Attachment 1: Research Protocol Template

(Interventional Study)

Project Name (Chinese): Efficacy and safety of PD-1 inhibitor combined with chemotherapy in locally advanced (III-IVB) poorly differentiated head and neck squamous cell carcinoma: a multi cohort, prospective phase II study

Project Name (English): Efficacy and safety of PD-1 inhibitor combined with chemotherapy in locally advanced (III-IVB) poorly differentiated head and neck squamous cell carcinoma: a multi cohort, prospective phase II study

Version Number: V1.3

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Department: Otolaryngology Head and Neck Surgery

Project Leader: Chen Xiaohong

I. Research Background and Project Basis

Please briefly describe the 'medical research content' of this study, explain the reasons for conducting intervention research, the current domestic and international research status, existing problems, and propose research hypotheses.

Background:

Head and neck squamous cell carcinoma (HNSCC) refers to a series of tumors that occur in the head and neck region, with more than 90% of the pathological types being squamous cell carcinoma, and most head and neck tumor patients are already in the locally advanced (III-IVB) or advanced stage when they seek medical treatment. Currently, data shows that the 5-year survival rates of patients with locally advanced and metastatic head and neck squamous cell carcinoma using standard treatment are 50% and 25% respectively. 50% to 60% of newly diagnosed subjects will experience recurrence or metastasis within 3 years.

Poorly differentiated squamous cell carcinoma is a pathological subtype of HNSCC, which has strong invasiveness, high rates of distant metastasis and regional metastasis, and shows completely different characteristics from well-differentiated squamous cell carcinoma in terms of biological behavior. The treatment effect is poor due to the high rate of distant metastasis, and the prognosis is very poor. Studies have shown that there is a 30% difference in the overall 5-year survival rate between patients with well-differentiated and poorly differentiated throat squamous cell carcinoma.

PD-L1 is a key negative regulatory factor of self-reactive T cells and plays a role in maintaining peripheral immune tolerance and inhibiting self-immunity in multiple ways, leading to T cell exhaustion and dysfunction, and tumor cells evading immune surveillance. PD-1/PD-L1 monoclonal antibodies restore the function of tumor-specific T cells by blocking the binding of PD-1 and PD-L1, and enhance anti-tumor immune

response. They have been used in the treatment of various tumors. In 2016, the FDA approved pembrolizumab for the treatment of platinum-resistant recurrent or metastatic head and neck squamous cell carcinoma. In 2020, pembrolizumab combined with chemotherapy can be used as a first-line treatment for recurrent/metastatic head and neck squamous cell carcinoma. Although the efficacy of PD-1 monoclonal antibodies as neoadjuvant therapy in head and neck squamous cell carcinoma is not yet clear, considering the good effect of immunotherapy in head and neck squamous cell carcinoma, PD-1 monoclonal antibodies are expected to have promising clinical application prospects for the induction therapy of poorly differentiated squamous cell carcinoma. In recent years, multiple studies have suggested that neoadjuvant therapy using nivolumab combined with cisplatin and paclitaxel has good tolerability and satisfactory efficacy.

Keywords (5): head and neck squamous cell carcinoma, poorly differentiated, locally advanced tumor, PD-L1, chemotherapy

II. Research objectives and research content

(I) Research objectives

By conducting comprehensive genomic testing and analysis on patients with poorly differentiated head and neck squamous cell carcinoma in stage III-IVB (excluding nasopharyngeal carcinoma), personalized guidance for the treatment of poorly differentiated squamous cell carcinoma can be provided. The combination of PD-1 inhibitors with platinum-based and paclitaxel-based regimens may be safer, more effective, and easier to apply in clinical practice. By combining tumor tissue CPS expression, TMB, gene expression profiles, and peripheral blood lymphocyte immune characteristics, we aim to find a more effective and safe treatment regimen for poorly differentiated squamous cell carcinoma.

(II) Research Content

Patients with locally advanced (III-IVB) poorly differentiated head and neck squamous cell carcinoma (excluding nasopharyngeal carcinoma) who meet the inclusion criteria will have their

blood samples collected, tumor tissue samples or patient paraffin tissue, and slides for comprehensive genomic sequencing and analysis. The study is divided into two groups. Arm1 group: Patients with stage IVB (T4bNxM0) poorly differentiated head and neck squamous cell carcinoma (excluding nasopharyngeal carcinoma) will receive PD-1 combined with platinum-based chemotherapy and albumin-bound paclitaxel (dose according to the drug instructions) for 2 to 3 cycles (determined by the researcher based on tumor shrinkage). If the imaging achieves complete response (CR) or partial response (PR), suitable patients will undergo surgical treatment. Patients who are not suitable for surgery or have stable disease (SD)/progressive disease (PD) will receive concurrent chemoradiotherapy or concurrent chemoradiotherapy combined with PD-1 treatment (up to a total of 17 cycles). Arm2 group: Patients with stage III and IVA (T3NxM0, T4aNxM0) poorly differentiated head and neck squamous cell carcinoma (excluding nasopharyngeal carcinoma) will receive PD-1 combined with platinum-based chemotherapy and albumin-bound paclitaxel (dose according to the drug instructions) for 2 cycles. Patients who undergo surgery within 2 weeks will receive PD-1 monotherapy maintenance treatment or low-dose radiotherapy followed by PD-1 monotherapy maintenance treatment based on pathological results. Patients who do not achieve pathological complete response (pCR) and have positive surgical margins or extracapsular extension will receive concurrent chemoradiotherapy followed by PD-1 maintenance treatment (up to a total of 17 cycles). Patients without high-risk factors will receive PD-1 maintenance treatment after radiotherapy (up to a total of 17 cycles). After completion of treatment, all patients will be followed up every 3 months for 1 year. Subsequently, patients will be followed up every 6 months for 3 years. Thereafter, patients will be followed up annually. Patient recurrence and survival data will be recorded.

