Study protocol and statistical analysis plan

一、 Research topics:

Application of MRD combined with personalized immunomodulatory diagnosis and treatment technology in epithelialPostoperative prevention of ovarian cancerStudy on the application of adjuvant therapy in relapse

二、 Purpose of the study:

evaluateApplication of tumor neoantigen peptide immunomodulation technology in preventing recurrence of MRD positive patients with epithelial ovarian cancer after surgeryThe efficacy and safety of

三、 Research background (research status at home and abroad)

Epithelial ovarian cancer (EOC) is the most common type of ovarian cancer. Its onset is concealed. 80% of patients are at advanced stage (stages III and IV) when they are found. It is characterized by invasiveness, high recurrence rate and significant chemoresistance. This is the main reason why ovarian cancer is the female reproductive system malignant tumor with the highest mortality, which seriously threatens women's health and life. Although the treatment mode of "surgery + platinum containing chemotherapy + targeted drug maintenance therapy" has been formed for the initial treatment of some stage II ~ IV EOC at present, 70% of patients who achieve complete remission will relapse within 2 years, and the 5-year survival rate is only 50% after diagnosis. Salvage strategies for patients with recurrent EOC are rarely curative, and only a few patients with isolated lesions and no ascites are suitable for secondary tumor cell reduction surgery. Patients who lose the opportunity of surgical treatment usually can only choose multiple chemotherapy, targetTo treatAnd non-surgical treatment including novel immunotherapy. Multiple chemotherapy often brings huge side effects and drug resistance; targetTo treatAt present, only a few subtypes have a good immune response, and the 2023nccn clinical practice guidelines for ovarian cancer including fallopian tube cancer and primary peritoneal cancer (version 1) has pointed out that PARP inhibitors have been used before or after relapseBevacizumab singleThere are limited data on the re use of PARP inhibitors in patients with relapse, and the combination of PARP inhibitors and shellfish is not recommended for maintenance therapy after relapse treatment in these patientsValgus chinensisAnti; EOC may respond to immunotherapy, but so far, immunotherapy for EOC has not met expectations. In the trial of programmed death-1 (PD-1) / programmed death ligand 1 (PD-L1) blockade as a monotherapy, the response rate is only 8.0 ~ 22.2%,First line and platinum sensitiveImmune checkpoint inhibitors combined with chemotherapy after relapse（Or bevacizumabThere was no additional benefit in progression free survival (PFS) of patients treated with anti -) therapy and maintenance therapy. These are perplexing the treatment of recurrent EOC, but if the high-risk group of recurrence can be screened early and intervened, it may significantly improve the quality of life and prolong the survival time of patients.

Neoantigen immunomodulatory diagnosis and treatment technology is a hot spot in tumor immunotherapy in recent years. The personalized immunomodulatory diagnosis and treatment technology of tumor neoantigen aims to trigger the body's specific T cell response against neoantigen, thereby killing tumor cells. A number of heavy studies have suggested that the combination of neoantigen immunomodulatory diagnosis and treatment technology based on tumor tissue mutant antigen preparation with standard treatment, targeted drugs, immune checkpoint inhibitors and other treatment methods is of great significance for tumor treatment.

In 2017, two research teams in the United States and Germany reported the results of phase I clinical experiments based on tumor neoantigen immunomodulatory diagnosis and treatment technology in nature. On December 29, 2018, Nature magazine published two important research results on the application of neoantigen immunomodulation technology in the treatment of refractory glioblastoma. The research showed that most patients could monitor the specific immune response to tumor neoantigen in vivo after the treatment of neoantigen immunomodulation technology. In the same year, nature medicine also published an article about the diagnosis and treatment technology of neoantigen immunomodulation. The researcher successfully cured a patient with advanced breast cancer by using neoantigen immunomodulation diagnosis and treatment technology combined with adoptive T cell therapy. According to the screened four mutant proteins, SLC3A2, kiaa0368, cadps2 and CTSB, the researchers designed immunomodulatory diagnosis and treatment technology to screen various T cell populations with specific reactivity in the patient, and adoptively reinfused them after culture and proliferation. 22 months after adoptive transfer of T cells, all target and non target lesions in the patient had disappeared. Strikingly, in 2018, an international team led by researchers at the University of Pennsylvania in the United States used neoantigen immunomodulatory diagnosis and treatment technology to treat patients with advanced ovarian cancer. In this study, 25 patients with ovarian cancer received this tumor immune regulation technology alone or in combination with shellfishValgus chinensisAnti ± cyclophosphamide treatment, the results showed that when tumor immune regulation diagnosis and treatment technology combined with other drugs can play a better curative effect, the results were published on Science Translational Medicine. At the same time, the team also found that adding acetylsalicylic acid and low-dose IL-2 to the original combination scheme can increase the efficacy of immunomodulatory diagnosis and treatment technology and trigger specific T cell responses of immunomodulatory diagnosis and treatment technology through the phase I trial of 28 cases of recurrent advanced ovarian cancer (nct01132014). These responses are positively correlated with the prolonged PFS and overall survival (OS) of patients. The research results have been published in npj vaccines in 2021. The latest research includes a phase I experiment on the treatment of patients with advanced metastatic solid tumors (including microsatellite stable colorectal cancer, non-small cell lung cancer, gastroesophageal adenocarcinoma) with individualized neoantigen immunomodulatory diagnosis and treatment technology combined with immune checkpoint inhibitors (CPI) reported by the American research team on Nature Medicine in August 2022. The results showed that all patientsBoth observedTo the induction of neoantigen specific CD8 T cell responses, including patients with microsatellite stable colorectal cancer whose tumors lacked immunoreactivity before vaccination with immunomodulatory diagnosis and treatment technology.CtDNAThe reduction of tumor burden and the reduction of tumor burden indicate that individualized neoantigen immunomodulatory diagnosis and treatment technology combined with CPI has early signs of potential therapeutic benefits in the refractory patient population. In December 2022, cancer cell published an article on personalized neoantigen immunomodulatory diagnosis and treatment technology neo-pv-01 combined with PD-1AsLate nonSquamous nonFirst line treatment of small cell lung cancer (NSCLC)IBPhase I clinical trial results. The results showed that after vaccination with immunomodulatory diagnosis and treatment technologyBoth observedTo neoantigen specific CD4 and CD8 T cell responses, and CD4 T cells generated after inoculation exhibited specific cytotoxicity in tumor tissues. We also noticed that the team of the second hospital of Sichuan University registered nct05270720 in 2022onetermSingle arm nonRandomized phase I studies are being recruited to evaluate the safety and feasibility of using novel dendritic cell immunomodulatory diagnostic and therapeutic techniques in 6 to 12 adult patients diagnosed

