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Survival in patients with glioblastoma at a first progression does not correlate with *isocitrate dehydrogenase (IDH)1* gene mutation status

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Abstract

Backgrounds: Mutations in the *isocitrate dehydrogenase (IDH)1* gene are favourable prognostic factors in newly diagnosed diffuse gliomas, whereas it remains controversial in the recurrent glioblastoma setting.

Methods: A total of 171 patients with newly diagnosed glioblastoma, either 'primary' glioblastoma or 'secondary' glioblastoma, treated at Kyorin University Hospital or Japanese Red Cross Medical Center from 2000 to 2015 were included. Patients with confirmed *IDH1* status and O^6-methylguanine-DNA methyltransferase promoter methylation status were retrospectively analysed for overall survival from the initial diagnosis ($n = 147$) and after the first progression ($n = 122$).

Results: *IDH1* mutation but not *IDH2* was noted in 19 of 147 patients with glioblastoma (12.9%). In patients with 'primary' glioblastoma ($n = 136$), median overall survival after the first progression was 13.5 and 10.5 months for mutant *IDH1* and wild-type *IDH1* glioblastoma, respectively ($P = 0.747$). Multivariate analysis revealed O^6-methylguanine-DNA methyltransferase promoter methylation, and Karnofsky Performance status 60 or higher, were independent prognostic factors for better overall survival after the first progression. When 'primary' glioblastoma and 'secondary' glioblastoma were combined, median overall survival from the first progression was not significantly different between the mutant *IDH1* group (10.1 months) and wild-type *IDH1* group (10.5 months) ($P = 0.559$), whereas median overall survival from the initial diagnosis was significantly different (47.5 months vs.18.3 months, respectively; $P = 0.035$).

Conclusions: These results suggest that *IDH1* mutation may not be a prognostic factor for survival at the first progression of patients with 'primary' glioblastoma and pretreated 'secondary' glioblastoma, and further warrant investigation in prospective studies.

Key words: IDH status, glioblastoma, recurrent glioblastoma, overall survival, MGMT

Introduction

Glioblastoma (GBM) is the most common malignant brain tumour in adults. The current standard treatment for GBM comprises maximal safe resection and administration of temozolomide (TMZ) combined with radiotherapy (RT), followed by 6–12 cycles of TMZ with or without tumour-treating fields as a maintenance therapy (1,2). Even after this standard treatment, progression occurs in almost all patients, resulting in a dismal survival outcome; median progression-free survival (PFS) and median overall survival (mOS) remain ~7 months and only 14–18 months, respectively (1–5). There is no established treatment method for recurrent GBM and it is an urgent task, as several ongoing clinical trials indicate.

It is well known that somatic mutations of the *isocitrate dehydrogenase* (*IDH*) *1* and *2* genes are important prognostic factors in gliomas. Parsons et al. reported that the *IDH1* mutation was associated with significantly longer OS in patients with newly diagnosed GBM (6). Yan et al. also showed a similar survival superiority in patients with m*IDH* GBM (mOS 31 months) over those with wild-type *IDH* (wt*IDH*) GBM (mOS 15 months) (P = 0.002) (7). These reports also revealed that *IDH* mutations are present in only 5–10% of 'primary' GBM (pGBM) cases, whereas *IDH* mutations are more frequently observed in cases of WHO Grade II or III gliomas which are also collectively known as lower grade glioma (LrGG)s, or in 'secondary' GBM (sGBM) cases, who were histopathologically diagnosed as GBM on relapse or progression following the initial diagnosis of LrGG (7–9).

With the revision of the WHO classification of tumours of the central nervous system (revised fourth edition) in 2016, GBM has been classified as either GBM, IDH-mutant (m*IDH* GBM) and GBM, IDH-wild type (wt*IDH* GBM). m*IDH* GBM corresponds almost exclusively to 'secondary GBM' and patients with m*IDH* GBM have significantly longer OS than those with wt*IDH* GBM which mostly corresponds to 'primary GBM' (10).

However, it remains unclear whether *IDH* gene mutation status also affects prognosis following the timepoint of progression. There are few reports that describe whether prognosis after progression of GBM is better in patients with m*IDH* GBM than those with wt*IDH* GBM. This clinical question may be an important issue, especially in the planning of clinical trials concerning recurrent GBM. In this study, we retrospectively analysed survival of the patients with GBM in our cohorts with regard to prognosis after the first progression of GBM.

Patients and methods

Patients

A total of 171 patients with pathologically confirmed GBM, who were treated at Kyorin University Hospital or Japanese Red Cross Medical Center from 2000 to 2015, were identified in our institutional database. Pathological diagnosis was reconfirmed by a pathologist at Kyorin University Hospital. Thirty-seven patients whose tumour samples were not available for *IDH1*gene mutation status were excluded. The remaining 147 patients were included in the study. As for survival analysis after progression, 25 patients were further excluded due to either lack of progression as GBM (n = 6) or inability to evaluate progression. In order to evaluate survival of the patients with pGBM, 11 patients with sGBM which had progressed from preceding LrGG albeit initial post-operative adjuvant therapy were excluded for the primary analysis (Fig. 1). The present study was approved by the Medicine Ethics Committee of Kyorin University Faculty of Medicine, and all subjects signed a specimen preservation and gene search study form.

