Journal of Neuro-Oncology (2020) 146:451–458
https://doi.org/10.1007/s11060-019-03339-0

CLINICAL STUDY

First-line bevacizumab contributes to survival improvement in glioblastoma patients complementary to temozolomide

Nobuhiro Hata[1] · Masahiro Mizoguchi[1] · Daisuke Kuga[1] · Ryusuke Hatae[1] · Yojiro Akagi[1] · Yuhei Sangatsuda[1] · Takeo Amemiya[1] · Yuhei Michiwaki[1] · Yutaka Fujioka[1] · Kosuke Takigawa[1] · Satoshi O. Suzuki[2] · Tadamasa Yoshitake[3] · Osamu Togao[3] · Akio Hiwatashi[3] · Koji Yoshimoto[1,4] · Koji Iihara[1]

Received: 24 September 2019 / Accepted: 9 November 2019 / Published online: 4 February 2020

Abstract

Introduction First-line bevacizumab (BEV) is now available as a treatment option for glioblastoma patients with severe clinical conditions in Japan. However, the survival benefits remain controversial. To elucidate these potential survival benefits, we retrospectively analyzed survival in glioblastoma patients receiving BEV.

Methods We analyzed survival in 120 patients with IDH-wild type glioblastoma treated from 2002 to 2018. Overall survival (OS) was assessed in three treatment era subgroups [pre-temozolomide (TMZ), TMZ, and TMZ–BEV], and the correlations of prognostic factors with survival were evaluated.

Results An improvement in survival was observed after BEV approval (median OS in the pre-TMZ, TMZ, and TMZ–BEV eras: 14.6, 14.9, and 22.1 months, respectively). A Cox proportional hazards model identified extent of resection and *MGMT* methylation status as significant prognostic factors in the TMZ era; however, these factors were not significant in the TMZ–BEV era. In subgroup analyses, patients with *MGMT* methylation had improved OS after TMZ introduction (pre-TMZ vs. TMZ, 18.5 vs. 28.1 months; $P=0.13$), and those without *MGMT* methylation had significantly increased OS after BEV approval (TMZ vs. TMZ–BEV, 12.2 vs. 16.7 months; $P=0.04$).

Conclusions Our findings imply that optional first-line administration of BEV can overcome the impact of conventional risk factors and prolong survival complementary to TMZ. The patient subgroups benefitting from TMZ and BEV did not seem to overlap, and stratification based on risk factors, including *MGMT* methylation status, might be effective for selecting patients in whom BEV should be preferentially used as a first-line therapy.

Keywords Bevacizumab · Glioblastoma · Radiotherapy · Survival · Temozolomide

Introduction

Glioblastoma (GBM) is well known to have one of the most dismal prognoses in cancer, and its outcome improvement has been relatively indolent in comparison with other malignant tumors. These issues seem to be at least partly due to the limited options for adjuvant treatment. Currently, temozolomide (TMZ) and NOVO-TTF, which have been proven to improve overall survival (OS) in GBM, are internationally approved as first-line standard treatments for GBM [1, 2]. Bevacizumab (BEV) has also shown the ability to improve progression-free survival in two randomized clinical trials, AVAglio and RTOG 0825 [3, 4]. This led to the approval of BEV in Japan as a first-line treatment for GBM in 2013. However, as clinical trials failed to show OS prolongation with BEV treatment, Japan is currently the only country in

✉ Nobuhiro Hata
hatanobu@ns.med.kyushu-u.ac.jp

1 Department of Neurosurgery, Graduate School of Medical Sciences, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan

2 Department of Neuropathology, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan

3 Department of Clinical Radiology, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan

4 Department of Neurosurgery, Graduate School of Medical and Dental Sciences, Kagoshima University, Kagoshima, Japan

which BEV is routinely available for treating newly diagnosed GBM (nd-GBM) in a clinical setting.

