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# A Multicenter Phase II Trial of Nimustine Hydrochloride Administered via Convection-Enhanced Delivery in Children With DIPG

Ryuta Saito[1,2] | Masayuki Kanamori[2,3] | Yoshiki Arakawa[4,5] | Yohei Mineharu[4] | Yasuo Aihara[6] | Kentaro Chiba[5] | Toshihiro Kumabe[6] | Ichiyo Shibahara[6] | Yukihiko Sonoda[7] | Kenichiro Matsuda[7] | Manabu Kinoshita[1] | Aya Sato[2] | Fumiaki Takahashi[8] | Teiji Tominaga[2]

[1]Department of Neurosurgery, Nagoya University Graduate School of Medicine, Nagoya, Japan | [2]Department of Neurosurgery, Tohoku University Graduate School of Medicine, Sendai, Miyagi, Japan | [3]Department of Neurosurgery, Asahikawa Medical University, Asahikawa, Hokkaido, Japan | [4]Department of Neurosurgery, Kyoto University Graduate School of Medicine, Sakyo-ku, Kyoto, Japan | [5]Department of Neurosurgery, Tokyo Women's Medical University, Tokyo, Japan | [6]Department of Neurosurgery, Kitasato University School of Medicine, Sagamihara, Kanagawa, Japan | [7]Department of Neurosurgery, Yamagata University, Yamagata, Japan | [8]Department of Information Science, Iwate Medical University, Iwate, Japan

**Correspondence:** Ryuta Saito (saito.ryuta.b1@f.mail.nagoya-u.ac.jp)

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## ABSTRACT

Diffuse intrinsic pontine glioma (DIPG) is a very challenging-to-treat pediatric malignant tumor, with a median survival time of < 12 months. Convection-enhanced delivery (CED) allows for direct drug administration into the tumor site, showing potential as a novel therapeutic approach. This study evaluated the efficacy of CED of nimustine hydrochloride (ACNU) in children with DIPG. This phase 2, single-arm, multicenter study enrolled patients aged 3–21 years and diagnosed with DIPG. The investigational treatment commenced 1 month after completing radiotherapy (local 50–60 Gy). The treatment involved stereotactic brain surgery for catheter placement, followed by ACNU administration via a CED catheter at a concentration of 0.75 mg/mL for 2–3 days until a cumulative dose of 7 (±0.3) mL was achieved. The primary endpoint was the 1-year survival rate. From April 2018 to March 2020, 21 children were enrolled in the trial and treated, with 20 evaluable for the primary endpoint. The 1-year survival rate from the start of radiotherapy was 60%, and the median survival time was 15 months. The response rate was analyzed in 20 patients, with one complete response (CR), six partial responses (PR), nine stable diseases, and four progressive diseases, resulting in a response rate of 35% (CR + PR). The CED of ACNU in the brainstem of children with DIPG after radiotherapy appears to be an effective therapeutic strategy. This approach warrants further development as a treatment for children with DIPG. This study is registered with jRCT (No. jRCT2021190003).

## 1 | Introduction

Diffuse intrinsic pontine glioma (DIPG) is a refractory brain tumor that originates in the brainstem, frequently affecting children, with many cases proving fatal within a year [1]. Given its location within the brainstem, DIPG cannot be surgically removed. In addition, temozolomide (TMZ) chemotherapy, which is a standard treatment for supratentorial

malignant gliomas, has been proven ineffective against DIPG [2]. Accordingly, radiotherapy remains the only effective treatment; however, it is typically associated with a median overall survival of < 1 year.

In chemotherapy for malignant glioma, a substantial challenge is the permeation of therapeutic drugs through the blood–brain barrier (BBB) when administered systemically [3]. In addition, achieving an effective drug concentration against malignant gliomas often results in systemic toxicity, which limits the dosage and, consequently, the therapeutic effect. To address this issue, convection-enhanced delivery (CED) has emerged as a promising drug delivery system designed to overcome the BBB. CED has gained attention recently, and clinical applications have been attempted for various diseases [4]. Unlike conventional intracerebral administration that relies on diffusion, CED delivers the drug continuously to the targeted site, applying positive pressure to distribute high concentrations into the interstitial space of the brain tissue without mechanically damaging brain structures. This method potentially allows for extensive drug distribution. However, despite its promise, many clinical studies using CED for brain tumor treatment have failed to achieve satisfactory outcomes. This may be due to inadequate drug distribution or questions about efficacy [5, 6]. For instance, when using chimeric proteins, tumor cells must express the antigen that the therapeutic antibody recognizes. Still, all tumor cells will unlikely express a specific antigen [6]. For brain tumors, alkylating agents, including temozolomide and nitrosourea compounds such as nimustine hydrochloride (ACNU), carmustine (BCNU), and lomustine (CCNU), have demonstrated efficacy in clinical practice. This study focused on using nitrosourea agents for their drug efficacy and targeting the brainstem for better drug distribution. ACNU, which is primarily used in Japan as an alternative to BCNU in the USA, is the most water-soluble nitrosourea agent, making it particularly suitable for CED [7]. Its dose-dependent effect indicates that even a single dose may provide a sustained therapeutic effect, and CED dosing has shown potential for achieving relatively wide distribution [7]. In 2001, the topical administration of nimustine hydrochloride (1-(4-amino-2-methyl-5-pyrimidL nyl)methyl-3-(2-chloroethyl)-3-nitrosourea hydrochloride) in humans was reported to be safe [8]. Currently, ACNU is approved for intravenous or intra-arterial administration for brain tumors in Japan and plays a central role in chemotherapy for malignant gliomas. Based on these findings, we investigated the toxicity and antitumor effects of ACNU using CED in a rat brain tumor model to confirm its safety and efficacy [9, 10]. In addition, clinical studies in patients with recurrent malignant glioma [UMIN000003983] have tested its efficacy and safety [11]. Encouraging results were observed in three patients with brainstem glioma, leading to further clinical studies to evaluate the safety of ACNU using CED catheters in recurrent patients with brainstem glioma. The results confirmed that a single continuous administration of a 7-mL dose of 0.75 mg/mL ACNU is expected to yield positive outcomes for brainstem gliomas [UMIN000005125] [12].

