TABLE 1: Characteristics of patients enrolled into study

Demographics & Clinical Characteristics	Value*		
	Initially Enrolled Patients (n = 27)	Study Cohort	
		Total (n = 22)	Newly Diagnosed GBM (n = 13)
age in yrs			
mean ± SD	47.1 ± 13.5	48.1 ± 13.5	46.0 ± 14.1
median (range)	50.0 (24–69)	50.5 (24–69)	49.0 (24–69)
sex			
male	13 (48.1)	11 (50.0)	6 (46.2)
female	14 (51.9)	11 (50.0)	7 (53.8)
histopathological type of tumor†			
GBM	13 (48.1)	13 (59.1)	13 (100.0)
gliosarcoma	1 (3.7)	1 (4.5)	0 (0)
anaplastic astrocytoma	3 (11.1)	3 (13.6)	0 (0)
anaplastic oligoastrocytoma	2 (7.4)	2 (9.1)	0 (0)
anaplastic oligodendroglioma	2 (7.4)	2 (9.1)	0 (0)
pilocytic astrocytoma w/ anaplastic features	1 (3.7)	1 (4.5)	0 (0)
oligodendroglioma	2 (7.4)	0 (0)	0 (0)
central review not performed‡	3 (11.1)	0 (0)	0 (0)
WHO grade†			
IV	14 (51.9)	14 (63.6)	13 (100.0)
III	8 (29.6)	8 (36.4)	0 (0)
II	2 (7.4)	0 (0)	0 (0)
central review not performed‡	3 (11.1)	0 (0)	0 (0)
tumor status			
newly diagnosed	26 (96.3)	21 (95.5)	13 (100.0)
recurrent	1 (3.7)	1 (4.5)	0 (0)
tumor location			
frontal lobe	16 (59.3)	13 (59.1)	7 (53.8)
temporal lobe	5 (18.5)	3 (13.6)	2 (15.4)
parietal lobe	4 (14.8)	4 (18.2)	3 (23.1)
occipital lobe	2 (7.4)	2 (9.1)	1 (7.7)
tumor side			
rt	13 (48.1)	12 (54.5)	8 (61.5)
lt	14 (51.9)	10 (45.5)	5 (38.5)
tumor functional grade			
located in eloquent area	13 (48.1)	12 (54.5)	7 (53.8)
adjacent to eloquent area	6 (22.2)	4 (18.2)	2 (15.4)
located in noneloquent area	8 (29.6)	6 (27.3)	4 (30.8)
PS before treatment§			
0	14 (51.9)	10 (45.5)	3 (23.1)
1	10 (37.0)	9 (40.9)	8 (61.5)
2	0 (0)	0 (0)	0 (0)
3	3 (11.1)	3 (13.6)	2 (15.4)
extent of tumor resection			
total	9 (33.3)	8 (36.4)	5 (38.5)
subtotal (>90% of lesion vol)	13 (48.1)	11 (50.0)	8 (61.5)
partial	5 (18.5)	3 (13.6)	0 (0)

* Unless otherwise stated, values represent cases (%).
† According to central review based on WHO criteria.
‡ These patients did not receive laser irradiation during surgery due to results of the intraoperative histopathological investigation of the resected tissue on the frozen sections and exclusion of the diagnosis of primary malignant parenchymal brain tumor.
§ According to the Eastern Cooperative Oncology Group Performance Status Scale.

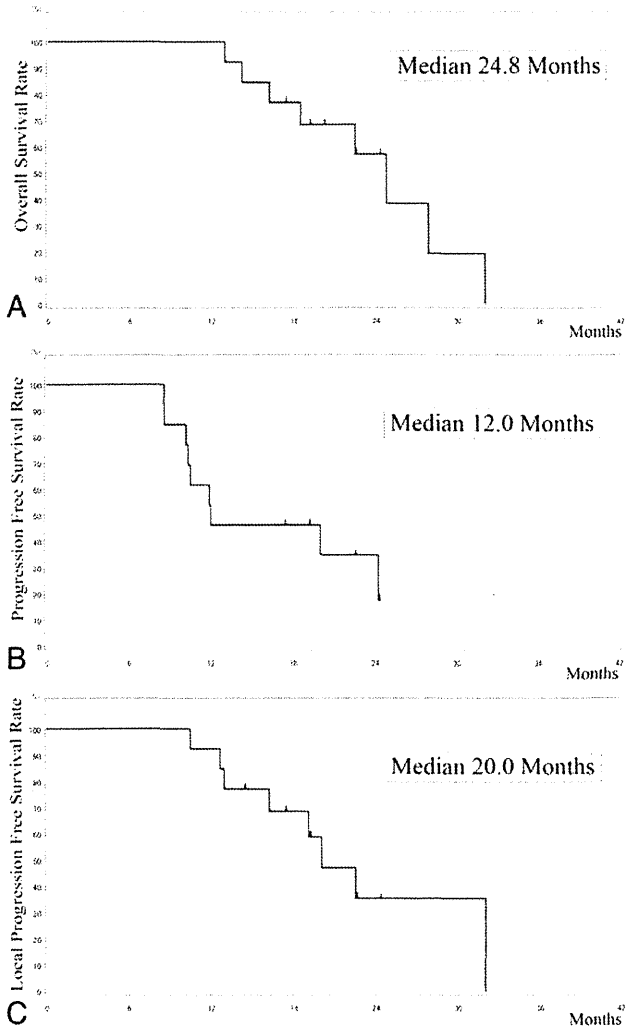

FIG. 1. Kaplan-Meier curves for OS (**A**), PFS (**B**), and local PFS (**C**) in the subgroup of patients with newly diagnosed GBM included in the study cohort. Censored observations are marked.

sidered as side effects after administration of talaporfin sodium. It included rash (2 cases), blister (1 case), and erythema (1 case).

Photosensitivity test results were relatively mild and most patients had a score of 1 (barely perceptible erythema) or 2 (distinct erythema); no patient had a score of 3 (marked erythema or edema). These reactions completely disappeared within 4, 8, and 15 days after administration of talaporfin sodium in 55.6%, 77.8%, and 100% of patients, respectively (Table 3).

Discussion

Management of primary malignant parenchymal brain tumors represents a significant challenge. According to the latest edition of the Japan Brain Tumor Registry, 1-, 2-, and 3-year survival rates of patients with high-grade gliomas constitute 64%, 37%, and 28%, respectively.[5] The poor survival rates are mainly due to an inability to perform complete removal of the neoplasm due to its infiltrative growth into functionally important neuronal structures, as well as the limited effectiveness of the postoperative radiotherapy and chemotherapy. Therefore, finding additional effective and safe treatment options in such cases is required.

As a highly selective treatment with minimal injury to the adjacent normal structures, PDT has demonstrated promising potential for management of the various cancers and nonneoplastic disorders, such as age-related macular degeneration, local infection, dermatological diseases, arteriosclerosis, and rheumatoid arthritis.[7] However, despite a large amount of basic and clinical research conducted during several decades and directed on testing of the various photosensitizers, light sources, irradiation types, and treatment regimens, PDT still was not approved to be used as a standard treatment for malignant brain tumors.[2,10] During the last decade there was considerable interest in the use of 5-aminolevulinic acid (5-ALA) in the surgical management of gliomas. Nevertheless, while its application for photodynamic diagnosis and fluorescence-guided resection was associated with a significant impact on effectiveness of tissue sampling, tumor resection rates, and clinical outcomes,[4,17] the attempts to use this photosensitizer for PDT were not so impressive.[2] These unimpressive results might be particularly caused by insufficient incorporation of the drug in the neoplastic cells, especially in necrotic regions and at the periphery of the neoplasm.[2]

In the present study PDT was based on administration of the relatively novel second-generation photosensitizer talaporfin sodium. This water-soluble compound is derived from plant chlorophyll. In the living body it binds to albumin and does not pass the blood-brain barrier. In neoplastic cells it is primarily distributed in the lysosomes.[14] Compared with conventional photosensitizers, talaporfin sodium is activated by light with longer wavelengths; therefore, its light absorption is not affected by hemoglobin and penetrates deeper.[13] Additionally, talaporfin sodium more selectively accumulates in glioma tissue, is rapidly eliminated from the normal tissues, and is less likely to cause adverse reactions.[14] It was demonstrated that PDT based on administration of talaporfin sodium with subsequent irradiation using a 664-nm laser led to necrosis and apoptosis of cultured human glioblastoma cells[13] and experimental tumors[14] in a dose- and time-dependent fashion. The adverse effects on the peritumoral brain were limited to mild temporary edema, and no damage to neurons or the myelin sheath was observed.[14] A pilot clinical study on 14 adult patients with unresectable malignant gliomas showed a median PFS of 23 months in newly diagnosed neoplasms.[1] In concordance, in our present prospective investigation, which included 21 patients with newly diagnosed high-grade gliomas treated according to strict research protocol, the median local PFS constituted 22.5 months.

