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ORIGINAL ARTICLE

Randomized trial of chemoradiotherapy and adjuvant chemotherapy with nimustine (ACNU) versus nimustine plus procarbazine for newly diagnosed anaplastic astrocytoma and glioblastoma (JCOG0305)

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Abstract
Purpose Glioblastoma (GBM) is one of the worst cancers in terms of prognosis. Standard therapy consists of resection with concomitant chemoradiotherapy. Resistance to nimustine hydrochloride (ACNU), an alkylating agent, has been linked to methylguanine DNA methyltransferase (MGMT). Daily administration of procarbazine (PCZ) has been reported to decrease MGMT activity. This study investigated the efficacy of ACNU + PCZ compared to ACNU alone for GBM and anaplastic astrocytoma (AA).

Methods Patients (20–69 years) who had newly diagnosed AA and GBM were randomly assigned to receive radiotherapy with ACNU alone or with ACNU + PCZ. The primary endpoint was overall survival (OS). This was designed as a phase II/III trial with a total sample size of 310 patients and was registered as UMIN-CTR C000000108.
Results After 111 patients from 19 centers in Japan were enrolled, this study was terminated early because temozolomide was newly approved in Japan. The median OS and median progression-free survival (PFS) with ACNU alone ($n = 55$) or ACNU + PCZ ($n = 56$) in the

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intention-to-treat population were 27.4 and 22.4 months ($p = 0.75$), and 8.6 and 6.9 months, respectively. The median OS and median PFS of the GBM subgroup treated with ACNU alone ($n = 40$) or ACNU + PCZ ($n = 41$) were 19.0 and 19.5 months, and 6.2 and 6.3 months, respectively. Grade 3/4 hematologic adverse events occurred in more than 40 % of patients in both arms, and 27 % of patients discontinued treatment because of adverse events.

Conclusions The addition of PCZ to ACNU was not beneficial, in comparison with ACNU alone, for patients with newly diagnosed AA and GBM.

Keywords Glioblastoma · Anaplastic astrocytoma · Nimustine · ACNU · Procarbazine · MGMT

Abbreviations

GBM	Glioblastoma
AA	Anaplastic astrocytoma
ACNU	Nimustine hydrochloride
BCNU	Carmustine
TMZ	Temozolomide
MGMT	Methylguanine DNA methyltransferase
WHO	World Health Organization
PFS	Progression-free survival
OS	Overall survival
RT	Radiotherapy
HR	Hazard ratio
AE	Adverse event
ND	Not determined
CR	Complete response
PR	Partial response
SD	Stable disease
PD	Progressive disease
WBC	White blood cell
3D-CRT	Three-dimensional conformal radiotherapy
CT	Computed tomography
IMRT	Intensity-modulated radiation therapy
BEV	Beam's eye views
DVH	Dose–volume histograms
GTV	Gross tumor volume
CTV	Clinical target volume
PTV	Planning target volume
ICRU	International Commission on Radiation Units
FLAIR	Fluid-attenuated inversion recovery
OAR	Organ-at-risk

Introduction

Glioblastoma (GBM) is one of the worst cancers in terms of prognosis, with almost all patients experiencing progression without cure. According to the report of the Brain Tumor Registry of Japan, the %5-year survival of World Health Organization (WHO) grade IV GBM is 6.9 % and that of WHO grade III anaplastic astrocytoma (AA) is 33.9 % [1].

Standard therapy against GBM consists of the maximal resection that is safely possible, with concomitant chemo-radiotherapy. Currently, temozolomide (TMZ) is the

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standard agent used in the treatment of GBM. However, before the TMZ era, nitrosourea had been widely used for GBM and AA. The Glioma Meta-analysis Trialists Group described that chemotherapy including nitrosourea showed significant prolongation of survival, with a hazard ratio of 0.85 ($p < 0.0001$) [2].

Nimustine hydrochloride (ACNU) was developed in Japan, and for more than 20 years since 1980, it has been the standard chemotherapeutic agent against gliomas [3]. Wolff et al. [4] analyzed 364 studies, including a total of 24,193 patients with high-grade glioma, and reported that the survival gain in the 15 ACNU-treated cohorts was 8.9 months, compared to those who received different drugs or no chemotherapy. Takakura et al. [5] reported that the overall survival (OS) of AA and GBM treated by radiotherapy (RT) and concomitant ACNU were 36 and 12 months, respectively. Furthermore, the response rate of a more than 50 % reduction in tumor size was 46.2 % in both AA and GBM. Alkylating agents, including ACNU and procarbazine (PCZ), confer cytotoxic effects on glioma cells by alkylation at the O^6-position of guanine in DNA. This results in the formation of DNA cross-links [6]. Methylguanine DNA methyltransferase (MGMT) removes methylation damage induced by nitrosourea from the O^6-position of DNA guanines before cell injury, and this enzyme was detectable in 76 % of glioma tissues [7]. MGMT in glioma cells is a primary defense against nitrosourea, but the cellular methyltransferase activity of MGMT is exhausted after MGMT takes effect. Daily administration of PCZ for 10 days was reported to cause the accumulation of O^6-methylguanine; it also decreased MGMT activity in rat liver [8] and lymphocytes in lymphoma patients [9]. Inhibition of MGMT by O^6-benzylguanine increased the cytotoxicity of TMZ and carmustine (BCNU) to tumor cells [10]. From these results, it can be predicted that daily administration of PCZ, by depleting MGMT activity, will increase the efficacy of ACNU against AA and GBM.

To prove this hypothesis and establish a more potent standard therapy for AA and GBM, the Brain Tumor Study Group of the Japan Clinical Oncology Group (JCOG) conducted this clinical trial. The study was terminated at the end of the phase II part. The current report describes the final outcome of the study.

Subjects and methods

Patient eligibility criteria

Patients aged 20 to less than 70 years of age who had newly diagnosed and histologically proven supratentorial GBM or AA were eligible for this study. Patients were enrolled between 3 and 14 days after their operation. To be eligible, a patient's preoperative MRI had to show that more than 50 % of the tumor was located in supratentorial areas, except the optic nerve, olfactory nerve, or pituitary gland. Eligible patients had Eastern Cooperative Oncology Group (ECOG) performance status (PS) of 0–2 or 3 (only in cases with neurologic symptoms caused by a tumor) and adequate hematologic, pulmonary, renal, and hepatic function, defined as follows: white blood cell (WBC) count $\geq 3.0 \times 10^3$/mL, hemoglobin level ≥ 8.0 g/dL, platelets count $\geq 1.0 \times 10^6$/mL, aspartate transaminase (AST) level ≤ 100 IU/L, alanine transaminase (ALT) level ≤ 100 IU/L, serum creatinine level ≤ 1.0 mg/dL. Additionally, written informed consent was obtained from all the participating patients. We excluded patients with multiple or disseminated tumors or large tumors in which the planned target volume for irradiation exceeded 1/3 of the whole-brain volume. Additionally, we also classified as ineligible any patient who was pregnant, had meningitis, pneumonia, diabetes mellitus with insulin injection, myocardial infarction, or unstable angina pectoris within the last 3 months, mental disorders, a history of pulmonary fibrosis or interstitial pneumonia, or other forms of active cancer occurring within 5 years of treatment. The study protocol was approved by JCOG Protocol Review Committee and institutional review board at each center.

Treatment

After the confirmation of the eligibility criteria, registration was made by telephone or fax to the JCOG Data Center. Patients were randomized within 14 days of surgery to either ACNU with RT (the control arm, A) or to ACNU + PCZ with RT (the experimental arm, B) (Fig. 1a) by a minimization method with adjustment factors consisting of histology (GBM vs. AA), age (younger than 60 vs. 60 years or older), residual tumor (presence vs. absence), and institution. Residual tumor was assessed using a gadolinium-enhanced MRI obtained within 72 h of the surgery.

