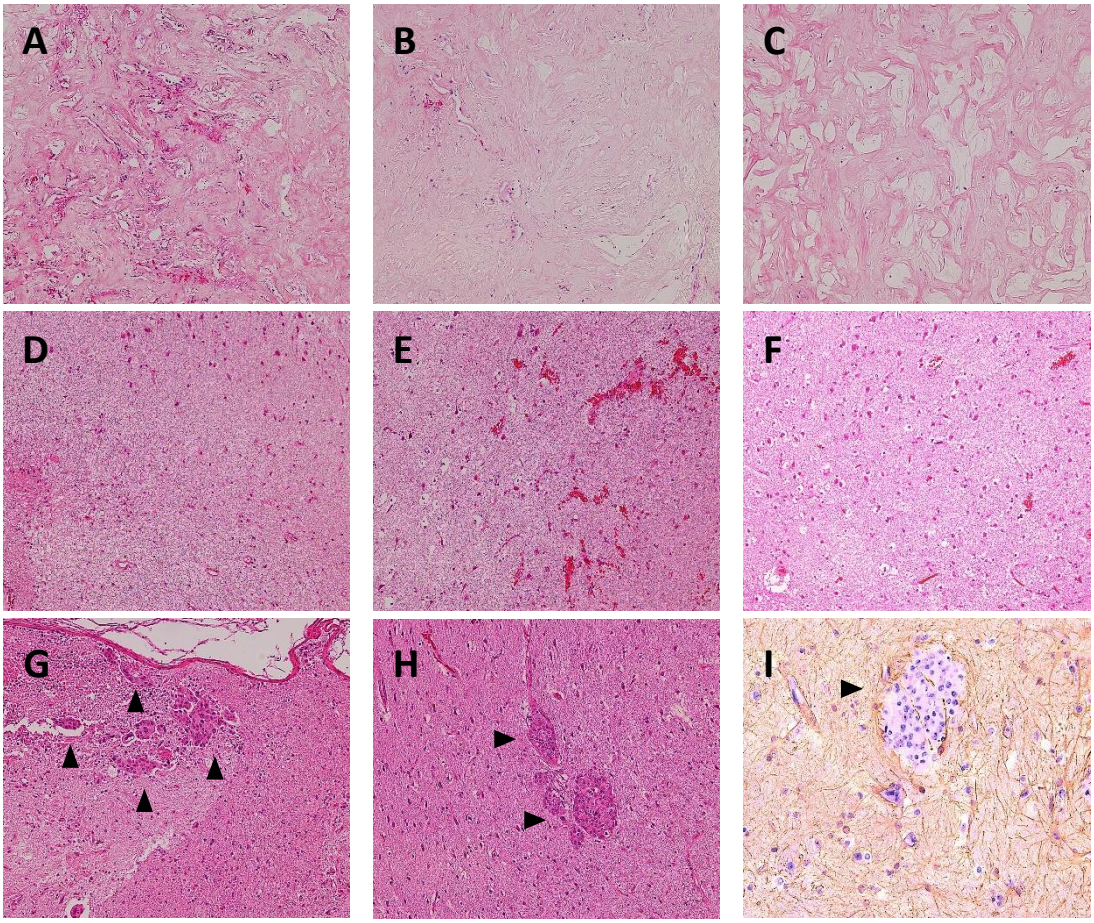
A prospective multicenter single-arm clinical trial of bevacizumab for patients with surgically untreatable symptomatic brain radiation necrosis," by Furuse et al.