The most impressive results of our study were obtained in patients with a newly diagnosed GBM. In this subgroup, the 12-month OS and 6-month PFS rates were 100%, and the median OS and median PFS were 24.8 and 12.0 months, respectively. These rates compare favorably

TABLE 2: Frequency of adverse events and side effects by grade*

System Organ Class†	No. of Patients (%)					
	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5	Total (n = 27)
adverse events						
investigations	3 (11.1)	12 (44.4)	10 (37.0)	2 (7.4)	0 (0.0)	27 (100.0)
gastrointestinal disorders	5 (18.5)	16 (59.3)	0 (0.0)	0 (0.0)	0 (0.0)	21 (77.8)
general disorders & administration site conditions	15 (55.6)	6 (22.2)	0 (0.0)	0 (0.0)	0 (0.0)	21 (77.8)
nervous system disorders	1 (3.7)	17 (63.0)	2 (7.4)	0 (0.0)	0 (0.0)	20 (74.1)
skin & subcutaneous tissue disorders	10 (37.0)	8 (29.6)	0 (0.0)	0 (0.0)	0 (0.0)	18 (66.7)
injury, poisoning, & procedural complications	9 (33.3)	6 (22.2)	0 (0.0)	0 (0.0)	0 (0.0)	15 (55.6)
eye disorders	7 (25.9)	1 (3.7)	1 (3.7)	0 (0.0)	0 (0.0)	9 (33.3)
infections & infestations	1 (3.7)	3 (11.1)	2 (7.4)	0 (0.0)	0 (0.0)	6 (22.2)
renal & urinary disorders	3 (11.1)	2 (7.4)	0 (0.0)	0 (0.0)	0 (0.0)	5 (18.5)
psychiatric disorders	4 (14.8)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	4 (14.8)
respiratory, thoracic, & mediastinal disorders	4 (14.8)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	4 (14.8)
vascular disorders	0 (0.0)	0 (0.0)	4 (14.8)	0 (0.0)	0 (0.0)	4 (14.8)
musculoskeletal & connective tissue disorders	1 (3.7)	2 (7.4)	0 (0.0)	0 (0.0)	0 (0.0)	3 (11.1)
blood & lymphatic system disorders	1 (3.7)	1 (3.7)	0 (0.0)	0 (0.0)	0 (0.0)	2 (7.4)
metabolism & nutrition disorders	0 (0.0)	0 (0.0)	2 (7.4)	0 (0.0)	0 (0.0)	2 (7.4)
cardiac disorders	1 (3.7)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (3.7)
ear & labyrinth disorders	1 (3.7)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (3.7)
side effects						
investigations	7 (25.9)	6 (22.2)	5 (18.5)	0 (0.0)	0 (0.0)	18 (66.7)
skin & subcutaneous tissue disorders	1 (3.7)	1 (3.7)	0 (0.0)	0 (0.0)	0 (0.0)	2 (7.4)

* According to the Cancer Therapy Evaluation Program.[3]
† According to the Medical Dictionary for Regulatory Activities version 14.1 (http://www.meddra.org).

with contemporary results obtained in such tumors with standard treatment. In a global Phase III randomized controlled study on radiotherapy with concomitant and adjuvant temozolomide for GBM, Stupp et al.[18] demonstrated a 12-month OS rate of 61%, a 6-month PFS rate of 54%, a median OS of 14.6 months, and a median PFS of 6.9 months. In the series by Stummer et al.[17] on fluorescence-guided resection of malignant gliomas with the use of 5-ALA, the 6-month PFS rate was 41% and the median PFS period was 5.1 months. Moreover, in our patients with a newly diagnosed GBM, the median local PFS was nearly two times longer than the median PFS (20.0 vs 12.0 months). It can therefore be speculated that prolonged survival was caused by improved local tumor growth control due to intraoperative PDT. It should be emphasized that in the present series all patients with newly diagnosed GBM underwent either total or subtotal resection. Aggressive removal of the tumor may be an important prerequisite for clinical effectiveness of intraoperative PDT, since the penetration depth of a laser is approximately 2.5–5 mm; therefore, the corresponding effective distance for irradiation is limited to 0.75–1.5 cm.[1,2] The limitations of the efficacy of PDT in bulky target tissues and recurrent tumors have been demonstrated.[1] It is also possible that metabolically active infiltrating tumor cells in the periphery of the GBM may be more sensitive to PDT because of incorporation of a greater amount of photosensitizer. It was reported that the tissue concentration of a photosensitizer directly correlates with the grade of malignancy of the neoplasm.[2]

In the present study PDT showed a high level of safe-

TABLE 3: Skin photosensitivity test results in 27 patients*

No. of Days†	No. of Patients (%)	Cumulative No. of Patients (%)
3	4 (14.8)	4 (14.8)
4	11 (40.7)	15 (55.6)
8	6 (22.2)	21 (77.8)
10	1 (3.7)	22 (81.5)
13	2 (7.4)	24 (88.9)
14	1 (3.7)	25 (92.6)
15	2 (7.4)	27 (100)

* For the skin photosensitivity test, between 11 a.m. and 2 p.m., the back of the individual's hand was exposed to direct sunlight for 5 minutes, and the occurrence of any photosensitivity reaction, such as erythema, was assessed. In cases in which photosensitivity reactions were detected, the subject was kept shielded from light until the reaction disappeared, and the skin photosensitivity test was subsequently repeated.
† From administration of talaporfin sodium to disappearance of reaction.

ty. While laboratory investigations have frequently revealed abnormalities likely attributable to the administration of talaporfin sodium, only 2 patients (7.4%) had definite symptoms on the skin, which did not exceed Grade 2 toxicity. In no case did we encounter brain edema or cerebral infarction, which may complicate PDT.[1,2] Therefore, the risk of clinically significant side effects caused by the administration of talaporfin sodium and intraoperative irradiation of the residual tumor and peritumoral brain with a 664-nm laser 22–27 hours thereafter may be considered low. Moreover, according to photosensitivity test results, any reactions completely disappeared in all patients within 15 days after administration of the drug.

The main limitations of the present study are related to its design. A nonrandomized noncontrolled prospective investigation was performed in just 2 neurosurgical centers with well-established neurooncology programs and enrolled a limited number of highly selected cases with rather heterogeneous histopathological diagnoses of malignant parenchymal brain tumors. It is evident that to prove clinical efficacy of the intraoperative PDT with talaporfin sodium and a semiconductor laser, further carefully designed Phase III studies should be performed in a sufficiently large number of patients with possible initial stratification according to tumor resection rate. Testing of the proposed treatment method is also planned in cases of low-grade gliomas and in incompletely resected benign extraaxial neoplasms, such as pituitary adenomas and meningiomas. Since appropriate use of equipment for PDT requires specific skills, the dedicated training program for neurosurgeons is currently under organization. Finally, advanced experimental investigations directed at further understanding the basic mechanisms of the therapeutic effectiveness of intraoperative PDT are also required, and additional studies to search for the most optimal treatment regimens should be continued as well.

Conclusions

The results of the present study demonstrate that novel PDT based on administration of talaporfin sodium and subsequent irradiation with a 664-nm semiconductor laser may provide an additional benefit to the combined management of primary malignant parenchymal brain tumors through possible improvement of their local growth control, which, in turn, may lead to prolongation of the patient's survival. The therapy seems sufficiently safe with a minimal risk of serious side effects. Therefore, application of the intraoperative PDT along with aggressive resection, radiotherapy, and chemotherapy may be of clinical significance, particularly in patients with newly diagnosed GBM.

Disclosure

This study was supported by grants of an open-label study of photodynamic therapy with ME2906 and PNL6405CNS in patients with malignant brain tumors by Center for Clinical Trials, Japan Medical Association, Funding Program for World-Leading Innovative R&D on Science and Technology (FIRST Program) by the Japan Society for the Promotion of Science (JSPS), and Strategic international standardization acceleration action plan by METI (Ministry of Economy, Trade and Industry).

Author contributions to the study and manuscript preparation include the following. Conception and design: Muragaki, Akimoto, Iseki, Maebayashi, Matsumura, Kuroiwa, Nakazato, Kayama. Acquisition of data: Muragaki, Akimoto, Ikuta, Nitta, Saito, Kaneko. Analysis and interpretation of data: Muragaki, Akimoto, Ikuta, Karasawa. Drafting the article: Muragaki. Critically revising the article: all authors. Reviewed submitted version of manuscript: all authors. Approved the final version of the manuscript on behalf of all authors: Muragaki. Statistical analysis: Muragaki, Ikuta. Administrative/technical/material support: Maruyama, Iseki, Nitta, Maebayashi, Saito, Okada, Kaneko, Matsumura, Kuroiwa, Karasawa, Nakazato, Kayama. Study supervision: Muragaki, Iseki, Maebayashi, Okada, Matsumura, Kuroiwa, Nakazato, Kayama.

Acknowledgments

The authors thank all of the patients who participated in this study and the investigators from both study sites. Special thanks are devoted to Drs. Masahiko Tanaka, Norio Mitsuhashi, and Mikhail Chernov, and Mr. Takashi Sakayori (Tokyo Women's Medical University) for valuable help with clinical work and data analysis.

