

Abbreviated Title: Neoantigen Vaccine in NSCLC

Protocol Number: HJ-N-1601

CLINICAL STUDY PROTOCOL

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Research Title :	Phase I clinical trial of personalized neoantigen peptide vaccine for non-small cell lung cancer
Sponsor:	Tianjin Beichen Hospital
Principal Investigator:	Xueming Du
Protocol Number:	HJ-N-1601
Version Date:	2016.11.10
Principal Investigator (signature):	Xueming Du

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Synopsis

Research Title	Phase I clinical trial of personalized neoantigen peptide vaccine for non-small cell lung cancer
Sponsor	Tianjin Beichen Hospital
Indication	non-small cell lung cancer
Study Design	prospective, open label, single arm, phase I study
Number of trial subjects	60 participants
Study Period	36 months
Study Objective	<ol style="list-style-type: none"> 1. Primary objective To investigate the safety and feasibility of personalized neoantigen peptides vaccine on non-small cell lung cancer. 2. Secondary objectives <ol style="list-style-type: none"> (1) Peripheral blood-specific T cell IFN-γ levels, immune repertoire diversity, and tumor marker levels after treatment. (2) Progression-free survival (PFS) and overall survival (OS). (3) Clinical efficacy evaluation: CR, PR, SD, PD.
Study Procedure	The enrolled subjects were screened and fresh tissue or pathological sections and blood were taken for neoantigen detection and analysis. Production of neoantigenic peptides: The neoantigenic peptide vaccine mixture was injected subcutaneously in the left and right upper limbs once a week for 12 weeks, and treatment was permitted to continue after 12 weeks. Imiquimod was applied to the injection site after each administration.
Eligibility Criteria	<ol style="list-style-type: none"> 1. Inclusion Criteria <ol style="list-style-type: none"> (1) Recurrent stage III or IV NSCLC (2) EGFR-TKI resistance or progression after frontline therapy (3) Age > 18 years (4) Expected survival of at least three months (5) Eastern Cooperative Oncology Group (ECOG) \leq 3 (6) Biopsy sample or pathology slice available (7) Presence of more than one genetic mutation (8) No previous immunotherapy (9) Be able to follow the research program and follow-up process (10) Be able to and willing to give written informed consent 2. Exclusion Criteria <ol style="list-style-type: none"> (1) Pregnancy or lactation (2) Patients with known or suspected autoimmune disease or other complicated immune system disease (3) Systemic cytotoxic chemotherapy or experimental drugs for metastatic NSCLC within 4 weeks prior to the first dose of personalized neoantigen vaccine (not including EGFR-TKI) (4) Participation in any other clinical trial involving another investigational agent within 4 weeks prior to first dose of personalized neoantigen vaccine (5) Liver and kidney dysfunction, severe heart disease, coagulation dysfunction and/or hematologic impairment (6) Systemic infection (7) Other malignancy

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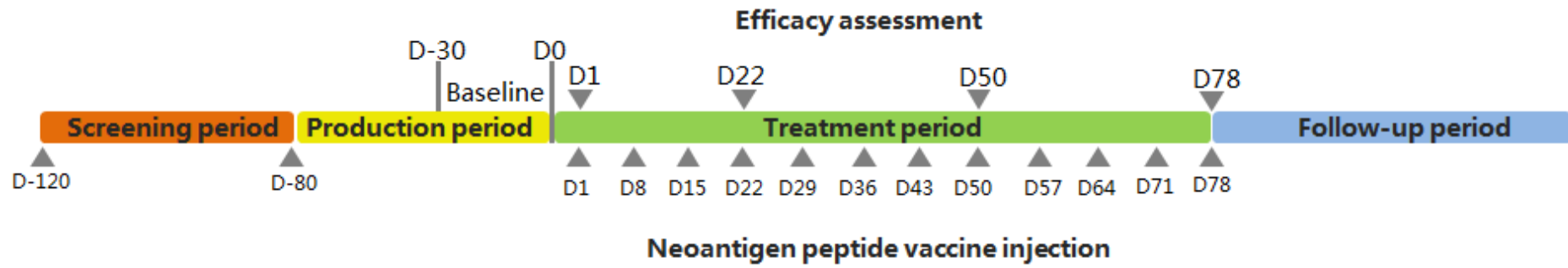
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Observation index	Blood was collected prior to treatment and after 4 weeks, 8 weeks, and 12 weeks of treatment. Clinical imaging, tumor marker evaluation, and immunologic efficacy test as well as ECOG score and basic clinical information were recorded. Patients were required to perform at least one pre-treatment scan for baseline measurements and another scan at 3 to 4 months post-vaccine for response assessment. Additional patient scans were taken monthly during the first 12 weeks of vaccination if feasible for OS and PFS follow-up. During the first 12 weeks of vaccine treatment and continued vaccination beyond 12 weeks, safety assessments were performed starting on the day of each vaccination until the next injection, and were performed every 2 months for patients with extended follow-up time. Disease-associated symptoms that were present at baseline (pre-treatment) were not reported unless they worsened after vaccination.
Safety assessment	The type, severity, frequency of occurrence, and relationship to the vaccine for all adverse events occurring during the study was described. Discontinuation due to adverse events and serious adverse events were noted. Treatment-associated adverse events were analyzed based on those categorized and graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE) version 4.0.
Statistical analyse	All statistical analyses were performed using the GraphPad Prism 5 (GraphPad Software Inc., La Jolla, CA). Survival curves and rates were calculated using the Log-rank (Mantel-Cox) Test, and overall survival was measured from the date of enrollment up to December 31, 2018 or the time of death. Two-tailed Student's t test or Mann-Whitney U test was used to analyze the statistical significance between groups. A P-value less than or equal to 0.05 was the threshold used to determine statistical significance.