(III) Study Outcome Measures

Primary Study Outcome Measures and Definitions

Overall Survival (OS), 1-year/2-year Distant Metastasis Rate: Kaplan-Meier analysis will be used to analyze the time from the start of PD-1 combined with platinum-based and albumin-paclitaxel treatment to the end of data collection (including distant metastasis, local treatment failure, and death), and Kaplan-Meier curves will be plotted. The difference in median OS (mOS) will also be calculated (95% bilateral confidence interval).

Secondary Study Outcome Measures and Definitions

Among the secondary efficacy measures, the proportion of infiltrating squamous cell carcinoma in the resected primary tumor specimen and all sampled lymph nodes (mPR) will be determined based on real-time results, with mPR defined as ≤10%. If further stratified analysis is desired for CPS≥1 and CPS＜1, weighted analysis can be performed using the Miettinen method with a 95% bilateral confidence interval. PFS analysis will be conducted similarly to OS.

Primary, secondary, and other outcome measures, including specific measurement variables (such as systolic blood pressure), quantitative analysis (such as changes from baseline),final values,time to occurrence of endpoint events, etc.), methods of data integration (such as median, proportion), and time points for each outcome measure. Strongly recommend explaining the relevance of the selected efficacy or harm outcome measures to clinical practice. Indicate whether there is involvement of biospecimen collection. Describe how various measures are assessed.

III. Study Design

(1) Overall Study Design

Provide a description of the study design, including the type of trial design (such as parallel, crossover, factorial design), allocation ratio of subjects in each group, and the study type (such as superiority, equivalence, non-inferiority, exploratory).

A prospective, multicenter, non-randomized phase II clinical trial.

√Complete CPS and HPV testing before treatment;

√Complete genetic testing before treatment: TMB, TP53BP1, KRAS, DDR, GEP, EGFR, and other related genes

√For patients who meet the inclusion criteria:

1. Locally advanced (stage IVB, T4bNxM0) poorly differentiated

head and neck squamous cell carcinoma (excluding nasopharyngeal carcinoma) patients receive PD-1 inhibitor combined with platinum-based chemotherapy and albumin-bound paclitaxel (dose according to the drug instructions) for 2 to 3 cycles (determined by the investigator based on tumor shrinkage),

2. Locally advanced poorly differentiated head and neck squamous cell carcinoma (excluding nasopharyngeal carcinoma) patients with stage III and IVA (T3NxM0, T4aNxM0) receive PD-1 inhibitor combined with platinum-based chemotherapy and albumin-bound paclitaxel (dose according to drug instructions) for 2 cycles. Patients who undergo surgery within 2 weeks based on pathological results are given PD-1 monotherapy maintenance treatment or low-dose radiotherapy followed by PD-1 monotherapy maintenance treatment if they achieve pathological complete response (pCR). Non-pCR patients with positive surgical margins or extracapsular extension after surgery receive PD-1 maintenance treatment after concurrent chemoradiotherapy (up to a maximum of 17 cycles). Patients without high-risk factors receive PD-1 maintenance treatment after radiotherapy (up to a maximum of 17 cycles).

(II) Duration of the study

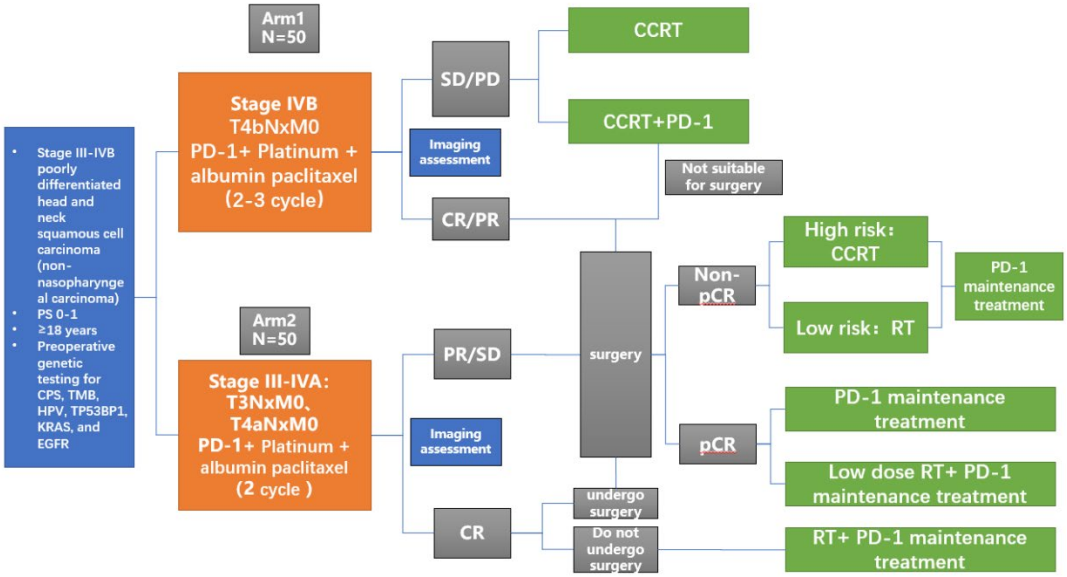
Start time, end time, follow-up status, and follow-up interval.

November 1, 2023 - October 31, 2025.