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Manuscript submitted February 28, 2013.
Accepted July 16, 2013.
Please include this information when citing this paper: published online August 16, 2013; DOI: 10.3171/2013.7.JNS13415.
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Neurol Med Chir (Tokyo) 53, 447 ~ 454, 2013

The 71st Annual Meeting Special Topics — Part III: Treatment Strategy of Low Grade Glioma

Updated Therapeutic Strategy for Adult Low-Grade Glioma Stratified by Resection and Tumor Subtype

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Abstract

The importance of surgical resection for patients with supratentorial low-grade glioma (LGG) remains controversial. This retrospective study of patients (n = 153) treated between 2000 to 2010 at a single institution assessed whether increasing the extent of resection (EOR) was associated with improved progression-free survival (PFS) and overall survival (OS). Histological subtypes of World Health Organization grade II tumors were as follows: diffuse astrocytoma in 49 patients (32.0%), oligoastrocytoma in 45 patients (29.4%), and oligodendroglioma in 59 patients (38.6%). Median pre- and postoperative tumor volumes and median EOR were 29.0 cm^3 (range 0.7–162 cm^3) and 1.7 cm^3 (range 0–135.7 cm^3) and 95%, respectively. Five- and 10-year OS for all LGG patients were 95.1% and 85.4%, respectively. Eight-year OS for diffuse astrocytoma, oligoastrocytoma, and oligodendroglioma were 70.7%, 91.2%, and 98.3%, respectively. Five-year PFS for diffuse astrocytoma, oligoastrocytoma, and oligodendroglioma were 42.6%, 71.3%, and 62.7%, respectively. Patients were divided into two groups by EOR ≥ 90% and < 90%, and OS and PFS were analyzed. Both OS and PFS were significantly longer in patients with ≥ 90% EOR. Increased EOR resulted in better PFS for diffuse astrocytoma but not for oligodendroglioma. Multivariate analysis identified age and EOR as parameters significantly associated with OS. The only parameter associated with PFS was EOR. Based on these findings, we established updated therapeutic strategies for LGG. If surgery resulted in EOR < 90%, patients with astrocytoma will require second-look surgery, whereas patients with oligodendroglioma or oligoastrocytoma, which are sensitive to chemotherapy, will be treated with chemotherapy.

Key words: low-grade glioma, treatment strategy, extent of resection, survival, residual tumor volume

Introduction

Low-grade glioma (LGG) has a more favorable prognosis than its malignant counterparts. The median survival for patients with LGGs is 5–10 years.[5,11,18] The slow growth of LGG requires extensive follow up, and therefore there are few evidence-based standards for guiding the medical and surgical treatment of patients with LGGs. Despite long-term survival, patients with LGG eventually die of either progression of the low-grade tumor or malignant transformation.[9] Compared with high-grade glioma, little is known about the factors that may predict survival of patients with LGGs. Historically, age, size of the tumor, tumor location, tumor subtype, and neurological deficit have been used as preoperative prog-

Received March 3, 2013; Accepted May 9, 2013

nostic factors for LGGs,[1,19] and the importance of surgical resection has not been emphasized.[9] However, emerging evidence suggests a strong correlation between extent of resection (EOR) and patient survival over 5 years.[12,21,23] The present retrospective study assessed the influence of EOR on outcome in patients with LGGs, and attempted to establish therapeutic strategies for LGG based on EOR and tumor subtype.

Methods

We conducted a retrospective review with long-term follow up of 153 patients (age ≥15 years, 84 males and 69 females) with supratentorial infiltrative LGG treated with surgical resection or biopsy at Tokyo Women's Medical University (TWMU) between January 2000 and August 2010. Patients were excluded if they had undergone prior resection of the tumor, with the exception of previous biopsy procedures performed as part of a diagnostic workup leading to eventual surgical removal in TWMU. Patients with neurofibromatosis type 1, pleomorphic xanthoastrocytoma, or infratentorial lesions were also excluded. Tumor grading and pathological diagnosis were performed based on World Health Organization (WHO) guidelines.[10] Clinical data were collected from patient records and telephone interviews. Two outcome measures were assessed: overall survival (OS) and progression-free survival (PFS). OS was defined as the time between initial surgery and death, whereas PFS was defined as the time between initial surgery and demonstration of unequivocal increase in tumor size on follow-up imaging, malignant progression, and/or death. Patients with no known progression/malignant progression were censored as of their last visit/scan date.

Tumor volumes as determined from axial T_2-weighted magnetic resonance (MR) imaging were calculated by importing Digital Imaging and Communications in Medicine (DICOM) images from the MR scanner to Leksell GammaPlan® software (Elekta AB, Stockholm, Sweden). EOR was calculated as: (preoperative tumor volume − postoperative tumor volume)/preoperative tumor volume.

OS and PFS were estimated using Kaplan-Meier method. A log-rank test was used to evaluate the importance of prognostic factors that might affect survival. Data analysis was performed using the JMP® statistical software (SAS Institute, Cary, North Carolina, USA). Univariate analyses for OS and PFS were performed using Cox proportional-hazards modeling. Variables that were statistically significant or showed borderline significance on univariate analysis were further analyzed with multivariate analysis using Cox proportional-hazards modeling. Hazard ratios (HRs) and 95% confidence intervals (CIs) are reported with the two-tailed probability values. The reported probability values in the Cox model are based on the World test, and values of <0.05 were considered significant.

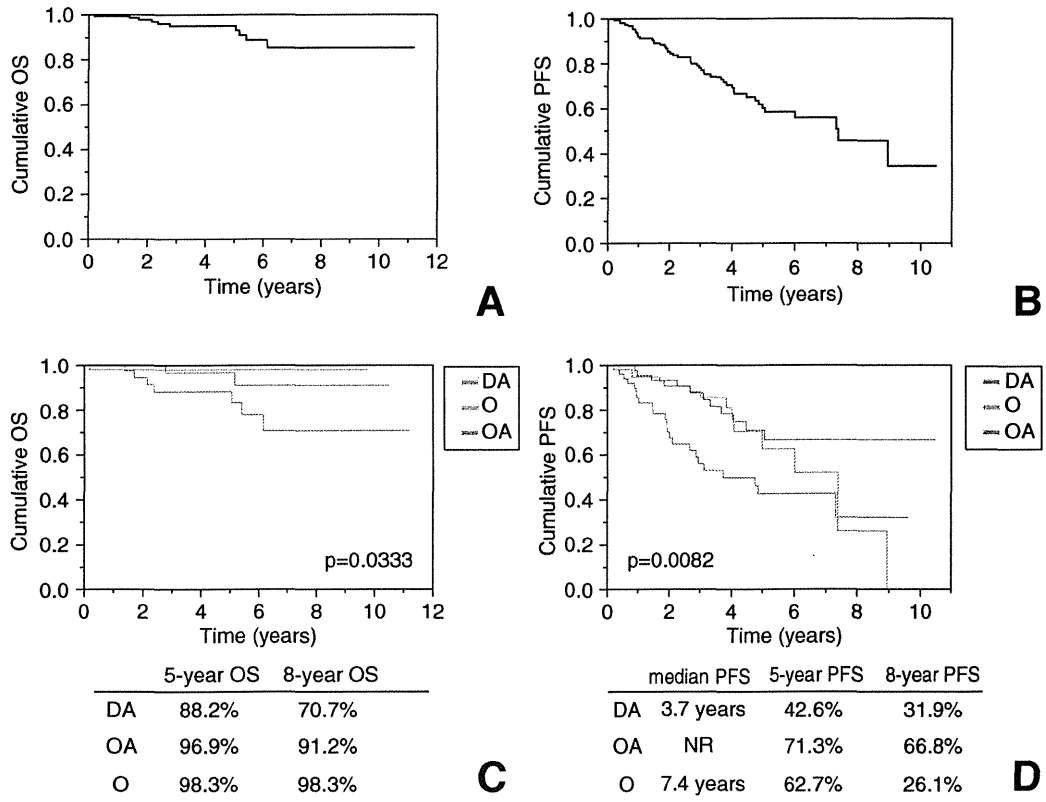
Results

Patient demographics and tumor characteristics are

Table 1 Clinical and tumor characteristics of patients with low-grade glioma (N = 153)

Characteristics	
Sex	
male	84 (54.9%)
female	69 (45.1%)
Age	
<50 yrs	116 (75.8%)
≥50 yrs	37 (24.2%)
median	37 yrs
range	15–76 yrs
KPS at diagnosis	
100	121 (79.1%)
90	27 (17.6%)
<90	5 (3.3%)
median	100
range	70–100
Tumor subtype	
diffuse astrocytoma	49 (32.0%)
oligoastrocytoma	45 (29.4%)
oligodendroglioma	59 (38.6%)
Postoperative radiation therapy	
yes	48 (31.4%)
no	105 (68.6%)
Postoperative chemotherapy	
yes	35 (22.9%)
no	118 (77.1%)
Preoperative maximum tumor diameter	
median	4.75 cm
range	1–10.5 cm
Preoperative tumor volume	
median	29.0 cm^3
range	0.7–162 cm^3
mean	38.4 cm^3
Postoperative tumor volume	
median	1.7 cm^3
range	0–135.7 cm^3
mean	9.3 cm^3
Extent of resection	
<90%	59 (38.6%)
≥90%	94 (61.4%)
median	95%
95% CI	77.0–85.7%
mean	81.4%

KPS: Karnofsky performance status.



