A Prospective Single Arm Phase II Exploratory Study on the Combination of Whole Brain Radiotherapy, Thiotepa Intrathecal Injection and Systemic Treatment of Primary Diseases in the Treatment of Solid Tumor Leptomeningeal Metastasis

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1 Research background

Tumor meningeal metastasis (LM) refers to the metastasis that occurs when tumor cells spread to the subarachnoid space and spread to the meninges with the flow of cerebrospinal fluid (CSF). The occurrence of LM is less common than brain parenchymal metastasis, but more deadly. LM can occur in non small cell lung cancer (NSCLC), breast cancer (BC), melanoma and other malignant solid tumors. Compared to the 20% to 65% incidence of brain metastasis in NSCLC patients, the proportion of LM occurring in NSCLC patients is only 3% to 5%. However, the prognosis of LM patients is worse, and for untreated patients, the median overall survival (mOS) is only 6-8 weeks. With tumor treatment, the survival can be extended to several months The mOS of LM BC is approximately 3.5 to 4.4 months, LM NSCLC is 3 to 6 months, and LM melanoma is 1.7 to 2.5 months.

LM can produce clinical manifestations such as brain parenchymal involvement, meningeal irritation sign, cranial nerve involvement, and increased intracranial pressure. However, considering that most clinical manifestations of LM are non-specific and overlap with brain parenchymal metastasis, they are easily overlooked. The diagnosis and treatment of LM are extremely important but also challenging. According to the EANO-ESMO guidelines, imaging is one of the diagnostic criteria, but the positivity rate is low and the patient's condition becomes severe when imaging features are present. With the development of detection technology for CSF circulating tumor cell DNA (ctDNA), the early detection rate of LM has been improved by 2. The treatment of LM includes comprehensive treatment methods such as intrathecal drug therapy, systemic drug therapy, and local radiotherapy. The purpose of treating leptomeningeal metastasis is to improve survival rate and maintain quality of life by delaying the deterioration of the nervous system. However, due to the presence of the Blood Brain Barrier (BBB), systemic drugs are unable to exert their effects on the meninges. Although activated T cells can pass through BBB, the blood brain barrier located in the choroid plexus and

ependymal epithelial cells restrict T cell infiltration through tight junctions, thus limiting the action of immune checkpoint inhibitors on tumor cells in cerebrospinal fluid. In the BLOOM trial, EGFR tyrosine kinase inhibitors (EGFR TKIs), third-generation TKI ositinib, with its outstanding ability to penetrate BBB, improved the median progression free survival (mPFS) and mOS (11.0 months) of LM NSCLC patients with EGFR mutations (EGFRm), demonstrating preliminary potential. Another retrospective study confirmed that ositinib significantly improved OS in EGFRm LM NSCLC patients, outperforming first/second generation EGFR-TKIs6. Local radiotherapy is more suitable for nodular lesions. Therefore, intrathecal injection of drugs has the advantage of directly acting on the affected area, making it the best choice for LM. The administration routes include injecting chemotherapy drugs into the subarachnoid space through lumbar puncture and intraventricular chemotherapy through the Ommaya reservoir. Repeated lumbar puncture and intrathecal administration can lead to complications such as infection and bleeding at the puncture site. At the same time, intracranial hypertension is a contraindication for lumbar puncture, and these patients cannot receive effective treatment through lumbar puncture and intrathecal administration. Metastatic and disseminated lesions cannot be effectively treated. Compared to lumbar puncture, Ommaya fluid sacs can prevent the delivery of drugs into the epidural or subdural space, resulting in uniform drug distribution and better patient comfort. The revision rates are less than 7.4%, but in a study, it was found that the incidence of ventricular infections in reservoir revisions was as high as 18%. In addition to the difficulties in drug administration, due to the difficulty in establishing a mouse model of solid tumor meningeal metastasis and the limited research on drugs for intrathecal injection of LM, the main intrathecal injection chemotherapy drugs in clinical practice are methotrexate, liposome cytarabine, and thiotepa. Shandong Cancer Hospital shared a case of HER2 (+) LM BC patients who achieved a 16 month survival benefit through a regimen of pyrrolitinib+vinorelbine rhythm chemotherapy+intrathecal injection of methotrexate. For LM patients whose condition worsens after treatment with methotrexate, Thiotepa can be used as a salvage treatment. A retrospective study included 30 LM patients

who failed methotrexate treatment and received 10mg of intrathecal injection twice a week. Among them, 14 cases (47%) had cytological reactions to intrathecal injection of cetepete. In addition to chemotherapy drugs, other drugs have also been actively added to the experimental scope. A prospective phase I/II trial (chiCTr1800016615) included 30 EGFRm LM NSCLC patients who failed to receive EGFR-TKIs treatment, proving that such patients can obtain mOS10 up to 9 months through intrathecal injection of 50mg of the recommended dose of pemetrexed. Trastuzumab has also been evaluated in the phase I study of LM patients with Her2 positive breast cancer (NCT01373710). The mOS of patients who received intrathecal injection and intravenous injection of the PD-1 inhibitor nivolumab (NCT03025256) was 4.9 months. However, it is worth noting that four patients survived to 74 weeks, 115 weeks, 136 weeks, and 143 weeks (i.e. 2.7 years), exceeding the mOS 11 that patients can achieve in all LM clinical trials. The EANO-ESMO guidelines recommend intrathecal drug therapy if tumor cells are found in CSF, regardless of MRI findings. Radiation therapy is also one of the treatment methods, but the bone marrow suppression caused by whole brain and spinal cord radiation therapy is significant, with limited dosage, which leads to interruption of systemic treatment and poor prognosis. The combination regimen in this study can quickly control symptoms by intrathecal injection of chemotherapy drugs, while opening the blood-brain barrier through whole brain radiotherapy, combined with systemic treatment for the primary disease, to achieve better therapeutic effects.

