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CLINICAL STUDY

Infusion of 5-Azacytidine (5-AZA) into the fourth ventricle or resection cavity in children with recurrent posterior Fossa Ependymoma: a pilot clinical trial

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Abstract

Background DNA methylation inhibitors are logical therapeutic candidates for ependymomas originating in the posterior fossa of the brain. Our objective was to test the safety of infusing 5-Azacytidine (5-AZA), a DNA methylation inhibitor, directly into cerebrospinal fluid (CSF) spaces of the fourth ventricle or tumor resection cavity in children with recurrent ependymoma originating in the posterior fossa.

Materials and methods In patients with recurrent ependymoma whose disease originated in the posterior fossa, a maximal safe subtotal tumor resection was performed. At the conclusion of the tumor resection, a catheter was surgically placed into the fourth ventricle or tumor resection cavity and attached to a ventricular access device. CSF flow from the posterior fossa to the sacrum was confirmed by CINE phase contrast magnetic resonance imaging (MRI) postoperatively. 12 consecutive weekly 10 milligram (mg) infusions of 5-Azacytidine (AZA) were planned. Disease response was monitored with MRI scans and CSF cytology.

Results Six patients were enrolled. One patient was withdrawn prior to planned 5-AZA infusions due to surgical complications after tumor resection. The remaining five patients received 8, 12, 12, 12, and 12 infusions, respectively. There were no serious adverse events or new neurological deficits attributed to 5-AZA infusions. All five patients with ependymoma who received 5-AZA infusions had progressive disease. Two of the five patients, however, were noted to have decrease in the size of at least one intraventricular lesion.

Conclusion 5-AZA can be infused into the fourth ventricle or posterior fossa tumor resection cavity without causing neurological toxicity. Future studies with higher doses and/or increased dosing frequency are warranted.

Keywords Fourth ventricle · Intraventricular chemotherapy · Ependymoma · 5-Azacytidine

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Introduction

Patients with posterior fossa ependymoma have very low survival rates when tumors recur after initial surgical resection and radiation therapy despite attempts at salvage therapy [1–4]. Novel approaches to treatment of recurrent ependymoma are needed to improve survival rates.

One such novel approach is direct drug infusion into the fourth ventricle of the brain or tumor resection cavity. The concept of direct drug infusion for posterior fossa ependymoma is appealing for a number of reasons. First, ependymoma frequently recurs within the fourth ventricle or at the site of disease origin in the cerebellopontine angle, and direct infusions will enable high drug concentrations at the site of recurrence [4]. Additionally, infusion into the fourth ventricle or tumor cavity will also enable drug distribution within the spinal subarachnoid space. This is important because ependymoma often disseminates to the subarachnoid spaces of the brain and spine [4]. Moreover, with this approach, catheter/reservoir placement can be performed concurrent with surgery to remove recurrent tumor from the posterior fossa, thereby sparing patients a separate procedure to place a catheter and reservoir in a separate site such as the lateral ventricle. Prior studies in piglets, non-human primates, and a pilot clinical trial have demonstrated that infusion of chemotherapeutic agents directly into the fourth ventricle of the brain is safe and may optimize local and regional drug concentrations [5–10].

Recent studies have demonstrated the antitumor effect of DNA methylation inhibitors 5-Azacytidine (5-AZA) and 5-AZA-2′-deoxycytidine on cancer cells in leukemia, medulloblastoma, glioblastoma, neuroblastoma, prostate cancer, pancreatic cancer and testicular germ cell tumors [11]. Most relevant to the current trial, Mack et al. defined epigenomic alterations in ependymomas and noted that hyperactivity of DNA methylation was associated with poor-prognosis ependymomas of the posterior fossa [12]. Moreover, a DNA-demethlating agent was cytotoxic in-vitro when assessed in patient-derived primary ependymoma cultures [12]. The authors concluded that drugs targeting DNA methylation should be considered for clinical trials for this currently incurable disease.