Recent reports have suggested that the benefits of BEV in real clinical settings might be different from those in clinical studies due to their discrepant patient backgrounds. An exploratory study in AVAglio showed prolongation of OS with first-line BEV in patients who did not undergo second-line treatments, which implied the possibility of a favorable impact of BEV in patients with severe conditions, who are generally not enrolled in randomized trials [5]. In terms of real-world data, the OS prolongation after the approval of BEV for recurrent GBM has been seen in population-based studies in the US [6–8]; however, there has been no such data for first-line BEV. As Japan is the only country in which first-line BEV has been approved, only clinical data from Japan can evaluate the impact of first-line BEV on OS in the real world.

In Japan, there is a general consensus that first-line BEV is a valid option for GBM patients with severe clinical conditions, such as unresectable tumors and poor performance status [9–11]. As BEV for recurrent malignant gliomas was also simultaneously approved in Japan, BEV has been predominantly administered as a second-line option for other non-aggressive cases. We previously reported that BEV addition can prevent early clinical deterioration of nd-GBM patients with unresectable tumors and contribute to prolonged survival, especially for those with a poor performance status [12]. Based on the further accumulation of clinical cases after our previous study, we retrospectively analyzed the outcomes of GBM patients over a long period including TMZ and BEV approval to elucidate the survival benefits of first-line BEV for patients in the real world.

Materials and methods

Patients

One hundred forty-one adult (over 18 years old) patients with nd-GBM were registered in our brain tumor database between 2002 and 2018. Patients who refused adjuvant treatment ($n=3$), who had infratentorial tumors ($n=5$), or whose genetic status was unknown due to a lack of available tissue samples ($n=3$) were excluded from our analysis. In addition, we also excluded patients with *IDH1* ($n=8$) and *H3F3A* ($n=2$) mutations because we and other groups have reported them as distinct biological subgroups of GBM [13–15]. In total, 120 patients (85.1%) were analyzed to evaluate their outcomes. We divided these enrolled patients into three subgroups according to the timing of TMZ and BEV approvals (in 2006 and 2013, respectively): (I) pre-TMZ era ($n=19$), (II) TMZ era ($n=51$), and (III) TMZ–BEV era ($n=50$). No significant bias of clinical or molecular factors was observed across each era (Table 1).

The present investigation was approved by the ethics committee. Research was conducted in accordance with the 1964 Declaration of Helsinki (as revised in Fortaleza, Brazil, October 2013).

Table 1 Clinical and molecular characteristics across treatment eras

Characteristics	Treatment era			*P*-value
	Pre-TMZ ($n=19$)	TMZ ($n=51$)	TMZ–BEV ($n=50$)	
Age, years (mean ± SD)	58.4 ± 12.0	60.8 ± 11.9	64.2 ± 13.9	0.2872
Gender, *n* (%)				
Male	10 (52.6)	23 (45.1)	29 (58.0)	0.4283
Female	9 (47.4)	28 (54.9)	21 (42.0)	
KPS score, points (mean ± SD)	64.7 ± 23.7	69.0 ± 19.5	76.8 ± 20.5	0.0517
Maximum tumor diameter, mm (mean ± SD)	53.4 ± 12.5	54.7 ± 17.5	47.9 ± 18.4	0.1385
Resection, *n* (%)				
GTR/STR	11 (57.9)	29 (56.9)	29 (58.0)	0.9926
PR/biopsy	8 (42.1)	22 (43.1)	21 (42.0)	
MGMT status, *n* (%)				
Methylated	8 (42.1)	25 (51.0)	24 (48.0)	0.8023
Unmethylated	11 (57.9)	24 (49.0)	26 (52.0)	
TERT status, *n* (%)				
Mutant	13 (68.4)	34 (70.8)	29 (58.0)	0.3893
Wild-type	6 (31.6)	14 (29.2)	21 (42.0)	

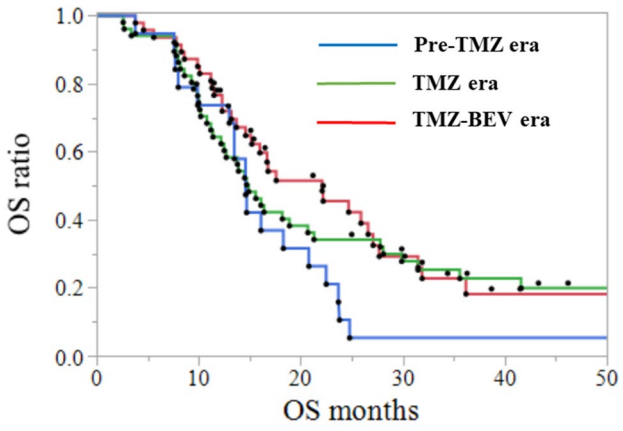
P-values across three eras were determined using one-way ANOVA test or Chi-Square

