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a median survival of 18.3 months (95% CI=14.6–22.5 months). When the 60 patients who were 18–70 years old on this trial were compared with the EORTC (RT+TMZ) data, the median survival (20.3 vs. 14.6 months) and percent surviving at 24 months (41.7% vs. 26.5%, $p=0.02$) appeared superior. Data on MGMT methylation and postprogression treatment with VEGF-targeted therapies for this population will be available for presentation. *Conclusion*: Talampanel was well tolerated and did not appear to increase the known hematologic or nonhematologic toxicities of TMZ. Talampanel can be added to RT+TMZ without significant added toxicity. These encouraging survival results in this study suggest that blocking AMPA receptors may be a useful strategy in glioblastoma.

O66. ONGOING CLINICAL TRIALS AND THE FUTURE DIRECTION OF GLIOMA TREATMENT

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Because of the proposed sensitivity to chemotherapy of oligodendroglial tumors both the RTOG and EORTC had investigated if these tumors benefit from adjuvant PCV chemotherapy. These studies, EORTC study 26951 and RTOG 9402, both showed that the addition of PCV chemotherapy (consisting of procarbazine, CCNU, and vincristine) to 59.4 Gy radiotherapy does increase progression-free survival without improving overall survival in anaplastic oligodendroglioma and anaplastic oligoastrocytoma. A major finding of both studies is the large difference in prognosis of patients with and without combined 1p/19q loss. Based on these differences in survival and the clear different outcome in anaplastic oligodendroglioma with 1p/19q loss, EORTC and the collaborative groups felt that it was no longer rational to treat these patients according to histology without taking the genotype of these tumors into account. For studies in anaplastic gliomas it was therefore proposed to classify into anaplastic glioma without 1p/19q loss and anaplastic oligodendroglial tumors with 1p and 19q codeletion. Another challenge is the definition of a proper end point for these trials. Overall survival seems to be the most relevant outcome parameter, even at progression. The outline and initiatives in grade III gliomas (EORTC 26053/22054, CATNON plus the codeleted trial) are presented. Standard therapy for glioblastoma is surgical resection aimed to be as complete as possible, respecting neurological function followed by chemoirradiation with temozolomide. TMZ given as concomitant and adjuvant therapy to RT has shown to increase progression-free survival (PFS) (rate at 6 months, 53.9% vs. 36.4%) and median survival (14.6 vs. 12.1 months) compared to adjuvant treatment with RT therapy only (EORTC 26981/22981 NCIC CE.3 trial). Still, many patients do not respond to therapy. The resistance of cells against DNA damage caused by nitrosoureas and temozolomide is at least in part mediated by the DNA-repair enzyme O^6-methylguanine-DNA methyltransferase (MGMT). Epigenetic silencing of the MGMT gene by promoter methylation compromises this DNA repair and has been associated with longer survival in (glioblastoma) patients who are treated with alkylating or methylating agents. An analysis of the EORTC 26981/22981 NCIC CE.3 trial showed, that indeed patients with glioblastoma containing a hypermethylated MGMT promoter benefited from TMZ (overall survival [OS] rate at 24 months, 46% vs. 23%), whereas those who did not have a methylated MGMT promoter did have a significantly worse survival rate and less benefit from the addition of temozolomide to RT (OS rate at 24 months, 14% vs.<2%). This raises the question if the small benefit from chemoirradiation observed in this group outweighs the toxicity and costs of the temozolomide treatment, and calls for the development of more effective drug regimens for this specific group of patients. Although there may be small numbers of patients with an unmethylated MGMT promoter that do benefit from combined chemoirradiation, for the entire subgroup of these molecularly defined GBM patients the overall benefit is questionable. Most interestingly, the phase II trial with the integrin inhibitor cilengitide also demonstrated a marked benefit mainly in the patients with glioblastoma containing a methylated MGMT promoter. Consequently, the current Merck/EORTC phase III trial is designated to delineate the role for cilengitide in glioblastoma with methylated MGMT. Even earlier, Eli Lilly took the approach to examine the protein kinase C-β inhibitor, enzastaurin, together with radiotherapy but without TMZ in patients with glioblastoma containing an unmethylated MGMT promoter. This raises the general question whether treatment in glioblastoma trials should not only be stratified according to MGMT but entry into those trials limited by MGMT status. This would call for different approaches of GBM patients, depending on the MGMT promoter gene status. The primary question to address in GBM with unmethylated MGMT promoter gene is the identification of drugs that provide more survival benefit compared to TMZ. The current EORTC trial initiatives are presented.

O67. THE RESULT OF A CLINICAL TRIAL FOR MALIGNANT GLIOMAS BY JCOG BRAIN TUMOR STUDY GROUP (JCOG 0305)

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Purpose: Japan Clinical Oncology Group (JCOG) Brain Tumor Study Group conducted a multiinstitutional randomized controlled trial on malignant gliomas entitled, a randomized controlled phase II study of chemoradiotherapy using ACNU versus procarbazine and ACNU for astrocytoma grade 3 and 4, with the support of the Health and Labour Sciences Research Grants of the Ministry of Health, Labour, and Welfare in order to establish a standard therapy for malignant gliomas in Japan. *Method*: The patients with newly diagnosed supratentorial astrocytoma grade 3 or 4 were enrolled and randomized into two groups. The patients in group A were treated with ACNU (80 mg/m^2 iv) during the postoperative radiotherapy (RT, 60 Gy local), while those in group B received procarbazine (80 mg/m^2 for 10 days per os) preceding administration of ACNU. Each regimen was continued every 8 weeks for 2 years if it was tolerable for the patients and their disease did not progress. The primary end point was the overall survival rate and the secondary end points were the response rate on the MRI and the frequency of the adverse events. Procarbazine is expected to reduce O^6-methylguanine-DNA methyltransferase (MGMT) and enhance the anticancer activity of nitrosoureas. The protocol was activated in April 2004 and 111 patients were registered by the end of August 2006 from 19 collaborating neurosurgical institutes of JCOG-BTSG. *Results*: The overall survival of the patients treated with ACNU+RT was 16.2 months and that of procarbazine+ACNU+RT was 18.7 months, while PFS of both groups were 6 months. CTCAE grade 3/4 was observed in 40–60% of the patients. *Conclusion*: ACNU-based chemoradiotherapy was an effective but toxic treatment.

O68. CURRENT CLINICAL TRIALS OF GLIOMA THERAPY AND SITUATIONS OF NEURO-ONCOLOGY PRACTICE IN KOREA

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There has been no qualified sponsor-investigator clinical trial program, and the standard therapies have been all we could do for the treatment of malignant glioma patients in the Korean Brain Tumor Society. We have just started to join two international clinical trials since 2008. In this article the past and current status of the neuro-oncology field in Korea as well as eastern and northern Asian countries will be introduced, and clinical outcomes of concurrent radiotherapy and temozolomide chemotherapy for 100 patients of four university hospitals of Korea (Advisory Board of S-P Korea) will be presented.

O69. HISTOGRAM ANALYSIS OF PERFUSION MRI DATA FOR THE ASSESSMENT OF TUMOR RESPONSE DURING GLIOMA THERAPY

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Purpose: A recently developed histogram analysis of relative cerebral blood volume (rCBV) from the entire tumor has been reported to offer excellent interobserver agreement for quantitative analysis and demonstrate the heterogeneous morphologic features of glioma vascularity. We aimed to determine whether histogram analysis can be adopted in the assessment of tumor response during glioma therapy. *Methods*: We retrospectively studied 51 dynamic susceptibility contrast 3-T MR imaging data of 29 patients (mean age 50.5 years, range, 18–76) with histologically confirmed gliomas (9 low grade, 20 high grade). rCBV maps were created and normalized to unaffected white matter. Histogram width (HW), peak height position (PHP), and maximum value (MV) of the entire tumor were measured from normalized histogram distribution. *Results*: The values (mean±SD) of HW, PHP, and MV were 4.64±2.03, 4.58±2.63, and 6.29±2.79 for the preoperative imaging of high-grade gliomas ($n=8$), and 3.83±1.96, 2.66±1.66, and 4.73±1.96 for the final imaging, which showed definite radiological tumor progression or confirmed tumor recurrence by biopsy ($n=8$). Thirty-two imaging data obtained during the median imaging follow-up of 3.7 months were divided into two groups (progression vs. stable/radiation necrosis) according to the follow-up result, and three parameters were compared. All three parameters were positively correlated with tumor progression (HW, 3.05±2.18 vs. 1.02±0.50; PHP, 2.39±1.71 vs. 0.94±0.28; MV, 4.13±2.83 vs. 1.56±0.52) and MV was the most predictive with multivariate analysis. *Conclusion*: Our results suggest that histogram analysis of rCBV can be a more objective and useful diagnostic

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ORIGINAL ARTICLE

O^6-Methylguanine DNA methyltransferase determined by promoter hypermethylation and immunohistochemical expression is correlated with progression-free survival in patients with glioblastoma

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Abstract
Objective The prognostic significance of O^6-methylguanine DNA methyltransferase (MGMT) was evaluated by analysis of both *MGMT* promoter methylation and protein expression in a series of patients with newly diagnosed glioblastoma.
Methods Seventy-three patients with glioblastomas treated with alkylating agents were analyzed for MGMT expression by immunohistochemistry. Genomic DNA was isolated from frozen surgical specimens obtained from 62 of 73 patients. *MGMT* promoter methylation was determined by methylation-specific polymerase chain reaction. The prognostic significance of MGMT was evaluated together with other well-known prognostic factors.
Results *MGMT* promoter hypermethylation was detected in 35 of 62 patients (56.4%). MGMT immunoreactivity was low in 26 (35.6%) tumors, moderate in 24 (32.9%), and high in 23 (31.5%). Significant correlation was observed between MGMT expression and *MGMT* promoter methylation ($P < 0.001$). Both *MGMT* promoter methylation and low MGMT expression were independently associated with better progression-free survival but not with longer overall survival. However, in the subgroup analysis, *MGMT* promoter hypermethylation was significantly associated with longer overall survival in patients treated with temozolomide (TMZ) after nimustine hydrochloride (ACNU) treatment.
Conclusions Low MGMT expression and *MGMT* promoter methylation are both predictive markers for slower tumor progression in patients with glioblastoma.

