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Clinical study

Long-term follow-up after BCNU wafer implantation in patients with newly diagnosed glioblastoma

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ABSTRACT

1,3-Bis(2-chloroethyl)-1-nitrosourea (BCNU, or Carmustine) wafers are intraoperatively implantable wafers used to achieve local tumor control. There is scarce data about the behavior of wafers in the long-term follow-up of implanted cases. We reviewed the data of 64 patients with newly diagnosed glioblastoma treated by surgery, BCNU wafers, radiation therapy, and temozolomide administration. This cohort included 55 patients who presented first recurrence, and 49 of them showed tumor progression to death. The MR imaging of each patient at the terminal stage and an autopsy case were used to elucidate the tumor progression pattern after the wafer implantation. We subdivided the first recurrence pattern into local, distant, and multifocal based on MR imaging or into infield, outfield, and marginal based on the radiation field. The first recurrence pattern was 33 patients (60%) with local, 13 (24%) with distant, and nine (16%) with multifocal recurrence, or 38 patients (69%) with infield, 13 (24%) with outfield, and four (7%) with marginal. The median and mean time intervals between MR imaging at the terminal stage and death were 2.0 and 2.3 months, respectively. Of note, 13 patients with first distant recurrence had no obvious radiological local tumor progression even at the terminal stage. Long-term follow-up after BCNU wafer implantation revealed that patients with first distant recurrence had long-lasting local tumor control until the terminal stage.

1. Introduction

Glioblastoma is a primary brain tumor with a dismal prognosis. The treatment of glioblastoma involves surgery, radiation therapy (RT), temozolomide administration, and tumor treating fields [1]. Also, lomustine-temozlomide combination prolonged the OS of glioblastoma with methylated O^6-methylguanine DNA methyltransferase (MGMT) promoter [2]. Despite aggressive treatments, almost all of these patients experienced a local recurrence as a first recurrence pattern, reaching up to 85% during their clinical course [3–6]. Therefore, 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU, or carmustine) wafers (Gliadel®; Eisai, Tokyo, Japan; intraoperatively implantable wafers) were created to obtain local tumor control. Giese et al. first reported about the recurrence patterns in patients with glioblastoma who were treated with BCNU wafers followed by RT. They found that > 90% of the patients experienced recurrence around the resection cavity [7], but their treatment protocol did not include temozolomide administration. Three retrospective studies reported that the first recurrence pattern changed with the addition of temozolomide administration to surgery, BCNU wafers, and RT, and the incidence of local recurrence decreased to 50%–64% [8–10], which strengthened the evidence of the wafers fulfilling their purpose. However, there is limited evidence of a long-term follow-up after BCNU wafer implantation and tumor progression pattern after the first recurrence.

Understanding the tumor progression pattern after the first recurrence is important to comprehend the effect of wafers and decide treatment strategies at subsequent recurrence. Repeat surgery can be performed in cases of local recurrence [11,12]; however, the treatment options are very limited in cases of distant or multifocal recurrence. To date, the pattern of post-BCNU wafer recurrence has been categorized into local, distant, diffuse, and multifocal as proposed by Giese et al., which is a classification sys-

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tem based on MR imaging and anatomical location [7]. Recent studies have discussed recurrence patterns based on the radiation field, which are briefly categorized into infield, outfield, and marginal [13,14]; however, this classification system has not been used for post-BCNU wafer recurrence.

In the present study, we retrospectively reviewed the data of patients with glioblastoma who underwent radical resection and BCNU wafer implantation followed by RT with concomitant temozolomide administration. First, we evaluated the first recurrence pattern based on MR imaging and the radiation field and then correlated this with patient characteristics, progression-free survival (PFS), and overall survival (OS). Second, we investigated MR imaging findings at the terminal stage of glioblastoma to radiologically observe tumor progression pattern. Finally, we histologically investigated an autopsy case to confirm the long-term effects of BCNU wafer implantation.

2. Patients and methods

2.1. Study design

In this single-center retrospective study, we reviewed the data of all patients with glioblastoma who were treated at the Kitasato University Hospital, and the Ethics Committee approved the study protocol. BCNU wafers were approved for clinical use in Japan in January 2013. We included all consecutive patients with newly diagnosed glioblastoma who underwent radical surgeries after January 2013 to April 2018 to perform long-term follow-up. BCNU wafers were routinely implanted, except in some patients with diffuse infiltration or those for whom rapid diagnosis was not possible such as diagnosis of malignant glioma by frozen section. We defined the BCNU wafer group as patients who underwent BCNU wafer implantation at the initial surgery followed by RT with concomitant temozolomide administration and maintenance. Data including patient characteristics, pre- and postoperative MR imaging, bevacizumab administration, and first recurrence pattern were collected from medical records. We also reviewed MR imaging findings at the last follow-up and an autopsy case to understand how the tumor progressed at the terminal stage. Tumor volume and extent of resection were calculated using OsiriX (Pixmeo SARL, Bernex, Switzerland), as reported previously [15–17].

2.2. RT

Pre- and postoperative MR imaging were fused with planned CT (2.5-mm slice thickness) for target delineation using gadolinium (Gd)-enhanced T1-weighted (GdT1) and fluid-attenuated inversion recovery (FLAIR) MR imaging by radiation oncologists (AS and HI) using Pinnacle (Philips HealthCare, Fitchburg, WI, USA).

We defined gross tumor volume (GTV) as the entire surgical resection cavity plus the Gd-enhanced residual tumor, initial clinical target volume (CTV1) as GTV plus the volume of hyperintense areas on FLAIR MR images plus margins of 1.5–2 cm, and boost clinical target volume (CTV2) as GTV plus margins of 1.5 cm or the volume of hyperintense areas on FLAIR MR imaging. We considered anatomical barriers such as the skull, ventricle, falx, and cerebellar tentorium for CTV margins. Initial planning target volume (PTV1) and boost planning target volume (PTV2) were generated by adding 3–5-mm margins to CTV1 and CTV2, respectively.

RT was administered using three-dimensional conformal RT or intensity-modulated RT selected based on the complexity of the target volume and the proximity of critical organs at risk. PTV1 was treated with 40–50 Gy in 20–25 fractions, followed by additional 10–20 Gy in 5–10 fractions to PTV2. Regardless of age, all patients received a total dose of 60 Gy, prescribed to D95 (i.e., minimum coverage dose of 95% of the target) for RT, which ensured at least 95% isodose coverage of PTV.