Supplementary Materials

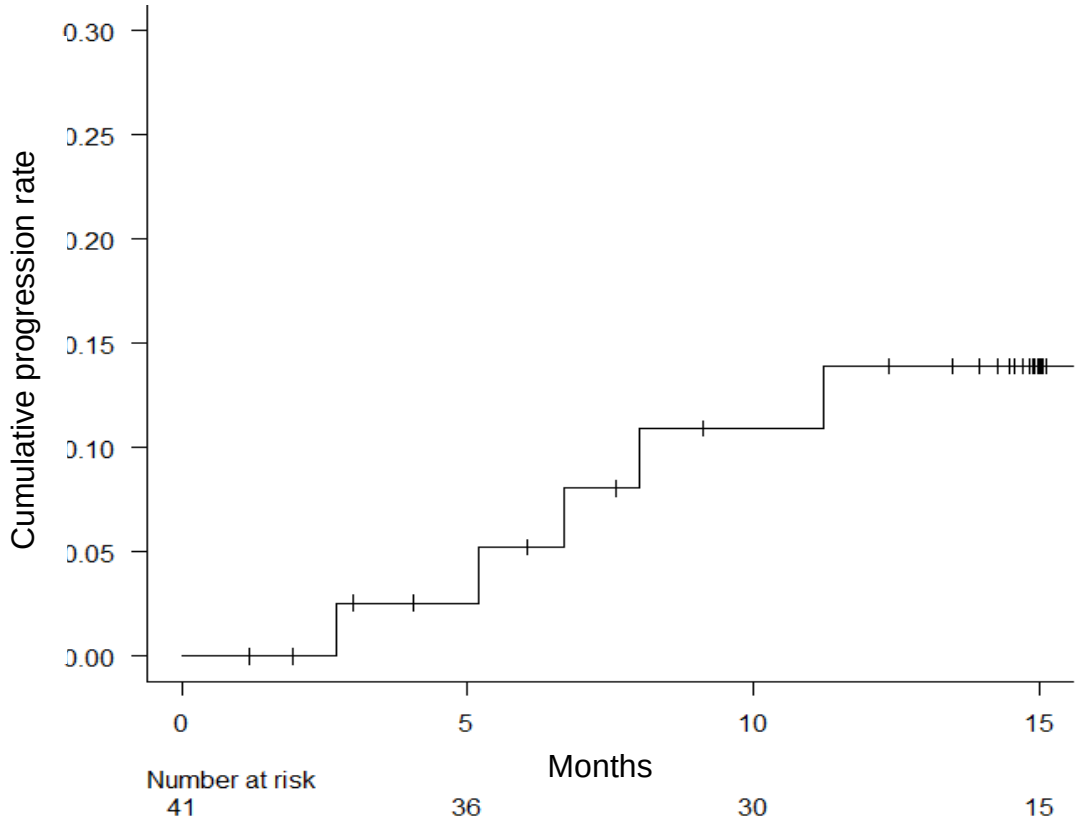


Supplementary Fig. 1. A representative sample of BRN removed by surgery. **A–H**: Hematoxylin and eosin (H.E.) findings for eight different sites in the same BRN specimen from a metastatic brain tumor (×100 magnification). Apparent tissue necrosis in A–C and damaged brain parenchyma in D–H are shown. The islet-like tumor clusters

can be seen only in G and H. **I**: Immunohistochemical staining for GFAP with hematoxylin counterstain (×200). A tumor islet consisting of viable tumor cells (GFAP-negative) is seen against the GFAP-positive background. If a biopsy is performed for this patient, the diagnosis may be “pure BRN” when tissue from areas A–F is sampled. However, the diagnosis can be changed to “necrotic tissue with viable cancer cells” when the area shown in G or H is sampled. As shown in this figure, it is not uncommon that residual tumor cells survive in and around the necrotic lesion in surgical specimens diagnosed as BRN. This is not surprising because not a few patients with a malignant brain tumor, especially those with a malignant glioma, eventually develop tumor recurrence after radiation therapy. In short, pure BRN is rather rare, and surviving tumor cells can be found by searching the lesion out.

In such cases, the predominant cause of perilesional edema, the relapsed tumor or the radiation necrosis must be thoroughly investigated. However, identification of the cause is not easy when only small biopsied specimens are available because, in BRN, islet-shaped residual tumor cells are strewn around the necrotic core, and not at the circumference (A–H). This means that the diagnosis for a biopsied specimen depends largely on the site from where it was removed. Moreover, in this case, marked interstitial edema could not be found just around the residual tumor islets. We presume

that in this case the cerebral edema was caused by BRN and not by tumor residue or tumor proliferation. We therefore used amino-acid PET to diagnose BRN with a specified cut-off value instead of a surgical biopsy in the present study.