Keywords Glioblastoma · O^6-Methylguanine DNA methyltransferase · Methylation-specific polymerase chain reaction · Immunohistochemistry

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Introduction

Glioblastoma is the most common primary malignant tumor in adults, and the median survival continues to be approximately 12 months despite therapeutic advances. Survival is related to age, preoperative Karnofsky performance status (KPS), more extensive tumor resection, radiotherapy, and adjuvant chemotherapies [1–8]. Our understanding of the genetic alterations in glioblastoma has progressed, but clinically useful molecular markers predictive of the therapeutic response and prognosis are still rare. Chloroethylnitrosourea such as nimustine hydrochloride (ACNU) was commonly used as the standard chemotherapeutic drug for glioblastomas in Japan. The mechanism of cytotoxic effect by ACNU is thought to be alkylation at the O^6 position of guanine, an important site of alkylation in DNA, resulting in the formation of DNA lethal cross-links. [9] More recently, temozolomide (TMZ) has been to shown to significantly prolong survival in patients with glioblastoma [10]. TMZ converts the cytotoxic methylating agent at physiologic pH, which forms methyl adducts at the O^6-position of guanine in DNA. The formation of O^6-methylguanine then results in GT

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mismatches during subsequent cycles of DNA replication, followed by DNA strand-break formation and eventually cell death [10, 11]. Although the mechanisms of antitumor effects by these drugs are not the same, expression of O^6-methylguanine DNA methyltransferase (MGMT) is critical to their effectiveness, as it removes alkyl adducts from the O^6 position. *MGMT* promoter methylation results in transcriptional silencing and inhibition of MGMT expression [12, 13]. *MGMT* promoter methylation is strongly associated with survival in patients treated with either ACNU or TMZ [8, 9, 11, 14–16].

MGMT promoter methylation status is commonly assessed by methylation-specific polymerase chain reaction (PCR) [11, 14]. However, methylation-specific PCR is a relatively complicated method not often available in local treatment centers. Immunohistochemistry is a widely used and reliable method in diagnostic histopathology and is available in most laboratories. In addition, immunohistochemistry allows evaluation of both staining degree and target factor localization in individual cells. MGMT protein can be visualized immunohistochemically, and commercial anti-MGMT antibodies are available. Several studies have reported significant associations of immunohistochemically assessed MGMT expression with outcome in patients with glioma [17–22]. However, the correlation between *MGMT* promoter methylation and MGMT protein expression in gliomas remains unclear, as contradictory findings have been reported [23–28].

This study evaluated the prognostic significance of MGMT by analyzing both *MGMT* promoter methylation and protein expression in a series of patients with newly diagnosed glioblastoma managed according to a common diagnostic and therapeutic protocol.

Patients and methods

Patients and tissue preparation

Seventy-three patients with glioblastoma, including 44 males (60.3%) aged 3–76 years (median 53 years), were admitted to the Department of Neurosurgery, Tohoku University Hospital. Tumor samples were obtained during the surgical procedure, formalin-fixed and paraffin-embedded for histological studies, quick-frozen in liquid nitrogen, and kept at −80°C until nucleic acid extraction. Sixty-seven patients (91.8%) underwent surgery (49 gross total resection, 18 partial or subtotal resection) and 6 (8.2%) underwent biopsy. Resection rate was estimated by postoperative magnetic resonance imaging (MRI) within 3 days after surgery. The Ethics Committee of Tohoku University Hospital approved this study. Informed consent for use of their tissues was obtained from all study participants. All patients received adjuvant radiotherapy (total dose of 60 Gy) and chemotherapy consisting of ACNU for 43 patients and TMZ for 30 patients. Twenty-three patients who received ACNU at initial therapy subsequently received TMZ at relapse. Twenty-nine patients underwent second surgery or radiosurgery at first or second relapse.

MGMT methylation analysis

Genomic DNA was isolated from 62 frozen surgical specimens with the Qiagen kit (Qiagen, Valencia, CA, USA). *MGMT* promoter methylation was analyzed by methylation-specific PCR, as described previously [16]. Tumor DNA (2 μg) was treated with sodium bisulfite using the CpG genome DNA modification kit (Qiagen). Primer sequences for the nonmethylated reaction were 5′TTTGTG TTTTGATGTTTGTAGGTTTTTGT3′ (forward) and 5′A ACTCCACACTCTTCCAAAAACAAAACA3′ (reverse), and for the methylated reaction 5′TTTCGACGTTCG TAGGTTTTCGC3′ (forward) and 5′GCACTCTTCCGAA AACGAAACG3′ (reverse). Annealing temperature was 60°C. PCR products were separated on 4% agarose gel. The investigators who selected and analyzed the glioblastoma samples were unaware of all clinical information.

Table 1 Association of O^6-methylguanine DNA methyltransferase (*MGMT*) promotor methylation and MGMT expression in human glioblastomas

	Total	Methylated	Unmethylated	Not done
3 groups				
Low (<20%)	26	18	1	7
Intermediate (20–50%)	24	12	10	2
High (≥50%)	23	5	16	2
2 groups				
Negative (<20%)	26	18	1	7
Positive (≥20%)	47	17	26	4

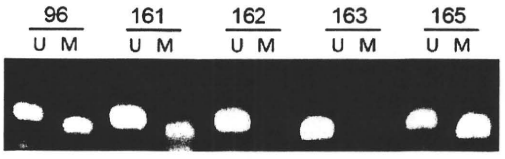

Fig. 1 Methylation-specific polymerase chain reaction (PCR) of O^6-methylguanine DNA methyltransferase (MGMT) promoter in glioblastomas. The presence of a PCR band under *lanes M* or *U* indicates methylated or nonmethylated genes, respectively. Cases 92, 161, and 165 are methylated, whereas cases 162 and 163 are nonmethylated

Immunohistochemistry of MGMT

Immunohistochemical procedures were routine, as previously described. Mouse monoclonal antibody (MT3.1; Chemicon, Temecula, CA, USA) was diluted 1:20. Sections were counterstained with hematoxylin. At least 1000 tumor-cell nuclei were individually reviewed and scored on the sections showing the highest density of immunopositive nuclei by two observers (MW, MY). Endothelial cells and perivascular lymphocytes were excluded from the positive cell count. MGMT protein immunoreactivity was evaluated semiquantitatively by estimating the fraction of positive

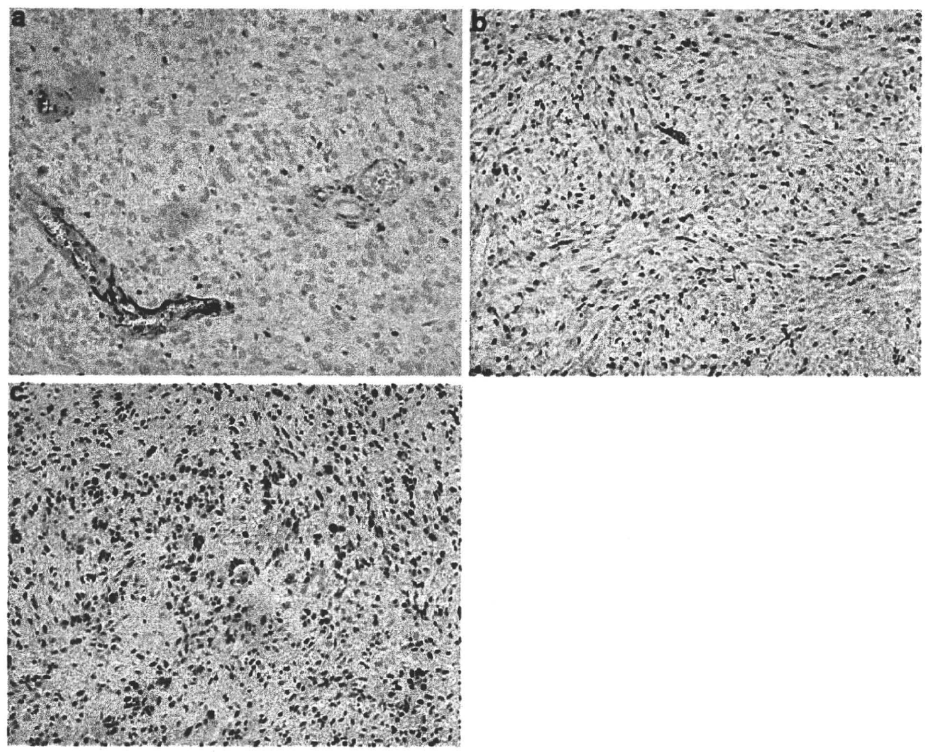

Fig. 2 Representative photomicrographs illustrating low (**a**), moderate (**b**), and high (**c**) immunoreactivity for O^6-methylguanine DNA methyltransferase (MGMT) in tumor samples (×200)

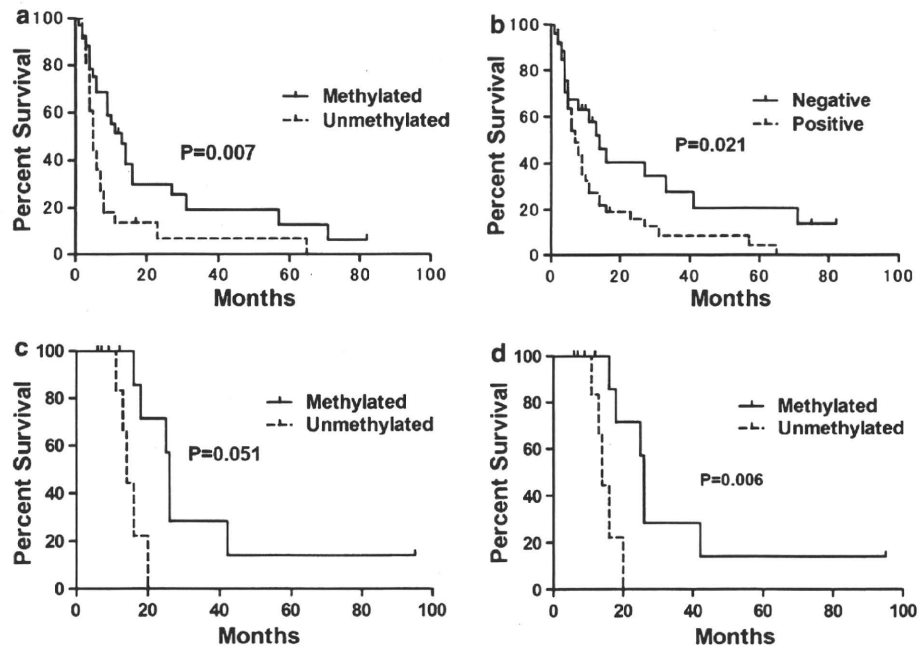

Fig. 3 Progression-free survival curves of patients with glioblastoma according to O^6-methylguanine DNA methyltransferase (MGMT) methylation status (**a**) or MGMT protein expression (**b**). Progression-free survival curves of patients treated with nimustine hydrochloride (ACNU) according to MGMT methylation status (**c**). Overall survival curves of patients treated with temozolomide (TMZ) after ACNU according to MGMT methylation status (**d**)

cells and defining <20% as low reactivity, 20–50% as moderate, and >50% as high.