Therefore, studying the effectiveness and safety of comprehensive treatment mainly consisting of whole brain radiotherapy combined with intrathecal injection of chemotherapy drugs for LM of different tumors can help guide clinical treatment decisions. This is a scientific problem that urgently needs to be solved in the field of cancer. Here, we conduct a single arm prospective exploratory study to provide treatment opportunities for more patients.

2 Research Objectives and Endpoints

2.1 Research Objectives

The aim of this prospective study is to evaluate the effectiveness and safety of whole brain radiotherapy combined with Thiotepa intrathecal injection for systemic treatment of primary diseases in patients with different solid tumor meningeal metastases in our hospital.

2.2 Primary endpoints

The primary safety analysis will be conducted based on subjects who experience toxicity (as defined by CTCAE standards). CTCAE version 5.0 will be used to evaluate safety through reported adverse events. The relationship between adverse events and drugs, onset time, duration of events, their resolution, and any concomitant medication will be recorded. Adverse events (AEs) will be analyzed, including but not limited to all AEs, SAEs, lethal AEs, and laboratory changes.

2.3 Secondary endpoints

overall survival (OS), progression free survival (PFS), 3-month OS rate, 6-month OS rate, 9-month OS rate, 12-month OS rate, the disease control rate (DCR), the objective response rate (ORR) (using RANO-LM criteria)

3 Research Plan

3.1 Research Design

This study is a prospective, one arm clinical study evaluating the efficacy of intrathecal injection of Thiotepa in patients with leptomeningeal metastases from different solid tumors in our hospital. This study plans to include 58 subjects in the intention to analyze (ITT) population for a duration of 2 years. Observe patient safety indicators and survival data during the research process.

The study is divided into three stages: screening period, intrathecal injection, and follow-up period. The screening period is 28 days before the first administration. Intrathecal injection: Subjects who meet the inclusion criteria but do not meet the exclusion criteria will undergo intrathecal injection after signing the informed consent form. The subjects received hippocampal avoiding whole brain radiotherapy (HA-WBRT). At the same time, the comprehensive treatment plan mainly includes intrathecal injection of Thiotepa, which is administered by qualified personnel (those holding training certificates in the radiotherapy department of our hospital). All eligible patients received intrathecal injection of Thiotepa twice a week for 3 week as induction treatment, followed by once every week as consolidation therapy for 6 week and then once bi-weekly as maintenance therapy, until progressive disease was observed or intolerance or adverse events (AEs) developed. Before each injection, cerebrospinal fluid pressure should be measured and an equal amount of cerebrospinal fluid containing the injection drug should be taken to detect cerebrospinal fluid routine, cerebrospinal fluid biochemistry, tumor markers, IgG and albumin content, exfoliated cells, and micro single-cell sequencing. After mixing with cerebrospinal fluid, inject slowly for 5-10 minutes. According to the Neurotumor Response Evaluation (RANO) - LM criteria for efficacy evaluation, if there is disease progression or serious adverse events, the study will be immediately withdrawn. Evaluate the efficacy and safety every 6 cycles according to RANO-LM criteria. Follow up period: After stopping the study treatment, the subjects enter the follow-up period. During the follow-up period, MRI enhanced scans will be performed every 3 months to observe PFS. For subjects who have not withdrawn their informed consent form, survival information (i.e. date and cause of death, subsequent tumor treatment, etc.) will be collected every month through telephone and/or clinical visits.

3.2 Research Steps

3.2.1 Screening period

The screening period starts from signing the informed consent form and ends with the start of the research process or the end of screening failure. Participants must sign an

informed consent form before proceeding with any screening procedures specified in the study.

Unless otherwise specified, the following screening should be completed within 28 days prior to the start of the study drug:

1) Sign an informed consent form.

2) Medical history collection, including past treatment history, current medical history, drug allergy history, concomitant diseases, and disease staging.

3) Collect adverse events and concomitant medications.

4) Physical examination, height, weight, vital signs (including body temperature, blood pressure, heart rate, breathing, blood oxygen saturation, and 5 minutes of sitting still).

5) ECOG score.

6) Blood, urine, and stool routine.

7) Blood biochemistry (including but not limited to ALT, AST, total bilirubin, direct bilirubin, indirect bilirubin, ALP, LDH, albumin, urea nitrogen or serum urea, creatinine, blood sugar, etc.) β 2 microglobulin).

8) Electrolytes (potassium, sodium, chlorine, calcium, magnesium, phosphorus).

9) Coagulation function (PT or INR), thyroid function, myocardial enzyme spectrum, serum creatine kinase, BNP.

10) Serum pregnancy test (only applicable to women of childbearing age).

