Sonodynamic Therapy Combined with Chemoradiotherapy for Glioblastoma: Clinical Study

1. Study Protocol Summary

Glioblastoma (GBM) is characterized by its high aggressiveness and short survival. Despite ongoing exploration of various treatments, effective therapeutic strategies remain elusive. Therefore, we are introducing Sonodynamic Therapy (SDT) to potentially offer new hope for glioma patients. SDT is a novel therapeutic technique derived from Photodynamic Therapy (PDT). Preclinical models have demonstrated promising therapeutic effects with SDT.

Studies in rat glioblastoma models have shown that SDT can effectively inhibit tumor growth and metastasis, and extend survival more significantly compared to standard temozolomide treatment. Furthermore, research both domestically and internationally exploring SDT in combination with immunotherapy and nanocarriers has also yielded encouraging results. The Harbin Medical University research team pioneered the use of SDT in treating patients with atherosclerosis, confirming its efficacy and safety in clinical settings. Multiple studies indicate synergistic effects between SDT and various tumor treatment regimens.

Preliminary results from our Phase I clinical trial have confirmed the safety and efficacy of SDT in treating recurrent glioblastoma. Consequently, we plan to conduct a single-center, two-arm, randomized, open-label, Phase II clinical trial to evaluate SDT for newly diagnosed glioblastoma. We plan

to enroll 220 patients with newly diagnosed GBM, randomly assigned to either the Experimental Group or the Control Group.

Experimental Group: Patients will receive SDT plus investigator-chosen treatment regimens (e.g., radiotherapy, temozolomide, bevacizumab, etc.).

Control Group: Patients will receive only the investigator-chosen treatment regimens.

Patients will be followed for 24 months, with monthly brain MRI scans. Treatment efficacy will be assessed according to the Response Assessment in Neuro-Oncology (RANO) criteria. The primary endpoint is Progression-Free Survival (PFS). The projected study duration is 10/01/2024 to 10/01/2028.

2. Research Background

Gliomas are the most common primary brain tumors, accounting for 81% of central nervous system malignancies. Glioblastoma (GBM), the highest-grade glioma, has a dismal prognosis, with a 5-year survival rate of only 2.5-5% and a median survival time (mOS) of 12-15 months. Post-recurrence, the mOS falls to 6.3-7.5 months[1]. Despite advancements in surgery, radiotherapy, chemotherapy, and targeted therapies, the overall survival for GBM patients has not significantly improved.

GBM's infiltrative growth pattern, with indistinct margins, makes complete surgical resection difficult, leading to high recurrence rates and extremely poor outcomes. The postoperative standard Stupp protocol extends

mOS to 14.6 months[2]. However, even with maximal safe resection followed by chemoradiation, the 5-year survival rate remains below 10%.

The NCCN guidelines recommend surgery as the primary intervention for high-grade gliomas, followed by adjuvant involved-field radiotherapy with concurrent and adjuvant temozolomide. Standard treatment includes a combination of concurrent chemoradiation and adjuvant chemotherapy. Tumor Treating Fields (TTFields), a relatively novel anti-cancer therapy using low-intensity energy fields to disrupt cancer cell division, showed only a 2-month extension in survival in its Phase III trial and is expensive.

Clinical trials are essential for medical research. After potential new anti-cancer approaches are developed and tested in laboratories, investigations in humans are necessary. If drugs, devices, or treatments prove safe and effective in clinical trials, they may receive regulatory approval (e.g., FDA).

The Chinese guidelines for childhood gliomas[4] state that for low-grade gliomas, surgery is first-line treatment. However, resection is often unfeasible for tumors involving deep midline supratentorial regions, optic pathways/hypothalamus, or brainstem. Extent of resection is a crucial factor for prolonging both progression-free survival (PFS) and overall survival (OS). Chemotherapy should commence promptly postoperatively, utilizing maximally tolerated doses with appropriate durations while monitoring toxicity. Chemotherapy regimens should be selected based on histopathological and molecular pathology findings. Patients should also be offered access to relevant and feasible clinical trials.

For childhood high-grade gliomas (HGG), maximal safe surgical resection remains the cornerstone of treatment. The utility of adjuvant cytotoxic chemotherapy combined with radiotherapy remains unclear. There is no standard chemotherapy regimen, and participation in relevant clinical trials is strongly recommended.

Sonodynamic Therapy (SDT) is a novel technique evolving from Photodynamic Therapy (PDT). PDT selectively destroys local tumors while sparing normal tissue. Its safety, minimal side effects, and repeatability have established it in clinical guidelines for skin, esophageal, biliary tract, and bladder cancers, among others[6]. Significant literature demonstrates that intraoperative PDT improves glioma outcomes. However, PDT's reliance on invasive surgery and the poor tissue penetration depth of light (<1 cm in soft tissue) limit its application, particularly for deep-seated tumors[7].

