

Clinical Study Protocol

Version No.: V2.0, Apr 7, 2020

**A multicenter, single-arm, phase II trial of camrelizumab and chemotherapy as neoadjuvant treatment for locally advanced esophageal squamous cell carcinoma**

**Institution responsible for the clinical trial** Shanghai Chest Hospital, Shanghai Jiao Tong University

**Participating institutions** Changhai Hospital Affiliated to The Second Military Medical University  
The First Affiliated Hospital of Nanchang University  
The First Affiliated Hospital of Anhui Medical University

**Principal Investigator** Zhigang Li

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**Protocol Summary**

<b>Study title</b>	A multicenter, single-arm, phase II study of camrelizumab and chemotherapy as neoadjuvant treatment for resectable esophageal squamous cell carcinoma
<b>Study phase</b>	Phase II
<b>Version No. (date)</b>	Version 2.0 (April 7, 2020)
<b>Study objectives</b>	To evaluate the safety and efficacy of preoperative PD-1 inhibitor camrelizumab combined with chemotherapy (weekly nab-paclitaxel and carboplatin) in the treatment for locally advanced esophageal squamous cell carcinoma (ESCC) with multi-station lymph node metastasis.
<b>Endpoints</b>	<p><b>Primary endpoint:</b></p> <p>Pathological complete response (pCR) rate</p> <p><b>Secondary endpoints:</b></p> <p>3-year recurrence-free survival rate</p> <p>R0 resection rate</p> <p>3-year overall survival rate</p> <p>Safety</p> <p>Surgical complication rate</p> <p><b>Exploratory endpoints:</b></p> <p>To identify the predictive value of potential biomarkers (PD-L1, TMB, dMMR/MSI-H, LDH) for pCR rate.</p>
<b>Study Design</b>	Patients with locally advanced (cT1b-4aN2-3M0) ESCC will be treated with two cycles of preoperative camrelizumab and chemotherapy. A fixed dose of 200 mg camrelizumab will be administered every 3 weeks. The selected dose of nab-paclitaxel will be 100 mg/m <sup>2</sup> (D1, 8, 15) and the dosage of carboplatin (D1) will be calculated according to AUC=5 mg/mL per min, with a 3-week administration cycle. Approximately 4-6 weeks after neoadjuvant treatment, the patient will be re-evaluated for surgery. Postoperative adjuvant treatment options will be decided by MDT discussion 1 month after surgery.
<b>Inclusion criteria</b>	<ol style="list-style-type: none"> <li>1. Age 18-75 years, male or female;</li> <li>2. Histologically confirmed T1b-4aN2-3M0 ESCC (AJCC/UICC TNM staging, 8th Edition. Lymph nodes with short diameter greater than 10 mm was defined as clinically metastasis. For lymph nodes located at recurrent laryngeal nerve (RLNs) and left gastric artery, short axis ≥ 6.5mm was considered as positive.);</li> <li>3. Without any previous treatment;</li> <li>4. ECOG performance score is 0-1;</li> <li>5. With no severe hematologic disorder, cardiac, pulmonary, hepatic or renal dysfunction, or immunodeficiency Signed consent prior to</li> </ol>