Treatments

Before TMZ approval, nd-GBM was treated with ACNU and INF-β based on the regimen designed in a previous Japanese cooperative study [16]. After TMZ approval in 2006 (eras II and III), we treated GBM using the Stupp regimen [1]. Maintenance TMZ treatments were performed for up to 2 years unless severe adverse events occurred. Since BEV approval, BEV combined with the Stupp regimen has been selected for patients with severe clinical conditions, mainly those with unresectable tumors, and the remaining patients were treated by the Stupp regimen and second-line BEV after recurrence. Subsequently, in the TMZ–BEV era, first-line BEV treatment was performed for 17 of 21 (80.9%) patients with partially resected tumors and for 5 of 29 (17.2%) patients with gross or subtotal removed tumors, defined as the removal of > 90% of the tumor using contrast-enhanced magnetic resonance imaging. Basically, BEV treatment was performed according to the AVAglio regimen [3]. Tapering or discontinuation of BEV after around 6 months of maintenance treatment was selected by the physician's decision according to the evaluation of improvements in clinical conditions and/or radiographic findings. Among first-line BEV-treated patients, the BEV-related toxicities that led to treatment being discontinued during concurrent chemo-radiation therapy, included Grade II neutropenia and thrombocytopenia that were each observed in 2 patients. With the exception of myelosuppression, discontinuation of maintenance treatment with BEV due to treatment-related toxicities occurred in 5 patients, including 2 patients with brain infarction and 2 patients with deep vein thrombosis and 1 patient who presented with gradually progressive brain atrophy after 1-year of maintenance with BEV. After the approval of carmustine implants in 2013, we provided them as an option for patients in whom subtotal or more removal was achieved. Concurrent radiotherapy was performed with fractionated extended local irradiation at a dose of 60 Gy delivered in 30 fractions over the entire treatment period; however, short-course radiotherapy of a total of 40 Gy in 15 fractions was selected for eight elderly patients (over 75 years old) since 2016 in accordance with the results of a recent randomized study [17].

Tissue samples and DNA preparation

A portion of each sample was retained for histopathological examination; the remainder was snap-frozen in liquid nitrogen and stored at − 80 °C. DNA was extracted from the tumor and matching normal tissue using the QIAamp® DNA Mini Kit (Qiagen Sciences Inc., Germantown, MD, USA) following the manufacturer's instructions.



No. at Risk (OS ratio)	12 mo.	24 mo.	36 mo.
Pre-TMZ era	15 (0.74)	3 (0.11)	2 (0.053)
TMZ era	33 (0.64)	18 (0.34)	10 (0.23)
TMZ-BEV era	35 (0.77)	16 (0.45)	6 (0.23)

Fig. 1 Kaplan–Meier estimates of overall survival (OS) in newly diagnosed glioblastoma patients ($n = 120$) according to treatment era. Pre-temozolomide (TMZ) era patients ($n = 19$) are represented by the blue line, TMZ era patients ($n = 51$) are represented by the green line, and TMZ–bevacizumab (BEV) era patients ($n = 50$) are represented by the red line

Genetic analyses

We analyzed the genetic alterations that we or other groups have shown to have prognostic potential in GBM [14, 15, 18–21]. The detection of hotspot mutations in the *IDH1*, *IDH2*, *BRAF*, and *H3F3A* gene bodies and the *TERT* promoter was performed as described previously [12, 22]. *MGMT* methylation status was assessed using a methylation-specific PCR-based method as described previously [23].

Statistical analyses

The main outcome of this study was postoperative OS.

