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Cerebrospinal fluid dissemination of high-grade gliomas following boron neutron capture therapy occurs more frequently in the small cell subtype of IDH^{1R132H} mutation-negative glioblastoma

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Abstract

We have used boron neutron capture therapy (BNCT) to treat patients in Japan with newly diagnosed or recurrent high-grade gliomas and have observed a significant increase in median survival time following BNCT. Although cerebrospinal fluid dissemination (CSFD) is not usually seen with the current standard therapy of patients with glioblastoma (GBM), here we report that subarachnoid or intraventricular CSFD was the most frequent cause of death for a cohort of our patients with high-grade gliomas who had been treated with BNCT. The study population consisted of 87 patients with supratentorial high-grade gliomas; 41 had newly diagnosed tumors and 46 had recurrent tumors. Thirty of 87 patients who were treated between January 2002 and July 2013 developed CSFD. Tumor histology before BNCT and immunohistochemical staining for two molecular markers, Ki-67 and IDH1^{R132H}, were evaluated for 20 of the 30 patients for whom pathology slides were available. Fluorescence in situ hybridization (FISH) was performed on 3

IDH1^{R132H}-positive and 1 control IDH1^{R132H}-negative tumors in order to determine chromosome 1p and 19q status. Histopathologic evaluation revealed that 10 of the 20 patients' tumors were IDH1^{R132H}-negative small cell GBMs. The remaining patients had tumors consisting of other IDH1^{R132H}-negative GBM variants, an IDH1^{R132H}-positive GBM and two anaplastic oligodendrogliomas. Ki-67 immunopositivity ranged from 2 to 75%. In summary, IDH1^{R132H}-negative GBMs, especially small cell GBMs, accounted for a disproportionately large number of patients who had CSF dissemination. This suggests that these tumor types had an increased propensity to disseminate via the CSF following BNCT and that these patients are at high risk for this clinically serious event.

Keywords

Boron neutron capture therapy; high-grade glioma; cerebrospinal fluid dissemination; small cell glioblastoma; anaplastic oligodendroglioma

Introduction

The prognosis of patients with high-grade gliomas remains poor despite surgical resection, followed by radiation therapy and adjuvant chemotherapy with temozolomide (TMZ). Tumor recurrence is almost inevitable and the median survival time (MST) of patients with newly diagnosed glioblastomas (GBMs) is only 14.6 months [1]. We have employed boron neutron capture therapy (BNCT) for the treatment of Japanese patients with either recurrent or newly diagnosed high-grade gliomas. A significant survival benefit of 23.5 months was seen in those patients with newly diagnosed GBMs who received BNCT, followed by photon boost [2, 3]. Furthermore, those patients with recurrent gliomas, especially those with poor prognosis who were classified as 3+7 by recursive partitioning analysis (RPA) [4], had a MST of 9.1 months compared to 4.4 months for those in the same RPA class [3] who had been treated by standard therapy.

BNCT is a form of tumor-selective particle radiation therapy consisting of two components [5, 6]. *First*, a boron-10 (¹⁰B)-containing drug is administered to the patient in order to obtain a sufficient tumor ¹⁰B concentration, and *second*, the tumor is irradiated with epithermal neutrons. The resulting ¹⁰B(n,α)⁷Li capture reaction produces alpha particles whose short path length (5–9 μm) results in selective killing of tumor cells with sparing of adjacent normal tissues. In our experience, with all of our clinical trials of BNCT, the most frequent cause of death following treatment has been cerebrospinal fluid dissemination (CSFD) [2, 3]. This is defined by us as tumor spread beyond the primary site of occurrence into the subarachnoid or intraventricular space, presumably secondary to tumor cell entry into the CSF.

Although CSF dissemination may be more common [7, 8, 9] than generally realized [10], it is unusual following current standard therapy for high-grade gliomas. Mandel et al. [11] have reported that in a series of 595 GBM patients treated in clinical trials at the Baylor College of Medicine, 36 (4%) developed dissemination. The MST of these patients from diagnosis to death was 16 months, the time from initial diagnosis to dissemination was 11.9 months, and the time to death following dissemination was 3.5 months.

Assuming that more than 85% of tumor recurrences following standard treatment is local, that is within 2 cm of the original margin of the contrast-enhancing lesion [12, 13], local control by BNCT has been significantly better than that obtained by conventional radiotherapy [12, 13]. The purpose of the present study was to determine if there was a correlation between CSFD and histopathology and molecular markers. We used two molecular markers: Ki-67, an indicator of cell proliferation rate, and isocitrate dehydrogenase-1 R132H mutation (IDH1^{R132H}), a useful prognostic marker. As has been reported in the literature [14, 15], patients whose tumors have this mutation survive longer than those with wild-type IDH. Co-deletion of chromosomes 1p and 19q has also been reported to be associated with a better prognosis in patients with oligodendrogliomas [16]. Based on this, we have attempted to determine what role, if any, these markers had in tumor dissemination.