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	<p>treatment;</p> <p>6. Hemoglobin <math>\geq 100</math> g/L; white blood cell (WBC) <math>\geq 4.0 \times 10^9/L</math>; neutrophil (ANC) <math>\geq 2.0 \times 10^9/L</math>, platelet (Pt) <math>\geq 100 \times 10^9/L</math>; serum bilirubin <math>\leq 1.5</math> times the upper limit of normal value (ULN); aspartate aminotransferase (AST) and alanine aminotransferase (ALT) <math>\leq 1.5 \times</math> ULN; serum creatinine <math>\leq 1.5 \times</math> ULN or creatinine clearance rate <math>\geq 60</math> ml/min; urea nitrogen <math>\leq 200</math> mg/L; thyroid stimulating hormone (TSH) <math>\leq</math> ULN (If abnormal, T3 and T4 levels should be referred to simultaneously. If T3 and T4 are normal, the patient can also be enrolled);</p> <p>7. Pulmonary function: baseline FEV1 at least 2 L; if baseline FEV1 <math>&lt; 2</math> L, the estimated postoperative FEV1 <math>\geq 800</math> ml; no myocardial infarction within 1 year; no unstable angina; no symptomatic severe arrhythmia; no cardiac insufficiency;</p> <p>8. Patients or their family members agree to participate in the study and sign the informed consent form.</p>
<b>Exclusion criteria</b>	<p>1. Previous exposure to any anti-PD-1 or anti-PD-L1 antibody;</p> <p>2. Patients with second primary malignant disease;</p> <p>3. Pregnant women or women preparing for pregnancy;</p> <p>4. Patients with concurrent autoimmune disease or a history of chronic or recurrent autoimmune disease;</p> <p>5. Patients who need to be treated with corticosteroid (dose equivalent to prednisone of <math>&gt;10</math> mg/day) or other immunosuppressive agents within 2 weeks prior to study drug administration;</p> <p>6. Patients infected with HIV, or with active hepatitis B or C (HBV DNA <math>\geq 10^4</math> copies/mL; HCV RNA <math>\geq 10^3</math> copies/mL);</p> <p>7. Patients with a history of pneumonitis or interstitial lung disease with clinical evidence, such as interstitial pneumonia and pulmonary fibrosis found by baseline CT scan;</p> <p>8. Patients with known or concurrent bleeding disorders or other uncontrolled diseases who cannot receive surgical treatment</p> <p>9. Physical examination or clinical trial findings that, in the opinion of the investigator, could interfere with the results or put the patient at increased risk for treatment complications</p> <p>10. Patients with concurrent systemic diseases (moderate to severe chronic obstructive pulmonary disease, poorly controlled hypertension, unstable angina, congestive heart failure, myocardial infarction within 6 months, unstable mental disorders requiring therapy);</p> <p>11. Patients who have allergic constitution or are allergic to multiple drugs;</p> <p>12. Patients who participated in other clinical trials within 30 days before enrollment;</p> <p>13. Patients who are not suitable for participation in clinical trials in the opinion of the investigator.</p>
<b>Discontinuation</b>	Trial discontinuation means the clinical trial is not completed according to

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<b>criteria</b>	<p>protocol and is prematurely discontinued.</p> <ol style="list-style-type: none"> <li>1. Identified unanticipated, significant, or unacceptable risk to subjects;</li> <li>2. Serious adverse events, or other events that affect subject safety, including but not limited to the occurrence of any symptomatic adverse events, laboratory abnormality, or other medical condition;</li> <li>3. Radiographic disease progression or metastasis, unless the subject meets the criteria for continued treatment after disease progression and agrees to continue treatment;</li> <li>4. Major protocol deviation is found in the implementation that make evaluation of drug efficacy difficult if the trial is to be continued;</li> <li>5. Other conditions that the investigator considers necessary to terminate the study drug treatment;</li> </ol>
<b>Sample size</b>	<p>Simon two-stage design method;</p> <p>The pCR rate of chemotherapy alone was set at 5% that had been reported previously, and the pCR rate of camrelizumab plus chemotherapy was assumed to be 15%. A total of 30 patients will be enrolled in the first phase. If pCR is obtained in less than or equal to one patient, the study will be terminated. Afterwards, 22 patients will be enrolled in the second phase as planned. An additional 8 patients will be enrolled to account for possible dropouts (15%). This leads to a planned sample size of 60 patients.</p>
<b>Statistical analysis</b>	<p>Statistical analyses were conducted using SPSS 25.0 software.</p> <p><b>Efficacy analysis</b></p> <p>The primary variables of this study are the completion of neoadjuvant regimen, pathological complete response rate (pCR). EFS and OS will be analyzed using the Kaplan-Meier method and log-rank test.</p> <p><b>Safety analysis</b></p> <p>The safety analysis was based on descriptive statistics. Adverse events and their most severe reaction grades will be summarized according to NCI-CTCAE version 5.0, and adverse events will also be summarized according to the association with study drug. The descriptive summaries of laboratory test values were mainly focused on abnormal values. Laboratory abnormalities will also be summarized by the worst grade according to the NCI-CTCAE version 5.0.</p>
<b>Trial schedule</b>	<p>Estimated start time of the trial: Oct. 2019</p> <p>Estimated time of enrollment completion: Oct. 2020</p> <p>Estimated end time of the trial: Dec. 2023</p>

## 1. Study Background

Esophageal cancer is one of the common malignant tumors in China, of which esophageal squamous cell carcinoma (ESCC) accounts for about 90% of patients and its incidence rate is increasing annually. Due to the atypical early symptoms of this disease, approximately half of patients presented with locally advanced disease at the time of diagnosis. Neoadjuvant chemoradiotherapy (nCRT) followed by esophagectomy is the recommended treatment modality for locally advanced disease, which could downstage the tumor, improve R0 resection rate and therefore improve the long-term prognosis. The pathological complete response (pCR) rate and safety outcomes are two key indicators for evaluating neoadjuvant therapy. It has been reported in the literature that the incidence of complications after nCRT combined with surgery ranges from 16% to 93%, and the mortality rate is 0-29%. For ESCC patients with multistation lymph nodes metastasis, the current AJCC classification has attributed it to stage IIIC and IVA, which shows that its prognosis is extremely poor. However, due to the wide range of lymph node metastasis in these patients, the radiation target area is difficult to be completely covered. And even if it is covered, it will lead to a wide field of radiation target area as well as expected high incidence of related adverse events. Therefore, how to reduce the toxicity of neoadjuvant therapy in these patients is worthy of investigation. Moreover, the pCR rate of ESCC is mostly maintained between 3-10% after neoadjuvant chemotherapy alone according to previous studies. Therefore, for patients with cN3 ESCC who cannot be treated with nCRT, how to identify a more effective neoadjuvant treatment is an important issue.

