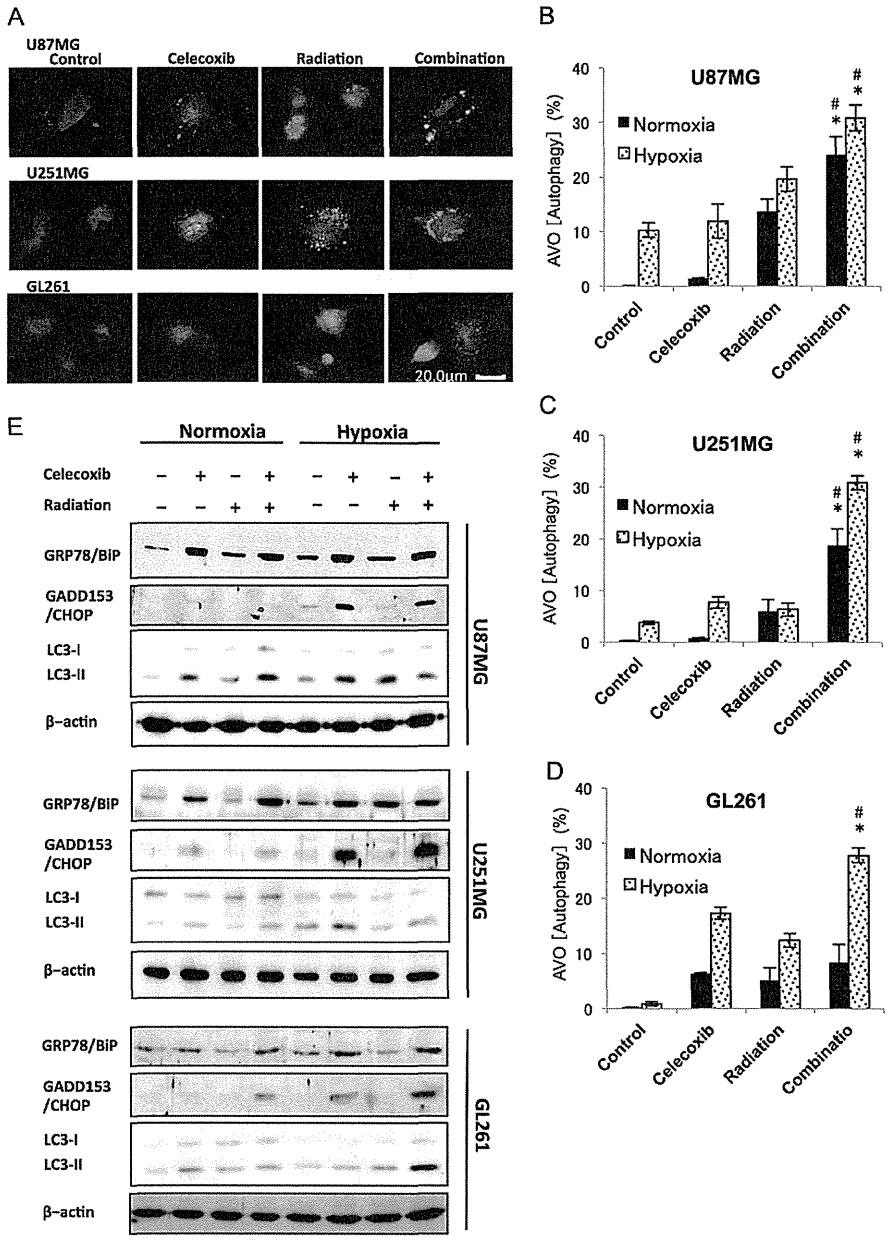

Fig. 6. Autophagy analysis. Immunofluorescent images of autophagosomes of 3 cell lines are shown in A. Autophagosomes of U87MG and U251MG were detected by Premo, and they were detected by Cyto ID in GL261. The nuclei were stained using Hoechst 33342. The images were captured 6 h after each treatment under hypoxic conditions. For quantitative analysis (B), autophagy was detected by acridine-orange staining, and the fractions were quantitated using flow cytometry 96 h after each treatment. The black bars indicate normoxia, and dotted bars indicate hypoxia. Error bars indicate standard deviations calculated from 3 independent experiments. $*P < .05$ in comparison between celecoxib alone and combined treatment. $\#P < .05$ in comparison between radiation alone and combined treatment. The results of immunoblotting evaluating expression of GRP78/BiP, GADD153/CHOP, and LC3-1 and LC3-II 6 h after each treatment in 3 cell lines are indicated in E. β-actin was used as a loading control. Data on GRP78/BiP, GADD153/CHOP, and β-actin are the same as those in Fig. 4.

promising approach is combination with other chemotherapeutic drugs.[9,10,17,18] To test the efficacy of these doses or combinations, an orthotopic mouse brain tumor model using GL261 cells would be suitable.

Another interesting strategy might be attributable to the effects on glioma stem-like cells, because Chen et al.[62] reported that celecoxib enhanced the radiosensitivity of cancer stem-like cells. Because hypoxia is

known to provide a niche for CD133-positive GBM stem-like cells,[63] a combination of celecoxib and radiation may be effective for tumor stem cells, which are the main culprits in local recurrence after radiotherapy.

Conclusions

The study results showed that celecoxib inhibited the growth of GBM cells and enhanced the radiosensitivity of these cells under not only normoxic but also hypoxic conditions. In addition, treatment with the celecoxib plus radiation combination caused cell cycle arrest and prominent autophagy in GBM cells under hypoxic conditions by ER stress loading. Our results suggest that celecoxib might contribute to overcoming the radioresistance of GBM cells under hypoxic conditions. These findings should be useful to further the clinical applications of celecoxib for improving outcomes in patients with GBM.

Supplementary Material

Supplementary material is available online at *Neuro-Oncology* (http://neuro-oncology.oxfordjournals.org/).

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Reirradiation for recurrent malignant brain tumor with radiotherapy or proton beam therapy

Technical considerations based on experience at a single institution

Malignant brain tumors are a major challenge for neurosurgeons and radiation-oncologists. The incidence of malignant brain tumors was reported to be 7.2 per 100,000 from 2004 to 2006 in the United States [1]. Despite progress with standard treatment comprising maximum surgical resection, conventional external beam radiotherapy, and various kinds of chemotherapy, long-term survival without recurrence is still rare in patients with malignant brain tumors. Furthermore, there is no standard treatment for relapsed tumors after initial treatment, and treatment options after conventional radiotherapy are usually very limited because of the tolerance dose of normal brain.

Several recent reports have indicated that stereotactic radiotherapy (SRT) for recurrent brain tumor may improve survival without causing severe toxicity [2, 3, 4, 5, 6, 7]. Fogh et al. found that stereotactic reirradiation of 35 Gy in 3.5-Gy fractions was effective for recurrent high-grade glioma [2, 4, 5, 7]. Single-fraction stereotactic radiosurgery (SRS) has also been reported as a palliative salvage modality for recurrent brain tumor [3, 6]. However, Mayer et al. found an increased risk of radiation-induced normal brain necrosis with an increasing total dose and treatment volume [8], which indicates the importance of avoiding irradiation of normal brain tissue by use of the latest technology. One of the promising methods is proton beam therapy (PBT). It has been reported that PBT is superior to X-ray radiotherapy in preserving the normal tissue volume [9, 10, 11, 12, 13].

In this study, we examined the efficacy of reirradiation including conventional radiotherapy (RT), SRT, and PBT for recurrent malignant brain tumors in a retrospective analysis of patients who underwent reirradiation at our hospital.

Patients and methods

Patients

A total of 26 patients with recurrent malignant brain tumor after radiotherapy received reirradiation at our hospital between January 2005 and September 2010. All patients had a recurrent tumor according to the findings of magnetic resonance imaging (MRI) with contrast enhancement. Written informed consent was obtained from all patients prior to reirradiation and the use of a particular treatment modality (RT, SRT, PBT) was determined according to each patient's condition.

