A prospective cohort observational study of whole-brain radiotherapy combined with immunotherapy for brain injury

Study Protocol

(Observational Study)

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Lead Unit:	
Research period:	
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II.Protocol Summary

This study aims to investigate the scientific controversies, potential mechanisms, and cutting-edge research on prophylactic cranial irradiation (PCI) in the treatment of

small cell lung cancer (SCLC) during the immunotherapy era. Patients diagnosed with SCLC are recruited and divided into limited-stage and extensive-stage groups according to the Veterans Administration Lung Study Group (VALG) staging system. Patients with extensive-stage disease are assigned to the observation group, while those with limited-stage disease serve as the control group. The drug treatment plan was carried out in accordance with the guidelines of (National Comprehensive Cancer Network, NCCN) and the standard first-line treatment protocols for small cell lung cancer in our department. For patients in the initial limited-stage who are not suitable for surgery, the first-line treatment consists of chemotherapy with paclitaxel and carboplatin, combined with or followed by radiotherapy.. Patients achieving complete or partial remission (CR/PR) receive prophylactic cranial irradiation (PCI), followed by immune checkpoint inhibitor (ICI) consolidation therapy if no progression occurs. For extensive-stage patients without local symptoms or brain metastases, the preferred initial treatment is ICI combined with EC/EP chemotherapy. Asymptomatic patients receive systemic therapy first, followed by whole-brain radiotherapy.Through clinical observations of magnetic resonance imaging and behavioral studies on two groups of patients, the impact of radiotherapy combined with immunotherapy on brain damage was evaluated. The novelty of this study lies in its prospective investigation of the "double-edged sword" effect of combined radiotherapy and immunotherapy. Theoretically, the immunogenic cell death induced by radiotherapy can synergize with immunotherapy to produce a "distal effect". However, this powerful immune activation may become out of control, leading to an excessive inflammatory response that exacerbates brain tissue necrosis within the radiotherapy field and may even trigger autoimmune reactions that attack normal brain tissue.

III. Research Background

Small cell lung cancer (SCLC) has a relatively high probability of brain metastasis. Therefore, prophylactic cranial irradiation (PCI) holds a unique position in the treatment of small cell lung cancer. Current evidence-based medical research indicates that for patients whose therapeutic efficacy after combined radiotherapy and chemotherapy has reached complete or partial remission, performing prophylactic

cranial irradiation on the entire brain can significantly reduce the incidence of brain metastasis and bring survival benefits [1]. PCI refers to brain radiotherapy conducted before the appearance of imaging results or clinical symptoms. Its essence should be to eliminate any tiny metastatic lesions that may already exist in the brain but have not yet been detected[2]. Numerous studies have demonstrated that PCI reduces brain metastasis incidence and improves long-term survival in limited-stage SCLC patients who achieve CR or PR after chemoradiotherapy [3]. For early-stage SCLC patients undergoing surgery, PCI is recommended for those with postoperative stage II or higher; however, the role of PCI in stage I patients and its efficacy in extensive-stage SCLC remain controversial. Many clinical trials indicate that PCI improves survival and reduces the likelihood of subsequent brain metastasis in SCLC patients [4]. Conversely, some studies suggest that although PCI reduces intracranial metastasis risk when brain MRI excludes metastases, it does not confer survival benefits [5]. Moreover, radiotherapy carries non-negligible treatment-related toxicities, such as radiation-induced brain injury (RIBI). RIBI refers specifically to pathological changes in brain tissue following exposure to ionizing radiation. Its pathological manifestations include hippocampal neuronal loss, white matter fiber demyelination, microvascular abnormalities, focal neurological deficits, and increased intracranial pressure. During long-term follow-up, most patients receiving cranial radiotherapy experience a progressive decline in neurocognitive function [6], manifesting as impaired learning and memory, distractibility, slowed information processing, executive dysfunction, and fine motor coordination disorders, which severely compromise long-term quality of life. Acute and early delayed injuries typically occur within days to months after irradiation, with clinical manifestations including headache, nausea, vomiting, mood swings, and recent memory impairment. Severe cases may rapidly progress to decreased consciousness. Late delayed RIBI, the most common clinical type, usually emerges gradually 6 months after radiotherapy completion. Imaging reveals irreversible structural damage, such as cerebral edema, focal brain necrosis, and cystic lesions. Severe cases can lead to brain herniation due to elevated intracranial pressure, resulting in coma or even death.

