Intensified Adjuvant Therapy for High-Risk Newly Diagnosed Glioblastoma With Subtotal Resection or Short-Term Progression: A Prospective, Single-Arm Phase II Clinical Study

Clinical Study Protocol

Principal Investigator：Ting Zhang

Version：1.0

Date：2025.12.1

1. Study Background

1.1 Epidemiological Background of Glioblastoma

Brain glioma refers to tumors originating from glial cells and is the most common primary intracranial tumor [1]. The 2021 WHO Classification of Tumors of the Central Nervous System classifies brain gliomas into grades 1-4; grades 1 and 2 are low-grade gliomas, and grades 3 and 4 are high-grade gliomas. The incidence of brain glioma in China is 5-8 per 100,000, and its 5-year mortality rate among systemic tumors is second only to pancreatic cancer and lung cancer. Brain glioma is characterized by high disability and high recurrence rates, seriously threatening patients' lives, impairing quality of life, and imposing heavy psychological and economic burdens on patients, families, and society.

Glioblastoma (GBM) is a WHO grade 4 high-grade glioma and is the most common primary malignant tumor of the adult central nervous system, accounting for approximately 57% of all brain gliomas and 48% of all primary malignant tumors of the central nervous system [2]. GMB is the most aggressive malignant brain tumor and has an extremely poor prognosis, with a 1-year survival rate of approximately 40.6% and a 5-year survival rate of only 5.6% [3].

1.2 Standard Treatment for Newly Diagnosed Glioblastoma

The treatment of newly diagnosed GBM is based primarily on surgical tumor resection, combined with radiotherapy, chemotherapy, and other comprehensive treatments. Maximal safe tumor resection combined with postoperative involved-field radiotherapy and concurrent and adjuvant temozolomide (TMZ) chemotherapy (the Stupp regimen) is the standard first-line treatment for newly diagnosed GBM [4]. In patients with newly diagnosed GBM treated with the Stupp regimen, the median overall survival (OS) is 14.6 months, the 2-year OS rate is 26.5%, and the 2-year PFS rate is 10.7%. Different from the Stupp regimen, in which oral TMZ begins on the first day of radiotherapy, the Department of Neurosurgery at Huashan Hospital in Shanghai conducted a multicenter clinical study of early postoperative TMZ chemotherapy (2 weeks after surgery), followed by concurrent chemoradiotherapy (the START regimen). The results showed that the median OS in the START regimen group reached 17.6 months, significantly superior to the Stupp regimen [5]. According to the 2024 NCCN, SNO-EANO, and the Chinese Guidelines for the Diagnosis and Treatment of Brain Glioma (2022 edition), surgery, postoperative radiotherapy, and TMZ chemotherapy are currently the standard treatment for newly diagnosed GBM. However, even with standard surgery and chemoradiotherapy, the median OS of GBM patients is approximately 15-18 months, and the 5-year OS rate still remains below 10%. Once GBM recurs, the median OS is estimated to be only 24-44 weeks. Therefore, exploring how to improve treatment efficacy and prognosis is of great significance for patients with GBM.

1.3 Treatment Advances in Newly Diagnosed Glioblastoma

In recent years, to further improve the prognosis of GBM patients, multiple clinical studies have continuously explored optimal comprehensive treatment strategies for newly diagnosed GBM, including prolonging the duration of adjuvant TMZ chemotherapy, combining other chemotherapeutic drugs, combining anti-angiogenic agents, combining immunotherapy, and tumor-treating fields. Concurrent chemoradiotherapy followed by 6 cycles of adjuvant TMZ chemotherapy is the standard treatment for GBM patients. A randomized phase II GEINO14-01 clinical trial explored whether extending adjuvant TMZ cycles could improve prognosis. A total of 159 patients were analyzed; the control group received the standard Stupp regimen, and the experimental group received more than 6 cycles of TMZ chemotherapy. The results showed no significant differences in 6-month PFS or OS between the two groups [6].

Several clinical studies have explored the efficacy of combination therapy based on the Stupp regimen. The AVAglio clinical study showed that bevacizumab combined with radiotherapy and TMZ prolonged the median PFS of newly diagnosed GBM patients by 4.4 months, but did not improve OS [7]. A randomized, open-label phase III CeTeG/NOA-09 clinical trial initiated by the Neuro-Oncology Working Group of the German Cancer Society analyzed the value of lomustine combined with TMZ in GBM patients. The results showed that the combination achieved an overall OS of 48.1 months in GBM patients with MGMT promoter methylation, compared with 31.4 months with TMZ alone [8]. On the basis of the Stupp regimen, adding tumor-treating fields (TTF) during adjuvant TMZ prolonged the median OS of patients with newly diagnosed GBM by 4.9 months and increased the 5-year survival rate to 13% [9]. Based on the