Methods

Patient eligibility criteria and BNCT clinical regimen

This clinical study was approved by the Ethics Committee of the Osaka Medical College, Takatsuki, Japan and the Kyoto University Committee for Radiation Therapeutics, Kyoto, Japan. The eligibility criteria for inclusion in the study have been described in detail elsewhere [2, 3]. Both newly diagnosed patients and those with recurrent gliomas had PET imaging using ¹⁸F-boronophenylalanine (BPA) prior to BNCT to assess the uptake and distribution of ¹⁸F-BPA by the tumor and to estimate the tumor boron concentrations in the primary or recurrent tumors [17, 18]. The lesion/normal brain (L/N) uptake ratios of ¹⁸F-BPA were determined using data obtained from the imaging studies, and dose planning was performed according to the L/N ratio, as previously described [19, 20]. If the tumors were deeper than 6 cm from the surface of the scalp, partial removal of the mass or cyst evacuation was carried out followed by air instillation via an Ommaya reservoir in order to increase the depth of penetration of neutrons to the deepest parts of the tumor [21, 22]. The general procedure used for BNCT has been described elsewhere [23].

As summarized in Table 1, BNCT was performed within 1 month following surgery for patients with newly diagnosed gliomas using three different protocols. In Protocol 3, a continuous infusion of BPA was administered before and during the irradiation with 500 mg/kg of BPA (400 mg/kg for 2 h+100 mg/kg for 1 h) and BSH was administered in the same way as indicated in Protocols 1 and 2 [23] (Tables 1, 3). For patients with recurrent gliomas, the neutron irradiation time was adjusted by simulation so as to not exceed 13 Gy-Eq to normal brain [3]. For patients with newly diagnosed tumors, the normal brain dose was adjusted to not exceed 13 Gy-Eq in Protocols 1 and 3, and 15 Gy-Eq in Protocol 2 [2]. Here, Gy-Eq (Gy: gray) corresponded to the biologically equivalent X-ray dose that would have produced the same effects on the tumor and normal brain.

Patient follow-up

Patients were followed up by Gd-enhanced MRI. When the presumptive tumor increased in size or new tumors appeared on MRI, BPA-PET imaging was performed to determine if this was indeed due to tumor progression or, alternatively, radiation necrosis [24]. If the PET

studies suggested tumor progression, additional treatments were offered to the patient, as summarized in Tables 2 and 4. The cause of death was analyzed with the following categories: local tumor progression (TP), leptomeningeal (subarachnoid) or intraventricular CSF dissemination, and other causes of death (OCD). These classifications were based on Gd-enhanced MRI, BPA-PET, cytology of CSF, histology of the surgical specimens or biopsy [2, 3]. Cerebrospinal fluid dissemination (CSFD) was defined as tumor spread beyond the primary site of occurrence into the subarachnoid or intraventricular space, presumably secondary to tumor cell entry into the CSF.

Histopathologic examination

Tumor histology was evaluated on formalin-fixed, paraffin-embedded (FFPE) hematoxylin and eosin (H&E) stained sections that were prepared using standard protocols for 20 of the patients whose paraffin blocks were available to us. Immunohistochemical staining for Ki-67 and IDH1^{R132H} also was evaluated in these same patients who developed CSFD following BNCT. Baked 4- μ m-thick FFPE sections were deparaffinized, rehydrated, blocked with 3% hydrogen peroxide for 5 min, and pretreated with pH 6.0 antigen retrieval solution (Dako, S1699) at 96 °C for 25 min in a steamer and cooled 15 min. Slides were then incubated with either anti-Ki-67 (Dako, M7240, diluted 1:400) or anti-IDH1^{R132H} (Dianova, clone H09, diluted 1:50) for 60 min. The detection system was MACH 3 mouse horseradish peroxidase (HRP) (Biocare, M3M530L) for 20 min followed by treatment with 3,3-diaminobenzidine (DAB) (Dako, K346811) for 5 min. All incubations were at ambient temperature unless otherwise specified.

The status of chromosome segments 1p and 19q was assessed on FFPE tumor sections using dual-color fluorescence *in situ* hybridization (FISH) probes (Vysis LSI 1p36/1q25 and LSI 19q13/1p13 SpectrumOrange and SpectrumGreen, Abbott, Abbott Park, IL) on the 3 tumors which were IDH^{R132H} immunopositive (patients 6, 7, and 18). Representative microscopic fields were selected and signals were detected and scored using the Bioview Accord Plus image analysis system (Abbott) followed by fluorescence microscopy. A 1p/1q ratio (or 19q/19p ratio) less than 0.8 was scored as positive for allelic loss. Tumors were categorized, and in some cases reclassified, according to histopathological type and grade using the new 2016 WHO criteria [25]. The majority of tumor samples were obtained at the time of initial surgery prior to BNCT (Table 2).