The 26 patients (12 men and 14 women) had a median age of 48 years (range, 4–76 years old). The performance status before reirradiation was 0 (n=6), 1 (n=8), 2 (n=4), 3 (n=5), and 4 (n=3). Eleven patients underwent tumor removal before reirradiation, including 8 with pathologically confirmed glioblastoma multiforme (GBM), 2 with grade 3 glioma, and 1 with anaplastic ependymoma. The other 15 patients were considered to have inoperable tumors before reirradiation, including 7 with pathologically confirmed GBM at the initial surgery, 1 with pineoblastoma (WHO grade 4), 4 with WHO grade 3 glioma, 1 with anaplastic meningioma, and 2 with atypical teratoid/rhabdoid tumors (AT/RT). The maximum tumor size before reirradiation was a medi-

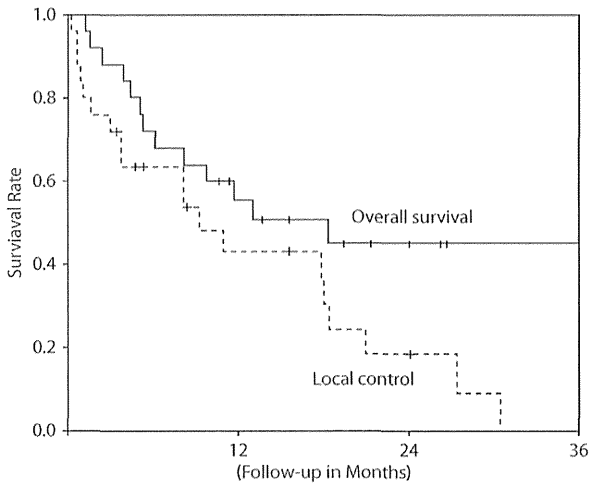

Fig. 1 ◀ Kaplan–Meier estimates of *overall survival* and *local control* for all 26 patients

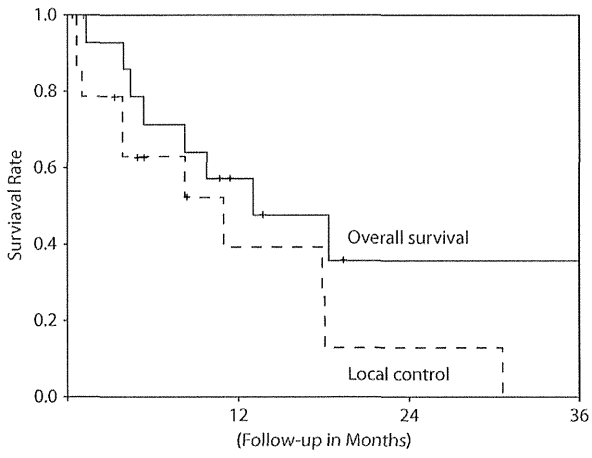

Fig. 2 ◀ Kaplan–Meier estimates of *overall survival* and *local control* for 15 patients with glioblastoma

an of 3.6 cm (range, 1.0–9.0 cm). The initial irradiation was performed using conventional RT (median dose of 60 Gy) in 21 patients, PBT in 4, and SRT in 1. The recurrent tumor was within the initial irradiation area in 21 patients and in the marginal region in 5 patients.

Treatment methods

The gross tumor volume (GTV) was defined as the area of contrast enhancement on MRI or the tumor bed. The planning target volume (PTV) was defined as the GTV plus a margin of 1–10 mm; edema was not included in the treatment area. The prescribed irradiated dose was chosen based on the organs at risk, such as the optic nerve, optic chiasma, and brain stem. Treatment planning for PBT was based on computed tomography (CT) images taken at 3-mm intervals in the treatment location. Proton beams with an energy of 250 MeV were generated by a booster synchrotron at the Proton Medical Research Center (PMRC). The treatment planning system provides dose distributions and settings for the collimator configuration, bolus, and range-shifter thickness. The relative biological effectiveness (RBE) for PBT was assumed to be 1.1 [14, 15]. Treatment planning for SRT was based on CT images taken at 1-mm intervals in the treatment location. SRT was delivered with an energy of 6 or 10 MV using photons from a linear accelerator with a multileaf collimator. The irradiation dose was calculated based on coverage of at least 95% of the PTV. Different radiation schedules were compared using the biologically effective dose (BED) [16, 17], which was calculated with a linear quadratic model according to the following equation: BED = nd (1+d/[α/β]), where n is the number of fractions, d is the fraction dose (Gy), and α/β is the tissue repair capacity (Gy). The actual radiation dose was converted to the equivalent dose in 2-Gy fractions (EQD2).

Follow-up procedures and evaluation criteria

Acute treatment-related toxicities were assessed weekly during treatment in all patients. After completion of reirradiation, physical examination, MRI, and blood tests were performed every 3 months for the first 2 years and every 6 months thereafter. The Kaplan–Meier method was used for calculation of local control and overall survival rates, and a log-rank test was performed for evaluation of differences between groups. Acute and late treatment-related toxicities were assessed using the National Cancer Institute Common Criteria, v.3.0, and the RTOG/EORTC late radiation morbidity scoring scheme [19].

Results

Reirradiation was completed in all patients at doses of 30–60 Gy (median, 42.3 Gy, EQD2). The dose of initial radiotherapy ranged from 34.5 to 94.4 Gy (median, 60 Gy, EQD2), and thus the total dose of all radiotherapy was 64.5–150.4 Gy (median, 100.0 Gy, EQD2). The total dose of all radiotherapy for cases with recurrence inside the area of the first radiotherapy ranged from 64.5 to 106.2 Gy (median, 100.0 Gy, EQD2).

The treatment modality of conventional RT (n=8), SRT (n=10), or PBT (n=8) for reirradiation was chosen based on the location, distribution, and size of the recurrent tumor. Treatment periods were 14–43 days (median, 29 days), 2–47 days (median, 19 days), and 14–51 days (median, 35 days) for RT, SRT, and PBT, respectively. The period between initial radiotherapy and reirradiation was 2.7–320 months (median, 16.2 months). The GTVs were 14.3–135.3 ml (median, 77.2 ml), 0.2–46.2 ml (7.4 ml), and 3.9–

217.3 ml (30.2 ml) for RT, SRT, and PBT, respectively.

Of the 21 patients with glioma, 13 received concurrent chemotherapy, including 8 treated with temozolomide (75 mg/m^2 daily or 150–200 mg/m^2 for 5 days), 4 who received nimustine hydrochloride (80 mg/m^2), and 1 treated with cisplatin and etoposide.

Toxicity

Acute treatment-related toxicity was generally mild. Headache, dermatitis, and nausea were all of grade 1 or 0. Ten patients needed temporary steroid therapy during reirradiation. Performance status remained unchanged from before to after reirradiation. Two patients showed radiation necrosis. One was treated by surgery and the other was treated by conservative therapy. Both are still alive and radiation necrosis was well controlled 13.7 and 19.4 months after reirradiation.

Survival and local control

The outcomes of reirradiation are summarized in Tab. 1. Eleven of the 26 patients died within 1 year and 13 died within 2 years. The 1- and 2-year overall survival rates of all patients after reirradiation were 55.4 and 45.1%, respectively (Fig. 1), and the median survival period after reirradiation was 18.3 months (95% CI, 0.0–38.3 months). At the time of analysis, 11 patients were alive and 15 patients were dead; the median follow-up period for survivors was 19.4 months. The cause of death in all patients was tumor recurrence, including 11 with local recurrence and 4 with recurrence outside the reirradiated area as the initial recurrence after reirradiation. The 1- and 2- year local control rates after reirradiation were 43.0 and 18.4%, respectively, and the median local control period after reirradiation was 9.3 months (95% CI, 5.5–13.1 months). Six of the 15 patients with GBM (n=15) died within 1 year and 8 died within 2 years. The 1- and 2- year overall survival rates for patients with GBM (n=15) after reirradiation were 57.1 and 35.7%, respectively (Fig. 2), and the median survival period was 13.1 months (95% CI,

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Reirradiation for recurrent malignant brain tumor with radiotherapy or proton beam therapy. Technical considerations based on experience at a single institution

Abstract

Background and purpose. Radiotherapy for recurrent malignant brain tumors is usually limited because of the dose tolerance of the normal brain tissue. The goal of the study was to evaluate the efficacy and feasibility of reirradiation for patients with recurrent malignant brain tumors.