with ovarian cancer after surgical resection and routinely treated with standard of care chemotherapy.

However, the immune response induced by tumor immunomodulatory diagnosis and treatment technology is generally weak. At present, the benefit of advanced tumors from immune modulatory diagnosis and treatment technology is still very limitedIn tumorThe treatment of postoperative and recurrence prevention can show higher benefits. Because pancreatic ductal adenocarcinoma is fatal in 88% of patients, in 2023, the American team published exciting research results on the use of anti-PD-L1 immunotherapy, personalized RNA neoantigen immunomodulation diagnosis and treatment technology, and sequential intervention of four drug chemotherapy regimens in the treatment of pancreatic ductal adenocarcinoma after surgery on nature. The results showed that during the median follow-up period of 18 months, patients vaccinated with t cells expanded by immunomodulatory technology had longer RFs compared with patients not vaccinated with t cells expanded by immunomodulatory technology. The large number of active t cells induced by this treatment may be related to delaying the recurrence of pancreatic ductal adenocarcinoma. From the perspective of the latest clinical research, the neoantigen immunomodulatory diagnosis and treatment technology entering clinical phase II mainly focuses on the adjuvant treatment of postoperative recurrence prevention. For example, the phase II clinical trial of bnt122 product, an individualized mRNA cancer immunomodulatory diagnosis and treatment technology of biontech, is planned to enroll about 200Cases of colorectal cancerPatients to evaluate the efficacy of bnt122 compared with the current standard treatment after undergoing surgical resection and completing adjuvant chemotherapy.

The recognition of patients with high recurrence risk after surgery and the grasp of the timing of immunotherapy are the new antigen immunomodulation diagnosis and treatment technologyartThe key of adjuvant therapy for posterior prevention of recurrence. At present, clinical recurrence monitoring is carried out by monitoring blood tumor indicators and imaging methods, but its sensitivity and specificity are not ideal, resulting in some patients missing the optimal treatment time. Minimal residual lesions refer to residual tumor cells that persist in the body after treatment and are below the conventional detection limit, which cannot be detected by traditional methods. Circulating tumor DNA（CtDNA）It is a short DNA fragment from tumor cells in plasma, which can be used as a non-invasive and sensitive biomarker to monitor by tracking personalized tumor mutations, especially in monitoring MRD after radical surgery. Several retrospective studies involving patients with solid tumors, including ovarian cancer, confirmed that it was detected after curative treatmentCtDNA Without further adjuvant treatment, the recurrence risk is very high. A prospective study found that compared with standard treatment,Treatment onlyWith detectableCtDNA Of patients can reduce the percentage of patients receiving adjuvant therapy and will not affect recurrence free survival. In addition, the researchers also confirmed that untreatedCtDNA The recurrence risk of negative patients is very low. Therefore, we assume that the MRD positive population after ovarian cancer surgery is an ideal population for neoantigen immunomodulation diagnosis and treatment.