Statistics

Statistical analyses were performed using IBM SPSS statistical analysis software (version 20). A simple binomial test was used to test the significance of the distribution of small GBM histology. The Mann–Whitney U Test was employed for continuous data, i.e., time to dissemination and time to death. The Spearman correlation was used for comparison of patient age and Ki-67 labeling index to time to dissemination and time to death. Non-parametric statistics were used since nearly all of the continuous variables of interest were not normally distributed. All tests were two-sided, with the exception of the binomial test which was one-sided. A p value of 0.05 was considered statistically significant.

Results

Patient demographics, treatment, and patterns of recurrence

Patient demographics have been summarized in Tables 2 and 4. The BNCT protocol, radiation doses, time until dissemination and death, and the cause of death for the 20 patients for whom histologic sections were available for analysis are summarized in Tables 2 and 3. Data for the additional group of ten patients who developed CSFD, for whom histologic sections were not available, are summarized in Tables 4 and 5. Patients who developed CSFD were treated in a variety of ways (Table 3). With regard to chemotherapy, starting in 2006 we began using temozolomide (TMZ) as standard therapy for high-grade gliomas. Prior to 2006, other cytoreductive agents such as nimustine (ACNU), combined with procarbazine and vincristine (PCV), were used most frequently. Since 2013, 13 patients have received bevacizumab, however it is noteworthy that only one of the 30 patients who developed dissemination had received it. Between January 2002 and July 2013, a total of 87 patients were enrolled in this study. Among them, 41 patients had newly diagnosed GBM and 46 had recurrent tumors who had received BNCT at the time of tumor recurrence. Thirty patients of the 87 developed CSFD and the pattern of dissemination was determined radiographically. Among these, ten patients had local recurrence plus CSFD to the subarachnoid space; seven had tumor dissemination only in the intracranial subarachnoid space; and two had CSFD to the spinal cord (thecal sac plus cord). While 11 patients developed intraventricular dissemination (Tables 3 and 5). Representative examples of CSFD are shown in Fig. 1a–c, d–f, g–i.

Histopathologic diagnoses and molecular markers

Histopathologic examination revealed that 10 of the 20 tumors (50%) were histologically the small cell subtype of IDH wild-type GBM (WHO Grade IV) [25] and two were anaplastic oligodendrogliomas (WHO grade III), together comprising 60% of the total. Since small cell GBM accounts for only about 10% of GBM diagnoses [26], the fact that up to 50% of our patients had small cell GBM at the time of presentation represents a statistically significant disproportionate number of cases than which would have been expected by chance ($p < 0.001$, $n = 20$). Seven of the eight remaining tumors were histopathologically classified as conventional GBM (four of which contained gemistocytes and one contained giant cells), and one was classified as the IDH wild-type glioblastoma variant gliosarcoma. No tumors examined were consistent with GBM with a primitive neuronal component by histology. Representative photomicrographs of tumors are shown in Fig. 2.

Three of the 20 tumors were immunopositive for IDH^{R132H}. Immunohistochemical staining for Ki-67 was quite variable, ranging from <5–75%, with the highest labeling indices noted in an anaplastic oligodendroglioma (patient 6) and a small cell GBM (patient 12, Fig. 2e). FISH studies were performed to identify allelic loss of 1p and 19q on the 3 tumors which were IDH^{R132H} mutant protein-immunopositive (patients 6, 7, and 18) and 1 control IDH^{R132H}-immunonegative tumor (patient 17; Table 2). Two patients, 6 and 7, showed loss of both 1p and 19q confirming the diagnosis of anaplastic oligodendroglioma (Fig. 2i). One tumor showed borderline loss of 1p and intact 19q (patient 18); and one had intact 1p and 19q (patient 17), consistent with high-grade astrocytoma (GBM) in both cases (Table 2).

Based on Gd-enhanced MRI, involvement of the ependymal layer was evaluated in the 30 patients who developed CSFD (Tables 2, 3, 4, 5). Of the 30 patients, 13 (43%) had extension of the tumor into the ependymal layer before BNCT and 9 (30%) of the 30 had surgical intervention that involved the ependymal layer (Tables 2, 4).