significant efficacy of combined anti-CTLA-4 and anti-PD-1 immunotherapy in preclinical GBM models, a phase I clinical study, NRG-BN002, evaluated the safety of immune checkpoint inhibitors alone or in combination in newly diagnosed GBM. Combined anti-CTLA-4 and anti-PD-1 immunotherapy had good tolerability and safety, with median OS and PFS of 20.7 months and 16.1 months, respectively [10], superior to the standard Stupp regimen. These findings support subsequent phase II/III clinical trials of combined immunotherapy in newly diagnosed GBM patients. The above clinical data suggest that adjuvant TMZ combined with other treatment modalities can improve patient prognosis to a certain extent.

In addition, because radiotherapy is an independent factor affecting survival in GBM patients, exploring whether increasing radiotherapy intensity can further improve prognosis has long been an important area of clinical research. In multiple exploratory studies based on metabolic imaging-guided focal high-dose irradiation, a meta-analysis including 22 prospective clinical studies showed that dose escalation to 62.5 Gy to 80 Gy, compared with the standard dose of 60 Gy, did not significantly improve prognosis [11]. This may be related to failure to distinguish gross total resection from subtotal resection or short-term recurrence, and to increased radiotherapy-related adverse reactions because the high-dose region included both residual lesions and the entire tumor bed. A small-sample retrospective analysis of 22 high-risk GBM cases at our center showed that a radiotherapy regimen delivering a high dose of 63-66 Gy to residual or recurrent lesions, standard doses of 54-60 Gy to high-risk regions, and 50-54 Gy to low-risk regions increased mPFS from 5.15 months to 12.3 months (unpublished data). Therefore, delivering small-field high-dose irradiation only to residual or recurrent lesions while delivering current standard-dose radiotherapy to the tumor bed and surrounding high-recurrence-risk areas may further improve survival benefit in GBM patients while controlling radiotherapy-related adverse reactions.

1.4 Patients With Subtotal Resection or Short-Term Recurrent/Progressive GBM Have a Worse Prognosis

The extent of surgical resection is an independent prognostic factor in GBM patients [12]. A retrospective analysis of 500 patients with newly diagnosed GBM evaluated the effect of extent of surgical resection on prognosis. The results showed that patients with 100%, 90%, 80%, and 78% resection had OS of 16 months, 13.8 months, 12.8 months, and 12.5 months, respectively [13]. This study suggests that patients with subtotal resection or short-term postoperative recurrence/progression require stronger postoperative adjuvant treatment strategies to further improve survival. However, at present, the postoperative adjuvant treatment regimen for GBM patients with subtotal resection or short-term recurrence/progression before postoperative radiotherapy is the same as that for patients with gross total resection. No clinical study has been conducted specifically in the population of patients with subtotal resection or short-term postoperative recurrence/progression to evaluate intensified postoperative adjuvant treatment. Therefore, the benefit of intensified adjuvant therapy in patients with subtotal resection or short-term postoperative recurrence/progression requires further exploration in prospective clinical studies.

In summary, among patients with newly diagnosed GBM with subtotal resection or short-term recurrent/progressive disease, investigating the benefits of intensified adjuvant therapy based on the Stupp regimen in terms of PFS rate, OS rate, quality of life, and other outcomes will help further optimize comprehensive treatment strategies for GBM patients. Here, we conduct a prospective, single-arm phase II clinical study to explore the efficacy and safety of an intensified treatment regimen consisting of focal high-dose radiotherapy to residual or recurrent lesions and TMZ combined with immunotherapy and anti-angiogenic therapy during the adjuvant phase, thereby providing evidence for optimal comprehensive treatment choices for more patients with GBM.

1.5 References

1. DeAngelis LM. Brain tumors. N Engl J Med. 2001;344(2):114-123. doi:10.1056/NEJM200101113440207

2. Ostrom QT, Gittleman H, Truitt G, et al. CBTRUS Statistical Report: Primary Brain and Other Central Nervous System Tumors Diagnosed in the United States in 2011-2015. Neuro Oncol. 2018;20(suppl_4):iv1-iv86. doi:10.1093/neuonc/noy131

3. Dirven L, Aaronson NK, Heimans JJ, et al. Health-related quality of life in high-grade glioma patients. Chin J Cancer. 2014;33(1):40-45. doi:10.5732/cjc.013.10214

4. Stupp R, Mason WP, van den Bent MJ, et al. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. N Engl J Med. 2005;352(10):987-996. doi:10.1056/NEJMoa043330