2.3. Immunohistochemistry and IDH1 sequencing

MGMT status was evaluated using immunohistochemistry, as reported previously [18,19]. MGMT protein immunoreactivity was evaluated semiquantitatively by estimating the fraction of positive cells; <20% was defined as low reactivity, 20%–50% as moderate, and > 50% as high. Further, isocitrate dehydrogenase (IDH) 1 status was evaluated using the IDH1-R132H antibody. Cases under 56-year-old underwent sequencing for IDH1 status using primers by Parsons et al [20]. Macrophage was stained using the CD68 (PGM1) antibody.

2.4. Recurrence pattern

All patients underwent routine GdT1 MR imaging every 2 months after the initial radiochemotherapy. Emergence and timing of any new enhanced lesions were recorded as recurrent lesions and recurrence day, respectively. If any patient became symptomatic, GdT1 MR imaging was performed immediately.

Recurrence pattern was analyzed according to the modified definition and classification reported previously [3,7,9]. We subdivided the first recurrence into local, distant, and multifocal. Local recurrence was defined as a new enhanced lesion at, adjacent to, or contiguous with the primary resection cavity. Distant recurrence included leptomeningeal dissemination and was defined as a new enhanced lesion not contiguous with the primary resection cavity. Multifocal recurrence was defined as a mixture of distant and local recurrences. We used the term ‘‘non-local recurrence” to include both distant and multifocal recurrence. Recurrence at the terminal stage was also analyzed to determine how each first recurrence pattern eventually progressed as local, distant, and multifocal.

We also subdivided the first recurrence into infield, marginal, and outfield based on the positional relationship between a new enhanced lesion and radiation field, as reported previously [13]. Marginal recurrence was defined as the presence of 20%–80% of the recurred lesion within the 95% isodose surface. In patients with multifocal recurrence, any tumors located outside the radiation field were classified as outfield recurrence.

2.5. Treatment at recurrence

At the first recurrence, we considered a second resection if the tumor was diffuse infiltrative with a localized enhanced lesion, i.e., resection of the enhanced lesion might contribute to tumor control, and if the Karnofsky Performance Status (KPS) of the patient was > 60%. If there was no indication for surgery, we treated recurrent cases by combination of maintenance or rechallenge of temozolomide, bevacizumab administration, stereotactic RT, or other/none medication.

2.6. MRI at the terminal stage and an autopsy case

The patients were followed at the outpatient clinic using routine MR imaging until they experienced end-of-life symptoms such as drowsiness, poor communication, and dysphagia [21,22]. The last follow-up MR imaging, which was used to determine the discontinuation of treatment, was used to assess tumor progression. Some of the patients died at our hospital, and the rest died at other nursing hospitals or their homes. Histological investigation of a 75-year-old male with first distant recurrence was performed by autopsy.

2.7. Statistical analysis

OS was determined from the date of the initial surgery to the time of death or the last follow-up examination. PFS was determined from the date of the initial surgery to the time of confirmed first recurrence. The probability of OS and PFS was calculated with the Kaplan–Meier method and compared with the log-rank test. For statistical convenience, low, moderate, and high MGMT positivities were classified as positive (≥20%; moderate and high) and negative (<20%; low) as previously reported [19]. All calculations were performed using Prism (GraphPad Software, Inc., San Diego, CA), R 3.3.0, or Stata 16 (StataCorp, Texas, USA). A *P*-value of < 0.05 was considered statistically significant.

3. Results

3.1. Patients

During the study period, we performed BCNU wafer implantation in 96 patients including 64 patients with newly diagnosed glioblastoma (Table 1). These 64 patients included 34 males and 30 females who were aged 23–80 (median, 67) years. Mean tumor volume was 41.1 ± 28.8 cm^3, and tumors were commonly located in the temporal, parietal, and frontal lobes. Forty patients (63%) involved the subventricular zone and 39 (61%) required ventricular opening during surgery (ventricular entry). Median follow-up period was 19 (range, 7–83) months. Bevacizumab was administered during the initial treatment in 14 patients, which included 10 from clinical trials and 4 with early progression resulting in a KPS of < 70%. Our cohort included negative prognostic radiological features such as the so-called butterfly glioblastoma in 5 patients [23], multicentric lesions in 7 [24–26], and dissemination in 4 [27,28]. 33 cases were MGMT positive, and none were IDH1-R132H positive. 11 cases younger than 56-year-old underwent *IDH1* sequencing, resulting in all wild-types.

Table 1
Clinical features of BCNU wafer group.

	BCNU wafer (n = 64)
Age, median (range), years	67 (23–80)
Sex, male	34 (53%)
Preoperative KPS, median (range), %	90 (30–100)
Tumor volume, mean ± SD, cm^3	41.1 ± 28.8
Tumor location	
Frontal	17 (27%)
Temporal	24 (37%)
Parietal	21 (33%)
Occipital/Others	2 (3%)
Extent of resection	
GTR (100%)	41 (64%)
STR (95–99%)	7 (11%)
PR (<95%)	16 (25%)
Involvement of subventricular zone	40 (63%)
Ventricular entry	39 (61%)
MIB-1 labeling index, mean	34.4%
IDH1-R132H, positive	0
MGMT, positive	33 (52%)
Bevacizumab at initial treatment	14 (22%)
Recurrent cases	55
The number of deaths	49
Butterfly glioblastoma	5
Multicentric glioblastoma	7
Disseminated glioblastoma	4

KPS, Karnofsky Performance Status; SD, standard deviation; IDH1, isocitrate dehydrogenase 1; MGMT, O^6-methylguanine DNA methyltransferase, MGMT, positive; immunohistological positivity of ≥ 20% (moderate and high)

3.2. Pattern of the first recurrence

During the follow-up period, 55 of the 64 patients presented first recurrence, 7 had no recurrence, and 2 refused follow-up. At the first recurrence, 25 patients underwent a second surgery, 41 received either temozolomide rechallenge or further temozolomide maintenance, one received nimustine hydrochloride (ACNU) administration, 24 received bevacizumab administration, 7 underwent stereotactic RT, 1 underwent immunotherapy, and 5 refused additional treatments.

1) Recurrence based on MR imaging

Local recurrence was observed in 33 of the 55 patients (60%), distant recurrence in 13 (24%) (Fig. 1A–C, G–I), and multifocal recurrence in 9 (16%) (Fig. 1D–F). Initial tumor volume was largest in patients with multifocal recurrence. The extent of resection and MGMT positivity did not differ among recurrence patterns. A second surgery was more frequently performed for local recurrence than for distant and multifocal recurrences (Table 2 and Fig. 2).

2) Recurrence based on the radiation field

Correlation of recurrence pattern with the radiation field showed that infield recurrence occurred in 38 patients (69%), outfield recurrence in 13 (24%) (Fig. 1B, E, and H), and marginal in 4 patients (7%) (Table 3). Initial tumor volume was the largest in patients with marginal recurrence. MGMT positivity was 55% in infield recurrence, 62% in outfield recurrence, and 75% in marginal recurrence and was not statistically significant ($P = 0.72$). A second surgery was frequently performed in patients with infield recurrence.