Efficacy of the different BNCT protocols, radiation doses and histopathology on survival

For patients treated in Protocol 1 (BPA, BSH and air instillation), the tumor absorbed dose (Max, Min and D95) following BNCT, and the dose and timing of other radiation therapy (SRS, XRT), time to dissemination, survival time after BNCT, and the cause of death are summarized in Tables 3 and 5. Following BNCT, the tumor radiation doses (D95) ranged from 19.3 to 94.6 Gy-Eq, but this did not affect the time until CSFD and death. Similarly, other types of radiation therapy did not appreciably affect the time until dissemination and death following BNCT, nor did instillation of air. Likewise, Ki-67 positivity did not correlate with the time of onset of CSF dissemination or death after BNCT ($p = 0.742$, $n = 17$ and $p = 0.442$, $n = 17$, respectively). Of the 20 patients included in our study, five survived more than 20 months (patients 3, 5, 7, 10 and 18) after BNCT. Two of these (patients 7 and 18), who each survived greater than 30 months after BNCT, were IDH^{R132H}(+). On the other hand, one patient (patient 6), with a IDH^{R132H}(+) anaplastic oligodendroglioma survived only 6 months after BNCT (Tables 2, 3). Extension of the tumor to the ependymal layer before BNCT did not appear to affect the time to CSFD ($p = 0.983$, $n = 29$) or survival time after BNCT ($p = 0.408$, $n = 30$). Surgical intervention involving the ependymal layer also did not significantly affect the time to CSFD ($p = 0.871$, $n = 29$) or time to death after BNCT ($p = 0.824$, $n = 30$), or the cause of death (Tables 2, 3, 4, 5).

Discussion

The purpose of the present study was to develop some understanding as to why a subset of patients with high-grade gliomas who had been treated by BNCT developed CSFD of their tumors. Thirty of 87 patients (34.5%) developed dissemination, which is higher than the 7–27% that has been reported elsewhere [10]. Patients with high-grade gliomas who received high-dose radiation therapy had a higher rate of CSFD than those who had received lower doses [27]. The most frequent pattern of recurrence for patients who had received hypofractionated, high-dose, intensity modulated radiation therapy (IMRT) was dissemination (45.6%) [28]. A higher rate of CSFD at recurrence in the high-dose groups seems somewhat paradoxical. However, it should be noted that, compared to conventional external beam photon radiation (total dose 60 Gy in 2 Gy fractions), high-dose radiotherapy (total dose 80 or 90 Gy) or hypofractionated high-dose IMRT was associated with an increased time to recurrence and prolonged survival [27, 28, 29]. Similar trends were found among patients treated with BNCT, namely, that there was better local control, but a higher incidence of CSFD [2, 3].

BNCT probably results in one of the highest tumor biological dose rates of any currently used types of radiation therapy and this might be associated with a higher rate of specific mutations that could result in a more aggressive pattern of dissemination. There are a number of reports relating photon radiation to the migration and invasiveness of gliomas [30,

31, 32]. Chakravarti and co-workers first reported that radiation could enhance the in vitro invasiveness of primary glioma cells via activation of the Rho signaling pathway [31]. It was concluded that radiation-mediated invasion was fundamentally distinct from invasion under normal physiologic controls.

At the present time there is relatively little information available on the effects of charged particles [33], and more specifically alpha particles [34], on the expression of transcription factors. Since alpha particles are tumoricidal if they discharge their energy within tumor cells, bystander effects to adjacent cells would be most relevant. As reported by Ghandi et al. [34], two major transcription factors regulating cellular response to ionizing radiation, the most important of which is NF κ B, could be implicated in bystander responses. If the balance in signaling pathways was affected in bystander cells, this might lead to increased survival and perhaps a greater propensity to develop a more aggressive phenotype. It may well be that small cell subtype of GBMs and oligodendrogliomas may be more susceptible to mutational events involving key transcription factors that are associated with the invasiveness of high-grade gliomas. EGFR amplification is present in approximately 70% of small cell astrocytomas and small cell GBMs compared to 40% of primary glioblastomas [35] and this is thought to be an important driver of glioblastoma invasiveness [36]. Small cell GBMs therefore may have a propensity to invade or disseminate regardless of radiation therapy, including X-rays or BNCT. From a more simplistic, but not necessarily invalid perspective, the cells of small cell GBMs and oligodendrogliomas may more easily escape into the CSF partly due to their smaller size compared to those in other high-grade gliomas. Interestingly, small cell lung carcinomas notoriously show much more extensive dissemination compared to “larger cell” adenocarcinomas. Whether integrity of the ependymal cell layer influences CSFD of tumors is the subject of ongoing discussion [37, 38]. In our study, tumors which invaded or did not invade the ependymal layer developed CSFD. It has been reported that surgical manipulation of the ependymal layer appeared to be related to CSFD [39]. However, this also was observed in patients who did not have surgical manipulation of the ependymal layer, suggesting that the integrity of the ependymal layer was not essential for CSFD.