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Trial Design



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1. Title

Phase I clinical trial of personalized neoantigen peptides vaccine for non-small cell lung cancer.

2. Study Objective

Primary objective

To investigate the safety and feasibility of personalized neoantigen peptides vaccine on non-small cell lung cancer.

Secondary objectives

- (1) Peripheral blood-specific T cell IFN- γ levels, immune repertoire diversity, and tumor marker levels after treatment.
- (2) Progression-free survival (PFS) and overall survival (OS).
- (3) Clinical efficacy evaluation: CR, PR, SD, PD.

3. Sponsor

3.1 Sponsor: Tianjin Beichen Hospital

Principal Investigator : Xueming Du

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3.2 Peptide vaccine prediction and design: MD Anderson Cancer Center

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3.3 Immune response monitoring design: MD Anderson Cancer Center

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3.4 Data collection and evaluation: Tianjin Beichen Hospital

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Postcode : 300400

Telephone : 86-22-26803516

4. Background

4.1 Research status of clinical treatment of lung cancer

The incidence of malignant tumors is increasing in China with elevated mortality rates

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and poor prognosis. The National Cancer Center National Cancer Research Office published a summary analysis of China's largest cancer survival data in the International Cancer Journal. These data show that the 5-year survival rate of cancer in China is 30.9%, far lower than that of developed countries. The five-year survival rate for rural cancer patients is 21.8%, only about half (39.5%) the urban patient survival rate.

Lung cancer is one of the most malignant tumors with the fastest growth in morbidity and mortality in China and the greatest threat to the health and life. In the past 50 years, the incidence and mortality of lung cancer have been significantly increased in many countries. The incidence and mortality of lung cancer ranks first in males and second in females among all malignant tumors. Therefore the treatment of lung cancer has increasingly become an area of intense focus in clinical oncology.

For early stage lung cancer, surgical resection combined with radiotherapy and chemotherapy is still the treatment of choice. A large meta-analysis has demonstrated that video-assisted thoracoscopic treatment is superior to open surgery in quality of life and long-term outcome. A meta-analysis of perioperative chemotherapy found that patients with stage IB-IIIa received survival benefit from perioperative chemotherapy and had a reduced risk of death. In patients with stage I NSCLC who have surgical contraindications or refuse surgery, high-dose stereotactic radiation may result in higher local control rates and lower toxicity. For patients with locally advanced NSCLC (stages IIIa to B) with good performance status who are not suitable for surgical resection, standard treatment includes 6 weeks of radiotherapy followed by platinum-based two-drug chemotherapy. However, the 5-year survival rate of these patients is still abysmally low.

For advanced lung cancer patients, 69% of advanced NSCLC have available molecular targets, and targeted therapy has progressed significantly in young non-smoking patients. Forty percent of Asian patients (mostly young, non-smoking adenocarcinoma patients) exhibit EGFR mutations. Nine randomized, controlled clinical studies in which EGFR-TKI was administered as first-line treatment of EGFR-mutant NSCLC demonstrated enhanced progression-free survival, objective response rate, and improved quality of life compared to chemotherapy. EGFR-TKI also improves OS. And while second-generation TKI showed further improvement in OS, adverse effects were also greater. The main adverse effects of afatinib include diarrhea (12.5%) and rash or acne (9.4%). In a separate study, four patients treated with gefitinib developed interstitial lung disease.

Almost all patients on EGFR-TKI treatment eventually develop resistance and disease progression. The T790M mutation is the most common secondary drug-resistant mutation, and this mutation is found in 50% to 65% of drug-resistant biopsies. The third-generation TKI drug AZD9291 (Osimertinib) targets T790M mutations and EGFR-TKI activating mutations. T790M-positive patients who have previously been treated with TKI can achieve an objective response rate of 61% and a progression-free survival of 9.6 months.

Platinum-based chemotherapy is a reasonable treatment option for patients without T790M mutations who are resistant to first-line EGFR-TKI therapy. First-line EGFR-TKI drug-resistant treatment is informed according to the patient's pattern of

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progression. Patients with local progression, are recommended to continue TKI therapy plus local treatment. Slowly progressing patients continue first-line TKI therapy with aggressive monitoring. Patients with rapid systemic disease progression, and drug resistance gene test results receive platinum-based chemotherapy. Platinum-free two-drug chemotherapy combined with gefitinib is not recommended.