To develop a new treatment for pediatric DIPG, where treatment options are currently limited, we investigated the efficacy and safety of a continuous 7-mL dose of 0.75 mg/mL ACNU using CED in pediatric patients with newly diagnosed DIPG. The primary endpoint was the 1-year survival rate. The overall survival and response rates were defined as secondary endpoints, and adverse events were assessed as the safety endpoint.

## 2 | Methods

### 2.1 | Study Design and Participants

This investigator-initiated, single-arm, multicenter, phase 2 clinical trial was conducted to evaluate the efficacy of the CED of ACNU. Eligible patients were aged 3–21 years and diagnosed with DIPG. The eligibility criteria were as follows: patients whose magnetic resonance imaging (MRI) at screening showed that at least two-thirds of the lesion in the brainstem was located in the pons and considered of pontine origin (tissue diagnosis was not required for study entry), patients who could begin treatment within 28–35 days after completing radiotherapy (local 50–60 Gy), patients who had not received any other antitumor therapy except for radiotherapy and temozolomide, patients with a Lansky or Karnofsky performance status score of at least 50 at screening, patients expected to have a prognosis of at least 2 months, patients in adequate general condition, and patients who provided free and voluntary written consent for participation in the clinical trial.

The study was first approved by the Ministry of Health, Labour and Welfare Certified Clinical Research Review Board at Tohoku University and then by the ethics committee of Kyoto University, Tokyo Woman's University Hospital, Kitasato University Hospital, and Yamagata University Hospital.

### 2.2 | Procedures

One month after completing radiotherapy (local 50–60 Gy; 28–35 days later; in cases of re-enrollment, treatment initiation was allowed up to 56 days later), the investigational treatment was started. The treatment consisted of stereotactic brain surgery (catheterization) followed by ACNU administered via a CED catheter at a concentration of 0.75 mg/mL for 2 or 3 days until the cumulative dose reached 7 (±0.3) mL. If resuming administration after an interruption due to syringe pump failure, adverse events, or other circumstances was deemed reasonable, resumption within 24 h was allowed (if not resumed within 24 h, administration was discontinued). In such cases, the dosing period could be extended to day 4, with the day of surgery as day 1. Fuji Systems Co. provided the catheters used in this study. A microcatheter with a silicone balloon, initially approved for neurovascular intervention, was modified and used in this study (Figure S1). The administration site was determined based on contrast-enhanced T1-weighted MR images (3D) and T2-weighted images (3D) obtained preoperatively using a stereotactic surgical planning device. The target was defined as the T2-weighted high-signal region on MRI. Using a stereotactic neurosurgical device, an inserter was introduced into the brain, with the tip positioned 1 cm before the target site. The catheter was then inserted 1 cm deeper than the tip of the inserter, positioning the catheter tip at the target

site. The position of the catheter tip was confirmed under X-ray imaging. After approximately 1 h of administration at a flow rate of 0.1 mL/h, the balloon was inflated to the specified volume (0.02 mL) to prevent leakage through the catheter tract. The dosing rate was as follows: when using one catheter, the dose was started at 0.1 mL/h and increased to 0.2 mL/h at any time on day 2. After 1 h of administration at 0.2 mL/h, increasing the rate to 0.3 mL/h was acceptable. When using two catheters, the dose was started at 0.1 mL/h per catheter and maintained at this rate until the end of administration. The following concomitant medications and therapies were allowed: TMZ could be administered at 150 mg/m$^2$ for 5 days, either orally or intravenously. If used, TMZ was administered from days 1 to 5 (days 2–6 were also permissible). Patients were allowed to receive up to 12 courses of maintenance TMZ. Non-antineoplastic drugs, such as antibiotics and anticonvulsants, were allowed. In addition, intracranial pressure-lowering agents such as glycerol, mannitol, isosorbide, and steroids were permitted to lower brain pressure.

### 2.3 | Outcomes

The primary endpoint was defined as the 1-year survival rate. Secondary endpoints included overall survival and response rate. For the analysis of overall survival, the cutoff date was set at 1 year after day 1 of study drug administration for the participant who received the last dose in this trial. The response rate was determined according to the efficacy criteria published by the response assessment in neuro-oncology (RANO) working group [13]. For participants with contrast-enhanced lesions on gadolinium contrast-enhanced T1-weighted MRI at baseline, these lesions were analyzed, whereas for participants without contrast-enhanced lesions, T2-weighted high-intensity regions were analyzed.

Adverse events were also analyzed as a safety endpoint. Adverse events were defined as any undesirable or unintended illnesses, disorders, or symptoms (including abnormal laboratory values; abnormal laboratory values of grade ≥ 2 were counted; however, if medical or surgical intervention was required, abnormal laboratory values of grade 1 were also counted) that occurred in participants and surgeons who received the study treatment. Symptoms resulting from exacerbating the underlying disease were not considered adverse events. This study's severity of adverse events was determined according to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE v4.0).

### 2.4 | Statistical Analysis

To demonstrate the efficacy of the investigational treatment for pediatric first-episode brainstem glioma, we verified that the 1-year survival rate, starting from the initiation of the investigational treatment, was substantially higher than the threshold of 30%. This 30% threshold was extrapolated from previously reported DIPG survival curves [2] at 15 months, accounting for the fact that the study treatment begins 1 month after the completion of radiation therapy. Asymptotic normality was assumed for the 1-year survival rates estimated by the Kaplan–Meier method, and one-tailed tests were performed for the following hypotheses: null hypothesis (H0), 1-year survival rate for the study treatment group ≤ 0.30, and alternative hypothesis (H1), 1-year survival rate for the study treatment group > 0.30. The significance level was set at 2.5% one-sided. For the analysis of the response rate, the frequency distribution of tumor shrinkage was assessed according to the RANO criteria, and the percentage of response (complete response [CR] + partial response [PR]) was calculated. Clopper–Pearson intervals were used to construct the confidence intervals for the proportions. Waterfall plots were created to represent the data visually.