5. Mao Y, Yao Y, Zhang LW, et al. Does Early Postsurgical Temozolomide Plus Concomitant Radiochemotherapy Regimen Have Any Benefit in Newly-diagnosed Glioblastoma Patients? A Multi-center, Randomized, Parallel, Open-label, Phase II Clinical Trial. Chin Med J (Engl). 2015;128(20):2751-2758. doi:10.4103/0366-6999.167313

6. Balana C, Vaz MA, Manuel Sepulveda J, et al. A phase II randomized, multicenter, open-label trial of continuing adjuvant temozolomide beyond 6 cycles in patients with glioblastoma (GEINO 14-01). Neuro Oncol. 2020;22(12):1851-1861. doi:10.1093/neuonc/noaa107

7. Chinot OL, de La Motte Rouge T, Moore N, et al. AVAglio: Phase 3 trial of bevacizumab plus temozolomide and radiotherapy in newly diagnosed glioblastoma multiforme. Adv Ther. 2011;28(4):334-340. doi:10.1007/s12325-011-0007-3

8. Herrlinger U, Tzaridis T, Mack F, et al. Lomustine-temozolomide combination therapy versus standard temozolomide therapy in patients with newly diagnosed glioblastoma with methylated MGMT promoter (CeTeG/NOA-09): a randomised, open-label, phase 3 trial. Lancet. 2019;393(10172):678-688. doi:10.1016/S0140-6736(18)31791-4

9. Stupp R, Taillibert S, Kanner A, et al. Effect of Tumor-Treating Fields Plus Maintenance Temozolomide vs Maintenance Temozolomide Alone on Survival in Patients With Glioblastoma: A Randomized Clinical Trial. JAMA. 2017;318(23):2306-2316. doi:10.1001/jama.2017.18718

10. Sloan AE, Winter K, Gilbert MR, et al. NRG-BN002: Phase I study of ipilimumab, nivolumab, and the combination in patients with newly diagnosed glioblastoma. Neuro Oncol. 2024;26(9):1628-1637. doi:10.1093/neuonc/noae058

11. Singh R, Lehrer EJ, Wang M, et al. Dose Escalated Radiation Therapy for Glioblastoma Multiforme: An International Systematic Review and Meta-Analysis of 22 Prospective Trials. Int J Radiat Oncol Biol Phys. 2021;111(2):371-384. doi:10.1016/j.ijrobp.2021.05.001

12. Stark AM, Hedderich J, Held-Feindt J, et al. Glioblastoma--the consequences of advanced patient age on treatment and survival. Neurosurg Rev. 2007;30(1):56-61; discussion 61-52. doi:10.1007/s10143-006-0051-7

13. Sanai N, Polley MY, McDermott MW, et al. An extent of resection threshold for newly diagnosed glioblastomas. J Neurosurg. 2011;115(1):3-8. doi:10.3171/2011.2.jns10998

2. Study Objectives

This is a prospective, open-label phase II study in high-risk newly diagnosed GBM patients with subtotal resection or short-term recurrence/progression before radiotherapy, aiming to evaluate the efficacy and safety of intensified postoperative adjuvant therapy.

2.1 Primary Study Objective

To evaluate the effect of intensified adjuvant therapy on mPFS in high-risk newly diagnosed GBM patients with subtotal resection or short-term recurrence/progression (using RANO 2.0 criteria).

2.2 Secondary Study Objectives

2.2.1 To evaluate the effect of intensified adjuvant therapy on mOS and quality of life in high-risk newly diagnosed GBM patients with subtotal resection or short-term recurrence/progression.

2.2.2 To evaluate the safety and incidence of radiation necrosis of intensified adjuvant therapy in high-risk newly diagnosed GBM patients with subtotal resection or short-term recurrence/progression.

3. Study Design and Methods

3.1 Overall Study Design

This is a prospective, open-label phase II study evaluating the efficacy and safety of intensified postoperative adjuvant therapy in high-risk newly diagnosed GBM patients with subtotal resection or short-term recurrence/progression. High-risk newly diagnosed GBM is defined as disease crossing the midline or a deep lesion that cannot be completely resected by surgery, or no enhancing lesion on enhanced cranial MR re-examination within 3 days after surgery but newly appearing enhancing lesion on enhanced cranial MR re-examination 4-6 weeks after surgery and before radiotherapy. Radiologists, neurosurgeons, and radiation oncologists will independently judge the diagnosis of postoperative residual disease or recurrent/progressive disease. High-risk newly diagnosed GBM patients with subtotal resection or short-term recurrence/progression (4-6 weeks after surgery and before radiotherapy), after signing the informed consent form and being screened according to the inclusion and exclusion criteria, will receive intensified postoperative adjuvant therapy if eligible.