Based on enrolling 4-5 patients per month, a total of 100 patients are planned to be enrolled. The enrollment period is estimated to be around 24 months. The treatment group closely monitors adverse reactions to chemotherapy, recording the timing, grading, management measures, outcomes, etc. All patients must undergo evaluation after receiving 2 cycles of PD-1 combined with platinum-based and albumin paclitaxel treatment (Arm 1 may receive

a third cycle of treatment based on tumor regression). If they meet the criteria for surgery, they must undergo surgical treatment within 2 weeks and complete the evaluation within 4 weeks after surgery. PD-1 monotherapy maintenance treatment (up to 14 cycles), with a follow-up examination every 3 months during the maintenance treatment period. After completion of treatment, all patients will have a follow-up examination every 3 months for 1 year. Afterwards, a follow-up examination will be conducted every 6 months for 3 years. Subsequently, an annual follow-up examination will be conducted. Record patient recurrence and survival data.

(3) Brief flowchart and visit table of the study



(4) Study subjects

1. Inclusion criteria

√Patients with locally advanced (III-IVB) poorly differentiated head and neck tumors (hypopharyngeal cancer, laryngeal cancer, oropharyngeal cancer, nasal cavity and sinus cancer, excluding nasopharyngeal cancer) with a confirmed diagnosis by histology and/or cytology;

√Patients who can receive systemic treatment or PD-1/L1 monotherapy before treatment;

√Patients in arm2 must be evaluated as having resectable tumors before treatment;

√The investigator believes that the patient can safely receive PD-1 combined with platinum-based and albumin-bound paclitaxel treatment;

√Age ≥ 18 years;

√ECOG 0-1;

√Measurable disease defined by RECIST v1.1;

√Adequate bone marrow reserve and organ function: absolute neutrophil count (ANC) ≥ 1,000/μL, platelets ≥ 75,000/μL, hemoglobin ≥ 8g/dL, no transfusion or erythropoietin (EPO) dependence (within 7 days of assessment);

√Renal function: serum creatinine ≤ 1.5X upper limit of normal (ULN) OR measured or calculated creatinine clearance ≥ 60mL/min, creatinine level > 1.5X institutional ULN. (GFR can also be used instead of creatinine or CrCl). Creatinine clearance should be calculated according to institutional standards;

√Liver function: For subjects with total bilirubin levels >1.5 ULN, serum total bilirubin ≤1.5X ULN or direct bilirubin ≤ULN; For patients with liver metastasis, aspartate aminotransferase (AST/SGOT) and alanine aminotransferase (ALT/SGPT) ≤2.5X ULN or ≤5X ULN; Albumin > 2.5 mg/dL;

√Coagulation function: International normalized ratio (INR) or prothrombin time (PT) ≤1.5X ULN, if subjects require anticoagulant therapy, PT or PTT should be within the allowable range of anticoagulant use;

√Women should agree to use contraception during the study and

for 6 months after the end of the study (such as intrauterine devices (IUDs), contraceptive pills, or condoms); Within 7 days before study enrollment, serum or urine pregnancy test should be negative, and patients must be non-lactating; Men should agree to use contraception during the study and for 6 months after the end of the study.

2. Exclusion criteria

√Patients who have previously received PD-1/L1 combined chemotherapy drugs;

√Patients with a history of other malignant tumors (including unknown primary) within the past 5 years. Note: Excluding stage 1 or 2 skin basal/squamous cell carcinoma or in situ carcinoma receiving potentially curative treatment;

√Patients who cannot tolerate postoperative radiotherapy;

√Patients known to be allergic to the study drug or its active ingredients or excipients;

√Patients with any unstable systemic diseases, including but not limited to: severe infection, uncontrolled diabetes, unstable angina, cerebrovascular accident or transient ischemic attack, myocardial infarction, congestive heart failure, severe arrhythmia requiring medication, liver, kidney, or metabolic diseases;

√Patients with underlying immune deficiencies, chronic infections, including HIV, hepatitis, tuberculosis (TB), or autoimmune diseases;

√Patients with potential hematologic issues, including bleeding diathesis, known prior gastrointestinal bleeding requiring intervention within the past 6 months, active pulmonary embolism or deep vein thrombosis (DVT) unstable on anticoagulation regimen;

√History or evidence of active non-infectious pneumonia;

√Known active central nervous system (CNS) metastases and/or carcinomatous meningitis or leptomeningeal disease. Subjects with prior treated brain metastases may participate as long as they are stable (no evidence of imaging progression for at least four weeks prior to the first trial treatment and any neurological symptoms have returned to baseline), no new or enlarging brain metastases, and no use of steroids for at least 7 days prior to trial treatment. This exception does not include carcinomatous meningitis, which will be excluded regardless of clinical stability;

√Within 7 days before the first day of the first cycle, at the same time (or receiving) treatment with drugs that may affect drug metabolism;

√Pregnant or breastfeeding, or expecting to become pregnant or give birth during the expected trial period;

√Any uncontrolled concurrent disease, including but not limited to persistent or active infection, symptomatic congestive heart failure, unstable angina, arrhythmia;

√Screening EKG> 475 msprolonged corrected QT (QTc) interval;

√Ejection fraction <40% by 2D echocardiogram (ECHO) during screening;

√Any serious medical or mental illness/symptoms, including substance use disorders, may interfere with or limit compliance with study requirements/treatments in the investigator's judgment;

√Having active autoimmune diseases requiring systemic treatment in the past 2 years (even with disease-modifying agents, corticosteroids, or immunosuppressive drugs). Alternative therapies (e.g., thyroid hormone, insulin, or physiological corticosteroid replacement therapy for adrenal or pituitary

insufficiency) are not considered a form of systemic treatment.

Exclusion criteria:

1) Does notmeet inclusion criteria;

2) Did not receive treatment according to the study protocol;

3) Withdrawal of informed consent.