	5-year OS	8-year OS
DA	88.2%	70.7%
OA	96.9%	91.2%
O	98.3%	98.3%

C

	median PFS	5-year PFS	8-year PFS
DA	3.7 years	42.6%	31.9%
OA	NR	71.3%	66.8%
O	7.4 years	62.7%	26.1%

D

Fig. 1 A, B: Kaplan-Meier plots showing overall survival (OS) (A) and progression-free survival (PFS) (B) for all patients with low-grade glioma. C, D: Kaplan-Meier plots showing poorer OS for diffuse astrocytoma than oligodendroglial subtypes (C), but no statistical difference in PFS between tumor subtypes (D). DA: diffuse astrocytoma, NR: not reached, O: oligodendroglioma, OA: oligoastrocytoma.

described in Table 1. Median age was 37.0 years (range 15–76 years). Median time between symptom onset and time of surgical resection was 6.4 months (range 21 days to 18.7 years). The histological subtypes of WHO grade II tumors were as follows: diffuse astrocytoma in 49 patients (32.0%), oligoastrocytoma in 45 patients (29.4%), and oligodendroglioma in 59 patients (38.6%). Median Ki-67 proliferation index was 4.2% (range 0.3–21.0%). Radiation was administered to 48 patients (31.4%) and ACNU-based chemotherapy was given to 35 patients (22.9%). Median time to progression was 2.94 years, and median time to malignant progression was 2.24 years.

Five-, 8-, and 10-year OS for all patients analyzed were 95.1%, 85.4%, and 85.4%, respectively (Fig. 1A). Median PFS, 5-, 8-, and 10-year PFS were 7.3 years, 60.2%, 45.7%, and 34.3%, respectively (Fig. 1B). Eight-year OS for diffuse astrocytoma, oligoastrocytoma, and oligodendroglioma were 70.7%, 91.2%, and 98.3%, respectively (Fig. 1C). Five-year PFS for diffuse astrocytoma, oligoastrocytoma, and oligodendroglioma were 42.6%, 71.3%, and 62.7%, respectively (Fig. 1D). Diffuse astrocytoma showed significantly poorer prognosis compared to oligoastrocytoma and oligodendroglioma ($p = 0.033$).

Median and mean volumes of the preoperative tumor were 29.0 cm^3 (range 0.7–162 cm^3) and 38.4 cm^3, respectively. Median and mean volumes of the postoperative tumor were 1.7 cm^3 (range 0–135.7 cm^3) and 9.3 cm^3, respectively. One perioperative death due to pulmonary embolization was encountered. A total of 146 patients (95.4%) underwent surgical resection and intraoperative MR imaging was used in 140 (95.9%) patients. Seven patients (4.6%) underwent biopsy. Overall, median and mean EOR were 95.0% and 81.4% (95% CI 77.0–85.7%), respectively. EOR of 90% or more was achieved in 94 patients (61.4%) and EOR of less than 90% in 59 patients (38.6%).

Patients were divided into two groups by EOR $\geq 90\%$ and $< 90\%$, and OS and PFS were analyzed. Both OS and PFS were significantly longer in patients with $\geq 90\%$ EOR (Fig. 2A, B). To see if less residual tumor volume is important for outcome, patients were divided by residual tumor volume < 5

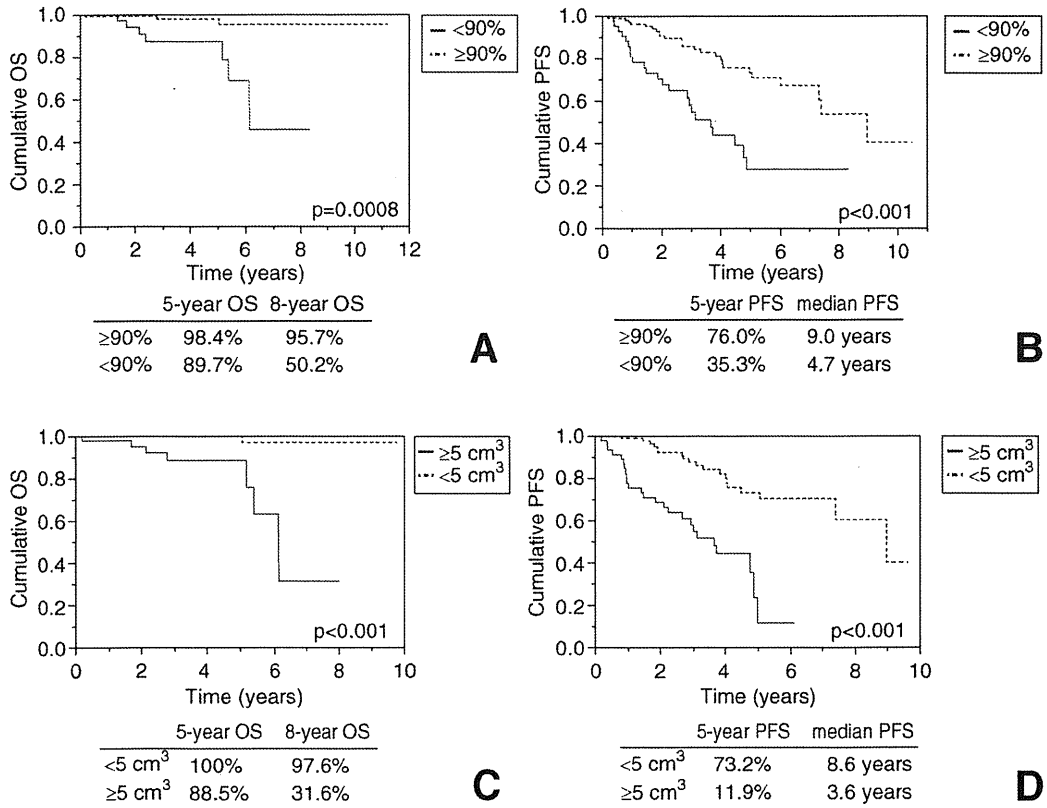


Fig. 2 Importance of extent of resection (EOR) for patient prognosis. A, B: Kaplan-Meier plots showing overall survival (OS) (A) and progression-free survival (PFS) (B) in patients with ≥90% and <90% EOR. C, D: Kaplan-Meier plots showing OS (C) and PFS (D) in patients with <5 cm³ and ≥5 cm³ residual tumor volume. Both EOR and residual tumor volume are strongly correlated with patient outcome.

cm³ and ≥5 cm³. As expected, patients with <5 cm³ of residual tumor volume showed better OS and PFS (Fig. 2C, D). These results strongly suggested that EOR ≥90% and decreased residual tumor volume are associated with better outcomes (Fig. 2).

We then addressed how EOR affects patient prognosis for each histological subtype. Because the number of patients was not adequate to analyze OS, the correlations between EOR, residual tumor volume, and PFS were investigated. Interestingly, patients with diffuse astrocytoma and oligoastrocytoma showed a significant difference in PFS between the group with ≥90% EOR and <90% EOR (Fig. 3A, B). Conversely, in patients with oligodendroglioma, EOR did not correlate with PFS (Fig. 3C). To confirm these results, the correlation between the volume of the residual tumor and patient prognosis was investigated. As expected, patients with astrocytoma and oligoastrocytoma, if residual tumor volume was <5 cm³, showed significantly longer PFS, whereas residual tumor volume did not affect PFS in patients with oligodendroglioma (Fig. 3D–F).

Univariate analysis on Cox proportional hazards model selected age (HR 5.43, 95% CI 1.55–21.30; p = 0.009), EOR (HR 8.25, 95% CI 2.26–38.73; p = 0.001), and tumor subtype (HR, 4.98, 95% CI 1.37–23.27; p = 0.0143) for OS (Table 2). Parameters selected for PFS were EOR (HR 3.40, 95% CI 1.88–6.12; p < 0.0001) and tumor subtype (HR 2.09, 95% CI 1.16–3.72; p = 0.014) (Table 2). Neither tumor diameter nor MIB-1 index affected the prognosis (Table 2).

Multivariate modeling was performed for each outcome measure using the following parameters: age, maximum tumor diameter, EOR, tumor subtype, and MIB-1 index. The parameters identified as significant for OS were age (HR 4.08, 95% CI 1.08–16.86; p = 0.038) and EOR (HR 4.75, 95% CI 1.07–26.48; p = 0.039) (Table 2). The only parameter selected for PFS was EOR (HR 2.69, 95% CI 1.43–5.04; p = 0.002) (Table 2). Thus, EOR showed the strongest correlation with patient survival (Table 2).

