A phase II clinical trial of sonodynamic therapy combined with radiotherapy for brainstem gliomas

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Case Report

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Abstract

Purpose

Brainstem gliomas (BSGs) are a class of clinically refractory malignant tumors for which there is no uniform and effective treatment protocol. Ultrasound and radiation can activate hematoporphyrin and produce sonodynamic and radiodynamic effects to kill cancer cells. Therefore, we conducted the first phase clinical trial of sonodynamic therapy (SDT) combined with radiotherapy (RT) for the treatment of BSGs to verify its safety and efficacy.

Methods

We conducted a study of SDT combined with RT in 11 patients with BSGs who received SDT and RT after hematoporphyrin administration. Magnetic resonance imaging (MRI) was performed during this period to assess the tumor, and adverse events were recorded.

Results

All adverse events recorded were grade 1–2; no grade 3 or more serious adverse events were observed. Treatment was well tolerated, and no dose-limiting toxicities were observed. There were no treatment-related deaths during the course of treatment. 2 of 7 (28.6%) patients with high-grade gliomas achieved partial response (PR), and the tumors were still shrinking as of the last follow-up date. 1 of 4 (25%) patients with low-grade gliomas achieved a minor response (MR), and 3 (75%) maintained stable disease (SD). The median progression-free survival (PFS) for patients was 9.2 (95% confidence interval [CI] 6.2–12.2) months, and the median overall survival (OS) was 10.5 (95% CI 5.1–15.9) months.

Conclusion

SDT combined with RT has a favorable safety and feasibility and shows a preliminary high therapeutic potential.

Introduction

Brainstem gliomas (BSGs) are a group of gliomas originating in the midbrain, pons, and medulla oblongata, which are commonly found in children, with an incidence of approximately 10–20% of intracranial tumors in children and 1.5–2.5% of intracranial tumors in adults[1]. Diffuse intrinsic pontine glioma (DIPG) is the most common brainstem glioma in pediatric patients, accounting for 80% of brainstem tumors[2]. DIPG is a highly aggressive malignant tumor with a poor prognosis and is the leading cause of death in children with brain tumors[3]. The fact that the brainstem is riddled with life-regulating centers (e.g., respiratory centers, etc.) and many important neuronal nuclei, and the infiltrative growth of DIPG makes it difficult to remove completely by surgery[4]. Radiotherapy (RT) combined with temozolomide (TMZ) is a common treatment method nowadays. Although RT can improve the treatment status of patients to a certain extent, tumors usually recur and develop rapidly after 6–9 months of treatment[5]. In addition, the existence of the blood-brain barrier (BBB) results in insufficient drug delivery to achieve an effective therapeutic concentration at the tumor site, leading to the unsatisfactory efficacy of chemotherapy[6,7]. As a result, the prognosis of patients with BSGs is extremely poor, with a median progression-free survival (PFS) of less than 6 months after clinical diagnosis, a median overall survival (OS) of 10 months, and a 2-year survival rate of less than 10%.[8–9]. In light of this, several organizations, such as the American Society of Clinical Oncology (ASCO), have recommended that clinical trials for BSGs should be conducted actively, in an attempt to find a safe, efficient and feasible therapy to maximize the survival time and improve the quality of life of such patients.

Sonodynamic therapy (SDT) is a non-invasive tumor treatment method derived from photodynamic therapy (PDT) using the synergistic action of ultrasound and sensitizer. Compared with PDT, SDT has the advantages of strong penetration ability up to 10 cm and low-frequency ultrasound without damage to normal tissues[10]. Its main principle is to use low-frequency, low-intensity ultrasound to irradiate the tumor site enriched with sensitizer, producing the cavitation effect and activating the sensitizer, which generates reactive oxygen species (ROS) and stimulates the key molecules, such as Bcl-2, Caspase-9, Caspase-3, Bax, etc., to trigger the apoptosis of the cells[11]. It has also been shown that SDT can interfere with mitochondrial structure and function[12,13] to trigger apoptosis through the mitochondrial apoptotic pathway[14]. Additionally, SDT can inhibit angiogenesis and so on[15]. For BSGs, ultrasound can reduce the aggregation of tight junction molecules such as claudin-1, claudin-5, etc., around the endothelial cells to reversibly open the BBB[16,17], which effectively solves the difficulty of chemotherapeutic drugs passing through the BBB. Therefore, SDT has a broad prospect in the treatment of brain tumors.