In recent years, immunotherapy which was represented by immune checkpoint inhibitors, has made great progress in the treatment of malignant tumors. In esophageal cancer, randomized trials such as Keynote-181, Attraction-3 and ESCORT had strongly demonstrated the therapeutic effect of immune checkpoint inhibitors as second-line treatment. Then, the ongoing phase 3 studies including Keynote-590, Attraction-4 and ESCORT-1<sup>st</sup> were designed to explore immunotherapy as first-line treatment for advanced esophageal cancer, with acceptable toxicity and satisfied short-term efficacy from the preliminary results. Camrelizumab is a checkpoint inhibitor targeting PD-1. The safety and efficacy of camrelizumab in the treatment of ESCC has been firstly described in 2018, which enrolled 30 heavily pretreated ESCC patients. Then, ESCORT study of camrelizumab versus chemotherapy as second-line therapy for ESCC patients revealed that patients with camrelizumab had a longer overall survival (OS) as well as comparable rate of grade 3-5 TRAEs to chemotherapy.

However, the efficacy of PD-1/PD-L1 inhibitors for neoadjuvant treatment of surgically resectable malignancies has not been determined. Generally, the immune function of patients with locally advanced disease is better than that of patients with advanced disease. Therefore, early application of immunotherapy to neoadjuvant treatment of patients with locally advanced disease may be able to better play the role of immunotherapy. In 2018, the study of Nivolumab neoadjuvant therapy for NSCLC opened the door to exploring the application of neoadjuvant immunotherapy. The results showed that neoadjuvant immunotherapy has fewer adverse effects and did not prolong the time to surgery. Twenty patients underwent surgical resection, of whom 8 (40%) patients achieved pathological downstaging and 9 (45%) patients achieved major pathological response, with an 18-month recurrence-free survival rate of 73%. Then, there have been prospective studies on neoadjuvant immunotherapy combined with chemotherapy for locally advanced gastroesophageal junction adenocarcinoma, and the preliminary results are encouraging.

Based on the above, we intend to carry out a prospective, single-arm, exploratory phase II clinical study of neoadjuvant immunotherapy combined with chemotherapy in ESCC patients with

multistation lymph nodes metastasis. The scientific question we want to solve is whether triple modality with neoadjuvant immunotherapy combined with chemotherapy followed by surgery can explore an effective approach for patients with multistation lymph node metastasis who are very difficult to achieve a satisfied treatment outcome.

## 2. Study Objectives

To investigate the safety and feasibility of neoadjuvant camrelizumab combined with chemotherapy (nab-paclitaxel and carboplatin) in the treatment of locally advanced thoracic esophageal squamous cell carcinoma with multistation lymph nodes metastasis.

### Study endpoints

#### Primary study endpoints

Overall pathological complete response (pCR) rate. PCR is defined as the proportion of subjects with no residual tumor cells (ypT0N0) in primary tumor and lymph nodes.

#### Secondary study endpoints

- 1) R0 resection rate;
- 2) 2-year, 3-year, and 5-year survival rates (OS rate);
- 3) Toxicities.

#### Exploratory study endpoints

To evaluate the relationship between tumor tissue/blood molecular markers (PD-L1 expression, TMB, dMMR/MSI-H, Tils, LDH) and pCR rate.

## 3. Study Design

A prospective, single-arm, phase II clinical trial.