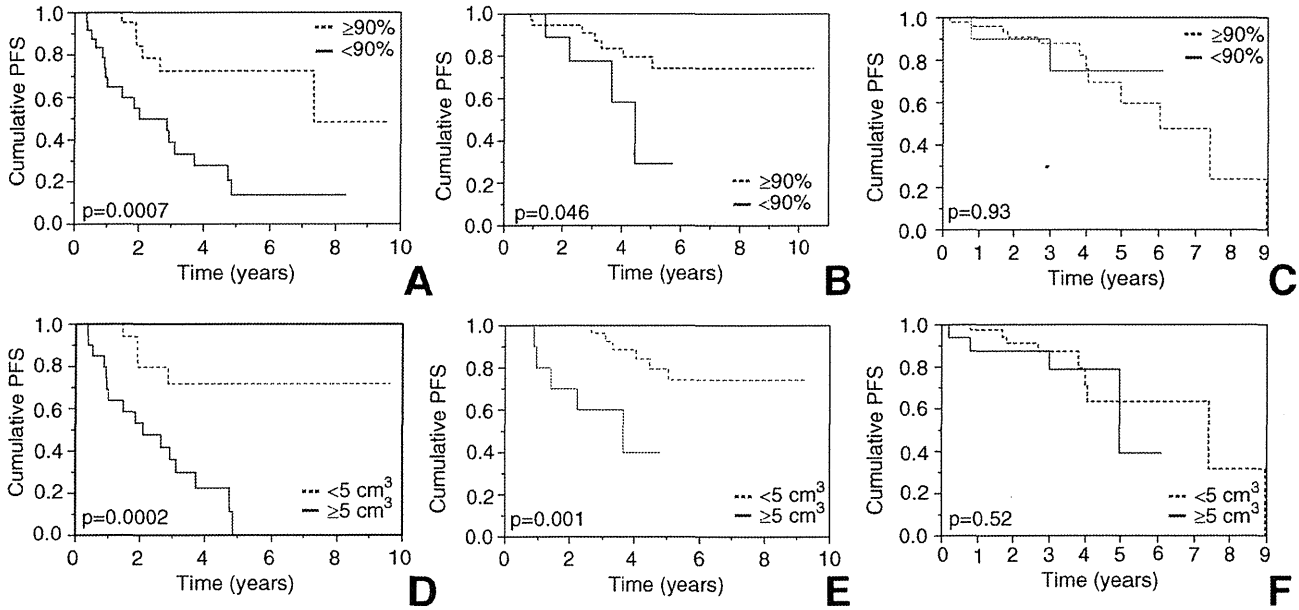


Fig. 3 Different effects of extent of resection (EOR) on patient progression-free survival (PFS) between tumor subtypes. A–C: Kaplan-Meier plots showing that ≥ 90% EOR resulted in better PFS in patients with diffuse astrocytoma (A) and oligoastrocytoma (B) but not in oligodendroglioma (C). D–F: Patients with < 5 cm³ of residual tumor volume showed longer PFS in patients with diffuse astrocytoma (D) and oligoastrocytoma (E) but not in oligodendroglioma (F).

Table 2 Univariate and multivariate analysis
For overall survival

Factor	Univariate			Multivariate		
	HR	95% CI	p Value	HR	95% CI	p Value
Age: ≥50 yrs vs. <50 yrs	5.43	1.55–21.30	**0.0089***	**4.08**	1.08–16.86	**0.0375***
Tumor diameter: ≥5 cm vs. <5 cm	1.2	0.43–5.97	0.5087	0.95	0.23–4.08	0.9468
Extent of resection: <90% vs. ≥90%	8.25	2.26–38.73	**0.0014***	**4.75**	1.07–26.48	**0.0391***
Tumor subtype: DA vs. O/OA	4.98	1.37–23.27	**0.0143***	2.64	0.62–13.79	0.1918
MIB-1 index: ≥5% vs. <5%	1.53	0.42–5.54	0.5024	1.69	0.45–6.38	0.4203

For progression-free survival

Factor	Univariate			Multivariate		
	HR	95% CI	p Value	HR	95% CI	p Value
Age: ≥50 yrs vs. <50 yrs	1.58	0.80–2.93	0.1757	1.36	0.68–2.56	0.3665
Tumor diameter: ≥5 cm vs. <5 cm	1.59	0.89–2.89	0.1122	1.42	0.78–2.62	0.2503
Extent of resection: <90% vs. ≥90%	3.4	1.88–6.12	**<0.0001***	**2.69**	1.43–5.04	**0.0022***
Tumor subtype: DA vs. O/OA	2.09	1.16–3.72	**0.0140***	1.7	0.92–3.12	0.0888
MIB-1 index: ≥5% vs. <5%	1.94	0.51–1.69	0.4328	0.94	0.51–1.70	0.8589

*p < 0.05. CI: confidence interval, DA: diffuse astrocytoma, HR: hazards ratio, O: oligodendroglioma, OA: oligoastrocytoma.

Discussion

The management of LGG remains controversial. Due to diffuse infiltration, LGG is usually not considered surgically curable,[20] and biopsy without resection is theoretically acceptable until better evi-

dence is available.[24] However, a recent report suggested that early surgical treatment of LGG is associated with better survival than observation, and more aggressive treatment thus appears warranted for optimal treatment of LGG.[8] Although the role of surgical resection for LGG remains controversial,[9,24] emerging evidences suggests EOR is important for survival in patients with LGG.[12,23] In our experience of 153 cases, EOR was strongly correlated with prognosis in patients with LGG. Both OS and PFS in our study were consistent with other reports that have shown benefits of surgical resection for patient survival.[12,23]

Given that LGG will eventually progress or undergo malignant transformation, reducing the number of tumor cells as far as possible to prevent progression or malignant transformation of the tumor appears reasonable. One of the limitations of previous studies that have not shown the benefit of surgical resection is that the EOR was based on the intraoperative interpretation of the surgeon or nonquantitative estimates.[17,24] Meticulous estimate of pre- and postoperative tumor volume and EOR using volumetric calculation is thus critical for accurate evaluation of the importance of EOR for patient prognosis.[7,23] Which sequence of MR imaging is most suitable for volumetric analysis of EOR remains unknown. Fluid-attenuated inversion recovery (FLAIR) imaging seems to provide better definition of spread of the tumor than T_2-weighted imaging. However, intraoperative MR imaging in our institute does not give adequate quality of FLAIR imaging, so we have been using T_2-weighted imaging for the volumetric analysis.

The boundaries of the tumor and normal brain tissue in LGG are difficult to distinguish compared to its malignant counterparts, and gross total resection of LGG is thus difficult. Use of intraoperative MR imaging can drastically reduce the amount of residual tumor.[13,22] Safe and maximum resection of LGG located in eloquent areas is difficult and intraoperative cortical mapping under awake craniotomy is useful.[2-4] Our department has introduced intraoperative MR imaging and has experienced more than 900 glioma surgeries since 2000.[13] We have also performed more than 250 cases of intraoperative functional mapping with awake craniotomy for gliomas located in eloquent areas to achieve both maximum resection and preservation of neurological function. Use of intraoperative MR imaging in the setting of awake craniotomy enables safe and maximum resection of LGG.[16] Thus information-guided surgery, integrating anatomical, functional, and histopathological data, permits the surgeon to achieve maximum resection with minimum risk of neurological deficit.[6,14,26]

Detailed characterization of resected tumor tissue during surgery is important in the accurate diagnosis of tumor border and maximum resection. We showed that EOR is strongly correlated with both OS and PFS in patients with astrocytoma. In other words, the prognosis for patients with astrocytoma who underwent partial resection was significantly worse. Furthermore, our analysis showed that neither radiation nor nitrosourea-based chemotherapy showed any survival benefit in patients with astrocytoma (data not shown). Maximum resection will be thus critical for successful treatment for patients with astrocytoma. Treatment for patients with astrocytoma in which surgery ended in partial removal is therefore difficult. A randomized study to evaluate the role of postsurgical treatments for partially resected astrocytoma will thus be necessary.

Interestingly, EOR did not affect PFS in patients with oligodendroglioma in our study, possibly because oligodendroglioma infiltrates beyond MR imaging-defined abnormalities.[15] Currently our strategy for oligodendroglioma is gross total resection of the high intensity lesion on T_2-weighted MR imaging, but more extensive resection may be required to prevent progression of oligodendroglioma.[15] However, concluding that surgical resection does not contribute patient survival would be premature, because patients with oligodendroglioma survive longer and OS may differ after longer follow-up periods. In fact, this study included relatively few death events for patients with oligodendroglioma. Careful long-term assessment of OS may be important to assess the importance of EOR in oligodendroglioma. This is the first report that showed different effects of EOR on patient PFS between tumor subtypes.