11) Virological testing (including HBV, HCV, HDV, HIV, syphilis markers, and HBV-DNA).

12) Cardiac ultrasound (mainly observing LVEF).

13) 12 lead electrocardiogram (QTc interval time should be indicated).

14) PET/CT or enhanced CT of the neck, chest, abdomen, and pelvic cavity, as well as head MRI.

15) Cerebrospinal fluid pressure measurement, cerebrospinal fluid biochemistry, cerebrospinal fluid routine, cerebrospinal fluid shed cells, and cerebrospinal fluid tumor markers

3.2.2 Treatment period

Laboratory tests and vital sign measurements specified in the protocol must be conducted within 72 hours before each cycle of administration (for the first cycle, laboratory tests can be conducted within 7 days before administration), and adverse event evaluations must be recorded at each visit.

3.2.3 Treatment after leaving the group

Patients who are excluded from the PD group due to disease should consider other treatment options, and the specific treatment plan will be determined by the researcher.

3.2.4 Follow up period

After stopping the study and treatment (whichever occurs first), the subjects enter the follow-up period, which is divided into three parts:

1) Safety visit: A safety follow-up is required within 30 days after the last administration.

2) Imaging follow-up: Except for subjects who stopped taking medication due to disease progression determined by imaging evaluation, imaging evaluation is conducted every 3 months until imaging disease progression occurs, new anti-tumor treatment begins, informed consent is withdrawn, death occurs, or the end of this study (whichever occurs first).

3) Survival follow-up: Within 2 years after the last administration, the subject receives a survival visit every 3 months. Within 3-5 years after the end of treatment, the subject receives a follow-up visit every 6 months. For more than 5 years after the end of treatment, the subject receives a follow-up visit every 12 months until the subject dies, is lost to follow-up, or terminates the study (whichever occurs first). Researchers can inquire about the survival status (date and cause of death) of the subjects, their family members, or local physicians by phone, and collect information on other anti-tumor treatments after the end of the study treatment. The details of each survival follow-up should be recorded in the original medical record.

3.2.5 End of Study

The end of the study is defined as the date of the last follow-up. The final analysis of the main research endpoint will be conducted up to 24 weeks after the last

participant is enrolled in this study. The study will continue until the last data point of the last subject is collected. Researchers can terminate the study at any time.

3.3 Case selection

3.3.1 Inclusion criteria

1) Voluntary participation in clinical research: fully understand and be informed of this study, and sign a written informed consent form; Willing to follow and capable of completing all experimental procedures.

2) Age: ≥ 18 years old, both male and female are acceptable.

3) Solid tumor patients diagnosed with leptomeningeal metastasis according to the EANO-ESMO guidelines

4) Expected survival time is at least 3 months.

5) Adequate organ and bone marrow function, no severe hematopoietic dysfunction, heart, lung, liver, kidney dysfunction, or immune deficiency (no blood transfusion, granulocyte colony-stimulating factor, or other related medical support received within 14 days prior to the use of the study drug):

a) Blood routine: Absolute neutrophil count (ANC) ≥ 1.5 ′ 109/L (1500/mm3), platelets ≥ 75 ′ 109/L, hemoglobin ≥ 9 g/dL (if bone marrow is involved, platelets ≥ 50 ′ 109/L, ANC ≥ 1.0 ′ 109/L, hemoglobin ≥ 8 g/dL).

b) Liver function: Serum bilirubin ≤ 1.5 times the upper limit of normal value, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) ≤ 1.5 times the upper limit of normal value (AST is allowed if there is liver involvement, ALT ≤ 5 times the upper limit of normal value).

c) Renal function: Serum creatinine ≤ 1.5 times the upper limit of normal value.

d) Coagulation function: INR ≤ 1.5 times the upper limit of normal value; PT and APTT are ≤ 1.5 times the upper limit of normal values (unless the subject is receiving anticoagulant treatment and PT and APTT are within the expected range of anticoagulant treatment at the time of screening).

6) Left ventricular ejection fraction (LVEF) ≥ 50% in cardiac function examination.

7) The serum pregnancy test is negative, and effective contraceptive measures have been taken from the signing of the informed consent form until 6 months after the last chemotherapy.

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

9) Ophthalmic examination: including dilated pupil fundus examination, slit lamp examination, and fundus color photography.

3.3.2 Exclusion criteria

1) I am currently participating in other clinical studies, or my first study drug was administered less than 4 weeks after the end of the previous clinical study treatment.

2) Suffering from other malignant tumors in the past 5 years.

3) Patients who have received CNS guided therapy for prevention.

4) Patients with a known history of human immunodeficiency virus (HIV) infection and/or acquired immunodeficiency syndrome.

5) Patients with active autoimmune diseases or a history of autoimmune diseases with a high risk of recurrence, including but not limited to immune related neuropathy, multiple sclerosis, autoimmune (demyelinating) neuropathy, Guillain Barr é syndrome, myasthenia gravis, systemic lupus erythematosus, connective tissue diseases, scleroderma, inflammatory bowel cancer (including Crohn's disease and ulcerative colitis), autoimmune hepatitis, toxic epidermal necrolysis, or Stevens Johnson syndrome.