Patients and methods. The subjects comprised 26 patients with recurrent malignant brain tumors treated with conventional radiotherapy (RT, n=8), stereotactic radiotherapy (SRT, n=10), and proton beam therapy (PBT, n=8) at our institute. Fifteen patients had glioblastoma, 6 had WHO grade 3 glioma, and 5 had other tumors. The dose of initial radiotherapy was 34.5–94.4 Gy. Different radiation schedules were compared using the equivalent dose in 2-Gy fractions.

Results. Reirradiation was completed in all patients without a severe acute reaction. The reirradiation doses were 30–60 Gy (median, 42.3 Gy) and the total doses for the initial and second treatments were 64.5–150.4 Gy (median, 100.0 Gy). Currently, 11 patients are alive (median follow-up period, 19.4 months) and 15 are dead. The median survival and local control periods after reirradiation of the 26 patients were 18.3 and 9.3 months, respectively. For the 15 patients with glioblastoma, these periods were 13.1 and 11.0 months, respectively. Two patients showed radiation necrosis that was treated by surgery or conservative therapy.

Conclusion. Reirradiation for recurrent malignant brain tumor using conventional RT, SRT, or PBT was feasible and effective in selected cases. Further investigation is needed for treatment optimization for a given patient and tumor condition.

Keywords
Glioblastoma · Proton beam therapy · Radiotherapy · Reirradiation · Recurrent

Erneute Bestrahlung mit üblicher Strahlen- oder Protonentherapie bei rezidivierendem bösartigem Hirntumor. Technische Aspekte basierend auf an einer Einrichtung gesammelten Erfahrungen

Zusammenfassung

Hintergrund und Zielsetzung. Bei bösartigen Hirntumoren ist eine operative Behandlung schwierig und bei rezidivierenden Tumoren schränkt die Dosistoleranz des normalen Hirngewebes eine Strahlentherapie häufig ein. Ziel der Studie war es, die Durchführbarkeit und Wirksamkeit einer erneuten Bestrahlung (Rebestrahlung) bei Patienten mit einem rezidivierenden bösartigen intrakraniellen Tumor zu bewerten.

Patienten und Methoden. Bei den Probanden handelte es sich um 26 Patienten, die mit konventioneller Strahlentherapie (RT, n=8), stereotaktischer Strahlentherapie (SRT, n=10) und Protonentherapie (PBT, n=8) in unserer Einrichtung behandelt wurden. Die Behandlung wurde in Abhängigkeit vom Tumorzustand ausgewählt. Ein Glioblastom hatten 15 Patienten, darunter 6 Patienten mit einem Gliom vom WHO-Grad III. Die Dosis der anfänglichen Strahlentherapie lag bei 34,5–94,4 Gy. Es wurden verschiedene Bestrahlungspläne anhand der Äquivalentdosis in 2-Gy-Fraktionen verglichen.

Ergebnisse. Bei allen Patienten wurde die Rebestrahlung ohne schwere akute Reaktion abgeschlossen. Die Rebestrahlungsdosen betrugen 30–60 Gy (Median 42,3 Gy) und die Gesamtdosen der ersten und zweiten Behandlung 64,5–150,4 Gy (Median 100,0 Gy). Gegenwärtig leben noch 11 Patienten (medianer Nachuntersuchungszeitraum 19,4 Monate), 15 Patienten sind bereits verstorben. Die mediane Überlebenszeit betrug 18,3 Monate und der Zeitraum für die lokale Kontrolle 9,3 Monate bezogen auf alle Patienten sowie 13,1 bzw. 11,0 Monate bezogen auf die Glioblastompatienten. Eine beherrschbare Strahlennekrose hatten 2 der 26 Patienten.

Schlussfolgerungen. Die Rebestrahlung bei rezidivierendem bösartigem Hirntumor anhand konventioneller RT, SRT oder PBT war durchführbar und wirksam. Weitere Untersuchungen sind notwendig, um die optimale Behandlung für einzelne Patienten bzw. einen bestimmten Tumorzustand herauszufinden.

Schlüsselwörter
Glioblastom · Protonentherapie · Strahlentherapie · Rebestrahlung · Rezidivierend

Tab. 1 Background and clinical characteristics of the patients

No.	Age (years)	Sex	Tumor	Initial dose (Gy)	Initial BED_2 (Gy)	Recurrence area	Tumor volume (cc)	Re-RT dose (Gy)	Re-RT BED_2 (Gy)	Cumulative BED_2 (Gy)	Reirradiation technique	Overall survival (months) [a]	Follow-up and status
1	58	M	Pineoblastoma	56.0	112.0	In-field	14.3	49.6	95.8	207.8	Conventional RT	15.6	Alive, extrafield recurrence
2	36	M	Glioma (WHO grade III)	50.0	100.0	In-field	116.1	49.6	95.8	195.8	Conventional RT	26.6	Alive, local recurrence
3	41	M	Glioblastoma	60.0	120.0	In-field	26.8	42.3	97.5	217.5	SRT	10.6	Alive, local recurrence
4	73	M	Glioma (WHO grade III)	60.0	120.0	In-field	30.1	40.0	80.0	200.0	Conventional RT	1.6	Death due to cancer
5	66	F	Glioblastoma	60.0	120.0	In-field	0.6	42.3	97.5	217.5	SRT	19.4	Alive, radiation necrosis
6	62	M	Glioblastoma	60.0	120.0	In-field	46.2	42.3	97.5	217.5	Conventional RT	1.1	Lost to follow up, local recurrence
7	67	M	Glioblastoma	56.0	168.0	Extrafield	No data	60.0	120.0	288.0	PBT	18.3	Death due to cancer
8	64	F	Glioblastoma	60.0	120.0	In-field	0.2	44.0	168.0	288.0	SRT	4.4	Death due to cancer
9	48	M	Glioblastoma	46.0	92.0	In-field	33.4	42.3	97.5	189.5	SRT	13.7	Alive, radiation necrosis
10	29	F	Glioma (WHO grade III)	54.0	108.0	In-field	73.7	32.5	75.0	183.0	Conventional RT	5.0	Death due to cancer
11	65	F	Glioma (WHO grade III)	60.2	116.3	In-field	11.5	30.0	60.0	176.3	SRT	40.3	Death due to cancer
12	73	F	Glioblastoma	60.2	116.3	Border	7.4	40.0	80.0	196.3	SRT	11.4	Alive, local recurrence
13	43	F	Glioma (WHO grade III)	63.8	123.1	In-field	No data	42.3	97.5	220.6	Conventional RT	2.5	Death due to cancer
14	39	F	Glioblastoma	53.1	102.6	In-field	80.6	53.1	102.6	205.2	Conventional RT	9.7	Death due to cancer
15	32	M	Glioblastoma	60.0	120.0	In-field	135.3	40.0	80.0	200.0	Conventional RT	5.3	Death due to cancer
16	72	F	Glioblastoma	63.8	123.1	In-field	17.4	40.0	120.0	243.1	SRT	1.3	Death due to cancer
17	6	M	AT/RT	34.5	63.0	In-field	6.5	30.0	60.0	123.0	SRT	11.8	Death due to cancer

Tab. 1 Background and clinical characteristics of the patients (continued)

