

Research Paper

Safety and efficacy of sonodynamic therapy combined with Stupp regimen in controlling postoperative residual lesions of glioblastoma: A prospective pilot phase I study

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ABSTRACT

Background: Postoperative residual disease in glioblastoma (GBM) drives early recurrence due to the blood–brain barrier (BBB), local hypoxia, and normal brain tissue dose constraints. Sonodynamic therapy (SDT) utilizes transcranial low-intensity ultrasound to selectively activate sonosensitizers (e.g., hematoporphyrin), inducing non-invasive oxidative tumor ablation, BBB permeabilization, and hypoxia alleviation. We evaluated the safety, feasibility, and preliminary efficacy of multi-session transcranial SDT combined with standard chemoradiotherapy in newly diagnosed GBM patients with MRI-confirmed residual lesions.

Methods: In this prospective, open-label Phase I trial (Ethics Approval No 2024-KY-0621–002), 18 newly diagnosed IDH-wildtype GBM patients received a three-phase regimen: (1) Induction: pre-radiotherapy SDT (hematoporphyrin 3 mg/kg, 0.84 MHz, 1.5 W/cm², 50% duty cycle, twice daily for 5 days) with temozolomide (TMZ, 75 mg/m² daily); (2) Concurrent: intensity-modulated radiotherapy (60 Gy in 30 fractions) with daily TMZ (75 mg/m² for 6 weeks); and (3) Maintenance: adjuvant TMZ with optional concurrent SDT (hematoporphyrin 5 mg/kg, n = 9) or TMZ alone (n = 9). Primary endpoints were safety (NCI-CTCAE v5.0) and feasibility. Secondary endpoints included progression-free survival (PFS), overall survival (OS), and objective response rate (ORR) via RANO criteria.

Results: Over a median follow-up of 26.23 months (95% CI: 21.97–27.47), primary safety and feasibility endpoints were met. No treatment-related deaths or grade 4/5 toxicities occurred. Treatment-related adverse events were mostly mild-to-moderate (grade 1/2) and transient, including 1 grade 1 photosensitivity reaction and 2 grade 1 cerebral edema events managed with osmotic therapy. For the entire cohort (n = 18), median OS was 19.00 months (95% CI: 17.27–23.97; 12-month OS rate: 88.9%), median PFS was 10.53 months (95% CI: 8.67–18.23), ORR was 66.7% (12 PR, 6 SD), and Disease Control Rate was 100%. In maintenance subgroup analysis, patients receiving SDT + TMZ showed a higher ORR (88.9% vs. 44.4%) and extended median PFS (11.80 vs. 9.27 months; P = 0.599) compared with TMZ alone, with comparable OS (19.00 vs. 20.10 months; P = 0.740).

Conclusions: Integrating transcranial SDT with standard chemoradiotherapy and maintenance temozolomide is safe, feasible, and well tolerated in newly diagnosed GBM with residual disease. Encouraging preliminary efficacy signals warrant further evaluation in multi-center Phase II trials.

1. Introduction

Glioblastoma (GBM; WHO grade 4) represents the most aggressive

and lethal primary intracranial malignancy in adults [1,2]. This malignancy constitutes a substantial public health burden [3]. According to recent statistics from the National Brain Tumor Registry of China

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(NBTRC) and World Health Organization (WHO), there are approximately 80,000 to 100,000 newly diagnosed central nervous system malignancies annually in China, with GBM accounting for nearly 50% of all malignant gliomas, and over 60,000 deaths attributable to brain malignancies are reported annually [4,5]. Despite the establishment of a multimodal standard of treatment nearly two decades ago—comprising maximal safe resection followed by chemoradiotherapy and adjuvant temozolomide (the Stupp regimen)—the prognosis for patients remains dismal, with a median overall survival of approximately 15 months and a 5-year survival rate below 5% [6,7]. A critical determinant of therapeutic failure is the inability to achieve gross total resection (GTR) in a substantial proportion of patients, largely due to the infiltrative nature of the tumor and its frequent proximity to eloquent brain structures [8,9]. For patients with postoperative residual disease, the prognosis is particularly poor, as these remaining foci serve as reservoirs for rapid recurrence and therapeutic resistance [10,11].

The management of residual GBM presents a formidable clinical challenge. Conventional adjuvant therapies are severely constrained by biological barriers; radiotherapy is dose-limited by the tolerance of surrounding healthy parenchyma [12], while the efficacy of systemic chemotherapy is frequently compromised by the blood-brain barrier (BBB) [13] and the hypoxic tumor microenvironment, which fosters resistance mechanisms [14]. While photodynamic therapy (PDT) has been investigated as an intraoperative adjuvant, its clinical utility is fundamentally restricted by the limited tissue penetration of light, rendering it ineffective for deep-seated or bulky residual lesions [15,16]. Consequently, there is an urgent unmet need for non-invasive therapeutic modalities capable of targeting deep intracranial lesions while synergizing with standard chemoradiotherapy.

Sonodynamic therapy (SDT) has emerged as a promising strategy to overcome these limitations [17]. By utilizing low-intensity ultrasound to activate sonosensitizers, such as hematoporphyrin, selectively accumulated within neoplastic tissue, SDT leverages the superior tissue penetration depth of ultrasound to ablate tumors situated deep within the brain [18,19]. Mechanistically, SDT induces tumor cell death through the generation of reactive oxygen species (ROS) and mechanical cavitation [20,21]. Furthermore, preclinical evidence suggests that SDT may transiently modulate the BBB and alleviate hypoxia, thereby potentially sensitizing residual tumor cells to subsequent chemoradiotherapy [22,23].

Despite the robust rationale and encouraging preclinical data, clinical translation of SDT for GBM remains in its nascency [24,25]. Specifically, prospective data evaluating the safety and efficacy of integrating SDT into the standard Stupp protocol for high-risk patients with residual disease are lacking. To address this knowledge gap, we designed a prospective, single-arm, pilot phase I clinical study. Herein, we report the safety profile and preliminary therapeutic outcomes of transcranial SDT combined with chemoradiotherapy in newly diagnosed GBM patients with MRI-confirmed postoperative residual lesions, aiming to validate a novel comprehensive strategy for this refractory patient population.

2. Patients and methods

2.1. Research design and ethics

This study was conducted as a single-center, single-arm, open-label, prospective pilot phase I clinical trial. The research protocol was reviewed and approved by the Ethics Committee of the First Affiliated Hospital of Zhengzhou University (Project No 2024-KY-0621; Ethics Approval No 2024-KY-0621-002). The study was performed in strict accordance with the Declaration of Helsinki [26] and Good Clinical Practice (GCP) guidelines. Prior to enrollment and inclusion screening, written informed consent was obtained from all patients or their legal representatives, ensuring they fully understood the sonodynamic therapy (SDT) procedures, potential risks, and expected benefits.