3) Comparison of first recurrence pattern between recurrence based on MR imaging and that based on the radiation field

Patients with infield recurrence included 33 with local recurrence, 2 with distant recurrence, and 3 with multifocal recurrence. Patients with marginal recurrence included 2 with distant recurrence and 2 with multifocal recurrence. Further, patients with outfield recurrence included 9 with distant and 4 with multifocal recurrence.

3.3. MR imaging at the terminal stage and an autopsy case

49 of the 64 patients died. MR imaging at the terminal stage was performed in 48 patients, and one patient refused follow-up MR imaging. Median and mean interval between the last MR imaging and death was 2.0 and 2.3 months, respectively. Among the 33 patients with first local recurrence, 23 died, eight are still alive, and two were lost to follow-up. MR imaging performed at the terminal stage in the 23 patients who died demonstrated local progression in 14 (64%), distant progression in four (18%), and multifocal progression in four (18%); further, follow-up MR imaging was not performed in one patient. Among the nine patients with multifocal recurrence, all patients died; two of the patients presented local progression and seven presented multifocal progression (Fig. 1F). Among the 13 patients with first distant recurrence, 12 died and one is still alive without further recurrence. Of note, all 12 patients who died demonstrated distant progression, including dissemination, without radiologically visible progression at the initial tumor location (Fig. 1C and I). Fig. 3 shows a case of first distant recurrence (Fig. 3A–E). MRI at the terminal stage showed tumor progression at the left temporal lesion but none at the left frontal initial resected area (Fig. 3F). Hematoxylin and eosin staining showed no apparent tumor but many macrophages at the initial resected

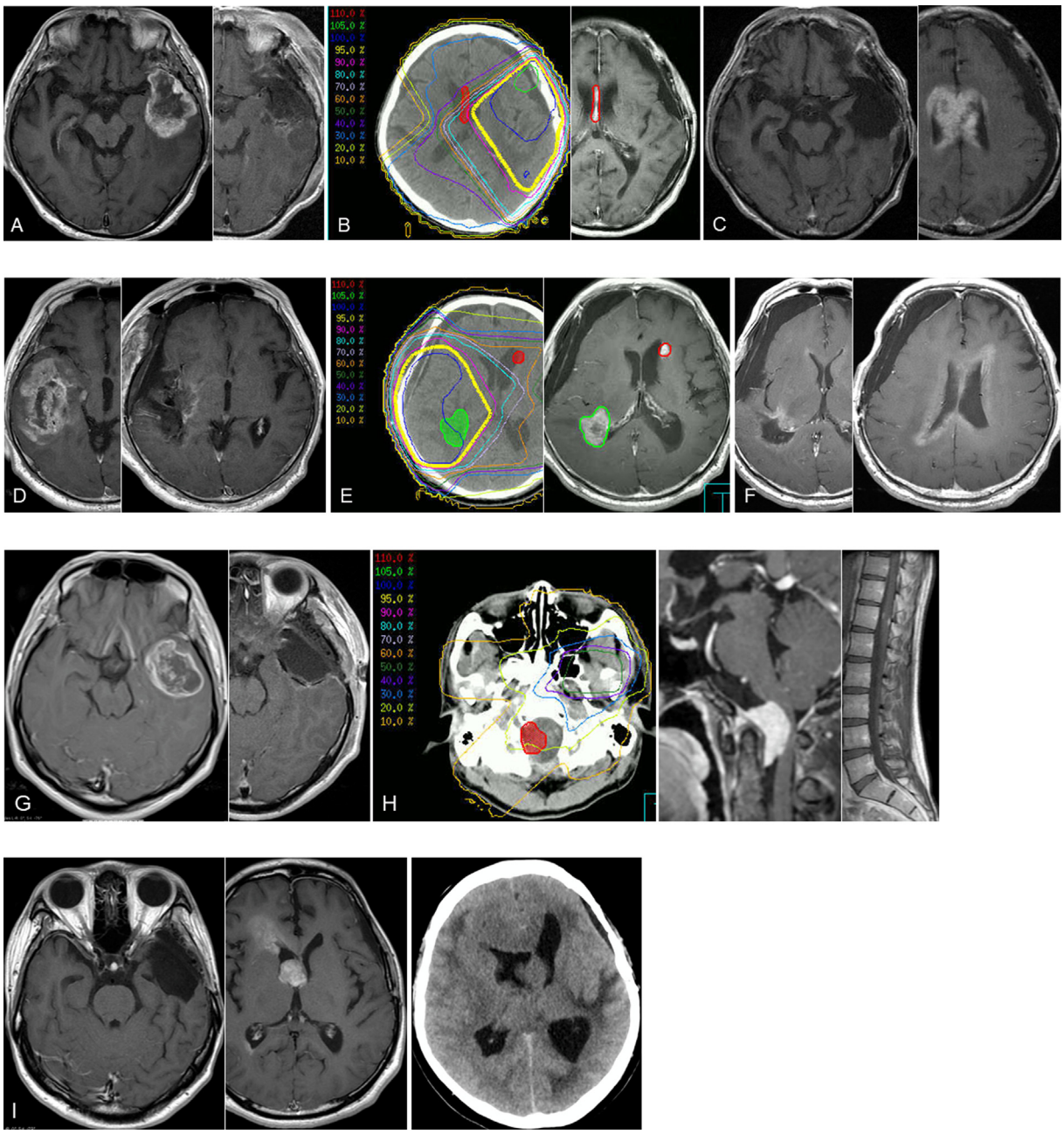

Fig. 1. Representative cases of distant and multifocal recurrence. A–C: A 77-year-old male with left temporal glioblastoma (A) underwent surgery with 97% resection and implantation of eight BCNU wafers, followed by the Stupp regimen. Distant recurrence outside the radiation field developed at the corpus callosum and cingulate gyrus 8 months postoperatively (B). No local recurrence was found at the terminal stage, but distant recurrence progressed to death (OS, 14 months) (C). D–F: A 64-year-old male with right temporal glioblastoma (D) underwent surgery with 100% resection (slight enhancement on postoperative gadolinium-enhanced T1-weighted MR image also appeared as high intensity on T1 MR image) and implantation of eight BCNU wafers, followed by the Stupp regimen. Local recurrence at the right trigone inside the radiation field and distant recurrence at the left frontal horn outside the radiation field emerged 6 months postoperatively, defined as multifocal or outfield recurrence (E). Both local and distant recurrences developed at the terminal stage and progressed to death (overall survival, 11 months) (F). G–I: A 23-year-old male with left temporal glioblastoma (G) underwent surgery with 100% resection and implantation of four BCNU wafers, followed by the Stupp regimen. Distant recurrence outside the radiation field developed at the medulla oblongata and spine 14 months postoperatively (H). No local recurrence was detected at the terminal stage, but distant recurrence progressed to death (MR image, 28 months postoperatively; CT, a day before death; OS, 30 months) (I).

area (Fig. 3G, original magnification x20; and 3H, original magnification x200). CD68 staining confirmed the presence of macrophages (Fig. 3I). On the other hand, hematoxylin and eosin staining showed many tumor cells at the distant lesion (Fig. 3J).