Statistical methods

The relationship between methylation-specific PCR findings and MGMT expression was evaluated by the χ^2 and Fisher's exact tests. Probabilities of overall and progression survival were calculated according to the Kaplan–Meier method and compared with the log-rank test. *MGMT* promoter methylation status and MGMT expression, together with demographic (age and sex), clinical (preoperative KPS), and therapeutic (extent of resection and initial chemotherapy) variables achieving $P < 0.1$ in the univariate analysis, were subsequently introduced in a backward stepwise proportional hazard analysis (Cox model) as independent predictors of survival. MGMT immunohistochemistry was reclassified as negative (low staining) and positive (moderate and high staining) for statistical purposes. All statistical methods adopted a significance level of $P = 0.05$ using statistical package software (SPSS, Inc., Chicago, IL, USA).

Results

MGMT protein expression and *MGMT* promoter methylation status

Results are summarized in Table 1. *MGMT* promoter hypermethylation was detected in 35 of 62 patients (56.4%) (Fig. 1). MGMT immunoreactivity was low in 26 tumors, moderate in 24, and high in 23 (Fig. 2). Heterogeneous immunostaining was observed in all positive tumor samples. Methylated *MGMT* promoter was associated with low MGMT protein expression in 18 tumors, moderate in 12, and high in 5. In contrast, only one case with low MGMT expression showed nonmethylated *MGMT* promoter. Therefore, significant correlation between *MGMT* promoter methylation and MGMT protein expression was observed ($P < 0.0001$).

Progression-free survival

At the end of the follow-up period, 18 patients (24.6%) remained progression free. Progression-free survival (PFS) was 1.7–96.7 (median 9.2) months. Univariate analysis showed significant prognostic factors were extent of resection, *MGMT* promoter methylation status, and MGMT protein expression (Fig. 3a, b; Table 2). Cox's regression model revealed that gross total resection, *MGMT* promoter methylation status, and MGMT protein expression were independent factors for longer PFS (Tables 3, 4). For subgroup analysis, we divided our patients into 2 groups based on the initial chemotherapy (ACNU or TMZ). In patients treated with ACNU, *MGMT* promoter methylation showed nearly significantly improved PFS compared with those with *MGMT* promotor nonmethylation (log-rank, $P = 0.051$; Fig. 3c), whereas those treated with TMZ showed no statistical difference of PFS between *MGMT* methylation status, as there were too few cases initially treated with TMZ (log-rank, $P = 0.077$).

Overall survival

At the end of the follow-up period of 103.2 months, 37 patients (50.7%) remained alive. Survival was 6.0–103.2 (median

Table 2 Predictors of progression-free and overall survival in the patients with glioblastoma

Variables	*n*	Progression-free survival		Overall survival	
		Median (months)	*P* value	Median (months)	*P* value
Age (years)					
≤60	50	8		26	
>60	23	10	0.853	18	0.083
Sex					
Male	45	10		26	
Female	28	9	0.632	19	0.207
Preoperative KPS					
≥80	38	10		26	
<80	35	8	0.396	17	0.174
Total resection					
Yes	49	10		43	
No	24	6	0.010	16	0.001
Chemotherapy (initial)					
ACNU	43	8		26	
TMZ	30	9	0.749	19	0.056
MGMT promoter status					
Methylated	35	12		26	
Unmethylated	27	5	0.019	17	0.473
MGMT expression					
Negative	26	13		43	
Positive	47	7	0.045	19	0.398
Second surgery					
Yes	22			19	
No	51			42	0.920
Radiosurgery					
Yes	10			103	
No	63			20	0.189
Second-line TMZ					
Yes	20			20	
No	53			43	0.478

KPS Karnofsky performance status, *MGMT* O^6-methylguanine DNA methyltransferase, *ACNU* nimustine hydrochloride, *TMZ* temozolomide

Table 3 Multivariate analysis of factors associated with survival

	Progression-free survival			Overall survival		
	P value	HR	95% CI	*P* value	HR	95% CI
Total resection	0.007	2.339	1.267–4.316	0.001	3.597	1.745–7.416
Age	NS			NS		
Chemotherapy	NS			NS		
MGMT promoter methylation	0.011	2.113	1.183–3.773	NS		

NS not significant, *HR* hazard ratio, *CI* confidence interval

Table 4 Multivariate analysis of factors associated with survival

	Progression-free survival			Overall survival		
	P value	HR	95% CI	*P* value	HR	95% CI
Total resection	0.006	2.265	1.265–4.055	0.001	3.597	1.745–7.416
Age	NS			NS		
Chemotherapy	NS			NS		
MGMT negative expression	0.049	1.777	1.002–3.151	NS		

NS not significant, *HR* hazard ratio, *CI* confidence interval

22.5) months. Univariate analysis showed factors affecting overall survival were sex and extent of resection, whereas salvage treatments (second-line TMZ, re-resection, and radiosurgery), *MGMT* methylation and expression, and overall prognosis had no correlation (Table 2). Multivariate analysis showed that total resection was independently associated with longer overall survival (Tables 3, 4). *MGMT* methylation was also a significant prognostic factor in patients treated with second-line TMZ (log-rank, $P = 0.006$; Fig. 3d); however, MGMT expression was not significantly associated with longer overall survival in these patients (log-rank, $P = 0.22$).

Discussion

The proportion of tumors exhibiting either absence of MGMT protein immunoreactivity or *MGMT* promoter hypermethylation did not differ from those previously reported [9, 23, 29–34]. Several studies of the relationship between *MGMT* promoter hypermethylation and protein expression in gliomas have observed contradictory results [16, 23–28]. In our study, 17 tumors had methylated *MGMT* promoter, of which 12 showed moderate immunoreactivity and 5 showed high immunoreactivity for MGMT protein. As methylation-specific PCR is a highly sensitive method, a methylated band might be detected in a small portion of tumor cells with *MGMT* promoter methylation [9]. In our study, the MGMT immunostaining patterns were heterogeneous in different regions of the same tumor. Although we cannot rule out the presence of contaminating normal cells, other explanations for the observed variability include monoallelic promoter methylation, methylation of a small portion of malignant cells, and loss of heterozygosity in 10q26 [9, 32]. However, we did observe a significant correlation between *MGMT* promoter methylation and MGMT protein expression, as almost all tumors with nonmethylated *MGMT* promoter showed positive MGMT expression.

Some studies found that *MGMT* promoter methylation was associated with improved time to progression or overall survival [9, 20, 22, 29, 31, 33], whereas other studies found no association between *MGMT* promoter methylation and prognosis [30, 32]. Immunohistochemical analysis of MGMT has shown negative MGMT expression was significantly associated with patient survival [15, 17, 19, 21, 34, 35] but no correlation between MGMT expression and prognosis [26–28, 36, 37].

This study found both low tumor MGMT expression and aberrant *MGMT* promoter methylation were independently associated with longer progression-free survival in patients with glioblastomas but not with longer overall survival. One reason is considered to be the effect of various treatments at recurrence. Despite no survival benefit, 29 patients received surgical resection and/or radiosurgery after recurrence. Moreover, 23 patients were initially treated with ACNU but received TMZ at relapse. Among this subgroup, MGMT methylation was still significantly associated with longer overall survival. This result suggests the effectiveness of TMZ might not be affected by preceding chemotherapy with ACNU, as previously reported [38].

Conclusion

The prognostic significance of MGMT protein expression or *MGMT* promoter methylation in patients with glioma remains unclear. The conclusion of this study relies mainly on evaluation of the MGMT predictive value after adjusting for well-recognized clinicopathologic prognostic factors. Further clinical studies are needed to clarify whether the MGMT predictor can discriminate between biologically distinct groups of tumors with different natural histories and treatment responses.

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Conflict of interest statement None.

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Glioblastoma treated with postoperative radio-chemotherapy: Prognostic value of apparent diffusion coefficient at MR imaging

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ABSTRACT

Purpose: To retrospectively evaluate whether the mean, minimum, and maximum apparent diffusion coefficient (ADC) of glioblastomas obtained from pretreatment MR images is of prognostic value in patients with glioblastoma.

Materials and methods: The institutional review board approved our study and waived the requirement for informed patient consent. Between February 1998 and January 2006, 33 patients (24 males, 9 females; age range 10–76 years) with supratentorial glioblastoma underwent pretreatment magnetic resonance (MR) imaging. The values of the mean, minimum, and maximum ADC (ADC_{mean}, ADC_{MIN}, and ADC_{MAX}, respectively) of each tumor were preoperatively determined from several regions of interest defined in the tumors. After surgical intervention, all patients underwent irradiation and chemotherapy performed according to our hospital protocol. The patient age, symptom duration, Karnofsky performance scale score, extent of surgery, and ADC were assessed using factor analysis of overall survival. Prognostic factors were evaluated using Kaplan–Meier survival curves, the log-rank test, and multiple regression analysis with the Cox proportional hazards model.

Results: Likelihood ratio tests confirmed that ADC_{MIN} was the strongest among the three prognostic factors. Total surgical removal was the most important predictive factor for overall survival ($P < 0.01$). ADC_{MIN} was also statistically correlated with overall survival ($P < 0.05$) and could be used to classify patients into different prognostic groups. Interestingly, ADC_{MIN} was also the strongest prognostic factor ($P < 0.01$) in the group of patients in whom total tumor removal was not possible.

Conclusion: The ADC_{MIN} value obtained from pretreatment MR images is a useful clinical prognostic biomarker in patients with glioblastoma.