2.2. Patient selection

Patients younger than 75 years (aged 15–74) with pathologically confirmed glioblastoma (CNS WHO grade 4, IDH-wildtype) [1,2] were screened for enrollment. Inclusion required newly diagnosed status without prior radiotherapy, chemotherapy, or targeted therapy; the presence of measurable postoperative residual tumor lesions confirmed by contrast-enhanced MRI; an estimated life expectancy of at least 3 months; and adequate organ function. Participants were excluded if they had any of the following: a known history of hypersensitivity to porphyrins or temozolomide; a history of porphyria; MRI-incompatible metal implants (eg, cardiac pacemakers) or other conditions preventing effective MRI evaluation; or severe comorbidities, including myocardial infarction within the past 6 months, severe arrhythmia, or uncontrolled active infection. Participants who had undergone major surgery other than the primary brain tumour resection within 4 weeks prior to enrolment were also excluded.

2.3. Procedures

Following maximal safe surgical resection and successful enrolment, eligible participants underwent a comprehensive therapeutic regimen integrating transcranial SDT with concurrent chemoradiotherapy. The treatment timeline was structured into three distinct phases: initial SDT combined with chemotherapy, concurrent chemoradiotherapy, and maintenance cycles.

Initial SDT combined with chemotherapy: The protocol commenced with an initial treatment phase initiated 7 days prior to the planned start of concurrent chemoradiotherapy. Participants received a single intravenous infusion of the sonosensitizer hematoporphyrin (3 mg/kg of body weight; infusion duration ≥ 30 min). Light-avoidance measures were implemented immediately following administration to prevent cutaneous photosensitivity [27]. The SDT procedure commenced 24–48 h post-infusion. A low-intensity ultrasound system was used, with the transducer coupled to the scalp surface corresponding to the surgical window. Target regions were identified based on postoperative contrast-enhanced T1-weighted and T2/FLAIR MRI sequences to encompass the tumor bed and surrounding measurable residual lesions. Acoustic parameters were fixed at a frequency of 0.84 MHz, a sound intensity of 1.5 W/cm², and a 50% duty cycle in pulse mode. Multi-target sonication was performed to cover the tumor bed and measurable residual areas, with the total treatment time per session controlled at 60 min. This phase consisted of SDT administered twice daily with a 6-h interval between sessions for a 5-day duration (5d). Concurrently, participants received oral temozolomide (75 mg/m² daily) alongside the SDT sessions.

Concurrent chemoradiotherapy: Following the pre-radiotherapy SDT cycle, participants proceeded to the concurrent chemoradiotherapy phase (weeks 2–7). Three-dimensional conformal or intensity-modulated radiotherapy was initiated, delivering 60 Gy in 30 fractions to the tumor bed (GTVtb). The clinical target volume (CTV), defined as the GTV plus a 1.5–2.0 cm margin, received 54 Gy in 30 fractions. Radiotherapy was administered concurrently with daily temozolomide (75 mg/m² of body surface area), 7 days per week for 6 weeks [6].

Chemotherapy phase: After a 3-week recovery interval following chemoradiotherapy, participants entered the chemotherapy phase (starting at week 11). This phase consisted of a combined-modality regimen in which SDT was administered in conjunction with chemotherapy. SDT was delivered twice daily for 5 consecutive days (days 1–5) within each 28-day temozolomide cycle (150–200 mg/m²), using an increased hematoporphyrin dose of 5 mg/kg based on individual tolerance. The protocol planned for up to four such combined cycles; however, participation in this phase was voluntary, allowing for flexibility based on participant preference and tolerance.

Concomitant Medications: Concomitant therapies were administered in accordance with institutional neuro-oncology guidelines and

standard clinical pathways. In newly diagnosed glioblastoma, bevacizumab is reserved for disease recurrence or progression; therefore, its administration was restricted during the initial protocol treatment (induction, concurrent chemoradiotherapy, and maintenance phases) and was initiated exclusively as a secondary salvage therapy upon verified disease progression. Corticosteroid use was judiciously managed; short-term, low-dose dexamethasone was prescribed solely for managing acute symptomatic cerebral edema and was rapidly tapered following symptom relief.

Patient management: To prevent cutaneous photosensitivity, a standardized light-protection protocol was rigorously enforced throughout the study [27]. Following hematoporphyrin administration, patients were managed in inpatient rooms with subdued ambient lighting and shielded from direct sunlight. Comprehensive patient education was conducted prior to each cycle. Patients were instructed to maintain strict light avoidance for 14–21 days following the 3 mg/kg induction dose, with precautions extended to 21–28 days following each 5 mg/kg maintenance cycle. Outdoor daylight activities were strictly minimized; when outdoor exposure was unavoidable, patients were required to wear broad-brimmed hats, UV-blocking sunglasses, and high-coverage protective clothing, supplemented by physical sunscreen. Compliance was actively reinforced through weekly telephone follow-ups by dedicated study personnel.

Monitoring and assessment: Vital signs were monitored, and participants were assessed for adverse events, with particular attention paid to photosensitive reactions and changes in intracranial pressure. SDT treatments could be paused or discontinued based on participant or investigator decision, including suspicion of radiographic disease progression or progression-related clinical deterioration. Post-procedure MRI was performed in all participants prior to SDT treatment, after the completion of the concomitant phase, and prior to the initiation of each chemotherapy phase to quantitatively evaluate therapeutic response using the Response Assessment in Neuro-Oncology (RANO) criteria [28]. All enrolled participants underwent long-term follow-up to evaluate progression-free survival and overall survival until study closure.

Assessment of Tumor Progression: To distinguish true tumor progression and post-chemoradiotherapy pseudoprogression, the updated RANO criteria were strictly adhered to [28]. When radiographic enlargement of enhanced lesions occurred—particularly within 12 weeks of completing chemoradiotherapy in asymptomatic or clinically stable patients—multidisciplinary consultations involving experienced neurosurgeons and neuroradiologists were routinely convened. In addition to conventional contrast-enhanced T1-weighted and T2/FLAIR sequences, advanced physiological MRI sequences, specifically diffusion-weighted imaging (DWI) and dynamic susceptibility contrast perfusion-weighted imaging (DSC-PWI), were utilized. Lesions demonstrating low or stable regional cerebral blood volume (rCBV) without concurrent clinical deterioration were considered treatment-related reactive changes [29]. For suspected pseudoprogression or indeterminate findings, a mandatory confirmatory repeat MRI was performed 4 to 8 weeks later, and true disease progression was confirmed only upon persistent or subsequent radiographic enlargement.