OS rates were calculated using the Kaplan–Meier method, and differences in survival distributions were compared using the log-rank test. Cox proportional hazards models were applied to estimate the hazard ratios and 95% confidence intervals of the putative prognostic factors. All statistical analyses were conducted in JMP Pro 14 Version 14.2.0 (SAS Institute Inc., Cary, NC, USA).

Results

OS in the three treatment eras

We analyzed the clinical contributions of the TMZ and BEV approvals by comparing outcomes across the three

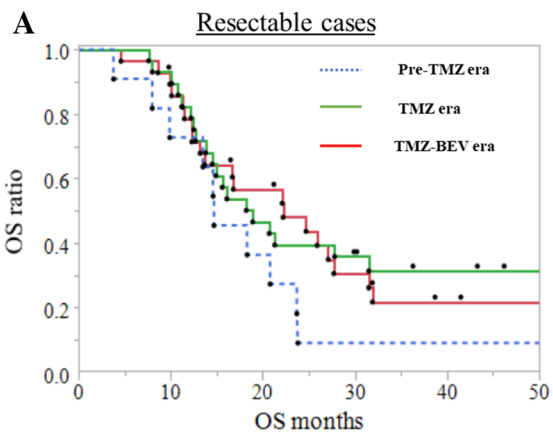
treatment eras. As shown in Fig. 1, the OS curves of the pre-TMZ and TMZ eras are similar during the earlier half of the curves, and the TMZ effect appeared only in the latter half, increasing the survival of long-term survivors; the 2.5-year survival rates in the pre-TMZ and TMZ eras were 5.3% and 27.8%, respectively. In contrast, the shift in the OS curve in the TMZ–BEV era from the TMZ era occurred around the median survival time. The median OS in the pre-TMZ, TMZ, and TMZ–BEV eras were 14.6, 14.9, and 22.1 months, respectively. These findings suggest that BEV seems to preferentially benefit short-term survivors, in comparison with the contribution of TMZ to long-term survival. This benefit of BEV seems to be particularly seen in patients with unresectable tumors, i.e., those who underwent partial resection or biopsy for their tumors: the median OS of the TMZ and the TMZ–BEV era for these patients were 10.1 and 16.0 months, respectively ($P=0.38$). However, in patients with resectable tumors, in whom BEV was predominantly selected for second-line treatment, the difference around the median OS was less clearly observed: the median OS of the TMZ and the TMZ–BEV era for these patients were 18.9 and 22.2 months, respectively ($P=0.80$). (Fig. 2). Accordingly, these findings might suggest that there was more OS improvement after BEV approval in our patient cohort with first-line BEV administration than with second-line administration.

Correlations of clinical and molecular prognostic factors with survival

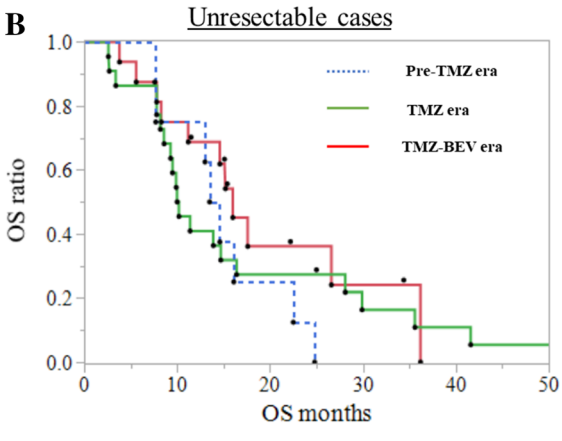
The correlations of prognostic factors with survival benefits by TMZ and BEV administration were evaluated using the Cox proportional hazards model for OS. In the TMZ era, extent of resection and *MGMT* methylation status were significant prognostic factors; however, they were not significant factors in the TMZ–BEV era (Table 2). Subgroup analysis revealed that patients with *MGMT* methylation had improved OS in the TMZ era (median OS; pre-TMZ vs. TMZ, 18.5 vs. 28.1 months; $P=0.13$), but those without *MGMT* methylation only saw improvement in OS after BEV approval (median OS; TMZ vs. TMZ–BEV, 12.2 vs. 16.7 months; $P=0.04$) (Fig. 3). These findings implied that BEV administration can overcome the negative impacts from unfavorable factors that lead to clinical conditions in which TMZ is less likely to be beneficial.