No.	Age (years)	Sex	Tumor	Initial dose (Gy)	Initial BED_2 (Gy)	Recurrence area	Tumor volume (cc)	Re-RT dose (Gy)	Re-RT BED_2 (Gy)	Cumulative BED_2 (Gy)	Reirradiation technique	Overall survival (months) [a]	Follow-up and status
18	4	M	AT/RT	49.5	92.2	In-field	6.2	49.6	95.8	188.0	SRT	19.9	Alive, local recurrence
19	46	F	Glioblastoma	94.4	180.1	Border	3.9	30.0	60.0	240.1	PBT	36.2	Death due to cancer
20	56	M	Glioblastoma	94.4	180.1	Extra-field	30.2	50.0	100.0	280.1	PBT	3.9	Death due to cancer
21	76	F	Glioblastoma	94.4	180.1	Extra-field	5.0	56.0	168.0	348.1	SRT	8.2	Death due to cancer
22	69	F	Glioblastoma	52.0	104.0	In-field	29.7	30.0	60.0	164.0	PBT	13.1	Death due to cancer
23	36	F	Glioblastoma	60.0	120.0	In-field	107.7	30.0	60.0	180.0	PBT	48.0	Alive, local recurrence
24	34	F	Glioma (WHO grade III)	53.1	102.6	In-field	88.9	30.1	58.1	160.7	PBT	26.2	Alive, local recurrence
25	68	F	Anaplastic meningioma	53.1	102.6	In-field	217.3	36.6	87.5	190.1	PBT	3.0	Death due to cancer
26	6	M	Ependymoma (WHO grade III)	49.6	95.8	In-field	5.4	49.6	95.8	191.6	PBT	20.5	Alive

AT/RT atypical teratoid/rhabdoid tumor, *Re-RT* reirradiation [a] *Overall survival* since reirradiation

2.1–24.1 months). The 1- and 2-year local control rates after reirradiation were 26.2 and 0.0%, respectively, and the median local control period was 11.0 months (95% CI, 1.7–20.2 months).

Typical cases

A typical clinical course of a patient treated with conventional RT is shown in **Fig. 3**. The patient was a 59-year-old man with pineoblastoma at the pineal region. Eighteen years before reirradiation, he received conventional RT of 56 Gy in 28 fractions for the pineal tumor. Two years before reirradiation, he had recurrence in the right occipital lobe, right cerebellar hemisphere, and corpus callosum. Although the lesions of the right occipital lobe and right cerebellar hemisphere were surgically treated, the lesion of the corpus callosum tumor, which was partly in the first irradiated area, was difficult to remove by surgery (**Fig. 3a**). In this case, we selected conventional RT because the tumor was larger than 3 cm and there was a small volume of normal brain tissue around the recurrent tumor. The GTV for reirradiation included only the Gd-enhanced area on MRI and the PTV included the GTV plus a 5-mm margin (**Fig. 3b**). The tumor was significantly shrunk at 10 months after reirradiation (**Fig. 3c**) and was well controlled at 16 months.

A typical clinical course of a patient treated with SRT is shown in **Fig. 4**. The patient was a 66-year-old woman with right occipital lobe glioblastoma. Nine months before reirradiation, she received conventional postoperative RT of 60 Gy in 30 fractions for the right occipital lobe glioblastoma. Two small recurrent tumors appeared in the right occipital lobe and right temporal lobe, and the tumor in the right occipital lobe was in the field of the initial irradiation (**Fig. 4a**). In this case, we selected SRT because the tumor was small. The GTV for reirradiation included only the Gd-enhanced area on MRI and the PTV included the GTV plus a 5-mm margin (**Fig. 4b**). The reirradiation dose was 39 Gy in 13 fractions to cover 95% of the PTV. Five months after reirradiation, radiation necrosis occurred and necrotomy was performed (**Fig. 4c**). At 15 months after reirradiation, the tumor was well controlled.

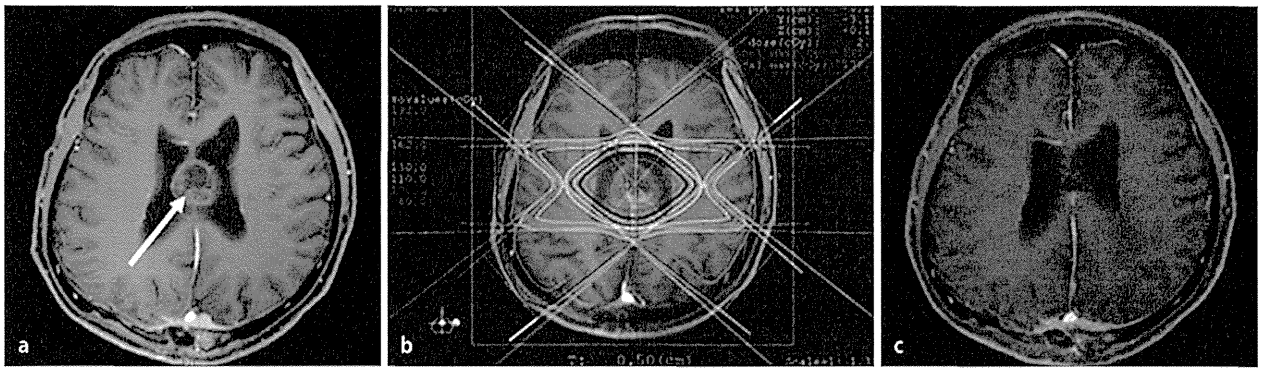

Fig. 3 ▲ Imaging for a patient treated with reirradiation with conventional RT. **a** Postcontrast T1-weighted MRI before reirradiation. **b** *Isodose curves* for conventional RT representing 100-10 % of the prescribed dose at 10 % intervals. **c** Postcontrast T1-weighted MRI at 10 months after reirradiation

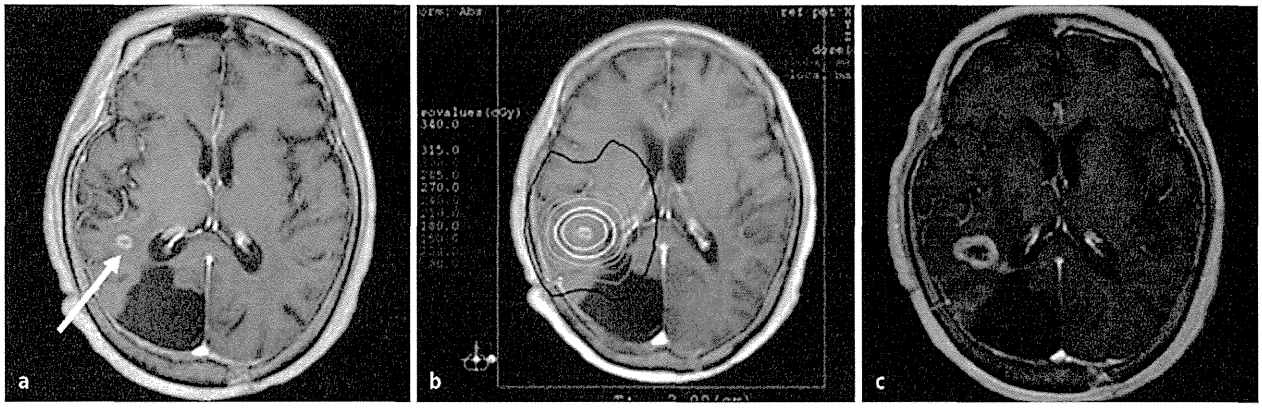

Fig. 4 ▲ Imaging for a patient treated with reirradiation with SRT. **a** Postcontrast T1-weighted MRI before reirradiation. **b** *Isodose curves for SRT representing 100-10 % of the prescribed dose at 10 % intervals.* **c** Postcontrast T1-weighted MRI at 5 months after reirradiation

