMZ	180	(65%)	8.6	0.001	17.0	0.000
	TMZ + BEV	43	(15.5%)	8.7		11.0	
	TMZ + Immunotherapy	39	(14.1%)	12.5		29.5	
	Others	15	(5.4%)	5.5		10.2	
Place after Discharge	Home	144	(52.0%)	10.5	0.000	23.5	0.000
	Trans	130	(46.9%)	7.1		10.8	
	Death during hospitalization	3	(1.1%)	2.7		2.7	

Table 3. A multivariate analysis of 7 factors from all 277 patient data and their relationship to patient prognoses (OS).

Factors	Groups	P values	Exp (95%CI)
Age	65 or less versus others	0.466	
Side	Right versus others	0.449	
pre KPS	70 or more versus others	0.093	
EOR	GTR versus others	0.000	2.14 (1.57-2.92)
p53	Negative/wild versus others	0.108	
RT	Proton therapy versus others	0.025	1.60 (1.06-2.40)
Combination therapy	Immunotherapy versus others	0.003	1.89 (1.23-2.88)

Table 4. A multivariate analysis of age, KPS and 2 candidate factors (RT, Adjuvant therapy) in 91 GTR cases and their relationship to patient prognoses (OS), and a multivariate analysis in 186 non-GTR cases and their relationships to patient prognoses (OS).

	Factors	Groups	P values	Exp (95%CI)
GTR cases (91 cases)	Age	65 or less versus others	0.336	
	pre KPS	70 or more versus others	0.414	
	RT	Proton therapy versus others	0.397	
	Adjuvant	Immunotherapy versus others	0.006	2.35 (1.27-4.33)
Non-GTR cases (186 cases)	Age	65 or less versus others	0.802	
	pre KPS	70 or more versus others	0.016	1.541 (1.083-2.192)
	RT	Proton therapy versus others	0.023	1.948 (1.094-3.469)
	Adjuvant	Immunotherapy versus others	0.135	

Table 5. A multivariate analysis of age, KPS and 2 candidate factors in 31 AFTV cases and their relationship to patient prognoses (OS).

Factors	Groups	P values	Exp (95% CI)
Age	65 or less versus others	0.120	
pre KPS	70 or more versus others	0.471	
EOR	GTR versus others	0.005	3.64 (1.48-8.93)
RT	Proton therapy versus others	0.780	