6) Patients with active chronic hepatitis B or active hepatitis C. Patients with positive hepatitis B surface antigen or hepatitis C virus antibody in screening period must further pass the hepatitis B virus DNA drop test (not more than 1000 iu/ml) and HCV RNA test (not more than the lower limit of the test method), and can be included in the test only after the active hepatitis B or hepatitis C infection requiring treatment is excluded. Hepatitis B virus carriers, hepatitis B patients who are stable after drug treatment and cured hepatitis C patients can be included in the group.

7) Suffer from active pulmonary tuberculosis.

8) Currently, there is interstitial lung disease or infectious pneumonia.

9) Active infections that require systematic anti infective treatment, including but not limited to bacterial, fungal, or viral infections.

10) Patients with heart failure, unstable angina, severe uncontrolled ventricular arrhythmias, acute ischemia or myocardial infarction as determined by the New York

Heart Association (NYHA) functional classification within the first 6 months of screening.

11) QTcF interval>480milliseconds, unless secondary to bundle branch block.

12) Suffering from uncontrollable comorbidities, including but not limited to uncontrolled hypertension, active peptic ulcers, or bleeding disorders.

13) Individuals with a history of mental illness in the past; Individuals without legal capacity or with limited legal capacity.

14) According to the researchers' assessment, the patient's underlying condition may increase their risk of receiving study drug treatment, or cause confusion regarding the occurrence of toxic reactions and their assessment.

15) Other researchers believe that patients who are not suitable to participate in this study.

3.3.3 Study drug discontinuation/withdrawal criteria

If any of the following occurs, the subject must discontinue the investigational drug:

1) AE related to treatment that meets the discontinuation criteria specified in the research protocol.

2) Disease progression.

3) During the study period, receiving any prohibited concomitant therapy may affect the assessment of safety and efficacy.

4) Any intermittent disease that prevents the subject from continuing to receive study drug treatment.

5) The subject is unwilling to continue the clinical study and may discontinue the investigational drug at any time with the consent of the researcher. According to the Helsinki Declaration and other applicable regulations, participants have the right to withdraw from this study at any time and for any reason, and researchers may not have any bias towards their future medical care.

6) The subjects had poor compliance, did not receive treatment on time, and communication and coordination with the researchers were ineffective, which may result in significant deviations in the trial results and cannot be remedied.

7) Other situations where researchers believe it is not appropriate to continue medication.

In any case, the primary reason for discontinuing the investigational drug will be recorded in the original medical record.

3.3.4 Follow up on withdrawal cases

Any patient who withdraws from this study for reasons other than progression should continue to undergo imaging evaluation at a predetermined time to collect information about disease progression, until imaging disease progression occurs, new anti-tumor treatment begins, informed consent is withdrawn, death occurs, or the study ends (whichever occurs first). After recording disease progression, researchers need to contact patients, their families, or their current treatment physicians by phone at least once a month to collect long-term follow-up information on survival.

4 Concomitant medication

4.1 Permissible drugs for use

1) Unconventional treatment (such as non anti-cancer Chinese herbal medicine or acupuncture and moxibustion), vitamins/trace elements are allowed to be used without affecting the observation of the study endpoint, and are controlled by the researcher.

2) When clinically significant bone marrow suppression occurs after treatment, therapeutic treatments such as colony stimulating factor, TPO, and blood transfusion can be used.

3) Subjects can use topical, ocular, intra-articular, intranasal, and inhaled corticosteroids.

4) Emergencies that occur during treatment allow patients to undergo surgery or other targeted treatments.

4.2 Research drug and dosage

In this study protocol, whole brain radiotherapy combined with intrathecal injection of 10mg of Thiotepa was administered twice a week.

5 Efficacy evaluation

It is recommended to undergo MRI and CSF testing during the screening period. The efficacy of intrathecal injection is evaluated every 3 months based on the Neuro Tumor Response Evaluation (RANO) - LM criteria.

6 Statistical analysis

6.1 Sample size estimation

The main endpoint of this study is safety. 58 patients are expected to be enrolled and their overall efficacy will be observed.

6.2 Analyzing the population

Full Analysis Set (FAS): An analysis set determined according to the principle of intention analysis. All subjects who received at least one medication record will be included in this analysis set. The full analysis set is the main effectiveness analysis set.

PPS: A subset of the complete analysis set. Subjects who violate the protocol and are judged to have a significant impact on the therapeutic effect will be excluded from this episode.

Safety Analysis Set (SS): All subjects who received intrathecal injection medication and had a post medication safety assessment.

6.3 Statistical analysis

6.3.1 Demography and Baseline

Demographic and baseline characteristics such as age, gender, baseline disease characteristics, ECOG physical condition, etc. will be summarized in the safety analysis set. The baseline measurement value refers to the last data obtained before the first administration of the study drug by the subject. Continuous data will be subjected to descriptive statistics (mean, median, standard deviation, and range), and frequency and percentage will be used as categorical data descriptions.

6.3.2 Efficacy analysis

1) Primary efficacy endpoint

Descriptive statistical analysis was used to summarize the exposure levels of the study drugs, including course of treatment, frequency of administration, etc.