## 4. Study Population

### 4.1 Inclusion criteria

- 1) Age 18-75 years, male or female;
- 2) Histologically confirmed T1b-4aN2-3M0 ESCC (AJCC/UICC TNM staging, 8th Edition. Lymph nodes with short diameter greater than 10 mm was defined as clinically metastasis. For lymph nodes located at recurrent laryngeal nerve (RLNs) and left gastric artery, short axis  $\geq 6.5$ mm was considered as positive.);
- 3) Without any previous treatment;
- 4) ECOG performance score is 0-1;
- 5) With no severe hematologic disorder, cardiac, pulmonary, hepatic or renal dysfunction, or immunodeficiency Signed consent prior to treatment;
- 6) Hemoglobin  $\geq 100$  g/L; white blood cell (WBC)  $\geq 4.0 \times 10^9$ /L; neutrophil(ANC)  $\geq 2.0 \times 10^9$ /L, platelet (Pt)  $\geq 100 \times 10^9$ /L; serum bilirubin  $\leq 1.5$  times the upper limit of normal value (ULN); aspartate aminotransferase (AST) and alanine aminotransferase (ALT)  $\leq 1.5 \times$  ULN; serum creatinine  $\leq 1.5 \times$  ULN or creatinine clearance rate  $\geq 60$  ml/min; urea nitrogen  $\leq 200$  mg/L; thyroid stimulating hormone (TSH)  $\leq$  ULN (If abnormal, T3 and T4 levels should be referred to simultaneously. If T3 and T4 are normal, the patient can also be enrolled);
- 7) Pulmonary function: baseline FEV1 at least 2 L; if baseline FEV1  $< 2$  L, the estimated postoperative FEV1  $\geq 800$  ml; no myocardial infarction within 1 year; no unstable angina; no symptomatic severe arrhythmia; no cardiac insufficiency;



- 8) Patients or their family members agree to participate in the study and sign the informed consent form.
- 9) No other severe cardiopulmonary diseases.

#### 4.2 Exclusion criteria

- 1) Previous exposure to any anti-PD-1 or anti-PD-L1 antibody;
- 2) Patients with second primary malignant disease;
- 3) Pregnant women or women preparing for pregnancy;
- 4) Patients with concurrent autoimmune disease or a history of chronic or recurrent autoimmune disease;
- 5) Patients who need to be treated with corticosteroid (dose equivalent to prednisone of >10 mg/day) or other immunosuppressive agents within 2 weeks prior to study drug administration;
- 6) Patients infected with HIV, or with active hepatitis B or C (HBV DNA  $\geq$  104 copies/mL; HCV RNA  $\geq$  10<sup>3</sup> copies/mL);
- 7) Patients with a history of pneumonitis or interstitial lung disease with clinical evidence, such as interstitial pneumonia and pulmonary fibrosis found by baseline CT scan;
- 8) Patients with known or concurrent bleeding disorders or other uncontrolled diseases who cannot receive surgical treatment
- 9) Physical examination or clinical trial findings that, in the opinion of the investigator, could interfere with the results or put the patient at increased risk for treatment complications
- 10) Patients with concurrent systemic diseases (moderate to severe chronic obstructive pulmonary disease, poorly controlled hypertension, unstable angina, congestive heart failure, myocardial infarction within 6 months, unstable mental disorders requiring therapy);
- 11) Patients who have allergic constitution or are allergic to multiple drugs;
- 12) Patients who participated in other clinical trials within 30 days before enrollment;
- 13) Patients who are not suitable for participation in clinical trials in the opinion of the investigator.

#### 4.3 Removal criteria

Subjects who do not meet the inclusion criteria but are enrolled should be rejected, including: (1) mis-diagnosis; (2) mistaken enrollment; (3) having not taken the medication; (4) having no evaluation records. The reason of rejection should be explained for all rejected subjects, and their medical history in the study should be reserved for future reference. However, subjects who have received at least one treatment and have at least one safety record can be included in the safety analysis.

#### 4.4 Drop-out/Withdrawal criteria

Subjects may withdraw their informed consent at any time and for any reason. In the event of an adverse event, the investigator may decide to withdraw the subject from study treatment. In addition, the investigator could withdraw a subject from the study including the following reasons: adverse events, trial protocol violation (poor compliance), lack of efficacy, trial discontinuation is determined to be necessary for subjects by the investigator due to medical concerns, and the patients themselves request to withdraw the trial.

When a patient drops out, the investigators must fill in the reasons for dropout in the case report form, try their best to contact the patient, and complete as much of evaluation items and related

examinations. The investigators should fill in the pathography in the last time the patient is contacted, including the patient's last medication time and efficacy. Those who drop out due to adverse events, whether investigational product-related or not, should be recorded in the case report form. The investigator should follow all existing or new adverse events that occur after the last treatment.

#### 4.5 Discontinuation criteria

Trial discontinuation means the clinical trial is not completed according to protocol and is prematurely discontinued. The main purpose of trial discontinuation is to protect the rights and interests of the subjects, ensure the quality of the trial, and avoid unnecessary economic losses.

- 1) If serious safety issues occur during the trial, the trial should be discontinued in time;
- 2) During the trial, major errors are found in the clinical trial protocol that make evaluation of drug efficacy difficult; or major deviations from the trial protocol are found in the implementation that make evaluation of drug efficacy difficult if the trial is to be continued;
- 3) The sponsor requests to discontinue the trial (because of economic or management factors or others);
- 4) The administrative department in charge rescinds the trial.