In view of the above research background and the foundation of the project team's preliminary work, we guess that by enrolling MRD positive high-risk groups with recurrence, and analyzing the sequencing results of their tumor tissues, we can prepare neoantigen personalized immunomodulatory diagnosis and treatment technology, and establish a clinical technology system of conventional scheme combined with personalized immunomodulatory diagnosis and treatment technology in the adjuvant treatment of postoperative recurrence prevention of EOC patients. Finally, we will realize the independent innovation of ovarian cancer neoantigen immunomodulation diagnosis and treatment technology, provide new

technical means for the adjuvant treatment of postoperative recurrence prevention of EOC, and minimize the risk of micrometastatic recurrence in the high-risk group of postoperative recurrence of EOC, so as to lay a solid theoretical and practical foundation for the adjuvant treatment of postoperative recurrence prevention of EOC.

四、 research contents

To establish the clinical technology system of conventional scheme combined with personalized immune regulation diagnosis and treatment technology applied to the anti relapse adjuvant treatment of MRD positive patients after EOC: MRD positive patients were enrolled, tumor tissue samples were sequenced, neoantigens were analyzed, personalized immune regulation diagnosis and treatment technology was prepared, and conventional scheme combined with personalized immune regulation diagnosis and treatment technology was applied to the anti relapse adjuvant treatment. The patients were followed up for blood routine, biochemical, immunological indicators, tumor indicators (CA125, HE4, CEA, CA199, etc.), imaging examination (CT, PET-CT, MRI, ultrasound, etc. for the resection site of the primary lesion or metastasis) and survival time (PFS, OS) after treatment, and their effectiveness and safety were evaluated, Objective to analyze the advantages of conventional regimens combined with personalized immunomodulatory diagnosis and treatment technology in the adjuvant treatment of MRD positive patients after EOC.

The new technology system was popularized and applied in six hospitals in the province: it was evaluated and verified by carrying out prospective multicenter clinical research to guide the continuous optimization of clinical treatment strategies.

五、 Research methods and technical routes

research method

(1) Patient recruitment and sample preparation

① Recruitment of ovarian cancer patients: selection 12-20Case compliancePatients with MRD positive EOC in stages II, III and IV as required by the experiment.

② Sample preparation: fresh and frozen tumor tissue or FFPE samples of patients were obtained by surgery, and peripheral blood cells of patients were obtained by intravenous blood sampling..

(2) Ovarian cancer-related precise gene detection

① Select tumor tissue samples from ovarian cancer patients, extract DNA, and use Agilent whole exome probe capture sequencing method (Illumina nova6000 ngs sequencing platform) to conduct deep sequencing detection on DNA. According to the sequencing results, detect tumor gene variation analysis and annotation according to the reference genome, analyze gene variation, and determine HLA typing.

② RNA was extracted from tumor tissues of patients with ovarian cancerTranscriptomeGene expression in tumors was analyzed by sequencing.

(3) Neoantigen prediction of ovarian cancer

① According to somatic SNV/IndelTumor neoantigens were selected from the analysis results.

② Neoantigens with high similarity of normal protein sequences were removed.

③ Using consensusNetmhcpanNetmhc iipanAnd other software to predict the avidity of neoantigens and HLA molecules.

④ Combined with the expression of tumor neoantigens.

⑤ Machine learning was used for comprehensive scoring to predict patients' tumor neoantigens.

⑥ According to the prediction results, peptide immunomodulation diagnosis and treatment technology was synthesized: the standard Fmoc protocol was used, and the double coupling reagent Fmoc Arg was used for arginine（PBF）-Oh. After peptide synthesis, 95% TFA lysate was used to remove a variety of protective groups. The purification and purity analysis of the polypeptide used RP-HPLC, reversed-phase C-18 column, water acetonitrile gradient elution, and MS to determine its molecular weight and identify. The final purity was > 98%, and the endotoxin concentration was less than 0.01eu/g. TFA(trifluoroacetic acid) with a residue of less than 1% of standard clinical grade polypeptides.

⑦ GMP level personalized immunomodulatory diagnosis and treatment technology preparation: divide all polypeptides into 2-4 pools (designated as 1-4 pools,Each pool contains3-5 peptides, 0.3mg/ peptide), and strict quality control, including sterility test, mycoplasma detection, pyrogen detection and abnormal toxicity test. Before injection, the neoantigen immunomodulation diagnostic and therapeutic technology pool was mixed with 0.5mg Polyinosine polycytidylic acid injection (adjuvant).

(4) Evaluation of in vitro effectiveness of individualized immunomodulatory therapy for ovarian cancer

① The peripheral blood of patients should be collected at an interval of more than 1 month after the end of chemotherapy treatment for more than 20 days after surgery, and the blood routine should be as follows: lymphocyte count greater than 1.0 × 109/l, monocyte count greater than or equal to 0.4 × 109/l.

② Isolation and culture of PBMC cells from peripheral blood of patients: transfer 10ml of whole blood into a 50ml centrifuge tube, add the same amount of normal saline to dilute, and mix well; Take the centrifuge tube and add 10ml firstFicollSolution. The diluted blood was then gently added to theFicollUpper layer, 2000rpm, 30min. Pipette the cells in the cell layer where PBMCs are located into another clean centrifuge tube. Add normal saline to 30ml, 1500rpm, centrifuge for 10min, remove the supernatant, and then add normal saline for the same cleaning operation; Add the medium to resuspend the cells, plate them, and place them in the incubator for 2h. Adherent cells were used for the induction culture of DC cells,Collect nonAdherent cells, T cells, and remaining cells were frozen for subsequent T cell induction and target cell preparation.