The use of immune modulating agents for the treatment of tumors is becoming increasingly popular. CTLA-4 inhibitors and PD-1/PD-L1 inhibitors are the most well-studied immunotherapeutic drugs and have showed promise in the treatment of various tumors including advanced lung cancer. A variety of anti-PD-1 and PD-L1 agents have been approved for use with additional agents in clinical trials or preclinical studies. These drugs can provide sustained remission in 14% to 20% of patients with advanced NSCLC. Although there is no significant improvement in progression-free survival, improvement in OS is considerable.

4.2 Personalized tumor neoantigens

The induced systemic killing of tumor cells by the body's immune system is a novel regimen for the treatment of tumors. The manner by which to induce re-engagement of T cell activity is one of the core issues of individualized immunotherapy. The presentation of patient-specific tumor neoantigens in a heightened inflammatory context is an exciting emerging strategy for immune system re-engagement.

Gene mutation initiates tumorigenesis, and dividing tumor cells continue to produce mutations and potential neoantigens that permit differentiation cells from non-transformed cells. "Neoantigens" - novel amino acid sequences in protein-encoding genes - are important because they provide rare tumor-specific targets for discernment of neoplastic from normal self by the immune system. Immune responses generated by vaccination with neoantigen is theoretically directed only to tumor cells, avoiding destruction of normal cells and tissues.

Following vaccination with tumor-specific neoantigen peptides, the neoantigen is enzymatically digested into antigenic peptides by antigen-presenting cells (DC), loaded onto MHC, and presented to T cells able to recognize tumor antigen in the context of MHC presentation. Subsequently, activated T-cells expand and infiltrate the tumor microenvironment. It is generally believed that CD8+ T cells play the most critical role in eradicating tumor cells; however, recent data indicate that CD4+ T cells also effectively exert tumor immunity through the production of cytokines that recruit other immune cells, positive regulation of CD8+ T-cells, and their own direct cytotoxic effect.

In July 2017, teams from Harvard University's Dana-Farber Cancer Center (Catherine Wu) and the University of Mainz (Ugur Sahin) in Germany used neoantigen technology for small-scale clinical trials in the treatment of melanoma patients, achieving excellent results. Up to 20 patient-specific neoantigenic peptides were administered to each patient, resulting in immune responses against 60% of administered epitopes. Of 6 patients, 4 had no signs of recurrence after two years of treatment. Two other patients showed signs of relapsed but achieved CR after receiving anti-PD-1 therapy.

Because each patient's tumor mutations are individualized, each patient's

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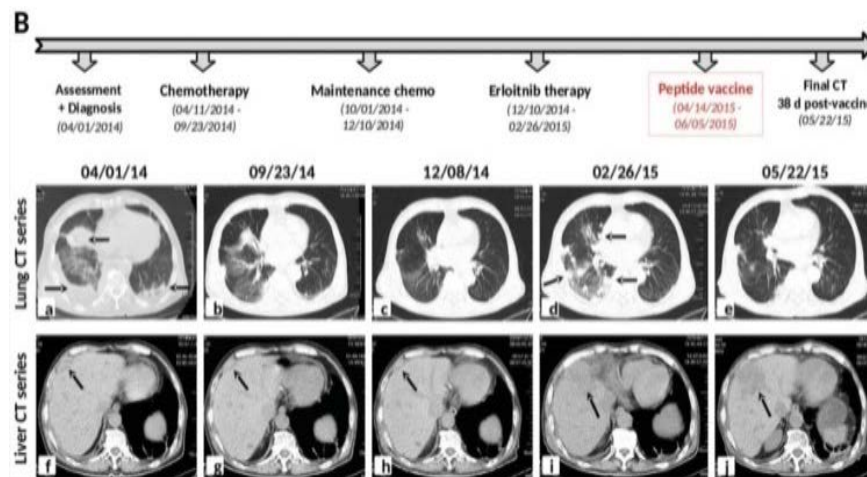
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"neoantigens" are different. Therefore the identification of personalized neoantigens requires whole exome sequencing of tumor and normal tissue as well as a sophisticated bioinformatics approach to identify the most likely antigenic peptides. With the advancement of genome sequencing technologies, bioinformatics analysis, and cancer immunotherapy methods, we have been able to quickly find mutations from the genome and select suitable targets to generate customized neoantigen vaccines for specific patients. Clinical trials have shown that such neoantigenic therapies are safe and effective and can generate immune response against individual mutations.

4.3 Clinical study of individualized neoantigen peptide in lung cancer

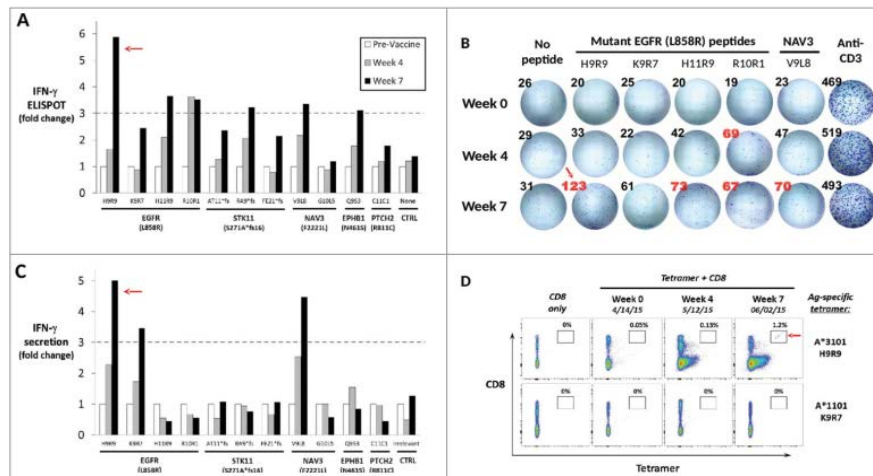
Our group previously published a case report *Rapid tumor regression in an Asian lung cancer patient following personalized neo-epitope peptide vaccination* upon application of individualized neoantigenic peptides in lung cancer at *Oncoimmunology*. This was the first report of a neoantigen vaccine for the treatment of lung cancer.