Currently, hematoporphyrin is the most commonly used and the only sensitizer approved by the Chinese Food and Drug Administration for the clinical treatment of a wide range of solid malignant tumors[18]. Hematoporphyrin can selectively accumulate in tumors and was initially applied as a photosensitizer in PDT[19]. In 1989, Yumita et al. found that hematoporphyrin could be activated to produce antitumor effects and kill tumor cells under ultrasound irradiation, and thus applied it to SDT[20]. Subsequently, it was found that hematoporphyrin could not only be activated by laser and ultrasound, it could also be activated by rays with a radiodynamic effect for sensitizing RT. Tumor cells treated with hematoporphyrin showed a significant increase in the amount of DNA damage, as well as greater inhibition of tumor growth and larger areas of necrosis after X-ray irradiation[21]. Wang et al. used X-rays as a source of energy to activate hematoporphyrin to kill tumor cells and proposed that X-ray-induced photodynamic therapy (X-PDT) might be used as a new method of treating cancer[22]. This idea was validated by several subsequent experiments that demonstrated the radiodynamic effect of hematoporphyrin as well[23–25]. Therefore, hematoporphyrin may also be used as a sensitizer to enhance the efficacy of RT.
After preliminary exploration, we rigorously initiated this first phase single-arm clinical trial of SDT combined with RT for the treatment of patients with recurrent or refractory BSGs in the world. This open, prospective preliminary study aims to evaluate the tolerability, safety and efficacy of SDT combined with RT to explore new directions for the future treatment of BSGs in the clinic.

Patients and Methods

1. Trial Information

This trial was a prospective, single-center, single-arm phase clinical trial. The trial protocol was approved by the Ethics Committee of the First Affiliated Hospital of Zhengzhou University (Project No.: TA2022-145, Ethics Approval No.: L/G2022-K001-003) before the study was started and ensured consistency with the requirements of the Declaration of Helsinki. All patients signed an informed consent for SDT combined with RT before screening and enrollment.

2. Medicines and equipment

We chose hematoporphyrin injection (trade name: Xipofen) produced by Chongqing Milelonge Biopharmaceutical Co., Ltd. as the sensitizer in this clinical trial. Based on previous clinical trials of hematoporphyrin used in PDT and the instructions for this drug, we chose to administer Xipofen at a dose of 5 mg/kg by intravenous infusion or 3 mg/kg by percutaneous arterial cannula infusion (interventional procedures). The dose of the drug was lowered for the interventional procedure because the scope of administration was limited. The equipment used in this clinical trial was a transcranial ultrasound therapy machine from Shijiazhuang Dukang Medical Instrument Co. The frequency of the machine was 800 KHz ± 10%, and the maximum output power at time was: 0 W/cm², 0.3 W/cm², 0.6 W/cm², 0.9 W/cm² and 1.2 W/cm²; the maximum sound intensity at time was: 0, 1.2 W/cm², 2.4 W/cm², 3.6 W/cm² and 4.8 W/cm². Previous experiments have shown that sound intensity ≥ 0.5 W/cm² and sound frequency around 800 KHz-1 MHz can stimulate the sonodynamic effect of hematoporphyrin, and ultrasound below 5.8 W/cm² is safe for brain tissue. Based on this, we set the SDT treatment parameters: sound intensity: 1-1.25 W/cm²; sound frequency: 840 KHz; treatment time per localization point: 15 min.