③ Induction culture of DC cells: human peripheral blood mononuclear cells were suspended in complete medium (RPMI1640 containing 10%fbs) and plated on 6-well plates at a concentration of 1107 cells / well. After incubation at 37 ° C and 5%co2 for 2h, the plate was shaken gently and the suspended cells were aspirated. The remaining cells were the obtained adherent monocytes. The adherent cells were incubated in 6-well plates containing human recombinantRhGM-CSF and human recombinant rhil-4 were cultured at 37 ° C with 5%co2. On the third day, the medium was supplemented and the culture continued.

④ DC cells derived from peripheral blood mononuclear cells cultured to the sixth day were collected and cultured in human DC cell culture medium (RPMI1640 complete medium, 10ng/ml rhil-4500u/mlRhGM-CSF), adjust the cell concentration to 2 × 105 cells /ml, divide into 24 well plates, add 20 μ m tumor polypeptide immunomodulatory diagnosis and treatment technology, collect the cells after 4 hours, discard the supernatant of the medium, centrifuge and wash the cells with RPMI1640 medium to remove the stimuli in the original medium, and finally suspend 2 × 105 cells in 0.5ml medium to stimulate the T lymphocytes of the same body.

⑤ T cell induction culture: the frozen T cells were resuspended and co cultured with peptide sensitized autologous DC cells at a concentration of 4 × 106/ml. On the fifth day of coculture, 20u/ml rhIL-2 was added. After 7 days of culture, lymphocytes were collected and incubated at a ratio of 10:1 withpeptideSensitized syngeneic DC cells were co cultured for a second round of stimulation. The same stimulation was performed three times a week. During this period, 20u/ml rhIL-2 was added every 3 days for 3 and a half daysMeasure and change

liquidCells were harvested 7 days after the last stimulation, and the collected T cells were subjected to IFN- γ ELISPOT assay.

⑥ ELISPOT detection: adjust the concentration of T cells to 5 × 106/ml, and transfer the cell suspension directly into the coating with anti IFN- γ ELISPOT detection plate for antibodies, 100 μ L/ well. Refer to IFN- γ Ifn- secreting T cell colonies were detected by the method described in the instructions of ELISPOT detection kit.

(5) Clinical treatment research and monitoring of patients with personalized immune regulation diagnosis and treatment technology

① Recruit patients for clinical treatment research of personalized immune regulation diagnosis and treatment technology: collect postoperative imaging and serum tumor markers failed to detect residual lesions but MRD positive stage II, III and IV EOC patients' surgical tumor tissue specimens, blood, sequence tumor tissue specimens, analyze neoantigens, and prepare personalized immune regulation diagnosis and treatment technology.

Peptide immunomodulatory diagnosis and treatment technology in vivo immune mode: the main purpose of clinical experiments is to study the safety and feasibility of individualized immunomodulatory diagnosis and treatment technology for ovarian cancer; Secondary objectives to study the effect of tumor neoantigens on inducing specific cellular immune responses and the control of tumor metastasis in patients with primary lesion resectionRate orPatient conversion treatment rate. Each patient synthesized 5-20 neoantigen polypeptides and injected 1ml polypeptide poly ICLC mixture with 1-4 injections each time. The first round of immunization was administered subcutaneously on days 1, 4, 8, 15, and 22, and the second round of immunization was on days 54 and 84.

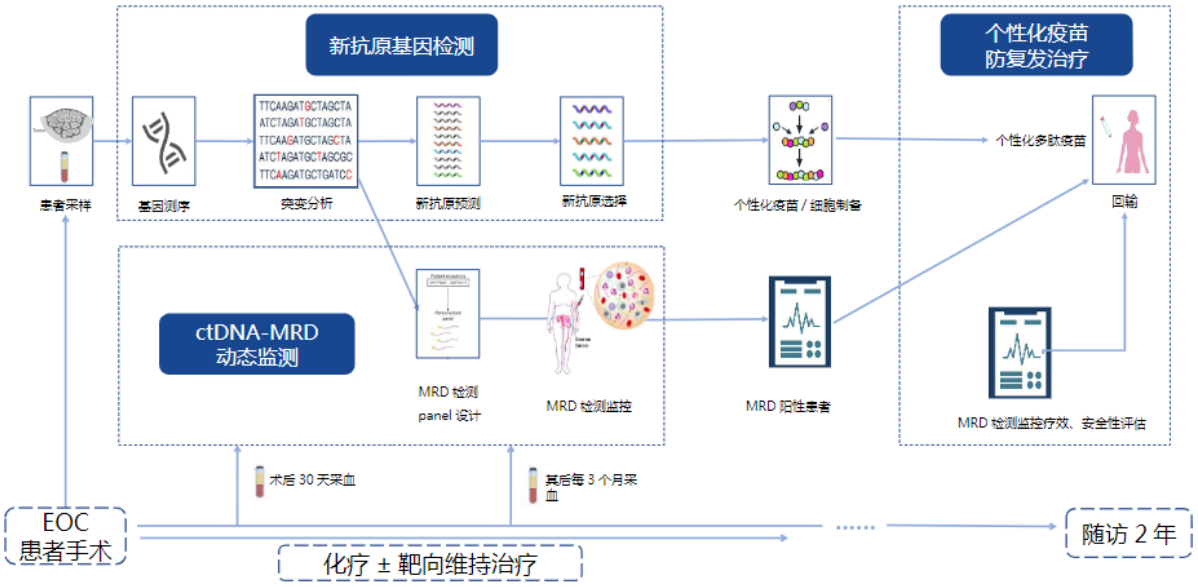
② Clinical treatment effect detection: follow up patients' survival time, blood routine, biochemical, immunological indicators, tumor indicators (HE4, CA125, CA199, etc.), imaging examination (CT, PET-CT, MRI, ultrasound, etc. for the resection site of the primary lesion or metastasis).