3.4. Survival

The study cohort had a median OS of 19 months and median PFS of 8 months (Fig. 4A). MGMT-negative patients had a significantly better prognosis ($P = 0.014$, data not shown). According to the first recurrence pattern based on MR imaging, PFS and OS were 8 and 25 months in patients with local recurrence, 11 and 14 months in those with distant recurrence, and 6 and 15 months in those with multifocal recurrence, respectively (Table 2 and Fig. 4B and C). OS was significantly longer in patients with local recurrence than in those with multifocal recurrence ($P < 0.0001$, Fig. 4C). Further, PFS was significantly longer in the patients with distant recurrence than in those with multifocal recurrence ($P = 0.0064$, Fig. 4B).

Table 2
Clinical features of local, distant, and multifocal recurrence.

	Local (n = 33)	Distant (n = 13)	Multifocal (n = 9)	*P*
Age, median (range), years	66 (47–80)	71 (23–71)	67 (58–75)	0.18*
Sex, male	17 (52%)	8 (62%)	6 (67%)	0.66
Tumor volume, mean ± SD, cm^3	37.3 ± 24.7	37.5 ± 19.0	63.3 ± 44.7	0.22*
Tumor location				
Frontal	11 (33%)	2 (15%)	3 (33%)	0.55
Temporal	8 (24%)	6 (46%)	3 (33%)	
Parietal	13 (39%)	5 (38%)	2 (22%)	
Occipital/ Others	1 (3%)	0 (0%)	1 (11%)	
Extent of resection				0.97
GTR (100%)	21 (64%)	7 (54%)	6 (67%)	
STR (95–99%)	4 (12%)	2 (15%)	1 (11%)	
PR (<95%)	8 (24%)	4 (31%)	2 (22%)	
Involvement of subventricular zone	17 (52%)	9 (69%)	7 (78%)	0.27
Ventricular entry	17 (52%)	9 (69%)	7 (78%)	0.27
MIB-1 labeling index, mean	34.6%	36.8%	34.4%	0.75*
IDH1 R132H, positive	0	0	0	NA
MGMT, positive	18 (55%)	8 (62%)	6 (67%)	0.78
Bevacizumab at initial treatment	7 (21%)	3 (23%)	3 (33%)	0.75
Treatment at initial recurrence				
Second surgery	20 (61%)	2 (15%)	3 (33%)	0.016
Temozolomide	23 (1 ACNU 70%)	10 (77%)	9 (100%)	
Bevacizumab	10 (30%)	6 (46%)	8 (56%)	
SRT	1 (3%)	4 (31%)	2 (11%)	
None/others	3 (9%)	3 (23%)	0 (0%)	
Cumulative Bevacizumab use	25 (76%)	12 (92%)	9 (100%)	NA
Median PFS	8 months	11 months	6 months	
Median OS	25 months	14 months	15 months	
Progression at the terminal stage				
Local	14	0	2	
Distant	4	12	0	
Multifocal	4	0	7	

GTR, gross total resection; STR, subtotal resection; PR, partial resection; SRT, stereotactic radiation therapy; PFS, progression free survival; OS, overall survival; ACNU, nimustine hydrochloride
* Kruskal-Wallis test.

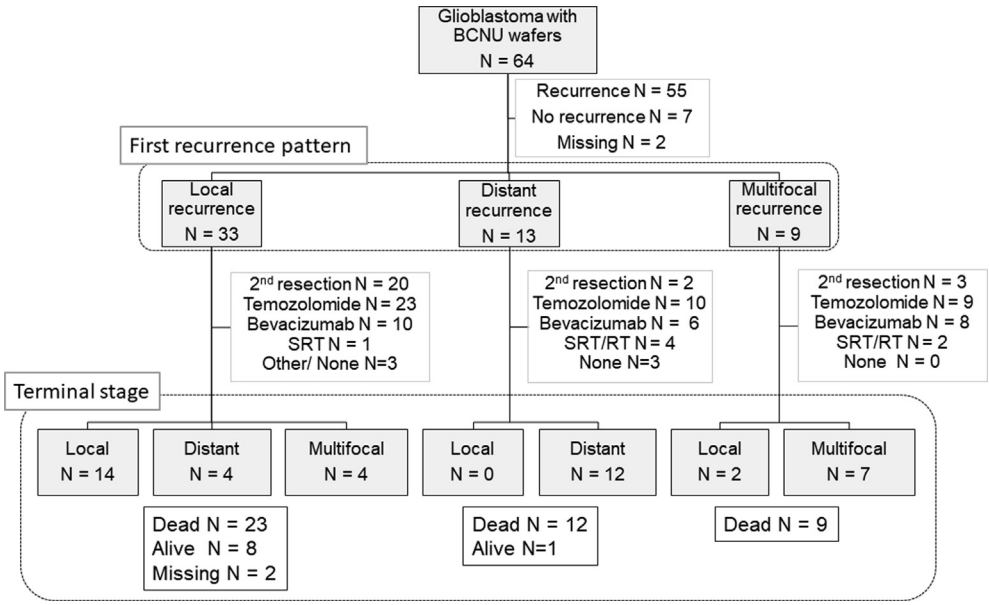

Fig. 2. Flow diagram of all patients in the BCNU wafer group (N = 64). All patients were classified based on the first recurrence pattern (local, distant, or multifocal). Next, tumor progression based on MR images at the terminal stage was assessed as local, distant, or local + distant progression. SRT, stereotactic radiation therapy.

According to the first recurrence pattern based on the radiation field, PFS and OS were 7.5 and 24 months in patients with infield recurrence, 8 and 12 months in those with outfield recurrence, and 6.5 and 21 months in those with marginal recurrence, respectively (Table 3 and Fig. 4D and E). Then, OS was significantly longer in patients with infield recurrence than in those with outfield recurrence ($P < 0.0001$, Fig. 4E).

Next, we analyzed the OS between local and non-local recurrence. OS was 25 months in local recurrence and 14.5 months in non-local recurrence ($P = 0.0023$, Fig. 4F).