The article reported a squamous cell lung carcinoma patient demonstrating frank disease progression following chemotherapy and EGFR inhibitor treatment. Based on tumor mutational profiling and HLA typing, a saline-based multi-epitope peptide vaccine was designed and administered along with topical imiquimod as an adjuvant. Weekly neo-epitope peptide vaccination was followed by a rapid and dramatic regression of multiple lung tumor nodules, while a much larger liver metastasis remained refractory to treatment. Peripheral blood immune monitoring showed that specific cytotoxic T lymphocytes (CTLs) were induced primarily against peptide targets encompassing the widely shared EGFR L858R mutation, particularly one restricted to HLA-A*3101.



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5. Study Design

5.1 Study design

This is a prospective, open label, single arm, phase I study, to investigate the safety and feasibility of a personalized neoantigen peptide vaccine on non-small cell lung cancer.

5.2 Sample size

The study planned to recruit 60 participants.

5.3 Study Procedure

The enrolled subjects were screened and fresh tissue or pathological sections and blood were taken for neoantigen detection and analysis. Neoantigenic peptides were then synthesized. The neoantigenic peptide vaccine mixture was injected subcutaneously in the left and right upper limbs once a week for 12 weeks. Imiquimod was applied to the injection site with each administration with treatment beyond 12 weeks permitted for responders. Follow-up continued through the end of the study during which the patient's disease progression and survival status were recorded.

Toxicities will be graded according to the NCI Common Toxicity Criteria v4.0. No further vaccinations will be given to patients who develop grade 3 or 4 hypersensitivity reactions or grade 3 injection site reactions.

For all other grade 3 or 4 toxicity reactions, no further vaccinations will be given if in the opinion of the investigator the toxicity is related to the vaccine administration.

If greater than 1 out of 3 patients experience \geq grade 2 allergic reaction, \geq grade 2 autoimmune reaction and any grade 3 or 4 toxicity, the protocol will be stopped.

5.4 Sample collection

1. Screening period

Collection of tissue and peripheral blood; Next-generation sequencing; HLA typing; CBC; clinical biochemistry; urinalysis; tumor biomarker.

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2. Treatment period

Peripheral blood samples were collected for immunological examination (ELISA and ELISOPT for detection of IFN- γ expression), CBC, clinical biochemistry; urinalysis; tumor biomarker, immune repertoire assay. Tissue samples were also collected at the same position before and after treatment (post-treatment biopsies were optional and required additional patient consent) for transcriptome analysis and immune repertoire assay.

3. Follow-up period

Peripheral blood samples were collected for immunological examination, immune repertoire assay, clinical biochemistry; urinalysis; tumor biomarker. Collection of additional blood samples beyond the 12 weeks of the trial period was optional and required additional patient consent.

4. Tissue sample requirements

60 mg fresh tissue from a minimum of two core biopsies, Tumor cell content of the biopsy had to exceed 20%, and the proportion of necrotic cells had to be less than 20%.

Peripheral blood: 15 ml EDTA anticoagulant tube

Treatment of peripheral blood for immunological examination: Ficoll separation then resuspend in bambanker™ cryopreservation medium and dispense into the cryotube. Each tube contained at least 5×10^6 cells and was stored at -80°C .

6. Research Objectives

6.1 Inclusion criteria

- (1) Recurrent stage III or IV NSCLC
- (2) EGFR-TKI resistance or progression after frontline therapy
- (3) Age > 18 years
- (4) Expected survival of at least three months
- (5) Eastern Cooperative Oncology Group (ECOG) ≤ 3
- (6) Biopsy sample or pathology slice available
- (7) Presence of more than one genetic mutation
- (8) No previous immunotherapy
- (9) Be able to follow the research program and follow-up process
- (10) Be able to and willing to give written informed consent

6.2 Exclusion criteria

- (1) Pregnancy or lactation
- (2) Patients with known or suspected autoimmune disease or other complicated immune system disease
- (3) Systemic cytotoxic chemotherapy or experimental drugs for metastatic NSCLC within 4 weeks prior to the first dose of personalized neoantigen vaccine (not including EGFR-TKI)

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- (4) Participation in any other clinical trial involving another investigational agent within 4 weeks prior to first dose of personalized neoantigen vaccine
- (5) Liver and kidney dysfunction, severe heart disease, coagulation dysfunction and/or hematologic impairment
- (6) Systemic infection
- (7) Other malignancy

6.3 Withdrawal criteria

- (1) Subject voluntarily withdraws
- (2) The patient's clinical condition deteriorates necessitating additional treatment
- (3) Development of additional diseases during the clinical study that could effected efficacy or adverse event assessors
- (4) Adverse events cannot be tolerated or ameliorated
- (5) Non-compliance
- (6) Subjects cannot complete follow-up on time

Subjects may withdraw from the trial because the investigator believes that it is medically necessary or the subject wishes to do so. Subjects are free to withdraw from the study at any time without impact on future treatment. For all subjects who withdraw from the trial, case report forms are retained and are listed separately in the clinical study summary.