## 3 | Results

### 3.1 | Patients

Informed consent was obtained from 24 participants, of whom 21 were enrolled. The 21 participants who underwent clinical trial treatment were defined as the safety analysis population. Patient demographics are detailed in Table 1. In comparison, the 20 participants who began receiving the investigational drug were defined as the efficacy analysis population, as one participant experienced a severe adverse event related to catheter implantation surgery and discontinued before administration of the investigational drug. In these 20 patients, who received ACNU, the scheduled dose (=complete dose) were given. In one case, the start of dosing was delayed due to intra-tumor hemorrhage associated with surgery, but after the initiation of infusion, the drug was administered to the final volume without interruption (Table 2). Of the participants who received ACNU, 2 completed the study period up to the post-observation period evaluation, whereas 18 discontinued the post-observation period evaluation. The reasons for discontinuation included the worsening of the primary disease in 17 participants and the occurrence of adverse events in 1.

### 3.2 | One-Year Survival Rates for Pediatric DIPG

One-year survival rates were calculated for 20 patients with newly diagnosed pediatric DIPG, starting from the treatment date in the clinical trial. The 1-year survival rate was 55.0% (95% confidence interval, 31.3%–73.5%, $p = 0.0123$), indicating a substantially higher survival rate than the 30% threshold. When the 1-year survival rate was evaluated from the start of radiotherapy, as often reported in historical data, it was 60% (95% confidence interval, 35.7%–77.6%).

### 3.3 | Overall Survival of Pediatric DIPG

The overall median survival time (MST) of 20 patients with newly diagnosed DIPG was calculated using the start date of the study treatment as the starting point. The MST was 386.0 (95% confidence interval, 261.0–466.0) days. When calculated using the start date of the initial radiation therapy as the starting point, this MST was 15 months (455 days). The survival curves, estimated by the Kaplan–Meier method from the start date of the initial radiation therapy, are shown in Figure 1.

**TABLE 1** | Patient demographics.

| | |
|---|---|
| Patients (*n*; number of subjects) | 20 |
| Sex *N* (%) | |
| Female | 10 (50.0) |
| Male | 10 (50.0) |
| Age | |
| Median | 8.5 |
| Min-Max | 4.0–17.0 |
| Mean | 8.4 |
| S.D. | 3.39 |
| Past History *n* (%) | |
| (−) | 4 (20.0) |
| (+) | 16 (80.0) |
| Complication *n* (%) | |
| (−) | 15 (75.0) |
| (+) | 5 (25.0) |
| Radiation dose | |
| Median | 54.00 |
| Min-Max | 50.00–60.00 |
| Mean | 53.72 |
| S.D. | 2.044 |
| Radiation dose per day | |
| Median | 1.80 |
| Min-Max | 1.20–2.00 |
| Mean | 1.81 |
| S.D. | 0.165 |
| Days of RT | |
| Median | 44.0 |
| Min-Max | 36.0–52.0 |
| Mean | 43.2 |
| S.D. | 3.58 |
| Radiation Field *n* (%) | |
| Local | 20 (100.0) |
| Use of steroids *n* (%) | |
| None | 20 (100.0) |
| KPS *n* (%) | |
| Over 70 | 13 (65.0) |
| Over 80 | 7 (35.0) |
| 50 | 6 (30.0) |
| 60 | 5 (25.0) |
| 70 | 2 (10.0) |
| 80 | 3 (15.0) |
| 90 | 3 (15.0) |
| 100 | 1 (5.0) |
| JCS *n* (%) | |
| 0 | 14 (70.0) |
| I-1 | 3 (15.0) |
| I-3 | 3 (15.0) |
| Aphasia *n* (%) | |
| (−) | 19 (95.0) |
| (+) | 1 (5.0) |
| Dysarthria *n* (%) | |
| (−) | 9 (45.0) |
| (+) | 11 (55.0) |
| Cranial nerve palsy *n* (%) | |
| (−) | 5 (25.0) |
| (+) | 15 (75.0) |
| Limb paresis *n* (%) | |
| No paresis | 10 (47.6) |
| Hemiparesis | 8 (38.1) |
| Tetraplegia | 3 (14.3) |
| CNS hemorrhage *n* (%) | |
| (−) | 19 (95.0) |
| (+) | 1 (5.0) |
| Memory disturbances *n* (%) | |
| (−) | 20 (100.0) |
| Cognitive deficit *n* (%) | |
| (−) | 16 (80.0) |
| (+) | 4 (20.0) |
| Seizure *n* (%) | |
| (−) | 19 (95.0) |
| (+) | 1 (5.0) |
| Somnolence *n* (%) | |
| (−) | 19 (95.0) |
| (+) | 1 (5.0) |
| Hydrocephalus *n* (%) | |
| (−) | 19 (95.0) |
| (+) | 1 (5.0) |

(Continues)

**TABLE 1** | (Continued)

Abbreviations: CNS, central nervous system; KPS, Karnofsky Performance Status Score; RT, Radiation Therapy.