In the era of immunotherapy, the drug treatment model combining chemotherapy with immunotherapy has brought significant survival benefits to patients with small cell lung cancer. Multiple clinical studies have confirmed the definite efficacy of immunotherapy in the field of small cell lung cancer. Immunotherapy has become the first-line drug treatment option for extensive-stage small cell lung cancer and the consolidation treatment mode after concurrent/synchronous radiotherapy for limited-stage small cell lung cancer [7]. Immunotherapy is a treatment strategy that enhances anti-tumor immune responses by mobilizing the host's own immune system. Radiotherapy can reshape the tumor immune microenvironment and, when combined with immunotherapy, can produce a synergistic anti-tumor effect. Numerous studies have confirmed that combined immunotherapy with radiotherapy for the primary tumor in the chest can improve the local control rate and survival rate of small cell lung cancer. However, the timing of chest radiotherapy intervention, the dose of chest radiotherapy, and the fractionation method need to be further explored, and the safety needs to be further evaluated [8]. Adding thoracic radiotherapy to the first-line immunotherapy has become an important research direction for the first-line treatment of SCLC. However, the efficacy and degree of brain damage of whole-brain radiotherapy when combined with immunotherapy have not yet been verified. In the immunotherapy era, the value of PCI in SCLC management is still debated, and safety data for PCI combined with immunotherapy are very limited. Two clinical trials are currently underway in Europe — EORTC-1901 (NCT04790253) and SWOG 1827 (NCT04155034)—to investigate the role of PCI in the immunotherapy era. However, these studies focus on whether MRI surveillance can replace PCI and on hippocampal protection. The ADRIATIC study, published in 2024, reported that 54% (n=285/530) of patients in the limited-stage SCLC cohort receiving PD-L1 inhibitors underwent PCI. Compared with patients not receiving PCI, the PCI group had significantly higher 3-year overall survival and progression-free survival (PFS). The incidence of grade $\geqslant 3$ serious adverse events was 28.4% and 19.8% in the two groups, respectively [9]. Patients receiving PCI experienced a higher incidence of grade 3 – 4 adverse events. However, this study did not pre-specify subgroup analyses for

efficacy and safety comparing PCI recipients versus non-recipients within the immune consolidation therapy group. Therefore, the mechanisms and safety profile of PCI in the immunotherapy context require further elucidation through prospective clinical studies.

1. Mechanisms of Radiation-Induced Brain Injury

The precise mechanisms of RIBI are not fully understood. Current evidence suggests that they involve complex, dynamic interactions among multiple injury mechanisms and cell types. These include the vascular injury hypothesis, inflammatory response hypothesis, neurological dysfunction hypothesis, neuronal and glial cell injury hypothesis, and oxidative stress hypothesis. As immunotherapy combined with radiotherapy gains prominence in clinical oncology, in-depth investigation of the pathophysiological mechanisms underlying brain injury in this treatment modality holds significant scientific and clinical value.

The direct effect of radiotherapy is that high-energy rays directly ionize or excite the key biological molecules within cells, causing direct damage to DNA; while the indirect effect is that high-energy rays act on water molecules, which are the most abundant in cells. The irradiation of water molecules leads to the generation of a large number of highly reactive oxygen free radicals. These free radicals attack and damage the surrounding biological macromolecules.Radiation-induced reactive oxygen species (ROS) cause widespread oxidative damage to cells, primarily targeting: ①DNA damage; ②lipid peroxidation; ③protein oxidation; ④mitochondrial damage; ⑤activation of signaling pathways. ROS play a dual role in radiotherapy-induced damage. Radiotherapy relies on ROS to inflict irreversible damage to key molecules such as DNA in tumor cells, thereby killing them. The presence of oxygen significantly enhances radiotherapy efficacy, whereas hypoxic tumor cells are relatively resistant. However, radiation beams inevitably pass through and irradiate normal tissues surrounding the tumor. In these tissues, substantial ROS are also generated, causing damage to DNA, lipids, and proteins in normal cells. This leads to acute reactions (e.g., radiation dermatitis, esophagitis, pneumonitis) and late complications (e.g., fibrosis, necrosis, secondary malignancies). Thus, ROS serve as a

central driver in the pathophysiology of radiotherapy damage.

2. Whether Radiotherapy Combined with Immunotherapy Exacerbates Brain Injury

The theoretical basis for combining local radiotherapy with immunotherapy is to achieve a synergistic effect. Radiotherapy directly ablates tumor cells via high-energy rays while simultaneously inducing immunogenic cell death (ICD).And immunotherapy works by removing the immune system's recognition of cancer cells, allowing the body's immune system to more effectively identify and attack cancer cells.In the ideal treatment scenario, the combined therapy of the two approaches aims to release tumor-associated antigens through radiotherapy, thereby activating the body's specific immunity. This not only enables the therapy to target cancer cells in the radiotherapy area but also achieves a "distant effect" by attacking other metastatic sites throughout the body.However, the risk is that this powerful synergy may become dysregulated and lose discrimination between tumor and normal tissues. When immune activation triggers an excessive inflammatory response, activated T cells and inflammatory factors can substantially amplify the pre-existing inflammatory response within the irradiated area. This excessive inflammation may attack not only cancer cells but also normal brain tissue in the field, causing vascular damage, edema, and necrosis, thereby markedly worsening radiation-induced brain injury (radiation necrosis). Furthermore, the activated immune system may mistakenly identify components of normal brain tissue as targets, triggering autoimmune reactions such as autoimmune encephalitis, even in non-irradiated areas. The blood-brain barrier (BBB) effectively restricts harmful blood-borne substances from entering the brain parenchyma [10]. Ionizing radiation can directly cause DNA damage to endothelial cells, inducing apoptosis. Acute-phase injury primarily manifests as BBB disruption, leading to increased vascular permeability, vasogenic edema, plasma protein extravasation, and elevated intracranial pressure. BBB damage further activates an inflammatory cascade, including peripheral immune cell infiltration into the brain parenchyma, release of pro-inflammatory cytokines (e.g., TNF-α, IL-1β, IL-6) and chemokines, and sustained microglial activation. These processes collectively initiate