Subsequent experiments performed at the National Institutes of Health established the safety of intraventricular infusions of 5-AZA. In four non-human primates, 10 mg of 5-AZA was infused into the lateral ventricle (n = 3) and fourth ventricle (n = 1) without any evidence of clinical or neurological toxicity [13]. Of note, these investigators also investigated intravenous and intranasal administration of 5-AZA. They reported that neither intravenous nor intranasal administration achieved adequate CSF exposure, but that intraventricular delivery resulted in sustained and substantial CSF drug levels [13]. This finding, along with the absence of reported neurological toxicity, supported the rationale for the current clinical trial investigating intraventricular infusions in children with recurrent ependymoma.

Additional preclinical safety data supporting the concept of intraventricular infusion of DNA methylation inhibitors comes from prior experiments testing fazarabine, a stereoisomer of 5-AZA. Heideman et al. studied four adult male rhesus monkeys that received intrathecal (n = 1) or intraventricular (n = 3) infusions of fazarabine (10 mg) [14]. The authors noted no neurological toxicity, and systemic toxicity was limited to one animal that had a mild and transient decrease in peripheral leukocyte count that was not associated with a change in hematocrit. As expected, intraventricular drug levels were dramatically higher (50-fold greater) than those achieved with systemic administration of much higher drug doses (1500–2400 mg).

The current study marks the first trial in humans of 5-AZA administration directly into the fourth ventricle or tumor resection cavity. Our primary objective was to establish the safety of these infusions based upon neurological examinations and post-infusion imaging studies. Our secondary objective was to assess the antitumor activity of 5-AZA infusions into the fourth ventricle in children with recurrent ependymoma.

Materials and methods

This study (clinicaltrials.gov ID NCT02940483) was performed in accordance with the ethical standards as outlined in the 1964 Declaration of Helsinki and its later amendments. The study was initiated at a single center after approval from the Food and Drug Administration (FDA IND Number 132873) and the institutional review board of McGovern Medical School/University of Texas Health Science Center at Houston/ Children's Memorial Hermann Hospital (Protocol HSC-MS-16-0739). All patients and/or their parents or legal guardians signed informed consent. All adverse events were reviewed by a Data Safety Monitoring Board (DSMB). After enrollment of the first patient, the second patient was not allowed to be enrolled until the first patient had completed the first four infusions of 5-AZA without dose-limiting toxicity. Once the first patient safely completed the first four infusions, two additional patients were allowed to be enrolled. After the first three patients completed all infusions, the DSMB reviewed the outcomes of these patients before permitting the remaining three patients in the study to be enrolled.

Eligible patients were between 1 and 80 years old with ependymoma that originated in the posterior fossa and subsequently recurred anywhere in the brain and/or spine. Patients were excluded if they were pregnant, receiving any other chemotherapy, or enrolled in another experimental protocol. An estimated life expectancy of at least 12 weeks and a Karnofsky or Lansky performance score of 50 or greater was required [15].

All patients enrolled in this trial came from outside institutions where they had previously been treated. All patients who met eligibility criteria were offered enrollment and enrolled consecutively. Enrolled patients underwent a posterior fossa craniotomy and maximal safe surgical resection if resectable tumor was within the operative field. A ventricular catheter was then placed under direct vision into the fourth ventricle or surgical resection cavity. The catheter was placed in the fourth ventricle if the tumor was located within the fourth ventricle, and it was placed in the tumor resection cavity if the tumor was located within the posterior fossa but outside of the fourth ventricle. The dura mater was closed in a water-tight fashion around the catheter, and the catheter was attached to a ventricular access device (VAD) that was implanted subcutaneously at the inferior aspect of the incision. Details describing this technique have been reported previously by our group [9].