Specific regimen:

1) Concurrent chemoradiotherapy: Concurrent TMZ (75 mg/m2) chemoradiotherapy will begin 4-6 weeks after surgery. Focal high-dose irradiation of PGTV 63-66 Gy/30F will be delivered to the subtotal resection lesion or the short-term postoperative recurrent/progressive lesion. The high-risk region around the tumor bed will receive PTV1 54-60 Gy/30F, and the low-risk region will receive PTV2 50-54 Gy/30F. Radiotherapy target volumes: residual tumor region GTV: GTV includes definite postoperative residual enhancing lesions or newly developed enhancing lesions; GTV is expanded by 0.3-0.5 cm to form PGTV. High-risk clinical target volume CTV1: tumor bed GTVtb includes the surgical cavity and any enhancing regions visible on postoperative MRI (T1-weighted contrast-enhanced images); GTV and GTVtb are expanded by 1.0-1.5 cm to form CTV1, and CTV1 is expanded by 0.3 cm to form PTV1. Low-risk clinical target volume CTV2: CTV1 is expanded by 1.0 cm to form CTV2, and CTV2 is expanded by 0.3 cm to form PTV2.

2) Adjuvant systemic whole-body therapy: Approximately 28 days after completion of concurrent chemoradiotherapy, adjuvant TMZ combined with sintilimab plus bevacizumab will be given as intensified adjuvant therapy. Sintilimab 200 mg plus bevacizumab 10 mg/kg will be administered once by intravenous infusion; 21 days constitutes one cycle, and treatment will be maintained for 1 year.

This study is divided into three stages: screening period, treatment period, and follow-up period. The screening period is 4-6 weeks after surgery. Treatment period: after signing the informed consent form, subjects who meet the inclusion criteria and do not meet the exclusion criteria will undergo intensified postoperative adjuvant therapy. Follow-up period: after discontinuation of study treatment, subjects enter the follow-up period. During follow-up, cranial MRI examinations will be performed at baseline, after surgery, before radiotherapy, and after radiotherapy for evaluation. During the follow-up period, an enhanced MRI scan will be performed once every month to observe PFS until the end of follow-up. For subjects who have not withdrawn informed consent, survival information (i.e., date and cause of death, subsequent tumor treatment, etc.) will be collected once every month by telephone and/or clinical visit.

The expected enrollment period for this study is 24 months, and the follow-up period is 24 months.

3.2 Study Design Flowchart

Flowchart translated from the original diagram:

Eligibility criteria	**Postoperative 4-6 weeks**	**Concurrent chemoradiotherapy**	**28 days after RT**	**Adjuvant therapy and endpoints**
Age: >=18 years;	Assessment after	High-dose	RT completed, then	Adjuvant therapy:

pathologically confirmed GBM; postoperative 4-6 weeks after surgery (before radiotherapy), with no sufficient organ or bone marrow dysfunction listed in the original figure	surgery	radiotherapy + TMZ concurrent chemotherapy: PGTV 63-66 Gy, PTV1 54-60 Gy, PTV2 50-54 Gy/30F; TMZ 75 mg/m2 PO qd	approximately 28 days later	TMZ combined with sintilimab and bevacizumab: TMZ from day 1 to 5; sintilimab 200 mg Q3W plus bevacizumab 10 mg/kg Q3W, for 1 year. Primary endpoint: mPFS. Secondary endpoints: mOS; changes in quality of life relative to baseline and incidence of severe AEs; safety; incidence of radiation necrosis.

3.3 Study Population

3.3.1 Inclusion Criteria

Eligible patients must meet all of the following criteria:

1) Voluntary participation in the clinical study: fully understands and is informed about the study and has signed the informed consent form in writing; willing to comply with and able to complete all trial procedures.

2) Age: >=18 years; male or female.

3) Pathologically confirmed GBM.

4) Subtotal resection or recurrent/progressive disease 4-6 weeks after surgery (before radiotherapy).

5) Adequate organ and bone marrow function, with no severe hematopoietic dysfunction or cardiac, pulmonary, hepatic, renal dysfunction, or immunodeficiency: a) Complete blood count: absolute neutrophil count (ANC) >=1.5*10^9/L (1500/mm3), platelets >=75*10^9/L, hemoglobin >=9 g/dL (if there is bone marrow involvement, platelets >=50*10^9/L, ANC >=1.0*10^9/L, hemoglobin >=8 g/dL). b) Liver function: serum bilirubin <=1.5 times the upper limit of normal (ULN), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) <=1.5 times ULN (if there is liver involvement, AST and ALT <=5 times ULN are allowed). c) Renal function: serum creatinine <=1.5 times ULN. d) Coagulation function: INR <=1.5 times ULN; PT and APTT <=1.5 times ULN (unless the subject is receiving anticoagulant therapy and PT and APTT at screening are within the expected range for anticoagulant therapy).