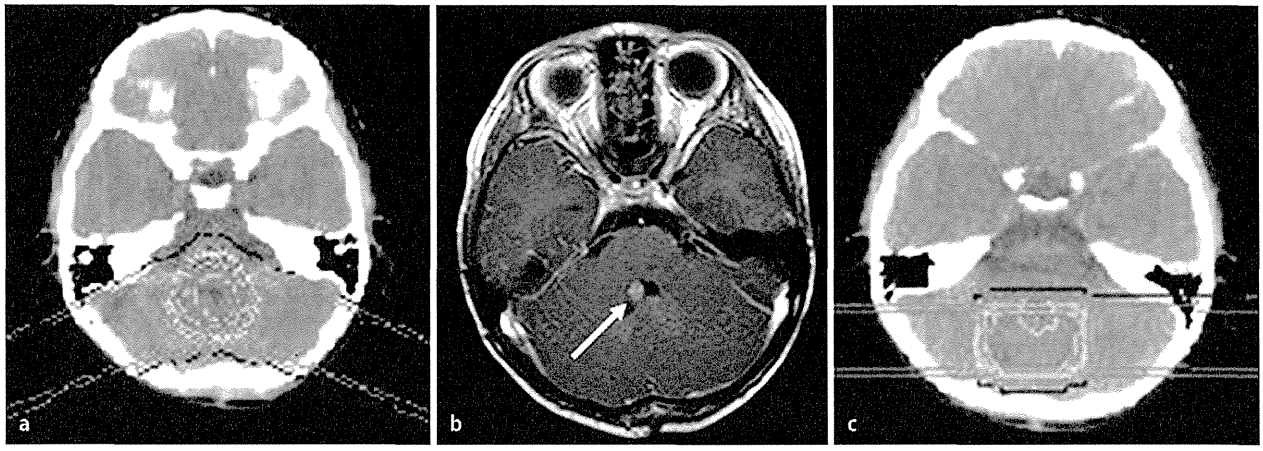

Fig. 5 ▲ Imaging for a patient treated with reirradiation with PBT. **a** *Isodose curves* for initial PBT representing 100-10 % of the prescribed dose at 10 % intervals. **b** *Postcontrast T1-weighted MRI before reirradiation.* **c** *Isodose curves for the second treatment with PBT representing 100-10 % of the prescribed dose at 10 % intervals*

A typical clinical course of a patient treated with PBT is shown in Fig. 5. The patient was a 6-year-old boy with a tumor in the fourth ventricle. Tumor resection was performed 7 months before reirradiation and pathological findings indicated ependymoma. Five months before reirradiation, initial PBT of 50.4 GyE in 28 fractions was performed (Fig. 5a). Three months before reirradiation, local relapse occurred (Fig. 5b) and re-resection was performed. In this case, we selected PBT because the brain stem was close to the recurrent tumor. The GTV for reirradiation was the tumor bed on MRI and the PTV included the GTV plus a 5-mm margin (Fig. 5c). The margin on the brain stem side was 0 mm at reirradiation. The reirradiation dose was 50.4 GyE in 28 fractions. At 24 months after reirradiation, the tumor was well controlled and no late toxicity had occurred.

Discussion

Several recent reports have indicated that reirradiation for recurrent glioma is a feasible and effective treatment option [2, 3, 4, 5, 6, 7]. In many cases, SRT or SRS was used for recurrent glioma after radiotherapy. In general, the dose of initial radiotherapy is about 60 Gy in 30 fractions for high-grade glioma and about 30–40 Gy in several fractions was frequently used as the reirradiation dose. Thus, the cumulative dose sometimes reached 100 Gy or more. Our results support the feasibility of reirradiation for recurrent malignant brain tumor using modern treatment modalities and depending on the dose concentration.

Although SRT or SRS is often used for treatment of recurrent brain tumors, these methods are usually not applicable to large or irregularly shaped tumors, since an increase in the treatment volume exposes large areas of normal brain tissue to the detrimental effects of a high-dose irradiation. In our hospital, PBT is available for various kinds of tumors, including large or irregularly shaped tumors [9, 10, 11, 12, 13, 18, 20, 21]; however, definitive selection criteria for the reirradiation method to brain tumors have not been established yet. Therefore, we select SRT, which is low in cost compared to PBT, for small tumors and conventional RT or PBT for larger tumors that are difficult to treat with SRT. Comparison of the outcomes of these methods was difficult at this point because of the small number of patients in this study. Several reports have shown overall survival of approximately 10 months after reirradiation for GBM [2, 3, 4, 5, 6, 7], and Combs et al. found a median survival period of 16 months for WHO grade 3 tumors [2] after reirradiation with SRT. In this study, the median survival after reirradiation for GBM was 13.1 months, indicating that the outcome using RT, SRT, or PBT was similar to that in patients treated with SRT or SRS.

Acute adverse events were generally mild in our patients. Although comparison with other reports is difficult because of the small number of events, all patients completed the planned reirradiation without change in the Karnofsky performance score suggesting that reirradiation is feasible at least in the short term. As for late adverse event, 2 patients demonstrated radiation necrosis. Although they were controllable in our series, these cases indicate that reirradiation to the recurrence at or close to critical regions such as the brain stem or the optic chiasma is very difficult to achieve while preserving functions. It is fundamental that the irradiated volume of normal brain must be minimized to reduce toxicity [8]. In this regard, PBT has an advantage of preserving normal brain tissue around the tumor. However, PBT is generally expensive, and compared with other radiation modalities such as SRT or SRS, significant clinical benefits of PBT in recurrent brain tumors have not been proved yet. Therefore, we currently consider that SRT as the first treatment option for a recurrent malignant brain tumor is acceptable.

Conclusion

In conclusion, reirradiation for recurrent malignant brain tumors using conventional RT, SRT, or PBT is feasible and effective in selective cases. Further investigation is needed for optimizing treatment modalities for each patient and tumor condition.

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Review

Bevacizumab for Glioblastoma—A Promising Drug or Not?

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Abstract: Two double blind, placebo-controlled, and randomized phase III studies were conducted, and the results including OS's were reported at the ASCO Meeting in June 2013, which was the beginning of confusion surrounding this topic. This is a review article not only summarizing the previous evidence, but also looking beyond.

Keywords: bevacizumab; glioblastoma; chemotherapy

1. Introduction

Studies have confirmed angiogenesis as a complex and dynamic process occurring during the growth of all solid tumors beyond 2–3 mm in size and that tumors are angiogenesis dependent [1]. Vascular endothelial growth factor (VEGF) is one of the most important factors regulating angiogenesis in the most aggressive malignant brain tumor, glioblastoma (GBM) [2]. Bevacizumab is a humanized form of a mouse monoclonal antibody against human VEGF, which binds to and neutralizes mainly VEGF-A. The interaction between GBM stem cells located in their perivascular niche and endothelial cells may also be disrupted by anti-VEGF agents such as bevacizumab, contributing to eventual cell death [3].

Bevacizumab was first reported as a treatment for recurrent GBMs in 2005 [4]. The results showed significant improvement in radiological scans as well as in the patients' symptoms and neurological function. In a retrospective analysis of 29 patients treated with a combination of bevacizumab and irinotecan, a topoisomerase-1 inhibitor, an overall response rate of 43% was observed, with one complete response (CR), eight partial responses (PR), and eleven patients with stable disease (SD).

There was one death that occurred secondary to intracranial hemorrhage and one due to gastrointestinal perforation [4]. This initial efficacy of bevacizumab led to a phase II trial by Vredenburgh *et al.* in a total of 35 patients with recurrent GBMs, using also bevacizumab and irinotecan [5]. The overall results of this trial included a PFS at 6 months (PFS-6m) of 46% (95% confidence interval [CI]; 32%–66%). A PR was observed in 20 of 35 patients (57%. 95% CI; 39%–74%), with the median OS in the study being 10.5 months. A historical comparator of PFS-6m for recurrent GBM should be the data of temozolomide by Yung *et al.* that was 18% [6]. The adverse events in this bevacizumab study included one intracranial hemorrhage and four patients who developed thromboembolic events. Thus, bevacizumab was seen as a promising new therapeutic for treating patients with gliomas. A subsequent study conducted by Friedman *et al.*, an open-label randomized phase II study, evaluated bevacizumab with and without irinotecan in 167 recurrent GBM patients (BRAIN study) [7]. A response rate of 37.8% and 28.2% in the bevacizumab plus irinotecan (n = 82) and the bevacizumab alone (n = 85) groups, respectively, and PFS-6m of 50.3% and 42.6%, respectively, was seen in this study.