3. Study design

Enrolled patients were required to perform magnetic resonance imaging (MRI) to evaluate the baseline of the tumor within 1 week prior to treatment, and the tumor treatment area was localized based on the MRI results. The projected area of the tumor in the direction of the closest distance to the skull was used as the ultrasound probe placement area and was shaved to outline the target area for SDT. The skin test with 5 mg of Xipofen was performed before treatment was carried out, if the skin test results showed no allergy, the drug was administered (5 mg/kg intravenous infusion or 3 mg/kg interventional procedure), and SDT was started after 40-48 hours of treatment with light avoidance. Parameters of SDT: sound intensity: 1 ~ 1.25 W/cm², sound frequency: 840 KHz. The ultrasound probe was placed on the outlined localization points after applying the appropriate coupling agent, and the treatment time for each localization point was 15 min twice a day, with an interval of 12 hours between each treatment, and consecutively treated for 5 days (it could be adjusted according to the actual situation of the subjects). The patient's condition was assessed after the completion of SDT, and RT was started one week later if the condition permitted. The total dose and frequency of RT were established according to the patient's condition. Since the half-life of hematoporphyrin in the body is about 9.28 days, and it takes at least 2 months to be completely cleared by the body, we set the treatment cycle of SDT + RT for about one month. During the treatment period and one month after the treatment, patients should pay attention to light protection. Besides, according to the patient's specific situation, we choose the appropriate treatment plan, such as combined chemotherapy (TMZ) or targeted therapy (bevacizumab), and so on. When TMZ is synchronized with RT, the dose is 75 mg/m²/day. For adjuvant chemotherapy with TMZ, the dose is 150 mg/m²/day orally for 5 days with 23 days of withdrawal, 28 days per cycle. Targeted therapy with bevacizumab at a dose of 7.5–10 mg/kg every 3 weeks for 4–6 courses. Cranial MRI was performed every 4 weeks during the treatment period to evaluate the tumor. The study was continued until one of the following criteria was met: (1) Confirmation of disease progression or patient death. (2) Intolerance to treatment. (3) Withdrawal of consent by the patient. (4) Change in the patient's condition that puts the patient at risk for further treatment.

4. Study assessments

The primary assessment endpoint of this study was to evaluate the safety and tolerability of SDT combined with RT in the treatment of patients with BSGs. In view of the extensive use of hematoporphyrin in PDT in the previous period, in order to avoid unnecessary waste of resources, we no longer set up excessive drug dose gradients to verify the dose-limiting toxicity of hematoporphyrin but instead focused on the adverse events related to its sonodynamic and radiodynamic effects. The safety assessment included the type, incidence, and severity of adverse events that occurred during treatment. Adverse events were faithfully documented on the basis of patient symptoms, physical signs, clinical tests, and examinations. Adverse events were analyzed according to the National Cancer Institute's Common Terminology Criteria for Adverse Events (CTCAE) version 5.0. The correlation between adverse events and SDT combined with RT will be assessed by the investigator according to the attribution assessment criteria specified in the protocol.

Secondary endpoints included the best overall response and PFS in patients with BSGs. Tumor response was assessed using the Response Assessment in Neuro-Oncology (RANO) criteria, including complete response (CR), partial response (PR), stable disease (SD), and progressive disease (PD). Objective response of high-grade gliomas (WHO Grade , ) includes CR and PR. It was determined by the investigators according to the RANO criteria or RECIST version 1.1. Because responses in low-grade gliomas (WHO Grade , ) are usually mild, the RANO working group considered that a 25%-50% reduction in tumor size from baseline had clinical significance and recommended that minor response (MR) be introduced as a secondary measure of treatment efficacy, defined as a 25%-50% reduction in the sum of the products of the diameters of the tumor in the T2/FLAIR sequence. Thus, objective response in low-grade gliomas includes CR, PR and MR. PFS was defined as the time from baseline to the first disease progression or death due to any cause, whichever occurred first.
5. Statistical analysis

Patient information was collected, including age, gender, treatment before enrollment, pathology type and grade, KPS score and treatment information of SDT combined with RT. Adverse events were registered according to CTCAE 5.0, descriptively analyzed and tabulated by type and grade. If a patient did not experience an endpoint event or was lost to follow-up at the end of the study, the patient's data should be recorded as censored, and the time of censoring should be the time of the last follow-up visit. PFS and OS were assessed using the Kaplan-Meier method.