1. Introduction

Glioblastoma is the most common malignant primary neoplasm of the central nervous system; median survival is approximately 1 year [1,2]. Conventional magnetic resonance imaging (MRI) can yield information on the gross anatomic structure of glioblastoma, but it provides little functional information. Diffusion-weighted (DW) MRI enables the volumetric intravoxel measurement of tissue characteristics based on the detection of changes in the random motion of water protons at the cellular or physiological level [3]. Although the usefulness of DW-MRI for preoperative grading and postoperative assessment of glial tumors has been investigated [4–7], its value for predicting survival has not been fully addressed [8–10]. Because the apparent diffusion coefficient (ADC) is inversely related to tumor cellularity and the glioma grade [4,6,11–14], we postulated that it reflects the biological viability and prognosis of glioblastomas. We therefore analyzed the ADC with respect to the surgical resection status and compared the mean, minimum, and maximum ADC (ADC_{mean}, ADC_{MIN}, and ADC_{MAX}, respectively) values as factors reflecting biological activity. We performed a retrospective study to determine whether these values obtained on preoperative MRI scans are of prognostic value in patients with glioblastoma. We discovered that the ADC_{MIN} value is a prognostic factor for survival in patients with glioblastomas that are not totally resectable.

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2. Materials and methods

The institutional review board of our hospital approved this retrospective study and waived the requirement for informed patient consent. Patient information was kept confidential by removing all identifiers from our records at the completion of our analyses.

3. Patients, diagnosis and treatment

Between February 1998 and January 2006, 49 patients (29 males, 20 females) with histologically confirmed supratentorial glioblastoma were treated at our institution. Of these, 16 were excluded from this study for reasons such as incomplete MRI, progression from anaplastic or low-grade glioma, infratentorial tumors, and incomplete- or no postoperative irradiation or chemotherapy. The remaining 33 patients (24 males, 9 females; age range 10–76 years) with new, histologically confirmed glioblastoma who underwent pretreatment MRI were included in this study. Maximum tumor resection was performed in all patients; it was followed by postoperative external-beam radiation therapy and chemotherapy. Histopathological diagnoses based on World Health Organization criteria were determined by consensus between two authors (V.J.A., Y.T.) who were blinded to the MRI results. Gadolinium-enhanced MRI performed within 1 week after surgery was used to categorize the surgical results according to the removed tumor proportion, i.e., biopsy, ≤50%; partial removal, 50–95%; subtotal removal, 96–99%; total removal, >99%. Nitrosourea-based chemotherapy and radiation therapy were administered concurrently. Patients were followed up to evaluate tumor control after postoperative radiation therapy. Follow-up included physical and neurological examinations and MRI study. Salvage surgery, additional radiation therapy, and/or chemotherapy were considered in patients with tumor recurrence or progression.

4. MRI study and image interpretation

All MRI scans were performed using a 1.5-T superconducting system (Signa Horizon; GE Medical Systems, Milwaukee, WI, USA) with a circularly polarized head coil. All patients underwent MRI studies that included at least unenhanced and contrast-enhanced transverse T1-weighted-, unenhanced transverse T2-weighted-, unenhanced transverse fluid-attenuated inversion-recovery (FLAIR)-, and unenhanced transverse DW images. The transverse T1-weighted spin–echo MR sequence was performed using the following parameters: repetition time ms/echo time ms, 400/8; field of view (FOV), 22 cm × 16 cm; matrix size, 256 (frequency), 192 (phase); section thickness, 5 mm; section gap, 2.5 mm; two signals were acquired. The contrast-enhanced T1-weighted sequences were obtained after administering 0.1 mmol of gadodinium compound per kg body weight. The transverse fast spin–echo T2-weighted sequence was performed using the following parameters: 3500/100; FOV, 22 cm × 16 cm; matrix size, 256 × 192; echo train length, 12; section thickness, 5 mm; section gap, 2.5 mm; 2 signals. Transverse FLAIR images were acquired using fast and interleaved multi-section sequences with the following parameters: 10,000/150; inversion time, 2200 ms; FOV, 22 cm × 22 cm; matrix size, 256 × 192; echo train length, 16; section thickness, 5 mm; section gap, 2.5 mm; 1 signal. Transverse DW images were acquired using a single-shot T2-weighted echo planar spin–echo sequence before contrast-enhanced T1-weighted imaging. We calculated ADC values according to the formula $ADC = -[\ln(Sb/S0)]/b$, where Sb is the signal intensity (SI) of the region of interest (ROI) obtained through three orthogonally oriented DW images, S0 the SI of the ROI acquired through reference T2-weighted images, and b is the gradient b factor with a value of 1000 s/mm^2. ADC maps were calculated on a pixel-by-pixel basis using software integral to the MR unit. The ADC was measured by manually placing ROI in tumor regions on the ADC map at the site of enhanced lesions on contrast-enhanced T1-weighted MRI. Cystic components were differentiated as areas of hyperintensity on T2-weighted- and hypointensity on FLAIR MRI scans. Necrotic components were differentiated on contrast-enhanced T1-weighted images as the interior of enhanced lesions. Hemorrhagic lesions were identified on unenhanced T1-weighted MRI as areas of hyperintensity and on unenhanced T2-weighted MRI as areas of hypointensity. We compared the ADC maps and other MR images, being careful to manually place the ROI only in the solid tumor components. Based on 6–10 ROI ranging in size from 40 to 60 mm^2 on the ADC maps, we obtained ADC_{mean}, ADC_{MIN}, and ADC_{MAX}, respectively. We performed DWI using the following parameters: before July 2003: 1600/107; diffusion gradient encoding in 3 (x, y, z) orthogonal directions; b values of 250, 500, 750, and 1000 s/mm^2; FOV, 24 cm × 24 cm; matrix size, 128 × 128; section thickness, 7.5 mm; section gap, 0 mm; one signal. After July 2003 the parameters were: 5000/107; diffusion gradient encoding in 3 (x, y, z) orthogonal directions; b values of 1000 s/mm^2; FOV, 24 cm × 24 cm; matrix size, 128 × 128; section thickness, 7.5 mm; section gap, 0 mm; 1 signal.

5. Statistical analyses

Survival was measured from the time of operation to the time of death or last follow-up (range, 3.6–54.4 months; median, 16.6 months). Of the 33 patients, 6 were alive at the time of the latest follow-up. We used the median of ADC_{mean}, ADC_{MIN}, and ADC_{MAX} as the cutoff value. We also applied a categorization cutoff of 1.0×10^{-3} mm^2/s because earlier studies used this value [4,10]. We analyzed the relationship between patient survival and prognostic factors determined from clinical and MRI data. Prognostic factors included the patient age, gender, duration of symptoms, Karnofsky performance scale (KPS) score, extent of surgery (biopsy, partial, subtotal or total resection) and ADC ($>1.0 \times 10^{-3}$ vs. $\leq 1.0 \times 10^{-3}$ mm^2/s). Survival curves were calculated with the Kaplan–Meier method, the log-rank test was used to analyze overall differences in the survival curves. The influence of prognostic factors was adjusted using multiple regression analysis with the Cox proportional hazards model. We applied the likelihood ratio test to make comparisons among ADC_{mean}, ADC_{MIN}, and ADC_{MAX} as prognostic factors. All statistical analyses were performed using computer software (StatView version 5.0; SAS

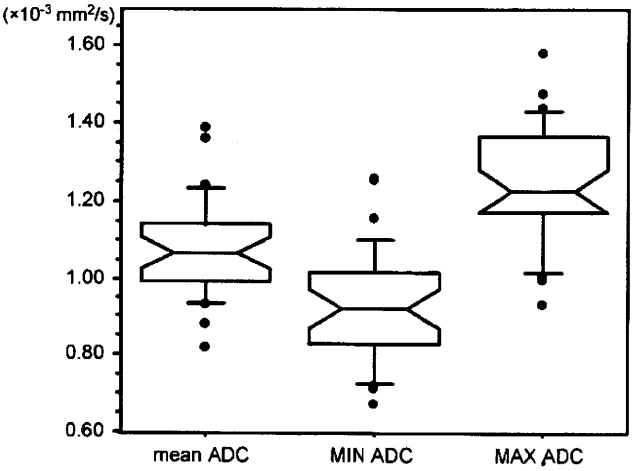

Fig. 1. Parallel boxplots showing the distribution of mean, minimum and maximum ADC values for all glioblastoma patients.

Table 1
Univariate analysis of specific prognostic factors.

Prognostic factor	No. of patients (n = 33)	Overall survival[a]	P-value[†]
Age (year)			
≤49	8	37.5%	NA
≥50	25	40.0%	
Sex			
Male	24	41.7%	NA
Female	9	33.3%	
Symptom duration (month)			
≤3	25	36.0%	NA
>3	8	50.0%	
Karnofsky performance score			
≥80	23	47.8%	<0.05
≤70	10	20.0%	
Extent of surgery			
Total	7	71.4%	<0.01
Not total (subtotal, partial and biopsy)	26	30.8%	
Apparent diffusion coefficient			
≤1.00 ($\times 10^{-3}$ mm^2/s)	23	30.4%	<0.05
>1.00 ($\times 10^{-3}$ mm^2/s)	10	60.0%	

[a] Data are 1.5-year overall survival rates, expressed as percentages. The 1.5-year overall survival rate for all 33 patients was 39.4% (13 patients).
[†] P-values calculated with the log-rank test. NA, not applicable; hazard ratio not calculated when $P \geq 0.05$.

Institute, Cary, NC). For all statistical tests, $P < 0.05$ was adopted as the significance level.

6. Results

6.1. Patient characteristics and imaging

The patients ranged in age from 10 to 76 years (mean ± standard deviation (S.D.): 57.3 ± 16.3; median 62). The KPS scores were 30 and 50 in 1 patient each, 60 and 70 in 4 each, 80 in 11, 90 in 9, and 100 in 3 patients. Surgery consisted of biopsy (n = 6), partial- (n = 12), subtotal- (n = 8), and total (n = 7) tumor removal. The ADC_{mean} of all tumors ranged from 0.716×10^{-3} to 1.389×10^{-3} mm^2/s (mean ± S.D. $1.070 \pm 0.141 \times 10^{-3}$ mm^2/s; median 1.066×10^{-3} mm^2/s). The ADC_{MIN} ranged from 0.676×10^{-3} to 1.260×10^{-3} mm^2/s (mean ± S.D. $0.934 \pm 0.144 \times 10^{-3}$ mm^2/s; median, 0.933×10^{-3} mm^2/s). The ADC_{MAX} ranged from 0.935×10^{-3} to 1.585×10^{-3} mm^2/s (mean ± S.D. $1.248 \pm 0.151 \times 10^{-3}$ mm^2/s; median, 1.230×10^{-3} mm^2/s) (Fig. 1). There was no statistical difference under our two imaging conditions.