Radiotherapy with concomitant chemotherapy was started within 3 weeks after the surgery. Patient positioning and immobilization with an individual head mask and computed tomography (CT)-based planning were required. Treatment was delivered using linear accelerators with nominal energies ≥ 4 MV. Intensity-modulated radiation therapy (IMRT) technique was not permitted. All fields were to be treated every day. Three-dimensional conformal radiotherapy (3D-CRT) planning including the use of beam's eye views (BEV) and dose–volume histograms (DVH) were recommended for volumetric dose evaluation. Quality assurance reviews were done at the Radiotherapy Support Centre in Tokyo, Japan, with feedback sent to each

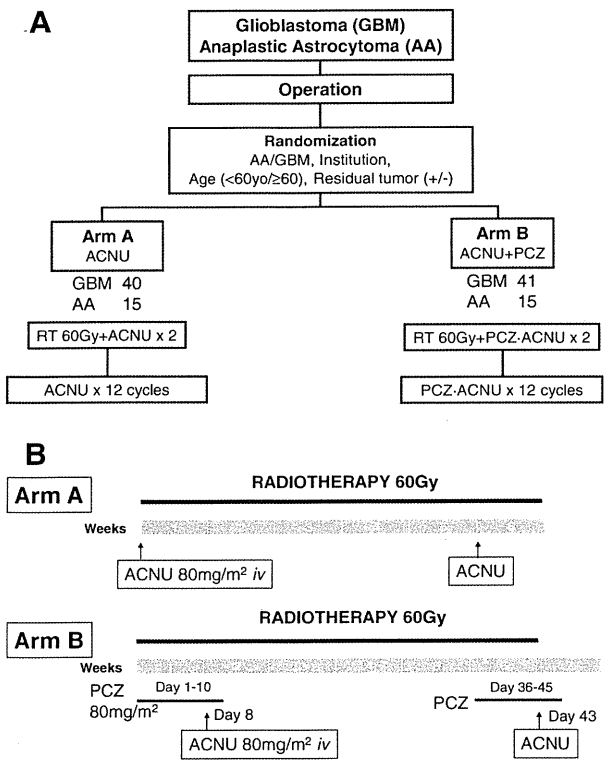

Fig. 1 **a** Study design of JCOG 0305: RT + ACNU versus RT + ACNU + PCZ; 40 patients with GBM and 15 patients with AA were assigned to *arm A*, and 41 patients with GBM and 15 patients with AA were assigned to *arm B*. **b** Treatment schedule of RT + ACNU (*Arm A*) and RT + ACNU + PCZ (*Arm B*)

institution by the radiotherapy study coordinator (Minako Sumi). The minimum and maximum dose to the PTV should be comprised between 95 and 107 % of the International Commission on Radiation Units (ICRU) reference point dose. The gross tumor volume (GTV) was defined as the primary tumor with or without enhancement on CT or magnetic resonance imaging (MRI). The clinical target volume 1(CTV1) included GTV, the resection cavity and surrounding edema (high-intensity area on T2-weighted or fluid-attenuated inversion recovery (FLAIR) image) plus a 1.5-cm margin. The CTV2 included GTV and the resection cavity plus a 1.5-cm margin. Planning target volume (PTV) was defined as CTV plus a margin of 0.5 cm or more. The doses for PTV1 and PTV2 were 50 and 10 Gy, respectively. The protocol required contouring organ-at-risk (OAR), including optic chiasm, brain stem, and retina. Cumulative doses to the optic chiasm and brainstem were limited to a maximum dose of 50 and 45 Gy for the retina.

In the control arm A, 80 mg/m^2 of ACNU was administered intravenously on days 1 and 36 during RT (Fig. 1b). In the experimental arm B, 80 mg/m^2 of oral PCZ was administered daily from days 1 to 10 and days 36 to 45, and given together with intravenous ACNU (80 mg/m^2) on days 8 and 43. Adjuvant therapy consisting of 80 mg/m^2 of ACNU alone in arm A or ACNU plus PCZ (PCZ: 80 mg/m^2 orally on days 1–10, ACNU: 80 mg/m^2 intravenously on day 8) in arm B started 56 days from the final administration of ACNU and was given every 8 weeks, for up to 12 cycles. Doses of ACNU and PCZ were calculated using actual body surface area, reduced for toxicity, and were not escalated.

Evaluations and follow-up

Baseline and follow-up examinations included vital signs, subjective symptoms, neurologic examination, MRI scan, and blood and serum laboratory examinations. For each patient, these examinations were performed weekly, with the exception of MRI scans, which were performed between the end of the initial chemoradiotherapy and the beginning of adjuvant therapy. All examinations were performed before each cycle of adjuvant chemotherapy, at a frequency of nearly every 2 months. After completion of the treatment protocol, patients were assessed every 3 months until progression. Toxicity was graded using the National Cancer Institute Common Toxicity Criteria (version 2). Findings of radiation necrosis were also assessed on MRI. Each patient was required to undergo a follow-up examination for at least 2 years from the date of randomization.

Tumor progression on MRI was defined according to Response Evaluation Criteria in Solid Tumors (RECIST), version 1.0 [11]. Progression of disease was defined as a 20 % increase in tumor size, as shown by contrast-enhanced imaging, or the development of new lesions, neurologic deterioration, or death by any cause. Further treatment at recurrence or progression was discretionary, but recorded.

A central pathology review by 3 independent pathologists (Yoichi Nakazato, a member of the Working Group for WHO 2007 classification; Nobuaki Funata; and Toru Iwaki) was performed and determinations given. A central review of radiological response was also performed.

Statistical analysis

When we planned this study, TMZ had been widely approved and was used worldwide. However, TMZ was not available in Japan. ACNU remained the standard therapy in Japan, but there was no sufficient data regarding this treatment. We planned a phase II/III clinical trial, with the phase II part designed to confirm the feasibility of ACNU and ACNU + PCZ.

The primary and secondary endpoints for the phase II part were %6-month survival and adverse events (AEs) in ACNU + PCZ arm. The primary endpoint of the phase III part was OS, while the secondary endpoints were PFS, response rate, complete response rate, and AEs.

Overall survival was calculated from the date of random assignment to the date of death from any cause and censored at the last follow-up for event-free patients. PFS was calculated from the date of randomization to the date of progression or death from any cause and censored at the last verifiable progression-free date for event-free patients. OS and PFS were estimated by the Kaplan–Meier method. OS was analyzed by the stratified log-rank test for eligible patients with adjustment factors, excluding institution. Unstratified log-rank tests were used for the analysis of PFS and subgroup analyses of OS and PFS. Fisher's exact test was used for categorical data. All p values are two-sided, except for primary analysis of OS.

We assumed %2-year survivals in AA and GBM for arm A were 50 and 20 %, and the ratio of those patients with AA or GBM enrolled in this study was expected to be 2:3. The phase III study was designed to enroll 155 patients per arm with 5 years of accrual and 2 years of follow-up, including those for the phase II part and about 10 % of ineligible patients, to achieve at least 75 % power to detect a hazard ratio (HR) of 0.74, with a one-sided alpha of 0.05 [12].

Three interim analyses were planned. The first was planned during phase II to test whether %6-month survival in arm A was superior to the predefined threshold (80 %), with a one-sided alpha of 0.1 and beta of 0.2, when 56 patients were included in ACNU + PCZ arm. The second and third interim analyses of OS were planned during phase III. For analyses of phase III part, multiplicity was adjusted by the Lan and DeMets alpha-spending function with the O'Brien and Fleming stopping boundary to control the type I error for primary endpoint.

In March 2007, protocol was amended to stop patient accrual after 111 patients had enrolled and to carry out the final analysis without planned interim analyses for both of phase II and phase III part. This was done because toxicity of both arms was unexpectedly high in phase II and because TMZ became available in Japan.