IDH gene mutation analyses

IDH1 gene mutation status was examined by direct sequencing using the Sanger method as previously described (8). Genomic DNA was extracted from frozen tumour specimens and a fragment of 129 bp in length spanning the catalytic domain of *IDH1* containing codon 132 was amplified using IDH1-f: CGGTCTTCAGAGAAGCCATT and IDH1-r: GCAAAATCACATTATTGCCAAC as primers. A fragment of 288 bp in length spanning the catalytic domain of *IDH2* containing R140 and R172 was amplified using IDH2-f AGCCCATCATCTGCAAAAAC and IDH2-r CTAGGCGAGGAGCTCCAGT as primers. GoTaq® DNA Polymerase (Promega, Madison, WI, USA) was used to perform polymerase chain reaction (PCR), and heat denaturation was performed at 95°C for 30 seconds, annealing at 56°C for 40 seconds, extension at 72°C for 50 seconds at 35 cycles (Supplementary Fig. 1A: Wild-type *IDH1*, Supplementary Fig. 1B: Mutated *IDH1*). Detection of IDH1R132H mutation by immunohistochemistry was performed with the mouse monoclonal antibody H09 (Dianova,1:25 dilution, Hamburg, Germany) (11). (Supplementary Fig. 1C: Wild-type *IDH1*, Supplementary Fig. 1D: Mutated IDH1).

O^6-methylguanine-DNA methyltransferase promoter methylation status

Promoter methylation status analysis of O^6-methylguanine-DNA methyltransferase (*MGMT)* gene was performed using methylation-specific PCR as described previously (12) (Supplementary Fig. 2).

Statistical analysis

PFS was measured from the date of initial surgery for GBM (GBM-PFS) or LrGG (LrGG-PFS) to the date of progression, death or otherwise the last follow-up date on which the patient was reported alive without disease progression. OS was measured from the date of initial surgery for GBM (GBM-OS) or LrGG (LrGG-OS) to the date of death, or otherwise the last follow-up date on which the patient was reported alive. In the pGBM cases, survival after first progression (Rec-pGBM-OS) was measured from the date of first progression to the date of death, or otherwise the last follow-up date on which the patient was reported alive. Rec-pGBM-OS in pGBM and sGBM-OS in sGBM were evaluated as OS after progression in the present study (Fig. 2).

The correlation between *IDH* mutation status and clinicopathological characteristics such as age, gender, extent of surgical resection, performance status (Karnofsky Performance Status; KPS), *MGMT* methylation status, adjuvant treatment after initial surgery, retreatment for first progression were evaluated using chi-squared test or Fisher's exact test. The Kaplan–Meier estimate was used for survival analysis, and univariate analysis was performed using a log-rank test with a significance level of P = 0.05 (two-tailed test). Multivariate analysis was performed by Cox proportional regression analysis, and variables included the presence or absence of *IDH* gene mutation, and variables which showed significant differences by univariate analysis. SPSS 18.0 J (SPSS, Inc., Chicago, IL, USA) software was used to perform all statistical analyses.