6.2. Comparison among ADC_{mean}, ADC_{MIN}, and ADC_{MAX} values by likelihood ratio analysis

We initially performed comparisons among ADC_{mean}, ADC_{MIN}, and ADC_{MAX} values to identify the most powerful prognostic factor

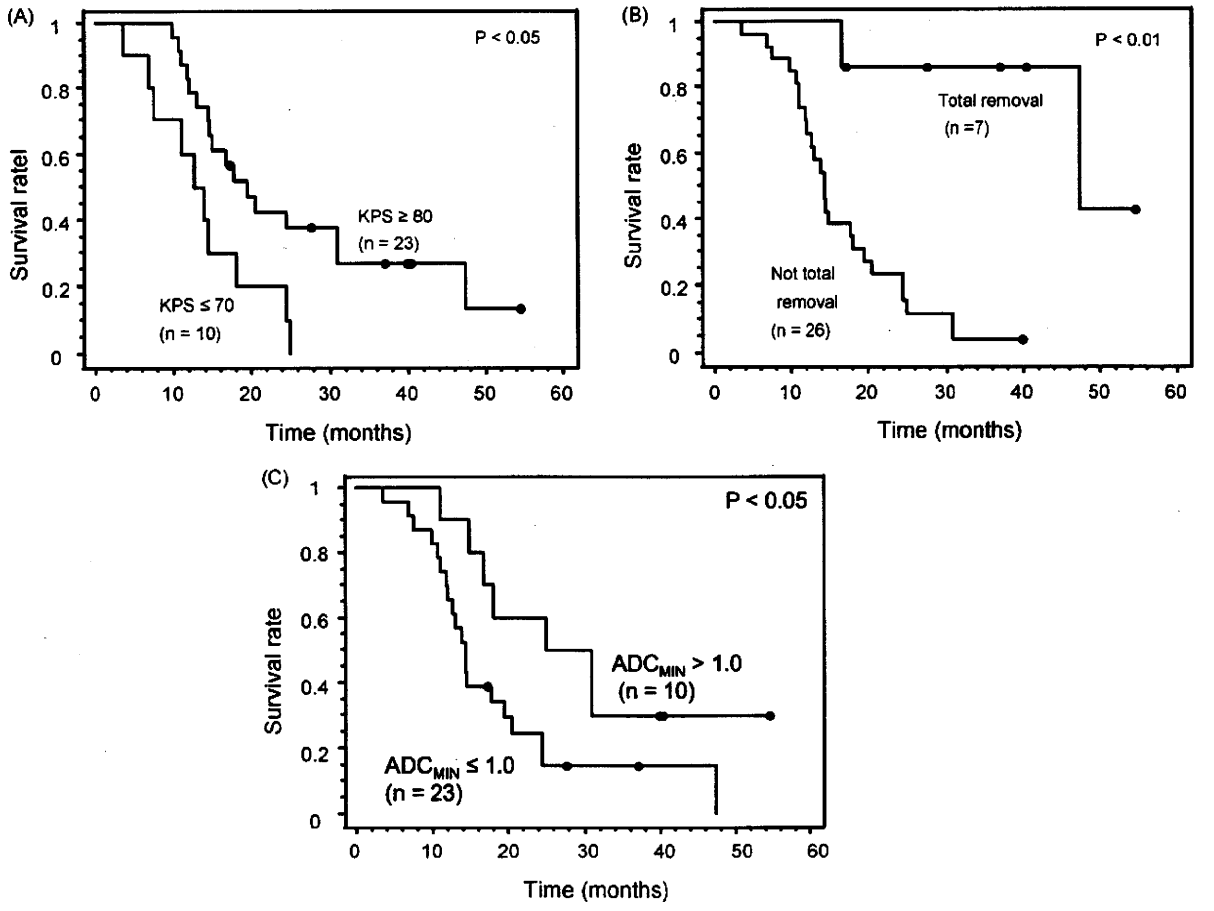

Fig. 2. Kaplan–Meier survival curves for all patients with glioblastoma (including six who remain alive) showing the relationship between minimum ADC and survival time measured from the date of surgery. Comparisons were done between KPS ≥80 and KPS ≤70 (A), between total and not total removal (B), and between ADC > 1.0×10^3 and ≤1.0×10^3 mm^2/s (C).

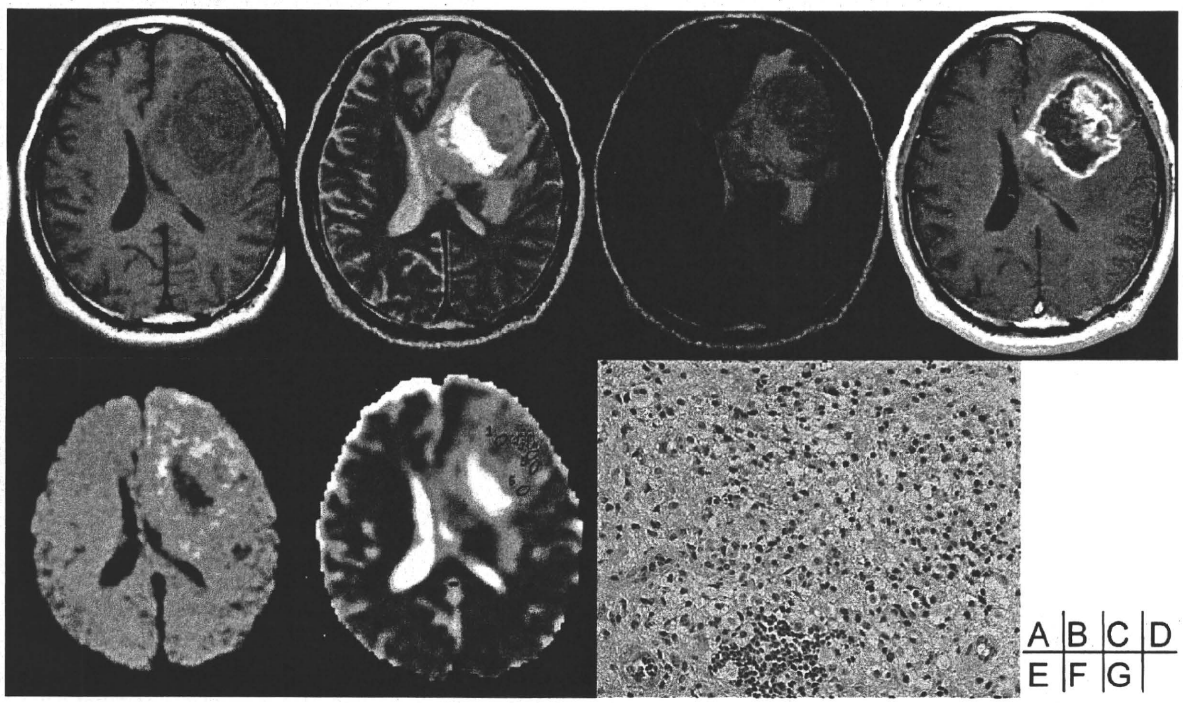

Fig. 3. MR images and pathological results obtained in 60-year-old man with glioblastoma. (A) T1-weighted-, (B) T2-weighted-, (C) FLAIR-, (D) contrast-enhanced T1-weighted images show an enhancing tumor and peritumoral edema. (E) On the DW image, the enhancing area exhibits moderately high signal intensity. (F) On the ADC map, the enhancing area manifests a minimum ADC of 1.05×10^{-3} mm^2/s. The tumor was partially removed and this patient survived for 31 months after the initial MRI study. (G) Histologic specimen (160×) shows the mildly hypercellular nature of the tumor.

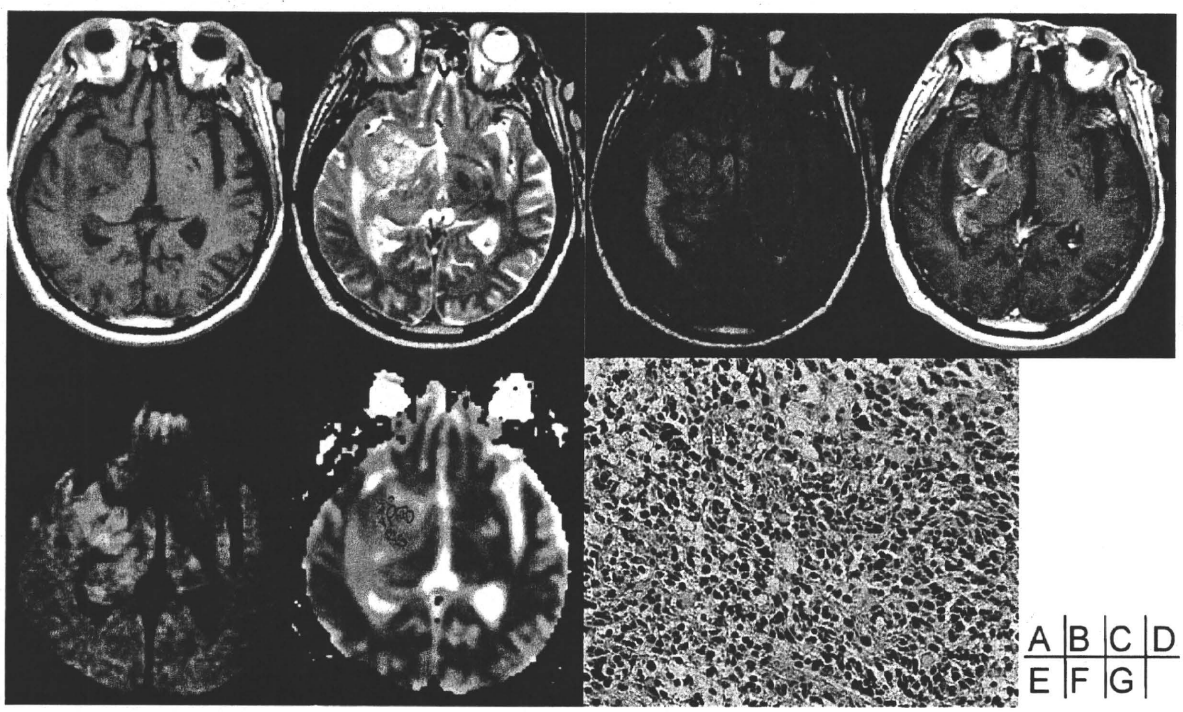

Fig. 4. Transverse MR images obtained in 67-year-old man with glioblastoma. (A) T1-weighted-, (B) T2-weighted-, and (C) FLAIR image. (D) Contrast-enhanced T1-weighted images show an enhancing tumor. (E) On the DW image, the enhancing area exhibits high signal intensity. (F) On the ADC map, the enhancing area has a minimum ADC of 0.902×10^{-3} mm^2/s. The tumor was subtotally removed and this patient died 13 months after the initial MRI study. (G) Histologic specimen (160×) shows marked hypercellularity of the tumor.