Given the present results, we have updated our therapeutic strategy for LGG stratified by EOR and tumor subtype (Fig. 4). Our findings suggest that EOR $\geq$ 90% is strongly correlated with the prognosis of astrocytoma, and the patient can be observed carefully without postsurgical treatment regardless of tumor subtype. If surgery resulted in EOR $<$ 90%, patients with astrocytoma will require second-look surgery, whereas patients with oligodendroglioma or oligoastrocytoma, which are sensitive to chemotherapy, will be treated with chemotherapy. We are now collecting genomic information including co-deletion of chromosome arms 1p and 19q, and mutation of IDH1 and IDH2. Integration of the genetic information into our updated strategy for the treatment of LGG will be necessary in the future. In conclusion, given the different prognosis and effects of EOR between tumor subtypes, treatment of

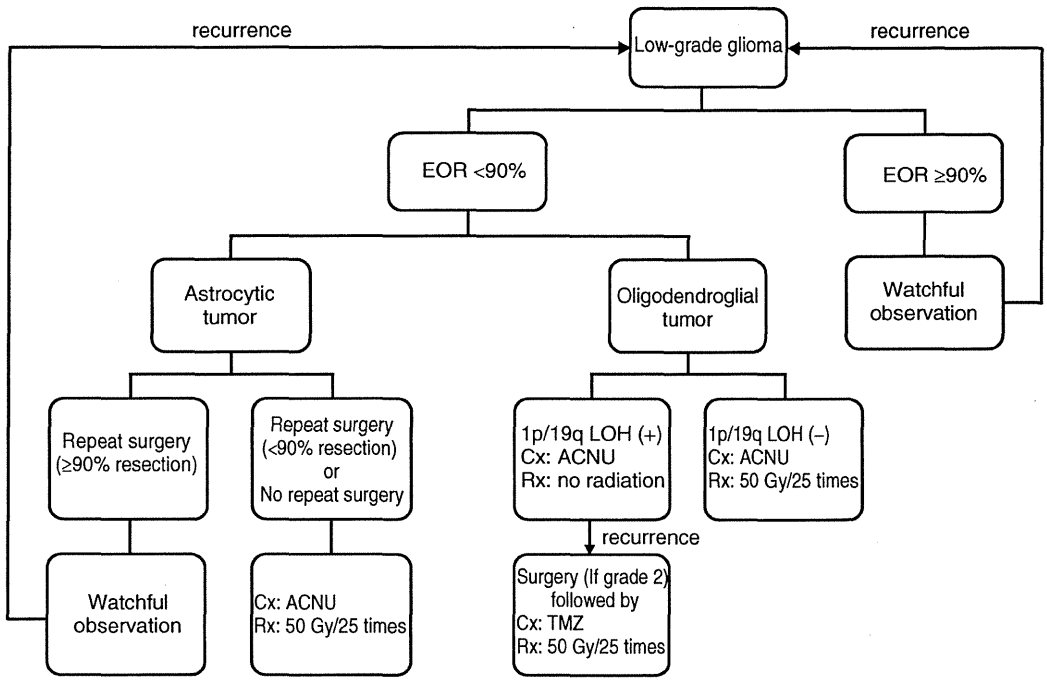


Fig. 4 Therapeutic strategy for low-grade glioma stratified by extent of resection (EOR) and tumor subtype. If the EOR is ≥90%, patients can be observed carefully without postsurgical treatment regardless of tumor subtype. If surgery resulted in EOR <90%, patients with diffuse astrocytoma will require second-look surgery, whereas those with oligodendroglioma or oligoastrocytoma, which are sensitive to chemotherapy, will be treated with chemotherapy. Cx: chemotherapy, LOH: loss of heterozygosity, Rx: radiation, TMZ: temozolomide.

LGG should be stratified by EOR and tumor subtype.

Conflicts of Interest Disclosure

The authors declare that they do not have any conflict of interests. All authors who are members of The Japan Neurosurgical Society (JNS) have registered online Self-reported COI Disclosure Statement Forms through the website for JNS members.

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J Neurooncol (2011) 104:573–577
DOI 10.1007/s11060-011-0529-1

CLINICAL STUDY – PATIENT STUDY

A multicenter phase I trial of combination therapy with interferon-β and temozolomide for high-grade gliomas (INTEGRA study): the final report

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Received: 20 July 2010 / Accepted: 31 January 2011 / Published online: 14 February 2011

Abstract Our previous study demonstrated that interferon-β markedly enhanced chemosensitivity to temozolomide; one of the major mechanisms is downregulation of O^6-methylguanine DNA-methyltransferase transcription via p53 induction. This effect was also observed in an experimental animal model. The results of these studies suggest that compared to temozolomide-based chemotherapy performed concomitantly with radiotherapy, chemotherapy with interferon-β and temozolomide and concomitant radiotherapy might further improve the clinical outcomes of patients with malignant gliomas. A multicenter phase I clinical trial—the Integrated Japanese Multicenter Clinical Trial: a Phase I Study of Interferon-β and Temozolomide for Glioma in Combination with Radiotherapy (INTEGRA Study)—was conducted in patients with high-grade gliomas in order to evaluate the safety, feasibility, and preliminary clinical effectiveness of combination therapy with interferon-β and temozolomide. The primary endpoint was the incidence of adverse events. The exploratory endpoints were progression-free survival time and overall survival time. The study population comprised 16 patients with newly diagnosed and 7 patients with recurrent high-grade gliomas. Grades 3–4 leukocytopenia and neutropenia were observed in 6.7 and 13.3% of patients, respectively. Overall, 40% of patients showed an objective response to therapy. In patients with newly diagnosed glioblastoma, the median overall survival time was 17.1 months and the rate of 1-year progression-free survival was 50%. We conclude that this regimen is safe and well tolerated and may prolong survival of patients with glioblastoma. A phase II clinical study is essential to corroborate our findings.

Keywords Glioma · Interferon-β · Temozolomide

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Introduction

Gliomas account for approximately 40% of all brain tumors and are thus the most common primary tumors of the central nervous system (CNS). Primary brain tumors are classified according to cell type and histological grade into categories defined by the World Health Organization

(WHO) [1]. High-grade (WHO grades 3 and 4) gliomas, including anaplastic astrocytoma (AA), anaplastic oligodendroglioma (AO), anaplastic oligoastrocytoma (AOA), and glioblastoma multiforme (GBM), are often resistant to treatment [2–4]. Temozolomide (TMZ), an oral alkylating agent, has been shown to possess antitumor activity against malignant gliomas with minimal additional toxicity; furthermore, in a previous study, the median survival time substantially improved from 12 to 15 months when radiotherapy was concomitantly used with TMZ-based chemotherapy followed by adjuvant TMZ therapy [5]. In 2006, TMZ was approved by the National Ministry of Health and Welfare of Japan as the treatment agent for malignant gliomas, and a combination of radiotherapy and TMZ-based chemotherapy is now used as first-line therapy. However, the clinical outcome of TMZ therapy depends on the methylation status of the O^6-methylguanine-DNA methyltransferase (MGMT) promoter; patients with GBM whose tumors had the methylated MGMT promoter benefitted from TMZ compared to patients whose tumors had the unmethylated promoter [hazard ratio: 0.45; 95% confidence interval (CI): 0.32–0.61] [6]. Thus, MGMT modification is one of the key factors that could enhance the clinical benefits of this treatment.

Interferon (IFN)-β exerts pleiotropic biological effects and has been widely used either individually or in combination with other antitumor agents to treat malignant gliomas and melanomas [7]. In the treatment of malignant gliomas, IFN-β can act as a drug sensitizer, and it enhances the toxicity of chemotherapeutic agents against various neoplasms when it is administered in combination with nitrosourea. Combination therapy with IFN-β and nitrosourea has been used primarily for the treatment of gliomas in Japan [8]. In a previous in vitro study in human glioma cells, we found that IFN-β markedly enhanced chemosensitivity to TMZ [9]; this finding suggested that one of the major mechanisms by which IFN-β enhances chemosensitivity is the downregulation of MGMT transcription via p53 induction. This effect was also observed in an experimental animal model [10]. The results of these two studies suggested that compared to chemotherapy with TMZ alone and concomitant radiotherapy, chemotherapy with IFN-β, and TMZ with concomitant radiotherapy might further improve the clinical outcome of malignant gliomas. Here, in order to evaluate the safety, feasibility, and clinical effectiveness of combination therapy with IFN-β and TMZ, we conducted a phase I clinical study, the Integrated Japanese Multicenter Clinical Trial: a Phase I Study of Interferon-β and Temozolomide for Glioma in Combination with Radiotherapy (INTEGRA Study). This study involved eight medical institutions that covered the entire Japanese population.

Patients and methods

Patient population

We included patients fulfilling the following eligibility criteria: (1) newly diagnosed or recurrent high-grade gliomas (AA, AO, AOA, or GBM) as confirmed by histological analysis; (2) pretreatment magnetic resonance imaging (MRI) showing a tumor with >50% volume located in the supratentorial region except for the optic nerve, olfactory nerve, and pituitary gland; (3) age 18–75 years at the time of registration; (4) performance status (PS) of 0–2, or a PS of 3 only in the case of neurological deficit; (4) newly diagnosed high-grade gliomas for which chemoradiotherapy had not previously been performed; and (5) recurrent high-grade gliomas for which the time lapse since the end of prior antitumor therapy (e.g., chemotherapy, radiotherapy, and immunotherapy) was at least 4 weeks regardless of the regimen used. Additional inclusion criteria included adequate organ function before initiation of chemotherapy as defined on the basis of the following criteria: WBC count $\geq$3,000/mm^3 or neutrophil count $\geq$1,500/mm^3; platelet count $\geq$100,000/mm^3; hemoglobin level $\geq$8.0 g/dL; bilirubin level $\leq$1.5 mg/dL; serum glutamic oxaloacetic transaminase (SGOT) level $\leq$100 IU; serum glutamic pyruvic transaminase (SGPT) level $\leq$100 IU; creatinine level $\leq$1.5 mg/dL; creatinine clearance rate $\geq$50 ml/min; electrocardiogram (ECG) showing no serious arrhythmia; and absence of serious ischemic heart disease. All the patients were informed of the investigational nature of the study and were required to sign an informed consent form. The protocol was reviewed and approved by the institutional review boards of each participating institution. The following patients were excluded from the study: (1) those who had developed cancer synchronously or metachronously at 2 sites in the past 5 years; (2) those with confirmed carcinoma in situ; (3) those with meningitis or pneumonia; (4) women who were pregnant, possibly pregnant, or breastfeeding; (5) those with psychological disorders; (6) those with untreated diabetes mellitus (DM) or under insulin treatment for DM; (7) those who had a myocardial infarction in the past 3 months; and (8) those with a history of pulmonary fibrosis or interstitial pneumonia.