**TABLE 2** | Treatment detail.

| | Age | Sex | No. of catheters | ACNU dose [mL] | Duration of infusion [hrs] | Interruption during infusion | Use of temozolomide | Severe adverse events | Best overall response | Overall survival[M]/ status | Note |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 7 | M | 2 | 7.0 | 35 | None | Yes | | PD | 18/Dead | |
| 2 | 16 | F | 2 | 7 | 35 | None | Yes | | CR | 18/Dead | Presented in Figure S3 |
| 3 | 5 | F | 2 | Not infused | Not infused | None | No | Intracranial hemorrhage | | | Presented in Figure S5 |
| 4 | 6 | M | 2 | 7.0 | 35 | None | Yes | | SD | 11/Dead | |
| 5 | 6 | M | 2 | 7.0 | 35 | None | Yes | | PR | 21/Dead | Presented in Figure 3 |
| 6 | 9 | M | 2 | 6.8 | 34 | None | No | | SD | 11/Dead | |
| 7 | 6 | F | 1 | 6.83 | 44.2 | None | Yes | | PR | 27/Dead | |
| 8 | 6 | F | 1 | 6.80 | 44.1 | None | No | | PD | 13/Dead | |
| 9 | 9 | M | 2 | 7.0 | 35 | None | Yes | | PR | 10/Dead | |
| 10 | 9 | F | 2 | 7.0 | 35 | None | No | | SD | 8/Dead | |
| 11 | 17 | M | 2 | 7.0 | 35 | None | Yes | | SD | 17/Dead | |
| 12 | 5 | F | 2 | 6.81 | 34.1 | None | Yes | | SD | 8/Dead | |
| 13 | 10 | F | 2 | 7 | 35 | None | Yes | | SD | 15/Dead | |
| 14 | 6 | M | 2 | 7.21 | 36.1 | None | Yes | | PR | 12/Dead | |
| 15 | 10 | M | 2 | 7.0 | 35 | None | Yes | | SD | 18/Dead | |
| 16 | 4 | F | 2 | 7.17 | 35.9 | None | Yes | Wound infection | PD | 12/Dead | |
| 17 | 11 | F | 2 | 6.84 | 34.2 | None | Yes | | PD | 19/Dead | |
| 18 | 8 | M | 2 | 6.9 | 34.5 | None | Yes | Intratumoral hemorrhage | PR | 11/Dead | Presented in Figures S2 and S6 |
| 19 | 9 | F | 2 | 7.01 | 35.1 | None | Yes | | PR | 16/Alive | |
| 20 | 5 | F | 2 | 7.02 | 35.1 | None | No | | SD | 16/Alive | |
| 21 | 9 | M | 2 | 7 | 35 | None | Yes | Nonbacterial infection & stroke | SD | 15/Alive | |

Abbreviations: CR, complete response; F, female; Fig, Figure; M, male; M, month; PD, progressive disease; PR, partial response; SD, stable disease.

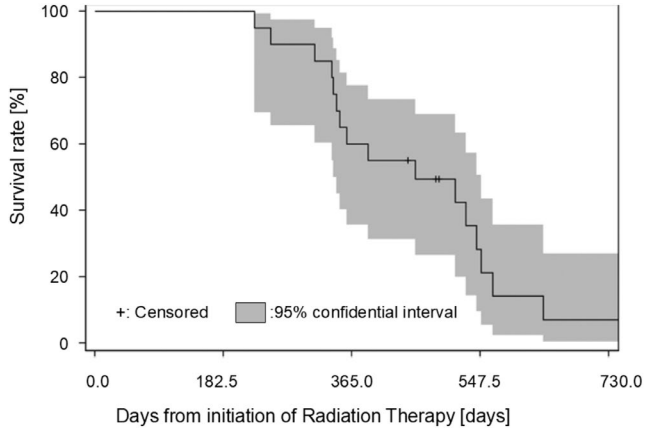


**FIGURE 1** | The overall survival time (MST) after the initiation of radiation therapy in 20 pediatric patients with newly diagnosed DIPG was calculated. The median overall survival was 455.0 (95% confidence interval, 339.0–549.0) days. The survival curves were estimated using the Kaplan–Meier method and are presented.

### 3.4 | Response Rate in Pediatric DIPG

Response rates were calculated according to the RANO criteria. Of the 20 pediatric patients in the efficacy analysis population, one patient achieved CR, 6 achieved PR, and 9 had stable disease (SD). The waterfall plot is shown in Figure 2A and the duration of response in Figure 2B. The estimated overall response rate (CR + PR) was 35%. Representative cases showing PR to the treatment are illustrated in Figure 3 and Figure S2. A representative case showing a CR is illustrated in Figure S3.

### 3.5 | Treatment Duration

In the safety analysis population, the median duration of catheter placement was 3.0 (range, 2.0–3.0) days. The median time for study drug administration was 35.00 (range, 34.00–44.20) h. The study drug's median dose was 7.000 (range, 6.800–7.210) mL.

### 3.6 | Adverse Events

In the safety analysis of 21 patients, all 21 (100%) experienced adverse events (Table 3). The most common adverse events were decreased lymphocyte count in 10 (47.6%) patients, constipation in 7 (33.3%), and fever in 4 (19.0%). However, no causal relationship with the investigational drug or device was established. The decreased lymphocyte count and constipation were considered side effects of TMZ, whereas the fever was likely due to the surgery. However, the participant's susceptibility to aspiration might have also contributed. Serious adverse events occurred in 4 (19.0%) patients. These included intracranial hemorrhage caused by catheter manipulation, hemorrhage within a brainstem tumor, wound infection, nonbacterial infection, and stroke. Although a causal relationship with the study drug was ruled out for all events, a link to the study device could not be excluded for the "intracranial hemorrhage due to catheter manipulation" and "intratumoral hemorrhage in the brainstem." In the case of "intracranial