All statistical analyses were performed using SAS software, release 9.1 (SAS Institute, Cary, NC).

This trial is registered with UMIN-CTR (www.umin.ac.jp/ctr/), number C000000108.

Results

Patient characteristics

A total of 111 patients from 19 centers were randomly assigned to arm A ($n = 55$) or arm B ($n = 56$) from March 2004 to September 2006. Primary analyses were performed in September 2007, and the updated analyses were completed in July 2009. All patients were eligible for this study. Baseline characteristics were well balanced between the arms (Table 1). The median ages for arms A and B were 56 and 54 years, respectively. Total numbers for AA and GBM patients were 15 (27.3 %) and 40 (72.7 %) in arm A and 15 (26.8 %) and 41 (73.2 %) in arm B. PS 0 to 1 in arms A and B were 45 (81.8 %) and 41 (73.2 %), respectively. Eighteen (32.7 %) patients in arm A and 21 (37.5 %) patients in arm B underwent gross total removal, and no residual tumor was confirmed on MRI scans. The median duration of follow-up was 20.2 (range 0–48.0 months) for all eligible patients.

Treatment

Patient compliance with the treatment regimen is depicted in Table 2. Among the 111 total patients, 1 patient in arm A died from pulmonary embolism before the beginning of initial chemoradiotherapy. Fifty-three (96.3 %) patients in arm A completed initial chemoradiotherapy and received ACNU twice. In arm B, 48 out of 56 (85.7 %) patients received 2 cycles of PCZ + ACNU, and 8 patients (14.3 %) received 1 cycle of PCZ + ACNU in initial chemoradiotherapy. Eighteen (32.7 %) patients in arm A and 20 (35.7 %) patients in arm B failed to start adjuvant chemotherapy. Furthermore, 14 (25.5 %) patients in arm A and 23 (41.1 %) patients in arm B discontinued protocol therapy by the fourth cycle of adjuvant chemotherapy. The numbers of patients who received 4 cycles of chemotherapy or more were 23 (41.8 %) and 13 (23.2 %) in arm A and B, respectively. Only 5 (9.1 %) patients in arm A and 2 (3.6 %) patients in arm B completed the full protocol therapy. Nineteen (34.5 %) patients in arm A and 22 (39.3 %) patients in arm B discontinued the protocol for reasons other than completion of protocol or disease progression [arm A: 31 (56.4 %), arm B: 32 (57.1 %)]. Reasons for discontinuation were as follows: AEs [arm A: 6 (10.9 %), arm B: 13 (23.2 %)]; patient refusal related to AE

Table 1 Baseline characteristics in the ITT population

	Arm A ($n = 55$) (RT + ACNU)	Arm B ($n = 56$) (RT + PCZ + ACNU)
Age	56 (24–69)	54 (24–69)
Sex		
Male	32 (58.2 %)	33 (58.9 %)
Female	23 (41.8 %)	23 (41.1 %)
PS		
0, 1	45 (81.8 %)	41 (73.2 %)
2, 3	10 (18.2 %)	15 (26.8 %)
Histology		
Grade 3 (AA)	15 (27.3 %)	15 (26.8 %)
Grade 4 (GBM)	40 (72.7 %)	41 (73.2 %)
Surgery		
Gross total removal	18 (32.7 %)	21 (37.5 %)
Partial removal	30 (54.5 %)	26 (46.4 %)
Biopsy only	7 (12.7′ %)	9 (16.1 %)

Table 2 Compliance

	Arm A ($n = 55$) (RT + ACNU)	Arm B ($n = 56$) (RT + PCZ + ACNU)
RT (completion)	54 (98.2 %)	56 (100 %)
Initial chemotherapy		
1 cycle	1 (1.8 %)	8 (14.3 %)
2 cycles	53 (96.4 %)	48 (85.7 %)
Adjuvant chemotherapy		
None	18 (32.7 %)	20 (35.7 %)
1–3 cycles	14 (25.5 %)	23 (41.1 %)
4–6 cycles	9 (16.4 %)	7 (12.5 %)
7–11 cycles	9 (16.4 %)	4 (7.1 %)
12 cycles (completion)	5 (9.1 %)	2 (3.6 %)

[arm A: 3 (5.5 %), arm B: 8 (14.3 %)]; and patient refusal not related to AE [arm A: 7 (12.7 %), arm B: 1 (1.8 %)].

After discontinuation of the protocol, 28 (50.9 %) patients in arm A and 29 (51.8 %) patients in arm B received TMZ as further treatment.

Central review of histology

The central pathology review diagnosis of all cases was performed according to the WHO 2007 classification (Table 3). Among 81 GBM in the intention-to-treat (ITT) population, 69 (85.2 %), 4 (4.9 %), 3 (3.7 %), 2 (2.5 %), and 3 (3.7 %) were diagnosed as GBM, anaplastic oligoastrocytoma (AOA), anaplastic oligodendroglioma (AO), AA, and others, respectively. Only 10 cases (33.3 %) were diagnosed as AA among the 30 AA in the ITT population, while 8 (26.7 %), 5 (16.7 %), 3 (10.0 %), 2 (6.7 %), and 2 (6.7 %) were confirmed as GBM, AOA, diffuse astrocytoma, pilocytic astrocytoma, and others, respectively. Among all 111 cases, phenotype change of astrocytic to oligodendroglial tumor occurred in 14 cases (12.6 %). Finally, 77 and 12 patients were diagnosed with GBM and AA, respectively, by central pathology review.

Response rate

The overall radiographic response rate for 66 measurable diseases after surgery, based on RECIST guideline, was assessed by Diagnostic Radiology Committee. The response rate was 21.2 % (7/33) in arm A and 6.1 % (2/33) in arm B. In GBM patients from the ITT population, response rates were 25.0 % in arm A [3 CR (complete response), 2 PR (partial response), 1 SD (stable disease), 14 PD (progressive disease)] and 9.1 % in arm B [1 CR, 1 PR, 1 SD, 19 PD] ($p = 0.23$). In AA patients from the ITT population, response rates were 15.4 % in arm A (2 PR, 1 SD, 10 PD) and 0 % in arm B (11 PD) ($p = 0.48$).

Progression-free survival

In the entire ITT population, PFS was 8.6 months [95 % confidence interval (CI); 5.1–20.5] in arm A ($n = 55$, 44 events), compared with 6.9 months (95 % CI 5.1–9.0, $p = 0.36$) in arm B ($n = 56$, 47 events) (Fig. 2a). According to grades in the ITT population, PFS of GBM in arm A ($n = 40$, 33 events) and B ($n = 41$, 35 events) was 6.2 (95 % CI 4.2–13.2) and 6.3 months (95 % CI 3.0–8.9), respectively ($p = 0.35$) (Fig. 2b). PFS of AA in arm A ($n = 15$, 11 PD) and B ($n = 15$, 12 events) was 8.6 (95 % CI 5.1–35.4) and 9.0 months (95 % CI 5.8–21.8), respectively ($p = 0.83$) (Fig. 2c). No difference was observed between the arms in any subgroup defined by histology, presence of remaining tumor, or age under or over 60.

In the subgroup defined by central pathology review, the PFS of GBM in arms A ($n = 37$, 33 events) and B ($n = 40$, 36 events) was 5.1 (95 % CI 4.2–10.3) and 5.7 months (95 % CI 2.7–8.4), respectively ($p = 0.49$) (Fig. 2d). The PFS of AA and AOA in arm A ($n = 9$, 4 events) and B ($n = 12$, 9 events) was ND (not determined) and 7.9 months (95 % CI 5.2–22.5), respectively ($p = 0.21$).