SDT addresses PDT's limitations by utilizing Low-Intensity, Low-Frequency Focused Ultrasound (LILFU), which provides superior tissue penetration (~10 cm depth). LILFU can non-invasively focus acoustic energy deep within tissues to activate sonosensitizing drugs, thereby inducing localized anti-tumor effects[8-10]. SDT thus combines PDT-like tumor selectivity and efficacy with significant depth penetration capability for precise treatment of deeper lesions. It offers targeted tumor destruction with minimal damage to normal tissues and has shown demonstrated efficacy with low toxicity.

SDT is a rapidly developing, highly promising treatment attracting significant research and clinical attention globally. Numerous preclinical studies confirm its efficacy against glioblastoma[11-15]. Research indicates SDT induces glioblastoma cell necrosis and potentiates the anti-tumor effect of temozolomide[16]. Studies using C6 glioblastoma models in mice showed SDT significantly inhibits tumor growth rate[17].

Critically, our Phase I clinical trial results demonstrated the safety and efficacy of SDT for recurrent glioblastoma, with significant tumor shrinkage observed in some patients. However, the Phase I trial was limited by significant patient heterogeneity, small sample size, and underpowered statistical analysis. Therefore, a larger Phase II trial is urgently needed to robustly validate both the safety and efficacy of SDT for glioblastoma.

In summary, glioblastoma's complex pathogenesis, characterized by deep location, infiltrative growth, incomplete surgical resection tolerance, and resistance to maximal radiotherapy doses, poses significant therapeutic challenges. The absence of truly effective treatments and extremely poor prognosis causes immense suffering for patients and families. Identifying safe, efficacious, and accessible therapeutic modalities to maximally extend survival and improve quality of life remains a critical unmet clinical need.

SDT, emerging from PDT, represents an effective, safe, and non-invasive therapeutic technology. Its promising preclinical results in glioma models and

the encouraging safety/efficacy signals from our Phase I trial justify further investigation. To address the limitations of our preliminary Phase I findings (heterogeneity, small sample size), we propose this single-center, randomized, open-label Phase II clinical trial. This study will further substantiate the efficacy and safety profile of SDT in glioblastoma treatment.

The successful clinical translation of SDT holds significant potential societal impact by alleviating patient suffering, enhancing quality of life, and extending overall survival.

3. Study Objectives

- To further substantiate the **efficacy and safety** of Sonodynamic Therapy (SDT) in the treatment of glioblastoma.
- To identify and characterize any **clinical issues** associated with SDT treatment for glioblastoma.

4. Study Design

This study is a **single-center, randomized, open-label, Phase II clinical trial** evaluating the efficacy and safety of SDT in the treatment of glioblastoma.

The trial plans to enroll **220 subjects** with **newly diagnosed glioblastoma or with residual disease post-surgery**.

Subjects will be **stratified based on baseline characteristics** such as tumor location and size.
Subjects will be **randomized in a 1:1 ratio** to either the **Experimental Group** or the **Control Group**.

- **Experimental Group:** Subjects will receive SDT **in addition to** the **investigator-chosen treatment regimen** (e.g., radiotherapy, temozolomide, bevacizumab, etc.).

- **Control Group:** Subjects will receive **only the investigator-chosen treatment regimen**.

5. Study Population

5.1 Inclusion Criteria

1. Provision of signed and dated written **Informed Consent Form (ICF)**.

2. Age < 75 years.

3. Newly diagnosed glioblastoma patients **with pathological confirmation** post-surgery.

4. Absence of severe hematopoietic, cardiac, pulmonary, hepatic, renal dysfunction, or immunodeficiency. Laboratory values within the following limits within the pre-enrollment period:

    - **Hematology**: White blood cell count ≥ 4.0 x 10^9/L; Absolute neutrophil count ≥ 1.5 x 10^9/L; Platelets ≥ 100 x 10^9/L; Hemoglobin ≥ 90 g/L.

    - **Renal Function**: Serum creatinine ≤ 1.2 mg/dL or Calculated creatinine clearance (CrCl) ≥ 60 mL/min.

    - **Hepatic Function**: Total bilirubin ≤ 1.5 x ULN (≤ 3.0 x ULN if liver metastases present); Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) ≤ 2.0 x ULN (≤ 5.0 x ULN if liver metastases present).

    - **Coagulation**: International Normalized Ratio (INR) ≤ 2.0; Prothrombin Time (PT), Activated Partial Thromboplastin Time (aPTT), and Thrombin Time (TT) within normal limits.