Postoperatively, patients underwent MRI of the brain with gadolinium to assess both baseline tumor burden and catheter placement within the fourth ventricle. Additionally, CINE phase contrast MRI sequences of the brain and total spine were performed to confirm CSF flow from the fourth ventricular outlets to the cervical, thoracic, and lumbar spine as previously reported by our group [16]. CSF flow was characterized as present or absent by the study neuroradiologist. The purpose of the CINE phase-contrast MRI sequences was to ensure that there was no overt blockage of CSF flow from the site of infusion to the spinal subarachnoid spaces. If CSF flow from the fourth ventricle to the lumbar spine was not definitively present, then a nuclear medicine CSF flow study with Indium-111 diethylenetriaminepenta-acetic acid (In-111 DPTA) was required prior to 5-AZA infusions. Patients additionally underwent lumbar puncture to assess CSF cytology before initiation of chemotherapy.

Intraventricular 5-AZA infusions were initiated at least 7 days after VAD placement. All infusions were performed on an outpatient basis. Neurological examinations were performed immediately before and after each infusion. Each patient had 12 planned consecutive weekly infusions of 5-AZA (10 mg prepared in 1 mL of preservative-free normal saline infused over a minimum of 30 s followed by a 1 mL flush of preservative-free normal saline infused over a minimum of 30 s). Prior to each infusion, 2 mL of CSF was aspirated from the VAD for cytology. Patients did not receive any simultaneous systemic chemotherapy.

MRI scans of the brain and total spine with gadolinium were obtained after the final infusion, and disease response was assessed by comparing theses studies to pre-treatment baseline MRI scans. Lumbar puncture to assess CSF cytology was planned after therapy was completed unless it was judged to be futile for assessing disease response due to obvious tumor progression. Table 1 outlines criteria used for assessing disease response.

Results

Six patients with a median age of 7.5 years (range 4–17 years) were enrolled. All six patients had ependymoma originating in either the fourth ventricle or cerebellopontine angle. All patients had progressive, recurrent disease at the time of enrollment despite at least two prior surgeries (range 2–5) and radiation therapy twice in five patients and once in the 6th patient. Five of six enrolled patients had received prior chemotherapy (various regimens), and the sixth patient had received intraventricular infusion of autologous natural killer cells as part of a clinical trial. Patient data and treatment responses are listed in Table 2.

All patients underwent subtotal surgical resection of recurrent posterior fossa tumor and successful surgical implantation of the catheter and VAD. Pathology analysis confirmed the diagnosis of recurrent ependymoma in all patients. Post-operative MRI scans in all six patients demonstrated accurate catheter placement into the fourth ventricle or surgical resection cavity. In all six patients, CINE phase contrast MRI sequences confirmed CSF flow from the fourth ventricular outlets to the cervical, thoracic, and lumbar spinal subarachnoid spaces. Nuclear medicine CSF flow studies were therefore not required in any patients.

Five patients had no new neurological deficits after surgery. One patient (patient 2) had new postoperative neurological deficits caused by tumor resection. After undergoing subtotal resection of two large, separate posterior fossa lesions and simultaneous catheter placement into the fourth ventricle, she was extubated in the operating room. Several hours later, she became stridorous and required reintubation. She was found to have bilateral vocal cord paralysis and eventually required tracheostomy. Additionally, she had worsened gait, new left ear deafness and medial deviation of her right eye postoperatively. Given these new findings and her worsened Karnofsky score, which were attributed to a challenging tumor surgery in the posterior fossa, she was withdrawn from the trial without ever receiving any infusions of the trial drug.

Table 1 Assessment of disease response

Selection of TARGET and non-target lesions

Target lesions: all measurable lesions were defined as "target" lesions. If multiple measurable lesions were present, up to five were selected as target lesions based upon size and suitability for accurate repeated measurements. Lesions were measured in whichever MRI sequence (i.e. T1, T2, FLAIR, etc.) which provided the most accurate measurement of tumor size

Non-target lesions: included lesions which could not be measured accurately, such as leptomeningeal disease. CSF cytology (e.g. CSF positive or negative for tumor cells) was followed as a non-target lesion

Response criteria for target lesions

Target lesions were measured in two dimensions: the longest diameter and perpendicular to the longest diameter. Response criteria were assessed based on the product of the longest diameter and its longest perpendicular diameter.