The presence of IDH mutations, the vast majority of which are IDH1^{R132H}, has been shown to correlate with a significantly better prognosis [14]. The majority of oligodendrogliomas, diffuse astrocytomas and secondary GBMs express the IDH1^{R132H} mutation, while primary GBMs are generally negative [14, 15, 16]. Overall, IDH mutations are present in 10 to 12% of GBMs [15]. In our study, only three tumors were positive for IDH1^{R132H}, two of which were WHO grade III anaplastic oligodendrogliomas (patients 6 and 7) and one of which was a GBM involving the frontal lobe of a 32-year-old patient (patient 18). To the best of our knowledge there has been no reported relationship between IDH mutations and dissemination, and this could not be evaluated in our small cohort of patients. It is noteworthy, however, that IDH wild-type GBMs are generally more clinically aggressive than IDH mutant GBMs. IDH mutations are absent in small cell GBMs [26, 40], and this was confirmed by us.

Of the 20 patients' tumors that demonstrated CSFD, two were anaplastic oligodendrogliomas, eight were histologically conventional GBMs or other GBM variants.

The remaining ten tumors were small cell GBMs. This is comparable to the overall percentage of high-grade gliomas that disseminate to the leptomeninges. One limitation of our study was that we were unable to review the histopathology of the patients' tumors following BNCT, and therefore we do not know what percent of them may have demonstrated transformed histopathology following treatment. Another limitation was that we had no information relating to CSFD of patients with small cell GBMs following standard therapy, and therefore we cannot state whether the incidence of post-treatment dissemination of small cell GBM is specific for BNCT. Furthermore, since the majority of GBMs did not have the IDH1^{R132H} mutation, we cannot state with certainty that absence of the IDH1^{R132H} mutation was specifically associated with CSFD following BNCT.

Fifty percent of tumors that underwent CSFD following BNCT were the small cell variant of GBM. Small cell GBMs often show similar histologic features to those of anaplastic oligodendrogliomas, although their clinical behavior is similar to that of primary GBMs [26, 40]. Coderre et al. have reported that the concentration of BPA in patients with GBMs correlated with cellularity and the higher the cellularity, the higher the boron concentration [41]. The significance of this is that if small cell GBMs and oligodendrogliomas indeed have higher boron concentrations, the constituent tumor cells could have received a higher radiation dose than non-small cell tumors, and possibly be at a higher risk for mutations that could enhance their invasiveness and penetrance into the CSF.

The migratory properties of GBM cells within the CNS are most evident when they reach a border that constitutes a barrier. More specifically, tumor cells line up and accumulate in the subpial zone of the cortex, in the subependymal region and around neurons and blood vessels. These patterns, referred to as secondary structures [42], result from the interaction of glioma cells with normal brain structures and are highly diagnostic of infiltrating gliomas such as oligodendrogliomas [43]. One feature of many GBMs, especially the small cell subtype, and anaplastic oligodendrogliomas, is extensive involvement of the cerebral cortex [25, 40]. Therefore it can be hypothesized that small cell GBMs located in the subpial zone of the cortex and possessing a strong intrinsic migratory propensity could more easily disseminate to the subarachnoid space following BNCT, which potentially could produce secondary damage to the pia membrane. Likewise, a high capacity for subependymal spread may enhance the chance of intraventricular seeding following treatment-related damage. Second only to anaplastic astrocytomas and GBMs, oligodendrogliomas, particularly anaplastic oligodendrogliomas, appear to show a high rate of metastasis outside the CNS compared to other gliomas. It has been suggested that a tendency for late CSF seeding and even extraneural metastasis of oligodendrogliomas may be secondary to the fact that these patients survive longer than those with high-grade astrocytomas [44]. Extended survival times, perhaps combined with mechanical and other tissue damaging effects of multiple surgeries and radiation, which may damage the leptomeninges, ependyma and vascular structures, might facilitate cell migration and eventually lead to the development of CSFD or systemic metastasis. This phenomenon is likely not specific to BNCT-treated patients. It should be noted that many of our BNCT-treated patients had advanced aggressive tumors that had been previously treated with other modalities (Table 3). By analogy, the combination of enhanced tumor aggressiveness and extended survival as a result of BNCT

could have been contributing factors for the high rate of dissemination we observed in our series.

However, in our study the overall MST of newly diagnosed GBM patients treated with BNCT was 23.5 months [2], indicating superior survival even in the face of tumor dissemination. This is not to minimize the significance of CSFD but to point out that despite it, the MST of patients treated with BNCT was increased. Be that as it may, BNCT is still an experimental modality, the utility of which for the treatment of high-grade gliomas has yet to be fully established. Since it provides a mechanism for selectively delivering high LET radiation to tumor cells for patients who have been treated to tolerance by external beam photon irradiation, BNCT may represent a useful salvage therapy [6]. The take-home message of our study is that patients with small cell GBMs and possibly anaplastic oligodendrogliomas may be at higher risk to develop CSF dissemination, and that this should be taken into account before treating them with BNCT.

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Fig. 1.