2. Bevacizumab as a Single Agent *vs.* Combination Therapy for Recurrent GBM

One of the practical issues in treating recurrent GBM with bevacizumab is that combination with other chemotherapeutic agents has not been proved to increase efficacy of bevacizumab monotherapy. This is distinct from other malignancies where bevacizumab is approved and used in combination with cytotoxic agents, due to the fact no appreciable benefit is observed by using bevacizumab alone. For recurrent GBM, similar rates of response (20%–40%) and PFS-6m (23%–50%) were reported for bevacizumab plus chemotherapy (including irinotecan, carboplatin, nitrosoureas such as carmustine [1,3-Bis(2-chloroethyl)-1-nitrosourea; BCNU], lomustine[1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea; CCNU] and fotemustine, temozolomide (TMZ), erlotinib, etoposide, and enzastaurin) relative to bevacizumab monotherapy [7–12], having argued that it is unclear whether additional agents contribute to enhance the benefit of bevacizumab in the treatment of recurrent GBM. Among those, irinotecan showed a slight increase in efficacy, albeit with limited single-agent activity in the recurrent setting [7,13–17].

The lack of significant enhancement of efficacy by bevacizumab combinations might be partially attributable to the brain-specific microenvironment constituted with the blood-brain barrier (BBB), as compared to other organs. One of the hypothetical mechanisms for therapeutic effects by bevacizumab is normalization of VEGF-induced dysregulated vascular structure causing excessive increase in fluid leakiness from vessels, resulting in high interstitial pressure and low perfusion in the tumor tissue, thereby hampering sufficient drug delivery. A consequence of bevacizumab action is neutralization of vascular permeabilizing effects of VEGF and reducing interstitial edema, thus it is postulated that perfusion within tumor tissue is normalized, leading to improved drug delivery to tumor cells. However, in the brain, this process may be accompanied with repair of disrupted BBB function as well, which in turn would restrict penetration of most chemotherapeutic agents into the brain parenchyma [18,19]. One example was shown in a mouse xenograft model where TMZ was effective in inducing apoptosis and eradication of tumors derived from human glioma U87 cells, but the addition of a multitargeting anti-angiogenic inhibitor vandetanib to TMZ conversely resulted in a decrease in apoptosis rate and tumor suppression compared to TMZ monotherapy [20].

To explore this issue directly, randomized comparator trials of bevacizumab have been initiated in patients with recurrent GBM by a Dutch study (BELOB) and European Organisation for Research and Treatment of Cancer (EORTC; EORTC 26101). Both studies use lomustine (CCNU) as the standard comparator to bevacizumab, either as a single agent or in combination with bevacizumab, to evaluate whether bevacizumab alone is superior in improving survival to lomustine that has served as the standard therapy at progression in Europe, and whether the combination has a higher activity than bevacizumab monotherapy as well. The phase II three-arm Dutch study BELOB has recently closed with 140 patients randomly allocated to either bevacizumab alone, lomustine alone, or combination of these two agents using the RANO criteria for response assay. The primary endpoint, OS at 9 months, was 38%, 43%, and 59%, and PFS-6m was 16%, 13%, and 41%, respectively, demonstrating better activity for bevacizumab/lomustine combination therapy [21]. While PFS-6m in the bevacizumab/lomustine arm (41%) was consistent with other bevacizumab combination studies, considerably lower PFS-6m in the bevacizumab single arm (16%) in this study than in historical data (25%–43%) appears to correlate with the number of cycles administered before progression determined by Response Assessment in Neuro-Oncology (RANO) criteria. Here, the median number of cycles was two, with 28% of patients in the bevacizumab arm deemed PD after the first cycle. This data contrasted strikingly with the previous phase II studies of bevacizumab as a single agent where the median number of bevacizumab doses was nine (BRAIN trial, NCT00345163) and six (JO22506) [7,22]. Whether this difference comes from different methods in measuring progression or other more complex reasons, remains to be elucidated.

An Australian randomized phase II trial (CABARET) compared bevacizumab with or without carboplatin to determine whether addition of another commonly used cytotoxic agent to bevacizumab may benefit survival in 122 patients with recurrent GBM. PFS-6m was 26% (combination) and 24% (bevacizumab monotherapy), and mOS was 6.9 months and 6.4 months, respectively, failing to result in clinical benefit with the combination [23].

A German randomized phase II trial (GLARIUS, NCT00967330) aimed to improve survival of patients with newly-diagnosed GBM having unfavorable unmethylated MGMT promoter, by treating with bevacizumab and radiotherapy followed by combination of bevacizumab plus irinotecan instead of TMZ. The primary endpoint of PFS-6m was 79.6% in the bevacizumab/irinotecan arm and 41.3% in TMZ arm ($p < 0.0001$). mPFS was 9.7 months (bevacizumab/irinotecan) *vs.* 6.0 months (TMZ), and mOS was 16.6 months (bevacizumab/irinotecan) *vs.* 14.8 months (TMZ). Among patients in the TMZ arm who had progressed or died (85%), the crossover rate (second line with bevacizumab/irinotecan) was 63% [24]. These results suggest that substitution of TMZ with irinotecan in combination with upfront bevacizumab use may benefit the survival of patients with MGMT-unmethylated newly diagnosed GBM, in contrast to the Radiation Therapy Oncology Group (RTOG) 0825 study (see below) where addition of bevacizumab to TMZ showed no survival benefits in this unfavorable prognostic population. These studies warrant further phase III trials investigating bevacizumab/irinotecan combination in the patients with MGMT-unmethylated newly diagnosed GBM.

3. Controversies

Following the results of the various trials in recurrent GBMs, there has been considerable interest in evaluating the benefit of bevacizumab in untreated newly diagnosed GBMs. There are two randomized phase III trials evaluating the role of bevacizumab in combination with TMZ and radiation therapy for newly diagnosed GBMs. RTOG 0825 is a large phase III trial targeting 720 patients and the other trial is the Effectiveness of Avastin in GBM (AVAglio) study targeting over 900 patients and sponsored by Hoffman-LaRoche (Basel, Switzerland). In November 2012 at the Society of Neuro-Oncology Meeting in Washington, D.C., Chinot *et al.* presented the interim results of the AVAglio study that included final PFS data and interim OS data. The result was positive for an improved PFS, with other promising outcomes in secondary endpoints such as quality of life (QoL). Specifically, the Investigator-Assessed PFS, that was one of the co-primary endpoints, had a 10.6 months median survival in the bevacizumab arm compared to median survival of 6.2 months for standard therapy with a hazard ratio (HR) of 0.64 (95% CI: 0.55–0.74, $p < 0.0001$). The central Independent Review Facility Assessed PFS (a secondary endpoint) demonstrated a median survival in the bevacizumab group as 8.4 months compared with 4.3 months in the standard therapy group; again statistically significant with stratified HR of 0.61 (95% CI: 0.53–0.71, $p < 0.0001$). The interim OS analysis demonstrated a small non-statistically significant benefit in the bevacizumab group with a one year survival rate of 72% (68–76) compared with 66% (62–71) with $p = 0.052$; with 254 events in the bevacizumab arm compared with 263 events in the standard therapy arm, with a stratified HR of 0.89 (0.75–1.07), $p = 0.2135$. In the arm receiving up-front bevacizumab, there were statistically significant benefits in the five pre-specified domains of health-related quality of life (HRQoL) (secondary endpoints), namely global health status, physical functioning, social functioning, motor functioning, and communication deficit; with longer median duration that patients were stable/improved from baseline. The median duration these patients maintained was a KPS higher than 70, with 9 months in the bevacizumab arm *vs.* 6 months in the standard arm. In the bevacizumab arm, 66% of patients who were on steroids at baseline discontinued their steroids compared with 47% in the standard arm. The time to steroid initiation for patients who had been off steroids at baseline was 12.3 months in the bevacizumab arm *versus* 3.7 months in the standard arm with a HR of 0.71 (95% CI;0.57–0.88, $p = 0.0018$). Overall, patients receiving bevacizumab had a diminished steroid requirement. There was no significant increase in intracranial hemorrhage (2.6% *vs.* 2.2% for all WHO tumor grades, I–IV, 1.5% *vs.* 0.7% for grade 3 or higher). There was more mucocutaneous bleeding in the bevacizumab arm (26.7% *vs.* 8.9%), but only 0.4% was grade 3 or higher. There were slightly more wound-healing complications (3.7% *vs.* 2.2%), as well as an increase in arterial thromboembolic events (5% *vs.* 1.6%) and a slightly lower incidence of venous thromboembolic events (7.8% *vs.* 9.6%). The incidence of hypertension was higher in the bevacizumab arm; 37.5% *vs.* 13.0%, and 10.3% *vs.* 2.0% for grade 3 or higher). Proteinuria was also higher (14% *vs.* 4%). There was a slight excess of gastrointestinal perforation at 1.7% *vs.* 0.2%; with abscesses or fistulae of 0.6% *vs.* 0.4%.