### 5. Therapeutic drugs

#### 5.1 Investigational product

All study medication will be prepared by qualified or experienced study personnel according to the package insert.

Drug name	Short name	Strength
Camrelizumab for injection	Camrelizumab	200 mg
Paclitaxel for injection (albumin bound)	Nab-paclitaxel	100 mg
Carboplatin Injection	Carboplatin	100 mg

#### 5.2 Administration method

PD-1 inhibitor camrelizumab: 200 mg iv drip, d1, q3w. Camrelizumab is recommended to be administered via an infusion pump as an intravenous infusion in approximately 60 mins. Do not administer as a bolus or bolus. At the end of the infusion, flush the infusion line with an adequate volume of normal saline or 5% dextrose solution (per site standard of care).

Chemotherapy: nab-paclitaxel: 100 mg/m<sup>2</sup>, ivgtt, d1/8/15; carboplatin: AUC = 5, ivgtt, d1, q3w.

#### 5.3 Dose modifications for camrelizumab

No increase or decrease at the dose of camrelizumab is allowed, and only postponement or suspension is allowed. The maximum allowable postponement for camrelizumab is 12 weeks (calculated from the actual time of last dose), otherwise, camrelizumab will be discontinued unless the subject still could benefit from camrelizumab which is deemed by the investigator.

The window for each dose during the neoadjuvant treatment was calculated from the date of first dose. Delays generally could not exceed 3 days (subsequent cycles were performed as originally planned) and could exceed 3 days after the reason was noted in exceptional cases.

Postpone or suspension of camrelizumab are recommended as described in Table 1. For more information, see the package insert for camrelizumab.

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**Table 1. Dose modification for camrelizumab**

Toxicities	Severity	Adjustment Scheme
Diarrhea/colitis	Grade 2-3	Postpone until recovery to Grade 0-1
	Grade 4	Suspension
Elevated AST/ALT	Grade 2, AST, ALT 3-5×ULN or TBIL 1.5-3×ULN	Postpone until recovery to Grade 0-1
	Grade 3 or 4, AST, ALT > 5×ULN or TBIL > 3×ULN	Suspension
Nephritis	Grade 2-3 blood creatinine increased	Postpone until recovery to Grade 0-1
	Grade 4 blood creatinine increased	Suspension
Endocrine disorders	Grade 2-3 hyperthyroidism Grade 2-3 hypophysitis Grade 2 adrenal insufficiency Grade 3 hyperglycemia or diabetes mellitus	Postpone until recovery to Grade 0-1
	Grade 4 hypothyroidism Grade 4 hyperthyroidism Grade 4 hypophysitis Grade 3-4 adrenal insufficiency Grade 4 hyperglycemia or diabetes mellitus	Suspension
Pneumonia	Grade 2	Postpone until recovery to Grade 0-1
	Grade 3-4 or recurrent Grade 2	Suspension
Infusion reaction	Grade 2	Reduce the titration rate or suspend the administration. Resume the administration and pay attention on the patient when the symptoms are relieved
	Grade 3-4	Suspension
Other	Grade 3-4 blood amylase increased or lipase increased Grade 2-3 pancreatitis Grade 2 myocarditis Grade 2-3 Initial Other immune-related adverse events	Postpone until recovery to Grade 0-1
	Grade 4 pancreatitis Grade 3-4 myocarditis Encephalitis Grade 3-4 Grade 4 Initial Other immune-related adverse events	Suspension

**5.4 Dose modifications for chemotherapy**

In case of toxicity, the dose of chemotherapy can be reduced and the toxicity is graded according to NCI-CTCAE version 5.0. Dose adjustment of chemotherapy is determined according to the

toxicity of the last course of chemotherapy. Treatment may be delayed for no more than 2 weeks to allow the patient to recover from the toxicity.

#### 5.4.1 Principles for dose modification of chemotherapy

- 1) Dose adjustment of chemotherapy was performed according to the dose-limiting toxicity (DLT).
- 2) Delay of chemotherapy for more than 1 week that caused by the failure to recover due to adverse events should not exceed twice, and the total delayed time should not exceed 2 weeks.
- 3) Each chemotherapy drug is only allowed to reduce the dose once, otherwise the chemotherapy will be discontinued.
- 4) If there is an allergic reaction related to chemotherapy, the chemotherapy will be discontinued.

#### 5.4.2 Dose modifications for chemotherapy

Table 2. Dose modifications based on hematologic toxicity.