Table 3
Clinical features of infield, outfield, and marginal recurrence.

	Infield (n = 38)	Outfield (n = 13)	Marginal (n = 4)	
Age, median (range), years	66 (47–80)	67 (23–77)	67 (66–73)	0.42*
Sex, male	20 (53%)	9 (69%)	2 (50%)	0.56
Tumor volume, mean ± SD, cm^3	37.7 ± 24.3	40.5 ± 25.3	82.8 ± 51.8	0.13*
Tumor location				
Frontal	12 (32%)	1 (8%)	3 (75%)	NA
Temporal	10 (26%)	7 (54%)	0 (0%)	
Parietal	15 (39%)	4 (31%)	1 (25%)	
Occipital/ Others	1 (3%)	1 (8%)	0	
Extent of resection				0.88
GTR 100%	25 (66%)	7 (54%)	2 (50%)	
STR (95–99%)	4 (11%)	2 (15%)	1 (25%)	
PR (<95%)	9 (23%)	4 (31%)	1 (25%)	
Involvement of subventricular zone	21 (55%)	9 (69%)	3 (75%)	0.55
Ventricular entry	21 (55%)	9 (69%)	3 (75%)	0.55
MIB-1 labeling index, mean	33.4%	39.6%	36.0%	0.23*
IDH1 R132H, positive	0	0	0	NA
MGMT, positive	21 (55%)	8 (62%)	3 (75%)	0.72
Bevacizumab at initial treatment	7 (18%)	4 (31%)	2 (50%)	0.29
Treatment at initial recurrence				
Second surgery	21 (55%)	4 (31%)	0	NA
Temozolomide	28 (1 ACNU 74%)	11 (84%)	3 (75%)	
Bevacizumab	14 (37%)	7 (54%)	3 (75%)	
SRT	4 (11%)	1 (8%)	2 (50%)	
None/others	3 (8%)	2 (15%)	1 (25%)	
Cumulative Bevacizumab use	29 (76%)	13 (100%)	4 (100%)	NA
Median PFS	7.5 months	8 months	6.5 months	
Median OS	24 months	12 months	21 months	
Progression at the terminal stage				
Local	15	1	0	
Distant	4	9	2	
Multifocal	6	3	2	

* Kruskal-Wallis test.

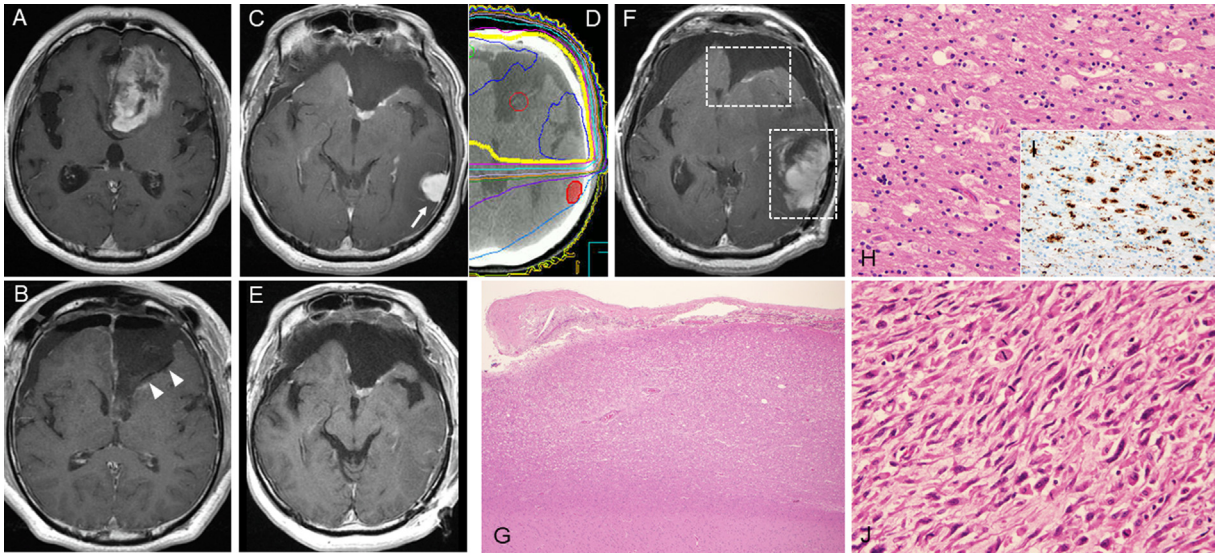

Fig. 3. An autopsy case. A 75-year-old male with left frontal glioblastoma underwent initial resection with BCNU wafer implantation, RT, and temozolomide, followed by maintenance temozolomide. A: Initial GdT1 MR imaging. B: Postoperative GdT1 MR imaging. Arrowheads indicate BCNU wafers. C and D: GdT1 MR imaging (C) and CT with radiation field (D) obtained 14 months after the initial surgery. The first distant recurrence occurred at the left temporal lobe (arrow: tumor). E: GdT1 MR imaging after the second surgery for distant recurrence. F: GdT1 MR imaging 3 months after the second surgery and 21 days before death. There was no tumor at the initial location but a massive tumor at the distant location. G- I: Hematoxylin and eosin staining of brain tissue from an autopsy. Tumor tissue obtained at the initial tumor location indicates no obvious tumor cells (G; original magnification × 20, H; original magnification x200) but many macrophages confirmed by CD68 staining (I). Tissue obtained at the distant location indicates many tumor cells without obvious macrophages (J).

4. Discussion

In this study, we investigated 64 patients with newly diagnosed glioblastoma treated with surgery, BCNU wafers, RT, and temozolomide administration. BCNU wafers were created to achieve local tumor control; therefore, to understand the effect of the wafers, it is worth investigating recurrence pattern and long-term follow-up after the first recurrence. We demonstrated the