6.4 Elimination criteria

Cases that should not be included and cannot be included should be eligible for exclusion. These include misdiagnosis, mis-submission (not meeting inclusion and/or meeting exclusion criteria), use of other drugs not approved by the investigator during the trial; use of pharmaceuticals that render evaluation impossible. Each eliminated case will be explained. The CRF will be kept for reference but not included in the statistical analysis of efficacy. However, patients receiving at least one treatment and one follow-up will be included in the safety analysis.

7 Test Substance

7.1 Personalized neoantigen peptide vaccine

7.1.1 Personalized neoantigen analysis

Collect primary or metastatic tissue samples and peripheral blood of tumor patients. Extract tissue DNA. Perform library construction and 508 gene panel sequencing. Draw peripheral blood for high resolution HLA typing. Neoantigen sequences were assessed for predicted binding affinity to patient HLA class I and class II molecules according to the HLA-peptide prediction algorithms NetMHC4.0, NetMHCpan3.0, NetMHCpan4.0, NetMHCII2.2 and NetMHCII2.3. Immunizing neoantigen peptides were chosen primarily based on highest predicted binding affinity to the patient's HLA class I and class II molecules. However, vaccines were also designed to maximize the number of different HLA molecules engaged and minimize

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intra-HLA peptide competition when possible. Certain biochemical properties (such as elevated hydrophobicity or the presence of multiple cysteines) which can negatively impact the synthesizability or solubility of the immunizing peptides were also considered. In addition, we aimed to design individual patient vaccines to contain an approximately 2:1 ratio of short to long vaccine peptides, or as close as the somatic mutation profiling and HLA/peptide binding predictions would allow. For each patient, up to 14 peptides of 9 to 20 amino acids in length were selected and prioritized.

7.1.2 Personalized neoantigen peptide production

7.1.2.1 Production Process

Individualized tumor neoantigen peptides were prepared by solid phase synthesis using Fmoc method and protected by N-terminal acetylation. Purification was carried out by reversed phase high performance liquid chromatography. Peptides were lyophilized and mixed according to the designed grouping (two subgroups per patient).

7.1.2.2 Quality control standard

- (1) Purity: > 98%
- (2) The molecular weight is consistent with the target product
- (3) Endotoxin: < 10Eu/mg
- (4) TFA content: < 1%

7.1.2.3 Storage Conditions

The lyophilized powder is stored at -20°C and at 4°C after reconstitution. Injection must be completed within 2 hours of reconstitution.

7.2 Treatment method

Dissolve each neoantigen vaccine (200 ug each peptide, two subgroups per patient, no more than 10 peptides in each subgroup) in 1 ml PBS and administer subcutaneously on each of the left and right upper limbs once a week for 12 weeks. Apply imiquimod to each injection site after administration and treatment was permitted to continue after 12 weeks.

7.3 Packaging label

Phase I clinical trial of personalized neoantigen peptide vaccine	
for non-small cell lung cancer	
(For clinical trial only)	
Patient Number:	Initials:
Product number:	gross weight:

7.4 Issuance, return and destruction of test objects

The cooperative unit shall directly deliver the test materials for clinical trials to the

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test center, establish a complete receiving procedure, and assign a dedicated location in the research unit to be kept by a coordinator other than the research doctor. The use of the test substance should be recorded in the corresponding test substance inventory record form. At the end of the intermediate phase of the study, all tested or unused samples should be determined and recorded. At the end of the study, all test materials shipped to the investigator, including all opened or unopened test articles and all recovered test materials must be returned to the partner unit with appropriate written documentation. Returned test object should be kept in its original packaging. When destroying the test object at the research site, the coordinator is responsible for ensuring the completion of this process, maintaining written authorization of the partner unit, and maintaining a written record of destruction.

8 Test Implementation

8.1 Test procedure

In order to complete the requirements of this research protocol, the researcher should follow the current Drug Clinical Trial Management Regulations (GCP) and related SOPs and perform clinical trial according to the following procedures:

- (1) Ethics committee review of clinical trial materials, including clinical trial protocols, informed consent forms, case report forms, and obtained ethical approvals
- (2) Preliminary screening of subjects interested in participating in clinical studies through outpatient or inpatient examinations
- (3) Enroll subjects after obtaining informed consent and conducting a comprehensive clinical examination
- (4) Receipt of personalized tumor neoantigen peptide therapy
- (5) Efficacy assessment
- (6) Follow-up observation
- (7) Data entry and statistical analysis
- (8) Clinical trial summary

8.2 Research process

The study enrolled 60 participants for a prospective, open label, single arm phase I clinical trial.