According to NCI CTCAE v5.0, adverse events are classified. All adverse events that occurred during or after the first study drug administration will be summarized according to NCI CTCAE grading. In addition, serious adverse events, severe adverse events (grades 3 to 5), investigational drug related adverse events, and adverse events leading to the termination or change of investigational drug treatment will be summarized accordingly. Multiple occurrences of the same event will be recorded as one event based on the highest severity. The proportion of subjects who reported at least one adverse event. We will summarize the deaths reported during the treatment period and during the follow-up period after treatment completion/termination. Specific laboratory test results, vital signs, physical examinations, as well as 12 lead ECG test values and their relative baseline changes will be summarized. When applicable, the baseline check values and post baseline check values are presented in a cross tabular form.

2) Secondary efficacy endpoint

PFS refers to the time (in months) from the initial use of medication to the initial confirmation of disease progression or death (whichever comes first). When researchers evaluate disease progression analysis based on the 2005 IPCG criteria, subjects without disease progression or death will be excluded on the last tumor assessment date. Subjects without baseline tumor evaluation results will be deleted on the first day of medication plus one day. Use the Kaplan Meier method to obtain estimates of the survival function and median PFS, and use the Greenwood formula to estimate the error of the survival function, resulting in a 95% CI of the median PFS value. And draw the Kaplan Meier survival curve.

OS refers to the time from the initial use of medication to death, with no limitation on the cause of death. For subjects who did not report death before the data deadline, the last known survival date will be deleted. If the subjects do not have post baseline data, they will be deleted on the first day of medication plus one day. The analysis method is the same as PFS.

6.3.3 Analysis of cerebrospinal fluid indicators

Record the routine, biochemical, albumin content, IgG content, exfoliated cells, and micro single-cell detection data of cerebrospinal fluid before and after each treatment. The discarded cerebrospinal fluid from our laboratory can be used as a control.

7. Adverse event

7.1 Definition

AE refers to any adverse medical event that occurs 90 days after the last use of the investigational drug, regardless of whether there is a causal relationship with the investigational drug, starting from the signing of the informed consent form by the clinical trial subjects.

7.2 Content

1) The worsening of the original medical condition/disease (including symptoms, signs, and laboratory abnormalities before entering clinical trials);

2) Any newly occurring adverse medical condition (including symptoms, signs, newly diagnosed diseases);

3) Abnormal laboratory test values or results with clinical significance.

Researchers should provide detailed records of any adverse events that occurred in the subjects, including the name of the event and a description of all related symptoms, time of occurrence, severity, correlation with the investigational drug, duration, measures taken, and final outcomes and outcomes.

7.3 Record of Adverse Events

Researchers should carefully observe any adverse reactions that occur in subjects during clinical research, require subjects to truthfully reflect changes in their condition after medication, avoid inducing questioning, and record in detail in the case table CRF, including the time of occurrence of adverse reactions, symptoms, signs, degree, duration, laboratory test indicators, treatment methods, process, results, etc., as well as the situation of combined medication.

7.4 Criteria for determining the severity of adverse events

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

7.5 Judgment criteria for the relationship between adverse events and investigational drugs

AE includes all unexpected clinical manifestations, as long as these events occur after signing the informed consent form, regardless of whether they are related to the investigational drug or whether the drug is used, they should be reported as AE. During the treatment period, any discomfort or abnormal changes in objective laboratory test indicators reported by the subject should be truthfully recorded, and the severity, duration, treatment measures, and outcomes of the AE should be indicated. The research physician should also comprehensively determine the relationship between the AE and the investigational drug, and evaluate the possible association between the AE and the investigational drug using the "definitely related, possibly related, possibly unrelated, definitely unrelated, and unable to determine" five level classification method "Definitely related", "possibly related", and "undeterminable" are all listed as adverse drug reactions, and the criteria for determination are shown in the table below:

Table 1 Criteria for Judging the Relationship between AE and investigational Drugs

Grading	Judging criteria
Definitely related	The occurrence of the event conforms to a reasonable time sequence after medication, and the event conforms to the known reaction type of the suspected drug; Or improve after discontinuation of medication, and the event may occur again after repeated administration.
May be related.	The occurrence of the event conforms to a reasonable time sequence after medication, and the event does not match the known reaction type of the suspected drug; The patient's clinical status or other treatment methods may also contribute to this event.
May not be relevant	The occurrence of the event does not conform to the reasonable time sequence after medication, the event does not match the known reaction type of the suspected drug, and the patient's clinical status or other

	treatment methods may have caused the event.
Definitely unrelated	The occurrence of the event does not conform to the reasonable time sequence after medication, the event does not match the known reaction type of the suspected drug, the patient's clinical status or other treatment methods may cause the event, the event disappears after disease improvement or cessation of other treatment methods, and the event of repeated use of other treatment methods occurs.
Unable to evaluate	The occurrence of the event has no clear relationship with the time sequence after medication, and is similar to the known reaction type of the drug. At the same time, the use of other drugs may also cause corresponding events.

7.6 Serious adverse events and recording and reporting

1) Definition of SAE

SAE refers to medical events that occur during clinical trials that require hospitalization or prolonged hospitalization, disability, affect work ability, endanger life or death, and result in congenital malformations.

Including the following unexpected medical events: events leading to death; A life-threatening event (defined as the risk of immediate death of the subject at the time of the event); Events that require hospitalization or extended length of stay; Events that can lead to permanent or severe disability/functional impairment/impact on work ability; Congenital abnormalities or birth defects; Other important medical events (defined as events that harm the subject or require intervention to prevent the occurrence of any of the above situations).