Table 2
Multivariate analysis of specific prognostic factors.

Prognostic factor	Hazard ratio	*P*-value
Age (≥50)	NA	NA
Gender	NA	NA
Symptom duration (≤3.0 months)	NA	NA
KPS score (≤70)	NA	NA
Extent of resection (not total)	19.187	<0.01
Minimum ADC ($\leq 1.0 \times 10^{-3}$ mm²/s)	3.915	<0.05

NA, not applicable; hazard ratio not calculated when $P \geq 0.05$.

according to likelihood ratio analysis (Fig. 1). We analyzed the ADC with surgical results because "total removal" was the most sensitive prognostic factor according to our analyses. The results of likelihood ratio analysis were ADC_{mean}, $\chi^2 = 16.291$ ($P = 0.003$); ADC_{MIN}, $\chi^2 = 19.739$ ($P < 0.0001$), and ADC_{MAX}, $\chi^2 = 13.633$ ($P = 0.0011$). Based on these findings we used ADC_{MIN} in further analyses.

6.3. Univariate analyses of prognostic factors

Univariate analysis (Table 1) revealed that the significant factors in overall survival were the KPS score ($P < 0.05$) (Fig. 2A), total surgical removal ($P < 0.01$; Fig. 2B), and ADC_{MIN} ($P < 0.05$; Figs. 2C, 3, and 4). Other factors were not associated with overall survival. Among our 33 patients, the ADC_{MIN} value was below 1.0×10^{-3} mm²/s in 23; it was higher in 10 patients. The group-specific survival rate at 1.5 years was 30.4% and 60.0% for patients in the low- and high ADC_{MIN} value groups, respectively ($P < 0.05$; Fig. 2C).

6.4. Multivariate analysis of prognostic factors

We evaluated the prognostic factors for overall survival using multivariate analysis. The results of multiple regression analysis with the Cox proportional hazards model (Table 2) confirmed that incomplete tumor removal was the most important prognostic factor (hazard ratio 19.187; $P < 0.01$). Our results also confirmed that a low ADC_{MIN} value was a statistical prognostic factor (hazard ratio 3.915; $P < 0.05$). No other factors were significantly associated with overall survival.

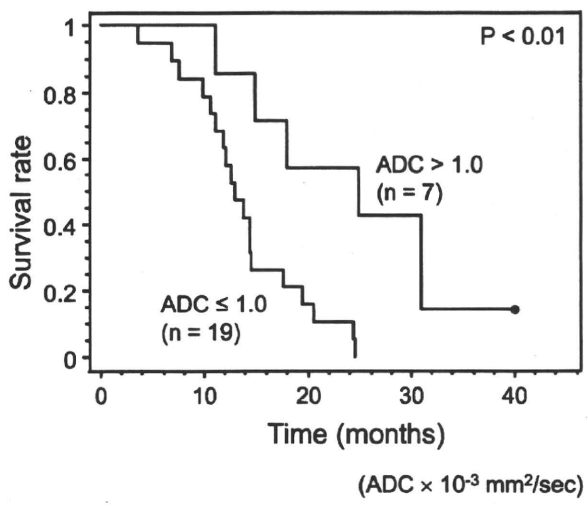

Fig. 5. Kaplan–Meier survival curves based on minimum ADC ($>1.0 \times 10^3$ vs. $\leq 1.0 \times 10^3$ mm²/s) in the subgroup of patients with incomplete tumor removal.

6.5. Survival analysis of patients with incomplete tumor removal

We analyzed the prognostic factors in patients whose tumors could not be totally removed because the biological features of postoperative residual tumors may affect overall survival. Univariate analysis with the log-rank test revealed that the only significant factor for overall survival was the ADC_{MIN} ($P < 0.01$; Fig. 5). Neither the patient age (≥50 year) nor the gender, symptom duration (≤3.0 months), KPS (≥80), subtotal tumor removal, nor surgical achievements were of prognostic value. The results of multivariate analysis with the Cox proportional hazards model also confirmed that low ADC_{MIN} was a powerful prognostic factor (hazard ratio 6.107, $P < 0.01$).

7. Discussion

Our results suggest that the ADC value of tumors, obtained from preoperative MRI scans, represents a prognostic factor in patients with glioblastoma. Although ADC_{mean}, ADC_{MIN}, and ADC_{MAX} were statistically significant prognostic factors in our patients, we confirmed that ADC_{MIN} was the most sensitive predictive factor for the overall survival of these patients. Others [9,10] who assessed the value of the ADC for predicting the prognosis of patients with malignant astrocytic tumors used ADC_{MIN} based on the hypothesis that this value reflects the sites of highest cellularity within heterogeneous tumors and that these sites are of prognostic importance. Studies of ADC that documented an inverse relationship between tumor cellularity and the glioma grade support this hypothesis [6,11,12]. Tissues with high cellularity manifest a low ADC because the mobility of water protons is impeded; cystic and necrotic regions, on the other hand, exhibit a high ADC due to the rapid diffusion of water protons [13,15]. However, no previous reports have compared the value of the ADC_{MIN} versus the ADC_{mean} and ADC_{MAX}. Our likelihood ratio analysis confirmed that ADC_{MIN} was the most sensitive prognostic factor and that it can be used to evaluate overall survival in patients with glioblastoma.

Our observation that a low ADC_{MIN} value is associated with a poor prognosis is consistent with previous studies. According to Higano et al. [9] who studied 37 patients with malignant astrocytic tumors including 22 glioblastomas, the outcomes were more favorable in groups with $ADC_{MIN} > 0.90 \times 10^{-3}$ mm²/s than $\leq 0.90 \times 10^{-3}$ mm²/s [9]. We also applied the cutoff values of 0.90×10^{-3} mm²/s of ADC_{MIN} and of the median 0.933×10^{-3} mm²/s (median of ADC_{MIN}) in our statistical analyses. At both cutoff values, groups with a lower ADC had a statistically poorer prognosis ($P < 0.01$, log-rank test; data not shown) and our results agree with their findings. Higano et al. [9] also found a significant negative correlation between ADC_{MIN} and the Ki-67 labeling index; this may explain why the group with the lower ADC_{MIN} value had a poor prognosis. Murakami et al. [10] studied 79 malignant supratentorial astrocytic tumors, including 50 glioblastomas; patients whose $ADC_{MIN} > 1.00 \times 10^{-3}$ mm²/s had better outcomes than patients with $ADC_{MIN} \leq 1.00 \times 10^{-3}$ mm²/s, a value they considered the most important factor in predicting a poor prognosis (hazard ratio 10.459). Although they did not provide information regarding the degree of surgical resection, our results coincide with theirs. Our findings and those of others confirm that the ADC_{MIN} statistically correlates with the prognosis of glioblastoma patients.

In our series, total, but not subtotal removal or less affected overall survival; an observation that coincides with that of others [16]. Interestingly, we found that the ADC_{MIN} of tumors on pretreatment MR images was a useful predictor of the overall survival of glioblastoma patients whose tumors could not be totally removed. Oh et al. [8] who evaluated the ADC_{mean} of glioblastomas on MR images obtained after surgery but before the start of radiation therapy

found that the survival of patients with a low ADC was substantially shorter. Our findings support their results.

In combination with conventional MRI findings, the ADC can yield additional useful information about physiological changes since the entire tumor can be assessed. Tissue sampling, on the other hand, may not yield information about the entire tumor. Furthermore, if the chosen biopsy site is suboptimal, the glioma grading may be incorrect because these tumors are histologically heterogeneous. Thus, because the ADC helps to identify areas of highest cellularity within a tumor, it is useful for selecting the biopsy target [9].

Our study has some limitations. DWI does not eliminate perfusion effects attributable to tumor vessels or white matter tracts. In addition, ADC changes due to cystic, necrotic, and/or hemorrhagic areas and the influence of artifacts caused by inhomogeneous structures such as the skull base, bone, and sinus air must be considered. To avoid the influence of susceptibility artifacts or ADC changes, we excluded patients with infratentorial tumors or gross hemorrhage from our study. Another limitation was the variability of the calculated ADC. Significant variability in ADC values reportedly reflects the coil systems and imagers used, the instrument vendors, and the field strengths applied for MR re-imaging [17,18]. We set the cut off value of ADC_{MIN} at 1.00×10^{-3} mm^2/s and this value would be affected, therefore, we suggest that the optimal absolute ADC be established by larger studies. The semiquantitative use of the ADC such as its ratio to the contralateral side, may help to eliminate these variabilities. However, as the ADC is inhomogenous in various brain lesions and is affected by aging [19–21], these ADC changes must be considered when the ratio to the contralateral normal-appearing side is to be established. Future studies are necessary to standardize ADC measurement methods. Although our retrospective study showed that ADC_{MIN} is one of the most important prognostic factors, due to the heterogeneous nature of glioblastomas, other variables related to the characteristics of the patients, the tumors, and the treatment strategies must be taken into account.

8. Conclusions

The ADC_{MIN} value of tumors obtained from preoperative MR images is a useful clinical prognostic biomarker for overall survival in patients with glioblastoma. Patients whose tumors have a low minimum ADC ($\leq 1.0 \times 10^{-3}$ mm^2/s) may have a poor prognosis, especially when the tumor cannot be completely resected. Thus, pretreatment DW-MRI and calculating the ADC values may be helpful for planning therapy in patients with glioblastoma.

Conflicts of interest

None.

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Standard Therapy for Glioblastoma— A Review of Where We Are

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Abstract

Glioblastoma is the most common primary malignant brain tumor in adults and is a challenging disease to treat. The current standard therapy includes maximal safe surgical resection, followed by a combination of radiation and chemotherapy with temozolomide. However, recurrence is quite common, so we continue to search for more effective treatments both for initial therapy and at the time of recurrence. This article will review the current standard of care and recent advances in therapy for newly-diagnosed and recurrent glioblastomas, based on the most authoritative guidelines, the National Cancer Institute's comprehensive cancer database Physician Data Query (PDQ®), and the National Comprehensive Cancer Network Clinical Practice Guidelines in Oncology™ for central nervous system cancers (V.1.2010), to elucidate the current position and in what direction we are advancing.