As on the first day of chemotherapy	Neutrophils $\geq 1.8 \times 10^9/L$ or platelets $\geq 100 \times 10^9/L$	Standard dose
As on the first day of chemotherapy	Neutrophils $< 1.8 \times 10^9/L$ or platelets $< 100 \times 10^9/L$	Postpone 1 week
If postponed 1 week	$1.5 \times 10^9/L < \text{neutrophils} < 1.8 \times 10^9/L$ or $75 \times 10^9/L < \text{platelets} < 100 \times 10^9/L$	Dose reduction by 20%
If postponed 1 week	Neutrophils $< 1.5 \times 10^9/L$ or platelets $< 75 \times 10^9/L$	Postpone another 1 week
If postponed 2 weeks	Neutrophils $\geq 1.5 \times 10^9/L$ and platelets $\geq 75 \times 10^9/L$	Dose reduction by 20%
If postponed 2 weeks	Neutrophils $< 1.5 \times 10^9/L$ or platelets $< 75 \times 10^9/L$	Suspension

#### 5.4.3 Postponed chemotherapy

- 1) If the patient has expired chemotherapy and does not meet the criteria for the next course of chemotherapy, the duration of chemotherapy can be postponed, but the maximum interval of postponement is 2 weeks. If the criteria for chemotherapy cannot be met after 2 weeks, the treatment should be terminated and the patient should withdraw the study.
- 2) If the patient recover to meet the criteria for the next course of chemotherapy within 2 weeks after postponement, chemotherapy can be continued. If necessary, dose adjustment can be performed according to the principle.

#### 5.4.4 Criteria for continuation of chemotherapy

- 1) Neutrophils  $\geq 1.8 \times 10^9/L$ ;
- 2) Platelet count  $\geq 100 \times 10^9/L$ ;
- 3) ALT, AST, total bilirubin  $\leq$  Grade 2;
- 4) Non-hematological toxicities (except alopecia) recovered to Grade 1 or baseline;
- 5) Neurologic toxicity  $\leq$  Grade 2.

#### 5.5 Concomitant medications

During the clinical trial, except systemic intravenous chemotherapy according to the protocol, patients should stop using other drugs related to systemic treatment of tumor, including other chemotherapy drugs, targeted therapy, immunomodulators, Chinese patent medicine and other drugs that affect the efficacy. Patients may receive supportive care measures including colony-stimulating

factors, blood product transfusions, antibiotics, antiemetics, antidiarrheals, etc., as appropriate, which should be recorded in the CRF. The subjects who receive concomitant medications that cause the difficulty of correct judgment of efficacy and safety should be treated as rejected subjects.

## **6. Outcome measures and laboratory tests**

### **6.1 Medical history and physical examination**

Patients should be inquired by the doctor about their present and previous medical history in detail before being enrolled in the study. Systematic physical examinations and related laboratory tests will be performed before the trial and after the end of treatment. The medical history, physical examinations and laboratory tests results will be recorded in the CRF.

### **6.2 Laboratory tests**

The enrolled patients will receive the following tests before treatment:

- 1) Blood routine test (HB + RBC + WBC), and coagulation function test;
- 2) Urine routine test (RBC, WBC, protein, and urine glucose), stool routine test;
- 3) Liver function (ALT and AST), renal function (BUN and Cr), electrolytes, and blood glucose;
- 4) Electrocardiogram, chest and abdominal CT, cervical ultrasound, PET-CT;
- 5) Blood tumor markers;
- 6) Thyroid function test;
- 7) Myocardial enzyme test.

All above tests on the subjects prior to treatment will be carried out in qualified laboratories, and the test results will be recorded in the CRF. Subjects with abnormality found in reexamination after the end of treatment should be followed up and reexamined, and the relation between the abnormality and the investigational product will be determined.

## **7. Outcomes evaluation**

### **7.1 Efficacy evaluation**

The primary endpoint was pathological complete response (pCR) rate, pCR was defined as the absence of viable tumour cells in the resected primary tumour specimen and all sampled regional lymph nodes (ypT0N0).

Secondary endpoints included toxicity profile of the combination, the proportion of patients who had completed the neoadjuvant treatment, the proportion of patients who had completed surgical resection, surgical outcome, pathological response, RFS and OS. Surgical outcome was defined as R0 resection rate (defined as the rate of negative margins microscopically), morbidity and mortality, and complications within 30 days after surgery. Toxicity, recorded during the period when patients signed their informed consent forms to 90 days after surgery, were graded according to the NCI-CTCAE version 5.0.