Supplementary Fig. 2. Kaplan-Meier analysis of the cumulative tumor-recurrence rate.

Enrollment inclusion and exclusion criteria details

Inclusion criteria:

Hematologic, hepatic, and renal function were adequate as follows: absolute neutrophil count ≥ 1500 mm^3; platelet count ≥ 100000 mm^3; hemoglobin ≥ 9.5 g/dL; serum glutamic oxaloacetic transaminase (GOT) and serum glutamic pyruvic transaminase (GPT) < 2.5-fold; total bilirubin ≤ 2.0 mg/dL; serum creatinine ≤ 1.2 mg/dL; urine protein $\leq 1+$; prothrombin time-international normalized ratio (PT-INR) ≤ 1.5-fold.

The PT-INR was stable at a range of 1.5–2.5 when anticoagulant was used.

Life expectancy was greater than 3 months.

Able to provide written informed consent (patient or legally authorized representative).

In cases of radiation necrosis with a metastatic brain tumor, no active lesion was detected by PET or other radiographic examinations, and the values of tumor markers were within normal limits (if they were examined).

Exclusion criteria:

- The patient was able to undergo surgery to remove necrotic tissue of the brain.
- The patient's intracranial tumor was active and recurrent (i.e., the L/N ratio on F-BPA-PET was >2.5, or the L/N ratio on C-MET-PET was >1.8.
- The patient required the intravenous administration of antibiotics, antiviral drugs, or antifungal drugs for infection.
- The patient was febrile (< 38°C body temperature or equal).
- The patient had:
  - a severe comorbidity such as heart disease, pulmonary fibrosis, interstitial pneumonia, hemorrhagic diathesis, or uncontrollable hypertension or diabetes
  - comorbid or history of unstable angina or myocardiac infarction within the prior 6 months
  - uncontrollable peptic ulcer
  - uncontrollable hypertension
  - unhealed advanced wound or fracture
  - comorbid or history of gastrointestinal perforation and fistula, or abdominal abscess within 6 months

- comorbid or history of hemoptysis and pulmonary hemorrhage > Grade 1
- comorbid or history of vascular diseases (venous and arterial thrombosis and embolism, aortic aneurysms) that required intervention within the prior 6 months
- congestive heart failure > NYHA class I
- a scheduled operation during this study or had been operated within the 4 weeks prior to registration
- hemorrhage (intracranial hemorrhage, gastrointestinal hemorrhage, urinary hemorrhage, hemoptysis)
- PT-INR > 2.5 or unstable during anticoagulant use
- history of severe hypersensitivity or allergy to bevacizumab

- The patient was or might be pregnant.
- The patient was breastfeeding.
- Investigators judged that the patient was inappropriate for enrollment in this study.

Treatment interruption or discontinuation

Treatment was interrupted or discontinued when the patient exhibited any of the adverse events (AEs) described below:

Adverse Event	**Grade**	**Remediation**
Hematologic	grade ≥3	Bevacizumab was stopped, and it was restarted if the AE improved to grade ≤2.
Non-hematologic (except for below AE)		
Venous thromboembolism	grade 3	Bevacizumab was stopped, and it was restarted if the AE improved to grade ≤2.
	grade 4	Bevacizumab was discontinued.
Arterial thromboembolism	grade ≥3	Bevacizumab was discontinued.
Hypertension	grade 3	Bevacizumab was stopped, and it was re-started when the hypertension was controllable.
	grade 4	Bevacizumab was discontinued.
Proteinuria	grades 2, 3	Bevacizumab was stopped, and it was restarted when proteinuria was

		downgraded to grade 1 or ≤ 2 g/24 hr.
	grade 4	Bevacizumab was discontinued.
Hemoptysis	grade ≥2	Bevacizumab was discontinued.
Intracranial hemorrhage	all grades	Bevacizumab was discontinued.
Gastrointestinal perforation, wound dehiscence, fistula	all grades	Bevacizumab was discontinued.