6) Left ventricular ejection fraction (LVEF) >=50% on cardiac function examination.

7) Negative serum pregnancy test, and effective contraceptive measures from signing the informed consent form until 6 months after the last chemotherapy.

8) Thyroid-stimulating hormone (TSH), free thyroxine (FT4), or free triiodothyronine (FT3) within the normal range ±10%.

9) Ophthalmologic examination: including dilated fundus examination, slit-lamp examination, and color fundus photography.

3.3.2 Exclusion Criteria

Subjects meeting any of the following criteria cannot be enrolled in this study:

1) Currently participating in another clinical study, or less than 4 weeks since completion of treatment in a previous clinical study.

2) History of malignancy other than GBM within the past 3 years, or another uncured primary malignancy.

3) Prior history of brain radiotherapy.

4) Pregnant or lactating women.

5) Patients assessed as having contraindications to radiotherapy.

6) Severe active comorbidity that would affect the study treatment.

7) Active infection requiring systemic anti-infective treatment, including but not limited to bacterial, fungal, or viral infection.

8) Within 6 months before screening, New York Heart Association (NYHA) class III or IV heart failure, unstable angina, severe poorly controlled ventricular arrhythmia, or electrocardiographic evidence of acute ischemia or myocardial infarction.

9) QTcF interval >480 msec, unless secondary to bundle branch block.

10) Uncontrolled concomitant disease, including but not limited to uncontrolled hypertension, active peptic ulcer disease, or hemorrhagic disease.

11) Prior history of mental illness; lack of capacity for civil conduct or limited capacity for civil conduct.

12) Any medical history or disease evidence, treatment, or abnormal laboratory value that may interfere with trial results or prevent the subject from fully participating in the study, or any other condition that the investigator considers unsuitable for enrollment.

3.3.3 Discontinuation of Study Treatment

Subjects may discontinue treatment at any time for any reason, or the investigator may decide whether to discontinue treatment when any adverse event occurs. In addition, the investigator may discontinue treatment if the subject is unsuitable for treatment, violates the protocol, or for administrative and/or other safety reasons.

For any of the following reasons, a subject must discontinue treatment but may continue to be monitored in the study:

- The subject or the subject's legal representative requests discontinuation of treatment.
- An adverse event requiring treatment discontinuation as specified in the protocol occurs.
- An intercurrent illness occurs that precludes further treatment.
- The investigator decides to withdraw the subject from the study.
- The subject has a positive serum pregnancy test.
- The subject has poor compliance and fails to receive treatment on time; communication and coordination by the investigator are ineffective, or the deviation may cause a substantial bias in trial results and cannot be remedied.
- Based on the subject's disease status or personal circumstances, the investigator believes continued study treatment would place the subject at unnecessary risk.
- Completion of the protocol-specified treatment.

3.3.4 Follow-up of Withdrawn Cases

If the subject or the subject's legal representative withdraws informed consent to participate in the study, the subject must withdraw from the study. If the subject withdraws from the study, the subject will no longer receive treatment or

planned visits. With the subject's consent, survival follow-up may be conducted after withdrawal. If the subject is lost to follow-up, the subject must withdraw from the study.

Any patient who withdraws from this study for reasons other than progression should continue to undergo imaging assessments at the scheduled times to collect information on disease progression until radiographic disease progression, initiation of new antitumor therapy, withdrawal of informed consent, death, or study completion, whichever occurs first. After documenting disease progression, the investigator should contact the patient, the patient's family, or the patient's current treating physician by telephone at least once every month to collect long-term survival follow-up information.

4. Sample Size Calculation

This study aims to evaluate the median progression-free survival (mPFS) of the experimental group receiving intensified adjuvant therapy. Based on retrospective studies and preliminary data from our center, the expected mPFS of the Stupp group is 6.9 months, the 1-year PFS rate is 40%, and the experimental group is expected to be 70%. To ensure the reliability of the study results, we set alpha at 0.05 and beta at 0.10 to ensure sufficient statistical power to detect a difference between the two groups. Based on these parameters, 27 patients are required. Considering a 15% loss-to-follow-up rate, we plan to recruit 31 patients to ensure a sufficient sample size for the final analysis.

PASS 15.0.5 output (translated/preserved): Tests for One Proportion. Numeric results for testing one proportion using the exact test. Alternative hypothesis: two-sided (H0: P = P0 vs. H1: P ≠ P0). Power = 0.90429; n = 27; proportion given H0 P0 = 0.4000; proportion given H1 P1 = 0.7000; difference P1-P0 = 0.3000; alpha = 0.0500; reject H0 if R <= 5 | R >= 17. Power was computed using the normal approximation method.