Complete response (CR): No evidence of disease

Partial response (PR) : ≥ 50% decrease in the sum of the products of the two perpendicular diameters of all target lesions (up to 5)

Stable disease (SD): Neither sufficient decrease in sum of the products of the two perpendicular diameters of all target lesions to qualify for PR nor sufficient increase in a single target lesion to qualify for PD

Progressive disease (PD): ≥ 25% increase in the product of perpendicular diameters of any target lesion or the appearance of any new lesions

Response criteria for non-target lesions

Complete response (CR): Disappearance of all non-target lesions

Stable disease (SD)/incomplete response (IR): The persistence of one or more nontarget lesions

Progressive disease (PD): The appearance of one or more new lesions and/or unequivocal progression of existing non-target lesions

Overall response assessment

Target lesions	Non-target lesions	New lesions	Overall response
CR	CR	No	CR
CR	IR/SD	No	PR
PR	CR, IR/SD	No	PR
SD	CR, IR/SD	No	SD
PD	Any	Yes or no	PD
Any	PD	Yes or no	PD
Any	Any	Yes	PD

Table 2 Patient data and treatment responses

Pt	Age/sex	Prior treatments	Recurrent disease sites at time of enrollment	Time interval from initial tumor diagnosis to enrollment (years)	Catheter implantation Site	No. of infusions received	Treatment response and time point of assessment
1	6/F	Surgery (n = 4), Radiation therapy (n = 2), Chemo	CPA	5.8	Tumor resection cavity	8	PD (2 months after enrollment)
2	17/F	Surgery (n = 5), Radiation therapy (n = 2), Chemo	4th ventricle, CPA, cerebellar hemisphere, vermis	8.0	4th ventricle	0	N/A
3	4/F	Surgery (n = 2), Radiation therapy (n = 2), Chemo	4th ventricle, CPA, lateral ventricles, cervical spine, thoracic spine, lumbar spine	2.0	4th ventricle	12	PD (3 months after enrollment)
4	9/F	Surgery (n = 3), Radiation therapy (n = 2), Chemo	4th ventricle and lateral ventricle	2.5	4th ventricle	12	PD (3 months after enrollment)
5	4/M	Surgery (n = 2), Radiation therapy (n = 2), Chemo	CPA	2.7	Tumor resection cavity	12	PD (3 months after enrollment)
6	12/M	Surgery (n = 2), Radiation therapy (n = 1), Intraventricular infusion of Autologus natural killer cells	4th ventricle, CPA, sacral	3.9	4th ventricle	12	PD (3 months after enrollment)

Pt Patient, *M* Male, *F* Female, *CPA* cerebello-pontine angle, *Chemo* Chemotherapy, *PD* Progressive disease, *N/A* Not applicable (patient received no infusions)

The five patients who underwent 5-AZA infusions received 8, 12, 12, 12, and 12 cycles respectively, totaling 56 infusions. There were no new neurological deficits after these infusions either immediately or subsequently for the duration of treatment or follow-up (at least 30 days after the final infusion). In all five patients who received 5-AZA infusions, MRI scans performed after the final infusion revealed no radiographic evidence of leukoencephalopathy or any damage to the brainstem, cerebellum, or cerebral cortex. One patient (patient 1) had infection of the implanted VAD which presented after 8 weeks of treatment with purulence associated with the incision. The patient was admitted to the hospital and started on broad-spectrum antibiotics. An MRI of the brain was performed and demonstrated not only infection around the VAD but also massive tumor progression. After discussion with the patient's parents, the decision was made to surgically remove the VAD and not proceed with further infusions. Besides this infection in patient 1, additional adverse events were all grade 1 or grade 2 and were either unrelated or possibly related to treatment (see Table 3).