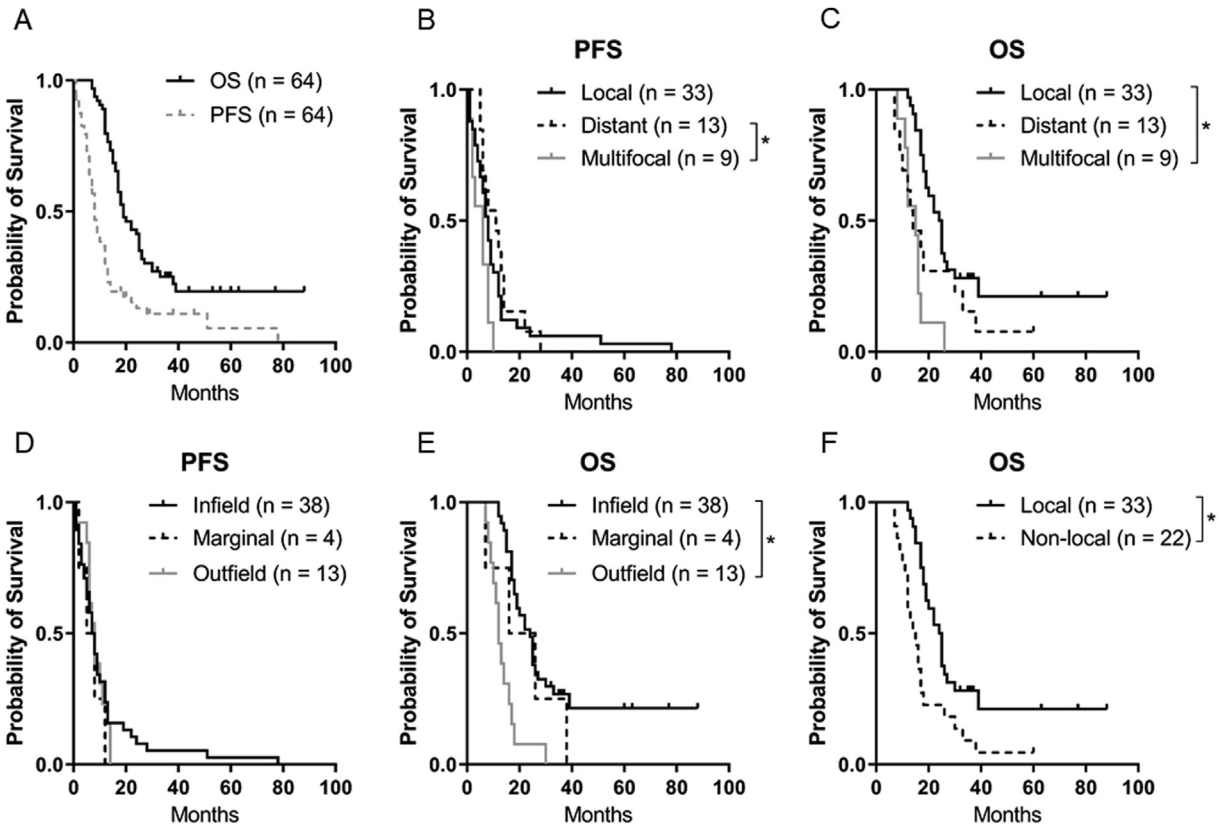

Fig. 4. Kaplan–Meier curves. A: OS and PFS of patients with glioblastoma treated by radical surgery and BCNU wafer implantation, followed by the Stupp regimen (BCNU wafer group). OS, 19 months; PFS, 8 months. B and C: PFS and OS of local, distant, and multifocal recurrence in the BCNU wafer group. Local, 8 and 255 months; distant, 11 and 14 months; multifocal, 6 and 15 months, respectively. (PFS, distant vs. multifocal, $^*P = 0.0064$; OS, local vs. multifocal, $^*P < 0.0001$). D and E: PFS and OS of patients with infield, outfield, and marginal recurrence in the BCNU wafer group. Infield, 7.5 and 24 months; outfield, 8 and 12 months; marginal, 6.5 and 21 months, respectively. (OS, infield vs. outfield, $^*P < 0.0001$). F: OS between local and non-local recurrence (distant + multifocal). 25 months for those who presented local recurrence, and 14.5 months for those who presented non-local recurrence ($P = 0.00023$).

first recurrence pattern, survival based on the first recurrence pattern, and progression pattern after the first recurrence using MR imaging findings at the terminal stage. To the best of our knowledge, for the first time, we presented how the tumor progressed after the BCNU wafer implantation at the terminal stage.

The first recurrence pattern based on MR imaging in the BCNU wafer group was local recurrence in 33 patients (60%), distant recurrence in 13 (24%), and multifocal recurrence in 9 (16%). The incidence of non-local recurrence reaches 40%, matching the rate of four previous reports that presented the non-local recurrence after the wafer implantation and the Stupp regimen as 24%-50% [8–10,29]. No clinical features, including the extent of resection, ventricular entry, and MGMT positivity, were correlated with the recurrence pattern.

Next, we investigated the first recurrence pattern based on the radiation field. Previous reports demonstrated that glioblastoma recurrence after RT and temozolomide administration was found infield in 72–93% of patients, outfield in 2–22%, and marginal in 0–15% [13,14,30–33]. In our series, we implanted BCNU wafers in addition to the RT and temozolomide administration, and infield, outfield, and marginal recurrences were found in 69%, 24%, and 7% patients, respectively. Therefore, the recurrence pattern based on the radiation field did not differ with or without BCNU wafers. In our series, we did not find any correlation between MGMT positivity and recurrence patterns. The correlation between MGMT status and recurrence pattern based on the radiation field has differed across studies [13,14,34], which can be explained by the different methodologies to assess the MGMT status. We used immunohistochemical staining, which is considered unreliable due to poor reproducibility and high interobserver variability [35].

MR imaging at the terminal stage could show that the first local and multifocal recurrences presented various progression patterns such as local, distant, and multifocal. However, all patients with first distant recurrence showed further distant or dissemination progression. Surprisingly, they remained free from local tumor progression until the terminal stage. MR imaging cannot visualize all tumors, but no obvious radiological local recurrence was observed at the initial tumor location in the first distant recurrent cases. Ogura et al. reviewed the initial and cumulative recurrence patterns of glioblastoma in the temozolomide era [36]. They showed that distant recurrence was cumulatively observed in 89% of patients with glioblastoma, and 88% of them had an uncontrollable local lesion before distant recurrence. Our findings of a controlled local lesion with distant progression are unique; therefore, the addition of BCNU wafers may affect recurrence pattern. Our previous findings indicated that CD8-positive and CD68-positive cells were introduced around the implanted BCNU wafers [16], which might indicate the induction of antitumor immunity and somehow affect long-term local tumor control. We also examined an autopsy case with first distant recurrence that remained free from local progression. The brain tissue from this autopsy contained many macrophages but no apparent tumor cells at the initial tumor location.