Table 3 Local diagnosis and central pathology review

Grade	Histology	Local diagnosis	Central pathology review		
			Total	Arm A	Arm B
IV	*Glioblastoma*	83	77	37	40
III	*Anaplastic astrocytoma*	30	12	6	6
III	Anaplastic oligoastrocytoma		9	3	6
III	Anaplastic oligodendroglioma		3	2	1
III	Anaplastic ependymoma		1	1	0
II	Diffuse astrocytoma		4	1	3
II	Oligoastrocytoma		1	1	0
II	Oligodendroglioma		1	1	0
I	Pilocytic astrocytoma		2	2	0
	Sarcoma		1	1	0
	Total	111	111	55	56

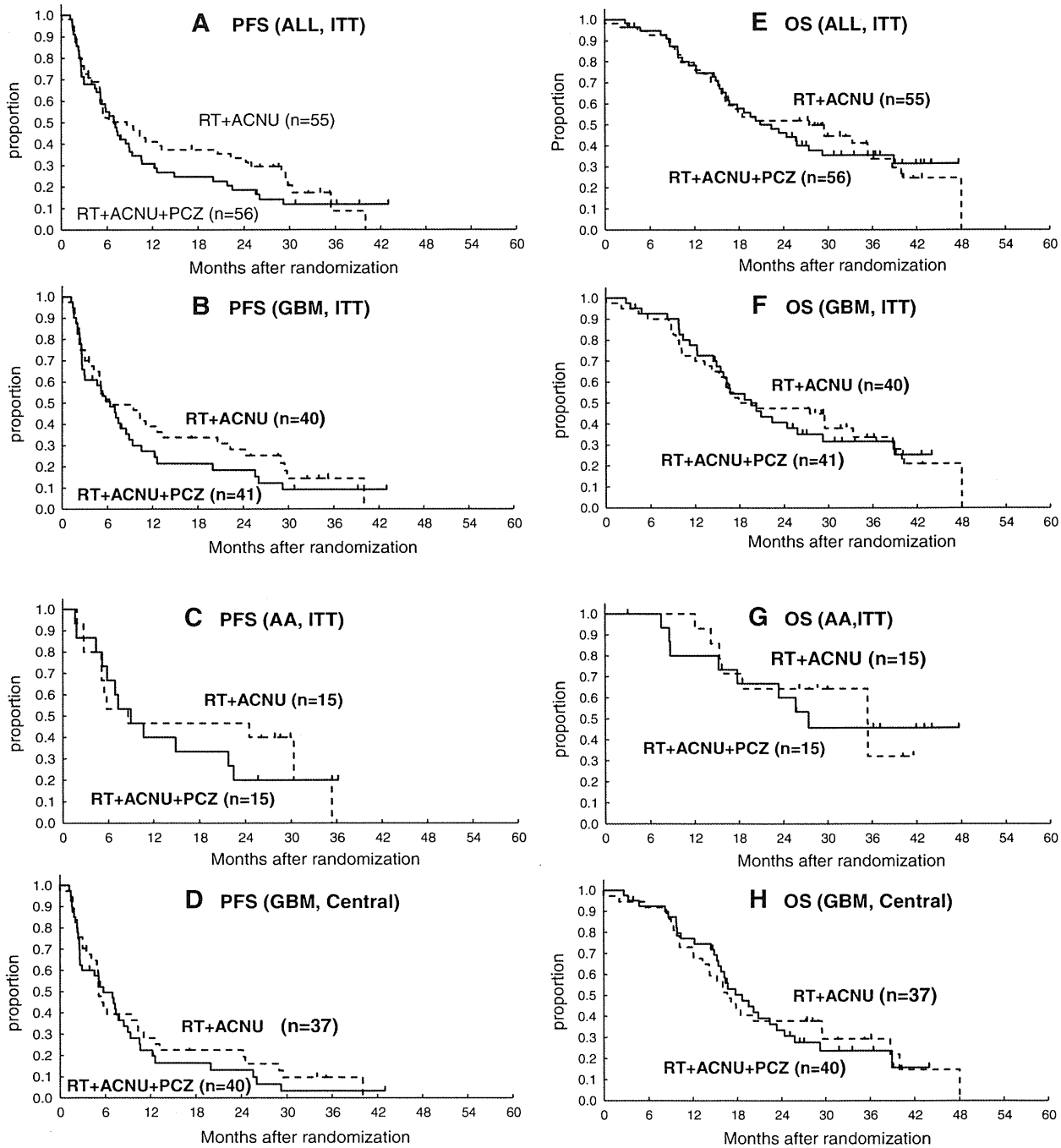

Fig. 2 Progression-free survival in the intention-to-treat (ITT) population (**a**), the GBM subgroup (**b**), the AA subgroup (**c**), and the GBM subgroup with central pathology review (**d**). Overall survival in the ITT population (**e**), the GBM subgroup (**f**), the AA subgroup (**g**), and the GBM subgroup with central pathology review (**h**). RT + ACNU alone (*solid line*), RT + ACNU + PCZ (*dotted line*)

Overall survival and cause of death

From the entire ITT population, 35 patients died in each group. In arm A ($n = 55$), OS was 27.4 months (95 % CI 16.2–35.4), compared with 22.4 months (95 % CI 16.4–29.2) in arm B ($n = 56$) (Fig. 2e). The %2-year survival in arms A and B was 51.9 % and 46.2 %, respectively. There was no difference between the 2 arms ($p = 0.75$ and pre-planned, one-sided $p = 0.62$, by stratified log-rank test).

The OS of GBM subgroup in arms A ($n = 40$, 28 death) and B ($n = 41$, 27 death) was 19.0 (95 % CI 15.2–33.3) and 19.5 months (95 % CI 15.8–29.2), respectively ($p = 0.90$) (Fig. 2f). The %2-year survival in arms A and B was 48 and 41 %, respectively. The OS of AA subgroup in arms A ($n = 15$, 7 death) and in arm B ($n = 15$, 8 death) was 35.4 [95 % CI 15.7–not estimated (NE)] and 27.4 months (95 % CI 17.8–NE), respectively ($p = 0.88$) (Fig. 2g). There were no differences between the arms of any subgroup.

In the subgroups defined by central pathology review, the OS of GBM in arm A ($n = 37$, 28 death) and in arm B ($n = 40$, 29 death) was 16.6 (95 % CI 13.3–29.5) and 18.7 months (95 % CI 15.4–23.4), respectively ($p = 0.92$) (Fig. 2h). The %2-year survival in arms A and B was 38 and 34 %, respectively. The OS of AA and AOA in arm A ($n = 9$, 3 death) and B ($n = 12$, 4 death) was 33.3 months (95 % CI 15.7–33.3) and NE, respectively ($p = 0.83$).

Among the 70 total deaths, 31/35 (88.6 %) patients in arm A and 32/35 (91.4 %) in arm B experienced neuronal death of an original tumor. One patient (2.9 %) in arm A and 2 (5.7 %) patients in arm B contracted treatment-related pneumonia and died from that illness. Other causes of death were pulmonary embolism (1), pneumonia (2), and unknown (1).

Toxicity

Toxicity was assessed in 110 patients receiving initial therapy and in 73 patients receiving adjuvant chemotherapy. The most frequent grade 3/4 toxicities, experienced by more than 10 % of patients, were hematologic, neurologic, gastrointestinal, and hepatic AEs (Table 4). Patients in both arms frequently experienced leukopenia and neutropenia; more than half of the patients in arm B experienced these AEs during adjuvant therapy as well as during initial therapy. More than 40 % of patients in arm A also experienced these hematologic events even during adjuvant therapy. Grade 4 neutropenia was observed in 5.6 and 39.3 % of patients in arms A and B during initial chemoradiotherapy and 11.1 and 15.6 % during adjuvant therapy. Grade 3/4 nausea and anorexia were seen in 10.7 and 16.1 % of patients in initial therapy in arm B, but were rare in the adjuvant-therapy subgroups in both arms. One patient in arm B had cerebral infarction. Extrapyramidal signs, including tremors or involuntary movements, occurred in 2 patients in each arm.