and sustain acute and chronic neuroinflammation [11].

Microglia (MG), the innate immune effector cells of the central nervous system, play a crucial role in brain injury. They can polarize into M1 or M2 phenotypes, possessing bidirectional immunomodulatory functions [12]. In the acute phase of injury, they may exacerbate BBB permeability by releasing inflammatory mediators. In the subacute phase, they may shift toward a neuroprotective phenotype by reducing oxidative stress and secreting anti-inflammatory factors. Intracranial irradiation rapidly activates microglia, mediating acute-phase cytokine responses and releasing various chemokines. Among these, CCL2/MCP-1 upregulates endothelial adhesion molecule expression and stimulates immune cells to release core inflammatory mediators such as TNF-α, nitric oxide (NO), and IL-1β. It also activates key transcription factors including AP-1, NF-κB, STATs, MAPK, and CREB [13]. Microglial activation involves multiple signaling pathways, such as Toll-like receptor, MAPK, Notch, glycogen synthase kinase-3β (GSK), and NF-κB pathways. Signal transduction through these pathways further induces reactive astrogliosis. Astrocytes can secrete neurotrophic factors and growth factors to promote neuronal survival, but they can also produce inflammatory cytokines that exacerbate neuroinflammation and disrupt vascular structures, thereby worsening nerve injury [14].

Beyond inflammatory factors, peripheral immune cells of the adaptive immune system—especially CD8+ T lymphocytes—also play a significant role in neurological disease progression. Studies show that CD8+ T cell infiltration in the brain parenchyma increases markedly in patients with RIBI. Microglia-derived CCL2/CCL8 chemokines can mediate CD8+ T cell migration into the brain [15] and contribute to tissue damage [16] via CCR2/CCR5 receptors. Pro-inflammatory microglial subtypes highly express CCL2 and CCL8, recruiting cytotoxic CD8+ T cells into ischemic brain areas, which causes axonal damage and myelin destruction, leading to neuronal degeneration. Concurrently, activation of interferon signaling pathways in activated microglia further amplifies inflammatory responses, creating a positive feedback loop that exacerbates cellular damage and neuroinflammation in the brain. Under normal physiological conditions, white blood cells are rarely distributed

in the brain tissue. However, after brain injury leads to an increase in the permeability of the blood-brain barrier and the upregulation of chemokines such as intercellular cell adhesion molecule-1 (ICAM-1) that attract white blood cells, peripheral white blood cells can be massively recruited into the brain to participate in the neuroinflammatory process. During brain injury, the expression of chemokine receptors and their ligands increases, and the chemokines released by activated microglia can further enhance the permeability of the blood-brain barrier and promote the transmigration of immune cells such as macrophages across the barrier [17]. Throughout the injury process, neurons and their microenvironmental cells—microglia, astrocytes, and pericytes—interact by secreting inflammatory mediators, jointly driving inflammation. Neurons undergo morphological changes after injury, leading to dysfunction. Dendritic spines, critical structures for excitatory synaptic transmission, directly influence synaptic plasticity and function through their density and morphology. Studies confirm that radiation reduces dendritic spine number and density and decreases dendritic complexity [18]. In the inflammatory microenvironment after radiation, there are also changes in the composition of synaptic proteins, including the upregulation of postsynaptic density protein-95 (PSD-95) in the dentate gyrus region and the downregulation of presynaptic protein synaptophysin in the pulmonary hilum region. These changes collectively have a profound impact on dendritic plasticity. It is particularly noteworthy that radiation causes a decrease in the expression of the ionotropic glutamate receptor GluR1 in the hippocampus. The AMPA receptors, which are involved in rapid excitatory synaptic transmission in the central nervous system and participate in the induction and maintenance of long-term potentiation effects, are mediated by the postsynaptic membrane. The absence of the GluR1 subunit may lead to a reduction in extrasynaptic AMPA receptors and a decrease in synaptic efficacy, thereby weakening basic neuronal functions such as action potential propagation and information processing. This is considered to be one of the direct mechanisms by which radiation causes neuronal dysfunction [19].