All five patients who received 5-AZA infusions were noted to have progressive disease on MRI scans performed after the final infusion. Two of the five patients were noted to have decrease in size of at least 1 lesion, with progressive disease elsewhere. Patient 3 was noted to have decrease in the size of tumors in the fourth ventricle and both lateral ventricles with progression of disease elsewhere in the brain (Fig. 1a) and spine (Fig. 1b). Patient 3 was the only patient who had a new lesion noted (new thoracic spine metastasis, as demonstrated in Fig. 1b2). Patient 4 had a decrease in the size of her fourth ventricle tumor but considerable increase in size of her lateral ventricle tumor (Fig. 2).

Four of five patients had negative CSF cytology on lumbar puncture prior to infusions, and one patient had positive CSF cytology. Lumbar puncture was not performed after the final infusion in any of the five patients because it was judged to be futile given the progressive disease noted on

Table 3 Adverse events in patients receiving infusions

Patient	Adverse event	Grade	Relationship to 5-AZA infusions
1	Nasal congestion	1	Unrelated
1	Sneezing	1	Unrelated
1	Cough	1	Unrelated
1	Fever	2	Unrelated
1	Ear Infection	2	Unrelated
1	Reservoir infection	3	Related
3	Unsteady gait	2	Unrelated
3	Pseudomeningocele	2	Unrelated
3	Fever	2	Unrelated
3	Cough	1	Unrelated
3	Sneezing	1	Unrelated
3	Urinary incontinence	1	Unrelated
3	Diarrhea	1	Unrelated
4	Pseudomeningocele	1	Unrelated
4	Headache	1	Unrelated
4	Nausea	1	Possibly related
4	Vomiting	1	Possibly related
4	Constipated	1	Unrelated
4	Stomach cramps	1	Possibly related
4	Vomiting	1	Possibly related
5	Nausea	1	Possibly related
5	Vomiting	1	Possibly related
5	Fever	1	Unrelated
5	Unsteady gait	1	Unrelated
6	Headache	1	Possibly related
6	Fever	1	Unrelated
6	Sneezing	1	Unrelated

post-treatment MRI scans. CSF samples obtained from the fourth ventricle were negative for cytology at all time points in all patients except for patient 6, who had one CSF result suspicious for malignant cells at the time of the 5th infusion.

Discussion

Drug delivery directly into the fourth ventricle of the brain has been shown to be safe in piglets, non-human primates, and in a previously published pilot clinical trial from our center [5–10]. These and other previously published reports have highlighted potential advantages of fourth ventricular chemotherapy administration over systemic chemotherapy, infusions via repeated lumbar puncture, and infusions into the lateral ventricle of the brain for patients with recurrent malignant brain tumors originating in the posterior fossa.

The primary objective of this study was to establish the safety of direct administration of 5-AZA into the fourth ventricle of the brain or resection cavity in patients with recurrent posterior fossa ependymoma. This objective was achieved based upon both radiographic and clinical criteria. Radiographically, no patient demonstrated encephalopathy or other evidence of brain injury. A total of 56 infusions of 5-AZA were administered in five patients without any new neurological deficits or other serious systemic toxicity. While one patient had an infection necessitating removal of the VAD, infections are an unfortunate but known complication of placing VAD's. An additional patient, unfortunately, was withdrawn from the study prior to receiving any infusions due to surgical complications associated with an especially challenging posterior fossa tumor removal.

The secondary objective of this study was to assess the antitumor activity of 5-AZA infusions into the fourth ventricle based upon imaging studies and lumbar cerebrospinal fluid (CSF) cytology. Unfortunately, all five patients who received infusions had progressive disease despite treatment. Two of five patients, however, were noted to have a decrease in the measured size of at least one lesion, with progression of disease elsewhere. In both of these patients, tumor burden in the fourth ventricle was decreased after infusions, and one of these patients had a decrease in the size of tumors in both lateral ventricles. In the context of overall progressive disease in all patients enrolled, and the small number of patients included in this study, definitive conclusions cannot be drawn regarding the potential efficacy of this treatment modality for recurrent posterior fossa ependymoma. Our group's prior study of methotrexate infusions into the fourth ventricle showed a decrease in the size of recurrent medulloblastoma tumors in fourth ventricle, lateral ventricle, and spinal subarachnoid spaces in several patients [10]. We are hopeful that similar responses can be obtained for recurrent posterior fossa ependymoma with the right agent infused at the appropriate dose. We suspect that lack of proper targeting is not the most likely reason that the anti-tumor response was not more robust in the current study. While this question could potentially be answered by co-infusion of gadolinium during 5-AZA infusions with immediate subsequent imaging, this was not performed during this trial due to challenging logistics associated with obtaining sedated MRI scans in children.