Grade 3/4 pneumonitis occurred in 1 patient in arm A and 2 in arm B during the entire treatment period. Opportunistic infections—including 2 cases of *Pneumocystis jirovecii* pneumonia (PCP), 1 case of oral candidiasis, and 2 case of herpes zoster—occurred in arm B. One patient (1.8 %) in arm A and 2 (3.6 %) patients in arm B died from treatment-related pneumonia, and 1 of these patients in arm B had PCP. One patient in arm B died from sepsis and acute respiratory distress syndrome after initial therapy. One patient in arm A died from pulmonary embolism before starting chemoradiotherapy, and 1 patient in arm A and 2 patients in arm B died from pneumonia following tumor progression.

Radiation necrosis was observed in 2 out of 54 (3.7 %) patients in arm A and 1 out of 56 (1.8 %) patients in arm B. During surgery, 1 patient in arm A was found to have radiation necrosis. Pseudo-progression within 3 months after chemoradiotherapy was not suspected in any patient.

Discussion

This study aimed to evaluate the efficacy and safety of treatment with ACNU + PCZ compared to ACNU alone as concomitant chemoradiotherapy against AA and GBM. We found no obvious differences in OS or PFS for AA and GBM between the treatment groups, but patients treated with ACNU + PCZ experienced more adverse effects than those treated with ACNU alone. TMZ is an effective regimen for malignant gliomas with less toxicity than our ACNU regimens, but it was not approved in Japan when this study began. At the end of the phase II part of this study, TMZ became available even in Japan, so this study was terminated at that point.

Methylguanine DNA methyltransferase is a major DNA repair protein and is implicated in resistance of glioma cells to alkylating agents [13]. Transcriptional silencing by MGMT promoter methylation results in inhibition of MGMT expression [14], and thus MGMT promoter methylation is strongly associated with survival in glioma patients treated with either nitrosourea or TMZ [15–17]. The status of the promoter of MGMT in primary tumors was frequently observed to change from methylated to unmethylated in recurrent tumors following ACNU or TMZ treatment [18, 19], which constitutes one of the mechanisms behind malignant gliomas' resistance to nitrosourea and TMZ. The rationale for treatment with ACNU + PCZ is that daily application of PCZ depletes MGMT activity, increasing sensitivity of AA and GBM to ACNU. Dose-dense TMZ therapy based on the theory of depletion of MGMT [20, 21], or BCNU or TMZ with direct inhibition of MGMT by O^6-benzylguanine [22, 23] has been shown in previous studies to be effective for GBMs. However, there was no difference in OS found between standard and dose-dense TMZ for newly diagnosed GBMs [20].

While we were conducting this study, the European Organization for Research and Treatment of Cancer (EORTC) Brain Tumor and Radiotherapy Groups and the

Table 4 Toxicity

Grade 3/4 adverse events	Initial therapy with RT ($n = 110$) (%)		Adjuvant therapy ($n = 73$) (%)	
	Arm A	Arm B	Arm A	Arm B
Hematologic				
Leukopenia	38.9	73.2	40.5	69.4
Neutropenia	38.9	76.8	44.4	56.3
Thrombocytopenia	5.6	50.0	40.5	50.0
Anemia	0	8.9	10.8	8.3
Neurologic				
Seizure	9.3	7.1	5.4	8.8
Speech impairment	11.1	10.7	5.4	2.9
Neuropathy-motor	11.1	12.5	0	0
Extrapyramidal sign	0	0	5.4	2.7
Pulmonary (pneumonitis)	0	3.6	2.7	0
Gastrointestinal				
Nausea	0	10.7	0	0
Anorexia	1.9	16.1	0	2.9
Hepatic				
AST	3.7	12.5	2.9	2.9
ALT	3.7	16.1	2.9	8.8
Total bilirubin	1.9	5.4	0	0
Renal (creatinine)	0	0	0	0
Metabolic				
Hyponatremia	1.9	8.9	5.9	2.9
Hypokalemia	1.9	7.1	2.9	2.9
Fever	0	3.6	0	0
Dermatologic: erythema	3.7	5.4	0	2.9

National Cancer Institute of Canada (NCIC) Clinical Trials Group (EORTC/NCIC TMZ study) reported, in 2005, that RT + TMZ significantly prolonged the survival of GBM patients compared to RT alone [24]. The median PFS, OS, and 2-year survival for RT + TMZ were 6.9, 14.6 months, and 26.5 %, respectively [24]. Although our results compared favorably with the EORTC/NCIC TMZ study, the PFS of RT + ACNU alone for GBMs in our ITT population and in GBM subgroups in central pathology review were 6.2 and 5.1 months, shorter than those from the EORTC/NCIC TMZ study. Since more than half of the patients in our study underwent TMZ treatment following disease progression, it is possible that TMZ rescued these patients with progression after ACNU regimens and prolonged the survival of these patients.

The incidence of grade 3/4 hematologic AEs—such as leukopenia, neutropenia, and thrombocytopenia—were reported to be 5, 4, and 11 %, respectively, in adjuvant TMZ therapy in the EORTC/NCIC TMZ trial [24]. Compared to TMZ, even ACNU alone caused severe hematologic AEs in 40 % of the patients in our study, and most of those patients in both arms discontinued the treatment protocol due to AEs or patient refusal related to AEs. It is noteworthy that approximately 30 % of patients in both arms failed to start adjuvant chemotherapy. The low completion rate of our protocol might explain the lack of differences in PFS and OS between the arms. After 2 patients in arm B experienced PCP, prophylactic use of cotrimoxazole (trimethoprim–sulfamethoxazole) against PCP was recommended in this study and was found to be useful.

Radiation necrosis has been reported in 2.5–21 % of patients undergoing chemoradiotherapy against malignant gliomas [25]. This complication was observed in 2.7 % of the patients in our study, but was tolerable. "Pseudo-progression" is the phenomenon of transient early disease progression after treatment with chemoradiotherapy consisting of TMZ for GBM progressive and enhancing lesions, as shown on MRI images taken immediately after treatment [25]. No patients in our study were suspected of pseudo-progression within 6 months after beginning chemoradiotherapy.

In general, the difference in histological diagnosis for local versus central pathology review is a major problem in the conduct of clinical trials on gliomas [26]. In our study, the concordance of GBM and AA between local and central diagnosis was low, but nearly identical to previous reports. In the EORTC/NCIC TMZ trial, central pathology review was performed in 85 % of cases, which confirmed the diagnosis of GBM in 93 % of the reviewed cases; 3 % had AA or AOA. In the phase III study of RT versus RT + BCNU + dibromodulcitol (EORTC 26882), of the 193 cases of AA diagnosed by the local pathologist, 176 were reviewed by the central pathologist. At review, 61 patients (35 %) were diagnosed with AA, 13 (8 %) with AOA, 4 (2 %) with AO, 44 (25 %) with GBM, 41 (23 %) with low-grade gliomas, and 13 (7 %) with another diagnosis [27].

The WHO classification system reflects the prognoses depending on grade I–IV tumors, or astrocytic or oligodendroglial tumors. However, it is based on morphological descriptions and contains subjective elements; thus, inter-observer variation occurs. The boundaries between grades II, III, and IV in gliomas are unclear, and there is a trend toward a more frequent diagnosis of oligodendroglial tumors [28]. Central pathological review before inclusion of a patient into clinical study is ideal, but it is very difficult to complete for aggressive grade III/IV tumors. Even if central review before enrollment is difficult in a multi-institutional setting, it is indispensable to perform post hoc central review at least in order to appropriately interpret the results of clinical studies of gliomas. A consensus meeting might also be useful before commencing clinical studies in order to gain concordance between local and central diagnoses. More objective classification of tumors based on

genotype, such the IDH1/2 mutation or 1p/19q codeletion, should be included in at least the stratification factor and subgroup analysis.