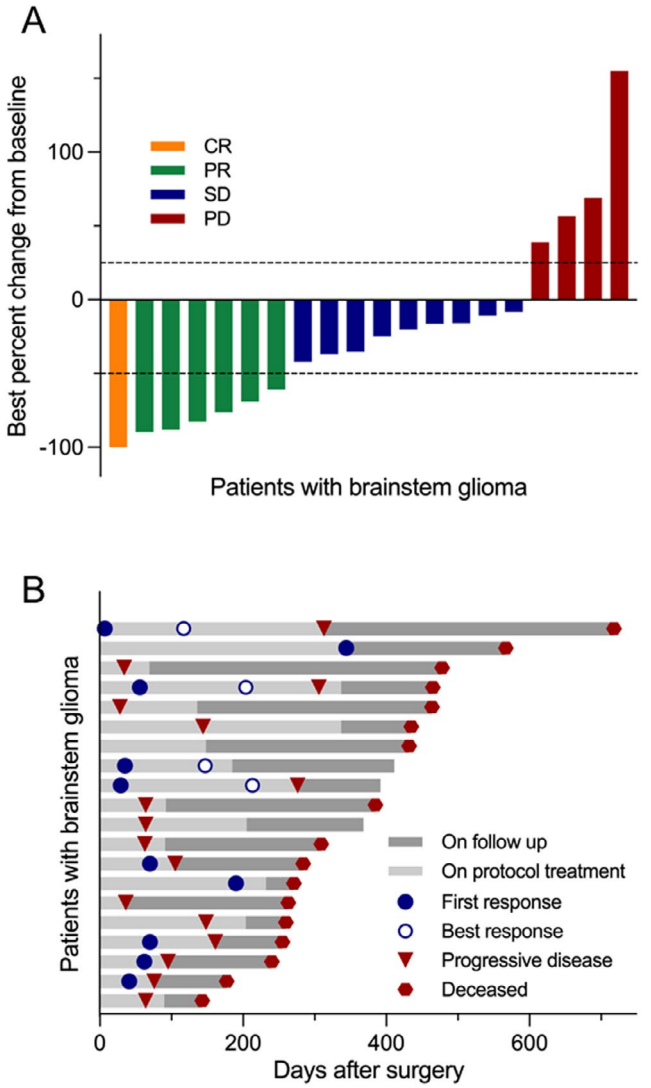


**FIGURE 2** | The frequency distribution of tumor shrinkage and response rates (CR + PR) were calculated according to the RANO criteria in 20 patients. Among the 20 pediatric patients in the efficacy analysis population, one achieved CR, 6 achieved PR, and 9 had SD. (A) The waterfall plot illustrates these results. The estimated overall response rate (CR + PR) was 35%. Panel B shows the duration of response with display of the occurrence of first and best response or progression.

hemorrhage due to catheter manipulation," a hematoma was identified in the right frontal lobe along the catheter tract on an MRI of a 6-year-old girl (Figure S5). Hematoma removal was performed, and the trial was terminated for her safety. She was transferred to another hospital, and her postoperative left hemiparesis improved, leading to the conclusion of the follow-up study with the outcome labeled as "minor improvement." "Intratumoral hemorrhage in the brainstem" occurred in an 8-year-old boy. Following catheter placement, a computed tomography (CT) scan revealed a hemorrhage within the brainstem tumor (Figure S6). The hemorrhage did not enlarge the next day, and the investigational drug administration was initiated. Although the patient developed right hemiparesis after surgery, this gradually improved, and he could walk with assistance. A wound infection was observed in a 5-year-old girl who had already been discontinued from the clinical trial

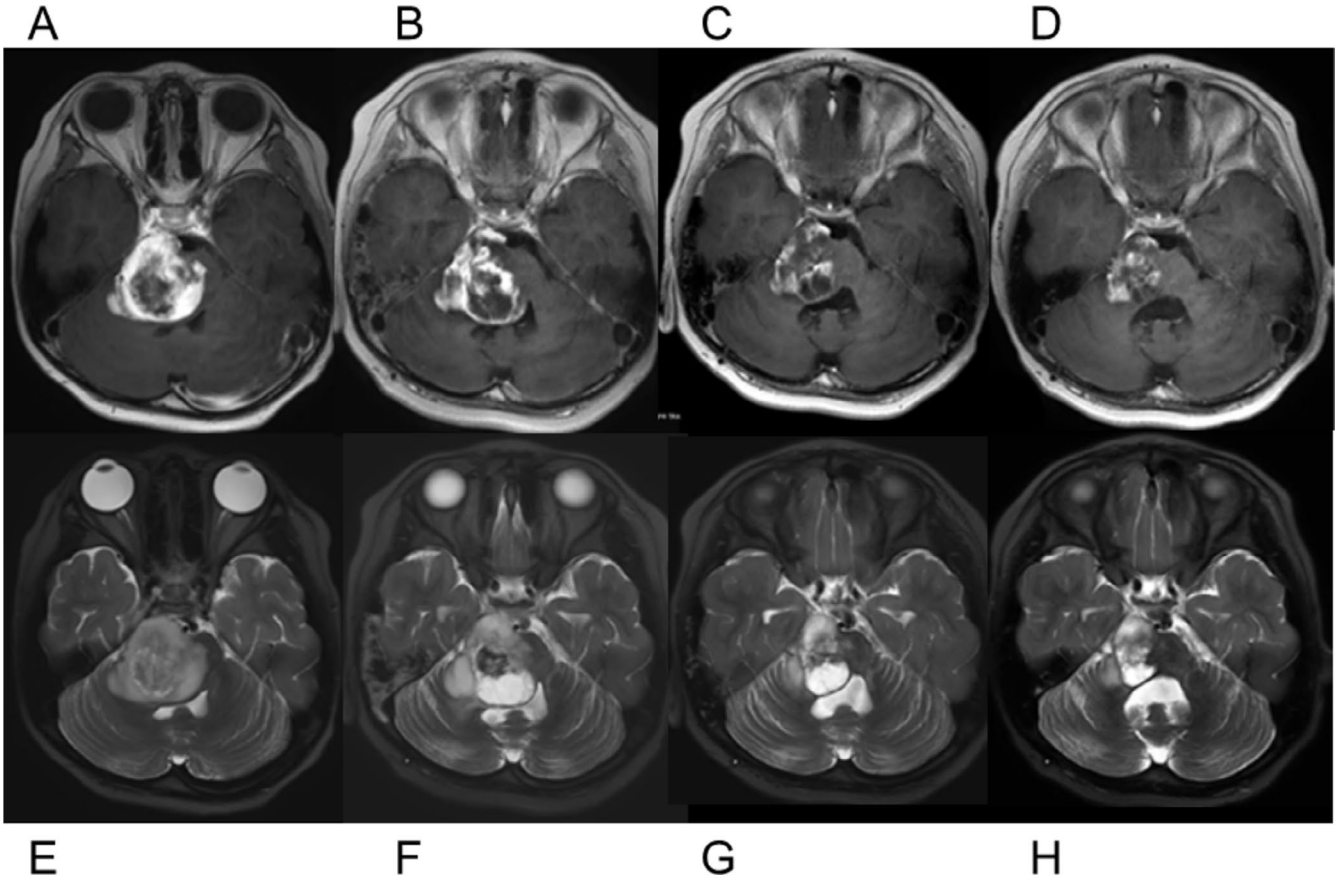


**FIGURE 3** | Case of a 6-year-old male with newly diagnosed DIPG who achieved a partial response after treatment. Images were obtained just before the CED of ACNU (A, E), 7 months after treatment (B, F), 8 months after treatment (C, G), and 11 months after treatment (D, H). Contrast-enhanced T1-weighted images (A–D) and T2-weighted images (E, F, G, and H) are shown.