Study design and treatment

This study was a phase I, open-label, preliminary multicenter trial for evaluating the safety, feasibility, and clinical effectiveness of combination therapy with IFN-β for the treatment of malignant gliomas. The primary endpoint for the trial was the incidence of adverse events and the exploratory endpoints were progression-free survival time and overall survival time. In addition, the objective tumor

response was evaluated in a subpopulation of patients with measurable disease by the committees for safety and efficacy retrospectively. The reduction rate of measurable tumors was calculated according to the response evaluation criteria in solid tumors (RECIST) as assessed by MRI. Unmeasurable tumors were classified as those showing complete response (CR), partial response (PS), or progression (PD) or those that could not be evaluated (NE). Subsequently, overall response was evaluated on the basis of the results obtained for measurable and unmeasurable tumors. Pseudoprogression was excluded by carefully reviewing serial MRIs and case report forms including the information on steroid use and dose.

Patients with newly diagnosed high-grade gliomas received radiotherapy at a total dose of 60 Gy, intravenous (IV) IFN-β at a dose of 3 MIU/body on alternate days, and TMZ at a dose of 75 mg m^{-2} day^{-1}) daily. After the induction period, all the patients went through a 4-week washout period. Subsequently, the adjuvant treatment was initiated; this comprised IFN-β (3 MIU/body on the first morning of every 4th week) and TMZ (150 mg m^{-2} day^{-1}] on days 1–5 of the first cycle and 200 mg m^{-2} day^{-1}] on days 1–5 of the second to the sixth cycle). When no hematologic toxicity was noted, the TMZ dosage was increased to 200 mg m^{-2} day^{-1}) from the second cycle to the sixth cycle. The cycle was repeated 6 times every 28 days when no tumor progression or serious adverse events such as grade 4 hematologic toxicity were noted, and the patient did not refuse therapy or deviate from the protocol.

Patients with recurrent high-grade gliomas received a combination of IFN-β (3 MIU/body on the first morning of every 4th week) and TMZ (150 mg m^{-2} day^{-1} on days 1–5 of the first cycle and 200 mg m^{-2} day^{-1} on days 1–5 of the second to the sixth cycle). When no hematologic toxicity was noted, the TMZ dose was increased to 200 mg m^{-2} day^{-1} from the second cycle to the sixth cycle. All patients received non-steroidal anti-inflammatory drugs 1 h prior to IV IFN-β. On the basis of the results of previous clinical studies, this regimen is considered to be the most promising option [8, 11–14]. In this trial, we did not determine the maximum tolerated dose (MTD) of IFN-β for several reasons. IFN-β is a cytokine that exerts pleiotropic biological effects and has been widely used either individually or in combination with other antitumor agents for treating malignant gliomas and melanomas [7]. Combination therapy with IFN-β and nitrosourea (3 MIU/body in clinical setting) has been used primarily for the treatment of gliomas in Japan [8]. The favorable effect of a cytokine depends on its dose, and overdosing might not only increase the adverse events but also decrease the antitumor cytotoxic effect of the drug; therefore, it is difficult to determine the drug's optimal dose. However, on the basis of our previous animal study and our experience in the clinical use of IFN-β and nitrosourea, we concluded that the dosage of IFN-β used in this study would be the most promising and feasible one.

Registration and monitoring

The participating researchers were instructed to send an eligibility criteria report to the data center at Nagoya University, a third-party institution with which the study director was not affiliated. Patients were registered for 6 months starting December 2007. Laboratory data, including those from MRI, blood tests, and pathological tests, were obtained at the data center. The data quality was checked and verified at the data center. The committees for safety and efficacy (spearheaded by Dr. Kazuo Tabuchi, Koyanagi Memorial Hospital, Saga), radiotherapy (spearheaded by Dr. Shinji Naganawa, Department of Radiology, Nagoya University School of Medicine), pathological review (spearheaded by Dr. Youichi Nagasato, Department of Pathology, Gunma University School of Medicine), and statistics (spearheaded by Dr. Kunihiko Hayashi, Gunma University School of Health Science) submitted their reports to the head office.

Follow-up and statistical analysis

Disease progression and the occurrence of new tumors were examined by MRI performed at baseline and after at least 4–5 weeks of treatment. Blood tests were performed and symptoms were assessed before treatment and after at least 2 weeks during treatment. Follow-up continued for 3 months after the end of treatment. In cases in which therapy was discontinued because of toxicity, clinicians followed up the patients until they recovered. In addition, overall survival, progression-free survival, and treatment success curves were constructed as time-to-event plots with the Kaplan–Meier method.

Results

Patient characteristics

Between November 2006 and May 2007, 23 patients with high-grade gliomas were enrolled in our study. Detailed patient demographic and clinical characteristics are shown in Table 1. In order to evaluate the toxicity profile during maintenance treatment with the TMZ and IFN-β combination, we have included patients with recurrent high-grade gliomas for whom the time lapse since the end of prior antitumor therapy (e.g., chemotherapy, radiotherapy, and immunotherapy) was at least 4 weeks regardless of the regimen used.

Table 1 Demographic and clinical characteristics of patients

Characteristic	Value
Total	23
Age, years	
Median	51
Range	29–70
Sex, *n* (%)	
Male	10 (43%)
PS	
Median	1
Range	0–2
Histology	
Newly diagnosed	
GBM	10
AA	3
AO	2
AOA	1
Recurrent	7
GBM	3
AA	3
AO	1

PS performance status, *GBM* glioblastoma, *AA* anaplastic astrocytoma, *AO* anaplastic oligodendroglioma, *AOA* anaplastic oligoastrocytoma

Toxicity evaluation

Table 2 summarizes the nature of therapy-induced toxicity occurring during initial chemoradiotherapy. Grades 3–4 leukocytopenia and neutropenia were observed in 6.7 and 13.3% of patients, respectively. Grade 4 neutropenia recovered within 2 weeks without granulocyte colony-stimulating factor rescue. Hematologic toxicity was minimal during maintenance treatment. The most common adverse event was grade 1 appetite loss (30.4%) followed by grade 1 SGOT/SGPT elevation (26%). Grade 1 fever was observed in 15% of patients.

Response and survival

15 patients (10, newly diagnosed; 5, recurrent) with measurable disease were assessed for objective tumor response. Of these, 3 patients (20%) exhibited CR after 3 cycles of chemotherapy; 3 patients (20%) exhibited PR after 6 cycles; 5 patients (33%) exhibited stable disease after 6 cycles; and 4 patients (27%) exhibited disease progression after 1 cycle. Overall survival was assessed from the date of diagnosis to the date of the last follow-up or death. The overall survival distribution among patients with newly diagnosed high-grade gliomas (grade 3: 6 patients; grade 4: 10 patients) was estimated using the Kaplan–Meier method (Fig. 1). Progression-free survival was assessed from the date of diagnosis to the date of disease progression or death, whichever occurred first. The median survival time in patients with newly diagnosed grade 4 tumors was 17.1 months and the 1-year progression-free survival rate (95% CI) was 50.0% (range, 18.2–81.2%).

Table 2 Grade 3 and 4 toxicities attributed drug treatment

	Grade 3 (%)	Grade 4 (%)
Induction Tx		
Leucopenia	6.7	6.7
Platelet	0	0
Neutropenia	0	13.3
SGOT	0	0
SGPT	0	0
Maintenance Tx		
Leucopenia	5.6	0
Platelet	0	0
Neutropenia	0	0
SGOT	0	0
SGPT	0	0

Tx treatment

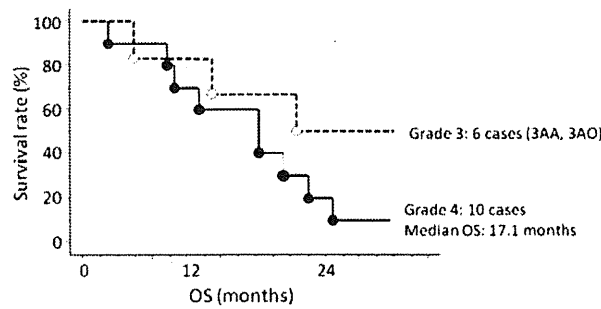

Fig. 1 The overall survival distribution among patients with newly diagnosed high-grade gliomas (grade 3: 6 patients; grade 4: 10 patients) was estimated using the Kaplan–Meier method. The median survival time in patients with newly diagnosed grade 4 tumors was 17.1 months. *AA* anaplastic astrocytome, *AO* anaplastic oligodendroglioma

Discussion

The results of this trial reveal that combination therapy with IFN-β and TMZ caused minimal toxicity. The most frequently observed toxic effect was the inhibition of hematopoiesis (e.g., with leukocytopenia); it took as long as 1 month after discontinuation of therapy for patients to recover from this effect. The overall response rate (CR+PR) was 40%, and the median survival time in newly diagnosed GBM patients was 17.1 months. Although the sample size was limited in this study, the median survival time was significantly longer than that in the EORTC 2698/22981 study

in which the median survival time in GBM patients under the Stupp regimen was 14.6 months.