This pilot trial offers further proof of principle that fourth ventricular drug delivery may be a safe potential treatment option for recurrent ependymoma and other malignant tumors originating in the posterior fossa of the brain. The challenge, of course, is determining the best agent or combination of agents to infuse as well as the

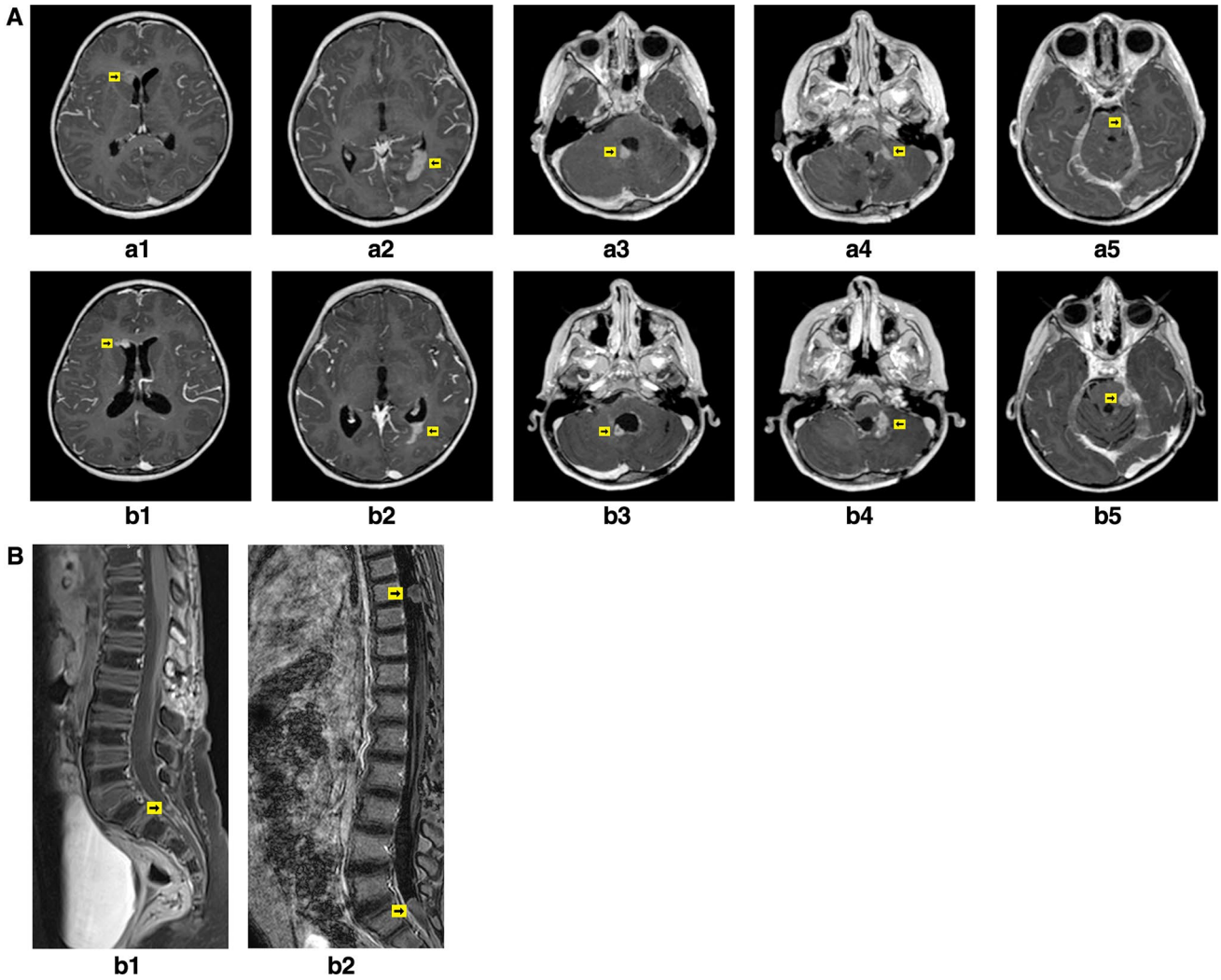

Fig. 1 **a** Pre-treatment and post-treatment brain MRI images (axial T1 with gadolinium) from patient 3. A decrease in 3hree tumors in the brain is noted when comparing pre-treatment images of the right frontal horn of the lateral ventricle (1a1), left atrium of the lateral ventricle (1a2), and fourth ventricle (1a3) with post-treatment images (1b1, 1b2, 1b3). Two separate tumors in the brain (left foramen of Luschka and left cerebellopontine angle) have increased in size (1a4, 1a5 vs. 1b4, 1b5). Tumors are marked by arrows. **b** Pre-treatment (1b1) and post-treatment (1b2) spine MRI images (sagittal T1 with gadolinium) from patient 3. A new tumor is noted in the thoracic spine and there has been considerable progression of a previously small lesion in the distal thecal sac. Tumors are marked by arrows

optimal dose and dosing frequency. In the current pilot trial, all patients were noted to have progressive disease despite infusions of 5-AZA. However, given the modest decrease in size of at least one tumor in two of five patients despite a low infusion dose, as well as the safety of these infusions, dose escalation of 5-AZA is appealing to test in a subsequent clinical trial. The chosen dose for this trial was the same as the dose utilized in prior non-human primate safety studies of intraventricular 5-AZA infusion [13]. A higher dose would likely be tolerated in pediatric patients, the majority of whom have considerably higher body weights than the non-human primates studied. The currently reported trial will form the basis for future trials to explore the safety and efficacy of higher doses of 5-AZA, as well as infusion of additional agents into the fourth ventricle, in an effort to expand treatment options and improve outcomes for patients with ependymoma and other malignant posterior fossa tumors.