Our research group has long been dedicated to studying the mechanisms and

protection of normal tissue damage caused by radiotherapy combined with immunotherapy. By establishing mouse models of radiation-induced heart and lung injury and intervening with PD-1 inhibitors, we have conducted a series of systematic investigations. Our previous work constructed a clear trajectory from clinical problems to molecular mechanisms: combined radiotherapy and PD-1 inhibitors, through key events such as HMGB1 release [20] and GSDMD-mediated pyroptosis [21], synergistically activate downstream pro-fibrotic pathways like TGF-β1/Smads. This process relies on CD8+ T lymphocyte infiltration and amplification by inflammatory factors (e.g., IL-6, IL-18) [22], ultimately leading to severe fibrosis and dysfunction of heart and lung tissues. These findings not only reveal a common mechanism of combined treatment toxicity but also suggest potential intervention strategies, such as targeting HMGB1 or inhibiting pyroptosis.

IV. Research Objectives

To investigate the mechanisms and protection of normal tissue damage induced by radiotherapy combined with immunotherapy. This study aims to elucidate the common molecular mechanisms underlying normal tissue injury caused by this combination and propose intervention strategies with translational potential, thereby providing a solid theoretical foundation and experimental basis for future research and clinical protection.

V. Trial Design

This study is a prospective cohort observational study. The patients were divided into the observation group and the control group based on the disease stage at the time of enrollment. The follow-up observation period was conducted, and the occurrence of the expected outcomes to be studied in each group was examined and registered. The incidence rates of the outcomes in each group were compared, thereby evaluating and testing the relationship between risk factors and outcomes.

VI. Research Methods

(1) Study Subjects

The study plans to enroll patients diagnosed with lung cancer. Participants are divided into observation and control groups according to disease stage at enrollment.

The occurrence, severity, and clinical efficacy of neurological damage are compared between the two groups to verify whether whole-brain radiotherapy combined with immunotherapy exacerbates neurological impairment.

(2) Sample Size Calculation

This prospective cohort observational study includes an observation group (PCI combined with immunotherapy) and a control group (PCI alone). The outcome is the incidence of treatment-induced brain injury. Based on relevant literature, the incidence of Fazekas grade 3 changes is 10% in the control group and is expected to be 20% in the experimental group [23–25]. Using biostatistical methods with a significance level (α) of 0.05, power of 80%, and margin (Δ) of -0.2, the calculated sample size is 18 cases per group. Accounting for a 20% loss to follow-up or refusal rate, at least 21 participants are required for each group, totaling a minimum of 42 subjects.

(3) Inclusion, Exclusion, and Withdrawal Criteria

1. Inclusion Criteria

(1) Histologically or cytologically confirmed limited-stage or extensive-stage SCLC, meeting indications for PCI (limited-stage patients achieving CR/PR after first-line treatment; extensive-stage patients with effective tumor control after systemic therapy and no clear evidence of brain metastasis).

(2) Age 18–75 years, Karnofsky Performance Status (KPS) score ≥70.

(3) Expected survival ≥6 months and able to complete at least 12 months of follow-up.

(4) No contraindications to radiotherapy, chemotherapy, or immunotherapy.

(5) Good compliance with the treatment protocol and follow-up, and provision of informed consent.

2. Exclusion Criteria

(1) MRI-confirmed brain metastases, cerebral infarction, cerebral hemorrhage, sequelae of traumatic brain injury, or other primary brain diseases (e.g., Alzheimer's disease, multiple sclerosis) before enrollment.

(2) Previous brain radiotherapy, brain surgery, or immunotherapy.

(3) Mental illness or cognitive dysfunction (Mini-Mental State Examination [MMSE] score <26) preventing cooperation with follow-up assessments.

(4) Concurrent other malignancies during or before treatment.

(5) Severe cardiac, pulmonary, or renal diseases; active autoimmune diseases; or uncontrolled active infections.

(6) Pregnant or lactating women.

3. Withdrawal Criteria

(1) The participant requests to withdraw from the study.

(2) For patients in the limited-stage, if extracranial lesions progress, a systemic treatment plan needs to be changed; for patients in the extensive-stage, if brain metastases or other organ metastases occur, palliative radiotherapy (such as SRS) or a change in systemic treatment is required; for patients with confirmed radiological signs of radiation necrosis or other severe brain lesions, glucocorticoid shock, bevacizumab, or surgical intervention is necessary.

(3) Occurrence of fatal or irreversible adverse events (AE) or serious adverse events (SAE), including but not limited to: fatal hematological toxicity; fatal or irreversible non-hematological toxicity (such as immune-related pneumonia, hepatitis, enteritis, or neurological toxicity); death or permanent neurological dysfunction due to intracranial hypertension, epilepsy, or consciousness disorders, and the investigator assesses that continuing participation in the study will increase the risk.