### **7.2 Exploratory analysis**

Tissue samples or paraffin sections must be provided as a patient was enrolled. Pretreatment (baseline) biopsy and surgical resected specimen collections were performed, when available, for exploratory biomarker analysis, including analysis of PD-L1 expression and tumor mutational burden (TMB). The baseline formalin-fixed paraffin-embedded (FFPE) sections were obtained by pretreatment endoscopy. The surgical FFPE sections were either ESCC or residual mucosa after

remission. PD-L1 expression was assessed by a central laboratory using Immunohistochemistry (PD-L1 IHC 22C3 pharmDx assay [Dako, Glostrup, Denmark]) in baseline FFPE. PD-L1 expression was evaluated using both combined positive score (CPS) and tumor proportion score (TPS). TMB was estimated by a Solid Tumor Comprehensive Test (Berry Oncology, Fuzhou, China), which is a targeted next-generation sequencing assay using capture single molecule amplification and resequencing technology (capSMART 2.0) in genetic profiles of 654 cancer-related genes.

### 7.3 Safety evaluation

**Adverse events (AEs):** All AEs occurring during the trial, including signs and symptoms during the screening period, whether or not related to the investigational product, should be recorded in the CRF. Changes in patients' subjective symptoms (nausea, vomiting, poor appetite, alopecia, etc.), PS scores before and after treatment; changes in indexes of blood routine, urine routine and stool routine tests, electrocardiogram, changes in physiological indexes (body temperature, blood pressure, heart rate, respiration) during medication, and comparison of hepatic and renal function before and after treatment.

**Postoperative complications:** All complications occurring within 30 days after surgery were recorded according to Clavien-Dindo Classification and ECG definition, respectively.

## 8. Adverse event (AE) reporting

### 8.1 Observation of AEs

#### 1) Clinical AEs

All subjects should be observed for any AEs that occur during the clinical trial. The clinical manifestations, severity, occurrence time, duration, management and prognostic measures of AEs should be recorded in time, and the correlation between AEs and the investigational product should be determined.

#### 2) Abnormal laboratory tests results

Subjects with abnormal results of the above tests after medication should be closely followed up and observed until they recover to normal or stable, and the correlation between abnormality and the investigational product should be determined.

### 8.2 Criteria for determining AE severity

Grade	Clinical description of severity
1	Mild: no or minimal clinical symptoms; clinical or laboratory abnormalities only; treatment not indicated.
2	Moderate: minimal, local, or noninvasive intervention indicated; limiting age-appropriate activities of daily living.
3	Seriously or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care activities of daily living.
4	Life-threatening; urgent treatment indicated.
5	Death due to AE.

Attention should be paid to distinguish the severity and seriousness of adverse events. For

example, "severe headache" may be serious in degree, but it should not be listed as a serious adverse event (SAE), unless it meets the criteria for an SAE.

### **8.3 Criteria for determining the relationship between AE and the investigational product**

According to the criteria for determining the causality between the drug and AEs, the correlation between AEs and the investigational product is divided into five grades: definitely related, probably related, possibly related, possibly unrelated, and definitely unrelated. The three conditions of definitely related, probably related, and possibly related are classified as adverse reactions caused by the investigational product. The investigator should determine whether there is a reasonable possibility that the investigational product caused or contributed to the AE through comprehensive evaluation. The factors to be determined include whether there is a reasonable temporal sequence between the occurrence of AE and the administration of investigational products, characteristics of investigational product, toxicological and pharmacological effects of investigational product, the use of concomitant medications, the subject's accompanied comorbidity, medical history, and family history.

### **8.4 Severe adverse event (SAEs) handling and reporting methods**

SAEs are AEs that occur during the study that meet one or more of the following criteria:

- 1) Death or life-threatening (the term "life-threatening" refers to an event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe);
- 2) Requires hospitalization or prolongation of existing hospitalization;
- 3) Persistent or significant disability/incapacity;
- 4) Other important medical events that are not immediately life-threatening or result in death or hospitalization, but the subject may require intervention [such as medical or surgical] based on appropriate medical evaluation.

SAEs should be treated promptly and actively based on symptoms to minimize the loss of patients, be immediately reported to the principal investigator, and be notified to the trial sponsor within 24 hours.

### **8.5 Immune-related adverse events (irAEs)**

irAEs refer to specific events (including pneumonia, diarrhea/colitis, hepatitis, nephritis/renal dysfunction, rash and endocrine lesions.) in subjects requiring treatment with immunosuppressive drugs. Endocrine lesion events (hypothyroidism/thyroiditis, hyperthyroidism, hypophysitis, diabetes mellitus, adrenal insufficiency) were generally exceptional, immune-related in nature, not typically treatment-related, and manageable without immunosuppressive intervention.

## **9. Data management**

### **9.1 Data collection**

Include but not limited to the following information:

- 1) Basic characteristics of subjects
- 2) Clinical data of subjects before randomization
- 3) Pathology of biopsy and PD-L1 expression
- 4) Laboratory tests and radiological data before and after treatment
- 5) Questionnaire of quality of life

- 6) Neoadjuvant treatment: Immune-related toxicity; chemotherapy toxicity; dose reduction of chemotherapy; dose and application interval of chemotherapy; data of subject's condition at the end of treatment; follow-up data.
- 7) Surgery-related information: Surgery-related complications; pathological reports.