5. Data Management and Confidentiality

5.1 Case Report Form

The primary purpose is to obtain the data required by the study protocol in a complete, accurate, clear, and timely manner. The data in the case report form should be consistent with the source documents. The case report form must be completed completely and clearly (using a black or blue ballpoint pen, in compliance with legal document requirements). The case report form is a regulatory document and must be suitable for submission to hospital authorities.

All revisions and corrections must be made and confirmed by the investigator, with the date of revision/correction indicated. Errors must remain clearly visible and must not be covered by corrected information (e.g., correction fluid must not be used). The investigator must indicate the reason for revising important data. Missing data/notes in the medical record should be represented in the case report form by striking through the blank entry, to avoid unnecessary follow-up investigation.

5.2 Study Documents and Retention

The investigator should maintain a file for the purpose of the study. This file should contain all relevant documents necessary for conducting the study. After the study is completed, these documents should be archived in accordance with hospital and national regulations.

5.3 Data Management and Data Traceability Requirements

The designed CRF is in triplicate (carbonless copy). The second copy of the CRF will be submitted to the data management personnel participating in this clinical trial to establish a unified database. After at least 5 CRFs are completed, they will be submitted promptly to the data manager through the clinical monitor to establish the corresponding database. All data will be entered in duplicate using a data entry program compiled with computer software.

During this period, query forms will be transferred to the investigator through the clinical monitor for data review, and the investigator should respond and return them as soon as possible. Locked data files cannot be changed. The database will be submitted to the statistical analysis personnel for statistical analysis according to the statistical analysis plan. The statistical analysis report will be submitted to the principal investigator of this trial to write the study report.

Investigators will be trained on relevant regulations and SOPs, and all investigators will strictly comply with them, ensuring that records are truthful, timely, accurate, and complete, preventing omissions and arbitrary modifications, and prohibiting falsification or fabrication of data. The monitor will strengthen verification of all records. Follow-up medical records of subjects, as source documents, should be retained together with the CRF. The laboratory department of each center will provide a certificate of participation in quality control by the Clinical Laboratory Center of the Ministry of Health, and inspection results will be stored in the computers connected to the testing equipment for at least three years for future review.

5.4 Data Confidentiality

All records related to subject identity will be kept confidential and will not be disclosed beyond the scope permitted by relevant laws and/or regulations.

6. Informed Consent

Relevant explanation of informed consent

1) Obtaining informed consent from patients: The study team will provide potential participants with detailed study information, including the study objectives, procedures, potential risks, and benefits. The informed consent process will be conducted in a private and quiet environment to ensure that patients are not under any pressure. Study personnel will answer all questions from patients and ensure that they fully understand the study. The informed consent form will be signed by the patient after the patient fully understands the study; if the patient is unable to sign personally, it will be signed by the legal guardian.

2) Measures for obtaining informed consent from vulnerable groups:

For patients without capacity, such as hospitalized patients with severe disease and patients with mental illness, the informed consent form will be signed by their legal guardian.

For illiterate individuals, oral consent will be used, and a witness will be present and sign to certify it.

For disadvantaged individuals such as prisoners and cognitively impaired persons, special attention will be paid to their autonomy, and it will be ensured that they are not subjected to any form of coercion or inducement.

For students and personnel of this hospital, it will be ensured that participation in the study is voluntary and that they will not obtain additional benefits due to participation. For patients with mental/cognitive disorders, a psychiatrist will evaluate whether they have self-awareness capacity and determine the method of obtaining informed consent accordingly.

Process of signing/obtaining the informed consent form

Provision of the informed consent form: The study team will provide potential participants with a written informed consent form, which will detail all relevant information about the study.

Explanation of informed consent: Study personnel will explain the contents of the informed consent form to participants item by item to ensure full understanding.

Questions and answers: Study personnel will give participants an opportunity to ask questions and will answer all questions during the explanation process.

Signing the informed consent form: After participants fully understand the study and agree to participate, they will be invited to sign and date the informed consent form.

Person responsible for obtaining informed consent: The informed consent form will be obtained by a trained research coordinator or the principal investigator (PI).

Time point and location: Informed consent will be obtained before the participant is enrolled in the study, at a designated location in the hospital.

Handling of special circumstances: For participants who cannot personally sign the informed consent form, appropriate measures such as video call or telephone recording will be taken to ensure that they can provide informed consent.

Throughout the study, we will respect each participant's rights and ensure that they can freely raise questions, opinions, or choose to withdraw from the study at any time. We will regularly review the informed consent process to ensure that it always complies with ethical standards and legal regulations.