5. Expected survival ≥ 3 months.

6. Resolution of toxicities from prior anticancer therapy to Grade ≤ 1 (Common Terminology Criteria for Adverse Events [CTCAE] v5.0), or complete recovery from prior surgery (Investigator judgment).

7. **Contraception Requirements**: Women of childbearing potential (WOCBP) and all male subjects must agree to use **highly effective**

contraception (e.g., condom, contraceptive sponge, gel, diaphragm, intrauterine device, oral or injectable contraceptives, subdermal implant) during the trial and for 12 months after the last dose of the sonosensitizer. WOCBP must have a negative pregnancy test within ≤7 days prior to the first SDT administration.

5.2 Exclusion Criteria

1. Patients with recurrent glioblastoma, brainstem tumors, or prior postoperative chemoradiotherapy.

2. History of **hypersensitivity to the sonosensitizer** (Hematoporphyrin/Xipofin).

3. **Uncontrolled conditions**: Active infection, poorly controlled seizures, elevated intracranial pressure, hypertension, or hyperglycemia.

4. Active infection with Human Immunodeficiency Virus (HIV), active Hepatitis B Virus infection (HBsAg positive and HBV DNA positive), or active Hepatitis C Virus infection (HCV antibody positive).

5. History of other **malignancy within 5 years**, except adequately treated carcinoma in situ of the cervix, cutaneous squamous cell carcinoma, or localized basal cell carcinoma of the skin.

6. Any other condition that, in the **Investigator's judgment**, would preclude safe participation in the study or interfere with study assessments.

5.3 Criteria for Exclusion from Efficacy/Safety Analysis

1. Subjects who fail to meet major protocol requirements such that **valid assessment of efficacy and/or safety endpoints** is precluded.

5.4 Withdrawal/Termination Criteria

1. **Subject Withdrawal**: Subject decides to withdraw consent and discontinues participation.

2. **Investigator Decision**: The Investigator determines that withdrawal is necessary for the subject's best interests (e.g., safety concerns, major protocol deviation, intercurrent illness).

6. Trial Procedures

- **Sonosensitizer**: Hematoporphyrin Injection (Xipofin®)
- **Device**: Transcranial Ultrasound Therapy Apparatus

Treatment Schema:

1. **Pre-treatment Evaluation**: Upon admission, subjects will undergo pre-treatment laboratory tests and assessments. A head MRI will be performed if not done within the previous month.
2. **Randomization & Stratification**: After completing baseline evaluations, subjects will be stratified and randomized 1:1 to the Experimental or Control Group based on clinical and pathological characteristics.
3. **Treatment Allocation**:
    - **Experimental Group**: SDT + Investigator-Chosen Regimen (Radiotherapy, TMZ, Bevacizumab, etc.)
    - **Control Group**: Investigator-Chosen Regimen only.
4. **Investigator-Chosen Treatment Regimen (Example - Standard Stupp-like Regimen +/- Bevacizumab):**
    - **Radiotherapy (RT)**: Total dose 50-54 Gy in daily fractions of 1.8-2.0 Gy, delivered 5 times per week over 6 weeks (approximately 25 fractions).
    - **Temozolomide (TMZ)**:
        - *Concurrent with RT*: 75 mg/m² orally daily throughout RT course.
        - *Adjuvant*: Starting post-RT, cycles consist of 150 mg/m²/day orally for 5 days, followed by 23 days without treatment

(28-day cycles). Dose may be escalated to 200 mg/m²/day for cycles 2-6 if tolerated.

- **Bevacizumab** (if included): 7.5-10 mg/kg intravenous infusion every 3 weeks for 4 to 6 cycles.

5. **SDT Procedure**:
   a. **Sonosensitizer Administration**:
   * Hematoporphyrin (Xipofin) administered intravenously at 5 mg/kg body weight.
   * Adults: Hematoporphyrin administered via percutaneous trans-arterial catheterization for locoregional drug perfusion.
   * Place subject in a room with controlled illumination post-administration.
   b. **SDT Treatment Initiation & Setup**:
   * Commence SDT approximately 40 hours post-sonosensitizer administration.
   * Before SDT, use MRI guidance for precise **ultrasound transducer placement and tumor targeting**. Shave hair in the transducer application area(s). c. **SDT Parameters**:
   * Ultrasound Intensity: 1.0 - 1.25 W/cm²
   * Ultrasound Frequency: 800 kHz - 1 MHz
   * Treatment Duration: 15 minutes per session.
   d. **Post-SDT Precautions**: Subjects must observe **strict light avoidance precautions** for one month following sonosensitizer administration. e. **SDT Schedule**: Treat subjects twice daily at 24 hours, 48 hours, and 72 hours after sonosensitizer administration.