Discussion

The present findings suggest that OS prolongation in nd-GBM patients can be achieved with first-line BEV administration. In addition to this study, real clinical data from other Japanese institutes also indicated improvement in survival with selective administration of first-line BEV for patients with severe clinical conditions [10–12]. One of the noteworthy insights of the present study is the comparison of the survival benefit from TMZ alone and TMZ + BEV, wherein the shifts in the survival curves occurred at different points.



No. at Risk (OS ratio)	12 mo.	24 mo.	36 mo.
Pre-TMZ era	9 (0.73)	2(0.091)	2 (0.091)
TMZ era	24 (0.82)	12 (0.39)	8 (0.31)
TMZ-BEV era	24 (0.79)	12 (0.49)	6 (0.22)



No. at Risk (OS ratio)	12 mo.	24 mo.	36 mo.
Pre-TMZ era	7 (0.75)	2 (0.13)	0
TMZ era	10 (0.41)	7 (0.27)	3 (0.11)
TMZ-BEV era	12 (0.72)	4 (0.38)	2 (0.25)

Fig. 2 Kaplan–Meier estimates of overall survival (OS) in patients with **a** resectable and **b** unresectable glioblastoma according to treatment era. Pre-temozolomide (TMZ) era patients are represented by the blue line, TMZ era patients are represented by the green line, and TMZ–bevacizumab (BEV) era patients are represented by the red line

Table 2 Comparison of prognostic factors for overall survival (OS) between glioblastoma patients on TMZ and TMZ–BEV era

Treatment era	TMZ era (n = 51)				TMZ–BEV era (n = 50)			
Subgroup	No. of Pt.	mOS (m)	HR (95% CI)	*P*-value	No. of Pt.	mOS (m)	HR (95% CI)	*P*-value
Age								
≥ 70 years	13	11.2	2.64 (1.17–5.97)	**0.0192***	18	13.2	2.87 (1.21–6.80)	**0.0163***
< 70 years	38	16.4			32	24.7		
KPS score								
< 80 points	29	13.9	0.83 (0.38–1.79)	0.6319	15	15.3	2.42 (0.86–6.81)	0.0951
≥ 80 points	22	16.0			35	25.9		
Maximum tumor diameter								
> 50 mm	24	12.5	1.89 (0.96–3.72)	0.0642	23	22.2	1.09 (0.46–2.57)	0.8381
≤ 50 mm	27	21.3			25	22.1		
Surgical status								
Unresectable	22	10.1	2.07 (1.08–3.96)	**0.0280***	21	16.0	1.25 (0.50–3.12)	0.6352
Resectable	29	18.9			29	22.2		
MGMT status								
Unmethylated	24	12.2	2.52 (1.22–5.18)	**0.0120***	26	16.7	2.08 (0.93–4.62)	0.0736
Methylated	25	28.1			24	24.7		

No. of Pt. number of patients, *mOS (m)* median OS (months), *CI* confidence interval, *HR* hazard ratio, *KPS* Karnofsky performance status

*Bold values indicate statistical significance ($P < 0.05$)