Figures

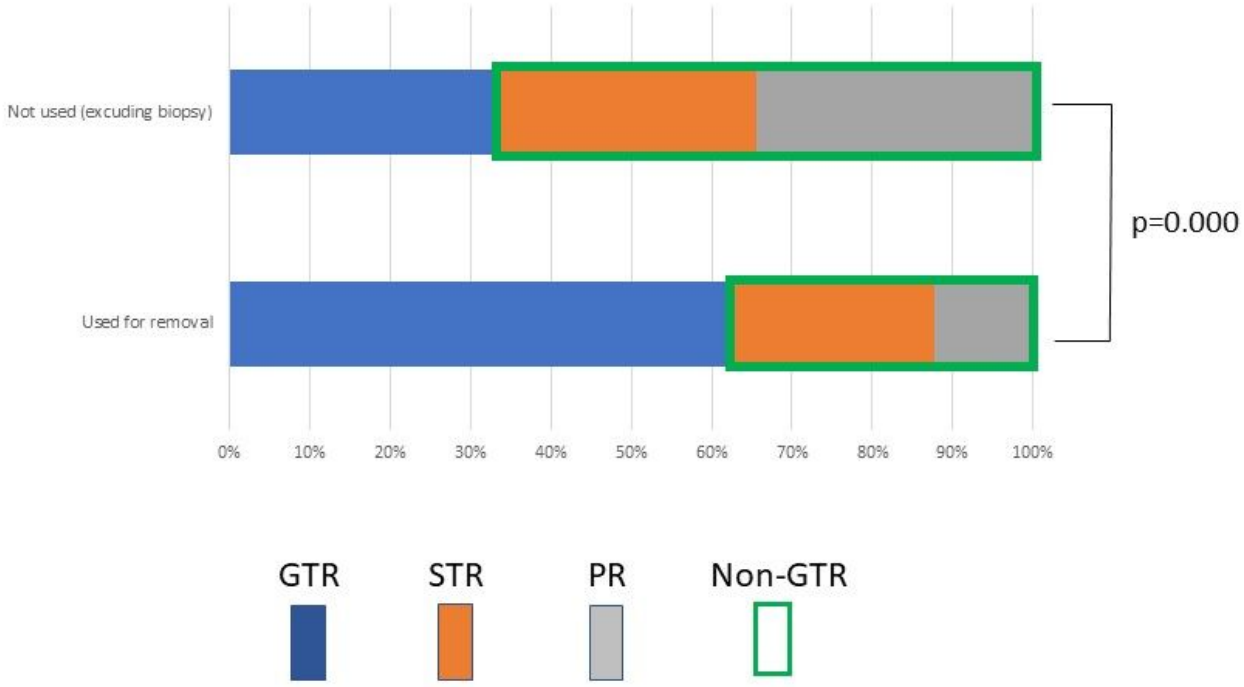

Figure 1

Extent of resection (EOR) of initially diagnosed glioblastoma (GBM) in tumor removal (non-biopsy) cases without intraoperative MRI and with intraoperative MRI.