Management of Adverse Events and Treatment Modification:
Monitor and manage potential adverse reactions including, but not limited to:

- **Systemic Symptoms**: Photosensitivity reactions, fatigue, asthenia, fever, pain.
- **Skin/Mucosa Symptoms**: Rash, ulceration, hemorrhage.
- **Gastrointestinal Symptoms**: Diarrhea, nausea, vomiting, anorexia, melena.
- **Respiratory Symptoms**: Cough, wheezing, hemoptysis, dyspnea.

- **Neurological and Cardiovascular Symptoms**: As reported.

Serious Adverse Reactions (e.g., anaphylaxis, shock):

- Immediately **discontinue sonosensitizer infusion**.
- Initiate **prompt anti-allergic** and **anti-shock management** protocols.

7. Study Endpoints

7.1 Efficacy Evaluation Endpoints

- **Primary Endpoint:** Progression-Free Survival (PFS)
- **Secondary Endpoints:**
    1. Overall Survival (OS)
    2. Disease Control Rate (DCR)
    3. Treatment-Emergent Adverse Event (TRAE) Rate

7.2 Safety Evaluation Endpoints

- Incidence, severity, and duration of **any adverse event (AE)**.
- Incidence, severity, and duration of **drug-related adverse events**.

8. Data Collection

8.1 Data Source

- This study plans to collect data from **50 patients** with newly diagnosed glioblastoma or with residual disease post-surgery.

8.2 Data Collection Content

- **Demographics/Baseline Characteristics:** Gender, age, height, weight, ethnicity, smoking history, alcohol history, allergy history, medical history, surgical history.
- **Clinical Diagnosis & Staging:** Pre-/post-treatment complete blood count (CBC), liver/kidney function tests, tumor location, tumor size, TNM stage (if applicable), clinical stage, Eastern Cooperative Oncology Group

(ECOG) Performance Status or Karnofsky Performance Status (KPS), EGFR expression status. Severity of adverse events graded per the **World Health Organization (WHO) Common Toxicity Criteria for Adverse Events** (see Appendix 1).

- **Prior Anti-tumor Therapy Information:**

    1. *Surgery:* Procedure name, details.

    2. *Radiotherapy:* Technique, total radiation dose, treated volume, fraction dose, number of fractions.

    3. *Chemotherapy:* Drug(s), dosage/regimen, number of cycles.

    4. *Other relevant anti-tumor therapies.*

- **Concomitant Medications/Therapies:** Name of therapy/medication, drug(s), dosage/regimen, start/end dates, reason for administration.

- **Imaging Data:** Magnetic Resonance Imaging (MRI). Baseline tumor assessment MRI must be performed within 2 weeks before treatment initiation. Post-treatment, MRI scans will be performed **every 4 weeks (±7 days)** for response assessment until disease progression, patient intolerance, or withdrawal occurs.

- **Clinical Follow-up Information:**

    1. *Safety:* Descriptive statistics of **all treatment-related adverse events**, including nature of event, duration, severity, and relationship to SDT. Calculation of AE and Serious Adverse Event (SAE) incidence rates.

    2. *Efficacy - Short-term:* Collection of imaging data for response assessment. Evaluation after **one cycle of SDT** for tumor response: Complete Response (CR), Partial Response (PR), Stable Disease (SD), Progressive Disease (PD). Calculation of Objective Response Rate (ORR) and Disease Control Rate (DCR) based on the **best overall response** within the first 12 months post-treatment.

    3. *Efficacy - Long-term:* Collection of survival data for enrolled patients. Documentation of date and cause of death. Assessment of survival status at **1 year, 2 years, and 3 years post-treatment**.

Calculation of 1-year OS rate, 1-year PFS rate, and safety outcomes. Recording of **median PFS (mPFS)** and **median OS (mOS)**.

9. Statistical Analysis

1. **Safety Analysis:** Descriptive analysis will be performed for the incidence of **all AEs and SAEs**.

2. **Efficacy Analysis:**

    - **PFS and OS curves** will be generated using the **Kaplan-Meier method**.

    - Differences in survival outcomes between groups will be assessed using the **Log-rank test**.

    - **Multivariate analysis** using the **Cox proportional hazards model** will be performed to evaluate the effect of prognostic factors. Hazard ratios (HRs) and their corresponding 95% confidence intervals (CIs) will be reported.

    - Unless otherwise specified, all statistical tests will be **two-sided**, conducted at the **α = 0.05 level of significance**. **Two-sided 95% confidence intervals** will be calculated.

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