Bevacizumab treatment was discontinued when the toxicity had not resolved within 6 wks or the same AE occurred after the re-start of bevacizumab. Bevacizumab should be interrupted or discontinued when investigators judge that the administration of bevacizumab is inappropriate.

Supplementary Table 1.

Radiation modality and dose information in all 41 cases in the study

Case	FAS	PPS	Radiation Modality	Gy/Fraction	Fractions
1	○	×	hypo-IMRT	7.4	10
2	×	×	SRS	22	1
3	○	×	XRT	2	30
4	○	×	SRS	22	1
			SRS	22	1
			SRT	10	3
			SRS	22	1
			SRS	22	1
5	○	×	WB-XRT	3	10
			SRS	20	1
			SRS	20	1
			XRT	2	18
			SRT	2	5

6	○	×	XRT	1.8	28
			SRS	16	1
			SRS	16	1
7	○	○	hypo-IMRT	2.5	28
			XRT	2	28
8	○	×	hypo-IMRT	4.5	10
			SRS	16	1
9	○	○	WB-XRT	3	10
			SRS	22.5	1
10	○	×	SRT	3.5	7
11	○	×	SRS	13.4	1
			SRS	14.6	1
12	○	○	SRT	5	8
			SRT	6	5
13	○	×	SRT	5	8
14	○	○	SRT	5	8
15	○	×	XRT	2	30

16	○	○	SRT	5	8
17	○	○	SRT	5	8
18	○	×	SRT	5	8
19	○	○	XRT	2	30
20	○	○	XRT	2	27
			SRT	10	3
21	○	○	SRT	11.7	3
22	○	×	SRS	20	1
			SRS	22	1
			SRS	20	1
23	○	○	XRT	2	30
			BNCT	10.5	1
24	○	○	SRT	1.8	28
			Proton	1.65	28
25	○	○	XRT	1.8	28
			Proton	1.5	28
26	○	○	XRT	1.8	28

			Proton	1.65	28
27	○	×	XRT	2	20
			hypo-IMRT	6	8
28	○	○	XRT	2.15	32
29	○	○	SRT	8.5	4
30	○	○	SRT	8.5	4
31	○	×	XRT	2	30
			XRT	2	10
32	○	×	SRS	12	1
			SRS	12	1
33	×	×	SRS	23	1
			SRT	7	5
			SRS	22	1
			SRS	22	1
34	○	○	XRT	2	30
			XRT	2	15
			SRT	9	3

35	○	○	SRS	22.5	1
36	○	×	XRT	2	25
			XRT	2	25
			SRT	16	8
			SRS	16	1
			SRS	16	1
37	×	×	SRT	15	3
38	○	×	XRT	2	30
			SRS	23	1
39	○	×	XRT	2	30
			SRS	20	1
			SRS	20	1
40	○	○	XRT	2	30
			SRS	18	1
41	○	○	SRT	10	3

○ : means included in FAS (Full analysis set) or PPS (Per protocol set)

X : means excluded in FAS or PPS

Hypo-IMRT: hypo-fractionated intensity modulated radiotherapy

SRS: stereotactic radiosurgery, SRT: stereotactic radiotherapy,

XRT:X-ray treatment, WB :whole brain,

Supplementary Table 2. Subgroup analysis of patients with remission and patients without remission in the full analysis set (FAS)

Variable	Remission (n=30)	Non-Remission (n=8)	p-value
Median age (yrs): median (range)	53.5 (17–72)	63 (22–73)	0.203
Sex			
Male	17 (56.7)	5 (62.5)	1.000
Female	13 (43.3)	3 (37.5)	
KPS: median (range)	70 (60–100)	60 (60–80)	0.434
Original tumor pathology: no. (%)			
Primary brain tumor	22 (81.5)	5 (18.5)	0.655*
Metastastic brain tumor	7 (70.0)	3 (30.0)	
Time from RT to diagnosis (mos): median (range)	24.5 (3.0–140.9)	16.0 (5.3–95.9)	0.535
Radiotherapy			
SRS	21	5	0.689*
RT other than SRS:	9	3	