Representative cases exhibiting good local control following BNCT that developed CSF dissemination. Gd-enhanced MRI images are shown. **a** Patient 1, newly diagnosed left temporo-occipital GBM prior to BNCT. **b** Tumor was locally well controlled. **c** Spinal cord dissemination (*arrows*) 7.1 months after BNCT. **d** Patient 10, recurrent right frontal GBM prior to BNCT. **e** Good local control by BNCT. **f** Dissemination to fourth ventricle (*arrow*) 17.5 months after treatment. **g** Patient 4, recurrent left frontal GBM prior to BNCT. **h** Patient 4, Tumor was locally well controlled by BNCT. **i** Patient 4, CSF dissemination to right lateral ventricle posterior horn (*arrow*) 2 months after BNCT with good local control

Fig. 2. Histopathology of tumors

a Patient 11, low-power photomicrograph of small cell GBM showing microvascular proliferation (MP, *arrows*) **b** Patient 11, high magnification illustrates small cells, a delicate blood vessel (*arrowheads*) and mitotic figures (*arrows*). **c** Patient 4, small cell GBM showing MP. Perinuclear clearing is evident. **d** Patient 12, small cell GBM demonstrating minimal pleomorphism and MP. **e** Patient 12, The Ki-67 labeling index was up to 75%. **f** Patient 19, prominent cellular pleomorphism in conventional GBM. **g** Patient 7, anaplastic oligodendroglioma showing perinuclear clearing, occasional minigemistocytes, delicate blood vessels (*arrowheads*) and mitotic figures (*arrows*). MP was also present (not shown). **h** Patient 7, tumors cells are IDH1^{R132H} immunopositive. **i** Patient 7, FISH analysis. The left panel shows several cells with only one orange-red 1p36 signal (*arrows*) and several cells with two green 1q25 signals within DAPI stained nuclei. The right panel shows several cells with only one orange-red 19q13 signal (*arrows*) and several with two green 19p13 signals. Images are of H&E stained sections unless otherwise specified. Original magnifications: **a**, 200×; **b**, 600×; **d–f**; **g–h**, 600×; **i**, enlarged from original 500×

Table 1

BNCT treatment protocols for patients with either newly diagnosed or recurrent gliomas

Protocol	Sodium borocaptate (BSH)	Boronophenylalanine (BPA)	Treatment
1	100 mg/kg, 12 h prior to BNCT	250 mg/kg, 1 h prior to BNCT	BNCT only
2	100 mg/kg, 12 h prior to BNCT	700 mg/kg, prior to BNCT	BNCT only for recurrent gliomas, BNCT+X-ray for newly diagnosed gliomas
3	100 mg/kg, 12 h prior to BNCT	500 mg/kg, (400 mg/kg for 2 h prior to BNCT+100 mg/kg for 1 h during BNCT)	BNCT+X-ray for newly diagnosed gliomas

Table 2

Patient demographics, treatments, pathologic diagnoses and involvement of the ependymal layer

Patient	Age	Sex	Initial diagnosis ^a	Treatments ^b	Histopathology	Molecular markers ^c			Invasion of ependymal layer before BNCT	Surgical intervention to ependymal layer
						Ki-67 (%)	IDH1 R132H	LOH ^d 1p/19q		
1	51	F	Lt. temporooccipital GBM (N)	S, BNCT+CN+SRS, SRS	Small cell GBM	4	(-)	(+)	(+)	(-)
2	73	M	Lt. frontal GBM (N)	S, BNCT, ACNU, BNCT, Biopsy	Small cell GBM	15	(-)	(-)	(-)	(-)
3	61	F	Lt. temporal GBM	Biopsy, SRS, S, BNCT +ACNU, Biopsy, TMZ, XRT	Small cell GBM	3	(-)	(+)	(+)	(+)
4	31	M	Lt. frontal GBM	S+SRS, S+XRT+ACNU, S, BNCT	Small cell GBM	NA	(-)	(-)	(-)	(-)
5	37	F	Rt. parietal GBM (N)	S, BNCT, SRT, ACNU, Biopsy, SRS, S	Small cell GBM	15	(-)	(-)	(-)	(-)
6	35	M	Rt. temporal astrocytoma (GII), GBM	S, S, BNCT, ACNU, XRT, Biopsy with laminectomy, VP shunt	Anaplastic oligodendroglioma	75	(+)	LOH (+)	(-)	(+)
7	51	M	Rt. parietooccipital AOA	S, XRT, S, 3rd rec BNCT	Anaplastic oligodendroglioma	35	(+)	LOH (+)	(+)	(+)
8	63	M	Lt. frontal GBM	S, XRT, S, BNCT, SRS, XRT	GBM with gemistocytes	4	(-)	(-)	(-)	(-)
9	67	F	Rt. frontal GBM	S, XRT, ACNU, BNCT, S (for RN)	Small cell GBM	10	(-)	(-)	(-)	(-)
10	57	F	Rt. frontal AO, GBM	S, SRS, PCV, S, BNCT +XRT, S (for RN)	Small cell GBM	30	(-)	(+)	(+)	(-)
11	46	F	Lt. temporal GBM	S, S, BNCT, XRT, TMZ	Small cell GBM	9	(-)	(+)	(+)	(+)
12	46	F	Rt. frontal GBM (N)	S+TMZ, BNCT	Small cell GBM	75	(-)	(-)	(-)	(-)
13	61	F	Rt. frontal gliosarcoma	S, SRS, TMZ, BNCT	Gliosarcoma	35	(-)	(-)	(-)	(-)
14	53	M	Lt. frontal AO	S, XRT, PCV, S, BNCT	GBM	NA	NA	(+)	(+)	(-)
15	73	F	Lt. frontal GBM	S, S, BNCT, XRT, SRS	Small cell GBM	15	(-)	(-)	(-)	(-)
16	61	M	Rt. temporal GBM (N)	S, BNCT+XRT+TMZ	GBM with gemistocytes	10	(-)	(-)	(-)	(-)
17	67	F	Rt. temporal GBM (N)	S, BNCT+XRT+TMZ	GBM with gemistocytes	20	(-)	LOH (-)	(+)	(+)
18	32	M	Rt. frontal GBM (N)	S, BNCT+XRT, TMZ Bevacizumab	GBM	NA	(+)	1p ^e /19q intact	(-)	(-)
19	18	F	Lt. parietal GBM	S, XRT+TMZ, SRS, S, S, BNCT	GBM with giant cells	60	(-)	(+)	(+)	(-)