Although the median age of our cohort is high (67 years old), the OS of local recurrent cases reached 25 months, and the OS of non-local recurrent cases was only 14.5 months. Dorner et al. investigated glioblastoma patients treated with BCNU wafer and

Stupp regimen and the OS of local, diffuse, and non-local recurrence based on the recurrence pattern was 54, 47, 45 weeks, respectively [29]. Their study included recurrent glioblastoma cases, but, consistent with our data, the BCNU wafer implantation may increase the non-local recurrent cases with shorter OS. One explanation is due to the retrospective nature of the study, and all consecutive cases were included. In our series, 41% of the patients with non-local recurrent cases had negative prognostic radiological features such as multicentric, butterfly, and dissemination [23–28], which are generally excluded in a prospective clinical study. We used a cox proportional hazard model to estimate the hazard ratios (HRs) and the 95% confidence intervals (CI) of the negative prognostic radiological features, and as expected, the result was HRs 2.1, 95% CI 1.1–3.8, $P = 0.023$ (data not shown). Therefore, cases with negative prognostic radiological features remained poor in OS even after wafer implantations, indicating that the wafer's effect was limited in such cases. Another possible explanation is that the wafer affects the biology of glioblastoma. Shorter survival and less local recurrence were observed in a study about high dose radiation therapy (90 Gy) in glioblastoma [37]. High-intensity treatment on the local region may change the biology of glioblastoma.

The present study has some limitations. This study is a single institute retrospective analysis with relatively small samples without a control cohort. The cohort included all consecutive cases treated by the BCNU wafers in the temozolomide era and received different treatments after recurrences; therefore, multiple factors may affect the survival and recurrence pattern. Indeed, several retrospective studies showed different survival and recurrence patterns based on MR imaging or the radiation field [5,6,33,38]. We showed that glioblastoma with non-local recurrence presented shorter OS, but non-local recurrence itself is a poor prognostic factor [39] without the BCNU wafers implantation. Also, how wafer implantations affect recurrence and survival cannot be elucidated by the retrospective data. Therefore, prospective studies with a large number of patients and analysis of molecular characteristics are warranted. Currently, a multicenter randomized phase III study for newly diagnosed maximally resected glioblastoma comparing BCNU wafer implantation followed by chemoradiotherapy with temozolomide or chemoradiotherapy alone is ongoing in Japan (Japanese Clinical Oncology Group (JCOG) 1703) [40] to answer all these limitations.

We evaluated the clinical features of patients with glioblastoma treated with surgery, BCNU wafers, RT, and temozolomide administration with particular consideration of recurrence pattern. BCNU wafers achieved local tumor control in some patients, which lasted until the terminal stage, but these patients developed distant progression. Future studies should elucidate who will obtain the most benefit from BCNU wafers.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

[1] Stupp R, Taillibert S, Kanner A, Read W, Steinberg DM, Lhermitte B, et al. Effect of Tumor-Treating Fields Plus Maintenance Temozolomide vs Maintenance Temozolomide Alone on Survival in Patients With Glioblastoma: A Randomized Clinical Trial. JAMA 2017;318(23):2306. https://doi.org/10.1001/jama.2017.18718.
[2] Herrlinger U, Tzaridis T, Mack F, Steinbach JP, Schlegel U, Sabel M, et al. Lomustine-temozolomide combination therapy versus standard temozolomide therapy in patients with newly diagnosed glioblastoma with methylated MGMT promoter (CeTeG/NOA–09): a randomised, open-label, phase 3 trial. The Lancet. 2019;393(10172):678–88.
[3] Shibahara I, Sonoda Y, Saito R, Kanamori M, Yamashita Y, Kumabe T, et al. The expression status of CD133 is associated with the pattern and timing of primary glioblastoma recurrence. Neuro Oncol. 2013;15:1151–9.
[4] Chamberlain MC. Radiographic patterns of relapse in glioblastoma. J Neurooncol. 2011;101(2):319–23.
[5] Konishi Y, Muragaki Y, Iseki H, Mitsuhashi N, Okada Y. Patterns of intracranial glioblastoma recurrence after aggressive surgical resection and adjuvant management: retrospective analysis of 43 cases. Neurol Med Chir (Tokyo). 2012;52(8):577–86.
[6] Petrecca K, Guiot M-C, Panet-Raymond V, Souhami L. Failure pattern following complete resection plus radiotherapy and temozolomide is at the resection margin in patients with glioblastoma. J Neurooncol. 2013;111(1):19–23.
[7] Giese A, Kucinski T, Knopp U, Goldbrunner R, Hamel W, Mehdorn HM, et al. Pattern of recurrence following local chemotherapy with biodegradable carmustine (BCNU) implants in patients with glioblastoma. J Neurooncol. 2004;66(3):351–60.
[8] Asano K, Kurose A, Kamataki A, Kato N, Ogawa K, Katayama K, et al. Importance and accuracy of intraoperative frozen section diagnosis of the resection margin for effective carmustine wafer implantation. Brain Tumor Pathol. 2018;35(3):131–40.
[9] Shimato S, Nishizawa T, Ohshima T, Imai T, Goto S, Yamamoto T, et al. Patterns of Recurrence After Resection of Malignant Gliomas With BCNU Wafer Implants: Retrospective Review in a Single Institution. World Neurosurg. 2016;90:340–7.
[10] Sonoda Y, Shibahara I, Matsuda K-I, Saito R, Kawataki T, Oda M, et al. Opening the ventricle during surgery diminishes survival among patients with newly diagnosed glioblastoma treated with carmustine wafers: a multi-center retrospective study. J Neurooncol. 2017;134(1):83–8.
[11] Chaichana KL, Zadnik P, Weingart JD, Olivi A, Gallia GL, Blakeley J, et al. Multiple resections for patients with glioblastoma: prolonging survival. J Neurosurg. 2013;118(4):812–20.
[12] Ringel F, Pape H, Sabel M, Krex D, Bock HC, Misch M, et al. Clinical benefit from resection of recurrent glioblastomas: results of a multicenter study including 503 patients with recurrent glioblastomas undergoing surgical resection. Neuro Oncol. 2016;18(1):96–104.
[13] Brandes AA, Tosoni A, Franceschi E, Sotti G, Frezza G, Amistà P, et al. Recurrence pattern after temozolomide concomitant with and adjuvant to radiotherapy in newly diagnosed patients with glioblastoma: correlation With MGMT promoter methylation status. J Clin Oncol. 2009;27(8):1275–9.
[14] Buglione M, Pedretti S, Poliani PL, Liserre R, Gipponi S, Spena G, et al. Pattern of relapse of glioblastoma multiforme treated with radical radio-chemotherapy: Could a margin reduction be proposed?. J Neurooncol. 2016;128(2):303–12.
[15] Chaichana KL, Jusue-Torres I, Navarro-Ramirez R, Raza SM, Pascual-Gallego M, Ibrahim A, et al. Establishing percent resection and residual volume thresholds affecting survival and recurrence for patients with newly diagnosed intracranial glioblastoma. Neuro Oncol. 2014;16:113–22.
[16] Shibahara I, Hanihara M, Watanabe T, Dan M, Sato S, Kuroda H, et al. Tumor microenvironment after biodegradable BCNU wafer implantation: special consideration of immune system. J Neurooncol. 2018;137(2):417–27.
[17] Sanai N, Polley M-Y, McDermott MW, Parsa AT, Berger MS. An extent of resection threshold for newly diagnosed glioblastomas. J Neurosurg. 2011;115(1):3–8.
[18] Shibahara I, Sonoda Y, Suzuki H, Mayama A, Kanamori M, Saito R, et al. Glioblastoma in neurofibromatosis 1 patients without IDH1, BRAF V600E, and TERT promoter mutations. Brain Tumor Pathol. 2018;35(1):10–8.
[19] Sonoda Y, Yokosawa M, Saito R, Kanamori M, Yamashita Y, Kumabe T, et al. O(6)-Methylguanine DNA methyltransferase determined by promoter hypermethylation and immunohistochemical expression is correlated with progression-free survival in patients with glioblastoma. Int J Clin Oncol. 2010;15(4):352–8.
[20] Parsons DW, Jones S, Zhang X, Lin J- C-H, Leary RJ, Angenendt P, et al. An integrated genomic analysis of human glioblastoma multiforme. Science 2008;321(5897):1807–12.
[21] Walbert T, Khan M. End-of-life symptoms and care in patients with primary malignant brain tumors: a systematic literature review. J Neurooncol. 2014;117(2):217–24.