Result

1. Patients

A total of 20 patients were screened. Eventually, 11 patients met all the eligibility criteria and were subsequently enrolled in the study. The detailed inclusion and exclusion criteria are listed in supplementary information. The 11 patients included 7 males and 4 females, with a median age of 27 years (range 3–59 years) and a median KPS score of 70 (range 70–90). 8 patients had newly diagnosed BSGs, and 3 patients had BSGs that had recurred after receiving systemic treatment. The 11 patients with BSGs were all wild-type isocitrate dehydrogenase (IDH), the specific pathologic types are shown in Table 1. 7 patients were high-grade gliomas and 4 patients were low-grade gliomas. Prior to receiving SDT combined with RT, 9 patients had received surgery, four had received at least 1 cycle of RT, five had received at least 1 systemic therapy such as chemotherapy, targeted therapy, or immunotherapy, and one (patient #11) had not received any treatment. Finally, 9 patients received 1 cycle of SDT + RT, and two received 2 cycles. Regarding the administration of hematoporphyrin, the majority of patients (8) received intravenous infusions, and only 3 patients received interventional procedures. Depending on the size of the patient’s tumor and individual condition, the total dose of RT was 35–60 Gy and the frequency was 10–25 sessions.

2. Safety

11 patients were evaluated for treatment-related adverse events after 1–2 cycles of SDT + RT. During the treatment period and one month after the completion of treatment, we performed clinical observation and follow-up of the patients’ adverse events, which are recorded in Table 2. The adverse events that occurred can be broadly categorized into three groups: (1) chemotherapy-related myelosuppressive effects, gastrointestinal reactions, and liver injury; (2) side effects caused by the therapeutic effects of SDT and RT; and (3) adverse events caused by other random events. Of these adverse events recorded, all were grade 1–2 and normalized with simple or untreated treatment, and no grade 3 or higher adverse events were observed. 2 patients had slight decreases in platelet counts, 3 patients had slight decreases in white blood cell and neutrophil counts, and 4 patients experienced elevated transaminases (with overlap in some patients), all of whom had received chemotherapy with TMZ, and thus they were considered to be side effects of TMZ. These indexes returned to normal after symptomatic treatment with platelet elevation, leukocyte elevation, or hepatoprotection. 2 patients experienced headaches, and 3 patients experienced dizziness with nausea and vomiting. These symptoms were also frequently seen in clinical RT alone and were attributed to local cerebral edema and increased intracranial pressure caused by the killing effect of SDT and RT on tumor cells. These symptoms were relieved or disappeared after treatment with mannitol dehydration. One patient experienced a seizure, which, in combination with the fact that the patient also had a history of seizures prior to treatment, was considered to be a symptom caused by the tumor itself, and the seizure was controlled after treatment with sodium valproate. One febrile patient was recorded with a maximum temperature of 38.6°C, which was attributed to pneumonia after examination, and after control of the infection, the temperature dropped to normal. Treatment with SDT combined with RT was continued after determining that there were no contraindications to treatment. Other adverse events, such as hypokalemia and constipation, were not considered to be related to the treatment. As all patients underwent skin tests before the treatment, no allergies were observed, as we feared. In addition, no dose-related adverse events were observed with either intravenous or interventional infusions. Therefore, SDT combined with RT has a good safety to be applied to clinical treatment.