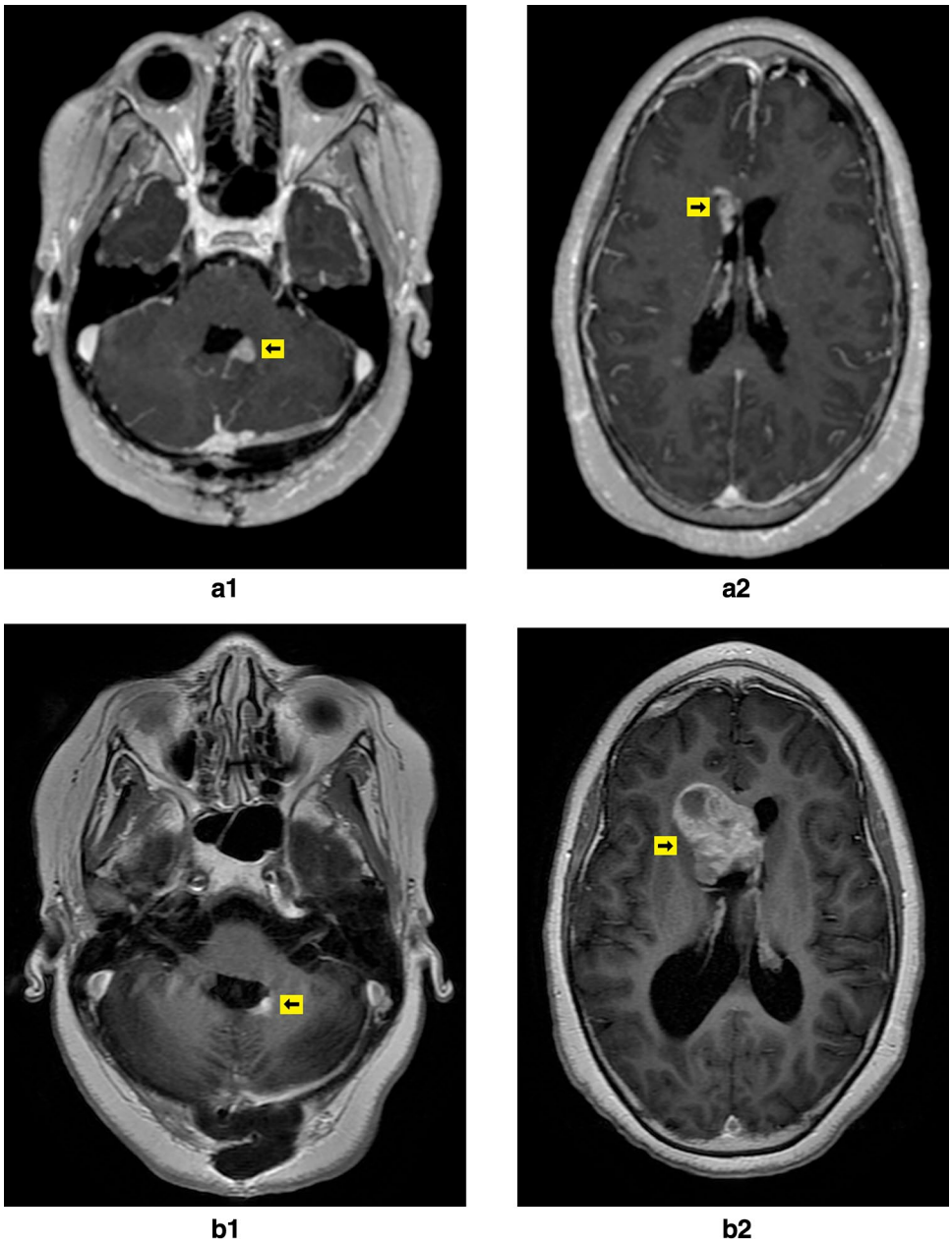

Fig. 2 MRI Scans (axial T1 with gadolinium) demonstrating treatment response in patient 4 in fourth ventricle with disease progression in lateral ventricle. Fourth ventricle tumor prior to treatment is demonstrated in a1, and post-treatment decrease is demonstrated in b1. Lateral ventricle lesion before treatment is demonstrated in a2, and post-treatment growth is demonstrated in b2. Tumors are marked by arrows

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Compliance with ethical standards

Conflict of interest All authors (Bangning Yu, M.D., Ph.D., Rajan Patel, M.D., Emilie Miesner, PA-C, Jennifer Sabin, PA-C, Sarah Smith, PA-C, Stephen Fletcher, D.O., Manish N. Shah, M.D., and Michael D. Taylor, M.D., Ph.D.) declare that they have no conflicts of interest. Author David Sandberg, M.D. received grant support from Ian's Friends Foundation to support this study as noted above. Dr. Sandberg also received a speaker honorarium from Aesculap in 2017 for a talk at the International Society for Pediatric Neurosurgery on a topic unrelated to this study. Author John Hagan, Ph.D., has received financial support from GlaxoSmithKline for research that is unrelated to this study. Author Rachael Sirianni, Ph.D., currently receives grant support from Ian's Friends Foundation and the National Institutes of Health, and she has received grant funding in the past from the Rick Oehme Foundation, the Ben and Catherine Ivy Foundation, the ALS Association, the Barrow Neurological Foundation, the National Science Foundation, and the Department of Defense. Author Rachael Sirianni, Ph.D. owns stock in the company NP Therapeutics, Inc. and works as a consultant for the Ian's Friends Foundation. None of these relationships for Dr. Sirianni have any connection to the current study.

Ethical Approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national search committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants in the study.

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