(4) Research Protocol

1.Anti-tumor Drug Treatment: First-line chemotherapy consists of etoposide plus platinum combined with PD-1/PD-L1 inhibitors. Immunotherapy agents are PD-1/PD-L1 inhibitors approved for SCLC. Dosage and cycle follow drug labels (for limited-stage, used as consolidation after concurrent chemoradiotherapy; for extensive-stage, used concurrently with chemotherapy and continued as consolidation). Cisplatin 75–100 mg/m² IV on day 1, or carboplatin 300–350 mg/m² IV on day 1, or lobaplatin 30 mg/m² IV on day 1; etoposide 100 mg/m² IV on days 1–3. Each cycle is 21–28 days. Supportive care includes antiemetics, hydration, and allergy prophylaxis. At least two chemotherapy cycles must be completed during

radiotherapy.

2.Radiotherapy Plan:

(1)Positioning: Supine position with thermoplastic mask fixation. Contrast-enhanced CT scan from cranial vertex to lower C1, slice thickness 3 mm. MRI T1 images are fused with CT in the planning system.

(2)**Target Delineation:** Whole-brain radiotherapy defines the entire brain parenchyma as the clinical target volume (CTV). The planning target volume (PTV) is generated by expanding the CTV by 3 mm in all directions.According to the requirements of the Radiation Therapy Oncology Group (RTOG) 0933, the hippocampal protection area is the granular cell subregion of the dentate gyrus and should be expanded outward by 5 mm. The CTV should exclude the hippocampal avoidance zone.

(3) **Planning and Evaluation:**ANAC Pinnacle treatment planning system with 5 coplanar or non-coplanar beams. Prescription: 100% dose covers 100% GTV; 95% dose covers 100% CTV; 90% dose covers 98%–100% PTV.

(4) Using 6MV X-rays and volumetric modulated arc therapy (VMAT), the prescription dose for the PTV is 30 Gy in 10 fractions (5 fractions per week). The radiotherapy should be initiated after 2 cycles of chemotherapy when the efficacy of the drug treatment has been evaluated.

3.Experimental Design:

Observation Group: Conventional chemotherapy per first-line SCLC regimen combined with immunotherapy. PCI begins after two cycles if efficacy is CR/PR.

Control Group: Conventional chemotherapy per first-line SCLC regimen plus thoracic radiotherapy. PCI begins after two cycles if efficacy is CR/PR.

4.Observation Indicators:

(1) Starting at the end of radiotherapy, patients undergo head plain MRI, enhanced MRI, DWI, and MRS every three months, along with chest enhanced CT. Treatment-related toxicity is graded per CTCAE 5.0.

(2) MMSE and MoCA are administered every three months.

(3) Venous blood is drawn the day before radiotherapy and every three months post-radiotherapy for white blood cell count, neutrophils, lymphocytes, platelets, hemoglobin, C-reactive protein, homocysteine (Hcy), and routine fecal and urine tests.

5.Evaluation Criteria:

(1) **RIBI Grading:** The current classification of radiation-induced brain injury follows the radiation therapy response evaluation criteria recommended by the National Cancer Institute's Common Terminology Criteria for Adverse Events. It classifies radiation-induced brain injury into 6 grades:

Grade 0: No symptoms;

Grade 1: Mild symptoms;

Grade 2: Moderate symptoms, with limitations in the ability to perform daily activities with the aid of tools;

Grade 3: Severe symptoms, with limitations in self-care ability;

Grade 4: Complications that threaten life, requiring medical intervention;

Grade 5: Death.

(2) **Modified Fazekas Grading:** The modified Fazekas classification provides a unified visual assessment for periventricular white matter (PVWM) and deep white matter (DWM) lesions:

0 grade: Normal, with no obvious white matter hyperintensity lesions.

1 grade: Spot-like lesions, presenting as scattered point-like hyperintensities, commonly seen in normal elderly individuals, often related to age, usually without obvious symptoms. Regular observation is recommended and a healthy lifestyle should be maintained.

2 grade: Plaque-like lesions, with partial fusion of the spots, suggesting possible small vessel diseases such as hypertension and diabetes, and active control of risk factors is necessary.

3 grade: Patch-like lesions, with large-scale fusion of the lesions, indicating more severe cerebral small vessel disease, which may increase the risk of cognitive decline, gait instability or stroke. Further assessment and management by a specialist are

recommended.

(3) **Deep white matter hyperintensity (DWMH)**

0 points: No abnormal signal.

1 point: Point-like abnormal hyperintensity, scattered distribution.

2 points: Patch-like abnormal hyperintensity, some lesions start to fuse.

3 points: Patch-like and irregular abnormal hyperintensity, large areas of lesions have fused.

(4) **Grade of Periventricular High Signal (PVH)**

0 points: No lesion.

1 point: Cap-like or pencil-like thin-layer lesion, distributed around the ventricles.

2 points: Smooth halo-like lesion, surrounding the ventricles.

3 points: Irregular periventricular high signal, extending to the deep white matter.