2.4. Outcomes

The prespecified primary outcomes were safety and feasibility. Safety was assessed by monitoring adverse events throughout the study period, graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE) version 5.0 [30]. The relationship between adverse events and the study treatments was clinically assessed by the investigators. Feasibility was evaluated based on the successful completion of the planned therapeutic regimen. Prespecified secondary endpoints included progression-free survival and overall survival. Therapeutic efficacy was evaluated using the Response Assessment in Neuro-Oncology (RANO) criteria [28], measuring the

product of the maximal cross-sectional diameters of measurable residual lesions on contrast-enhanced T1-weighted MRI. Objective response rate (ORR) was defined as the proportion of patients achieving a complete response or partial response ($\geq 50\%$ reduction in tumor size). Progression-free survival was defined as the time from the date of pathological diagnosis to the first documentation of radiological disease progression or death from any cause. Overall survival was defined as the time from the date of pathological diagnosis to death from any cause. For surviving patients, data were censored at the date of the last follow-up.

2.5. Statistical analysis

This pilot phase 1 study was designed primarily to assess safety and feasibility. Descriptive statistics were used to describe baseline characteristics and safety data. Continuous variables were reported as medians with interquartile ranges (IQRs), and categorical variables as frequencies and percentages. Survival endpoints (progression-free survival and overall survival) were estimated using the Kaplan-Meier method, and median survival times with corresponding 95% CIs were calculated. Survival curves were generated for the entire intention-to-treat population. All statistical tests were two-sided, and a p value of <0.05 was considered statistically significant. Data analyses were performed using R software (version 4.2.0) and SPSS software (version 26.0; IBM, Armonk, NY, USA).

3. Results

3.1. Workflow of pilot phase I clinical study

To understand the safety and efficacy of SDT combined with the concurrent chemoradiotherapy for GBM patients, we designed a pilot phase I clinical study. The study workflow is shown in Fig. 1. Patients with brain tumors received surgical treatment. After histological examination of tumor tissues, a total of 47 GBM patients were determined and evaluated with MRI. Among these patients, 29 were excluded with the criteria of gross total resection ($n = 25$), refusal of radiotherapy ($n = 2$), history of WHO grade 2 glioma ($n = 1$), or expected survival of <3 months ($n = 1$). Qualified with the criteria of measurable postoperative residual tumors, 18 patients were recruited and enrolled into the study. As summarized in Table 1, the median age of the cohort was 58.5 years (IQR, 56.2–65.8), and 11 patients (61.1%) were male and 7 were female. The median Karnofsky Performance Status (KPS) score was 90 (IQR, 80.0–90.0). The median cross-sectional area of residual tumors was 164.5 mm^2 (IQR, $129.4\text{--}275.5 \text{ mm}^2$). The median Ki-67 labeling index was 30.0% (IQR, 20.0%–40.0%). All 18 enrolled patients initially received SDT combined with chemotherapy, followed by concurrent chemoradiotherapy. Subsequently, the patients were assigned into two subgroups for the maintenance treatment SDT + chemotherapy ($n = 9$) or chemotherapy only ($n = 9$). Data of efficacy and safety were analyzed for the study.

3.2. Protocol of SDT, chemoradiotherapy, and chemotherapy

As depicted in Fig. 2, we detailed the phase I study. The treatment timeline was stratified into three distinct phases: 1, pre-radiotherapy SDT combined with chemotherapy (induction); 2, concurrent chemoradiotherapy; and 3, maintenance chemotherapy with and without SDT. After a 3–4-week postoperative recovery period, patients were entered into the induction phase of treatment that consisted of a single intravenous infusion of hematoporphyrin (3 mg/kg) on the day 1, followed by transcranial ultrasound activation (0.84 MHz, 1.5 W/cm^2) administered twice daily, along with oral temozolomide (75 mg/m^2) daily for 5 days (days 2 to 6) in week-1. Then, patients received chemoradiotherapy that consisted of fractionated intensity-modulated radiotherapy (delivering 60 Gy in 30 fractions to the tumor bed and residual lesions) and