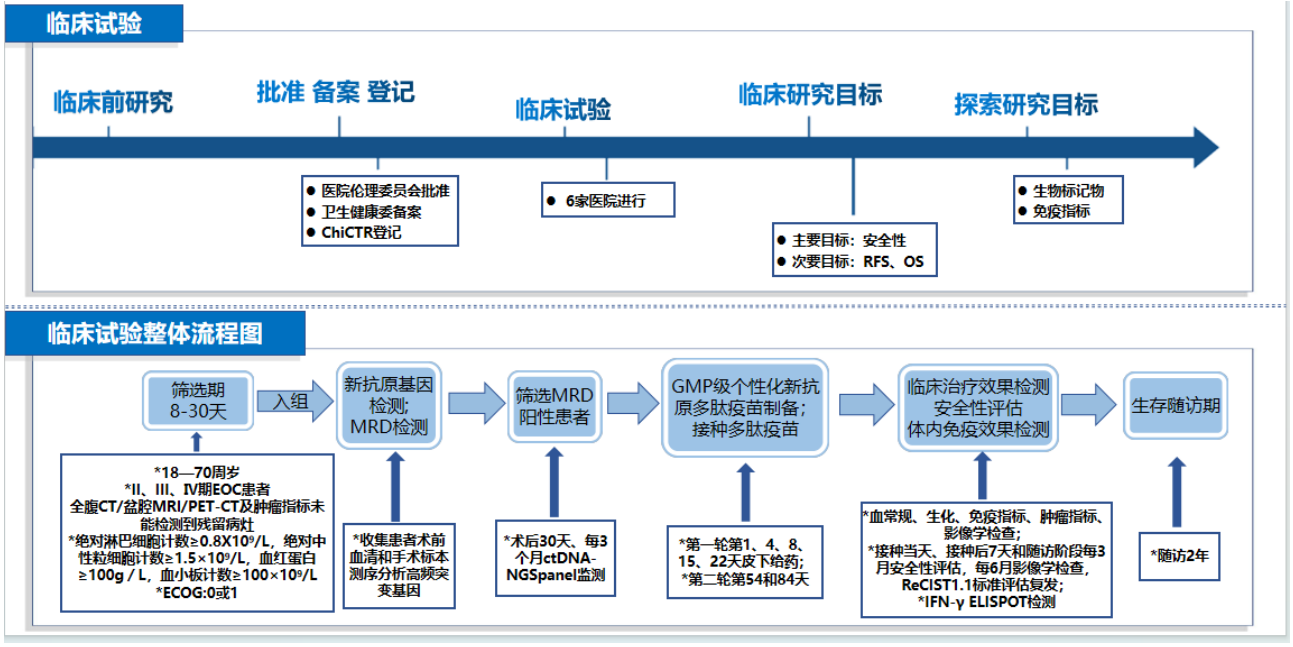
③ Safety evaluation: the safety of the study was evaluated according to the occurrence of adverse events, which were classified and graded according to the National Cancer Institute common adverse event criteria (version 4). In the treatment phase, the safety assessment was carried out on the day of vaccination and 1 week after vaccination of immunomodulatory diagnosis and treatment technology. During the follow-up phase, safety assessments were conducted every 3 months. Systematic imaging examination (CT, MRI or PET-CT, etc.) was performed every 6 months; The standard recist1.1 criteria were used to assess disease recurrence.

④ In vivo immune effect detection: the peripheral blood of patients was collected after 16 weeks of immunization with individualized immune regulation diagnosis and treatment technology, PBMC cells were isolated, DC and T cell culture, peptide sensitization and IFN- γ ELISPOT test refers to the in vitro effectiveness evaluation of individualized immunomodulatory diagnosis and treatment technology for ovarian cancer.