3. Withdrawal criteria

(1) Subjects withdraw informed consent to participate in the study and refuse further follow-up;

(2) The investigator determines other necessary reasons to withdraw from the study, such as the subject losing the ability to express their will due to imprisonment or isolation;

(3) Lost to follow-up;

(4) Subject death;

(5) Sponsor terminates the study.

Indicate whether healthy volunteers are accepted and whether vulnerable groups are involved.

(V) Definition of treatment/intervention groups and controls

Group A: Locally advanced poorly differentiated head and neck squamous cell carcinoma (stage IVB, T4bNxM0) patients receive PD-1 inhibitor combined with platinum-based chemotherapy and albumin-bound paclitaxel (dose according to drug instructions) for 2 to 3 cycles (determined by the investigator based on tumor shrinkage). Patients achieving complete response (CR) or partial response (PR) on imaging are suitable for surgical treatment. Patients who are not suitable for surgery or have stable disease (SD)/progressive disease (PD) receive concurrent chemoradiotherapy or concurrent chemoradiotherapy combined with PD-1 treatment (up to a maximum of 17 cycles).

Group B: Locally advanced (III-IVA) poorly differentiated head and neck squamous cell carcinoma patients (excluding nasopharyngeal carcinoma) with stage T3NxM0 or T4aNxM0 receive PD-1 inhibitor combined with platinum-based chemotherapy and albumin-bound paclitaxel (dose according to drug instructions) for 2 cycles. Patients who undergo surgery within 2 weeks based on pathological results are given PD-1 monotherapy maintenance treatment or low-dose radiotherapy

followed by PD-1 monotherapy maintenance treatment if they achieve pathological complete response (pCR). Non-pCR patients with positive surgical margins or extracapsular extension after surgery receive PD-1 maintenance treatment after concurrent chemoradiotherapy (up to a maximum of 17 cycles). Patients without high-risk factors receive PD-1 maintenance treatment after radiotherapy (up to a maximum of 17 cycles). After completion of treatment, all patients are followed up every 3 months for 1 year. Afterwards, patients are followed up every 6 months for 3 years. Subsequently, patients are followed up annually. Record patient's recurrence and survival data.

(VI) Randomization, allocation concealment, and blinding method

1. Methods for generating random sequence allocation

Describe the specific randomization method used. If stratification is used, provide details on the stratification factors.

No randomization method was employed.

2. Concealment of random allocation

Describe the method used for executing random allocation, such as central randomization, sealed opaque envelopes, etc.

No random allocation method was employed.

3. Blinding and unblinding

Specify who was blinded after implementing the intervention (e.g., subjects, healthcare providers, outcome assessors, data analysts) and how blinding was implemented. If blinding was implemented, describe the circumstances under which unblinding can occur and the procedures for emergency unblinding during the trial.

Not implementedBlind method and unblinding.

(VII) Study procedures

1. Study treatment/intervention period

Describe the interventions in each group, including how and when the intervention is administered; It is recommended to include a

study schedule, which should include screening period, treatment period, and follow-up period, etc.

Patients with locally advanced (III-IVB) poorly differentiated head and neck squamous cell carcinoma (hypopharyngeal cancer, laryngeal cancer, oropharyngeal cancer, nasal cavity and paranasal sinus cancer, oral cancer, excluding nasopharyngeal cancer) receive PD-1 inhibitor combined with platinum-based chemotherapy and albumin-bound paclitaxel (dose according to the drug instructions) for 2 to 3 cycles (determined by the investigator based on tumor shrinkage in patients). If the imaging reaches CR/PR, suitable patients undergo surgical treatment, while patients unsuitable for surgery or with SD/PD receive subsequent concurrent chemoradiotherapy or concurrent chemoradiotherapy combined with PD-1 treatment (total of no more than 17 cycles).

Patients with locally advanced poorly differentiated head and neck squamous cell carcinoma (III-IVA stage, T3NxM0, T4aNxM0), excluding nasopharyngeal carcinoma, including hypopharyngeal cancer, laryngeal cancer, oropharyngeal cancer, and nasal cavity and paranasal sinus cancer, receive PD-1 inhibitor combined with platinum-based chemotherapy and albumin-bound paclitaxel (dose according to drug instructions) for 2 cycles. Patients who undergo surgery within 2 weeks based on pathological results are given PD-1 monotherapy maintenance treatment or PD-1 monotherapy maintenance treatment after low-dose radiotherapy for pathological complete response (pCR) patients. For non-pCR patients with positive surgical margins or extracapsular extension after surgery, PD-1 maintenance treatment after concurrent chemoradiotherapy (up to a maximum of 17 cycles) is given. If there are no high-risk factors, PD-1 maintenance treatment after radiotherapy (up to a maximum of 17 cycles) is given. After

completion of treatment, all patients are followed up every 3 months for 1 year. Afterwards, patients are followed up every 6 months for 3 years. Subsequently, patients are followed up annually. Record patient's recurrence and survival data.

2. Administration method and dose adjustment

Pembrolizumab in combination with cisplatin and albumin-bound paclitaxel, with 3 weeks (21 days) as 1 cycle. On the first day (D1) of each cycle, intravenous infusion of pembrolizumab injection 200mg is given, and chemotherapy can be initiated after completion of pembrolizumab infusion. For subsequent adjuvant or maintenance therapy, pembrolizumab is administered at 200mg IV Q3W, with a maximum duration of 1 year.

Chemotherapy drug dose adjustment: Dose adjustment of cisplatin and albumin-bound paclitaxel should refer to the approved product instructions for standardized chemotherapy regimens. During the study, if the investigator determines that the subject cannot tolerate the toxicity of chemotherapy drugs (such as grade 4 hematologic toxicity, grade ≥3 febrile neutropenia, and grade ≥3 non-hematologic toxicity excluding nausea and vomiting), the dose of chemotherapy drugs can be adjusted based on the investigator's judgment. In principle, two dose adjustments are allowed. It is recommended to adjust the dose to 75% of the original dose for the first adjustment, and 50% of the original dose for the second adjustment.