3. Efficacy

During the course of treatment, 2 of 7 (28.6%) patients with high-grade gliomas achieved PR, which was sustained for 3.9 and 13.9 months, respectively, and the tumors were still shrinking at the cut-off date for follow-up (Fig. 1). 1 of 4 (25%) patients with low-grade gliomas achieved MR, and 3 (75%) maintained SD (Fig. 2), and 3 patients are currently alive except one who died of sepsis unrelated to disease. Overall, the median PFS for all patients was 9.2 (95% CI 6.2–12.2) months and the median OS was 10.5 (95% CI 5.1–15.9) months. A total of six patients died during this period, five from disease progression, including three recurrent patients, and one from sepsis. In the eight newly diagnosed patients, the median PFS was 9.3 (95% CI 9.0–9.6) months and the median OS was 11.7 (95% CI 8.2–15.2) months, and with more than half of the patients currently alive, the actual values must have been longer than those observed (Fig. 3). Also, the late enrollment of some patients had an impact on the statistics of the data. Of course, due to the small number of patients included in this clinical trial, strong evidence that SDT combined with RT is effective in prolonging patients’ PFS cannot be concluded yet.

In terms of clinical symptoms, patient 1 had bucking when drinking or eating, emotional instability, and an inability to stand before enrollment. After one cycle of SDT + RT, his swallowing function was better than before, his mood was more stable than before, and he could stand up for 3 minutes with appropriate support. Patient 2 had grade 5 muscle strength of the left upper and lower limbs, impaired eye movement of the left eye, and an inability to close the left eye before enrollment. After one cycle of treatment, the muscle strength of the limbs was restored to grade 5, and the eye movement was normalized. However, due to personal reasons, patient 2 withdrew from the study group after one cycle of treatment, and the patient’s disease progressed after 3 months and died after 4 months. Some patients showed varying degrees of clinical symptomatic relief, although there was no significant reduction in tumor size on imaging.

Discussion

BSGs have always been a challenge for clinical treatment. Currently, there is no standard treatment for patients with BSGs, and there has been no significant improvement in the survival time and quality of life of patients. The emergence of SDT has provided new hope for the treatment of BSGs. Many early cellular
or animal experiments have already explored the treatment of gliomas with SDT in some depth. Lv et al. successfully inhibited the growth of in situ gliomas in mice in their SDT experiments with a good long-term safety profile[26]. Qu et al. significantly prolonged the survival time of tumor-bearing mice by using a novel nanosensitizer in combination with ultrasound and showed good therapeutic efficacy[37]. In addition, the low attenuation coefficient of ultrasound allows it to penetrate the skull to focus on the tumor site deep in the biological tissues, achieving safety and non-invasiveness[28]. Focused ultrasound (FUS) can open the BBB reversibly and non-destructively, minimizing damage to surrounding normal brain tissue[39]. These properties provide unique advantages for the clinical application of SDT. Currently, there are also some experiments on the radiodynamic effect of hematoporphyrin for the treatment of gliomas. It has been shown that the metabolic activity of human glioblastoma cells treated with Photofrin (a second-generation photosensitizer of the hematoporphyrin derivative) was severely inhibited after irradiation with rays (> 90% compared to the control group)[40]. In addition, the conclusion of hematoporphyrin sensitizing RT for the treatment of malignant tumors has been demonstrated in many trials[41]. An experiment combining chemotherapy, radiodynamic, and photodynamic therapy even achieved complete tumor elimination[42]

This clinical trial is the first study in the world to validate the sonodynamic and radiodynamic effects of hematoporphyrin by combining SDT and RT for human BSGs. Our phase trial demonstrated that SDT combined with RT is sufficiently safe for the treatment of BSGs. Moreover, the dose of hematoporphyrin at 5 mg/kg was well tolerated by the patients, with no dose-limiting toxicity and occasional adverse effects within acceptable limits. The efficacy of the therapy was also reflected to some extent by the relief of the patients' clinical symptoms and the reduction in the extent of their tumors during the course of treatment. However, our sample size was too small to conclude that SDT combined with RT was effective in improving patient survival, and it is still necessary for further phase and clinical trials to prove it. Nevertheless, the present study has provided proof of safety and a reliable basis to support the next trial.