⑤ The new technology system has been promoted and applied in five hospitals in the province by carrying out prospective multicenter clinical research, evaluating and validating it, and guiding the continuous optimization of clinical treatment strategies.

technology roadmap





六、 The name and address of the sponsor, the site where the trial was conducted, and the name, qualification, and address of the investigator;

Sponsor: the Second Affiliated Hospital of Wenzhou Medical University (Yuying children's Hospital of Wenzhou Medical University)

Address: 109 Xueyuan West Road, Lucheng District, Wenzhou, Zhejiang Province

Name of researcher: Duanping

Researcher qualifications: chief physician, Professor, doctoral supervisor

七、 Type of trial design, randomization grouping methodAnd blindingThe level of;

Type of trial design: prospective, multicenter, single arm clinical study

八、 The inclusion criteria, exclusion criteria, the steps of selecting subjects, and the method of assigning subjects;

- **Inclusion criteria**

1. voluntarily join this study;

2.18-70 years old;

3. be able to comply with the research protocol in the judgment of the researcher;

4. patients with stage II, III and IV EOC who can undergo surgical resection and provide sufficient tumor materials;

5. sufficient tumor material must be provided in formalin fixed paraffin embedded (FFPE) blocks or sectioned tissues (only approved by the sponsor), preferably from resection. Specimens should be submitted together with the relevant pathology report. Multiple samples can be provided according to the situation, but priority should be given to the tissue with the highest tumor content and the lowest necrosis area;

6. patients should meet the following biochemical indicators: total bilirubin ≤ 2 × upper limit of normal (ULN); AST and alt ≤ 2 × upper limit of normal (ULN); Creatinine clearance ≥ 60 ml/min;

7. patients should meet the following hematological indicators: neutrophil count ≥ 1.5 × 109 /l; Hemoglobin ≥ 10.0 g/dl; Platelet count ≥ 100 × 109 /l;

8. expected survival ≥ 3 months;

9. Eastern Cooperative Oncology Group (ECoG) performance status is 0 or 1;

10. postoperativeCtDNAMRD was positive, routine blood indicators were negative, and imaging was negative.

- **Exclusion criteria**

1. the patient has HIV infection, HBV infection, HCV infection, uncontrollable coronary artery disease or asthma, uncontrollable cerebrovascular disease or other diseases that the investigator believes are not eligible;

2. those with a history of bone marrow or organ transplantation;

3. coagulation dysfunction;

4. those with gastrointestinal bleeding or gastrointestinal bleeding tendency;

5. subjects with immunodeficiency or autoimmune diseases;

6. Patients who have received other immunotherapies within 1 month (such as immune checkpoint inhibitor therapy, therapeutic antibody therapy, immune cell therapy and immune system regulator therapy);

7. those who may be allergic to immunotherapy;

8. patients whose informed consent or research implementation is affected by drug abuse, clinical or psychological or social factors;

9. pregnant and lactating women;

10. patients who are participating or have participated in other clinical trials within 1 month;

11. any uncertain factors affecting the safety or compliance of patients.

九、 Calculate the number of cases needed to achieve the intended purpose of the trial according to the statistical principle; According to clinical experience and relevant references, the target value P0 of this trial is 79%, and the expected effective rate PT is 99%. According to the degree of grasp (1- β） 80%, inspection level α It is 0.025 (one-sided), and the number of subjects calculated by assuming that the shedding rate is 20% is at least 20.

十、 Study risks and risk disposal plans;

Research risk: potential slight impact on patients' immune system and blood system;

In order to minimize the degree of damage, protect patients' life and health, reducemedicineThis plan is formulated to prevent the occurrence of serious consequences due to the loss of both parties.

1. Eliminate the harmful factors immediately: once the technical damage occurs, the first discoverer shall immediately try to stop the harmful factors; When it is difficult to identify and determine the harmful factors, the superior medical staff should be called immediately for guidance and treatment without delay.

2. Take remedial measures quickly: pay close attention to the patient's vital signs and disease changes, make every effort to take effective remedial measures, reduce the degree of technical

damage, and protect the patient's life and health.

3. Report to relevant leaders as soon as possible: once technical damage occurs, it must be reported truthfully immediately. First, report to the superior physician and the director of the Department. If the circumstances are serious, report to the medical office, the leader of the competent hospital or the chief on duty at the same time. Major technical damage must be reported to the president at the same time. No one is allowed to conceal or conceal it.

十一、 Criteria for discontinuing clinical trials and provisions for ending clinical trials;

1. subjects have the right to voluntarily withdraw from this clinical study

2. in case of relapse of the original disease, adverse events, violation of the treatment plan, management or other reasons, the investigator has the right to terminate the subject's clinical research, and at the same time, the reason for terminating the patient's clinical research shall be described in detail in the case report

The patient will discontinue the study treatment in advance for any of the following reasons:

a. Met the inclusion criteria, signed the informed consent, and received treatment but not completedMake a planSubjects with prescribed observation periods
b. The subject was unwilling to continue the clinical trial and proposed to withdraw from the clinical trial.
c. Although the subject did not explicitly propose to withdraw from the trial, the subject had poor compliance and could not adhere to the treatment or follow-up specified in the protocol
d. The subject had serious adverse events, and the investigator judged that it was not appropriate to continue the trial.
e. The pregnant women during the clinical trial should not continue the trial according to the judgment of the investigator.
f. During the trial, the investigator believed that the subject had factors that could not continue to participate in the trial.
g. The subject has major protocol violation, which affects the safety and effectiveness evaluation.
h. Other circumstances that the investigator believes are no longer suitable for continuing the trial.