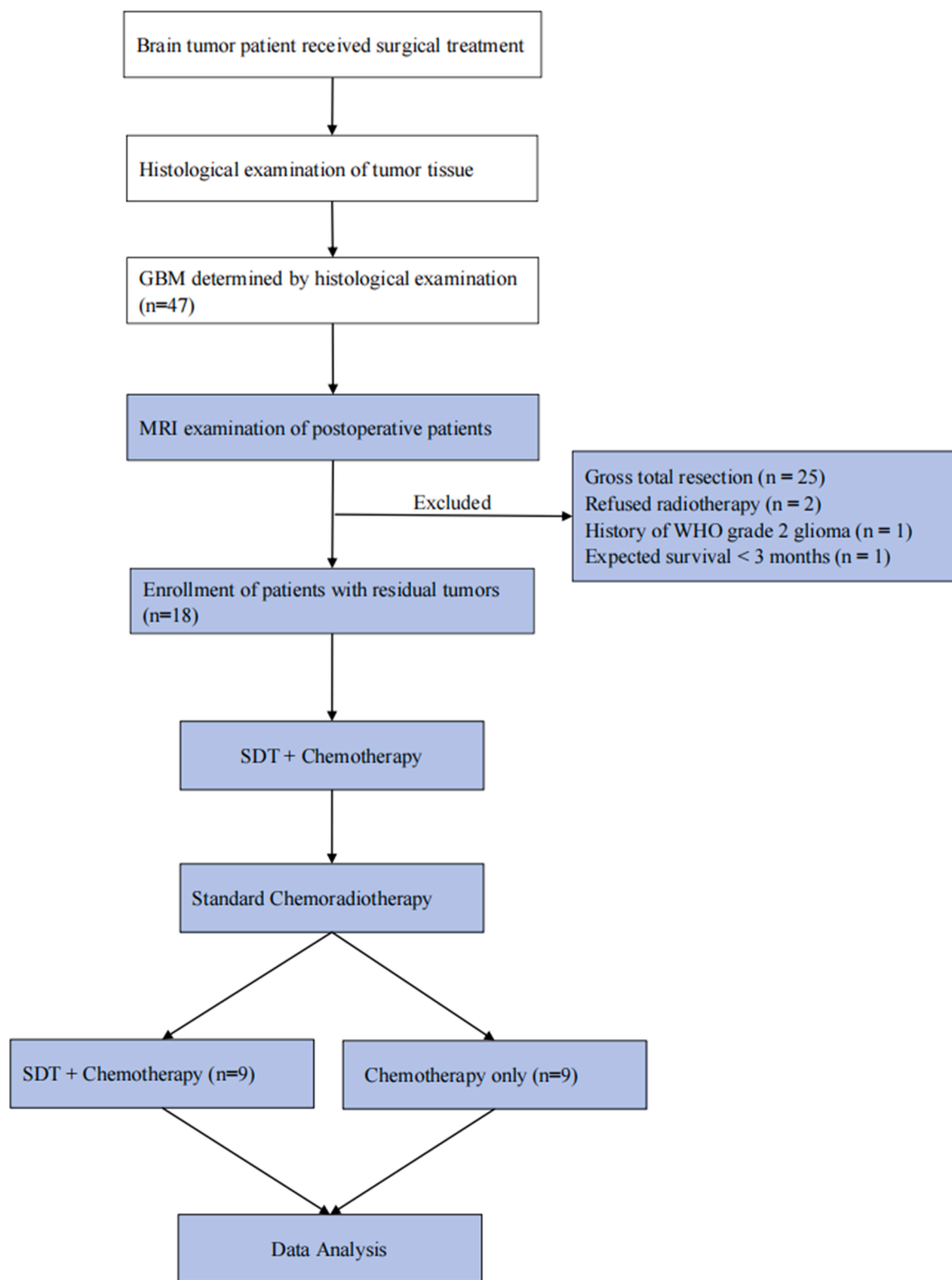


Fig. 1. Flowchart of clinical study. After surgical treatment, removed tumor tissues were histologically examined to identify patients with glioblastoma (GBM). A total of 47 GBM patients were evaluated with MRI examination and criteria for the study, and 18 patients carrying postoperative residual tumors were recruited and enrolled into the study. Patients were initially treated with sonodynamic therapy (SDT) combined with chemotherapy, followed by standard chemoradiotherapy. Subsequently, the patients were assigned into two subgroups for the maintenance treatment SDT + chemotherapy (n = 9) or chemotherapy only (n = 9). Data of efficacy and safety were analyzed. Exclusion criteria: gross total resection (n = 25), refusal of radiotherapy (n = 2), history of WHO grade 2 glioma (n = 1), and expected survival < 3 months (n = 1).

Table 1
Baseline characteristics of enrolled patients.

Characteristic	No. (%) / Median (IQR)
Total Patients	18
Age (years old)	58.5 (56.2 - 65.8)
Gender	
Female	7 (38.9%)
Male	11 (61.1%)
BMI (kg/m ²)	21.8 (20.3 - 23.9)
KPS Score	90.0 (80.0 - 90.0)
Comorbidities	
No	6 (33.3%)
Yes	12 (66.7%)
Tumor Laterality	
Bilateral	1 (5.6%)
Left	8 (44.4%)
Midline/Other	3 (16.7%)
Right	6 (33.3%)
Preoperative tumor area (mm ²)	1613.0 (994.5 - 2188.8)
Residual Tumor Area (mm ²)	164.5 (129.4 - 275.5)
IDH Status	
Wildtype	18 (100.0%)
MGMT Promoter Methylation	
Methylated	4 (22.2%)
Unknown/Not tested	11 (61.1%)
Unmethylated	3 (16.7%)
Ki-67 Index (%)	30.0 (20.0 - 40.0)

Abbreviations: BMI, Body Mass Index; KPS, Karnofsky Performance Status; SDT, Sonodynamic Therapy; IDH, Isocitrate Dehydrogenase; MGMT, O6-methylguanine-DNA methyltransferase; IQR, Interquartile Range. Note: Continuous variables are presented as median (IQR), and categorical variables are presented as number (percentage).

temozolomide (administered with 75 mg/m²) daily for 6 weeks (weeks 2–7). After a 3-week recovery period (weeks 8–11), patients were entered into the maintenance phase. During this phase, temozolomide was administered for 5 days per 28-day cycle, starting at 150 mg/m² for the first cycle and escalating to 200 mg/m² for cycles 2–4 if no grade >2 hematologic toxicity was observed. Concurrently, to accommodate individual patient tolerance, voluntary SDT sessions were offered twice

daily using an increased hematoporphyrin dose of 5 mg/kg.

3.3. Analysis of survival

To evaluate the long-term survival outcomes following SDT combined with concurrent chemoradiotherapy, patients were followed up for a median duration of 26.23 months (95% CI, 21.97–27.47; data cutoff date: July 31, 2026). Kaplan-Meier estimates were utilized to determine the survival metrics for the entire cohort. As shown in Fig. 3, the median overall survival (mOS) was 19.00 months (95% CI, 17.27 to 23.97) (Fig. 3A). The overall survival rates were robust, with 88.9% (16 of 18) of patients surviving beyond 12 months, and a projected 24-month OS rate of approximately 29% based on the survival curve. The median progression-free survival (mPFS) was 10.53 months (95% CI, 8.67 to 18.23) (Fig. 3B), with a 6-month PFS rate of 94.4% (95% CI: 66.6% to 99.2%) and a 12-month PFS rate of 44.4% (95% CI: 21.6% - 65.1%). These results indicate a substantial survival extension compared to the classic Stupp protocol historical data (mOS 14.6 months, mPFS 6.9 months) [6,31].

To further delineate the impact of the maintenance treatments, an exploratory subgroup analysis was performed between patients who received SDT + Chemotherapy (n = 9) and those who received Chemotherapy only (n = 9) during the maintenance phase. The SDT + Chemotherapy subgroup demonstrated a median PFS of 11.80 months (95% CI, 8.50 to 23.33) and a median OS of 19.00 months (95% CI, 11.53 to 26.23). Conversely, the Chemotherapy only subgroup experienced a shorter median PFS of 9.27 months (95% CI, 6.73 to 22.20) and a median OS of 20.10 months (95% CI, 17.27 to 27.47). No statistically significant differences were observed between the two subgroups for OS (P = 0.740, Fig. 3C) or PFS (P = 0.599, Fig. 3D), primarily due to the limited sample size of this pilot study.

3.4. Adverse events

To evaluate the safety and tolerability of incorporating SDT into the standard regimen, treatment-related adverse events (AEs) were