The other phase III study, RTOG 0825, also reported a longer PFS in the bevacizumab arm compared to the standard therapy; 10.7 months, and 7.3 months, respectively, HR = 0.79 (95% CI; 0.66, 0.94, $p = 0.007$), which actually did not reach the pre-specified statistical endpoint of $p = 0.004$. The mOS could not achieve the pre-specified statistical endpoint of $p = 0.046$, as that in the bevacizumab arm was 15.7 months and that in the placebo arm was 16.1 months (HR = 1.13, 95% CI; 0.93, 1.37). One of the

striking differences between the two phase III trials was in the analyses of HRQoL data. In the RTOG 0825 trial, patients on the bevacizumab arm experienced significant worsening in cognitive function, motor dysfunction, and communication deficits measured by EORTC/BN20 QoL scale. Global symptom burden, interference and multiple factor groups measured by MDASI-BT were also significantly worse with bevacizumab compared to placebo. If longer PFS but equal OS compared to placebo, rather worse HRQoL (if the analyses of HRQoL for RTOG 0825 was appropriate), and some side effects are realistic, it would suggest no room for bevacizumab to be used in an upfront GBM treatment setting.

4. Treatment Options after Progression on Bevacizumab

It is also challenging to determine treatment options for patients with recurrent GBM who progress after bevacizumab treatment. Currently there is no active regimen in this setting, since previous studies that employed continuing bevacizumab plus another agent, for example irinotecan, carboplatin, etoposide, or dose-intensified TMZ, or discontinuing bevacizumab and treating with an alternative agent failed to show efficacy: mPFS was 1.0–2.6 months, PFS-6m was 0–16%, and mOS was 3–6 months [9,25–27].

There is a concern that discontinuation of bevacizumab after failure in patients with recurrent GBM, may give rise to rapid tumor re-growth with accelerated clinical deterioration, which is recognized as a rebound phenomenon [28]. Mikkelson and his colleagues reported that 28% of patients who did not respond to bevacizumab showed rebound progression and mOS was only 7 weeks [28]. Some of these patients exhibited a partial response to re-administration of bevacizumab post rebound progressive disease (PD). It is, however, argued that the incidence of rebound phenomenon after bevacizumab discontinuance is relatively rare in other studies, and it may be a matter of interpretation as to the definition [29–31].

Another critical issue that has been addressed is whether continuation of bevacizumab beyond progression (BBP) could enhance survival of patients with recurrent GBM, although its net benefit appears limited as described above. This therapeutic approach has been exploited and proved meaningful in metastatic colorectal cancer in ML18147 randomized phase III study [32]. Patients who received bevacizumab plus standard first line chemotherapy (either oxaliplatin or irinotecan) and exhibited PD were randomly allocated to standard second line chemotherapy either with or without bevacizumab until PD. OS from randomization, the primary endpoint, was significantly longer in patients with bevacizumab continuation than those without, with HR = 0.81 and $p = 0.0062$. PFS was also longer in the bevacizumab group (HR = 0.68, $p < 0.0001$) [32]. According to the positive results in colorectal cancer, and the fact that outcome after bevacizumab failure remains dismal, BBP has also drawn attention for recurrent GBM [22,25]. Reardon *et al.* analyzed outcome among patients with recurrent GBM who received subsequent therapy after initial progression on bevacizumab regimens of one of five consecutive, single-arm phase II trials; the bevacizumab partners were either irinotecan, daily TMZ, etoposide, bortezomib, or erlotinib [33]. mOS and OS at 6 months for patients who continued bevacizumab therapy beyond progression ($n = 55$) were 5.9 months and 49.2%, compared with 4.0 months and 29.5% for those treated with a non-bevacizumab regimen ($n = 44$; $p = 0.014$), and bevacizumab continuation was an independent predictor of improved OS (HR = 0.64; $p = 0.04$) [33]. OS for patients who did not receive further therapy after initial PD was only 1.5 months. To confirm whether BBP strategy is beneficial in recurrent GBM, a phase IIIb randomized trial (MO28347) has been planned

to initiate in 2013 with estimated enrollment of 510 patients (NCT01860638). Patients with newly diagnosed GBM who are treated with standard TMZ and radiotherapy plus upfront bevacizumab will be randomized upon PD to receive second line therapy either with bevacizumab continuation *vs.* placebo, which will further proceed to the third line therapy. The primary endpoint is OS from randomization.

5. Should Bevacizumab Be Used at First Indication of PD?

Another issue in treating recurrent GBM with bevacizumab that has not been treated with bevacizumab upfront is whether to apply it at the first relapse or to defer until other second line therapies have failed. As discussed above, despite its potent and rapid antitumor or anti-edema effects, tumors will eventually regrow and, at the time of bevacizumab failure, survival expectation is quite limited because of lack of effective follow-up therapies [9,25,26,33]. In the BRAIN trial (bevacizumab *vs.* bevacizumab + irinotecan), 85 patients in the bevacizumab alone arm comprised 69 (81%) at the first relapse and 16 (19%) at the second. mOS after initiation of bevacizumab was similar in both settings, 9.1 months and 9.2 months, respectively [7]. Omuro *et al.* performed a clinical trial of protracted TMZ regimen in patients with recurrent TMZ-pretreated GBM with or without a history of bevacizumab use. Patients with previous bevacizumab exposure (18 cases) survived significantly less than bevacizumab-naïve patients (19 cases; nine of them received bevacizumab after progression on protracted TMZ) (mOS was 4.3 months *vs.* 13 months, HR = 3.2; $p = 0.001$) [34], suggesting that bevacizumab may benefit patients with recurrent GBM given even at the second relapse. Piccioni *et al.* analyzed retrospectively patients with recurrent GBM treated with bevacizumab (388 cases) to determine whether the timing of bevacizumab initiation would affect time to progression (TTP) and post-bevacizumab survival after initiation of bevacizumab. Analysis of this data showed that there were no significant differences in any survival periods as median TTP was 4.1, 4.2, and 3.4 months for those treated with bevacizumab at the first recurrence ($n = 264$), at the second recurrence ($n = 88$), and at the third recurrence ($n = 36$), respectively ($p = 0.165$), and median post- bevacizumab survival was 8.5, 8.9, and 6.2 months, respectively ($p = 0.330$) [35]. Since these studies are not intended to evaluate this issue primarily, efficacy and its duration of chemotherapy may be reduced with an increase in number of recurrence. Prospective studies are warranted to address this question. Nonetheless, these results may pose a potential strategy to withhold bevacizumab until further recurrence occurs by applying novel exploratory therapeutics in front at the initial recurrence prior to bevacizumab to benefit survival in cases with good performance status.