because of disease progeression. She was hospitalized because of effusion and crust formation at the surgical wound site on her right forehead. After receiving cephazolin sodium, her symptoms improved, and she was discharged. Nonbacterial infection and stroke occurred as adverse events in the same patient, a 9-year-old boy. Fifty-six days after receiving the study drug, he showed no abnormalities but later developed tachypnea, tachycardia, impaired consciousness, and fever. He was intubated and placed on a ventilator; however, his condition progressed to multiple-organ failure and disseminated intravascular coagulation syndrome. Eventually, his condition stabilized enough that weaning from the ventilator was considered, and he was judged to have "recovered." Because the event was likely caused by a cytokine storm triggered by a nonbacterial infection, and more than 2 months had passed since the investigational treatment, a causal relationship with the investigational drug and device was ruled out. After recovering from the described symptoms, CT revealed extensive cerebral infarctions in bilateral cerebral hemispheres, sparing the brainstem. Despite 3 months of follow-up, the consciousness disorder or quadriplegia did not improve. The patient remained dependent on ventilatory support, and ischemia findings on CT persisted. The patient was judged to be in a steady state with minimal prospects for further recovery, leading to the termination of the follow-up investigation with the outcome labeled "not recovered." The adverse events in this patient, particularly the infection and stroke, led to the discontinuation of the clinical trial evaluation.

During the investigation period, no participants died. However, among those who discontinued study evaluation and participated in survival studies, 10 deaths were recorded during the post-observation period, all attributable to the underlying disease. In addition, during the 13-month follow-up period after treatment, seven more cases resulted in death, also due to the progression of the underlying disease.

### 3.7 | Effects of Concomitant Temozolomide

TMZ was concomitantly used in 16 patients (Table 2). The primary efficacy endpoint of 1-year survival with and without TMZ was 62.5% and 25.0%, respectively, and the secondary endpoints of overall survival were 433 days and 285.5 days, respectively, with response rates of 37.5% and 50.0%, respectively. In any of these endpoints, no statistical difference was observed between the presence or absence of TMZ concomitant use.

### 3.8 | Effects of Post-Treatment

Post-treatment disease progression was not specified in this study. However, 15 patients received bevacizumab after progression. Of these, seven also underwent a second course of radiation. The effect of re-irradiation on the 1-year overall survival after the initiation of initial radiation therapy was minimal; two

**TABLE 3** | Summary of the adverse events observed in this study. The severity of adverse events in this study was determined according to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE v4.0).

| | Adverse events | | Patients *N* (%) | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | Related | | | Non-related | | | |
| Severity | SOC (system organ class) | PT (preferred term) | Grade1 | Grade2 | Grade3 | Grade1 | Grade2 | Grade3 | Total |
| Non-severe | Ear and labyrinth disorders | Middle ear inflammation | 0 | 0 | 0 | 1 (4.8) | 0 | 0 | 1 (4.8) |
| | Endocrine disorders | Adrenal insufficiency | 0 | 0 | 0 | 0 | 2 (9.5) | 0 | 2 (9.5) |
| | Eye disorders | Dry eye | 0 | 0 | 0 | 0 | 1 (4.8) | 0 | 1 (4.8) |
| | | Keratitis | 0 | 0 | 0 | 0 | 1 (4.8) | 0 | 1 (4.8) |
| | | Periorbital oedema | 0 | 0 | 0 | 1 (4.8) | 0 | 0 | 1 (4.8) |
| | Gastrointestinal disorders | Constipation | 0 | 0 | 0 | 6 (28.6) | 1 (4.8) | 0 | 7 (33.3) |
| | | Cyclic vomiting syndrome | 0 | 0 | 0 | 1 (4.8) | 0 | 0 | 1 (4.8) |
| | | Dysphagia | 1 (4.8) | 0 | 0 | 0 | 0 | 1 (4.8) | 2 (9.5) |
| | | Nausea | 0 | 0 | 0 | 1 (4.8) | 0 | 0 | 1 (4.8) |
| | | Stomatitis | 0 | 0 | 0 | 0 | 2 (9.5) | 0 | 2 (9.5) |
| | General disorders and administration site conditions | Catheter site pain | 0 | 0 | 0 | 0 | 1 (4.8) | 0 | 1 (4.8) |
| | | Infusion site extravasation | 0 | 0 | 0 | 0 | 1 (4.8) | 0 | 1 (4.8) |
| | | Pyrexia | 0 | 0 | 0 | 1 (4.8) | 3 (14.3) | 0 | 4 (19.0) |
| | Hepatobiliary disorders | Drug-induced liver injury | 0 | 0 | 0 | 0 | 1 (4.8) | 0 | 1 (4.8) |
| | Infections and infestations | Gingivitis | 0 | 0 | 0 | 1 (4.8) | 0 | 0 | 1 (4.8) |
| | | Influenza | 0 | 0 | 0 | 0 | 1 (4.8) | 0 | 1 (4.8) |
| | | Lip infection | 0 | 0 | 0 | 0 | 1 (4.8) | 0 | 1 (4.8) |
| | | Otitis media acute | 0 | 0 | 0 | 0 | 1 (4.8) | 0 | 1 (4.8) |
| | Injury, poisoning and procedural complications | Post procedural hemorrhage | 0 | 0 | 0 | 1 (4.8) | 0 | 0 | 1 (4.8) |
| | | Procedural headache | 0 | 0 | 0 | 1 (4.8) | 0 | 0 | 1 (4.8) |
| | | Wound complication | 0 | 0 | 0 | 2 (9.5) | 0 | 0 | 2 (9.5) |