Key words: glioblastoma, standard therapy, chemotherapy, temozolomide, clinical trial

Definition of Standard Therapy

Standard therapy is the treatment that experts agree is appropriate, accepted, and widely used, and is also called best practice and standard of care.[14] Health care providers are obligated to provide patients with standard therapy. Physicians are not allowed to provide patients with non-standard therapy without explaining the reason why standard therapy will not be provided and obtaining informed consent. Every clinical trial should have a convincing scientific basis to indicate that testing the treatment is worthwhile, and the patients should be informed that the test treatment is not a standard therapy, which is requisite from an ethical point of view. The Institutional Review Board (IRB) will examine the protocols, case report forms, and related documents from both scientific and ethical points of view. In randomized phase 3 studies, the control arms are always standard therapies of the diseases.

Standard Therapy for Glioblastoma in Physician Data Query (PDQ®)

PDQ® is the National Cancer Institute's comprehensive cancer database, and is the most authoritative guideline.[15] PDQ® is written in an itemized manner, so needs some commentary and explanations (Table 1).

Table 1 Standard therapy for glioblastoma in PDQ®

Surgery plus radiation therapy for elderly glioblastoma patients
No additional benefit from brachytherapy added to external-beam radiation therapy and carmustine (BCNU)
BCNU-impregnated polymer (Gliadel wafer) implanted during initial surgery
Radiation therapy and concurrent chemotherapy with temozolomide

PDQ®: Physician Data Query.

The first point is the treatment of glioblastoma (GBM) in the elderly population. Since the landmark European Organization for the Research and Treatment of Cancer (EORTC)/National Cancer Institute of Canada (NCIC) study published in 2005, the standard therapy for GBM has been post-operative adjuvant radiotherapy with concomitant and adjuvant temozolomide (TMZ) (so-called Stupp's regimen).[25] However, the patients eligible for this study were aged from 18 to 70 years, so the standard therapy for GBM patients aged over 70 years remains undetermined. Because the frequency of severe adverse events of TMZ is less than 10%, and the pharmacokinetic profile of TMZ is not age-dependent, investigators surmise that Stupp's regimen would be applicable for elderly patients, but this notion has not actually been proven yet.

A randomized phase 3 study comparing postoper-

ative supportive care and postoperative radiation therapy plus supportive care was performed for GBM patients over 70 years old.[10] The median survival time was 29.1 weeks for the 39 patients who received radiation therapy plus supportive care and 16.9 weeks for the 42 patients who received only supportive care. The hazard ratio of death in the radiation therapy arm was 0.47 (95% confidence interval [CI] 0.29–0.76; p = 0.002). This study was discontinued prematurely at the first interim analyses, because the radiotherapy plus supportive care arm was superior to the only supportive care arm with a preset boundary of efficacy. Post-operative radiotherapy resulted in a robust improvement in survival in elderly patients with GBM, and is now the standard therapy for this population. To prove that a full dose of 60 Gy/30 fractions was necessary for elderly GBM patients, a randomized study of patients 60 years and older comparing post-operative radiotherapy of 60 Gy/30 fractions (standard course) and 40 Gy/15 fractions administered over the course of 3 weeks (short course) was performed. Overall survival (OS) was similar for the two groups; 5.1 months for the standard course arm, and 5.6 months for the short course arm (p = 0.57).[22] Although there was concerns about the power of the study, which was discontinued prematurely at the first interim analysis when 100 patients were recruited, the results showed the outcomes of the two arms were statistically equivalent, so the short course of radiotherapy seemed to be the reasonable treatment option for elderly patients with GBM.

Deterioration of cognitive function is a well known adverse effect of radiotherapy, especially in the elderly population. Treatment with only TMZ, without radiotherapy, may be equivalent in OS and would provide better health-related quality of life (QoL), which is a reasonable hypothesis to be tested in elderly GBM patients. Three randomized phase 3 studies for elderly GBM patients are on-going: a three-arms study by the Nordic Clinical Brain Tumor Group assessing the efficacy of short course radiotherapy and only TMZ arms with standard course radiotherapy of 60 Gy; a study by the German Neuro-Oncology Working Group simply testing the efficacy of only TMZ treatment versus the standard course of radiotherapy, and the study by NCIC and EORTC aiming at the assessment of the additive effect of TMZ to short course radiotherapy. Three institutes from Japan, Kitano Hospital, Hiroshima University, and the International Medical Center, Saitama Medical University are members of the international study group for the NCIC/EORTC study, CE.6. Conclusions from these studies will decide if only TMZ is equivalent to radiotherapy, and provides better QoL, and if concomitant and adjuvant TMZ with short course radiotherapy would be valuable for elderly GBM patients.

The second point in PDQ® is evaluation of the efficacy of brachytherapy for GBM. A randomized cooperative study showed no additional benefit from brachytherapy added to external-beam radiation therapy and carmustine (BCNU) (NIH Trial 87-01).[23] Interstitial brachytherapy is one of the techniques to deliver high doses of irradiation to the tumor beds. Stereotactic radiotherapy is another high-dose local radiotherapy technique, which also failed to show survival advantage compared to external beam irradiation in a phase 2 study by the Radiation Therapy Oncology Group (RTOG 0023).[4] Because of the highly invasive nature of GBM, however high the irradiated dose is, the effect of radiotherapy would be limited as the irradiated field is restricted to the enhanced lesion.

The third point in PDQ® is the evaluation of BCNU-impregnated polymer (Gliadel® wafer) implanted during surgeries. A multicenter randomized double-blinded controlled trial with 240 patients with high-grade glioma including 207 GBM and 21 anaplastic glioma reported significantly longer OS for patients who had Gliadel® wafer placed intraoperatively (13.8 months for Gliadel® wafers vs. 11.6 months for placebo; HR 0.73, 95% CI 0.56–0.95; p = 0.0018).[32] However, a subanalysis of 207 GBM patients could not show significantly longer survival with Gliadel® wafer (13.1 months in the Gliadel®-treated group and 11.4 months in the placebo-treated group, p = 0.08). The spacial and temporal distribution of BCNU released from the polymer was calculated by a mathematical simulation model.[31] The penetration depth of BCNU from a polymer was estimated to be 0.5 cm. The penetration depth was defined as the average distance measured from the surface of a polymer at which the drug concentration is 1% compared to that of the polymer surface. The distance of penetration is short because BCNU has a high transvascular permeability and, therefore, is very easily absorbed into the systemic circulation. BCNU molecules, being lipid-soluble and very permeable, enter the bloodstream before they can travel far. Together with the short half life of BCNU (1.5 hours), the short distance of penetration would limit the efficacy of the therapy.

The last point in PDQ® is radiation therapy and concurrent chemotherapy. A randomized study performed by EORTC and NCIC was a landmark of GBM treatment. Radiation therapy plus TMZ followed by 6 months of adjuvant TMZ in patients with newly diagnosed GBM demonstrated a statistically significant survival advantage over simple

radiotherapy.[25] The median OS was 14.6 months with radiotherapy plus TMZ and 12.1 months with only radiotherapy (HR 0.63, 95% CI 0.52 to 0.75; $p < 0.001$). The treatment is relatively safe and well tolerated. The combined treatment regimen consists of concomitant and adjuvant TMZ with radiotherapy. While the dose of TMZ for adjuvant phase is 150–200 mg/m^2 for 5 days every 4 weeks, TMZ of 75 mg/m^2 is administered daily during the concomitant phase with radiotherapy. The rationale for the small dose and continuous administration of TMZ with radiotherapy are radiosensitization induced by TMZ and chemosensitization by O^6-methylguanine-deoxyribonucleic acid-methyltransferase (MGMT) depletion induced by TMZ. Radiosensitivity enhancement of tumor cells by TMZ was shown in vitro and in vivo. The enhancing effect involves inhibition of deoxyribonucleic acid (DNA) repair leading to increased mitotic catastrophe.[11] MGMT depletion induced by protracted TMZ schedules was shown in the peripheral blood mononuclear cells. MGMT decreased significantly after 7, 14, and 21 days of treatment with low-dose and protracted TMZ administration, thus resulting in autosensitization to TMZ.[27] MGMT is the key enzyme to determine the sensitivity for TMZ. In the clinical setting, promoter methylation of MGMT is one of the factors contributing to better outcomes for GBM patients treated by TMZ.[9]

In the combined treatment arm with TMZ and radiotherapy, patients with GBM with methylated MGMT promoters showed significantly better OS than those with unmethylated MGMT promoters.[9] In the only initial radiotherapy arm, patients with methylated MGMT promoter also showed improvement in OS. This was expected because more than 70% of the patients in the radiotherapy arm received chemotherapy, most likely with an alkylating agent at progression, and 60% of these patients received TMZ at progression. When progression-free survival (PFS) was analyzed, so eliminating second-line therapies as a confounding factor, PFS was only prolonged in patients with methylated MGMT promoters who were treated with radiotherapy plus TMZ. However, a close look at the Kaplan Meyer survival curves of patients in the radiotherapy arm with methylated or unmethylated MGMT promoters finds that the PFS of patients with methylated MGMT promoter showed slightly longer survival than that of patients with unmethylated MGMT promoter. In another set of patients who received radiotherapy without any alkylating agents, MGMT promoter methylation was predictive of response to radiotherapy and good prognosis.[21] Although the mechanism of radiation resistance by MGMT activation is not clear, methylated MGMT promoter may be a surrogate marker of as yet unidentified processes, other than TMZ resistance, that contribute to the overall aggressive biology of GBM. Furthermore, cancer-associated DNA methylation may affect the expression of many CpG island-associated genes including MGMT, either modifying sensitivity to radiation or resulting in less aggressive phenotype.[18]

Is ACNU Only a Memory?

In 2000, the Japan Clinical Oncology Group (JCOG) brain tumor study group was discussing how to initiate clinical studies for brain tumors in Japan. One of the foci of the discussion was the standard therapy for GBM at that time. 1-(4-Amino-2-methyl-5-pirimidinyl)-methyl-3-(2-chloroethyl)-3-nitrosourea hydrochloride (ACNU) was widely used based on a small randomized study in Japan in the 1980s.[26] The response rate was better in the combined treatment arm with ACNU and radiotherapy (47.5% in the combined treatment arm, 13.5% in the simple radiotherapy arm), but the OS was not significantly different (median OS was 14 months and 12 months, respectively). Although the combined therapy with ACNU and radiotherapy was promising, a randomized phase 3 study had not been performed. Nevertheless, without a phase 3 study, the combination of ACNU and radiotherapy was adapted as the standard therapy for malignant gliomas in Japan. The brain tumor study group of JCOG considered this as the community standard of GBM therapy that was made the starting point.