Overall, the lower the grade of the tumor, the less malignant it is and the relative better the prognosis it has. However, during the course of treatment, we observed that only 1 of the patients with low-grade BSGs reached MR, although 3 patients (75%) are currently alive (1 patient died of sepsis unrelated to the BSGs). The therapeutic effect does not seem to be as significant as we expected. We speculate that the reason for this phenomenon may be related to the uptake of hematoporphyrin by tumor cells. It has been found that the higher the malignancy of the brain tumor, the higher the uptake of hematoporphyrin, with the highest levels in glioblastomas (tumor to brain tissue uptake ratio of 30:1), followed by high-grade astrocytomas (12:1) and low-grade astrocytomas (8:1) [43]. The uptake of sensitizers is directly correlated with the sonodynamic and radiodynamic effects, and therefore SDT combined with RT would be more effective in high-grade gliomas than in low-grade gliomas.

Brain tissue is more fragile than tissues in other parts of the body, and the requirements for the dose of RT are also more stringent. For the brainstem, which is an important structure full of life-regulating centers, the dose of RT should be more carefully controlled during treatment. Studies have shown that the maximum single irradiation dose to the brainstem should be kept below 10–12 Gy[44]. Once this dose is exceeded, the incidence of complications in the surrounding normal tissues will increase rapidly[45]. Therefore, the treatment of BSGs has always been a dilemma for conventional RT, with a high dose causing damage to normal tissues but a lower dose being much less effective in controlling the tumor. The emergence of hematoporphyrin as a radiosensitizer has brought new hope for RT to break through this shackles. A study of hematoporphyrin sensitizing RT for nasopharyngeal carcinoma showed that at the end of the treatment, the rate of total elimination of nasopharyngeal primary foci in the experimental group (hematoporphyrin + 50 Gy RT) was 100%, which was much higher than that of the control group (60–70 Gy RT alone)[46], and in the later 20 years of follow-up, it was found that the incidence of dry mouth, mouth opening limitation, cervical fibrosis, and radiation-induced cerebral necrosis were much lower than that of the patients treated with high doses of RT[47]. Therefore, the hematoporphyrin-mediated radiosensitization effect for the treatment of BSGs can not only solve the difficult problem of dose limitation of RT in the brain but also provide a certain degree of guarantee for the improvement of patients' quality of life in the future.

Although SDT combined with RT has unique advantages in the treatment of BSGs, it also has certain shortcomings. Hematoporphyrin, as the sensitizer in this experiment, not only can be activated by ultrasound and radiation but also has photosensitivity, which leads to the need for patients to be strictly protected from light for a period of time after treatment until the hematoporphyrin is metabolized by the body to the point where it does not cause phototoxicity. However, hematoporphyrins are metabolized slowly in the body[32], which causes inconvenience to patients. Therefore, there is still a need to develop a highly selective and fast-metabolizing sensitizer to improve the treatment. In the course of treatment, the sequence of starting SDT and RT after hematoporphyrin administration is also a debatable issue. It has been shown that the efficacy of rays + hematoporphyrin seems to be a little more obvious than ultrasound + hematoporphyrin[25], but the rays themselves have a killing effect on the tumor cells, so it is difficult to compare which is more effective between the sonodynamic effect and the radiodynamic effect. How to correctly arrange the timing of the two therapies in order to achieve the best therapeutic effect still needs to be further explored. In addition, considering the metabolism of hematoporphyrin in the body, the need for additional doses of hematoporphyrin during the long treatment period needs to be verified by more experimental data.