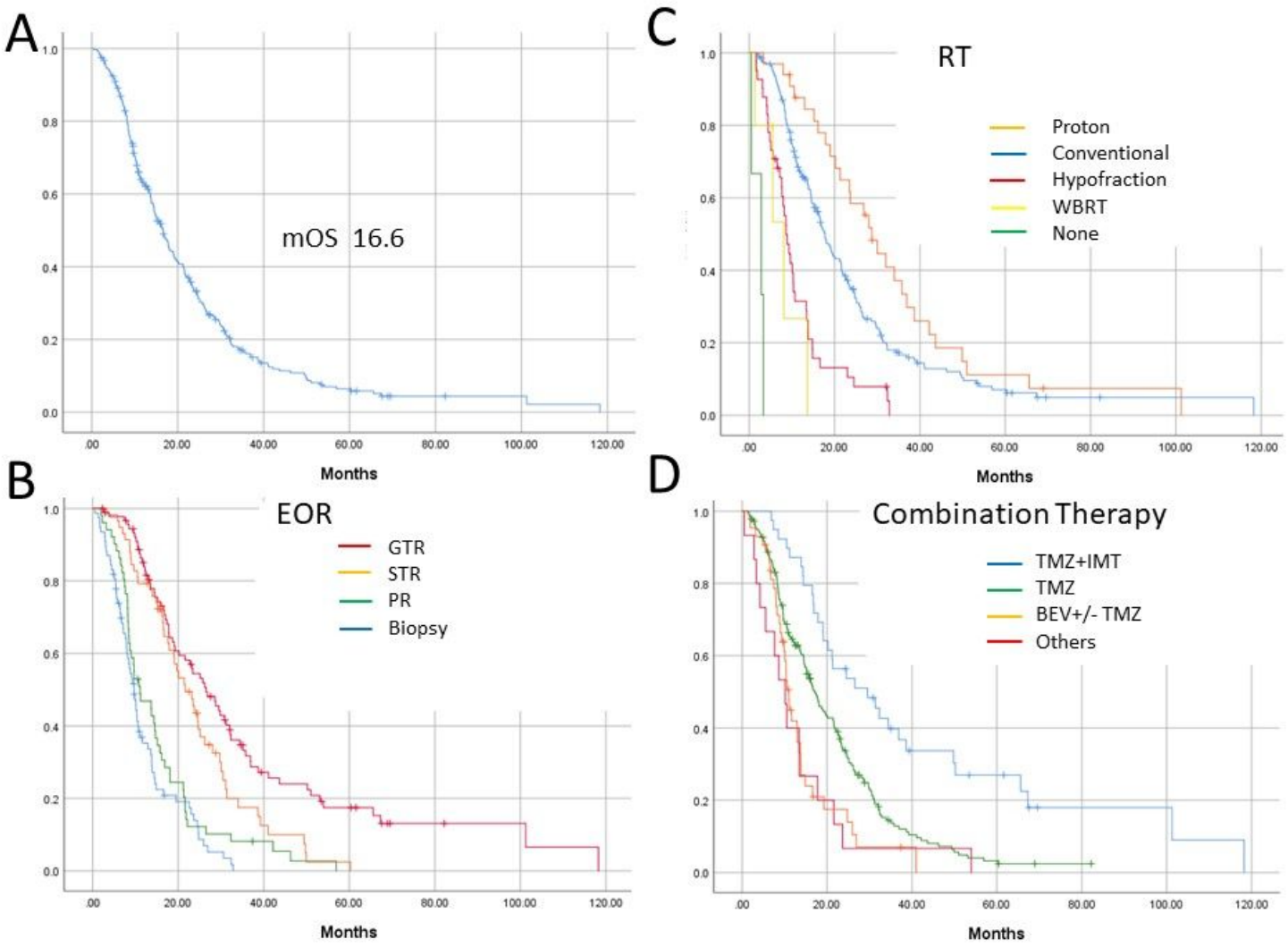

Figure 2

The overall survival (OS) curve for the entire 277 GBM cohort (A) and this curve divided by EOR consisting of gross total removal (GTR), subtotal removal (STR), partial removal (PR) and biopsy (B); type of radiotherapies (RT), including proton therapy, conventional RT, hypofractionated RT (Hypofraction), whole brain RT (WBRT), and no RT (non) (C); and type of combination therapy including temozolomide (TMZ) +immunotherapy (IMT), TMZ, Bevacizumab (BEV) with or without TMZ, and others (D).

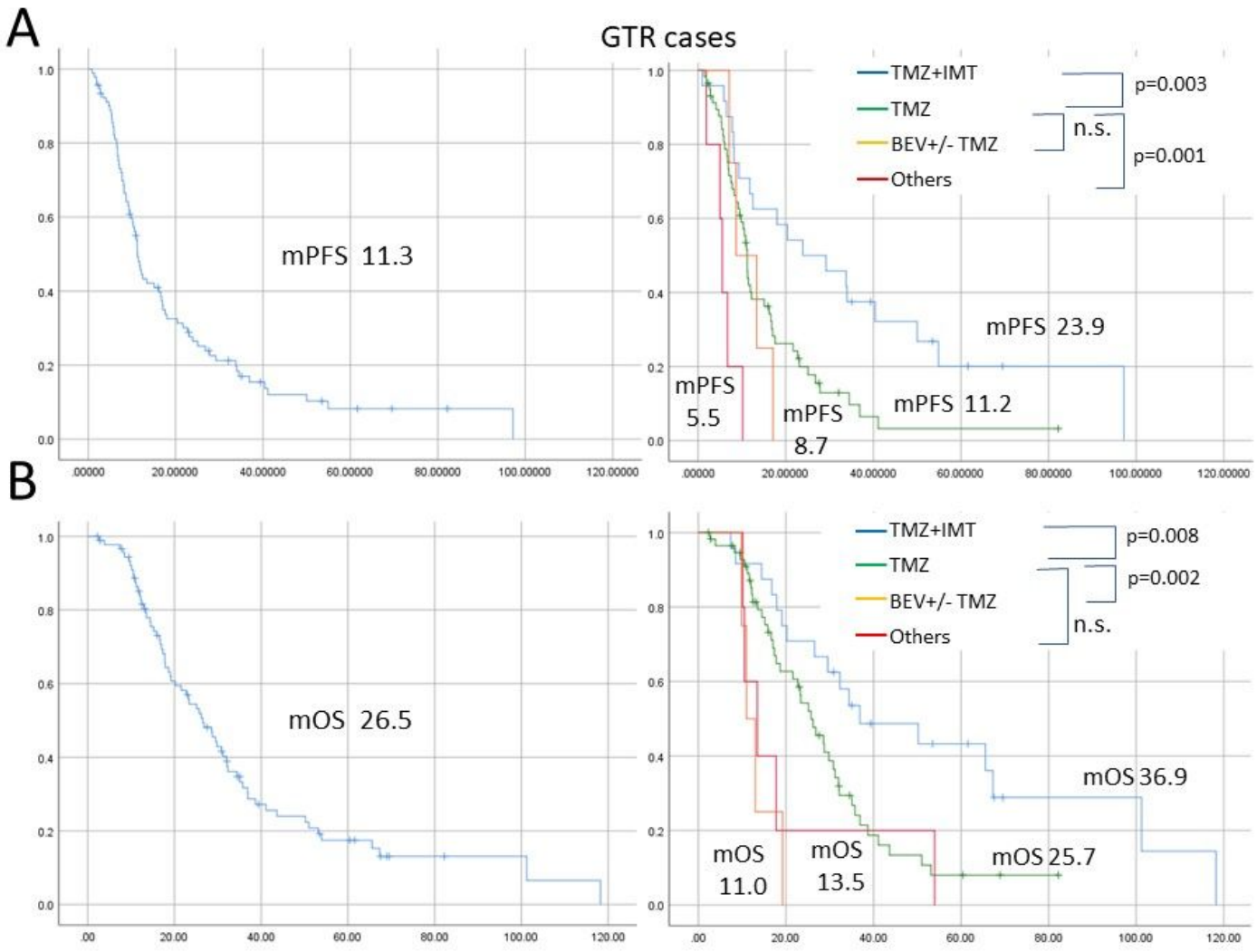

Figure 3

The progression-free survival (PFS) (A) and OS (B) curves for 91 cases with GTR (left) and each curve of the cases divided by type of combination therapy consisting of TMZ+ IMT, TMZ, BEV with or without TMZ, and others (right). The logrank test is used for analysis.