The JCOG 0305 study was a randomized phase 2 study comparing two combined-treatment protocols, ACNU with radiotherapy and procarbazine (PCZ) plus ACNU with radiotherapy. In the PCZ plus ACNU arm, PCZ was administered before ACNU aiming to deplete MGMT and to enhance the chemosensitivity to ACNU.[24] When the phase 2 study cleared a preset boundary of efficacy, the phase 3 study would begin, which was the original design. However, an interim analysis revealed there was no survival advantage of the test arm. This study was discontinued and did not proceed to phase 3. The control arm, ACNU with radiotherapy, achieved a median OS of 16.6 months for GBM, which should have been the basic data for the clinical trials for GBM thereafter in Japan. In 2005, the results of the combination of TMZ and radiotherapy were published as previously mentioned, and the combination of TMZ and radiotherapy became the standard therapy of GBM worldwide. No randomized comparison of ACNU and TMZ has been considered so far, based on the following reasoning.

The HR for death in the radiotherapy plus TMZ arm was 0.63 (95% CI 0.52–0.75; $p < 0.001$).[25] Meta-analysis evaluating the effectiveness of nitrosoureas (mainly BCNU) found the HR was 0.85 (95% CI 0.78–0.91; $p < 0.0001$). Considering the 95% CI, nitrosoureas were not thought to be as good as TMZ. The OS and PFS for GBM patients treated with concomitant and adjuvant TMZ with radiotherapy were 14.6 months and 6.9 months, respectively, whereas those with ACNU and radiotherapy were 16.6 months and 5.1 months based on the JCOG study, respectively. The better OS with ACNU and radiotherapy in Japan was possibly related to salvage therapies including repeat surgeries and stereotactic radiosurgeries, and to elaborate supportive care. The better OS with ACNU treatment was not due to better tumor control by ACNU because the PFSs were similar. Another retrospective report comparing BCNU and TMZ also showed that PFS was not significantly different between the two groups.[29] Severe adverse events (CTCAE grade 3 or higher) are more frequent in ACNU-treated patients than in TMZ-treated patients; leucopenia in 39% and 3% of the patients, respectively.

As the next step, the JCOG brain tumor study group has just started a randomized phase 2 study comparing a combination therapy of interferon-β plus TMZ with radiotherapy and a combination therapy of TMZ with radiotherapy, considering the latter as the standard therapy for GBM (JCOG 0911). This strategy is based on data showing the sensitizing effect of IFN-β for TMZ was possibly due to attenuation of MGMT expression via induction of the protein p53.[16]

When GBM Recurs

The National Comprehensive Cancer Network (NCCN) Clinical Practice Guidelines in Oncology™ is available on-line (http://www.nccn.org). The guideline for central nervous system cancers (V.1.2010) states surgical resection should be considered first if recurrent or progressive tumors are resectable. Systemic chemotherapies are indicated if allowed by the performance status of the patients.

Bevacizumab with or without chemotherapy is the first in the list of possible second line chemotherapies for GBM (Table 2). As we learn more about the biology of GBM and its aberrant signaling pathways, the neuro-oncology community has begun to investigate the role of molecular targeted therapies. The angiogenesis pathways and their associated antiangiogenic agents are the most promising topic recently. Bevacizumab, a humanized monoclonal antibody that targets vascular endothelial growth factor (VEGF), was first approved in combination with chemotherapy for colorectal, lung, and breast cancers. Despite initial reluctance to evaluate bevacizumab in patients with brain tumors because of concerns with intracranial hemorrhage, the combination of bevacizumab and irinotecan was studied in a single-arm phase 2 study for recurrent GBM.[30] The response rate was 57%, and PFS at 6 months was 46%. These results compared quite favorably with historical data of response rate of 8% and PFS at 6 months of 21% by TMZ for recurrent GBM.[34] To clarify the contribution of irinotecan, a large phase 2 study randomized 167 patients with recurrent GBM to either single agent bevacizumab or bevacizumab plus irinotecan. The response rates were 28% in the single treatment arm with bevacizumab and 38% in the combination arm of bevacizumab plus irinotecan, and the PFS at 6 months was 43% and 50%, respectively.[8] Curiously enough, the randomized design of the trial was not designed to compare outcomes in the two treatment groups, but to evaluate their superiority to the historical results of salvage chemotherapies, 15% of PFS at 6 months, without bias in treatment assignment. Bevacizumab is usually well tolerated, with the most common adverse effects being hypertension and minor bleeding, such as epistaxis. Intracranial hemorrhage occurred in less than 4% of patients and was severe in only approximately 1% of patients.

Individual infiltrative tumor cells tend to grow along preestablished normal cerebral vasculature, so there is no need for tumor-associated angiogenesis from the tumor cells in the central core. Indeed, there is at least a theoretical concern that inhibiting malignant glioma angiogenesis may have little effect on the infiltrative component of the disease and so little impact on the overall survival of the patient. Furthermore, recent laboratory evidence suggests that inhibition of VEGF may actually increase the invasive nature of tumor cells.[19] There seems to be a proinvasive adaptation to anti-angiogenic therapy, as suggested by magnetic resonance imaging in a subset of GBM patients who developed multifocal

Table 2 The second-line chemotherapies for recurrent glioblastoma in NCCN guideline

Bevacizumab with/without chemotherapy
Temozolomide
Nitrosourea or PCV
Cyclophsphamide
Platinum-based regimens

NCCN: National Comprehensive Cancer Network; PCV: procarbazine, lomustine, and vincristine.

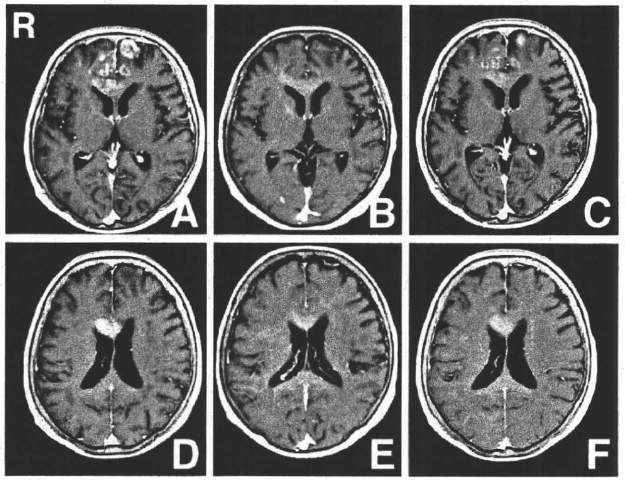

Fig. 1 Initial (A, D) and two follow-up magnetic resonance images 3 days (B, E) and 10 days (C, F) after bevacizumab treatment of a 68-year-old female patient with glioblastoma showing the heterogeneously enhanced tumor regressed 3 days after bevacizumab treatment, but reappeared 7 days later.

recurrence of tumors during the course of therapy with bevacizumab.[6,13,17] The infiltrative tumor cells are most often responsible for clinical relapse and ultimately the death of patients with gliomas. Early results from phase 2 trials showed that incorporation of bevacizumab into the standard initial treatment for newly diagnosed GBM increased median PFS, but prolongation of OS is still unclear. Two large phase 3 trials for newly diagnosed GBM are currently randomizing patients to standard radiotherapy and TMZ with or without bevacizumab.

A unique advantage of bevacizumab is the ability to decrease peritumoral edema. Patients treated with bevacizumab often have decreased corticosteroid dependence secondary to neutralization of VEGF, a known vascular permeability factor. Vascular permeability is decreased in and around the tumor, so decreasing both cerebral edema and the uptake of gadolinium within the tumor. An illustrative case (Fig. 1) showed marked decrease of enhancement on MRI after three days of bevacizumab administration. The decrease in enhancement was not due to tumor shrinkage as the enhancement was regained 7 days later (Fig. 1). As such, the remarkable radiographic response rates and PFS by bevacizumab should be interpreted cautiously.

A couple of successful regimens suggested low dose and continuous TMZ administration as a rechallenge was effective for recurrent disease.[20,33] Due to its usage as the first-line treatment of GBM, TMZ has been no longer considered by many investigators to be a reasonable choice for patients with recurrent GBM. However, alternative schedules of TMZ addressing different pathophysiological mechanisms could be effective even after progression during standard TMZ regimens.[33] There are several rationales supporting TMZ rechallenge. Firstly, there may be a benefit from alternative modes of action, such as antiangiogenic properties of a metronomic regimen. Secondly, as MGMT is inactivated after each reaction of removal of methyl bases (suicide enzyme), exposure to continuous and low-dose TMZ depletes MGMT activities. Thirdly, the schedule of temozolomide permits a greater drug exposure than the conventional schedule of 5 days every 28 days, with comparable or even lower toxicities.

A "one week on/one week off" scheme (150 mg/m^2 at days 1–7 and days 15–21, in a 28-day cycle) has been associated with considerable efficacy and was tolerated by patients. Another alternative is an intensified three out of four weeks approach (75–100 mg/m^2 at days 1–21, in a 28 day cycle). This regimen may yield similar results with respect to efficacy, but a higher rate of toxicity, specifically lymphopenia and infection, has been reported. Another regimen is a metronomic administration of TMZ, 20 mg daily.

The third optional treatment for recurrent GBM is regimens containing nitrosoureas, such as procarbazine, lomustine, and vincristine (PCV) chemotherapy. A randomized trial by the Medical Research Council Brain Tumour Working Party showed no benefit to PCV chemotherapy for newly diagnosed GBM.[12] However, PCV has certain activity, especially for malignant glioma with oligodendroglial component.[3,28] Therefore, this regimen may be important for recurrent GBM.

Cyclophosphamide is among the list of possible chemotherapies for recurrent GBM. However, the reference cited in the NCCN guideline is a report of recurrent anaplastic astrocytomas, and the efficacy of cyclophosphamide for recurrent GBM is not known.[5] Lastly, platinum-based regimens are reported to show modest activities for recurrent GBM.[1,2,7]

On-going Phase 3 Trials for Future Revision of the Standard Therapy

Table 3 shows four on-going randomized phase 3 confirmatory trials for GBM. Another schedule of TMZ administration (RTOG 0525), additive effect of bevacizumab (RTOG 0825, AVAglio), and also possible additive effect of cilengitide, integrin $\alpha v\beta 5$ inhi-