7. Adverse Event Reporting

7.1 Definition

An AE refers to any unfavorable medical event that occurs in a clinical trial subject from the time the subject signs the informed consent form until 90 days after the last use of study treatment. Regardless of whether there is a causal relationship with the trial treatment, it will be determined as an adverse event.

7.2 Contents

1) Aggravation of a pre-existing (before entering the clinical trial) medical condition/disease (including worsening of symptoms, signs, or laboratory abnormalities).

2) Any newly occurring AE: any newly occurring unfavorable medical condition (including symptoms, signs, or newly diagnosed disease).

3) Abnormal clinically significant laboratory values or results.

Study personnel should record in detail any AE occurring in a subject, including: AE name and description of all related symptoms, time of occurrence, severity, relationship to the study drug, duration, measures taken, and final outcome and resolution.

7.3 Adverse Event Recording

The investigator should carefully observe any adverse reaction that occurs during the clinical study, require subjects to truthfully report changes in their condition after treatment, avoid leading questions, and record in detail in the CRF, including time of onset, symptoms, signs, severity, duration, laboratory test indicators, management methods, course, results, and concomitant medication use.

7.4 Criteria for Determining Severity of Adverse Events

Refer to the NCI-CTCAE version 5.0 grading criteria for drug-related AEs.

7.5 Criteria for Determining Relationship Between Adverse Events and Study Drug

AEs include all unexpected clinical manifestations. As long as these events occur after signing the informed consent form, regardless of whether they are related to the trial treatment, they should be reported as AEs. Any discomfort reported by the subject during treatment or any abnormal change in objective laboratory test indicators should be truthfully recorded, and the severity, duration, management measures, and outcome of the AE should be noted. The study physician should also comprehensively determine the relationship between the AE and the study drug, and assess the possible association between the AE and the trial treatment according to a five-level classification: definitely related, possibly related, possibly unrelated, definitely unrelated, and unable to assess. “Definitely related”, “possibly related”, and “unable to assess” are all classified as adverse drug reactions. The judgment criteria are shown in the following table:

Table 1. Criteria for Determining the Relationship Between AE and Trial Treatment

Grade	Judgment Criteria
Definitely related	The event occurs in a reasonable chronological sequence after treatment, and the event is consistent with the known reaction type of the suspected drug; or it improves after drug withdrawal and recurs after re-administration.
Possibly related	The event occurs in a reasonable chronological sequence after treatment, but the event is not consistent with the known reaction type of the suspected drug; the patient's clinical status or other treatment methods may also have caused the event.
Possibly unrelated	The event does not occur in a reasonable chronological sequence after treatment, and the event is not consistent with the known reaction type of the suspected drug; the patient's clinical status or other treatment methods may have caused the event.
Definitely unrelated	The event does not occur in a reasonable chronological sequence after treatment, and the event is not consistent with the known reaction type of the suspected drug; the patient's clinical status or other treatment methods may have caused the event; the event resolves after disease improvement or discontinuation of other treatment methods, and the event appears after repeated use of other treatment methods.
Unable to assess	There is no clear chronological relationship between the event and treatment; the event is similar to the known reaction type of the drug, and other concomitant drugs may also cause the corresponding event.

7.6 Serious Adverse Events and Recording and Reporting

1) Definition of SAE

SAE refers to a medical event occurring during a clinical trial that requires hospitalization or prolongation of hospitalization, causes disability, affects working ability, is life-threatening or results in death, or causes congenital malformation.

It includes the following unexpected medical events: events resulting in death; life-threatening events (defined as events in which the subject is at immediate risk of death at the time of the event); events requiring hospitalization or prolongation of hospitalization; events that may result in permanent or severe disability/incapacity/impact on working ability; congenital anomalies or birth defects; other important medical events (defined as events that endanger the subject or require intervention to prevent any of the above situations).

2) Disease progression

Disease progression is defined as deterioration of the subject's condition caused by the primary tumor targeted by the study treatment. It includes radiographic progression and progression of clinical symptoms and signs. New metastatic lesions relative to the primary tumor, or progression of pre-existing metastatic lesions, are considered disease progression. Events caused by symptoms and signs of disease progression that are life-threatening, require

hospitalization or prolongation of hospitalization, or result in permanent or severe disability/incapacity/impact on working ability, congenital anomaly or birth defect are not subject to expedited SAE reporting. Death caused by symptoms and signs of disease progression is subject to expedited SAE reporting as an SAE.

3) Other antitumor therapy

AE recording begins at the signing of informed consent and continues until 90 days after the last use of study drug. Within 90 days after the last dose, if the subject begins other antitumor drugs and a serious adverse event occurs, it must be reported.