Prior to enrollment, the investigator explained the study to the patient in detail and signed the informed consent form approved by the ethics committee.

Screening was performed with reference to the eligibility criteria, and neoantigen detection analysis and laboratory tests were performed to explain the treatment procedure to subjects who met the enrollment criteria.

Each subject was assigned a subject number. Individualized tumor neoantigen peptide preparations were synthesized according to the analyzed neoantigen peptide information. The patient number remained unchanged throughout the trial and appeared on the test bottle and was recorded in the case report form for each subject.

Subjects underwent observation and efficacy evaluation during treatment, which the

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investigator recorded in the case report form.

The investigator decided whether to use other combined medications according to the condition of the subject to protect the interests and safety of the subject. All medications including research medications were recorded in the case report form.

8.2.1 Screening period (Day -120 ~ -80)

- (1) Sign written informed consent
- (2) Verification of inclusion/exclusion criteria
- (3) Demographic data collection: gender, age
- (4) Collect complete medical history
- (5) Physical examination, vital signs
- (6) Laboratory analysis
- (7) Tumor examination: Pathological examination and imaging (PET/CT, MRI)
- (8) Negative urine pregnancy test for women of childbearing age
- (9) ECOG score
- (10) Neoantigen detection: Gene mutation detection, HLA typing

8.2.2 Personalized tumor neoantigen peptides preparation (day-80 ~ 0)

- (1) Neoantigen analysis
- (2) Neoantigen peptide synthesis
- (3) Neoantigen peptide purification
- (4) Neoantigen peptide QA/QC
- (5) Neoantigen peptide lyophilization and packaging

8.2.3 Baseline evaluation (day -30 ~ 0)

- (1) Physical examination, vital signs
- (2) Laboratory analyses
- (3) Clinical imaging
- (4) ECOG score

8.2.4 Treatment period (day 1 ~ 78)

- (1) ECOG score
- (2) Physical examination, vital signs
- (3) Laboratory analyses
- (4) Clinical imaging
- (5) Peripheral blood immunological examination, immune repertoire assay
- (6) Tissue immune repertoire
- (7) Record adverse events

8.2.5 Follow-up period

- (1) ECOG score

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- (2) Physical examination, vital signs
- (3) Laboratory analyses (if available)
- (4) Clinical imaging (if available)

- (5) Peripheral blood immunological examination, immune repertoire assay (if available)
- (6) Record adverse events

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8.2.6 Trial design

Time Content	Screening	preparation	Baseline	Treatment period												Follow-up
	-120~-80day	-80~0day	-30~0day	1day	8day	15day	22day	29day	36day	43day	50day	57day	64day	71day	78day	
Informed consent	X															
Demographic characteristics	X		X													
Physical examination, vital signs	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X
Laboratory analyses			X				X				X				X	X
Tumor markers			X				X				X				X	X
ECOG score	X		X				X				X				X	X
Urine pregnancy check ¹	X															
Pathological examination	X															
Clinical imaging	X		X												X	X

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Time Content	Screening	preparation	Baseline	Treatment period												Follow-up
	-120~-80day	-80~0day	-30~0day	1day	8day	15day	22day	29day	36day	43day	50day	57day	64day	71day	78day	
Neoantigen detection	X															
Neoantigen analysis	X															
Neoantigen peptide preparation		X														
Neoantigen peptide injection				X	X	X	X	X	X	X	X	X	X	X	X	
Blood collection ²			X				X				X				X	X
Peripheral blood immunologic examination ²			X				X				X				X	X
Immune repertoire assay/ Transcriptome analysis ⁴			X												X	X
Adverse event				X	X	X	X	X	X	X	X	X	X	X	X	X

1. Must be performed by a female caregiver
2. Blood collection is performed before injection of the neoantigen vaccine in 15 ml EDTA anticoagulant tube
3. The immunological examination was performed by the partner unit after the end of the 12-week administration .
4. Transcriptome analysis and immune repertoire assay: if available
5. Determination during follow-up period: if available

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9 Evaluation index

9.1 Primary endpoint

The safety and feasibility of personalized neoantigen peptides vaccine on non-small cell lung cancer.

The primary objective of this study was to observe the treatment-related adverse events of each subject after treatment. Record vital signs data before and after treatment, record any discomfort, observe local injection reaction: fatigue, rash, fever, flu-like symptoms, arthralgia, headache, nausea, pruritus, etc.

The classification criteria for adverse event levels are in accordance with CTCAE, version 4.0.

9.2 Secondary endpoints

- (1) Peripheral blood T-cell IFN- γ secretion, immune repertoire diversity, tumor marker levels after treatment.
- (2) Progression-free survival (PFS) and overall survival (OS).
- (3) Clinical efficacy evaluation: CR, PR, SD, PD.

Objective tumor response assessments were made according to the Response Evaluation Criteria in Solid Tumors (RECIST version 1.1) guidelines. We utilized computerized tomography (CT) and/or magnetic resonance imaging (MRI) scans to measure selected target lesions. A maximum of two target lesions per organ were measured, with the two largest lesions selected, up to a maximum of five lesions in total. Tumor burden was calculated as the sum of the diameters of all target lesions.