(Continues)

**TABLE 3** | (Continued)

| | Adverse events | | Patients *N* (%) | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | Related | | | Non-related | | | |
| **Severity** | **SOC (system organ class)** | **PT (preferred term)** | **Grade1** | **Grade2** | **Grade3** | **Grade1** | **Grade2** | **Grade3** | **Total** |
| | Investigations | ALT increased | 0 | 0 | 0 | 0 | 1 (4.8) | 0 | 1 (4.8) |
| | | AST increased | 0 | 0 | 0 | 0 | 1 (4.8) | 0 | 1 (4.8) |
| | | Lymphocyte count decreased | 0 | 0 | 0 | 0 | 5 (23.8) | 5 (23.8) | 10 (47.6) |
| | | Neutrophil count decreased | 0 | 0 | 0 | 0 | 0 | 1 (4.8) | 1 (4.8) |
| | | Weight decreased | 0 | 0 | 0 | 0 | 1 (4.8) | 0 | 1 (4.8) |
| | | White blood cell count decreased | 0 | 0 | 0 | 0 | 3 (14.3) | 0 | 3 (14.3) |
| | Metabolism and nutrition disorders | Decreased appetite | 0 | 0 | 1 (4.8) | 0 | 1 (4.8) | 0 | 2 (9.5) |
| | Musculoskeletal and connective tissue disorders | Back pain | 0 | 0 | 0 | 0 | 1 (4.8) | 0 | 1 (4.8) |
| | Neoplasms benign, malignant and unspecified (incl cysts and polyps) | Intracranial tumor hemorrhage | 0 | 1 (4.8) | 0 | 0 | 0 | 0 | 1 (4.8) |
| | Nervous system disorders | Depressed level of consciousness | 2 (9.5) | 0 | 0 | 0 | 0 | 0 | 2 (9.5) |
| | | Dyslalia | 2 (9.5) | 0 | 0 | 0 | 0 | 0 | 2 (9.5) |
| | | Facial paralysis | 1 (4.8) | 0 | 0 | 0 | 0 | 0 | 1 (4.8) |
| | | Headache | 0 | 0 | 0 | 0 | 1 (4.8) | 0 | 1 (4.8) |
| | | Hydrocephalus | 0 | 0 | 0 | 0 | 0 | 1 (4.8) | 1 (4.8) |
| | | Lethargy | 0 | 0 | 0 | 2 (9.5) | 0 | 0 | 2 (9.5) |
| | | Pyramidal tract syndrome | 1 (4.8) | 2 (9.5) | 0 | 0 | 0 | 0 | 3 (14.3) |
| | | Somnolence | 0 | 1 (4.8) | 0 | 0 | 0 | 0 | 1 (4.8) |
| | Renal and urinary disorders | Dysuria | 0 | 0 | 0 | 0 | 1 (4.8) | 0 | 1 (4.8) |
| | Reproductive system and breast disorders | Penile pain | 0 | 0 | 0 | 0 | 1 (4.8) | 0 | 1 (4.8) |
| | Respiratory, thoracic and mediastinal disorders | Hypoxia | 0 | 0 | 0 | 0 | 1 (4.8) | 0 | 1 (4.8) |
| | | Pneumonia aspiration | 0 | 0 | 0 | 0 | 0 | 2 (9.5) | 2 (9.5) |

(Continues)

**TABLE 3** | (Continued)

| | Adverse events | | Patients *N* (%) | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | Related | | | Non-related | | | |
| **Severity** | **SOC (system organ class)** | **PT (preferred term)** | **Grade1** | **Grade2** | **Grade3** | **Grade1** | **Grade2** | **Grade3** | **Total** |
| Non-severe | Skin and subcutaneous tissue disorders | Dermatitis allergic | 0 | 0 | 0 | 1 (4.8) | 0 | 0 | 1 (4.8) |
| | | Dermatitis contact | 0 | 0 | 0 | 2 (9.5) | 0 | 0 | 2 (9.5) |
| | | Erythema | 0 | 0 | 0 | 1 (4.8) | 1 (4.8) | 0 | 2 (9.5) |
| | | Pruritus | 0 | 0 | 0 | 1 (4.8) | 0 | 0 | 1 (4.8) |
| | | Rash | 0 | 0 | 0 | 0 | 1 (4.8) | 0 | 1 (4.8) |
| | | Toxic skin eruption | 0 | 0 | 0 | 0 | 1 (4.8) | 0 | 1 (4.8) |
| | Vascular disorders | Vasculitis | 0 | 0 | 0 | 1 (4.8) | 0 | 0 | 1 (4.8) |
| Severe | General disorders and administration site conditions | Catheter site hemorrhage | 0 | 0 | 1 (4.8) | 0 | 0 | 0 | 1 (4.8) |
| | Infections and infestations | Infection | 0 | 0 | 0 | 0 | 0 | 1 (4.8) | 1 (4.8) |
| | | Postoperative wound infection | 0 | 0 | 0 | 0 | 0 | 1 (4.8) | 1 (4.8) |
| | Neoplasms benign, malignant and unspecified (incl cysts and polyps) | Intracranial tumor hemorrhage | 0 | 1 (4.8) | 0 | 0 | 0 | 0 | 1 (4.8) |
| | Nervous system disorders | Cerebrovascular accident | 0 | 0 | 0 | 0 | 0 | 1 (4.8) | 1 (4.8) |
| | | Hemorrhage intracranial | 0 | 0 | 1 (4.8) | 0 | 0 | 0 | 1 (4.8) |

patients died before reaching the 1-year mark, four received re-irradiation 12 months or later after the initial treatment (specifically at 12 months for two patients, 14 months for one, and 16 months for another), and only one patient received re-irradiation at 6 months after initial radiation.