Dose adjustment of pembrolizumab: Depending on the individual patient's safety and tolerability, administration may need to be temporarily suspended or discontinued. It is not recommended to increase or decrease the dose. Pembrolizumab may be interrupted for reasons other than treatment-related adverse events (such as

medical/surgical events or logistical reasons unrelated to the study treatment). Unless otherwise discussed with the investigator, subjects should resume study treatment within 3 weeks after planned interruption. The reason for interruption should be documented in the subject's study records .

3. Concurrent therapy, follow-up visits

Describe the relevant interventions allowed or prohibited during the trial.

Concomitant therapy: If it is deemed necessary for the health of the subjects to receive a certain drug treatment and it is expected not to interfere with the evaluation of pembrolizumab or interact with it, it can continue to be used during the study. All concomitant medications must be reported in the Case Report Form (CRF). In addition, any diagnoses, treatments, or surgeries performed during the trial should be recorded, including the dates, indications, interventions, and descriptions of any clinical findings. Patients are not allowed to receive any other anticancer treatments during the trial.

Follow-up visit: All patients need to be evaluated after receiving 2 cycles of PD-1 combined with platinum-based and albumin paclitaxel treatment (Arm 1 will receive a third cycle of treatment based on tumor regression). If the surgical indication is met, surgery should be performed within 2 weeks and evaluation should be completed within 4 weeks after surgery. PD-1 monotherapy maintenance treatment (up to 14 cycles), during the maintenance treatment re-evaluation every 3 months. After completion of treatment, all patients will be re-evaluated every 3 months for 1 year; Subsequently, re-evaluation will be conducted every 6 months for 3 years; Afterwards, re-evaluation will be conducted annually;

Record patient recurrence and survival data.

For specific treatment methods of irAEs, please refer to NCCN Guidelines Version 3.2023 Management of Immune Checkpoint Inhibitor-Related Toxicities.

4. Patient compliance and withdrawal

Patients who meet the requirements and participate in this study will enjoy the following benefits:

(1) The professional head and neck malignant tumor MTB (Molecular Tumor Board, including experts from head and neck surgery, pathology, oncology, radiation therapy, imaging, and bioinformatics) team will individually analyze your relevant test results and provide professional guidance for your treatment plan.

(2) You can enjoy a free genomic sequencing analysis service once (worth about 12,000 yuan/time, 100 cases).

(3) You can enjoy a free CPS test once (worth 1500/ case)

(4) PD-1 inhibitor cost discount program: Taking pembrolizumab as an example: Induction immunotherapy: 4 courses at own expense + 13 courses as a gift (worth 460,000); Maintenance immunotherapy: 4 courses at own expense + 31 courses as a gift (worth 1.06 million).

5. Deviation from the protocol

When there is a deviation from the protocol, it should be first discussed with the relevant parties. If no agreement is reached, the trial can be terminated according to the rights of the trial sponsor. During the negotiation, the Ethics Committee (EC)/Institutional Review Board (IRB) must be notified. If the trial needs to be terminated or the trial center needs to be closed prematurely, all research materials (except for the documents that must be archived at

the trial center) must be returned to the trial sponsor. The investigator will retain all other documents until notified by the trial sponsor to destroy them. Events that may lead to early termination of the trial or closure of the trial center include, but are not limited to: new drug toxicity results, trial benefits and follow-up completion of subjects, non-compliance with the trial protocol, changes in the research and development plan of the investigational drug, slow progress in subject recruitment, or poor data quality.

(VIII) Evaluation

1. Evaluation of therapeutic efficacy and intervention effects

1.1 Primary efficacy indicators

The primary efficacy indicator of this study is ORR, which is the proportion of subjects with objective relief evaluated based on RECIST 1.1 criteria (Appendix III).

Objective response rate (ORR): The proportion of subjects with best overall response (BOR) assessed as complete response (CR) or partial response (PR) according to RECIST 1.1 criteria.

1.2 Secondary efficacy indicators

Progression-free survival (PFS): Defined as the time from randomization to the date of first disease progression or death due to any cause, whichever occurs first.

Disease Control Rate (DCR): According to RECIST v1.1 criteria, it assesses the proportion of subjects with complete response (CR), partial response (PR), and stable disease (SD) in terms of overall best response (BOR).

Time to objective response (TTR): Defined as the duration from the start of medication to the date of first documented CR or PR

(whichever occurs first). Tumor response is based on confirmed tumor response, and the response date is from the first observation, not the confirmed response.

Duration of Response (DOR): The duration from the date of first assessment as CR or PR to the date of first assessment as disease progression or death for subjects who achieved complete response or partial response (CR or PR).

2. Safety assessment

2.1 Baseline signs and symptoms

Vital signs, including temperature, respiratory rate, heart rate, blood pressure. If there are any relevant discomfort symptoms during treatment, additional tests can be performed at any time.

Physical examination and weight measurement, including general condition, skin, head and neck (including ears, eyes, nose, throat), respiratory system, cardiovascular system, lymph nodes, thyroid, abdomen, skeletal muscles (including spine and limbs), nervous system. Targeted physical examination can be performed if clinically indicated during treatment. Weight measurement is performed at each follow-up visit during each cycle.