(5). Definition of Endpoints

Primary Endpoint:

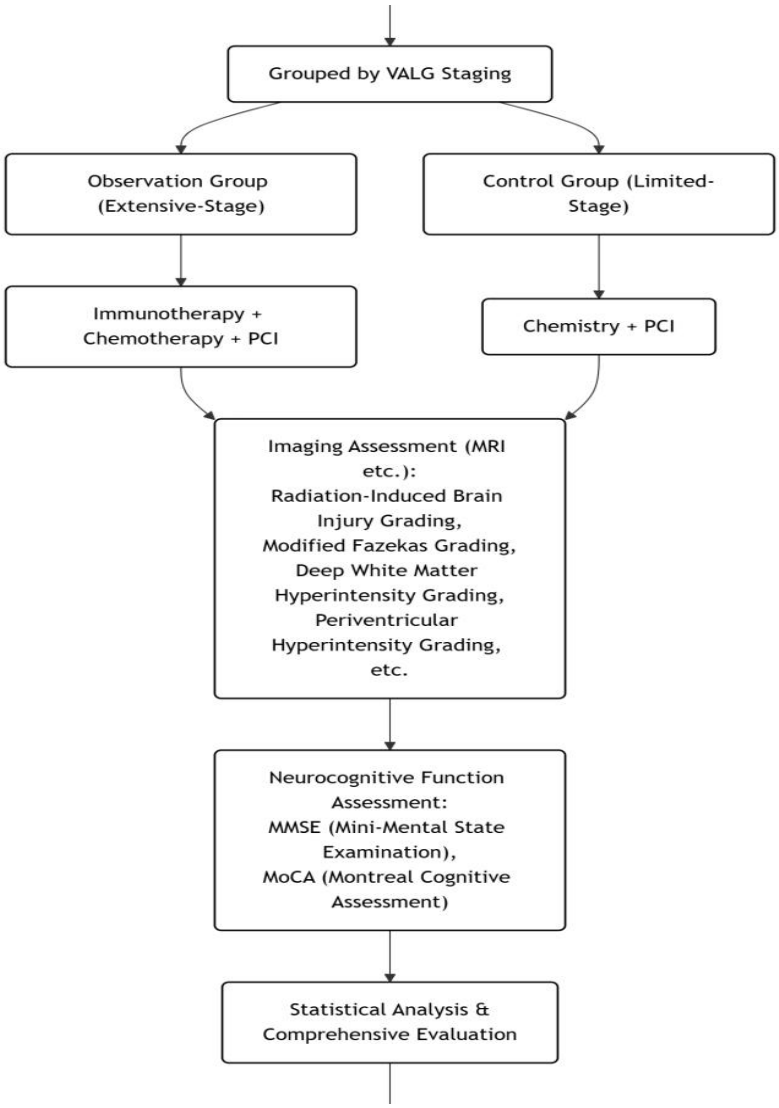
● Progression-Free Survival (PFS): Time from enrollment to tumor progression or death.

Secondary Endpoints:

● Safety of radiotherapy combined with immunotherapy.

● Incidence of adverse events.

VII. Flowchart



VIII. Data Collection, Management, and Quality Control

The screening codes for the subjects are uniformly numbered by our department; the center codes are represented by letters; the sequence numbers start from 001 and go up to 060. The case report forms are kept in a dedicated cabinet by a designated person, and can only be used with the approval of the designated person during the trial or each follow-up visit. The clinical trial data follow the principles of de-identification and confidentiality, and are stored and managed by a designated database by a designated person, without involving any third parties. After the trial is completed, all the clinical trial data obtained by the research will be saved by the central ethics unit until more than 5 years after the end of the trial.

1. Construction of the case report form (CRF) or research record form

The data manager constructs the CRF based on the research plan, original

medical record forms, etc.

2. Data entry and modification

Data entry and modification are completed by the researchers. The data should come from the original documents such as the original record forms and laboratory test reports, and should be consistent with the original documents. Any observations or test results during the trial should be promptly, accurately, completely, clearly, standardly, and truthfully entered into the form or database. The data manager is responsible for reviewing and managing the entered data. For any doubts in the data, the data manager will send corresponding questions to the researchers. The researchers should promptly reply to the questions sent by the data manager, and the data manager can re-raise questions if necessary.

IX. Statistical Methods

1.Enrolled Case Review: Checks whether enrolled cases meet inclusion/exclusion criteria, and counts actual enrollments, exclusions, and dropouts per center.

2.Incidence Evaluation: Efficacy grades are compared using the Wilcoxon rank-sum test; incidence rates use Fisher's exact test. Efficacy grades adjusted for center effect use the CMH method.

3.Safety Evaluation:(1) Safety is assessed mainly by descriptive statistics. Adverse events with incidence >10% are compared using chi-square or Fisher's exact test; those with lower incidence are described only. (2) Laboratory data are analyzed descriptively for pre- and post-treatment changes.