2) Disease progression

Disease progression is defined as the deterioration of the subject's condition caused by the primary tumor targeted by the study medication. Including the progression of imaging and clinical symptoms and signs. The appearance of new metastases relative to the primary tumor or the progression of existing metastases are considered disease progression. Events that endanger life due to symptoms and signs

of disease progression, require hospitalization or extended hospital stay, or result in permanent or severe disability/functional impairment/impact on work ability, congenital abnormalities or birth defects are not reported as SAE for accelerated reporting. Death caused by symptoms and signs of disease progression is reported as accelerated SAE.

3) Perform other anti-tumor treatments

The AE record starts from the signing of the informed consent form until 90 days after the last use of the study drug. Within 90 days after the last administration, if the subject begins to use other tumor drugs and experiences serious adverse events, a report is required.

4) Hospitalization treatment

Adverse events that lead to hospitalization or prolonged hospital stay in clinical studies should be considered as SAEs. Any initial hospitalization by a medical institution meets this standard. Hospitalization does not include the following situations: rehabilitation institutions; Sanatorium; Routine emergency room admission; Same day surgery (such as outpatient/same day/non bed rest surgery); Hospitalization treatment or prolonged hospital stay that is not related to the deterioration of AE itself is not an SAE. For example, if admitted due to an existing disease, there have been no new adverse events or worsening of the existing disease (such as in order to check for laboratory test abnormalities that have persisted since the trial); Hospitalization for management reasons (such as annual routine physical examinations); Hospitalization specified in the trial protocol during the clinical trial period (such as operating according to the requirements of the trial protocol); Selective hospitalization unrelated to the worsening of adverse events (such as elective surgery); The scheduled treatment or surgery should be recorded in the entire trial protocol and/or individual baseline data of the subjects.

Diagnostic or therapeutic invasive (such as surgery) and non-invasive procedures should not be reported as AEs, but when the disease condition that caused this procedure meets the definition of AE, it should be reported. For example, acute appendicitis that occurred during the AE reporting period should be reported as AE,

and the appendectomy performed as a result should be recorded as the treatment method for that AE.

5) SAE's reporting system

The reporting period for SAE should start from the signing of the informed consent form by the subject and continue until 90 calendar days (including 90 days) after the last use of the study drug. If SAE occurs, whether it is the first or follow-up report, the researcher must immediately fill out the Serious Adverse Event Report Form for Clinical Research of New Drugs, sign and date it, and report it to the relevant provincial, autonomous region, or municipality drug regulatory department, NMPA (via EMS), health administrative department (fax to the Medical Administration Bureau), and notify the applicant (via email) within 24 hours of the researcher's knowledge, And report to the ethics committee in a timely manner.

SAE occurring 90 days after the last use of the investigational drug, unless suspected to be related to the investigational drug, is generally not reported. SAE should provide detailed records of symptoms, severity, correlation with the investigational drug, occurrence time, treatment time, measures taken, follow-up time and method, and outcome. If the researcher believes that a certain SAE is not related to the investigational drug but potentially related to the study conditions (such as termination of original treatment or complications during the trial), this relationship should be detailed in the narrative section of the SAE report form. If the intensity of an ongoing SAE or its relationship with the test drug changes, a follow-up report should be submitted immediately.

6）Follow up of SAE

All SAEs should be followed up until disappearance, remission to baseline level, or ≤ level 1, stable.

Researchers should follow up until the end of AE, stable status, reasonable explanation, loss of follow-up, or death; Follow up information should be provided in a timely manner according to the requirements of the sponsor.

If there are no special requirements in the protocol, AE/SAE collection and follow-up usually begin after the subject signs the informed consent form, and each AE/SAE must be followed up during the study period; At the end of the study, the

principles for collecting and following up on AE/SAE that occurred after the completion of the last medication by the subjects can be referred to in the table below:

Classify, collect	and record.	Follow up.
Unrelated AE.	90 days after the end of treatment.	End treatment for 90 days.
Related AEs.	90 days after the end of treatment.	Until the event is resolved or the situation stabilizes when it returns to baseline.
Unrelated SAE.	Unrelated SAE. 90 days after the end of treatment.	End treatment for 90 days.
Related SAE.	Unlimited.	Until the event is resolved and the condition or stability is restored to baseline.

8. Research management

8.1 Case Report Form

The main purpose is to obtain the necessary information for the research plan in a complete, accurate, clear, and timely manner. The data in the medical record report form should be consistent with the original file. The filling out of the medical record report form must be complete and clear (using black or blue ballpoint pens, in compliance with legal requirements). The medical record report form is a regulatory document that must be suitable for submission to hospital authorities.

All revisions and corrections must be carried out and confirmed by researchers, indicating the date of revision/correction. Errors must be clearly preserved and cannot be covered with corrected information (such as using correction fluid). The researcher must indicate the reasons for his/her revision of important data. Missing information/notes in the medical record should be replaced by underlined spaces in the medical report form to avoid unnecessary follow-up investigations.

8.2 Research Documents and Preservation

Researchers should have a document regarding the research objectives. This file should include all relevant documents necessary for conducting research. After the

research is completed, these documents should be archived in accordance with relevant regulations of the hospital and the country.