2.2 Laboratory Safety Assessment

Samples such as blood and urine will be collected according to the experimental flowchart and analyzed in the local laboratory. The detailed laboratory examination items are shown in Table 2:

Table2: Laboratory Examination Items

Item	Content
Complete Blood Count	Hemoglobin, red blood cells, white blood cells, neutrophil count, lymphocyte count, and platelet count

Blood Biochemistry/Electrolytes	Total bilirubin, conjugated bilirubin, ALT, AST, ALP, γ-GGT, total protein, albumin, urea or urea nitrogen, creatinine, uric acid, lactate dehydrogenase, fasting blood glucose, triglycerides, cholesterol, potassium, sodium, chloride, calcium, phosphorus, lipase (only when there are suspected symptoms of pancreatitis during screening and follow-up), amylase (only when there are suspected symptoms of pancreatitis during screening and follow-up)
Urinalysis	Urinary protein, glucose, occult blood (red blood cells, white blood cells). If the semi-quantitative method during the screening phase shows urinary protein 2+ , a 24-hour urinary protein quantitative test is required. If the semi-quantitative method during the treatment phase shows protein 2+/3+ in two consecutive visits, a 24-hour urinary protein quantitative test is required; If the semi-quantitative method shows urinary protein > 3+ , a 24-hour urinary protein quantitative test should be directly performed.
Virological examination	Hepatitis B five-item test (HBsAg, HBsAb, HBcAb, HBeAg, HBeAb). If hepatitis B surface antigen (HBsAg) is positive, viral load HBV-DNA quantitative test should be performed; For individuals with positive anti-hepatitis C virus antibodies (anti-HCV antibodies), HCV viral load (HCV RNA) testing should be conducted; HIV antibody test.

2.3 Physical examination and vital signs

Vital signs, including temperature, respiratory rate, heart rate, blood pressure. If there are any relevant discomfort symptoms during treatment, additional tests can be performed at any time.

Physical examination and weight measurement, including general condition, skin, head and neck (including ears, eyes, nose, throat), respiratory system, cardiovascular system, lymph nodes, thyroid, abdomen, skeletal muscles (including spine and limbs), nervous system. Targeted physical examination can be performed if clinically indicated during treatment. Weight measurement is performed at each follow-up visit during each cycle.

(9) Adverse event reporting

1. Adverse events

Description of the collection, assessment, reporting, and

handling plan for any adverse events and other unexpected reactions during the intervention measures or implementation process. Adverse event assessment includes type and severity, timing, relevance, and outcome.

2. Definition of adverse events (AE)

AE refers to any adverse medical event that occurs in a clinical trial subject after receiving a drug, but it does not necessarily have a causal relationship with the treatment. AE can be any unfavorable unexpected symptom, sign, laboratory abnormality, or disease, including at least the following situations:

(1) Existing medical conditions/diseases that were present before entering the clinical trial are only considered adverse events if they worsen (including worsening of symptoms, signs, and laboratory abnormalities) after starting the investigational drug;

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

(3) Clinically significant abnormal laboratory test results.

Diagnostic or therapeutic invasive procedures (such as surgery) and non-invasive procedures should not be reported as adverse events, but if the disease condition that led to such procedures meets the definition of an adverse event, it should be reported. For example, acute appendicitis occurring during the reporting period should be reported as an adverse event, while the appendectomy performed as a result should be recorded as the treatment method for that adverse event.

Researchers should carefully record any AEs that occur in subjects, including: AE name, onset and end time, severity (graded according to version <5.0> of NCI CTCAE), AE relatedness to the investigational drug, duration, measures taken for the AE with the investigational drug, AE outcome, and whether it is a serious adverse event.

3. Abnormal examination results

If AST and/or ALT levels are abnormal and accompanied by abnormal elevation of total bilirubin levels, and if the following (1), (2), (3) conditions are met without any other causes for the abnormalities, the SAE reporting process should be followed.

Meeting the criteria	Criteria for judgment
(1) Abnormal ALT or AST	Baseline normal: ALT or AST during treatment > 3×ULN; Baseline abnormal: ALT or AST during treatment > 2×baseline level, and value > 3×ULN; or value > 8×ULN.
(2) TBIL abnormal	Baseline normal: TBIL during treatment > 2×ULN; Baseline abnormal: TBIL during treatment increased > 1×ULN or its value > 3×ULN.
(3) No hemolysis, and alkaline phosphatase < 2×ULN (or information not available)	

ULN: Upper limit of normal

If the subject has abnormal levels of AST and/or ALT and concurrent abnormal elevation of total bilirubin levels during the safety follow-up period, it is recommended to return to the study center for evaluation and confirmation as soon as possible (preferably within 48 hours) after learning about the abnormal results.

4. Serious Adverse Events (SAE)

SAE refers to adverse events that meet one or more of the following criteria during the clinical research process:

- Events leading to death;
- Events that are life-threatening (the term 'life-threatening' refers to the presence of a risk of death at the time of the event/reaction; it does not imply that worsening of the condition may lead to death);
- Events requiring hospitalization or prolongation of hospital stay;
- Events leading to permanent or severe disability/loss of function;
- Congenital abnormalities or birth defects;
- Other important medical events (referring to events/reactions that may not immediately endanger life, cause death or hospitalization, but may harm the subjects or may require intervention [such as medication or surgery] to prevent the serious consequences listed in the above definition, based on reasonable medical and scientific judgment).

5. Severity assessment

Refer to the grading criteria for drug-related adverse events in NCI-CTCAE version 5.0. If the observed adverse event is not listed in the CTCAE version 5.0 grading criteria, refer to Table 3:

Table3: Criteria for assessing the severity of adverse events

Grading	Criteria for judgment
Grade 1	Mild; No clinical symptoms or mild clinical symptoms; Only clinical or laboratory abnormalities; No treatment required
Grade 2	Moderate; Requires minor, local, or non-invasive treatment; Limitation in activities of daily living using age-appropriate tools*
Grade 3	Severe illness or medically significant symptoms that are not immediately life-threatening; Results in hospitalization or prolonged hospital stay; Results in disability; Limitation in activities of daily living (ADL) self-care**.
Grade 4	Life-threatening; Requires urgent treatment.
Grade 5	Adverse events leading to death.