Conclusions

No significant differences in OS or PFS were found between ACNU alone and ACNU + PCZ in either AA or GBM. We found that ACNU + PCZ treatment was more toxic in our treatment schedule. Therefore, we conclude that the addition of PCZ to ACNU was not beneficial for newly diagnosed, high-grade astrocytomas as compared to ACNU alone. Considering the greater number of AEs associated with ACNU regimens, RT + TMZ should serve as a standard therapeutic regimen in the treatment of newly diagnosed AA and GBM.

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Conflict of interest The authors declare that they have no conflict of interest.

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ORIGINAL ARTICLE

Secondary glioblastomas with *IDH1/2* mutations have longer glioma history from preceding lower-grade gliomas

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Abstract Isocitrate dehydrogenase (*IDH*)*1/2* mutations have been proposed as a genetic marker for secondary glioblastoma (sGBM). This study aimed to evaluate the impact of the *IDH1/2* mutations on the clinical course and genetic alterations of sGBMs, which histopathologically progressed from lower-grade gliomas. We investigated 18 sGBMs, including 8 sGBMs with *IDH1/2* mutations (sGBM-Mut) and 10 with wild-type *IDH1/2* (sGBM-Wt). The median overall survival time of patients with sGBM-Mut was significantly longer than that of patients with sGBM-Wt (68.2 vs. 25.3 months). The median time from initial diagnosis to sGBM diagnosis was also significantly longer for sGBM-Mut than for sGBM-Wt (50.1 vs. 13.4 months). There was no difference in the median survival time from the sGBM diagnosis between sGBM-Mut and sGBM-Wt (6.75 vs. 6.8 months). All sGBM-Mut (7 of 7) and 6 of 9 sGBM-Wt had *TP53* mutations, and the remaining one-thirds of sGBM-Wt had neither *TP53* mutations nor 1p/19q codeletion. These observations suggest that *IDH1/2* mutations have an impact on the glioma history of sGBM with different genetic pathway. The aggressive progression to sGBM-Wt suggest the need for more intense treatment to the *IDH1/2* wild-type tumors.

Keywords Glioblastoma · Glioma · *IDH* · Malignant progression

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Introduction

Glioblastomas (GBM) are the most frequent and malignant brain tumors. Conventionally, they have been divided into two subtypes: one develops through progression from grade II or III gliomas (secondary GBM: sGBM) and the other arises de novo without such an antecedent (primary GBM: pGBM) [1, 2]. The discrimination between primary and sGBMs is dependent on the clinical history of evolution from less malignant gliomas as evidenced by histological studies. Genetically, pGBMs are characterized by *epidermal growth factor receptor* (*EGFR*) amplification/overexpression and *phosphatase and tensin homologue* (*PTEN*) mutation, whereas sGBMs more frequently contain p53 mutations, overexpression of *platelet-derived growth factor receptor*, and loss of heterozygosity (LOH) at 19q [2].

Isocitrate dehydrogenase (*IDH*) gene mutations are predominantly identified in World Health Organization (WHO) grade II and III gliomas as well as sGBMs, while being very rare in pGBMs [3–7]. *IDH1/2* mutations have been proposed as a molecular diagnostic marker for sGBMs [8, 9]. Gliomas with *IDH1/2* mutations were associated with better prognosis than those without *IDH1/2* mutations [5, 7, 10–12]. Moreover, Turcan et al. [13] revealed that *IDH1/2* mutations trigger CpG island methylator phenotype, and tumors with *IDH1/2* mutations showed different patterns of both genetic and epigenetic alterations from those without *IDH1/2* mutations [7, 14–18].

These findings suggest that the presence of *IDH1/2* mutation status may define a biologically distinct tumoral entity [16]. In pGBMs, clinical features and genetic alterations were shown to be different according to the *IDH1/2* mutations status: pGBM with *IDH1/2* mutations were associated with better outcome, predominance of frontal lobe locations, presentation and maintenance of proneural expression signature, less extent of necrosis and edema, presence of non-contrast-enhancing component, and greater oligodendroglial content compared to those with wild-type *IDH1/2* [16]. However, there have been few reports on the difference of clinical history and genetic features of sGBMs according to *IDH1/2* mutations status [9, 19].

We have previously reported the histopathological changes of 53 tumors initially diagnosed as grade II or III that later recurred. We noted 18 tumors that recurred as GBM, and of these, 8 tumors carried *IDH1/2* mutations (sGBM-Mut) and the other 10 did not (sGBM-Wt) [20]. In the current study, we investigated the clinical course and genetic alteration of these 18 cases of histopathologically defined sGBMs, in order to understand the impact of *IDH1/2* mutations on clinical features and mechanisms of malignant progression to sGBM.

Patients and methods

Patient characteristics

This study included 18 patients who were histopathologically diagnosed as grade II or III glioma that recurred as a GBM and treated at National Cancer Center Hospital, Tokyo, Japan, from January 1990 to June 2010. All tumor samples obtained at the first, second, and subsequent surgeries if performed, were histopathologically diagnosed by neuropathologists at our hospital according to the WHO classification.

Clinical records of the patients were reviewed and the following data were collected: clinical history; date of initial, second, and subsequent operations; postoperative adjuvant therapy; date of death or last hospital visit; tumor histopathology at each operation; and extent of resection. The definition of the extent of resection was based on the surgeon's operative notes and postoperative imaging studies. Time from initial diagnosis to the diagnosis of sGBM was calculated from the date of initial operation to the date of subsequent operation at which the GBM of the tumor was confirmed. Survival time from sGBM was calculated from the date of operation in which the GBM of the tumor was confirmed to the date of death. Overall survival time was calculated from the date of initial operation to the date of death. This study was approved by the internal review board of the National Cancer Center.

IDH1/2 mutations analysis

Previously, we analyzed *IDH1/2* mutations using tumor samples obtained at the surgery of sGBM diagnosis in 12 cases and at surgery of the preceding lower-grade tumors in 4 cases either by sequence analysis or by immunohistochemistry (IHC) [20].

TP53 mutation analysis

Polymerase chain reaction (PCR) for the *TP53* exon 5–8 was done with a GeneAmp PCR System 9700 (Applied Biosystems, Foster City, CA, USA) using the following primers, exon 5 (1): 5-TTATCTGTTCACTTGTGCCC-3 and 5-TCATGTGCTGTGACTGCTTG-3, 5 (2): 5-TCCC ACACCCCCGCCCGGCA-3 and 5-ACCCTGGGCAACC AGCCCTG-3, exon 6: 5-ACGACAGGGCTGGTTGCCC A-3 and 5-CTCCCAGAGACCCCAGTTG-3, exon 7: 5-G GCCTCATCTTGGGCCTGTG-3 and 5-CAGTGTGCA GGGTGGCAAGT-3, and exon 8: 5-CTGCCTCTTGC TTCTCTTTT-3 and 5-TCTCCTCAACCGCTTCTTGT-3. Thermocycling conditions consisted of 5 min at 95 °C, 35 cycles of 30 s at 95 °C, 30 s at 60 °C, and 60 s at 72 °C, followed by 10 min at 72 °C. After purification of the PCR products by QIAquick PCR Purification Kit (Qiagen, Maryland, USA), DNA sequencing for the *TP53* gene was done with an ABI PRISM 310 Genetic Analyzer (Applied Biosystems) using the same primers [21].