4.Software and Parameters: The data are statistically analyzed and relevant charts are drawn using R (version 4.3.1) software. Quantitative data are described using $X \pm S$, and inter-group comparisons are conducted using the independent sample t-test; for quantitative data that do not meet the conditions for parametric tests, statistical description is conducted using M(Q1, Q3), and inter-group comparisons are conducted using the independent sample rank sum test。For count categorical data, the number (%) is used for description. The chi-square test is employed for comparisons between groups. When the chi-square test conditions are not met, the Fisher's exact

probability method is adopted. For ranked data, the number (%) is used for description, and the rank sum test is used for comparisons between groups. All tests are two-tailed, and a P value less than 0.05 indicates a statistically significant difference.

5.Correction Analysis:A multivariate regression model was used to conduct a correction analysis on the main outcome indicators. Based on clinical expertise and previous literature, factors that might affect prognosis (such as age, KPS score, baseline number of brain metastases, tumor gene typing, etc.) were included as covariates in the Cox proportional hazards model or Logistic regression model to evaluate the independent effect of whole brain radiotherapy combined with immunotherapy.

6.Subgroup Analysis: Predefined subgroups include age (<65 vs. ≥65), immunotherapy timing (concurrent vs. sequential), and GPA score (high vs. low). Results are shown in forest plots with HR/OR and 95% CI.

7.For cases that deviate from the protocol (such as withdrawal, loss to follow-up, or incomplete treatment), for patients who prematurely withdrew or were lost to follow-up, the available data before their withdrawal will be included in the analysis. The main analysis will adopt the ITT principle to retain the patients in their original groups.

X. Adverse Events

1.Prevention of Adverse Reactions

(1) Before determining the anti-tumor treatment plan, the doctor should inform the patient about the manifestations of the adverse reactions of the anti-tumor drugs used, and inform the patient to report any adverse reactions to the medical staff promptly so that appropriate treatment can be provided.

(2)Researchers should be aware of the adverse reactions of anti-tumor drugs. If necessary, they should promptly transfer the patient to a higher-level medical institution.

(3)Before the treatment, the doctor should be aware of the patient's and his/her family's history of drug allergies, and avoid using drugs with the same ingredients that

may cause serious adverse reactions. At the same time, the doctor should also be informed of the patient's liver and kidney functions, blood and urine routine tests, and the patient's general condition.

(4) For high-risk populations, prophylactic measures (e.g., liver protection) should be implemented; cisplatin should be avoided in renal impairment.

(5) Avoid concomitant drugs that increase anti-tumor adverse reactions.

(6) After stopping the medication and when the functions of all organs have returned to normal, when resuming chemotherapy, the drugs with the least possibility of causing adverse reactions should be used first, and the dosage should be gradually increased under close observation.

(7) Increase monitoring frequency of liver/kidney function and blood/urine routine in high-risk groups.

(8) This principle does not apply to non-drug-related risks (e.g., psychological discomfort during visits).

2.Management Principles for Common Adverse Reactions

(1) Taking serious measures to identify and monitor adverse reactions caused by anti-tumor drugs is the best prevention to prevent or avoid serious adverse events. Therefore, it is necessary to detect them early and provide timely treatment.

(2) For mild reactions, symptomatic treatment can be given while continuing anti-tumor therapy, with close observation. If necessary, withdraw from this trial and switch to another regimen.

(3) For severe reactions (e.g., high fever, severe rash, jaundice, hearing changes, oliguria, seizures), seek immediate medical attention, discontinue relevant drugs, and hospitalize for diagnosis and treatment.

3.Adverse Events

(1) Definition: Any adverse medical event reported by the patient or by the research physician between the time the patient signed the informed consent form and the last follow-up should be recorded. Whether it has a causal relationship with the drugs in the research protocol or not, it is classified as an adverse event. Any abnormal changes in physical examination or laboratory tests after treatment should

also be recorded as adverse events.

(2) Record of adverse events: During the study period, the occurrence time, relationship with the study drugs, severity, duration, measures taken and outcome of adverse events should be truthfully recorded. Adverse events should be recorded in the designated CRF adverse event report form.

(3) Judgment of causal relationship with the drug: The causal relationship between adverse events and the trial drugs is judged according to the following principles, divided into five levels: definitely related, likely related, possibly related, possibly unrelated and unrelated.

a) Is there a reasonable chronological relationship between the start of medication and the occurrence of the adverse event?

b) Does the suspected adverse event conform to the known adverse reaction types of the drug?

c) Can the suspected adverse event be explained by the combined use of drugs, the patient's clinical condition or the influence of other therapies?

d) Does the adverse event disappear or alleviate after discontinuation or reduction of the medication?

e) Does the same reaction occur again after taking the medication?

4.Severe adverse events

(1) Determination of severe adverse events: When an adverse event meets one or more of the following criteria, it is classified as a severe adverse event (SAE, Serious Adverse Event):

a) Death; life-threatening (such as: immediate danger of death);

b) Hospitalization or prolonged hospital stay;

c) Impairment of work ability: permanent or severe disability: congenital malformations or defects;

d) Some medical events that have not yet caused death, life-threatening or hospitalization, but are judged by the research physician to be likely to cause harm to the patient or require drug or surgical treatment to avoid the occurrence of the above situations, should also be regarded as SAE.