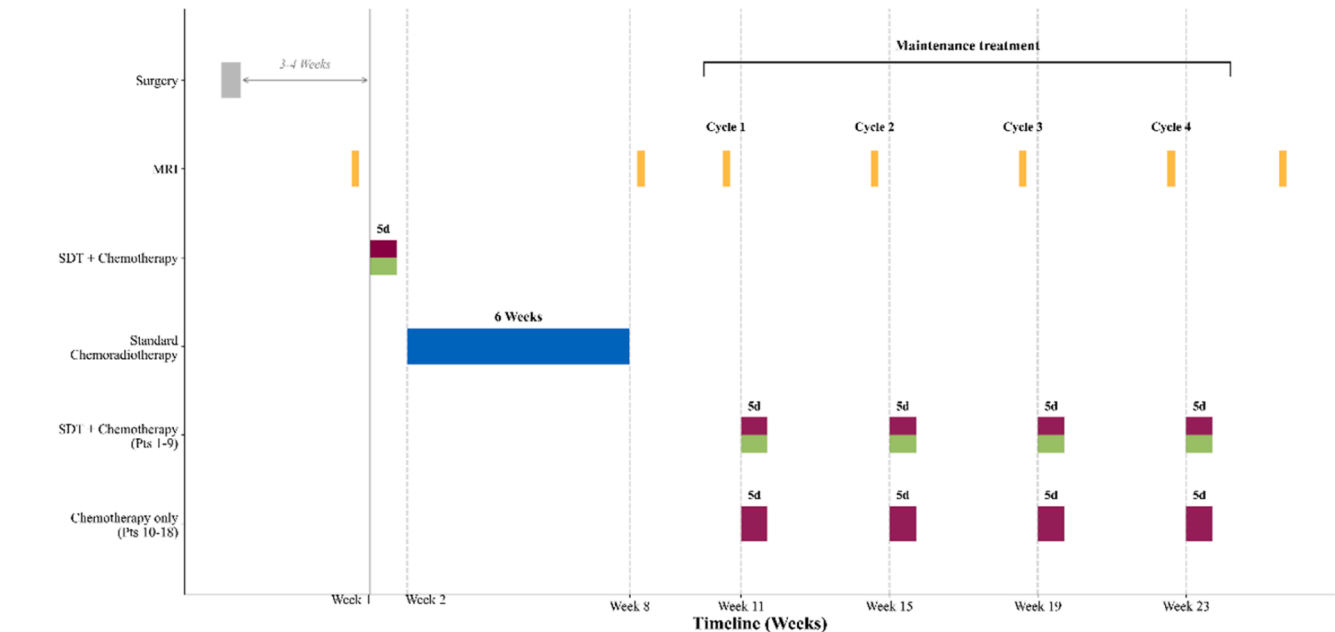


Fig. 2. Protocol of clinical study. After 3–4 weeks of recovery from surgical treatment (grey bar), all the 18 enrolled patients were treated with SDT (green bars) combined with chemotherapy (red bars) daily for 5 days (5d) (week 1), followed by standard chemoradiotherapy consisting of radiotherapy and chemotherapy with temozolomide (blue bar) daily for 6 weeks (week 2 to 7). Then, 18 patients were divided into 2 subgroups (9 each) for 4 cycles of maintenance treatment: 9 patients received both SDT and temozolomide (green and red bars), while the remaining 9 patients received only temozolomide (red bars), daily for 5 days in the weeks of 11, 15, 19, and 23. Adverse events were monitored, and tumor response was assessed with MRI (orange bars).

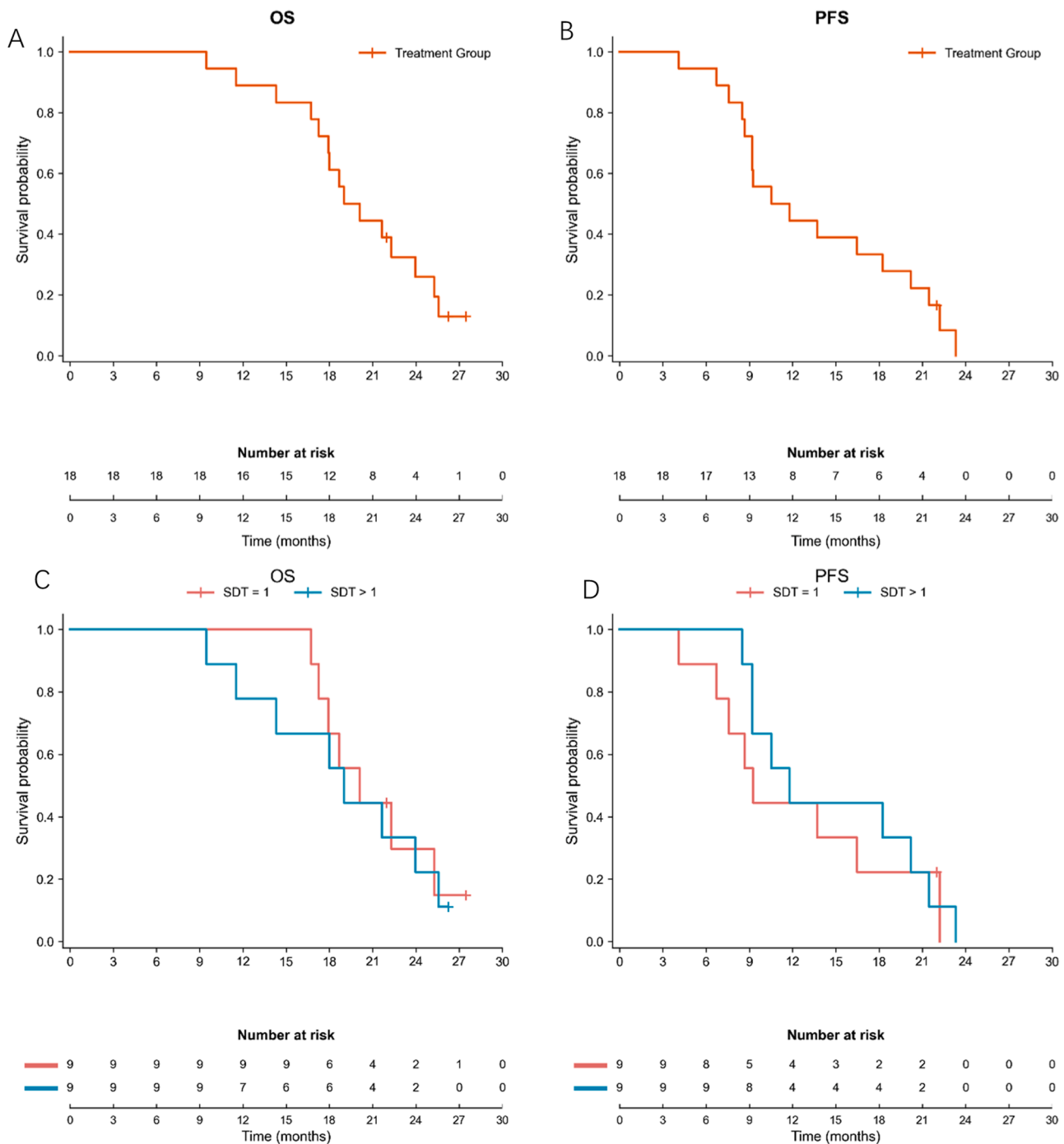


Fig. 3. Kaplan-Meier study of overall survival (OS) and progression-free survival (PFS). Data analysis of (A) OS (median 19.00 months) and (B) PFS (median 10.53 months) with the Kaplan-Meier curves for the entire cohort ($n = 18$) after the indicated treatments. Data analysis and comparison of (C) OS and (D) PFS between subgroups of patients treated with chemotherapy ($n = 9$) and patients treated with SDT+ chemotherapy ($n = 9$). Dotted lines indicate median values.

continuously monitored and stratified chronologically by treatment phase (Table 2). Overall, the safety profile was highly manageable, with the majority of AEs being mild (grade 1 or 2) and transient. No grade 4 life-threatening toxicities or treatment-related deaths occurred.