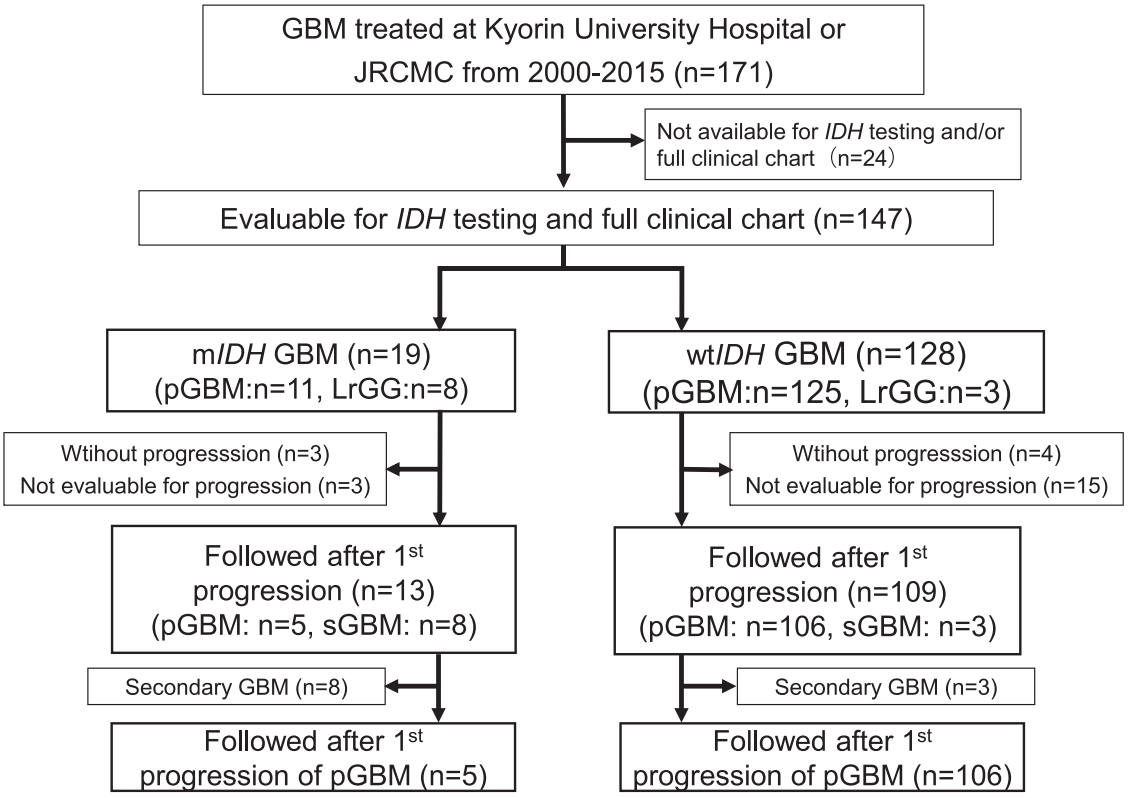

Figure 1. Study diagram. GBM patients (171 cases) treated at Kyorin University Hospital or Japanese Red Cross Medical Center (JRCMC) from 2000 to 2015 were retrospectively identified. Those whose *IDH1* status and clinical history were not available were excluded (24 cases). Investigated 147 patients consisted of 19 with mutated *IDH1* GBM and 128 with wild-type *IDH* GBM. Six m*IDH1* GBM patients were excluded because three patients had not shown tumour progression, whereas three patients were not evaluated in detail at progression, thus remaining 13 m*IDH1* GBM patients were investigated for survival after first progression. Nineteen wt*IDH1* GBM patients were excluded because four patients had not shown progresssion, whereas 15 patients were not evaluated in detail at progression, resulting in remaining 109 wt*IDH1* GBM patients being eligible for survival analysis after first progression. In the primary analysis, 11 patients with secondary GBM were excluded, and 106 patients with wt*IDH1* pGBM and five patients with m*IDH1* pGBM were eligible for survival analysis after the first progression.

Results

Patient backgrounds

Among the 147 patients, 19 (12.9%) had an *IDH1* gene mutation (m*IDH* GBM). All of these mutations were *IDH1* mutations. There was no *IDH2* mutation detected in all but 12 patients whose specimens were insufficient for conclusive judgement (data not shown). Of the remaining 128 patients with *wtIDH1* GBM, 125 accounted for pGBM (97.7%) and only three patients had sGBM (2.3%). In contrast, among the 19 patients with m*IDH1* GBM, 11 accounted for pGBM (57.9%) and eight patients had sGBM (42.1%). Therefore, the incidence of *IDH1* mutation was significantly higher in the sGBM group ($P < 0.0001$).

Among patients with pGBM ($n = 136$), the median age of those with m*IDH1* was 42 years (range 25–65), which was significantly younger than those with wt*IDH1* (65 years, range 16–86) ($P < 0.0001$). There were more patients with age younger than 50 years old ($P < 0.001$) and more females ($P = 0.022$) in the m*IDH1* pGBM group. The proportion of patients who received gross total resection (GTR), patients with KPS 60 or higher on initial diagnosis, or *MGMT* promotor methylation were not significantly different between the wt*IDH1* pGBM group and m*IDH1* pGBM group. Nearly all patients received first-line therapy consisting of RT and chemotherapy. The combination of RT and TMZ was the mostly used regimen and was well-balanced between wt*IDH* and m*IDH* groups (Table 1). At the first progression, variable treatments were given dependent on the history of the prior therapy and conditions of the patients. Repeated surgery or RT was used infrequently, whereas chemotherapy including TMZ, bevacizumab (BEV) or nimustine was administered in most of patients (>80%) regardless of *IDH1* status. No significant differences were noted between w*tIDH1* GBM and m*IDH1* GBM group (Table 2).

Characteristics of *mIDH1* GBM patients

Among the 19 patients with m*IDH1* GBM, there were 11 patients with pGBM, and eight patients with sGBM. All but one patient among the 11 patients with pGBM were initially treated with RT and concomitant TMZ. Three patients were in poor general condition with KPS 40 or lower. One of them could not receive RT nor chemotherapy due to perioperative death. Among the eight patients with sGBM, six of them had already received RT as the treatment for initial LrGG. Various treatments were given after the diagnosis of sGBM. The preceding tumours consisted of diffuse astrocytoma (WHO 2007 classification Grade II) in five patients (62.5%), gliomatosis cerebri (Grade III) in two (25%) and one anaplastic astrocytoma (AA; Grade III). The median time from initial diagnosis to the diagnosis of sGBM was 47.0 months (range 12.9–75.0) (Table 3).