High-dose RT			
(HF IMRT, proton beam, BNCT)	7	1	0.660*
(RT except for high dose RT)	23	7	
Chemotherapy for tumor			
Temozolomide	15 (50.0)	5 (62.5)	0.697
Other chemotherapeutic agents	9 (30.0)	1 (12.5)	0.653
Treatment for radiation necrosis			
Corticosteroids	30 (100.0)	8 (100.0)	–
Vitamin E	13 (43.3)	5 (62.5)	0.438
Anticoagulants/antiplatelets	14 (46.7)	3 (37.5)	0.709
Necrotomy	3 (10.0)	1 (12.5)	1.000
Hyperbaric oxygen	3 (10.0)	0 (0.0)	1.000
Bevacizumab	1 (3.3)	0 (0.0)	1.000
Osmotic diuretics	1 (3.3)	0 (0.0)	1.000
L/N ratio (median; range)			
^{11}C-methionine	1.5 (0.61–1.78)	1.56 (0.98–1.80)	1.000
^{18}F-boronophenylalanine	1.93 (1.64–2.30)	1.5	–
Volume of BRN (mL) median (range)			

Perilesional edema	136.23 (1.94–359.06)	134.30 (91.42–222.25)	0.816
Contrast-enhanced lesion	7.29 (1.59–40.25)	9.03 (1.66–33.64)	0.816

BNCT, boron neutron capture therapy; BRN, brain radiation necrosis; HF-IMRT, hypofractionated intensity-modulated radiotherapy; KPS, Karnofsky Performance Status; L/N, lesion/ normal tissue; SRS, stereotactic radiosurgery.

The Wilcoxon rank sum test was used for the statistical analysis. *Fisher's exact test was used.

Supplementary Table 3. Serious and frequent adverse events

Event	No. of patients	Percent
Grade ≥ 3:		
Hypertension	2	4.9
Elevated ALT	2	4.9
Convulsion	2	4.9
Cellulitis	1	2.4
Pneumonia	1	2.4
Urethritis	1	2.4
Anemia	1	2.4
Ulcerative colitis	1	2.4
Duodenal obstruction	1	2.4
Maculo-papular rash	1	2.4
Elevated GGT	1	2.4
White blood cell count abnormal	1	2.4
Undernutrition	1	2.4
> 5%:		

Hypertension	14	34.1
Elevated ALT	13	31.7
Anemia	12	29.3
WBC count abnormal	11	26.8
Fibrin D dimer abnormal	10	24.4
Platelet count abnormal	10	24.4
Pyrexia	8	19.5
Elevated AST	8	19.5
Prothrombin time prolonged	7	17.1
Convulsion	5	12.2
Headache	5	12.2
Diarrhea	5	12.2
Activated partial thromboplastin time Abnormal	5	12.2
Blood fibrinogen abnormal	5	12.2
Fibrin degradation products abnormal	5	12.2
Proteinuria	5	12.2
Neutrophil count abnormal	5	12.2

Epistaxis	4	9.8
Nausea	4	9.8
Fatigue	4	9.8
Decreased appetite	3	7.3

ALT, Alanine aminotransferase; AST, Aspartate aminotransferase; GGT, gamma-glutamyltransferase.

The search strategy used in the systematic review of the literature regarding treatments for BRN

We conducted a systematic literature review to identify previous research evidence about conventional medical treatments for brain radiation necrosis (BRN).

Eligibility Criteria

Our aim was to identify any studies describing the management for delayed cerebral radiation necrosis with a nonsurgical treatment and to provide a comprehensive review of the evidence related to this topic.

Definition

We defined BRN as late-phase brain radiation injuries with a necrotic lesion detected by radiographical examination or histopathological diagnosis.

Literature Search

The searches were performed by two independent investigators (authors M.F. and N.N.), using combinations of the terms shown in the table below. Studies in English

were retrieved from PubMed and the Cochrane Library, dated from 1 January 1946 to 21 February 2015. The searches included all article types (original articles, reviews, letters, and case series); only single case reports were eliminated. References were also checked for the inclusion of potential papers related to the topic, and duplicate results were deleted.