## 4 | Discussion

In this study, the 1-year survival rate after enrollment was 55.0%. Compared with the historical control [2], which was the prespecified statistical benchmark for this trial, this rate exceeded the lower threshold of the 95% confidence interval set at 30% (95% confidence interval, 31.3%–73.5%, $p = 0.0123$). Among various studies reported in the literature and summarized in reviews [1], the study from the Children's Oncology Group, reported in 2011, was selected as the historical control because it was one of the largest studies ($n = 63$) available when this trial was initiated [2]. When this 1-year survival rate was evaluated from the start of radiotherapy, as often reported in historical data, it was 60% (95% confidence interval, 35.7%–77.6%). Data from the International DIPG Registry, which included 372 cases with central radiological review from January 2004 to January 2014, were reported in 2017 [14, 15]. The median (min–max) age at diagnosis was 6.3 (4.6–9.1) years; 55% were female, 42.7% were Caucasians, 9.9% were Africans, and 2.4% were Asians. The reported median survival was 11.2 months, with no significant differences by age, sex, or race. The 1-year survival rate was 45.3%, and the median progression-free survival was 7.0 months. The median time from progression to death was 2.3 months. Another study from Japan summarized 99 cases retrospectively enrolled from 27 Japanese centers between January 2009 and November 2014 [16]. Of the 99 patients, 47 (47%) were male, 52 (53%) were female, and the median (min–max) age at diagnosis was 6 [2–15] years. Of these patients, 99% received radiation therapy, and 74 received temozolomide chemotherapy. The resulting 1-year survival rate for the cohort was 40.6%, with a median survival of 11 months. Compared with these data, our treatment strategy indicates effectiveness (Figure S4).

The median overall survival from accrual, a secondary endpoint, was 386.0 (95% confidence interval, 261.0–466.0) days. However, the median overall survival from the start of radiotherapy, which is comparable to the aforementioned historical data, was 15 months. This result is also clearly better than the reported historical data [1, 2, 14, 15, 16]. Another secondary endpoint was the response rate in pediatric patients with first-episode brainstem glioma, estimated at 35.5%, indicating a certain level of efficacy in this challenging disease. All these data support the efficacy of the current strategy against pediatric DIPG.

No deaths were reported during the investigation period for adverse events from the start of the clinical trial to the 3-month observation period. However, during the post-observation period, extending up to 12 months after treatment initiation, 10 deaths were recorded, all of which were associated with disease progression. The frequency of hemorrhage was 9.5%, observed in 2 of the 21 children, which is comparable to the frequency of hemorrhage typically seen in surgery [17]. One patient experienced an intratumoral hemorrhage in the brainstem; however, the drug could still be administered afterward, and the patient recovered. Consequently, 20 of the 21 patients could receive the drug as planned. In addition, one participant experienced multiorgan failure and cerebral infarction after being discharged from the hospital (2 months after treatment) because of a sudden deterioration of his condition, believed to be caused by an infection. Although treatment efficacy was recognized in this trial, no relationship was found between the treatment and the patient's condition deterioration, which began with infectious symptoms, nor with the cerebral infarction, which occurred in a way that preserved the brainstem, the treatment target. Other adverse events, including wound infection, were within the expected range, confirming the safety of this treatment.

A limitation of this study is its single-arm study design. Randomization was considered difficult because of the rarity of DIPG, and radiotherapy is the only standard treatment despite the poor survival outcomes, with nearly all patients succumbing to the disease after treatment. In addition, the diagnosis of DIPG was based solely on diagnostic imaging. Nonetheless, 17 out of 20 patients died, with 10 deaths occurring during the post-observation period and 7 during the follow-up period after 13 months of study treatment, reflecting the grim prognosis of this disease. Considering the efficacy and safety profile, the CED of ACNU was regarded as a treatment of high medical significance for patients with brainstem gliomas, with very high unmet medical needs and limited effective treatment options.

### Author Contributions

**Ryuta Saito:** conceptualization, data curation, formal analysis, investigation, methodology, project administration, writing – original draft, writing – review and editing. **Masayuki Kanamori:** conceptualization, data curation, formal analysis, investigation, writing – review and editing. **Yoshiki Arakawa:** data curation, investigation, writing – review and editing. **Yohei Mineharu:** data curation, investigation, writing – review and editing. **Yasuo Aihara:** data curation, investigation, writing – review and editing. **Kentaro Chiba:** data curation, investigation, writing – review and editing. **Toshihiro Kumabe:** data curation, investigation, writing – review and editing. **Ichiyo Shibahara:** data curation, investigation, writing – review and editing. **Yukihiko Sonoda:** data curation, investigation, writing – review and editing. **Kenichiro Matsuda:** data curation, investigation, writing – review and editing. **Manabu Kinoshita:** formal analysis, methodology, writing – original draft, writing – review and editing. **Aya Sato:** data curation, investigation, writing – review and editing. **Fumiaki Takahashi:** formal analysis, methodology, writing – original draft, writing – review and editing. **Teiji Tominaga:** data curation, funding acquisition, investigation, writing – review and editing.

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### Disclosure

Registry and the registration no. of the trial: This study is registered with jRCT, number jRCT2021190003.

## Ethics Statement

Approval of the research protocol by an institutional review board: The study was first approved by the Ministry of Health, Labour and Welfare Certified Clinical Research Review Board at Tohoku University and then by the ethics committee of Kyoto University, Tokyo Woman's University Hospital, Kitasato University Hospital, and Yamagata University Hospital.

## Consent

Informed consent: Informed consent was obtained from all study participants.

## Conflicts of Interest

R.S. and Y.A. have received clinical research funding from Dai-ichi Sankyo. R.S., Y.A., and Y.S. received honoraria from Dai-ichi Sankyo. Other authors do not have a conflicts of interest.

## Data Availability Statement

The data supporting this study's findings are available from the corresponding author (RS) upon reasonable request.

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## Supporting Information

Additional supporting information can be found online in the Supporting Information section.