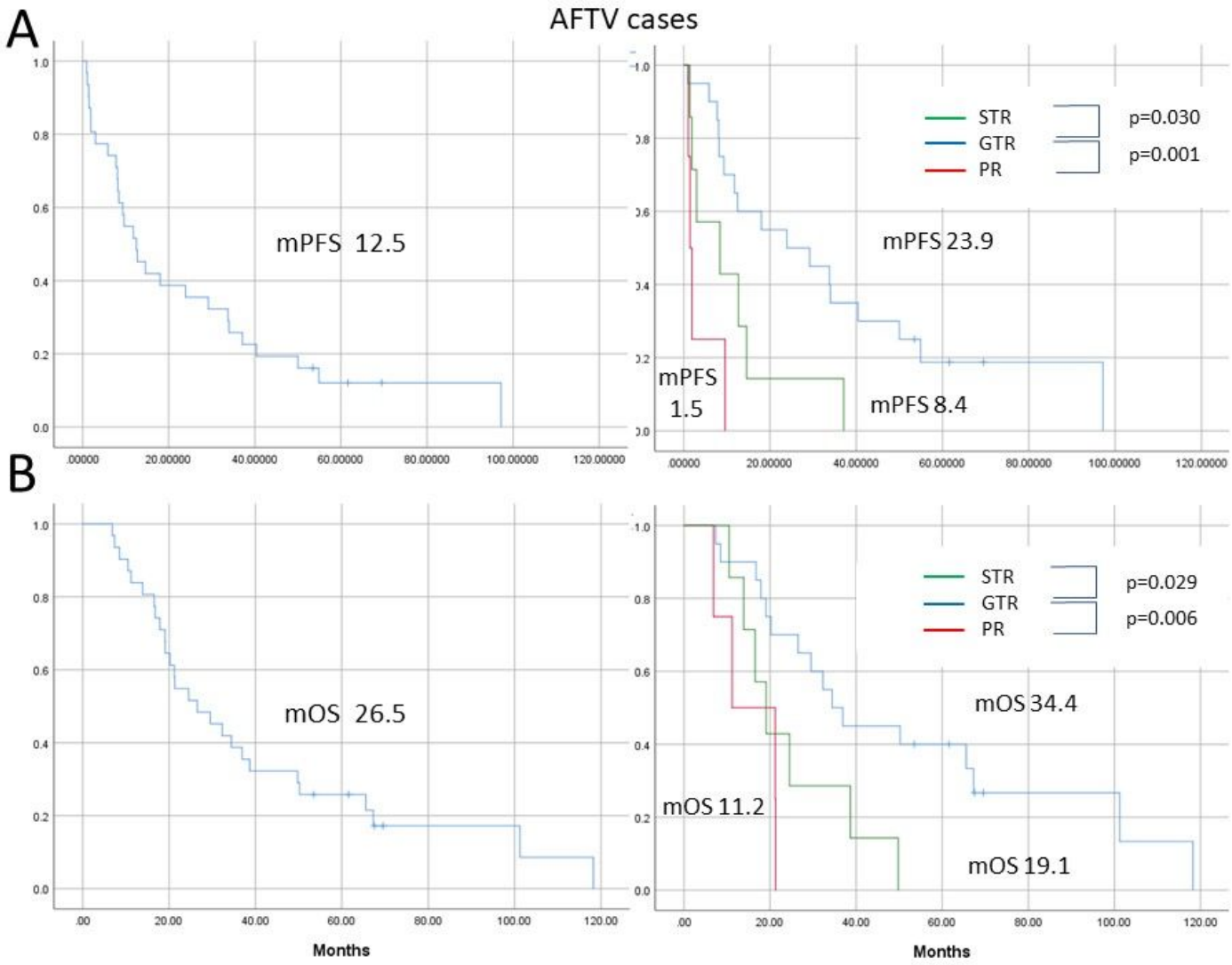

Figure 4

The PFS (A) and OS (B) curves for 31 cases with AFTV immunotherapy (left) and each curve of the cases divided by EOR consisting of GTR, STR, and PR (right). The logrank test is used for analysis.