1p and 19q, *CDKN2A*, *PTEN*, and *EGFR* status by multiplex ligation-dependent probe amplification analysis

We used the SALSA P088 kit for 1p and 19q and SALSA P105-C1 kit for *PTEN*, *cyclin-dependent kinase inhibitor 2A* (*CDKN2A*), and *EGFR* (MRC Amsterdam, The Netherlands). Multiplex ligation-dependent probe amplification analysis was performed as described previously [22]. The 1p36 or 19q deletions were considered to be present when 5 of 6 markers for 1p36 and 5 of 8 markers for 19q in each of the chromosome arms had normalized ratios of <1.5 [11]. The *PTEN* deletion was considered to be present when more than 8 of 11 markers had normalized ratios of <1.6. *CDKN2A* homozygous deletion was considered to be present when more than 2 of 5 markers had normalized ratios of <0.8. *EGFR* amplification was considered to be present when more than 3 of 11 markers had normalized ratios of >4.0.

Statistical analysis

The Chi-squared test or Fisher's exact test was used to compare *IDH1/2* mutated and wild-type tumors for

correlations with sex, initial tumor grade, location, and age at diagnosis of the initial tumor, and age at recurrence as sGBM. The frequencies of *TP53* mutations, 1p/19q codeletion, *CDKN2A* homozygous deletion, *PTEN* deletion and *EGFR* amplification between sGBM with and without *IDH1/2* mutations were also analyzed. Time to the diagnosis of sGBM, survival time from sGBM diagnosis, and overall survival time were calculated using to the Kaplan–Meier method, and the differences were compared using the log-rank test. All analyses were conducted using JMP 8® software (SAS Institute Inc., Cary, NC, USA). In all cases, probability values <0.05 were considered statistically significant.

Results

Patient population and *IDH1/2* mutations

The clinical characteristics and genetic profiles of the 18 patients with sGBM are shown in Table 1. The study group comprised 12 male and 6 female patients with a median age of 30.5 years (range 20–64) at initial tumor diagnosis and of 36 years (range 21–65) at the time of recurrence as sGBM. Initial histopathological examination identified 8 diffuse astrocytomas (DA), 7 anaplastic astrocytomas (AA), 1 anaplastic oligodendroglioma (AO), and 2 anaplastic oligoastrocytomas (AOA). After the initial surgical resection, 14 patients received chemoradiotherapy, 2 received radiotherapy alone, and 2 received no adjuvant therapy. For the treatment of sGBM upon recurrence, after surgical resection, 6 patients were administered chemoradiotherapy, 10 were given chemotherapy alone, and 2 received no therapy because of poor performance status. The chemotherapy regimen for sGBM comprised of temozolomide (TMZ), nimustine hydrochloride, vincristine, carboplatin, and etoposide. Three of the patients had a distant tumor recurrence from initial tumor location, and they could receive chemoradiotherapy during the treatment of the sGBM (AA083, DA048, and AO004).

IDH1/2 mutations were found in the tumors of 8 patients. All detected *IDH* mutations were R132H, and no *IDH2* mutant tumors were seen. *IDH1/2* mutations were not observed in the tumors of 9 patients, confirmed by sequence analysis. For patient AA009, the tumor was negative for anti-*IDH1* R132H antibody on IHC [23]. Frozen tissue or paraffin-fixed specimens suitable for direct sequencing were not available for this patient, and further screening for *IDH1* mutations and *IDH2* mutations were not performed. Because the possibility of *IDH* mutations other than the *R132H IDH1* mutation was reported to be <10 % [4, 24], we classified this case as tumor with wild-type *IDH1/2* for further analyses.

IDH1/2 mutations and clinical presentation

Clinical data for our patient cohort is summarized in Table 2. The median age at initial tumor diagnosis for patients with wild-type and mutant *IDH1/2* tumors was 39.5 and 29.5 years, respectively. Further, the median age at diagnosis of the sGBM was 40.5 and 32.5 years for patients with wild-type and mutant *IDH1/2* tumors. Patients with wild-type *IDH1/2* tumors were thus marginally older than those with *IDH1/2* mutated tumors, although these differences were not statistically significant (age at initial diagnosis: $p = 0.12$, age at sGBM diagnosis: $p = 0.25$). Seventy percent (7 of 10) of the sGBM-Wt progressed from grade III gliomas, whereas 37.5 % (3 of 8) of sGBM-Mut developed from grade III gliomas ($p = 0.17$). A frontal location was more common for tumors with mutant *IDH1/2* (7 of 8, 87.5 %), than for those with wild-type *IDH1/2* (5 of 10, 50 %, $p = 0.08$). The median overall survival time was 68.2 months for patients with sGBM-Mut and 25.3 months for those with sGBM-Wt (Fig. 1a, $p = 0.029$). The median time from initial diagnosis to sGBM diagnosis was 50.1 months for patients with sGBM-Mut and 13.4 months for those with sGBM-Wt (Fig. 1b, $p = 0.021$). In sGBMs-Mut, the median overall survival time was significantly longer in initial grade II tumors than in initial grade III tumors (Fig. 1c, 76.9 vs. 34.2 months, $p = 0.0042$), and the median time to the diagnosis of sGBM was significantly longer from initial grade II diagnosis than from initial grade III diagnosis (Fig. 1d, 69.3 vs. 31.7 months, $p = 0.022$). In contrast, sGBMs-Wt, no differences were noted in the median survival times and the median time to the diagnosis of sGBM between initial grade II and III tumors (Fig. 1e, median overall survival time: 21.5 vs. 29 months: $p = 0.51$, and Fig. 1f, median time to the diagnosis of sGBM: 15.2 vs. 12.6 months: $p = 0.34$). The median survival for patients from the diagnosis of sGBM was not found to vary according to the *IDH1/2* mutation status (Fig. 2, wild-type *IDH1/2,* 6.8 vs. mutant *IDH1/2*, 6.75 months: $p = 0.93$).

IDH1/2 mutations and molecular alterations

We investigated the following genetic alterations: *TP53* mutations, 1p loss, 19q loss, *CDKN2A* homozygous deletion, *PTEN* deletion, and *EGFR* amplification. These results are summarized in Tables 1 and 3. Among the 16 sGBMs examined, 13 had *TP53* mutations (81.3 %), while none, including the 3 cases that were initially diagnosed as grade III oligodendroglial tumors, had 1p/19q codeletion. All the sGBM-Mut (7 of 7) were found to have *TP53* mutations. Three carried *CDKN2A* homozygous deletion, one had *PTEN* deletion. None of the tumors (0 of 7) showed *EGFR* amplification. Among the sGBM-Wt,

Table 1 Clinical and genetic characteristics of patients with secondary glioblastomas