Clinical responses were evaluated as follows: CR, complete disappearance of all target lesions; PR, partial response, defined as a 30% decrease in the sum of diameters of target lesions; PD, progressive disease, defined as a minimum 20% increase in the sum of diameters of target lesions or the appearance of new lesions; and SD, stable disease, defined as a change in tumor burden insufficient to qualify for PR, or PD. Clinical responses were assessed 3 to 4 months following the date of the first immunization.

10 Adverse Events

10.1 Adverse events

Adverse events (AE): Refers to adverse medical events (including any adverse or unintended signs, symptoms, or diseases, including abnormal laboratory findings) that occur after a subject has received a drug, regardless of whether that symptom is related to the test drug. An increased severity of pre-existing disease is also considered an adverse event. Pay special attention to treatment-related adverse events.

10.2 Record adverse events

- (1) All adverse events must be recorded in detail in the CRF.

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- (2) Adverse event content: The name of the symptom or the name of the sign or the diagnosis of the disease or the laboratory test for an abnormality.
- (3) Adverse event start date: The date the subject first experienced AE or the AE related symptoms.
- (4) Adverse event end date: The date on which the symptoms associated with AE stopped. If the AE still exists, do not fill in the end date. If an adverse event lasts less than 24 hours, record the number of hours the AE lasts.
- (5) Assess the severity of adverse events.
- (6) Measures taken
- (7) End result

10.3 Judgment criteria for severity of adverse events

According to the National Cancer Institute (NCI) Common Adverse Event Scoring Criteria Version 4.0 (CTCAE 4.0) Level 5 Scale (Level 1 to 5), the intensity of all adverse events is graded and reported in detail on the case report form. The following grading methods apply to adverse events listed in CTCAE 4.0:

Grade 1 Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.

Grade 2 Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental ADL*.

Grade 3 Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self care ADL**.

Grade 4 Life-threatening consequences; urgent intervention indicated.

Grade 5 Death related to AE.

* Instrumental ADL refer to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.

** Self care ADL refer to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden.

10.4 Precautions

- (1) Researchers should be familiar with the data and literature related to clinical trials. All participants in the clinical trial are comprised of GCP-trained physicians with extensive clinical experience.
- (2) Researchers should have a good understanding of the pharmacology and toxicology of the test substance, and be aware of possible adverse events and their rescue measures.
- (3) Abnormalities in laboratory tests are determined by the investigator as to whether or not they are adverse events, depending on whether they are accompanied by clinical manifestations.
- (4) Researchers should maintain accurate, complete, legal, and timely inclusion of data in case report forms.
- (5) Training and guidance for the staff participating in the trial must be provided by the principal investigator.

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10.5 Treatment and follow-up investigation after adverse events

If an adverse event occurs during the trial, regardless of whether it is causally related to the test substance, the primary investigator should immediately give necessary and appropriate treatment with adequate explanation to the subject. At the same time, follow-up investigations should be conducted for adverse events until normalization or until the principal investigator judges that there is no need to continue the investigation. The principal investigator or investigator must record all adverse events in the case report form.

10.6 Serious adverse event (SAE)**10.6.1 Definition of serious adverse event**

Incidents requiring hospitalization, prolonged hospitalization, disability, ability to work, life-threatening or death, congenital malformations, etc., occurring during a clinical trial may be considered SAEs.

10.6.2 Serious adverse event reporting

When serious adverse events occur, the investigator must notify the clinical trial/ethics committee, partner, and the Serious Adverse Events Form by phone or fax within 24 hours.

10.7 Measures taken after the subject has become pregnant

If a subject becomes pregnant during the study, treatment must cease. Pregnancy must also be reported to the investigator within 3 months of the end of the treatment study. The investigator should provide counseling to discuss the risk of continuing pregnancy and the impact on the fetus. Subjects should be tested continuously until the end of pregnancy. The final outcome of pregnancy should be provided to the principal investigator and reported. Similar procedures and counseling shall be provided to the partners of participating men in the event that the partner of a male participant becomes pregnant.

11 Ethics**11.1 Ethical considerations in research design**

This study was designed to examine the safety and efficacy of subjects receiving individualized tumor neoepitope vaccination. Invasive procedures for the purposes of this study, including blood samples for hematology, clinical chemistries, tissue/cytopathology, and imaging studies are based in GCP and RECIST1.1 solid tumor efficacy evaluation criteria.

11.2 Protection of subject's rights

Clinical trials must comply with the ethical guidelines of the Helsinki Declaration. The subject's rights, safety, and will are higher than the needs of the study investigators. All trial results shall be kept confidential and all personal privacy rights shall be maintained and respected.

11.3 Ethical norms

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11.3.1 Researcher responsibility

- (1) It is the responsibility of the investigator to ensure that the clinical study is implemented according to the protocol, the GCP and related regulatory requirements.
- (2) GCP is an internationally recognized ethical theory and scientific quality standard for the design, implementation, documentation, and reporting of research on humans.

11.3.2 Ethics committee approval

Before the study begins, the researcher must submit the study protocol, patient informed consent, researcher's manual and other materials to the ethics committee. Clinical trial may be conducted only after obtaining approval from the ethics committee. At the conclusion of the study, the investigator will inform the ethics committee that the study has ended.