十二、 Recording requirements for adverse events and reporting methods, treatment measures, follow-up methods, time and outcome of serious adverse events;

Definition of serious adverse event (SAE): refers to the death, life-threatening, permanent or serious disability or loss of function of the subject after receiving the investigational drug, the subject needs hospitalization or prolongation of hospitalization, as well as congenital anomalies or birth defects and other adverse medical events.

Suspected unexpected serious adverse reactions (susar): all unexpected and serious adverse reactions definitely related to or suspected of the test drug or post marketing drug.

Immune related adverse events（Irae）: adverse drug reactions at all levels related to immune mechanism in clinical trials of antitumor drugs / treatments.

Once serious adverse events occurThe（SAE), the investigator should report to the medical ethics committee of our hospital within 24h after being informed. For minor and moderate adverse events, measures should be taken in time to ensure the safety of the experimenter, and the possible impact of subsequent trials should be fully evaluated.

Treatment measures: subjects with SAE received clinical treatment in time and were followed up until their condition improved or died.

十三、 Establishment and preservation of drug codes for trials,Unblinding methodAnd the provisions for breaking the blind in case of emergency; (double blind test)

This experiment is not blind, and the establishment and storage methods of drug codes are as follows:

Common name: neoantigen polypeptide immunomodulatory diagnosis and treatment technology;

Specification: each polypeptide (purity ≥ 98%) contains 0.3mg, 0.5mg poly (i:c) and less than 1% DMSO (necessary reagent for immune regulation diagnosis and treatment technology);

Product batch number: it consists of the patient's initials, preparation date, inoculation / reinfusion times and serial number; For example, hki/20230921300/01/001 corresponds to: initials + date + 1st input +001 serial number;

Production date: the production date of peptide immunomodulatory diagnosis and treatment technology is the time when the peptide and adjuvant are mixed to make a complete

immunomodulatory diagnosis and treatment technology;

Validity period: no more than 7d after the preparation of peptide immunomodulatory diagnosis and treatment technology;

Storage: polypeptide immunomodulatory diagnosis and treatment technology room temperature storage or immediate inoculation;

Immunomodulatory diagnosis and treatment technology packaging: research protocol number, cell number, usage, storage conditions, cell provider, and marked with "for clinical research only".

Storage and transportation management: after the polypeptide immunomodulatory diagnosis and treatment technology is prepared and qualified, it will be transported to the clinical department by a specially assigned person within a limited time and kept by a trained full-time administrator. All links of the storage and transportation of each batch of immunomodulatory diagnosis and treatment technology have complete handover procedures;

Regulations on the use of immunomodulatory diagnosis and treatment technology researchers and immunomodulatory diagnosis and treatment technology administrators must ensure that the research on immunomodulatory diagnosis and treatment technology is only transplanted and infused to the subjects specified in the research protocol. And accurately and timely record the distribution and recovery of research immunomodulatory diagnosis and treatment technology in the "research neoantigen immunomodulatory diagnosis and treatment technology distribution and recovery record table". At the end of the study, the acceptance, distribution, recovery and remaining quantity of immunomodulatory diagnosis and treatment technology should be re verified. Researchers are not allowed to use the research immune regulation diagnosis and treatment technology in any occasions and under any circumstances other than those specified in the research protocol.

十四、 Statistical analysis method

The software used for statistical analysis is SAS 9.4 or above, and the sample size is calculated by pass 2021 software. The effectiveness index of statistical analysis of test results simultaneously analyzes the full analysis data set and the scheme compliance data set, and the safety index analyzes the safety data set. All statistical analysis tests adopt two-sided hypothesis test, that is,

the difference between the tests will be considered statistically significant if the p value is less than 0.05 (unless otherwise specified).

十五、 Provisions on data management and data traceability;

This research group is responsible for the data management of this study to ensure the authenticity, integrity, privacy andcanTraceability. The project leader or other authorized researchers fill in the information in the CRF form, and only researchers with medical qualifications can fill in the original clinical evaluation / safety data. After the original data is entered, any modifications made by the project leader or other authorized researchers on the CRF form will be recorded. Any modification of approved data willmakeThe name of the modified researcher or other authorized researcher, the date of modification and the reason for modification (if the change is not significant).

十六、 Quality control of test

Researchers will adopt standard operating procedures to ensure the quality control of clinical trials and the implementation of quality assurance system. All observations and findings in the clinical trial will be verified to ensure the reliability of the data and ensure that all conclusions in the clinical trial are derived from the original data. Quality control is adopted at each stage of data processing to ensure that all data are reliable and processed correctly.