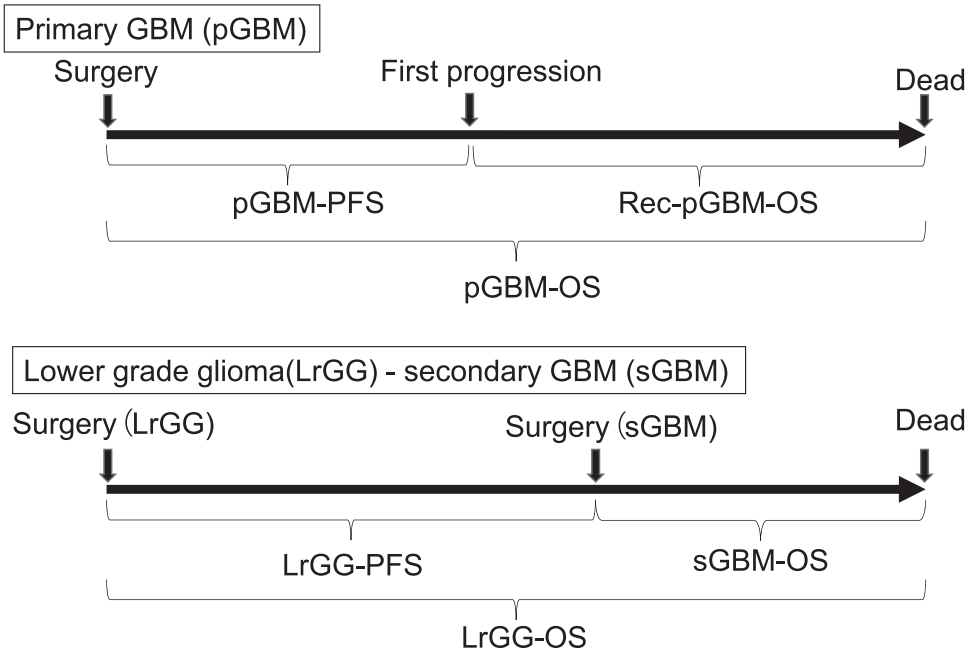

Figure 2. Definitions of survival analysis. pGBM-PFS; time from initial surgery of primary GBM to the date of either first progression, death, or the final confirmed survival date in case that the date of progression cannot be determined. Rec-pGBM-OS; time from first progression of primary GBM to either death or the final survival date confirmed. pGBM-OS; time from initial surgery of primary GBM to either death or the final survival date confirmed. LrGG-PFS; time from initial surgery of glioma to the diagnosis date of secondary GBM. sGBM-OS; time from the diagnosis date of secondary GBM to either death or the final survival date confirmed. LrGG-OS; time from initial surgery of lower grade glioma to either death or the final survival date confirmed.

Table 1. Patient characteristics and treatments of primary GBM according to *IDH1* mutation status

Variable	Result	*IDH1* mutation status		*P* value
		wt*IDH1* GBM (*n* = 125) Number of cases (%)	m*IDH1* GBM (*n* = 11) Number of cases (%)	
Age	<50y/≧50y	19 (15.2)/106 (84.8)	7 (63.6)/4 (36.4)	<0.001
Gender	Male/female	73 (58.4)/52 (41.6)	3 (27.3)/8 (72.7)	0.022
Extent of resection	GTR/non-GTR	51 (40.8)/74 (59.2)	6 (54.5)/5 (45.5)	0.393
KPS	≧60/<60	91 (72.8)/31 (24.8%)	7 (63.6)/4 (36.4)	0.444
	Unknown	3 (2.4%)	0 (0)	
MGMT	Met/UM	58 (46.4)/67 (53.6)	8 (72.7)/3 (27.3)	0.089
IDH2 mutation	Mutant/wildtype	0/113(90.4)	0/11(100)	
	Unknown	12 (9.6)	0 (0)	
First-line treatment	Received/not received	124 (99.2)/1 (0.8)	10 (90.9)/1 (9.1)	0.156
	Radiotherapy (RT)	118 (94.4)	10 (90.9)	0.543
	(RT alone)	11 (8.9)	0 (0)	
	Chemotherapy	113 (90.4)	10 (90.9)	0.684
	(TMZ alone)	6 (4.8)	0 (0)	
	RT + chemotherapy	107 (85.6)	10 (90.9)	0.526
	(RT + TMZ)	90 (72.6)	9 (90.0)	
	(RT + TMZ + BEV)	8 (6.5)	1 (10.0)	
	(RT + ACNU)	8 (6.5)	0 (0)	
	(RT + BEV)	1 (0.8)	0 (0)	

GBM, glioblastoma; *IDH*, *isocitrate dehydrogenase*; wt*IDH1* GBM, wild-type *IDH1* glioblastoma; m*IDH1* GBM, mutant *IDH1* glioblastoma; KPS, Karnofsky Performance Score; GTR, gross total resection; *MMT*, O^6-methylguanine-DNA methyltransferase; Met, methylated; UM, unmethylated; RT, radiotherapy; ACNU, nimustine; TMZ, temozolomide; BEV, bevacizumab.

Survival analysis

The primary analysis included only the 'primary' GBM cases to avoid any potentially confounding effects by incorporating both 'primary' and 'secondary' GBMs. mOS after the first progression was 13.5 months (95% confidence interval [CI] 3.7–23.2) for patients with m*IDH1* pGBM (*n* = 5) and 10.5 months(95% CI 8.6–12.4) for those with wt*IDH1* pGBM (*n* = 106) (Fig. 3A). Although there is a potential bias due to the small number of patients with m*IDH1*

Table 2. Treatment for the first progression of primary GBM according to *IDH1* mutation status