In conclusion, although there are shortcomings in SDT, its unique advantages of safety, non-invasiveness, and high efficiency in the treatment of BSGs cannot be ignored. SDT has great potential in the treatment of BSGs and is worthy of further study in phase and clinical trials.
Table 1
General Characteristics of patients

<table>
<thead>
<tr>
<th>Patients</th>
<th>Age</th>
<th>Gender</th>
<th>Diagnosis</th>
<th>WHO Grade</th>
<th>IDH</th>
<th>KPS at Baseline</th>
<th>Type</th>
<th>Prior Treatments</th>
<th>SDT + Radiotherapy Number</th>
<th>Method of Administration</th>
<th>Ra Dc</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5</td>
<td>Male</td>
<td>DIPG, H3 K27-altered</td>
<td>High-grade</td>
<td>Wild</td>
<td>70</td>
<td>Newly diagnosed</td>
<td>TMZ Bev</td>
<td>1</td>
<td>Intravenous infusion</td>
<td>45</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>Female</td>
<td>DIPG, H3 K27-altered</td>
<td>High-grade</td>
<td>Wild</td>
<td>70</td>
<td>Newly diagnosed</td>
<td>Surgery</td>
<td>1</td>
<td>Intravenous infusion</td>
<td>50</td>
</tr>
<tr>
<td>3</td>
<td>34</td>
<td>Male</td>
<td>Astrocytoma</td>
<td>Wild</td>
<td>Mutant</td>
<td>70</td>
<td>Recurrent</td>
<td>Surgery RT TMZ Bev</td>
<td>1</td>
<td>Intravenous infusion</td>
<td>35</td>
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<tr>
<td>4</td>
<td>48</td>
<td>Female</td>
<td>Diffuse low-grade glioma, NOS</td>
<td>Wild</td>
<td>Wild</td>
<td>80</td>
<td>Newly diagnosed</td>
<td>Surgery</td>
<td>1</td>
<td>Intervention(left vertebral artery)</td>
<td>50</td>
</tr>
<tr>
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<td>Wild</td>
<td>90</td>
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<td>Surgery TMZ</td>
<td>1</td>
<td>Intervention(right vertebral artery)</td>
<td>50</td>
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<td>Ganglioglioma</td>
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<td>90</td>
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<td>Surgery</td>
<td>1</td>
<td>Intravenous infusion</td>
<td>50</td>
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<tr>
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<td>27</td>
<td>Male</td>
<td>DIPG, H3 K27-altered</td>
<td>Wild</td>
<td>Wild</td>
<td>70</td>
<td>Newly diagnosed</td>
<td>Surgery</td>
<td>2</td>
<td>Intervention(left vertebral artery)</td>
<td>50</td>
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<td>8</td>
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<td>Male</td>
<td>Glioblastoma</td>
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<td>Wild</td>
<td>80</td>
<td>Newly diagnosed</td>
<td>Surgery</td>
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<td>Intravenous infusion</td>
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<td>Wild</td>
<td>70</td>
<td>Newly diagnosed</td>
<td>NA</td>
<td>1</td>
<td>Intravenous infusion</td>
<td>45</td>
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Table 2
Summary of overall adverse events (AEs)