8.3 Regulations on Data Management and Data Traceability

The designed CRF is in triplicate (carbon free copy), and the second copy of the CRF is submitted to the data management personnel participating in this clinical trial to establish a unified database. After completing at least 5 CRFs, the trial will be promptly submitted to the data administrator through clinical monitors for the establishment of the corresponding database. All data will be entered in duplicate using computer software to develop a data entry program.

During this period, any questions will be forwarded to the researcher for data review by the clinical monitor, and the researcher should answer and return as soon as possible. The locked data file cannot be changed again. The database will be handed over to statistical analysts for statistical analysis in accordance with the requirements of the statistical plan. The statistical analysis report shall be submitted to the main researcher of this experiment to write the research report.

Provide training to researchers on relevant regulations and SOPs, and ensure that all researchers strictly adhere to them, recording truthfully, timely, accurately, and completely, preventing omissions and arbitrary modifications, and not forging or fabricating data; The inspector strengthens the verification of various records. The follow-up medical records of the subjects should be kept together with the CRF as raw data. Each central laboratory provides a certificate of conformity for participating in the quality control of the Clinical Laboratory Center of the Ministry of Health, and the test results are stored in the computer connected to the testing equipment for at least three years for future reference.

8.4 Quality control and assurance of clinical trials

Applicants and researchers should fulfill their respective responsibilities and strictly follow the clinical trial protocol, adopting standard operating procedures to ensure the quality control of the clinical trial and the implementation of the quality assurance system.

All observations and findings related to clinical trials should be verified, and quality control must be carried out at each stage of data processing to ensure data completeness, accuracy, authenticity, and reliability.

This study was commissioned by the drug regulatory department and the sponsor to conduct a systematic inspection of the activities and documents related to this clinical trial, in order to evaluate whether the trial was conducted in accordance with the trial protocol, standard operating procedures, and relevant regulatory requirements, and whether the trial data was recorded in a timely, truthful, accurate, and complete manner. The inspection should be carried out by personnel who are not directly involved in the clinical trial. This study is subject to inspection by the drug regulatory authorities on the tasks and execution status of the researchers and sponsors during the implementation of this trial.

9. Ethical and regulatory aspects

9.1 Responsibilities of Researchers

Researchers are responsible for ensuring that clinical studies are conducted according to the research protocol, ethical and ethical principles are based on the Helsinki Declaration (the World Medical Association Declaration of Helsinki, current revised version) and Good Clinical Practice (GCP) for drug trials, and in accordance with China

Adjust the national conditions appropriately. The above documents must be stated on the informed consent form of the subjects, which is a basic prerequisite for inclusion in clinical studies.

9.2 Subject Information

Signing a written informed consent form is a necessary prerequisite for patient selection. Before signing, researchers must provide sufficient information to the subjects. If it complies with hospital regulations, information can be provided to the subject by the designated executor appointed by the subject. In addition to written information, researchers or practitioners should verbally inform patients. Pay attention to choosing wording so that the subjects can fully and easily understand. Whenever important new information is discovered that involves the patient's informed consent form, the subject information form should be modified.

9.3 Informed consent form

Before engaging in all research related behaviors, a written consent form from the patient must be obtained to participate in the clinical study. The informed consent form needs to be personally signed by the patient and the designated executor/researcher, indicating the signing date.

Researchers should confirm again in the CRF whether to sign the consent form. The signed and dated informed consent form should be stored at the researcher's location and must be securely archived by the researcher for supervision, review, and inspection at all times.

The original copy of the informed consent form should be provided to the patient before conducting the study. If patients or legally recognized representatives are unable to read the informed consent form, a reliable and independent witness should attend the entire process of discussing the informed consent form. The selection of witnesses must ensure the patient's right to confidentiality. Reliable and independent witnesses should be independent individuals, rather than affiliated with research institutions or participants in the study. Family members or acquaintances are a suitable choice for independent witnesses. If possible, after the subject, patient, or legally recognized representative verbally agrees and signs the informed consent form, the witness should personally sign and date the informed consent form to prove that the information is accurate, because only the patient or legally recognized representative fully understands the content of the informed consent form is the true informed consent form.

9.4 Compensation for subjects

The drugs used in this study were all domestically marketed drugs. During the treatment process of the subjects, the researchers closely monitored the treatment-related adverse reactions and actively handled them according to clinical routine.

9.5 Ethics Committee

Researchers must provide all documents as required by the hospital ethics committee before starting the study. Before obtaining approval from the ethics committee, participants are not allowed to participate in the study.

10. Publication and Achievements

After completing the research summary, researchers can publish papers, participate in domestic and international academic conferences, and receive awards for clinical research papers. The first author and corresponding author are enjoyed by the Second Affiliated Hospital of Zhejiang University School of Medicine**.**