11.3.3 Informed consent

Each subject must give written consent after being fully explained the purpose and content of the study. Subjects must sign an informed consent form before performing any research-related operations. The informed consent form used must be approved by the partner unit and approved by the ethics committee. Informed consent must be consistent with the principles of the Helsinki Declaration, current GCP guidelines, and current regulations.

Before the subject enters the study, the investigator or authorized researcher must explain to the prospective subject the purpose, method, expected benefit, and potential risk of the study and any possible side-effects. Subjects must be told that their participation in the study is voluntary and may withdraw at any time. Whether or not to participate in the study will not have any effect on future treatment. Subjects must be informed that their medical records will be kept for long-term follow-up and that these records can be viewed by the drug administration or authorized partner units with the permission of current laws and regulations. Once the subject has signed the informed consent, the subject is authorized to act as described above.

Subjects should have sufficient time to read the informed consent document and have the opportunity to ask questions. After the explanation and before enrollment, the informed process was recorded by the subject signing and dating the informed consent form. Subjects received a copy of this informed consent. If the subject (or legal representative) is unable to read and write, there should be a fair witness to participate in the entire process of informed consent (including reading and interpreting all written information).

11.3.4 Privacy of personal data

The collection and processing of personal data for subjects enrolled in the study must be limited to studies of the efficacy, safety, quality, and use of the treatment. Adequate measures must be taken to ensure the confidentiality of data and compliance with existing laws and regulations protecting privacy when collecting and processing such data.

Before collecting personal data, the investigator will ask the subject to agree to the collection of personal data. This consent also includes data that can be transmitted to

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other agencies. Appropriate technical methods and management measures should be taken to protect personal data from unauthorized or unauthorized access, accidental or illegal damage, and accidental loss or tampering.

12 Data Management And Statistical Analysis

12.1 Data management and quality control

The case report form must be filled out by the study investigators, and each report must be completed. After the completed case report form is reviewed by the clinical inspector, the original is handed over to the data administrator for data entry and management.

A database will be created to record the information in the CRF table. The format of the database will be as close as possible to the format of the CRF table for easy entry. The data items of the CRF are double-entered by two data entry clerks. The data in the database is then systematically checked by the data administrator application validator. All errors or omissions will be filled in the data question form and returned to each research center for verification.

When the database is considered complete and accurate, the database will be locked. Any changes to the database after this must be carried out through clinical trials by the principal investigator, the participating statistical statistician, and the data administrator.

12.2 Statistical analysis

12.2.1 Data set

Intent to treat population (ITTP): Refers to all cases that express treatment intentions and sign informed consent.

Per-protocol population (PPP): Refers to cases that meet the inclusion criteria, are not excluded, and complete the treatment plan, that is, analyze the cases that meet the test plan, have good compliance, and complete the CRF requirements (PP analysis).

Safety assessment population (SAP): Cases with safety indicators recorded. The incidence of adverse reactions is based on SAP as the denominator.

12.2.2 Statistical analysis

A statistical analysis plan was developed by principal investigator post hoc based on the research protocol. All statistical analyses were performed using the GraphPad Prism 5 (GraphPad Software Inc., La Jolla, CA). Survival curves and rates were calculated using the Log-rank (Mantel-Cox) Test, and overall survival was measured from the date of enrollment up to December 31, 2018 or the time of death. Two-tailed Student's t test or Mann-Whitney U test was used to analyze the statistical significance between groups. A P-value less than or equal to 0.05 was the threshold used to determine statistical significance.

Safety assessments will be descriptive, and safety indicators include adverse events, laboratory test data, and vital signs. The type, severity, frequency of occurrence, and relationship to the test article for all adverse events occurring during the study will be described. Cases of discontinued studies due to adverse events and serious adverse events are noted.

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13 Test Management

13.1 Compliance with the program

The study shall be carried out in accordance with the approved plan, and any deviation from the plan shall be recorded.

13.2 Case report form (CRF)

The CRF is filled in by the principal investigator's authorized personnel on the original record. Fill in the CRF according to the GCP principle to ensure the accuracy of the data. The main investigator signs the CRF signature page to confirm that the data in the CRF is true, accurate, and complete. The medical records of each subject are kept as hospital records. The CRF will be kept in the drug clinical trial facility of the research unit.

13.3 Data processing and record keeping

The researcher should save all the detailed original documents of the test, and record the contents of the test results, subject information, etc. in the information collection form. The recorded data should be complete, timely, clear, detailed, and easily recognized by personnel participating in the clinical trial.

The information collection form and the original documents can only be modified by the principal investigator, and the original data must not be obfuscated by modification. The correct modification method is to draw a single line through the original data, and then write the modified data next to the original data, and sign the date and the name of the modified person.

If the principal investigator leaves the study institution or retires or ceases to perform his research duties, he must notify the partner (in writing) to develop appropriate contingencies for the test data.

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Appendix

ECOG Performance Status

Grade	Description
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.
1	Symptomstic but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (e.g., light housework, office work).
2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.
4	100% bedridden. Completely disabled. Cannot self-care. Totally confined to bed or chair.
5	Deceased.