Patient	Age	Sex	Initial diagnosis ^a	Treatments ^b	Histopathology	Molecular markers ^c			Invasion of ependymal layer before BNCT	Surgical intervention to ependymal layer
						Ki-67 (%)	IDH1 R132H	LOH ^d 1p/19q		
20	47	M	Lt. frontal GBM	S, XRT, S, BNCT	GBM with gemistocytes	40	(-)		(+)	(+)

^aInitial diagnosis indicates the diagnosis at the time of treatment

^bTreatments: 'S' indicates surgery; 'S' indicates the surgery from which the histopathologic diagnosis was made; 'SRS' indicates stereotactic radiosurgery; 'XRT' indicates X-ray radiotherapy; 'TMZ' indicates temozolomide

^cNA indicates not available. (-) indicates an absence of staining and (+) the presence of staining

^dLOH (+) indicates loss of both 1p and 19q, and LOH (-) indicates that both are intact

^e1p borderline/likely loss

Table 3

BNCT protocols, radiation doses, time to dissemination, and causes of death

Patient	BNCT protocol		Tumor dose (Gy-Eq) ^a			Other RT ^b (dose, timing)	Time until dissemination (months)	Time until death (months)	Cause of death/location of dissemination
	BPA (mg/Kg)	BSH (mg/Kg)	Max	Min	D95				
1	250	100	52.6	18.6	26.0	SRS 15, 15 Gy (after BNCT)	7.1	9.2	Spinal cord dissemination
2	250	100	106.4	44.0	51.2		3.9	7.2	Contralateral ventricle
3	250	100	73.6	21.3	25.8	SRS 16 Gy (after 1st biopsy), XRT 60 Gy (for tumor progression)	24.4	28.4	Contralateral ventricle + local recurrence
4	250	None	75.5	29.3	32.2	SRS 15 Gy (after 1st S), XRT 52 Gy (after 1st REC)	2.1	4.4	Contralateral ventricle
5	520	100	100.0	30.9	52.9	XRT 10 Gy (after BNCT), SRS 20 Gy (for disseminated mass)	16.5	22.7	Inferior horn of right lateral ventricle
6	700	100	94.2	34.2	43.8	XRT 10 Gy (after BNCT)	3.5	6.0	Spinal cord dissemination
7	700	100	51.2	22.2	27.7	XRT 66 Gy (after 1st S)	15.1	32.4	Temporal, parietal lobe and spinal cord
8	700	100	58.5	31.7	37.3	XRT 60 Gy (after 1st S), SRS (24 Gy, 24 Gy for D), XRT 50 Gy (after BNCT)	1.8	11.0	Remote dissemination to rt. temporal lobe
9	700	100	91.3	50.5	62.6	XRT 60 Gy (after 1st S)	7.7 (time until extension)	12.3	Extension to contralateral hemisphere
10	500	100	72.9	31.2	41.4	SRS 15 Gy (after 1st REC), XRT 40 Gy (after BNCT)	17.5	22.0	Fourth ventricle
11	700	None	56.6	27.5	41.7	XRT 60 Gy (after BNCT)	5.6	12.8	Contralateral inferior horn
12	700	100	64.0	44.6	48.7		15.3	17.1	Contralateral ventricle
13	700	100	129.3	84.8	94.6	SRS 50 Gy (after 1st S)	4.2	6.0	Brain stem
14	700	100	103.2	73.9	81.1	XRT 55 Gy (after 1st S)	0 (just after)	6.9	Radiation necrosis or dissemination
15	700	100	53.4	31.4	33.6	XRT 20 Gy (after BNCT), X knife 20,20,24 Gy (for disseminated mass)	0.8	7.8	Contralateral frontal lobe and pons
16	500	100	71.9	32.0	34.8	XRT 8,16,24 Gy (after BNCT)	2.0	2.8	Contralateral parietal lobe, multiple lesions
17	500	100	80.7	9.3	19.3	XRT 8,16,24 Gy (after BNCT)	3.0	11.8	CSFD ^c
18	500	100	91.3	30.1	41.2	XRT 8,16,24 Gy (after BNCT)	35.9	37.3	Fourth ventricle
19	500	None	114.0	16.4	20.0	XRT 56 Gy (after 1st S), SRS 24 Gy (for REC)	1.9	8.87	Contralateral ventricle
20	500	None	48.5	27.1	36.3	XRT 30 Gy (after first S)	2.1	3.9	Ipsilateral and contralateral ventricles