During the Induction Phase (Initial SDT + Chemotherapy, Week 1): The combination was well tolerated early in the treatment course. Only 1 patient (5.6%) developed a grade 1 cutaneous photosensitive reaction (skin peeling) on day 2 post-SDT due to inadequate light protection. Following the reinforcement of strict light-avoidance measures, the reaction resolved fully within 7 days without scarring [27].

During the Concurrent Chemoradiotherapy Phase (Weeks 2–7): As

anticipated, most systemic and constitutional AEs emerged during this concurrent phase. Early in this period (weeks 2–3), constitutional symptoms including fatigue ($n = 6$, 33.3%) and anorexia ($n = 6$, 33.3%) were frequently reported. Gastrointestinal symptoms, such as mild nausea/vomiting ($n = 3$, 16.7%) and constipation ($n = 2$, 11.1%), also occurred during this time; all were successfully managed with standard supportive care. Neurologically, 2 patients (11.1%) experienced grade 1 brain edema/headaches during the first week of radiotherapy, which resolved within 24 h following mannitol dehydration. Additionally, fever/infection ($n = 3$, 16.7%) and an isolated case of grade 1 cardiac arrhythmia ($n = 1$, 5.6%) were observed and controlled with

Table 2
Adverse events.

Adverse Events	Grade 1	Grade 2	Grade 3–4	Total	After Treatment
Leukopenia	3 (16.7%)	3 (16.7%)	1 (5.6%)	7 (38.9%)	0 (0%)
Thrombocytopenia	2 (11.1%)	0 (0%)	0 (0%)	2 (11.1%)	0 (0%)
Anemia	2 (11.1%)	0 (0%)	0 (0%)	2 (11.1%)	0 (0%)
Nausea/Vomiting	3 (16.7%)	0 (0%)	0 (0%)	3 (16.7%)	0 (0%)
Constipation	2 (11.1%)	0 (0%)	0 (0%)	2 (11.1%)	0 (0%)
Anorexia	6 (33.3%)	0 (0%)	0 (0%)	6 (33.3%)	0 (0%)
Liver Dysfunction	2 (11.1%)	0 (0%)	0 (0%)	2 (11.1%)	0 (0%)
Fatigue	6 (33.3%)	0 (0%)	0 (0%)	6 (33.3%)	0 (0%)
Fever/Infection	3 (16.7%)	0 (0%)	0 (0%)	3 (16.7%)	0 (0%)
Brain Edema/ Headache	2 (11.1%)	0 (0%)	0 (0%)	2 (11.1%)	0 (0%)
Skin Reaction	1 (5.6%)	0 (0%)	0 (0%)	1 (5.6%)	0 (0%)
Cardiac Arrhythmia	1 (5.6%)	0 (0%)	0 (0%)	1 (5.6%)	0 (0%)

After treatments, adverse events were determined during the period of supportive care and graded based on NCI-CTCAE v5.0 severity definitions (described in the Patients and methods section). The "After Treatment" column indicates the status of adverse events, and the value of 0 (0%) indicates that all the adverse events were transient and became undetectable by the end of the study.

appropriate medical interventions. Hematological toxicities began to emerge during the middle-to-late stages of this phase (weeks 4–5). Mild (grade 1 or 2) leukopenia was observed in 4 patients (22.2%) and mild anemia in 2 patients (11.1%), which were safely managed with supportive agents. Furthermore, transient grade 1 liver function abnormalities were detected in 2 patients (11.1%) at the end of this phase, normalizing within one week after hepatoprotective therapy.

During the Maintenance Phase: As expected with prolonged chemotherapy exposure, delayed hematological toxicities emerged during the late maintenance cycles. Leukopenia was observed in 3 patients (16.7%), including one case of grade 3–4 leukopenia that was swiftly reversed within 3 days using recombinant human G-CSF. Mild thrombocytopenia also occurred in 2 patients (11.1%) and was effectively managed. Importantly, repeated cycles of SDT combined with chemotherapy did not result in any severe cumulative neurotoxicity, confirming the overall safety and feasibility of long-term repeated SDT treatments.

3.5. Analysis of efficacy

To determine the objective clinical efficacy of incorporating SDT into the concurrent chemoradiotherapy regimen, tumor responses were evaluated longitudinally using RANO criteria based on MRI assessments [28]. As shown in Fig. 4A, the entire cohort achieved a 100% Disease Control Rate (DCR), with 12 of 18 patients demonstrating a Partial Response (PR) and 6 patients maintaining Stable Disease (SD). Consequently, the Objective Response Rate (ORR) for the overall population was 66.7%.

To further elucidate the specific contribution of SDT during the extended treatment period, therapeutic outcomes were stratified by maintenance therapy subgroup. Patients assigned to the SDT + Chemotherapy subgroup (n = 9) exhibited a highly favorable response, achieving an ORR of 88.9% (8 PR, 1 SD). In contrast, the Chemotherapy only subgroup (n = 9) demonstrated a lower ORR of 44.4% (4 PR, 5 SD).

A comparison of these results indicates that the addition of repeated SDT cycles to maintenance chemotherapy suggests a potential pattern toward deeper radiographic response.