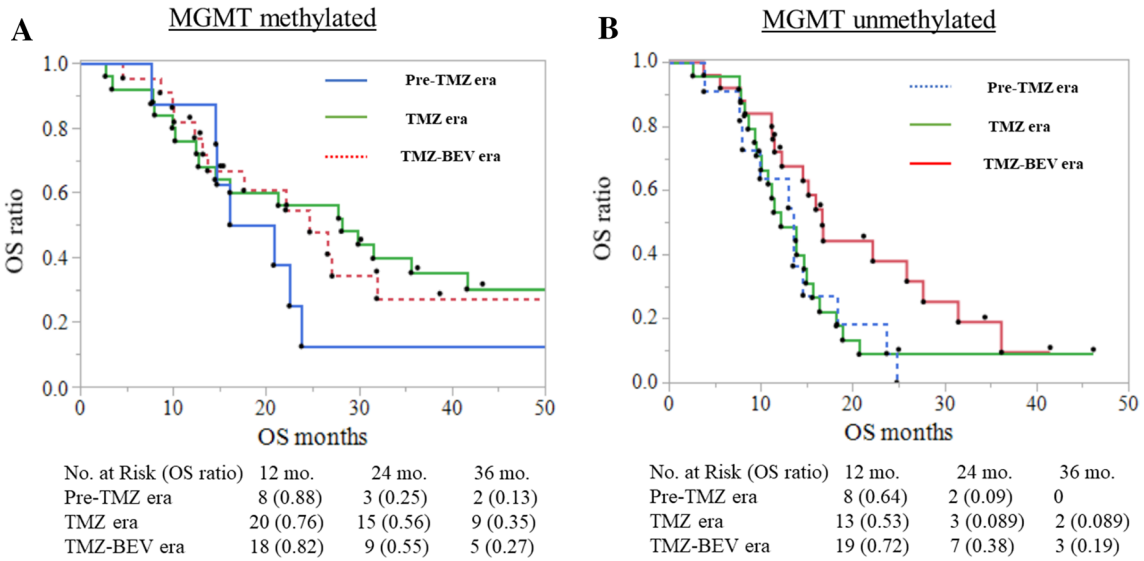


Fig. 3 Kaplan–Meier estimates of overall survival (OS) in patients with **a** *MGMT* methylated and **b** unmethylated glioblastoma according to treatment era. Pre-temozolomide (TMZ) era patients are represented by the blue line, TMZ era patients are represented by the green line, and TMZ–bevacizumab (BEV) era patients are represented by the red line

The survival curve from Stupp's study [1] showed the trend that TMZ increased long-term survivorship. Our study also showed a similar pattern after TMZ approval (Fig. 1a). Patients with favorable prognostic factors, e.g., therapeutic sensitivity typified by *MGMT* methylation, seem to be those preferentially receiving a benefit from TMZ approval, with only a limited contribution to the remaining patients. In contrast, the impact of first-line BEV administration on OS appeared as a different pattern from that of TMZ. The AVAglio study, which failed to show OS prolongation, remarked that the 1-year survival rate was significantly improved in the BEV arm, but no significant difference was observed in the 2-year survival rate [3]. The AVAglio study also provided its exploratory analysis revealing a significant OS improvement in the BEV arm among patients who did not receive second-line treatment [5], and the shift in their survival curve was

similar to our pattern. They speculated that inclusion of a greater proportion of patients with poor prognostic features in the BEV arm might have led to this new pattern. Taken together, our results suggest that, in a real clinical setting like that in Japan, BEV can improve OS in patients who conventionally fail to receive sufficient benefits from the Stupp regimen, which would appear as an improvement in survival, but only in the short term. Nonetheless, clinical trials are not expected to verify our result because of the discrepancy of patient backgrounds between clinical trials and real clinical settings [8, 9]. Although BEV is only approved in the US for recurrent cases, recent population-based data from the US revealed a similar result to the present study; only the 1-year survival rate improved significantly after BEV approval, and there was no significant difference in the 2-year survival rate [8]. Several retrospective studies derived from Japan provided clinical data suggesting OS impact of first-line BEV treatment (Table 3). As Japan is the only country in which first-line BEV for malignant glioma is currently approved, further real-world data from Japan are warranted to validate our result.

Another noteworthy finding in the present study is that survival benefits from BEV were preferentially observed among patients without *MGMT* methylation. A subgroup analysis of the AVAglio data revealed that patients with proneural GBM may derive survival benefit from first-line BEV [24]; however, predictive molecular markers indicating good clinical benefit from BEV treatment have not been previously examined. Our study suggested that *MGMT* methylation status might be an effective marker for prediction of benefit from first-line BEV treatment. Previously, we showed significant survival prolongation by BEV addition to the Stupp regimen after partial removal of poor PS cases [12]. As a possible interpretation of that result, BEV contributed to OS prolongation by decreasing the number of patients experiencing early deterioration, which led to sufficient continuation of TMZ therapy without interruption, i.e., BEV merely provides an environment in which TMZ benefits are maximized. However, the present study is not likely to support this hypothesis, considering the effectiveness of BEV was demonstrated preferentially in patients without *MGMT* methylation. As the improvement in survival by TMZ and BEV occurred at different points, groups that benefit from TMZ and BEV are unlikely to overlap.