6. Recurrence with Diffuse Infiltrative Pattern after Bevacizumab Therapy

It has been postulated that anti-angiogenic therapy, such as bevacizumab treatment of GBM, results in an increased incidence of distant and diffuse patterns of radiographic recurrence. One of the potential explanations for this phenomenon is that antiangiogenic therapy only targets the angiogenic-dependent contrast-enhancing components of tumor, but does not target the angiogenic-independent, highly-infiltrating glioma cells at the leading edge demonstrated by FLAIR image [29,36]. Another reason is that after normalization of VEGF-induced abnormally developed tumor vasculature and interstitial edema, remaining glioma cells migrate through preexisting vessels by vessel cooption [37]. Iwamoto *et al.* reported on patterns of relapse in 37 adult patients with recurrent GBM treated with

bevacizumab [38]. Following progression on therapy, 35% of patients showed non-enhancing tumor progression. Norden *et al.* retrospectively evaluated 28 patients with high-grade gliomas treated with bevacizumab and irinotecan with respect to the pattern of disease progression after bevacizumab failure; 18% were diffuse and 18% were distant. However, they also found similar patterns of radiographic recurrence (18% diffuse and 6% distant) in those not treated with bevacizumab [27,39]. Pope *et al.* retrospectively analyzed the data of the prospective BRAIN trial of 167 patients with recurrent GBM treated with bevacizumab and with or without Irinotecan. There were 17% diffuse and 2.4% distant patterns of recurrence after the Stupp regimen prior to bevacizumab. Following bevacizumab treatment, 82% of patients maintained the same in disease pattern, and 16% of patients in the baseline local disease group were converted to a diffuse pattern [40]. Chamberlain *et al.* retrospectively reviewed 80 patients with GBM who were treated with the Stupp regimen initially, followed by bevacizumab monotherapy at first recurrence as for patterns of radiographic presentation. At initial diagnosis, 87.5% of GBM were local and 2.5% were diffuse. At first recurrence after the Stupp regimen, 80% were local and 6.25% were diffuse. At second recurrence following progression on bevacizumab, 71% were local and 11.25% were diffuse, and at third recurrence, they were 71% and 14%, respectively, suggesting that the majority of patients with GBM manifest local disease and maintain the same pattern regardless of multiple recurrence and use of bevacizumab [41]. This issue has been addressed in a prospective manner in a large international phase III AVAglio trial for patients with newly diagnosed GBM as discussed earlier. Patterns of radiographic progression were assessed in 65% of patients enrolled in the study as compared to the baseline. At baseline, a diffuse pattern was observed in 60% of placebo arm and 70% of bevacizumab arm. Non-diffuse tumors (placebo arm 40%; Bev arm 30%) changed to a diffuse pattern at progression in 22.8% and 24.7% cases, respectively, indicating no significant increase in induction of an invasive pattern of recurrence following upfront bevacizumab treatment [42]. Based on these data from clinical trials and experiences, it does not seem a universal phenomenon that anti-angiogenic therapies induce preferentially diffuse invasive progression in GBM, although a number of basic research studies have implicated it through several distinct mechanisms including a shift of major pro-angiogenic factors from VEGF to others, such as c-Met or SDF (discussed later), and it is still challenging to define response and progression radiographically following bevacizumab treatment which may result in different interpretation of patterns of recurrence.

7. Bevacizumab Dosing

As bevacizumab is usually administered at 10 mg/kg, every 2-week interval, it is still unclear which dosing schedule is the most optimal for bevacizumab monotherapy in recurrent GBM. To analyze its dose-response effect, Wong *et al.* performed a meta-analysis of 15 studies published from 2005 to 2009, involving 548 patients treated with bevacizumab for recurrent GBM and showed that there was no difference in bevacizumab dose-response benefit between 5 mg/kg and 10 to 15 mg/kg [43]. If the dosing could be lowered to 5 mg/kg, reduced bevacizumab use results in a substantial savings of medical cost. The cost-effectiveness of 5 *vs.* 10 mg/kg of bevacizumab dose in GBM patients needs to be prospectively examined.

8. Next Steps and Future Directions

Disease progression, as reflected by tumor growth and, probably enhanced, metastasis/invasion during treatment with inhibitors of VEGF signaling, is attributed to multiple interacting mechanisms. Among them are activation of pathways that favor epithelial-mesenchymal transition, promotion of invasiveness, and induction of tolerance and activation of cancer stem cells. Disease progression during treatment with bevacizumab paired with chemotherapy does not necessarily mean that the inhibitor has lost efficacy [44]. The resistance could be due to the chemotherapy. Evidence of better OS in metastatic colorectal cancer, when bevacizumab is continued beyond progression in the presence of diverse types of chemotherapy, reflects the continued involvement of VEGF as mentioned before [45].

However, some effects of VEGF inhibitors that slow tumor growth can still promote invasion and metastasis in certain models [46,47]. Tumors with high MET expression or activating mutations of MET are generally more aggressive and have a less favorable prognosis [48]. The activation of MET can promote epithelial-mesenchymal transition and tumor invasiveness [49,50], partly by increasing the activity of transcriptional repressors, such as snail homolog 1 (*SNAI1*; also known as *SNAIL*), *ZEB1* and *TWIST1*, that reduce E-cadherin expression, increase N-cadherin expression and turn on the expression of other mesenchymal markers [51]. Inhibition of VEGF signaling can result in decreased expression of epithelial markers and increased expression of mesenchymal markers such as SNAI1, TWIST1 in preclinical models [52,53]. The expression of the mesenchymal marker fascin increases in GBMs after treatment with bevacizumab [54].

Targeting angiogenesis and tumor progression/invasion/metastases together has recently shown promise as a strategy for preventing escape from inhibitors of VEGF signaling [54]. One approach is the inhibition of MET and VEGF signaling together, either by concurrent administration of selective inhibitors or by single agents that block both receptors [cabozantinib (also known as XL184) or foretinib (also known as XL880)] [55,56]. Concurrent inhibition of MET and VEGF signaling can slow tumor growth, decrease invasion and metastasis, and change invasive tumors into a shape with a ball-like appearance in certain models. The therapeutic benefit of this approach is currently being evaluated in clinical trials of multiple tumor types.

Cabozantinib, which inhibits MET, AXL and VEGF receptors, as well as multiple other receptor tyrosine kinases, is a potent inhibitor of invasion and metastasis in spontaneous and xenograft tumors in mice [55,56]. Cabozantinib has better effects on tumor angiogenesis and survival than those found with combinations of selective inhibitors of MET and VEGF signaling in the same model, suggesting that AXL or other targets (such as RET, KIT and TIE2) contribute to the efficacy of cabozantinib [57]. Cabozantinib is showing promising results in clinical trials of castration-resistant metastatic prostate cancer, medullary thyroid cancer, breast cancer, NSCLC, malignant melanoma, liver and ovarian cancers [58,59]. It remains to be determined if clinical trials with such dual or multiple inhibitors will effectively impact GBM recurrence and invasion. Blocking both angiogenesis and escape pathways is now an attainable step in the evolution of the use of agents that inhibit VEGF signaling together with other targets. The current knowledge of tumor vascular biology and mechanisms of tumor growth, invasion and metastasis, will enable these approaches. Steps that still need to be taken include learning more about escape mechanisms, identifying additional targeted drugs that act synergistically with angiogenesis inhibitors. Predictive biomarkers to identify patients who will have benefit from such approaches is also important.