Variable	Result	*IDH1* mutation status		*P* value
		wt*IDH1* GBM (*n* = 125) Number of cases (%)	m*IDH1* GBM (*n* = 11) Number of cases (%)	
First progression	Progression/progression-free	106 (84.8)/4 (3.2)	5 (45.4)/3 (27.3)	0.053
	Censored	15 (12.0)	3 (27.3)	
Treatment for first progression	Received/not received	71 (67.0)/35 (33.0)	5 (100)/0	0.177
	Surgery	18 (25.4)	2 (40.0)	0.396
	Re-radiotherapy (RT)	27 (21.6)	0 (0.0)	
	(Re-RT alone)	11 (45.5)	0 (0.0)	
	Chemotherapy	60 (84.5)	5 (100)	0.447
	(TMZ)	18 (25.4)*	3 (60.0)	
	(BEV)	14 (19.7)*	0 (0.0)	
	(Platinum-based)	11 (15.5)	0 (0.0)	
	(ACNU-based)	6 (8.5)	1 (20.0)	
	(ddTMZ)	4 (5.6)	0 (0.0)	
	(BEV + ACNU)	3 (4.2)	1 (20.0)	
	(Others)	4 (5.6)	0 (0.0)	

ddTMZ, dose-dense temozolomide.

*Containing a case treated by BEV combined with TMZ.

pGBM, no statistically significant difference in OS by the *IDH1* mutation status was observed (P = 0.747). Also, there was no significant difference in OS from the initial diagnosis of pGBM between wt*IDH1* GBM (n = 125, 18.3 months, 95% CI 15.0–21.5) and m*IDH1* GBM (n = 11, 36.4 months, 95% CI 10.6–62.2) (P = 0.224) (Fig. 3B; pGBM-OS by *IDH1* mutation status). However, when patients whose initial KPS was <60, including three out of 11 patients with m*IDH1* pGBM, were excluded, there was significant difference in mOS from the initial diagnosis between patients with wt*IDH1* pGBM (20.8 months, 95% CI 17.1–24.5) and those with m*IDH* pGBM (82.9 months, 95% CI not determined) (P = 0.039).

In addition, we evaluated survival in patients with both 'primary' and 'secondary' GBM as an ancillary analysis. OS after the first progression by *IDH1* status was also similar irrespective of *IDH1* status. mOS after the first progression in pGBM (Rec-pGBM-OS) or OS after the time of GBM diagnosis in sGBM (sGBM-OS) was 10.1 months (95% CI 4.4–15.7) in patients with m*IDH1* and 10.5 months (95% CI 8.9–12.1) in those with wt*IDH1* (P = 0.559) (Fig. 3C). Similarly, survival from the first progression was almost equivalent between patients with pGBM (mostly wt*IDH1*; mOS 10.5 months, 95% CI 8.7–12.3) and sGBM (mostly m*IDH1*; mOS 10.1 months, 95% CI 5.7–14.4; P = 0.478), and mOS of m*IDH1* sGBM was only 9.2 m (95% CI 5.0–13.4). In contrast, survival from initial onset of primary GBM and LrGG (pGBM-OS/LrGG-OS) was different by *IDH1* mutation status. mOS of m*IDH1* patients [47.5 months, 95% CI 36.0–59.0] was significant longer than that of wt*IDH1* patients (18.3 months, 95% CI 15.1–21.6; P = 0.035) (Fig. 3D).

Prognostic factors associated with survival

We also examined relationships between survival and other prognostic factors including methylation status of *MGMT*, age, extent of resection and KPS. By univariate analyses, there was a significant difference in OS from initial diagnosis of pGBM between the *MGMT* methylated group (27.9 months, 95% CI 24.6–31.2) and the unmethylated group (15.5 months, 95% CI 13.7–17.3) (Fig. 3E; P < 0.0001). Regarding survival following first progression (Rec-pGBM-OS), a significant difference was observed as well (*MGMT* methylated group, 16.0 months, 95% CI 9.2–22.7; unmethylated group, 9.2 months, 95% CI 7.8–10.6) (Fig. 3F; P < 0.001). KPS 60 or higher was significantly associated with better survival from initial diagnosis (P = 0.004) and first progression (P < 0.0001) (Fig. 3G). Extent of resection at the initial surgery was a significant prognostic factor only for OS from initial diagnosis (P < 0.001). Young age (<50 years old) was not associated with better survival.

By multivariate analyses using Cox regression analysis (Table 4A and B), *IDH1* mutation status was not a significant prognostic factor for OS both from initial diagnosis of pGBM (pGBM-OS) and from first progression of pGBM (Rec-pGBM-OS). *MGMT* promoter methylation status, GTR and KPS (all P < 0.001) were found to be independent prognostic factors for newly diagnosed GBM (Table 4A), whereas only *MGMT* status (P = 0.019) and KPS at progression (P < 0.001) were independently associated with favourable OS after first progression (Rec-pGBM-OS) (Table 4B).

Discussion

The present study shows that in patients with recurrent GBM, *IDH1* mutation status was not significantly associated with OS from the first progression. This result suggests that the better prognostic property derived from *IDH1* mutation in newly diagnosed settings (7) does not translate into outcomes in recurrent settings for GBMs. It might also reflect malignant progression with acquisition of additional highly aggressive alterations that could override the survival benefit of *IDH1* mutation for the initial gliomas.