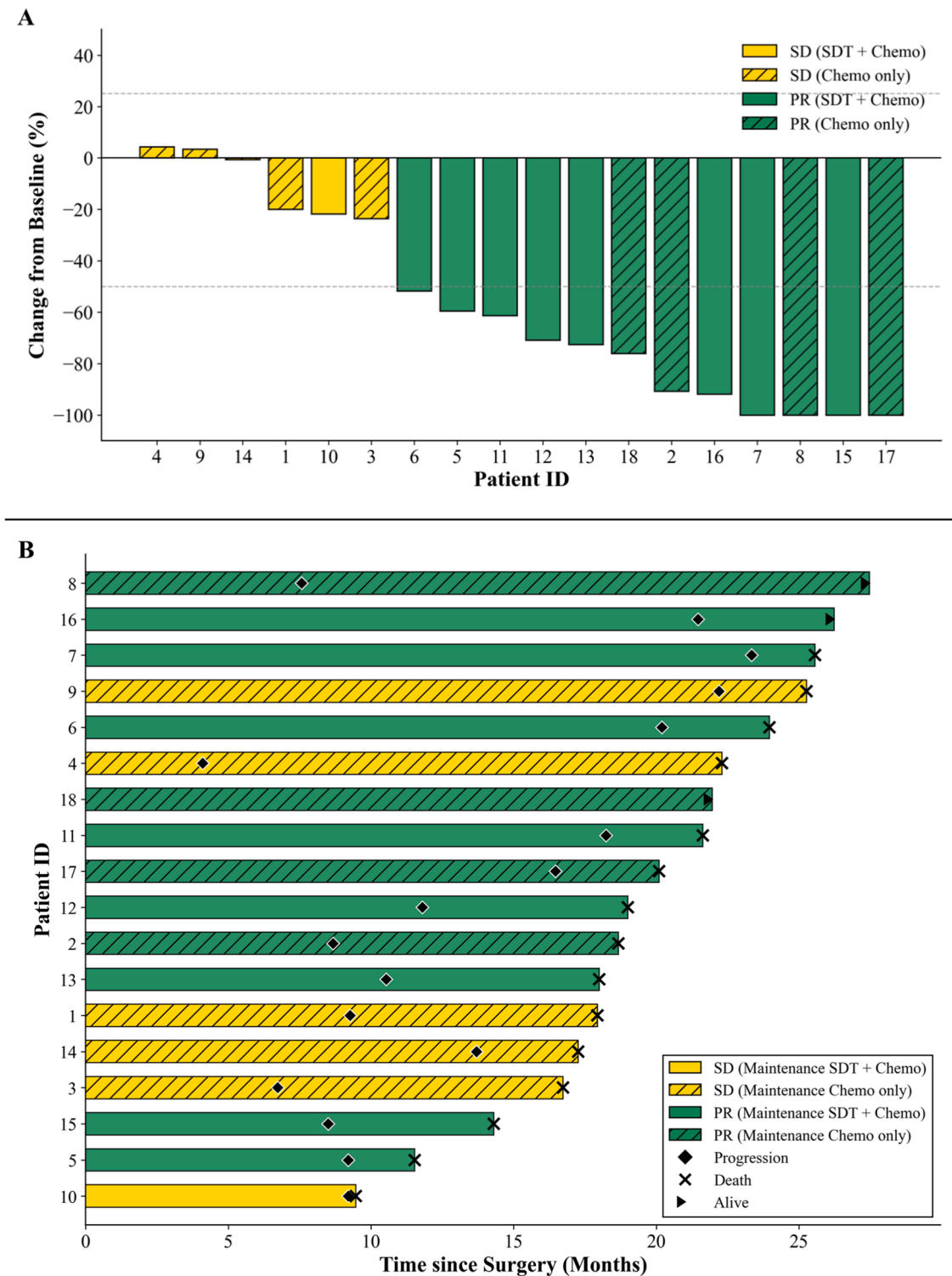
The clinical timelines and durability of these responses are detailed in the swimmer plot (Fig. 4B). Sustained therapeutic benefits were evident across the cohort, with 94.4% (17 of 18) achieving a progression-free interval of at least 6 months, and 44.4% (8 of 18) remaining progression-free beyond 12 months. Representative contrast-enhanced MRI scans (Fig. 5) visually corroborated these quantitative findings, demonstrating pronounced regression of measurable residual lesions in representative patients following combined modality treatment.

4. Discussion

In glioblastoma (GBM), postoperative residual disease remains a primary driver of therapeutic failure and rapid recurrence, as infiltrative neoplastic foci reside within protected niches behind an intact or partially intact blood–brain barrier (BBB) and within hypoxic, radio-resistant microenvironments [13,14]. Conventional adjuvant approaches, including the standard Stupp regimen, are fundamentally constrained by the dose tolerances of surrounding eloquent parenchyma and inadequate tissue penetration of systemic agents [6,12]. Sonodynamic therapy (SDT) has emerged as a compelling non-invasive strategy to overcome these physical and biological barriers. By deploying transcranial low-intensity focused ultrasound to selectively activate sonosensitizers like hematoporphyrin preferentially accumulated within neoplastic cells, SDT achieves deep tissue ablation via reactive oxygen species (ROS) generation and mechanical cavitation [18–21]. Preclinical evidence further indicates that SDT transiently permeabilizes the BBB and alleviates local tissue hypoxia, establishing a strong biological rationale for its integration as a sensitizing modality prior to and during standard chemoradiotherapy [22,23].

In the present prospective pilot phase I trial, the primary endpoints of safety and feasibility were successfully met. Transcranial SDT integrated with the Stupp regimen was well tolerated across all treatment phases, with no treatment-related deaths or grade 4/5 life-threatening toxicities observed. The safety profile was highly manageable, with the majority of adverse events being mild-to-moderate (grade 1 or 2) and transient. Importantly, despite repeated transcranial acoustic energy delivery, no severe cumulative neurotoxicity or acute intracranial hypertension occurred; the transient grade 1 cerebral edema reported in two patients during the early radiotherapy phase was rapidly controlled with osmotic therapy. Cutaneous photosensitivity—a recognized risk of systemic porphyrin administration—was limited to a single grade 1 event that resolved without sequelae following strict light-avoidance protocols [27]. These clinical observations confirm that integrating multi-session transcranial SDT with full-dose chemoradiotherapy and maintenance temozolomide is feasible without compounding the known toxicity profile of standard-of-care treatments.

Although this pilot study was primarily designed to assess safety rather than to formally establish efficacy, preliminary clinical and survival outcomes provide encouraging signals of therapeutic activity in patients with high-risk postoperative residual GBM. The entire cohort achieved a median overall survival (mOS) of 19.00 months (95% CI: 17.27–23.97) and a median progression-free survival (mPFS) of 10.53 months (95% CI: 8.67–18.23), alongside a 12-month OS rate of 88.9%. These metrics compare favorably with historical benchmarks established by the classic Stupp protocol (mOS 14.6 months, mPFS 6.9 months) [6,31] in unselected newly diagnosed populations, particularly considering that our trial specifically enriched for patients with measurable postoperative residual tumor—a subgroup traditionally associated with a significantly worse prognosis [10,11]. Furthermore, longitudinal MRI evaluations according to RANO criteria [28] yielded an overall objective response rate (ORR) of 66.7% and a disease control rate (DCR) of 100%. In exploratory subgroup analyses, patients who