[22] Walbert T. Integration of palliative care into the neuro-oncology practice: patterns in the United States. Neurooncol Pract. 2014;1:3–7.
[23] Dziurzynski K, Blas-Boria D, Suki D, Cahill DP, Prabhu SS, Puduvalli V, et al. Butterfly glioblastomas: a retrospective review and qualitative assessment of outcomes. J Neurooncol. 2012;109(3):555–63.
[24] Lasocki A, Gaillard F, Tacey MA, Drummond KJ, Stuckey SL. The incidence and significance of multicentric noncontrast-enhancing lesions distant from a histologically-proven glioblastoma. J Neurooncol. 2016;129 (3):471–8.
[25] Pope WB, Sayre J, Perlina A, Villablanca JP, Mischel PS, Cloughesy TF. MR imaging correlates of survival in patients with high-grade gliomas. AJNR Am J Neuroradiol. 2005;26:2466–74.
[26] Patil CG, Yi A, Elramsisy A, Hu J, Mukherjee D, Irvin DK, et al. Prognosis of patients with multifocal glioblastoma: a case-control study. J Neurosurg 2012;117(4):705–11.
[27] Pietschmann S, von Bueren AO, Henke G, Kerber MJ, Kortmann R-D, Müller K. An individual patient data meta-analysis on characteristics, treatments and outcomes of the glioblastoma/gliosarcoma patients with central nervous system metastases reported in literature until 2013. J Neurooncol. 2014;120 (3):451–7.
[28] Shibahara I, Saito R, Osada Y, Kanamori M, Sonoda Y, Kumabe T, et al. Incidence of initial spinal metastasis in glioblastoma patients and the importance of spinal screening using MRI. J Neurooncol. 2019;141(2):337–45.
[29] Dörner L, Mustafa A, Rohr A, Mehdorn HM, Nabavi A. Growth pattern of tumor recurrence following bis-chloroethylnitrosourea (BCNU) wafer implantation in malignant glioma. J Clin Neurosci. 2013;20(3):429–34.
[30] Sherriff J, Tamangani J, Senthil L, Cruickshank G, Spooner D, Jones B, et al. Patterns of relapse in glioblastoma multiforme following concomitant chemoradiotherapy with temozolomide. Br J Radiol. 2013;86 (1022):20120414. https://doi.org/10.1259/bjr.20120414.
[31] Minniti G, Amelio D, Amichetti M, Salvati M, Muni R, Bozzao A, et al. Patterns of failure and comparison of different target volume delineations in patients with glioblastoma treated with conformal radiotherapy plus concomitant and adjuvant temozolomide. Radiother Oncol. 2010;97(3):377–81.
[32] McDonald MW, Shu H-K, Curran WJ, Crocker IR. Pattern of failure after limited margin radiotherapy and temozolomide for glioblastoma. Int J Radiat Oncol Biol Phys. 2011;79(1):130–6.
[33] Milano MT, Okunieff P, Donatello RS, Mohile NA, Sul J, Walter KA, et al. Patterns and timing of recurrence after temozolomide-based chemoradiation for glioblastoma. Int J Radiat Oncol Biol Phys. 2010;78(4):1147–55.
[34] Holmes JA, Paulsson AK, Page BR, Miller LD, Liu W, Xu J, et al. Genomic predictors of patterns of progression in glioblastoma and possible influences on radiation field design. J Neurooncol. 2015;124(3):447–53.
[35] Mansouri A, Hachem LD, Mansouri S, Nassiri F, Laperriere NJ, Xia D, et al. MGMT promoter methylation status testing to guide therapy for glioblastoma: refining the approach based on emerging evidence and current challenges. Neuro Oncol. 2019;21:167–78.
[36] Ogura K, Mizowaki T, Arakawa Y, Ogura M, Sakanaka K, Miyamoto S, et al. Initial and cumulative recurrence patterns of glioblastoma after temozolomide-based chemoradiotherapy and salvage treatment: a retrospective cohort study in a single institution. Radiat Oncol. 2013;8(1):97. https://doi.org/10.1186/1748-717X-8-97.
[37] Nakagawa K, Aoki Y, Fujimaki T, Tago M, Terahara A, Karasawa K, et al. High-dose conformal radiotherapy influenced the pattern of failure but did not improve survival in glioblastoma multiforme. Int J Radiat Oncol Biol Phys. 1998;40(5):1141–9.
[38] Tejada S, Díez-Valle R, Aldave G, Marigil M, de Gallego J, Domínguez PD. Factors associated with a higher rate of distant failure after primary treatment for glioblastoma. J Neurooncol. 2014;116(1):169–75.
[39] Burger M, Breuer S, Cieplik H, Harter P, Franz K, Bähr O, et al. Bevacizumab for Patients with Recurrent Multifocal Glioblastomas. Int J Mol Sci. 2017;18 (11):2469. https://doi.org/10.3390/ijms18112469.
[40] Kadota T, Saito R, Kumabe T, Mizusawa J, Katayama H, Sumi M, et al. A multicenter randomized phase III study for newly diagnosed maximally resected glioblastoma comparing carmustine wafer implantation followed by chemoradiotherapy with temozolomide with chemoradiotherapy alone; Japan Clinical Oncology Group Study JCOG1703 (MACS study). Jpn J Clin Oncol. 2019;49:1172–5.