In this trial, we did not assess the MGMT status of patients because it was not a part of the protocol [15]. However, we retrospectively reviewed the cases of 68 consecutive patients with newly diagnosed GBM. Of these patients, 57.4% received a combination of IFN-β and TMZ. When this combination was administered at a similar dosage in our phase I trial, a median survival time of 19.9 months was achieved, whereas the median survival time achieved with TMZ therapy alone was 12.7 months. Notably, in patients whose tumors had the unmethylated MGMT promoter, the median survival time increased to 17.2 months after TMZ with IFN-β therapy compared to 12.5 months after TMZ without IFN-β therapy. This finding suggests that combination IFN-β and TMZ therapy may improve the clinical outcomes in patients with tumors expressing MGMT with an unmethylated MGMT promoter [16]. This finding indirectly supports the hypothesis that downregulation of MGMT expression might have contributed to the clinical efficacy of the combination used in this study.

The phase I trial of combination therapy with IFN-β and TMZ reported in this paper has defined the therapeutic approach for our ongoing phase II trial for the same; patients with newly diagnosed GBM will be included in the phase II trial. This trial will provide the data required to determine whether IFN-β inclusion will enhance the clinical efficacy of TMZ-based chemotherapy performed concomitantly with radiotherapy.

Acknowledgment This work was supported by grants from the Japan Brain Foundation (A.N.).

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Original Article

Benefits of Interferon-β and Temozolomide Combination Therapy for Newly Diagnosed Primary Glioblastoma With the Unmethylated MGMT Promoter

A Multicenter Study

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BACKGROUND: The aim of the current study was to catalog genomic and epigenomic abnormalities in newly diagnosed glioblastoma patients and determine the correlation among clinical, genetic, and epigenetic profiles and clinical outcome. **METHODS:** This study retrospectively included 68 consecutive patients who underwent surgical treatment and received standard radiotherapy with temozolomide (TMZ)-based chemotherapy. Of a total of 68 patients, 39 patients (57.4%) received interferon (IFN)-β in combination of TMZ. **RESULTS:** The genetic and epigenetic alterations frequently observed were *EGFR* amplification (51.5%), *TP53* mutation (33.8%), *CDKN2A* loss (32.4%), *TP53* loss (16.2%), methylation of the MGMT promoter (33.8%) and *IDH1* mutation (5.9%). Multivariate analysis revealed that methylated MGMT promoter and the combination of TMZ and IFN-β were independent prognostic factors associated with survival. The median survival time (MST) of the patients who received the combination of IFN-β and TMZ was significantly greater with 19.9 months as compared to the TMZ alone group (12.7 months). Notably, in even patients whose tumors had unmethylated MGMT promoter, the MST prolonged to 17.2 months when receiving TMZ with IFN-β, compared to 12.5 months in those receiving TMZ without IFN-β. **CONCLUSIONS:** Taken together, addition of IFN-β for newly diagnosed primary GBM achieved a favorable outcome, particularly in patients with unmethylated MGMT promoter. ***Cancer* 2010;000:000–000.** © *2010 American Cancer Society.*

KEYWORDS: IDH1, MGMT methylation, glioblastoma, interferon-β, temozolomide.

Glioblastoma multiforme (GBM) is one of the most frequent primary brain tumors in the central nervous system in adults and is highly malignant, with a median survival time of about one year from diagnosis. This is despite aggressive treatment, surgery, postoperative radiotherapy, and adjuvant chemotherapy. An international randomized trial by the European Organization for Research and Treatment of Cancer/National Cancer Institute of Canada (EORTC/NCIC) comparing radiotherapy alone and concomitant radiotherapy and temozolomide (TMZ) clearly attested the benefits of adjuvant TMZ chemotherapy for GBM patients.[1] Since then, TMZ has been the current first-line chemotherapeutic agent for GBM.

A subanalysis in this trial showed the effectiveness of epigenetic silencing of the MGMT gene by promoter methylation with longer survival in patients with primary GBM; it also suggested the benefits of combining chemotherapy using TMZ with radiotherapy.[2]

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We thank Mr. Akiyoshi Sakai (Clinical Laboratory, Kariya Toyota General Hospital, Kariya, Japan), Mr. Hideaki Maruse, Mr. Takafumi Fukui, and Mr. Yosuke Furui (FALCO biosystems, Kyoto, Japan) for wonderful technical assistance.

DOI: 10.1002/cncr.25637, **Received:** April 22, 2010; **Revised:** July 14, 2010; **Accepted:** August 2, 2010, **Published online** in Wiley Online Library (wileyonlinelibrary.com)

Furthermore, there have been recent attempts to comprehensively profile GBM genes by The Cancer Genome Atlas (TCGA) project and other groups.[3,4] Some genetic aberrations in GBM, such as *TP53* mutation or deletion, *NF1* deletion or mutation, and *ERBB2* mutation, have been found to be more common than previously reported. In addition, novel molecular markers, such as frequent mutations of the *IDH1* and *IDH2* genes in secondary GBM have been discovered.[5-7] These findings on mutations, genomic and epigenomic aberrations, and transcriptomal features in GBM might aid in understanding the classification of GBM and its further potential clinical implications.

However, the TCGA project included GBM patients who received surgical treatment, and detailed information on adjuvant chemoradiotherapy was not provided. Therefore, the close relationship between the gene profile provided by TCGA and chemotherapy regimens remains unknown.[3]

In this current study, we aimed to determine the correlation between clinical, genetic, and epigenetic profiles, and clinical outcome in newly diagnosed GBM patients who received TMZ-based chemotherapy. Interestingly, we found a significant beneficial outcome in patients receiving TMZ in addition to IFN-β. Moreover, our study discovered that GBM patients with the ummethylated O^6-methylguanine-DNA methyltransferase (MGMT) promoter, in particular, showed benefits from IFN-β.

MATERIALS AND METHODS

Patient population

We retrospectively reviewed 68 consecutive patients with newly diagnosed primary GBM who underwent surgical treatment at several academic tertiary-care neurosurgical institutions: Nagoya University Hospital, Hamamatsu University Hospital, Oita University Hospital, and Shizuoka Cancer Center from May 2006 through June 2010 after TMZ was approved as the treatment agent for malignant gliomas by the National Ministry of Health and Welfare of Japan. The diagnosis of GBM was established by histological confirmation according to the WHO guidelines[8,9] independently by at least two expert neuropathologists. The clinical, operative, and hospital course records were reviewed. Information collected from clinical notes included patient demographics, pre- and postoperative neuroimaging, and adjuvant therapy. Preoperative Eastern Cooperative Oncology Group performance status (ECOG PS) scores were assigned by the clinician at the time of evaluation and were available in the chart for review for all patients. The study was approved by the institutional review board at each participating hospital and complied with all provisions of the Declaration of Helsinki.

Treatment

Radiotherapy

After undergoing surgery, the patients received focal external-beam radiotherapy by conventional radiation planning to approximately 60 Gray (Gy) (±5% total dose), with daily concurrent TMZ at 75 mg/m^2 throughout the course of radiotherapy.

Chemotherapy

All patients received the standard Stupp regimen.[1] In the absence of grade 3 or 4 hematological excessive toxicity, TMZ administration was continued until clinical or radiological evidence of disease progression was observed. Of these 68 patients, 39 patients (57.4%) received adjuvant IFN-β treatment (Table 1). Patients in Nagoya University and Oita University received chemotherapy consisting of IFN-β. There were no significant differences in any of the clinical parameters and genetic, epigenetic parameters (i.e., age, sex, preoperative PS, tumor location, extent of resection, genetic and epigenetic alterations between the institutions using regimen with and without IFN-β. The IFN-β chemotherapy regimen comprised 3 million international units (MIU)/body administered intravenously on alternate days during radiotherapy and TMZ-induction chemotherapy.[10,11] At the end of the induction period, after a 4-week interval, the patients were administered 3 MIU/body of IFN-β on the first morning every 4 weeks during TMZ maintenance chemotherapy. In the case of tumor progression, salvage or second-line therapy was administered at the investigators' discretion; most patients received additional chemotherapy.

Response Evaluation During Treatment

Both radiological and clinical findings were used to evaluate the response. Follow-up magnetic resonance imaging (MRI) was performed for alternate cycles. If the MRI showed continued increase in enhancement, the case was considered as tumor progression. If re-resection was performed for a recurrent mass lesion, histological interpretation formed the basis for definitive diagnosis (treatment-related necrosis vs recurrent tumor).