c CSFD indicates cerebrospinal fluid dissemination

q RT indicates radiation therapy

p Tumor dose was determined by analyzing the dose volume histograms (DVH)

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Table 4

Additional patient demographics, treatments, and involvement of the ependymal layer

Patient	Age	Sex	Initial diagnosis	Treatments	Invasion of ependymal layer before BNCT	Surgical intervention to ependymal layer
21	29	F	Rt. front-temporal unclassified malignant glioneural tumor	<u>S</u> , SRS, BNCT, S, SRS	(+)	(+)
22	48	M	Rt. temporo-occipital GBM	<u>S</u> , ACNU+VCR, XRT BNCT	(-)	(-)
23	65	F	Lt. temporal GBM	<u>S</u> , BNCT, BNCT, necrotomy	(-)	(+)
24	42	M	Rt. frontal AA, GBM	<u>S</u> , SRS, S, BNCT, S, S, PCV, SRS	(-)	(-)
25	49	F	Lt. temporo-parietal GBM	<u>S</u> , ACNU+interferon β , S, BNCT, BNCT	(+)	(-)
26	16	F	Lt. frontal, corpus callosum small cell GBM (Ki-67 70%)	S, BNCT, XRT	(+)	(-)
27	59	M	Lt. temporal GBM	<u>S</u> , BNCT, SRS, ACNU, TMZ	(-)	(-)
28	15	M	Rt. frontal GBM	<u>S</u> , BNCT, S, XRT, TMZ	(-)	(-)
29	41	M	Rt. temporo-occipital GBM	<u>S</u> , XRT, TMZ, BNCT	(+)	(+)
30	63	F	Rt. frontal GBM	<u>S</u> , BNCT+XRT, TMZ, VP-shunt, WT-1 vaccine, S	(-)	(-)

Table 5

Additional patient BNCT protocols, radiation doses, time to dissemination, and causes of death

Patient	BNCT protocol		Tumor dose (Gy-Eq)		Other RT (dose, timing)	Time until dissemination (months)	Time until death following BNCT (months)	Cause of death/Location of dissemination
	BPA (mg/Kg)	BSH (mg/Kg)	Max	Min				
21	250	100	44.6	29.6	SRS 18 Gy, 18 Gy (after BNCT)	6.5	9.6	Brain stem and contralateral anterior horn + local recurrence
22	250	100	48.3	27.2	XRT 80 Gy	7.1	7.8	CSFD ^a +local recurrence
23	250	100	69.6	15.4		8.7	10.8	Contralateral ventricle
24	250	None	53.8	30.9	SRS 27 Gy (after BNCT)	40.7	43.1	CSFD+local recurrence
25	250	None	38.9	17.1	XRT 32 Gy (after BNCT)	6.4	13.4	CSFD+local recurrence
26	700	100	149.0	39.7	XRT 24 Gy (after BNCT)	9.1	15.7	CSFD
27	700	100	90.6	61.4	XRT 30 Gy (after BNCT)	7	9.5	Contralateral anterior horn
28	700	100	122.0	43.1	XRT 50 Gy (after BNCT)	Unknown	8.5	CSFD /necrosis + local recurrence
29	700	100	105.3	54.3	XRT 60 Gy (after 1st S)	5.8	10.3	CSFD+local recurrence
30	500	100	81.5	51.7	XRT 24 Gy (after BNCT)	3.1	15.4	Contralateral anterior horn+local recurrence +RN ^b

^a CSFD indicates cerebrospinal fluid dissemination^b RN indicates radiation necrosis