Activities of daily living (ADL)

*Activities of daily living using tools refer to cooking, shopping, making phone calls, counting money, etc.

**Activities of daily living self-care refer to bathing, dressing and undressing, eating, using the toilet, taking medication, etc., excluding bedridden individuals.

Pay attention to the severity and seriousness of adverse events. For example, "severe headache" may be severe in terms of severity, but it cannot be classified as a serious adverse event (SAE) unless it meets the criteria for SAE.

6. Assessment of relevance

Researchers should determine the reasonable possibility of the investigational drug causing or contributing to adverse events through comprehensive evaluation. Factors to consider include the reasonable temporal sequence between the administration of the investigational drug and the occurrence of the adverse event, characteristics of the investigational drug, its toxicological and pharmacological effects, concomitant medication use, subjects' underlying diseases, medical history, family history, as well as provocation and rechallenge reactions. Assess the potential association between adverse events and the investigational drug using a five-level classification system: definitely related, probably

related, possibly unrelated, definitely unrelated, indeterminate. Refer to Table 4 for assessment criteria.

Table 4 : Criteria for judging the relationship between AE and drugs

Association evaluation	Time-relatedness	Is it known	De-excitation	Re-excitation	Other explanations
Definitely related	+	+	+	+	-
Possibly related	+	±	±?	?	±?
Possibly unrelated	±?	-	±?	?	±?
Definitely unrelated	-	-	-	-	+
Unable to evaluate	Required evaluation data cannot be obtained				

1. ＋ indicates positive or affirmative; - indicates negative or negation; ± indicates difficult to judge; ?Indicates unknown.

2. Time-relatedness: Is there a reasonable time relationship between drug use and adverse reactions.

3. Is it known: Do the adverse reactions correspond to the known types of adverse reactions for the drug.

4. De-excitation: After discontinuation or dose reduction, do the adverse reactions disappear or alleviate.

5. Re-excitation: Whether the same adverse reactions occur again when the suspicious drug is used again.

6. Other explanations: Whether adverse reactions can be explained by the effect of the drug, the progression of the subject's condition, the influence of other treatments, etc.

(10) Data management and statistical analysis

1. Quality management: The principle of double data entry, quality control during data collection in multicenter studies, data cleaning and verification work.

(1) All trial processes are established SOPs.

(2) Trial unit qualification: Clinical trial units are drug clinical trial bases with clinical conditions determined by the National Medical Products Administration.

(3) Qualifications of trial participants: Trial participants must be physicians who have received clinical trial training and work under the guidance of senior professionals.

(4) Quality control measures in the laboratory: The laboratory should establish standard operating procedures and quality control programs for experimental observation indicators.

2. Statistical analysis

2.1 Sample size determination

The number of subjects required to achieve the research objectives and the calculation method should include any clinical and statistical hypotheses. Based on the research objectives and relevant parameters from pre-experiments or previous studies, sample size estimation work should be conducted using statistical software or specific formulas. Consider appropriate expansion of sample size considering attrition rate or design effect.

According to the calculation of enrolling 4-5 patients per month, it is planned to enroll 100 patients, and the enrollment time is expected to be about 24 months. Close monitoring of chemotherapy adverse reactions in the treatment group, recording the time, grading, treatment measures, outcomes, etc.

2.2 Analysis Population

Descriptive statistical analysis of the population definition, non-compliant subjects with the study protocol should be specifically described in which analysis set they are included, and how to handle missing data.

The following analysis sets will be involved in this study:

- Full Analysis Set (FAS): defined as all subjects who have been enrolled and have taken the investigational drug at least once.
- Evaluable Set (ES): The Evaluable Set (ES) is a subset of the Full Analysis Set (FAS), defined as subjects who have received the study drug at least once after enrollment and have at least one post-baseline efficacy assessment.
- Safety Set (SS): The Safety Set (SS) consists of all subjects who have received at

least one dose of the investigational drug in this study.

2.3 Efficacy Analysis and Statistical Methods

Please describe the statistical analysis methods for the primary and secondary outcome measures, as well as any additional analyses (such as subgroup analysis). What statistical software (such as SAS or SPSS) was used for data processing and analysis, and provide a detailed statistical description of the primary and secondary outcome measures. Specify whether one-sided or two-sided statistical tests were used, as well as the choice of α level.

Principles of basic data analysis: quantitative data is summarized using mean, standard deviation, median, maximum value, and minimum value; Qualitative data is summarized using frequency and percentage; Time-event data is analyzed using Kaplan-Meier estimation to calculate survival rate and median survival time, and survival curves are plotted.

2.3.1 Analysis of primary outcome measures

Overall Survival (OS), 1-year/2-year Distant Metastasis Rate: Kaplan-Meier analysis will be used to analyze the time from the start of PD-1 combined with platinum-based and albumin-paclitaxel treatment to the end of data collection (including distant metastasis, local treatment failure, and death), and Kaplan-Meier curves will be plotted. The difference in median OS (mOS) will also be calculated (95% bilateral confidence interval).

2.3.2 Analysis of secondary outcome measures

Among the secondary efficacy measures, the proportion of infiltrating squamous cell carcinoma in the resected primary tumor specimen and all sampled lymph nodes (mPR) will be determined based on real-time results, with mPR defined as ≤10%. If further stratified analysis is desired for CPS≥1 and CPS＜1, weighted analysis can be performed using the Miettinen method with a 95% bilateral confidence interval. PFS analysis will be conducted similarly to OS.