These findings also raise the possibility that the combination of TMZ and BEV, as in the AVAglio regimen, might not always maximize their therapeutic potential. Considering the increment of treatment-related adverse events [25], the combination of these two drugs is likely to lead to more frequent discontinuation of treatment, sacrificing the maximum benefit of each monotherapy regimen. In other words, BEV could have a negative impact on patients who are expected to benefit from TMZ, and vice versa. For patients without *MGMT* methylation, first-line BEV monotherapy can be a beneficial option, especially for elderly patients or those with poor PS, in whom combination chemotherapies are assumed to be more harmful. Further accumulation of such treatment experiences is warranted.

Our study has several limitations. First, it has a non-randomized retrospective design and only a small number of

Table 3 Clinical trials/studies of first-line BEV treatment for malignant gliomas

Ref. no	Article	Study category	Inclusion criteria	mOS in BEV-treated group	mOS in control group	P-value
[3]	Chinot OL, N Engl J Med, 2014	Phase III trial (AVAglio)	GBM, WHO PS: 0–2	16.8 months	16.7 months	0.10
[4]	Gilbert MR, N Engl J Med, 2014	Phase III trial (RTOG)	GBM, KPS: ≥ 70	15.7 months	16.1 months	0.21
[5]	Chinot OL, Neuro Oncol, 2016	Subanalysis of AVAglio	GBM, WHO PS: 0–2 Without receiving second-line therapy	11.6 months	8.0 months	**0.012***
[10]	Yamaguchi S, J Neurosurg Sci, 2018	Retrospective clinical study	GBM/AA Progressive disease during TMZ/RT	20.2 months	10.5 months	**0.018***
[11]	Yonezawa H, Mol Clin Oncol, 2017	Retrospective clinical study	GBM/AA/AO Unresectable (biopsy) cases	18.9 months	8.1 months	**0.003***
[12]	Hata N, Onco Targets Ther, 2017	Retrospective clinical study	GBM, IDH-wildtype, KPS: ≤ 70 Unresectable (biopsy or PR) cases	17.4 months	9.8 months	**0.017***

Ref. no. reference number, *GBM* glioblastoma, *AA* anaplastic astrocytoma, *AO* anaplastic oligodendroglioma, *TMZ/RT* concurrent temozolomide and radiotherapy, *mOS* median OS, *BEV* bevacizumab, *KPS* Karnofsky Performance Status, *PR* partial resection

*Bold values indicate statistical significance ($P < 0.05$)

enrolled patients from a single institution. Second, the differences in OS among the calendar periods could be influenced by confounding factors. Use of historical controls might be problematic because insights and techniques of treatment for glioma have changed over time, resulting in the great amount of heterogeneity of treatment regimens for the patients across three "eras". Carmustine implants were also approved in 2013, almost simultaneous to BEV approval, which might have had a positive impact on our patients; however, we administered carmustine only after total removal of the tumor, and these patients were predominantly treated with the Stupp regimen in our institute. Considering our result that OS prolongation was more evident in unresectable cases, carmustine seems to have had a relatively limited confounding effect. To obtain more credible results and to elucidate the precise benefits of BEV treatment in real clinical settings, further accumulation of clinical cases is warranted.

Conclusions

In conclusion, our findings imply that optional first-line administration of BEV can overcome the impact of conventional risk factors and prolong survival complementary to TMZ. The treatment of GBM in Japan is unique because it is the only country in which BEV is available as a first-line treatment. In the future, it is expected that accumulation of real-world clinical data like ours can derive an original impact of BEV treatment that might affect the worldwide approval of this chemotherapeutic regimen. In addition, development of other cytotoxic treatments that are suitable for combination with BEV could improve the outcome of patients with GBM.

Acknowledgements This work was supported by a Japanese Society for the Promotion of Science Grants-in-Aid for Scientific Research (JSPS KAKENHI) Award (Grant No. JP16K10779). We thank Ms. Aki Sako for technical assistance.

Compliance with ethical standards

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

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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