A subanalysis for prognostic factors in the randomized phase II BELOB trial, exploring a potential benefit of an anti-angiogenic monoclonal antibody, BEV, when added to an alkylating agent, lomustine (CCNU), in patients with recurrent GBM, identified seven *IDH1* mutations out of 114 patients (6.1%). Univariate analysis highlighted that *IDH1* mutation status was a factor which significantly correlated with OS (P = 0.04). However, multivariate analysis revealed no statistically significant impact of *IDH1* mutation on

Table 3. Characteristics, treatment and outcome in patients with m*IDH1* glioblastoma

Case No.	Diagnosis	*MGMT* status	Initial KPS	Age	Sex	Diagnosis for LrGG	EOR for LrGG	RT for LrGG (Gy)	Chemo-therapy for LrGG	LrGG-PFS (m)	EOR for GBM	RT for GBM (Gy)	Chemo-therapy for GBM	GBM PFS (m)	Treatment at the time of second progression	Rec-pGBM-OS/ sGBM- OS (m)	LrGG-OS/ pGBM-OS (m)	GBM-OS(m)	Status
1	pGBM	Met	20	51	M						G	60	TMZ	12.9	–	–	12.9	12.9	Censored
2	pGBM	Met	40	38	F						G	60	TMZ	5.8	TMZ	3.8	9.5	9.5	Dead
3	pGBM	Met	70	31	F						N	60	TMZ	25.7	no pro.	–	25.7	25.7	Alive
4	pGBM	Met	70	51	M						N	60	T + BEV	13.4	TMZ, ICE	5.5	18.8	18.8	Dead
5	pGBM	Met	80	31	F						N	60	TMZ	6.9	A	15	27.5	27.5	Alive
6	pGBM	Met	90	51	F						N	60	TMZ	28.3	no pro.	–	28.3	28.3	Alive
7	pGBM	Met	100	25	F						G	60	TMZ	29.9	no pro.	–	29.9	29.9	Alive
8	pGBM	Met	100	49	F						G	60	TMZ	69.4	TMZ, BEV, CK	13.5	82.9	82.9	Dead
9	pGBM	UM	10	34	M						N	–	–	0.5	–	–	0.5	0.5	Dead
10	pGBM	UM	60	65	F						G	60	TMZ	10.5	–	–	10.5	10.5	Dead
11	pGBM	UM	90	42	F						G	60	TMZ	13.3	A + BEV, CK, ITK	23.2	36.5	36.5	Dead
12	sGBM	Met	30	65	F	GC	N	-	PAV	46.1	N	–	TMZ	2.4	–	2.4	48.5	2.4	Dead
13	sGBM	Met	70	28	F	GC	N	50	TMZ	52.1	G	–	CE	9.2	CE, BEV	9.2	60.3	9.2	Dead
14	sGBM	Met	70	52	M	DA	N	56	–	75.0	N	–	T + BEV	8.6	SRT, BEV	15.9	90.9	15.9	Dead
15	sGBM	Met	90	23	M	DA	N	–	–	12.9	N	60	PAV	2	PAV, TMZ	10.1	22.9	10.1	Dead
16	sGBM	Met	90	35	F	AA	N	60	AE	48.4	U	–	TMZ	8.1	TMZ, CE	11.9	60.4	11.9	Dead
17	sGBM	Met	90	38	F	DA	N	54	–	34.2	N	–	TMZ	1.4	TMZ, ICE	7.1	41.4	7.1	Dead
18	sGBM	UM	70	34	F	DA	G	54	–	41.2	N	–	TMZ	4.3	–	6.3	47.6	6.3	Dead
19	sGBM	UM	70	42	M	DA	N	60	A	65.5	N	–	TMZ	7.3	BEV, ICE, A	19.4	84.9	19.4	Dead

pGBM, primary glioblastoma; sGBM, secondary glioblastoma; M, male; F, female; AA, anaplastic astrocytoma; DA, diffuse astrocytoma; GC, gliomatosis cerebri; G, gross total removal; N, non-gross total removal; ND, not known dose; *MGMT*: O6-methylguanine-DNA methyltransferase, Gy, grey; A, ACNU(nimustine); PAV, procarbazine, ACNU and vincristine; AE, ACNU and etoposide; LrGG-PFS, lower grade glioma-progression-free survival; T + BEV, temozolomide and bevacizumab; CE, carboplatin and etoposide; CK, cyberknife; ITK, personalized peptide vaccine; ICE, low dose ifosfamide, carboplatin and etoposide; rec., recurrence.