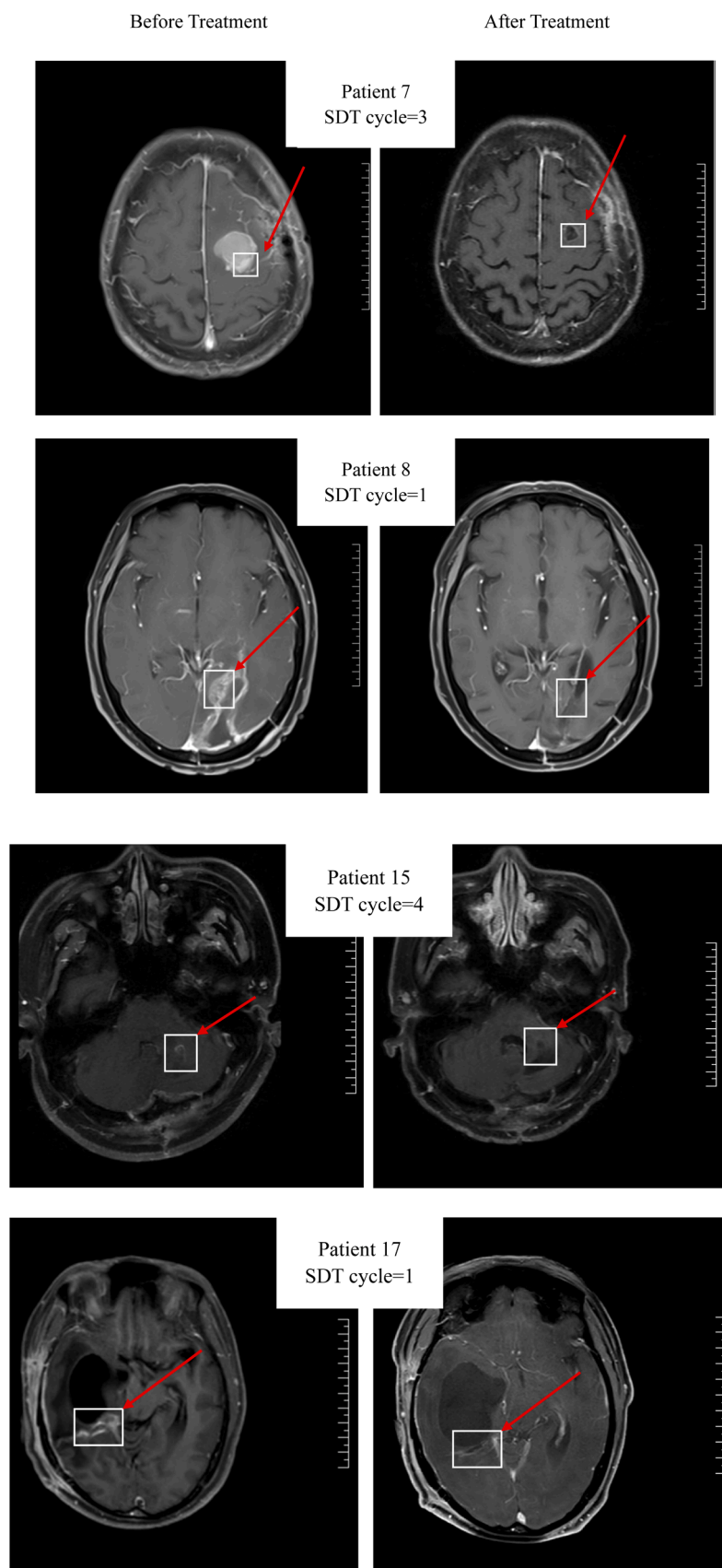


Fig. 5. Magnetic resonance imaging (MRI) of tumor response to treatment. Representative images of contrast-enhanced T1-weighted MRI of 4 patients (Patients 7, 8, 15 and 17) with tumor regression after combined treatment with SDT and Stupp regimen. Boxed areas with red arrows indicate tumors before and after treatment.

elected to receive repeated SDT cycles during maintenance chemotherapy exhibited a numerically higher ORR (88.9%vs. 44.4%) and extended mPFS (11.80 vs. 9.27 months) compared to those receiving chemotherapy alone, suggesting a potential pattern toward deeper radiographic response upon extended treatment exposure.

The clinical efficacy observed likely stems from the multi-modal synergy among SDT, radiotherapy, and temozolomide. The pre-radiotherapy induction SDT exerts a multi-faceted priming effect. First, ultrasound-mediated sonoporation transiently opens the BBB/BBB to facilitate intratumoral penetration of concurrent temozolomide, establishing an early bridging cytoreduction that preemptively suppresses rapid repopulation of residual clones prior to radiation [13,22,23]. Second, hematoporphyrin is selectively retained within neoplastic tissues with a prolonged clearance cycle, triggering a radiodynamic effect that amplifies radiation-induced free radical generation and cytotoxicity during subsequent fractionated radiotherapy [32]. Third, SDT-induced microvascular modulation alleviates intratumoral hypoxia, restoring the critical oxygen fixation effect required for radiation-induced DNA double-strand breaks and overcoming local radioresistance [14,18]. During the maintenance phase, repeated SDT cycles further eliminate quiescent residual reservoirs and suppress clonal evolution, contributing to durable disease control [24,25].

This study has several limitations inherent to early-phase clinical development. First, as a single-center pilot phase I trial with a modest sample size ($n = 18$), the study was not statistically powered to perform definitive comparative efficacy analyses or to detect subtle survival differences between maintenance subgroups. Second, assignment to the maintenance SDT subgroup was voluntary and unblinded, introducing potential selection bias regarding patient performance status and treatment compliance. Third, molecular biomarkers—such as longitudinal MGMT promoter methylation status, baseline TME immune cell profiling, or circulating tumor DNA dynamics—were not sequentially evaluated to correlate specific molecular signatures with long-term survival. Finally, post-treatment tissue biopsies were not routinely performed due to procedural risks in deep intracranial sites, precluding direct histological or transcriptomic confirmation of SDT-induced BBB opening and microenvironmental remodeling.

5. Conclusion

This prospective pilot phase I trial demonstrates that transcranial SDT combined with the standard Stupp regimen is safe, feasible, and clinically manageable in newly diagnosed glioblastoma patients with postoperative residual lesions. Preliminary efficacy signals, characterized by prolonged PFS/OS and substantial radiographic tumor regression, support the therapeutic potential of this novel combination strategy. These findings provide a strong foundation and strong biological rationale for our ongoing and planned multi-center Phase II trials to rigorously evaluate the survival benefits and molecular correlates of SDT-integrated multimodal therapy in high-risk glioblastoma.

Ethics approval and consent to participate

The study protocol was reviewed and approved by the Ethics Committee of The First Affiliated Hospital of Zhengzhou University (Ethics Approval No 2024-KY-0621-002). Written informed consent was obtained from all participants or their legal guardians.

Consent for publication

Not applicable.

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CRedit authorship contribution statement

Long Wang: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Jiayin Ding:** Writing – review & editing, Writing – original draft, Visualization, Software, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Xiaohao Liu:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Tianqi Li:** Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Yahang Liu:** Writing – original draft, Methodology, Formal analysis, Data curation, Conceptualization. **Yanan You:** Writing – original draft, Visualization, Validation, Methodology. **Yue Yu:** Writing – review & editing, Writing – original draft, Visualization, Resources. **Yingjuan Zheng:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no competing interests.

Data availability

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

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