2.4 Safety analysis and statistical methods

Descriptive statistics are used to summarize safety data, including but not limited to: treatment-emergent adverse events (TEAE) defined as adverse events occurring on or after the day of drug treatment. Only TEAEs will be summarized. All adverse events will be graded according to NCI-CTCAE version 5.0. Descriptive statistics will mainly summarize data on TEAEs, treatment-related adverse events (TRAEs), grade ≥3 adverse events, grade ≥3 treatment-related adverse events, serious adverse events (SAEs), treatment-related serious adverse events, TEAEs with an incidence ≥5%, treatment-related adverse events with an incidence ≥5%, TRAEs leading to dose interruption or adjustment, and TRAEs leading to treatment discontinuation.

2.5 Interim Analysis

Describes the criteria for interim analysis and stopping analysis, including who can access the results of these interim analyses and the final decision-making power to terminate the trial.

2.6 Final Analysis

(XI) Data Collection and Management

1. Case Report Form/Electronic Data Recording

Describes the plan for assessing and collecting outcome measures, baseline, and other trial data, and describes the reliability and accuracy of the data entry tool.

This study uses electronic case report forms (eCRF) for the collection and management of clinical research data.

Study Case Report Completion:

Study case reports, as original documents of clinical trials, should be kept intact. The study case report is filled out and kept by the investigator. Before each entry, the subject's information on the cover of the case report should be checked, with neat handwriting that is easy to recognize, and facilitate data verification with the eCRF.

eCRF filling

Use HRTAU EDC to collect clinical research data.

Fill in: The data in the eCRF comes from original documents such as medical records and laboratory test reports and should be consistent with the original documents. Any observations or examination results in the trial should be promptly, accurately, completely, clearly, standardized, and truthfully filled in the eCRF, and should not be arbitrarily changed. All items in the eCRF must be filled in, and there should be no empty or missing items.

Modification: If necessary, when making data corrections to the eCRF, the reason for the data modification should be filled in according to the system prompt. The system's logic check program will perform integrity and logic checks on the clinical trial data entered into the EDC system, and will issue information prompts for problematic data, allowing the Principal Investigator (PI) to modify or explain the problematic data. If necessary, multiple challenges can be issued until the problematic data is resolved.

Data Monitoring:

The Clinical Research Associate (CRA) is responsible for monitoring whether the center complies with relevant regulations, Good Clinical Practice (GCP) for drug clinical trials, and the execution of this study; Whether all eCRF entries are correct, complete, and consistent with the original documents such as medical records and laboratory test reports, and whether there are any errors or omissions in the data. The monitor will review the integrity, consistency, and accuracy of the trial data in the clinical database according to the monitoring plan, and discuss problematic data with the researchers. If necessary, the researchers will make supplements or corrections. Ensure the consistency of data in the eCRF with the original data, a process also known as Source Data Verification (SDV).

2. Data management

Description of the scheme for data entry, coding, confidentiality, and storage, including any relevant measures to improve data quality (such as double data entry, range checks, etc.)

- Establish EDC database

Database design: The data administrator designs the database and tests it with simulated data or real eCRF data to ensure its accuracy.

- Data entry

Data entry and management are the responsibility of the data administrator designated by the statistical unit. To ensure data accuracy, two data administrators should independently enter and verify the data.

- Data query

For any questions regarding the case report form, the data administrator will generate a Data Query Form (DRQ) and send it to the investigator through the clinical monitor. The investigator should respond promptly and return the answers. The data administrator will then modify, confirm, and enter the data based on the investigator's response. If necessary, another DRQ may be sent.

- Data lock

After confirming the correctness of the established database, the primary investigator, sponsor, and statistical analyst will lock the data. The locked data file will no longer be modified. Issues discovered after data lock will be corrected in the statistical analysis program after confirmation.

- Data processing

After all research data has been entered and locked, the database will be handed over to the statistical analyst for statistical analysis according to the statistical plan. After completing the statistical analysis, the statistical analyst will write a statistical analysis report, and the principal investigator of this clinical trial will write a trial summary report.

3. Protection of subject rights and informed consent

Researchers have a responsibility to maintain the anonymity of the subjects. Only uppercase letters, numbers, and/or codes should be used to identify subjects in case report forms or other documents, not the subject's name. Researchers must keep a record of subject codes, names, and home addresses in the subject enrollment table. Researchers must strictly maintain the confidentiality of documents that can reveal the identity of the subjects.

Researchers must inform the relevant information about this trial in both oral and written forms. Subjects, guardians, and the legally authorized representatives of the subjects (if necessary) have the right to know detailed information about this trial.

The informed consent form (along with the trial protocol) must be reviewed and approved by the ethics committee. If necessary, the researcher has the responsibility to explain the content of the informed consent form to the subject in a way and wording that the subject can understand. Subjects and their representatives should have sufficient time to read before formally signing the informed consent form.

The final text of the informed consent form should include the following: the purpose of the trial, the process and duration of the trial, examination procedures, potential benefits and risks to the subjects; Treatment and corresponding compensation that the subject can receive in case of trial-related harm; Principles of confidentiality of subject's personal information.

The informed consent form must be signed by the subject (and the subject's legal guardian), and the date must be indicated. The researcher who conducted the informed consent process must also sign their name and date on the informed consent form. The informed consent form may also be signed by an independent witness who can attest to the subject's agreement to participate in the trial. One copy of the informed consent form should be retained by the researcher and one by the subject. If significant new information regarding the investigational drug is discovered, the informed consent form must be revised in writing and submitted to the ethics committee for approval before obtaining the subject's consent again.

4. Definition of study completion

1. The end of the first phase is defined as the completion of the study's primary endpoint-related indicators and data statistics.
2. The overall study is defined as including all relevant indicators and data statistics, including secondary study endpoints and exploratory endpoints, and publishing papers.