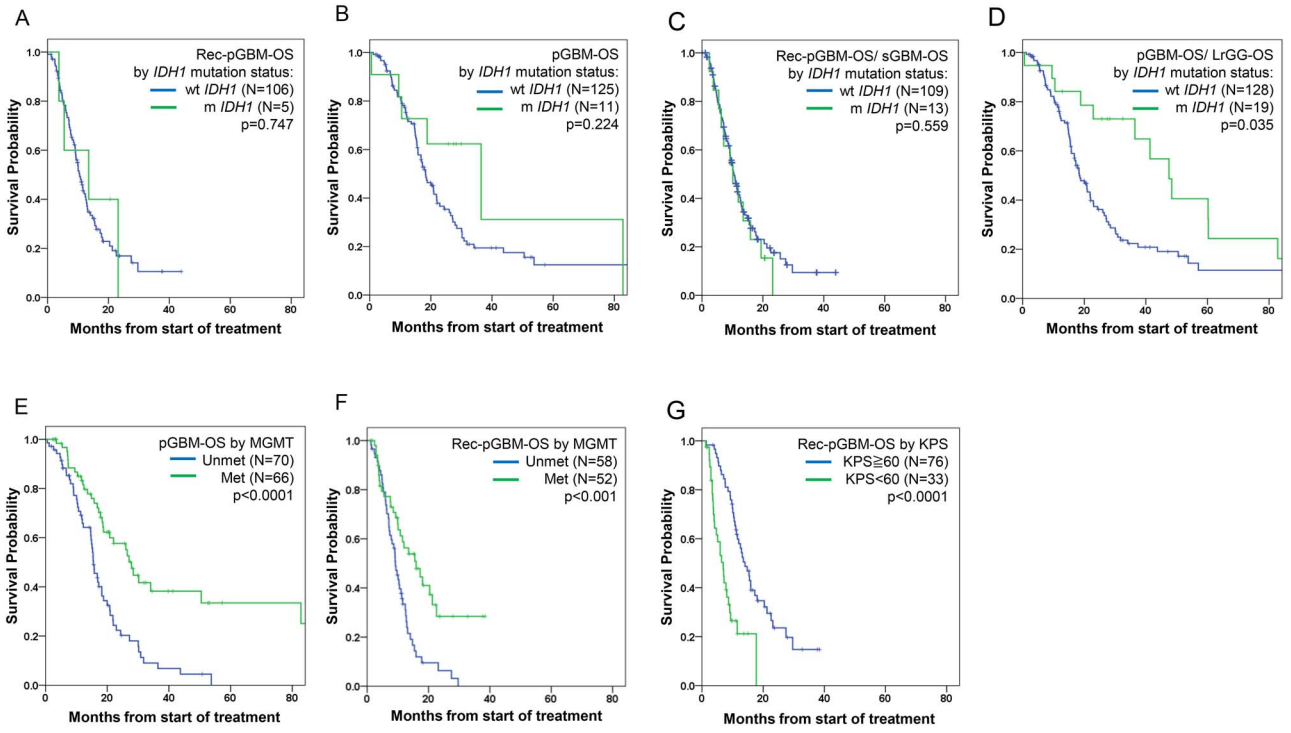

Figure 3. Kaplan–Meier plots of survival analysis for 147 patients with glioblastoma. (A) OS from the first progression of primary GBM (Rec-pGBM-OS) by *IDH1* mutation status. (B) OS from initial diagnosis of primary GBM (pGBM-OS) by *IDH1* mutation status. (C) OS from first progression of primary GBM or diagnosis of secondary GBM (Rec-pGBM OS/sGBM-OS) by *IDH1* mutation status. (D) OS from initial diagnosis of glioma (OS from initial diagnosis of primary GBM or initial diagnosis lower grade glioma; pGBM-OS/LrGG-OS) by *IDH1* mutation status. (E) OS from initial diagnosis of GBM (pGBM-OS) by *MGMT* status. (F) OS from first progression of primary GBM (Rec-pGBM-OS) by *MGMT* status. (G) OS from first progression of pGBM (Rec-GBM-OS) by KPS score of under 60 or 60 or greater.

Table 4. Multivariate analysis of factors associated with overall survival for pGBM (pGBM-OS) and recurrent pGBM (Rec-pGBM-OS)

Parameter		Parameter estimated	Standard error	*P* value	Hazard ratio	95% CI	
A. pGBM (pGBM-OS)							
IDH1	wt*IDH1* vs. m*IDH1*	0.105	0.453	0.817	1.111	0.457	2.702
MGMT	Methylated vs. unmethylated	−1.010	0.2	<0.001	0.364	0.227	0.586
Age	<50y vs. ≧50y	0.379	0.290	0.192	1.461	0.827	2.581
EOR	GTR vs. non-GTR	0.844	0.239	<0.001	2.326	1.455	3.719
KPS	<60 vs. ≧60	0.821	0.229	<0.001	2.272	1.450	3.560
B. Recurrent pGBM (Rec-pGBM-OS)							
IDH1	wt*IDH1* vs. m*IDH1*	−0.137	0.606	0.821	0.872	0.266	2.859
MGMT	methylated vs. unmethylated	−0.617	0.263	0.019	0.539	0.322	0.903
Age at recurrence	<50y vs. ≧50y	−0.356	0.401	0.375	0.701	0.319	1.537
KPS at recurrence	<60 vs. ≧60	1.132	0.287	<0.001	3.101	1.768	5.438

EOR, extent of resection; wt, wild-type; m, mutant; CI, confidence interval.

OS (P = 0.144), besides other favourable prognostic factors (13). There are no other studies that evaluated prognostic effects of *IDH1* mutation in recurrent primary GBMs with a greater number of m*IDH1* GBMs.

In the present study, multivariate analysis revealed that KPS at progression and *MGMT* methylation status were independent prognostic factors for OS after the first progression, while age at progression, and *IDH1* status were not associated with outcome (Table 4B). Both high KPS and *MGMT* promoter methylation are well-established prognosticators for better survival of GBM patients (14,15), and were identified as significant independent prognostic factors for OS in the newly diagnosed setting as well (Table 4A and B). Notably, *MGMT* promoter was methylated in 4/5 (80%) m*IDH1* pGBMs, suggesting that m*IDH1* GBM patients were further enriched with a prognostically favourable population in addition to *IDH1* mutation. As we had not explored other molecular alteration profiles including *CDKN2A/B* deletion, *EGFR* gene amplification, mutations of the *PTEN* gene and the *TERT* promoter, there remains a possibility that m*IDH* GBMs analysed in the present study carried these alterations at a higher incidence potentially

leading to aggressive behaviour, despite having better prognostic factors.