十七、 Ethical requirements;

1. before the clinical trial, the trial plan needs to be reviewed by the ethics committee. The review result is consent, and the approval document can be signed before implementation.

2. during the trial, the wma declaration of Helsinki (2013), CIOMS international ethical guidelines for human biomedical research (2016) and the national health and Family Planning Commission ethical review measures for biomedical research involving human beings (2016) were followed.

3. during the trial, any modification of the clinical research protocol, informed consent, recruitment materials, etc. must be reviewed and approved by the ethics committee before implementation.

4. before each subject is selected for this study, the researcher must introduce to the subject in detail the purpose of this study, the test process and duration, the inspection operation, the

expected possible benefits and risks of the subject, the money and time that may be spent, and the subjects' allocation to different groups. In addition, the researcher needs to inform the subjects that participating in this trial is entirely voluntary, and has the right to withdraw from the trial at any stage of the trial without discrimination and retaliation, and their medical treatment and rights will not be affected.

5. after the investigator has fully and in detail explained the situation of the test, the subject or his legal representative (for the incapacitated subject) should sign and date the informed consent form. The investigator performing the informed consent process should also sign the name and date on the informed consent form. The informed consent form is in duplicate, which should be kept by the subject and the investigator respectively.

十八、 Expected progress and completion date of clinical research;

time	Research plan
2024.01 ～ 2024.06	(1) Complete the ethical review and start the trial after ethical consent; (2) First recruit 3~5Case satisfactionConditional EOC patients, tumor tissues and blood samples of patients were collected; (3) Complete the whole exome sequencing, mutation analysis and HLA analysis of the tissues and blood cells of these 3-5 patients with ovarian cancer.
2024.07 ～ 2024.12	(1) Continue to recruit 3-5 eligible EOC patients; (2) Complete the prediction, design and synthesis of individualized immunomodulatory diagnosis and treatment technology for recruited patients; (3) Complete the in vitro cellular immune induction effect detection of individualized immune regulation diagnosis and treatment technology for recruited patients; (4) Patients have been recruited for clinical experiments of individualized immune regulation diagnosis and treatment technology for ovarian

	cancer; (5) GMP level individualized tumor immune regulation diagnosis and treatment technology preparation, patients' body reinfusion, and safety index monitoring. Blood tumor indicators and imaging data were collected, and the clinical treatment effect and survival time of patients were followed up.
January 2025 to June 2025	(1) Continue to recruit 3-5 eligible EOC patients; (2) Complete the prediction, design and synthesis of individualized immunomodulatory diagnosis and treatment technology for recruited patients; (3) Complete the in vitro cellular immune induction effect detection of individualized immune regulation diagnosis and treatment technology for recruited patients; (4) Patients have been recruited for clinical experiments of individualized immune regulation diagnosis and treatment technology for ovarian cancer; (5) GMP level individualized tumor immune regulation diagnosis and treatment technology preparation, patients' body reinfusion, and safety index monitoring. Blood tumor indicators and imaging data were collected, and the clinical treatment effect and survival time of patients were followed up.
July 2025 to December 2025	(1) Continue to recruit 3-5 eligible EOC patients; (2) Complete the prediction, design and synthesis of individualized immunomodulatory diagnosis and treatment technology for recruited patients; (3) Complete the in vitro cellular immune induction effect detection of individualized immune regulation diagnosis and treatment technology for recruited patients; (4) Patients have been recruited for clinical experiments of individualized

	immune regulation diagnosis and treatment technology for ovarian cancer; (5) GMP level individualized tumor immune regulation diagnosis and treatment technology preparation, patients' body reinfusion, and safety index monitoring. Blood tumor indicators and imaging data were collected, and the clinical treatment effect and survival time of patients were followed up.
2026.01 ~ 2026.06	(1) Complete the prediction, design and synthesis of individualized immunomodulatory diagnosis and treatment technology for recruited patients; (2) Complete the in vitro cellular immune induction effect detection of individualized immune regulation diagnosis and treatment technology for recruited patients; (3) Patients have been recruited for clinical experiments of individualized immune regulation diagnosis and treatment technology for ovarian cancer; (4) GMP level individualized tumor immune regulation diagnosis and treatment technology preparation, patients' body reinfusion, and safety index monitoring. Blood tumor indicators and imaging data were collected, and the clinical treatment effect and survival time of patients were followed up.
2026.07 ～ 2026.12	(1) The blood routine, biochemical, immunological, tumor indicators and imaging examination of the treated patients. (2) The patients were followed up and monitored, and the survival time of patients was analyzed. (3) Summarize the experimental results in time and publish 1 paper. (4) Complete the subject summary, identification, patent application, achievement declaration, etc.

十九、 **Follow up and medical measures after the end of the study;**

The follow-up plan lasted for 24 months and was divided into 8 times, that is, follow-up every 3 months after the end of treatment to track the clinical treatment effect and survival time of patients.

二十、 Responsibilities of each party and other relevant provisions;

In case of any damage related to this experimental study, the project research team will compensate and compensate according to relevant national laws and regulations.

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