(2) Handling of cases when severe adverse events occur: If a severe adverse event unrelated to the drugs in the study protocol and without significant impact on the patient occurs, and the patient can continue the follow-up on schedule, the trial can continue; otherwise, the patient should be withdrawn. Before withdrawal, an attempt should be made to complete one follow-up.

Record and reporting of severe adverse events: Any severe adverse event during the clinical research process should be reported to the health administrative department, the ethics committee, and the principal investigator within 24 hours. At the same time, the researcher must fill out the severe adverse event report form, recording the occurrence time, severity, duration, measures taken and outcome of the adverse event.

XI. Ethical Issues

This trial will be conducted after obtaining the approval from the Ethics Committee of the Affiliated Hospital of Guizhou Medical University. All participants will be enrolled after obtaining their informed consent. The trial will follow the principles of GCP and be subject to the follow-up review by the ethics committee. The trial will strictly comply with all relevant national laws and regulations.

XII. Protocol Amendments

This unit is responsible for the overall design of the project, the formulation of optimization plans. Data analysis and the final writing of the summary report of the project. Supervision of the collaborating units and the final decision on the inclusion of cases. Collection and preservation of the trial information and data of the enrolled patients from each center. Consultation on serious adverse reactions and the formulation of guidance principles. Purchase and distribution of free medicines for the included cases. Payment of the project implementation funds to the collaborating units in accordance with the requirements of the agreement.

XIII. Informed Consent

The subjects were screened and recruited by the members of the central trial team during clinical diagnosis and treatment. After meeting the inclusion and exclusion criteria, the members of each center trial team signed the informed consent

form with the subjects. The informed consent form must be approved by experts from all parties and the ethics committee. During the signing process, the detailed information about the clinical trial must be explained to the subjects, including:

(1) The participation of the subjects in the trial is voluntary, and they have the right to withdraw from the trial at any stage without being discriminated against or retaliated against, and their medical treatment and rights will not be affected.

(2) The personal information of the subjects participating in the trial is confidential.

(3) The purpose of the trial, the process and duration of the trial, the examination procedures, the expected benefits and risks for the subjects, and the possibility of being assigned to different groups of the trial are informed to the subjects.

(4) The subjects must be given sufficient time to consider whether they are willing to participate in the trial. For subjects who are unable to express consent, their legal representatives should be provided with the above introduction and explanation. The informed consent process should be conducted in a language and text that the subjects or their legal representatives can understand. During the experiment, the participants were able to access any information and materials relevant to themselves at any time..

(5) In case of any damage related to the trial, the subjects can receive treatment and corresponding compensation.

After fully and detailed explanation of the trial situation, the informed consent form can be obtained. During the signing process, the subjects or their legal representatives sign and date the informed consent form, and the researcher who conducts the informed consent process also needs to sign their name and date.

XIV. Confidentiality

1. Information Confidentiality Plan for Research Participants (Subjects)

All information of the research participants (subjects) must be strictly confidential. Personal information related to participation in the research and during the research is within the scope of confidentiality. The information of the research participants (subjects) and research data will be identified by the research number.

Information that can identify their identities will not be disclosed to members outside the research team. All research members are required to keep the identities of the research participants (subjects) confidential. The archives of the research participants (subjects) are stored in locked filing cabinets and are only accessible to researchers. To ensure compliance with regulations, when necessary, members of the government management department or the ethics review committee can consult the personal data of the research participants (subjects) at the research institution. When the research results are published, no personal information of the research participants (subjects) will be disclosed.

2. Confidentiality Plan for Research Data

Research data also fall within the scope of confidentiality. All research members are required to keep the research data confidential and shall not disclose the research data to members outside the research group without the permission of the main researcher, nor transfer the research data to external units without the permission of the hospital. In case of serious safety issues, the main researcher has the right to terminate the research after notifying the ethics committee.

XV. Declaration of Interests

The main researcher and all members of the research group declare that this research (project name: Prospective Cohort Study of Whole Brain Radiotherapy Combined with Immunotherapy on Brain Injury) has no economic or non-economic conflicts of interest that may affect the research results. During the trial, if any new conflicts of interest arise, the researcher promises to submit an updated statement within 30 days to the ethics committee.

XVI. Additional and Post-Test Supplementary Notes

The follow-up stage after 12 months of treatment for the subjects is included in the research process; if there are no special circumstances, the follow-up will be stopped after the end of the study.

XVII. Release of Results

The researchers report the research results to the subjects, the public, and other relevant organizations by publishing articles to convey the trial results.

XVIII. Funding

The trial funds, items, and other sponsorships are coordinated by the research institution itself.

XIX. References

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