In addition to the primary analysis on survival after progression with only pGBMs, we further investigated the mixed cohort of pGBM and sGBM as an ancillary analysis to see if the poor outcome after progression of pGBM could be observed for GBM in general. In the mixed cohort of pGBM and sGBM, mOS from the first progression was not statistically different between m*IDH1* and wt*IDH* GBMs. OS from the diagnosis of 'secondary' GBMs (sGBM-OS), mostly pre-treated with adjuvant therapy, was also unfavourable (10.1 months, n = 11), albeit most of them harboured m*IDH1*. It was almost same as the survival time from the first recurrence for 'primary GBM' (Rec-pGBM-OS). These results suggest that the better prognostic property derived from *IDH1* mutation in newly diagnosed settings (7) does not translate into outcomes in recurrent settings for GBMs, perhaps due at least partially to malignant progression with acquisition of additional highly aggressive alterations that could override the survival benefit of *IDH1* mutation for the initial gliomas. Along this line, Ohno et al. reported that time from initial diagnosis of glioma to sGBM diagnosis was significantly longer for m*IDH* sGBM than for wt*IDH* sGBM (50.1 vs.13.4 months), but there was no difference in mOS from the sGBM diagnosis between m*IDH* sGBM and wt*IDH* sGBM (6.75 vs. 6.8 months) (16). Mandel et al. also showed that there was no significant difference in OS from either the first progression of pGBM, or from the timepoint of diagnosis of progressed GBM from sGBM between m*IDH1* (9.6 months) and wt*IDH1* (8.7 months) groups (17).

Given that *IDH1* mutations have consistently been associated with better prognosis in adult patients with newly diagnosed diffuse gliomas WHO grades II–IV (GBM) (6,7, 18–21), it would be expected to see a similar tendency in the present study cohort. Along this line, there was also a trend towards better survival from the initial diagnosis in patients with m*IDH1* pGBM (n = 11; 36.4 months; 95% CI: 10.6–62.2) compared with those with wt*IDH1* pGBM (n = 125: 18.3 months, 95% CI 15.0–21.5), although not reaching statistical significance (P = 0.224). The non-significance might be due to the inclusion of patients with poor initial performance status in the m*IDH1* group with a small patient number (n = 11); one patient (Table 3, Case 9) immediately died post-operatively without any adjuvant therapies, and another (Table 3, Case 2) had relatively early tumour progression (Table 3). When the patients with KPS <60 who might do worse regardless of the molecular status were excluded from both groups, there was a significant difference in OS from the initial diagnosis between m*IDH1* and wt*IDH1* pGBM patients (82.9 vs. 20.8 months; P = 0.039). Indeed, as shown in Table 4, multivariate analysis showed that KPS (60 or higher vs. <60) was an independent strong prognostic factor in the newly diagnosed setting.

Limitations of this study include that this is a retrospective analysis and allowed a long inclusion period encompassing 16 years since 2000, which was partly due to the rarity of *IDH1* mutations in GBM (22). In the 122 eligible patients collected throughout this period, there were only 13 m*IDH1* GBMs identified, which rendered low statistical power. In addition, TMZ and BEV were approved in Japan for GBM in 2006 and 2013, respectively, during this period resulting in a variety of upfront treatment regimens used among patients dependent on their treated era, especially for the initial LrGG treatment in those with 'secondary' m*IDH1* GBM (Table 3). With regard to the treatment for recurrent GBM, patients were also treated heterogeneously with either TMZ, BEV, nimustine or platinum derivatives, with or without salvage RT. To overcome these limitations, it is necessary to perform a large-scale prospective trial for recurrent pGBM including the m*IDH* subset with uniform treatment methods. However, it might be difficult to plan such a study in the future since the cIMPACT NOW update 5 has recently advocated a new term 'astrocytoma, IDH-mutant, grade 4' for the *IDH* mutated glioma carrying GBM features, distinct from 'GBM, IDH-mutant', to be incorporated in the next revision of WHO brain tumour criteria (23).

In conclusions, although *IDH1* mutation has consistently been demonstrated as a prognosticator for better survival in patients with diffuse gliomas including GBM, it may not be the case for recurrent GBMs that have progressed after initial treatments including TMZ and RT. OS of the patients with m*IDH1* GBM after the first progression from the 'primary' GBM as well as from preceding LrGG was similar to that of those with wt*IDH1*, despite a higher proportion of methylated *MGMT* in m*IDH1* GBM. Since this is a retrospective, single institutional study with heterogeneous patient backgrounds, it is important to evaluate a larger number of recurrent m*IDH* 'primary' GBM population for survival after the first progression in prospective trials to confirm the impact of *IDH* status.

Supplementary data

Supplementary data can be found at *Japanese Journal of Clinical Oncology* online.

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Conflict of interest statement

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The remaining authors declare no potential conflicts of interest.

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