11. Treatment process

Table 1 Research Process Table

Research period	Screening period	Treatment period							Follow up period		
Treatment cycle or visit name.	screening period	1	2	3	4	5	6	Study treatment termination visit 1	Safety follow-up 2	Imaging follow-up 3	Survival follow-up 4
Visit time and window period	Day-28 to Day-1	Day ±3	Day ±3	Day ±3	Day ±3	Day ±3	Day ±3	7	30 days ± 3 days after the last treatment		Every 12 weeks ± 7 days after the last treatment
Research management process											
Informed consent form	×										
Emission standards	×										
Demography and	×										

medical history											
Previous treatment and concomitant medication 5	×	×	×	×	×	×	×	×	×	×	
Clinical operation evaluation											
Adverse Event 6	×	×	×	×	×	×	×	×	×	×	×
Twelve lead electrocardiogram 7	×	×	×	×	×	×	×	×	×		
Height, weight, and vital signs 8	×	×	×	×	×	×	×	×	×		
Physic	×	×	×	×	×	×	×	×	×		

al examination and ECOG score 9											
Subsequent anti-tumor treatment									×	×	×
existence									×	×	×
Research treatment											
Chemotherapy 10		×	×	×	×	×	×				
Laboratory operations/evaluations											
Blood routine, biochemistry, stool and urine routine 11	×		×	×	×	×	×	×	×		

coagulation12	×		×	×	×	×	×				
Infectious Diseases 13	×										
EBV--DNA14	×		×	×	×	×	×	×			
HBV-DNA15	×		×	×	×	×	×	×			
HCV-RNA16	×										
thyroid function	×		×	×	×	×	×				
Myocardial enzyme spectrum, creatine kinase, BNP	×		×	×	×	×	×				

	×		×	×	×	×	×				
pathology	×										
Efficacy evaluation											
Imaging Examination 17	×		×		×		×			×	
Detection of circulating tumor cells in cerebrospinal fluid 18	×										

1. Study treatment termination visit: The date of study treatment termination is defined as the date on which the researcher confirms that the subject needs to terminate the study treatment for any reason. The time window for study treatment termination visit is 7 days from this date.

2. Safety follow-up: The time window for safety follow-up is 30 days+-3 days after the last study treatment; If the termination of the study treatment visit occurs within the time window of safety follow-up, the same examination items do not need to be repeated.

3. Imaging follow-up: Except for subjects who stopped taking medication due to disease progression determined by imaging evaluation, imaging evaluation will continue at the established time until imaging disease progression occurs, new anti-tumor treatment begins, informed consent is withdrawn, death occurs, or the study ends (whichever occurs first).

4. Survival follow-up: Follow up the survival status of the subjects by phone in the 12th week after the last study treatment.

5. Combination medication: Record the combination medication within 30 days prior to the screening visit until imaging follow-up is completed.

6. Adverse events: Starting from the signing of the informed consent form, all AEs and SAEs are recorded until 90 days after the last study treatment or the start of new anti-tumor treatment (whichever occurs first), after which only AEs and SAEs related to the study treatment are recorded. AE after safety follow-up visits can be recorded through telephone inquiries on the 60th and 90th days after the last treatment.

7. Electrocardiogram: The electrocardiogram will be analyzed during the screening period, treatment period, study treatment termination visit, and safety follow-up. If necessary, an electrocardiogram examination can be added.

8. Height, weight, vital signs: will be conducted during the screening period, treatment period, study treatment termination visit, and safety follow-up.

9. Physical examination and ECOG score: During the screening period, at the end of the study treatment visit and safety follow-up, a full body physical examination will be conducted. During the treatment period, a lymphoma specific physical examination will be conducted.

10. Research treatment: Administration should start on the first day of each cycle after all clinical and laboratory procedures/evaluations have been completed.

11. Blood routine, blood biochemistry, stool and urine routine: Blood routine examination must include whole blood cell classification count and hemoglobin; The blood biochemistry test items must include urea nitrogen, creatinine, sodium, potassium, magnesium, chlorine, calcium, phosphorus, total bilirubin, direct bilirubin, indirect bilirubin, ALT, AST, lactate dehydrogenase, total cholesterol, total protein,

and albumin; Screening period (within 7 days prior to initial administration), within 3 days prior to weekly administration, study treatment termination visits, and safety follow-up will be conducted.

12. Coagulation function: Coagulation function testing should be conducted during the screening and treatment periods.

13. Infectious disease detection: hepatitis B, hepatitis C, HIV, syphilis and other antibodies were detected during the screening period.

14. EBV-DNA testing: EBV-DNA testing will be conducted during the screening period, treatment period, and study treatment termination visit.

15. HBV-DNA test: Regardless of the results of infectious disease test during the screening period, HBV-DNA test must be carried out during the screening period, the treatment period, and the termination of the study treatment visit. For patients with positive hepatitis B B surface antigen or positive hepatitis B core antibody, the follow-up period should be reviewed once a month.

16. HCV-RNA testing: HCV antibody positive subjects must undergo HCV-RNA testing during the screening period, followed by monthly follow-up.

17. Imaging examination: PET/CT examination is recommended during the screening period, after the 4th and 6th cycles of chemotherapy. After the second cycle, it is recommended to perform CT enhanced examination of the lesion site. During the follow-up period, except for subjects who stopped taking medication due to disease progression determined by imaging evaluation, CT enhancement examinations will be performed every 3 months until imaging disease progression occurs, new anti-tumor treatment begins, informed consent is withdrawn, death occurs, or the end of this study (whichever occurs first). If PET/CT examination is not possible, head MRI and whole body enhanced CT examination should be performed instead.

18.Testing of cerebrospinal fluid tumor markers, protein content, IgG and albumin content, albumin index, circulating tumor cells, and micro single-cell sequencing.

19. Retain blood samples: for biomarker exploration.