4) Hospitalization

Adverse events that lead to hospitalization or prolongation of hospitalization during a clinical study should be considered SAEs. Any first admission to a medical institution meets this criterion. Hospitalization does not include the following: rehabilitation facilities; nursing homes; routine emergency room admission; same-day surgery (such as outpatient/same-day/ambulatory surgery); hospitalization or prolongation of hospitalization unrelated to AE worsening is not itself an SAE. For example: hospitalization due to a pre-existing disease without a new adverse event and without worsening of the pre-existing disease (e.g., to examine laboratory abnormalities that have persisted since before the trial); hospitalization for administrative reasons (e.g., annual routine physical examination); hospitalization specified in the study protocol during the clinical trial (e.g., procedures required by the protocol); elective hospitalization unrelated to adverse event worsening (e.g., elective surgery); scheduled treatment or surgery should be recorded in the entire protocol and/or the subject's individual baseline data.

Diagnostic or therapeutic invasive (e.g., surgical) or non-invasive procedures should not be reported as AEs, but the disease condition that led to the procedure should be reported if it meets the definition of an AE. For example, acute appendicitis occurring during the AE reporting period should be reported as an AE, whereas the appendectomy performed for it should be recorded as the treatment method for that AE.

5) SAE reporting system

The reporting period for SAE should begin when the subject signs the informed consent form and continue until 90 calendar days (including day 90) after the last use of the study drug. If an SAE occurs, whether as an initial report or a follow-up report, the investigator must immediately complete the “Serious Adverse Event Report Form for New Drug Clinical Research,” sign and date it, and report it within 24 hours of the investigator becoming aware of the event to the relevant provincial, autonomous regional, or municipal drug regulatory authority, NMPA (via EMS), the health administrative department (fax to the Medical Administration Bureau), notify the sponsor (by email), and report promptly to the ethics committee.

SAEs occurring more than 90 days after the last use of the study drug generally do not need to be reported unless suspected to be related to the study drug. SAEs should be recorded in detail, including symptoms, severity, relationship to the study drug, time of occurrence, time of management, measures taken, follow-up time and method, and outcome. If the investigator considers an SAE unrelated to the study drug but potentially related to study conditions (e.g., discontinuation of original treatment or comorbidities during the trial), this relationship should be described in detail in the narrative section of the SAE report form. If the intensity of an ongoing SAE or its relationship to the study drug changes, a follow-up report should be submitted immediately.

6) Follow-up of SAE

All SAEs should be followed until disappearance, improvement to baseline level or <= grade 1, or stabilization.

The investigator should follow up until the AE ends, the condition is stable, a reasonable explanation is obtained, the subject is lost to follow-up, or death occurs; follow-up information should be provided in a timely manner according to the sponsor's requirements.

Unless otherwise specified in the protocol, AE/SAE collection and follow-up usually begin after the subject signs the informed consent form, and every AE/SAE during the study should be tracked and followed up. At the end of the study,

the principles for collection and follow-up of AE/SAE occurring after the subject completes the last treatment may refer to the table below:

Category	Collection/Recording	Follow-up
Unrelated AE	90 days after end of treatment	90 days after end of treatment
Related AE	90 days after end of treatment	Until the event resolves, or returns to baseline status or stabilizes
Unrelated SAE	90 days after end of treatment	90 days after end of treatment
Related SAE	Indefinitely	Until the event resolves, or returns to baseline status or stabilizes

- All adverse events: take timely measures for management and record them in the case report form.
- Serious adverse events (SAEs): take timely measures for management, record them in the case report form, and the investigator will decide whether to discontinue or reduce the dose. Immediately report to the ethics committee, drug clinical trial institution, and sponsor, and report to the national and provincial food and drug administration within 24 hours. SAEs must be reported through the hospital intranet “Adverse Event and Near-Miss Non-Punitive Reporting System.” The specific process is shown below.

SAE reporting flowchart (translated from the original figure): Serious adverse event occurs (requires emergency treatment, prolongation of hospitalization, disability, affects working ability, life-threatening or death, causes congenital malformation, etc.) -> determine whether emergency treatment is required. If no emergency treatment is required: report within 24 hours. If emergency treatment is required: provide emergency treatment and report immediately -> Hospital Medical Department 3880, 3881 (note: night duty telephone: 660000) -> responsible proper management -> report to Ethics Committee 87783969, Sponsor/CRO, National Bureau for Monitoring Adverse Drug Reactions 010-88363228, Provincial Food and Drug Administration Registration Office 88903275 -> follow-up and summarize handling opinions -> report via the hospital intranet adverse event and near-miss non-punitive reporting system -> report to the competent hospital leadership.