<table>
<thead>
<tr>
<th>AEs</th>
<th>All AEs</th>
<th>Grade 1</th>
<th>Grade 2</th>
<th>Grades 3–4</th>
</tr>
</thead>
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<tr>
<td>Anemia</td>
<td>2(18.2%)</td>
<td>0(0.0%)</td>
<td>0(0.0%)</td>
<td></td>
</tr>
<tr>
<td>White blood cell count decreased</td>
<td>0(0.0%)</td>
<td>3(27.3%)</td>
<td>0(0.0%)</td>
<td></td>
</tr>
<tr>
<td>Neutrophil count decreased</td>
<td>0(0.0%)</td>
<td>3(27.3%)</td>
<td>0(0.0%)</td>
<td></td>
</tr>
<tr>
<td>Platelet count decreased</td>
<td>1(9.1%)</td>
<td>1(9.1%)</td>
<td>0(0.0%)</td>
<td></td>
</tr>
<tr>
<td>Alanine aminotransferase increased</td>
<td>2(18.2%)</td>
<td>2(18.2%)</td>
<td>0(0.0%)</td>
<td></td>
</tr>
<tr>
<td>Aspartate aminotransferase increased</td>
<td>1(9.1%)</td>
<td>2(18.2%)</td>
<td>0(0.0%)</td>
<td></td>
</tr>
<tr>
<td>Hypokalemia</td>
<td>1(9.1%)</td>
<td>0(0.0%)</td>
<td>0(0.0%)</td>
<td></td>
</tr>
<tr>
<td>Nausea</td>
<td>1(9.1%)</td>
<td>0(0.0%)</td>
<td>0(0.0%)</td>
<td></td>
</tr>
<tr>
<td>Vomiting</td>
<td>0(0.0%)</td>
<td>2(18.2%)</td>
<td>0(0.0%)</td>
<td></td>
</tr>
<tr>
<td>Constipation</td>
<td>2(18.2%)</td>
<td>0(0.0%)</td>
<td>0(0.0%)</td>
<td></td>
</tr>
<tr>
<td>Dizziness</td>
<td>1(9.1%)</td>
<td>2(18.2%)</td>
<td>0(0.0%)</td>
<td></td>
</tr>
<tr>
<td>Headache</td>
<td>0(0.0%)</td>
<td>2(18.2%)</td>
<td>0(0.0%)</td>
<td></td>
</tr>
<tr>
<td>Epilepsy</td>
<td>0(0.0%)</td>
<td>1(9.1%)</td>
<td>0(0.0%)</td>
<td></td>
</tr>
<tr>
<td>Fever</td>
<td>0(0.0%)</td>
<td>1(9.1%)</td>
<td>0(0.0%)</td>
<td></td>
</tr>
</tbody>
</table>

Declarations

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Author Contributions: All authors contributed to the article and approved the submitted version. Linkuan Huangfu conducted data collection, analysis and article writing; Linkuan Huangfu, Boya Zha, Long Wang, Xiaohao Liu and Yingjuan Zheng worked out the treatment plans of every patient; Peihong Li, Jingjing Wu, Shuling Shi, Haiyang Cui and Yuxin Li collected the data; Linkuan Huangfu, Yuchuan Yang, Xiaocong Sun, Shibo Gao and Daoke Yang participated in the imaging evaluation.

Data Availability: The datasets generated during and analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval: This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of the First Affiliated Hospital of Zhengzhou University, China (Ethics number: L/G2022-K001-003). Trial registration: Chinese Clinical Trial Registry (http://www.chictr.org.cn/): ChiCTR2200065992. November 2, 2022.

Consent to participate: Informed consent was obtained from all individual participants included in the study.

Consent to publish: The authors affirm that human research participants provided informed consent for publication of the images in Figure1.

References

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**Figures**

**Patient #8**

![Baseline](Baseline.png)  ![One month after one treatment cycle](One_month_after_one_treatment_cycle.png)  ![Before the deadline](Before_the_deadline.png)

**Patient #11**

![Baseline](Baseline.png)  ![One month after one treatment cycle](One_month_after_one_treatment_cycle.png)  ![Before the deadline](Before_the_deadline.png)
MRI of patient #8 and patient #11 at baseline, 1 month after one treatment cycle, and before cut-off date

Figure 2

The graph shows the best response in tumor size of patients during treatment and follow-up, and the data are the ratio of the SPD of the tumor with the best response to that at baseline. Two patients achieved PR and one patient achieved MR. MR was only available for patients with low-grade gliomas, i.e., those marked by gray shading in the figure. (SPD: sum of products of the diameters)
Figure 3

The time points at which patients experienced disease remission, disease progression, or death are labeled in the figure. The length of the bar represents the OS of the patients (5 patients were still alive at the end of follow-up and are indicated by ▶), and the data in gray shading at the end of each bar is the PFS of the patient.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryInformation.docx