Case	Sex	Initial tumor					Histological change	Secondary GBM		Time to the diagnosis of sGBM (months)	Survival time from sGBM diagnosis (months)	Overall survival time (months)
		Diagnosis	Age (years)	Tumor location	Extent of resection	Treatment		Age (years)	Treatment			
DA048	M	DA	28	Hypothalamus	Biopsy	RT 50 Gy/Chemo (ACNU)	DA → GBM	29	RT 60 Gy/Chemo (ACNU)	9.9	6.6	16.5
DA065	F	DA	37	Fronto-Temporal	Partial	RT 54 Gy/Chemo (ACNU)	DA → GBM	38	None	15.2	6.3	21.5
DA085	M	DA	30	Temporal	Subtotal	RT 60 Gy/Chemo (ACNU)	DA → GBM → GBM	37	Chemo (TMZ)	86.4	10.3	96.7
AA017	F	AA	64	Parietal	Partial	RT 56 Gy	AA → GBM	65	Chemo (ACNU, VCR)	12.6	24.1	36.7
AA009	M	AA	42	Frontal	Partial	RT 60 Gy/Chemo (ACNU, VP16)	AA → GBM	43	Chemo (ACNU, PCZ)	11.4	6.6	18.0
AA043	M	AA	64	Temporal	Partial	Observation	AA → GBM → GBM	65	RT 60 Gy/Chemo (ACNU)	7.2	6.8	14.0
AA020	M	AA	26	Frontal	Total	RT 63 Gy/Chemo (ACNU, VCR)	AA → AA → GBM	28	Chemo (CBDCA, VP16)	22.6	6.3	29.0
AO004	M	AO	20	Frontal	Partial	RT 60 Gy/Chemo (ACNU, VCR)	AO → GBM	21	RT 60 Gy/Chemo (ACNU)	9.8	6.2	16.0
AOA010	M	AOA	62	Parietal	Total	RT 60 Gy/Chemo (ACNU, VCR)	AOA → GBM	63	Chemo (CBDCA, VP16)	14.2	17.6	31.8
AOA025	F	AOA	47	Fronto-Parietal	Biopsy	RT 60 Gy/Chemo (ACNU, VCR)	AOA → AOA → GBM	49	Chemo (CBDCA, VP16)	29.1	6.8	35.9
DA093	M	DA	33	Frontal	Subtotal	Observation	DA → AA → GBM	38	RT 60 Gy/Chemo (TMZ)	54.9	12.3	67.3
DA042	M	DA	26	Frontal	Biopsy	RT 60 Gy/Chemo (ACNU)	DA → AOA → GBM	32	None	75.7	1.2	76.9
DA030	F	DA	31	Fronto-temporal	Partial	RT50 Gy	DA → N → DA → GBM	35	Chemo (TMZ)	45.2	16.6	61.8
DA082	M	DA	50	Frontal	Biopsy	RT 60 Gy/Chemo (ACNU)	DA → GBM	61	Chemo (TMZ)	124.0	20.1	144.2
DA094	F	DA	24	Frontal	Subtotal	RT 60 Gy/Chemo (ACNU)	DA → GBM	30	Chemo (CBDCA, VP16)	69.3	5.5	74.8
AA083	M	AA	29	Frontal	Partial	RT 60 Gy/Chemo (TMZ)	AA → GBM	30	RT 60 Gy/Chemo (TMZ)	16.1	6.3	22.4
AA015	F	AA	30	Putamen	Biopsy	RT 60 Gy/Chemo (ACNU, VP16)	AA → GBM	33	RT 30 Gy/Chemo (ACNU, VCR)	31.7	2.4	34.2
AA048	M	AA	27	Frontal	Partial	RT (UK)/Chemo (ACNU, IFN)	AA → GBM → GBM	31	Chemo (TMZ)	45.3	7.2	52.5

Case	Sex	Outcome	*IDH1/2* status	*IDH* analysis	1p/19q codeletion	1p loss	19q loss	*TP53* mutations	*CDKN2A* homozygous deletion	*PTEN* deletion	*EGFR* amplification
DA048	M	Dead	Wt	Seq	No	No	No	Yes	No	No	No
DA065	F	Dead	Wt	Seq	No	No	Yes	Yes	Yes	No	Yes
DA085	M	Dead	Wt	Seq	No	No	Yes	Yes	No	No	No
AA017	F	Dead	Wt	Seq	No	No	No	No	No	No	No
AA009	M	Dead	Wt	IHC	N/A	N/A	N/A	N/A	N/A	N/A	N/A

Table 1 continued

Case	Sex	Outcome	*IDH1/2* status	*IDH* analysis	1p/19q codeletion	1p loss	19q loss	*TP53* mutations	*CDKN2A* homozygous deletion	*PTEN* deletion	*EGFR* amplification
AA043	M	Dead	Wt	Seq	No	No	No	No	Yes	Yes	Yes
AA020	M	Dead	Wt	Seq	No	No	No	Yes	No	No	No
AO004	M	Dead	Wt	Seq	No	No	No	Yes	No	Yes	No
AOA010	M	Dead	Wt	Seq	No	No	No	No	No	No	No
AOA025	F	Dead	Wt	Seq	No	No	No	Yes	No	No	No
DA093	M	Alive	Mut	IHC	No	No	Yes	Yes	No	No	No
DA042	M	Dead	Mut	Seq	No	No	No	Yes	Yes	Yes	No
DA030	F	Dead	Mut	Seq	No	No	No	Yes	No	No	No
DA082	M	Alive	Mut	Seq	No	No	No	Yes	No	No	No
DA094	F	Dead	Mut	Seq	No	No	No	Yes	No	No	No
AA083	M	Dead	Mut	IHC	No	No	No	Yes	Yes	No	No
AA015	F	Dead	Mut	IHC	N/A	N/A	N/A	N/A	N/A	N/A	N/A
AA048	M	Dead	Mut	IHC	No	No	No	Yes	Yes	No	No

M male, *F* female, *DA* diffuse astrocytoma, *AA* anaplastic astrocytoma, *GBM* glioblastoma, *sGBM* secondary glioblastoma, *OL* oligodendroglioma, *OA* oligoastrocytoma, *AO* anaplastic oligodendroglioma, *AOA* anaplastic oligoastrocytoma, *N* necrosis, *RT* radiation therapy, *Chemo* chemotherapy, *ACNU* nimustine, *TMZ* temozolomide, *VP16* etoposide, *VCR* vincristine, *IFN* interferon, *PCZ* procarbazine, *CBDCA* carboplatin, *IDH* isocitrate dehydrogenase, *Wt* wild-type, *Mut* mutation, *IHC* immunohistochemistry, *Seq* sequence analysis, *N/A* not available, *CDKN2A* cyclin-dependent kinase inhibitor 2A, *PTEN* phosphatase and tensin homologue, *EGFR* epidermal growth factor receptor

Table 2 Summary of clinical characteristics of patients with wild-type and mutant *IDH1/2* secondary glioblastomas

	Patients with sGBM-Wt ($n = 10$)	Patients with sGBM-Mut ($n = 8$)	p
Median age at initial tumor diagnosis (years)	39.5 (20–64)	29.5 (24–50)	0.12
Median age at sGBM diagnosis (years)	40.5 (21–65)	32.5 (30–61)	0.25
Sex			
Male	7	5	0.74
Female	3	3	
Initial tumors grade			
II	3	5	0.16
III	7	3	
Location			
Frontal	5	7	0.08
Other	5	1	
Median overall survival time (months)	25.3	68.3	0.029*
Initial grade II	21.5 ($n = 3$)	76.9 ($n = 5$)	0.33
Initial grade III	29 ($n = 7$)	34.2 ($n = 3$)	0.3
Median time to sGBM diagnosis (months)	13.4	50.1	0.021*
Initial grade II	15.2 ($n = 3$)	69.3 ($n = 5$)	0.45
Initial grade III	12.6 ($n = 7$)	31.7 ($n = 3$)	0.048*
Median survival time after sGBM diagnosis (months)	6.8	6.75	0.93

sGBM secondary glioblastoma, *IDH* isocitrate dehydrogenase, *sGBM-Wt* secondary glioblastoma with wild-type *IDH1/2*, *sGBM-Mut* secondary glioblastoma with *IDH1/2* mutation

* Statistically significant

66.7 % tumors (6 of 9) had *TP53* mutations. Further, one of these 6 tumors had both *CDKN2A* homozygous deletion and *EGFR* amplification (DA065), and another one was associated with *PTEN* deletion (AO004). Three of the sGBMs harbored neither alterations in *IDH1/2*, or *TP53*, or 1p/19q codeletion. One of them (AA043) showed *EGFR* amplification, *PTEN* deletion, and *CDKN2A* homozygous deletion. The other 2 tumors (AA017 and AOA10) had no genetic alterations among those examined.

Discussion

This study showed that sGBM-Wt and sGBM-Mut were different in overall survival time and time from initial diagnosis to sGBM diagnosis, but the survival time from sGBM diagnosis was not different according to the *IDH1/2* mutations status. Molecular analysis showed all the sGBM-Mut and two-thirds of sGBM-Wt had *TP53* mutations, but