Summarizing the evidence

We summarized the evidence extracted from the above literature search as described below. Corticosteroids and anticoagulants have been routinely used in the treatment for BRN patients, but we were unable to find any randomized studies of these conventional therapies. Thus, there is limited evidence regarding conservative treatments for BRN.

Literature search (21, February, 2015)

Search	Query	items found
#1	Search (radiotherapy[ti] OR radiotherapies[ti] OR radiation therapy[ti] OR “radiation injuries” [ti] OR “radiation injury”[ti] OR radio*[ti] OR radiation*[ti] OR irradiation*[ti] OR Cyberknife[ti] OR “Gamma knife” [ti] OR Sterotactic*[ti] OR Radiosurgery[ti] OR Radiotherapy[ti] OR “Sterotactic radiosurgery” [ti] OR “Sterotactic radiotherapy” [ti]) AND (necrosis[ti] OR necroses[ti] OR "necrotic"[ti] OR “radiation necrosis” [ti] OR radiation necroses[ti] OR radionecrosis[ti] OR "cerebral radiation necrosis"[ti] OR "cerebral radionecrosis"[ti] OR "cerebral radio-necrosis"[ti] OR "delayed brain injury"[ti] OR "late brain injury"[ti] OR "delayed cerebral injury"[ti] OR "late cerebral injury"[ti] OR "late injury"[ti] OR "delayed injury"[ti] OR Injury*[ti] OR Damage*[ti] OR Edema*[ti])	8830
#2	Search (“drug therapy”[tw] OR “drug treatment”[tw] OR Pharmacotherapy[tw] OR Pharmacotherapies[tw] OR Medication[tw] OR “Medical treatment”[tw] OR Medicine[tw] OR Radioprotective[tw] OR Melatonin[tw] OR Vitamin[tw] OR Anti-platelet[tw] OR Antiplatelet[tw] OR Anticoagulant[tw] OR Steroid[tw] OR Edaravone[tw] OR “Hyperbaric oxygen”[tw] OR Dexamethasone[tw] OR Betamethasone[tw] OR Glucocorticoid*[tw] OR Conservative[tw])	2978698
#3	Search (brain[tw] OR cerebral[tw] OR cerebrum[tw] OR “cerebral white matter”[tw] OR “central nervous system”[tw] OR glioma*[tw] OR arteriovenous malformation* OR arteriovenous fistula* OR meningioma*[tw] OR “Brain metastasis”[tw] OR “Brain metastases”[tw] OR “Metastatic brain tumor*”[tw] OR “Metastatic cerebral tumor*”[tw])	1452981
#4	Search (#1 AND #2 AND #3) AND Humans[mh]	111
#5	Search (bevacizumab[ti] OR Avastin [ti])	5391
#6	Search (#4 NOT #5)	**92**

Response rate of steroids for BRN reported in the literature

Steroid	Author	Number of treated	Clinical and radiographical improvement	Response rate	PMID	Year	Article title
	Lorenzo ND et al.	10	1	10%	364708	1978	Late cerebral radionecrosis.
	Glass JP et al.	9	3	33%	6478431	1984	Cerebral radiation necrosis following treatment of extra cranial malignancies.
	Woo E et al.	7	3	43%	3694200	1987	Cerebral radionecrosis: is surgery necessary?
	Tang Y et al.	65	25	38%	25142813	2014	Effect of edaravone on radiation-induced brain necrosis in patients with nasopharyngeal carcinoma after radiotherapy: a randomized controlled trial.
Total		91	32	**35.2%**			

Response rate of anticoagulants for BRN reported in the literature

Anticoagulants	Author	Number of treated	Clinical and radiographical improvement	Response rate	PMID	Year	Article title
	Rizzoli HV et al.	2	2	100%	6699703	1984	Treatment of delayed radiation necrosis of the brain. A clinical observation.
	Glantz MJ et al.	8	5	63%	7969953	1994	Treatment of radiation-induced nervous system injury with heparin and warfarin.
	Happold C et al.	8	3	38%	18716713	2008	Anticoagulation for radiation-induced neurotoxicity revisited.
Total		18	10	**55.6%**			