## 9.2 Data management

The investigators should record the data in the case report form in an accurate, complete, clear and timely manner according to the original observation records of subjects. The clinical research associate (CRA) should monitor the trial according to the trial protocol. And confirm that all CRFs are filled correctly and completely and are consistent with the original data. If there are any errors and omissions, the investigators should correct them in time. For any modifications, the original records should be clearly visible, and the corrections should be signed and dated by the investigator. After inspection by the CRA, the CRFs should be checked and signed by the CRA, and submitted to the clinical trial data manager in time. For the transmission of the completed CRFs among investigators, CRA and data managers, there should be special records. The records should have corresponding signatures when receive and be kept properly.

## 10. Statistical analysis

### 10.1 Data analysis set

#### Intent-to-treat population

Statistical analysis will be performed on the intent-to-treat (ITT) population, which is defined as all randomized patients.

#### Safety analysis population

All patients who have received at least one treatment and have actual safety assessment data will be included in the safety analysis. Safety outcomes will be analyzed and presented according to the treatment received by the subject.

#### Per-protocol population

Per-protocol (PP) population includes subjects who meet the inclusion criteria, do not meet the exclusion criteria and have completed the treatment regimen, that is, those who meet the trial protocol, have good compliance and have filled all required sections of the CRF are included in the PP analysis. In this trial, subjects who drop out after half of the treatment course due to aggravation should be included as invalid subjects in the PPS analysis.

### 10.2 Statistical analysis plan

Statistical analysis should employ the two-sided test, and  $P \leq 0.05$  indicates a statistically significant difference (unless otherwise specified). The Continuous data should be statistically described by mean, median, standard deviation, maximum value, and minimum value; the categorical data or ranked data should be statistically described by frequency. SPSS 25.0 software should be employed for statistical analysis, and the analysis process should be all programmed.

Efficacy evaluation: the evaluation of primary efficacy evaluation indicator: the pathological complete response (pCR) rate should be described. The Kaplan-Meier method will be used for



comparative analysis of recurrence-free survival and overall survival. Logistic analysis will be performed using the Cox proportional hazards model.

Safety evaluation: For patients in the safety population, the following safety indicators should be described: adverse events, serious adverse events, normal/abnormal changes in laboratory test results, and vital signs before and after treatment should be described.

### 10.3 Determination of sample size

The primary end point was the proportion of patients with pCR following surgery. In this study, pCR rate was used to calculate the sample size according to the Simon two-stage design method, and unilateral test was performed (Class I error 5%, accuracy 80%). The pCR rate of chemotherapy alone was set at 5% that had been reported previously (13), and the pCR rate of camrelizumab plus chemotherapy was assumed to be 15%. A total of 30 patients will be enrolled in the first phase. If pCR is obtained in less than or equal to one patient, the study will be terminated. Afterwards, 22 patients will be enrolled in the second phase as planned. An additional 8 patients will be enrolled to account for possible dropouts (15%). This leads to a planned sample size of 60 patients. Recruitment will be conducted during a period of 2 years. If pCR is obtained in no more than five patients, the treatment regimen will be regarded as non-effective; otherwise, it will be regarded as feasible.

### 11. Ethics requirements

- 1) The clinical trial protocol shall be approved by the ethics committee of the participating institution before it can be implemented. The approval document of the ethics committee on the clinical trial protocol will be copied to each clinical trial institution for the record.
- 2) Informed consent form: The investigator should explain the objectives and process of the study to the enrolled subjects, and the informed consent form will be signed after obtaining the consent of the subjects.

This clinical trial will be conducted in accordance with the Declaration of Helsinki and relevant Chinese Good Clinical Practice. The protocol should be reviewed and approved by the ethics committee of the hospital before the start of the trial. Any necessary modifications to the trial protocol during the clinical study should be reported to the ethics committee for the record.

### 12. Quality control of the clinical trial

- 1) The preparation of test vesicle packaged chemotherapy drugs provided by the sponsor should conform to the relevant regulations and conditions and be subject to strict quality control.
- 2) In the process of clinical trial, the clinical research associate assigned by the sponsor should visit the study institution regularly and faithfully make the inspection records to ensure that the study protocol is strictly followed and all CRFs can be filled in accurately.
- 3) The laboratory tests should be conducted in accordance with the standard operating procedures. The testing methods and quality control of different study sites should be unified.
- 4) The clinical laboratory of the study site should carry out internal quality control according to relevant regulations and obtain the quality assessment certificate from National Center for Clinical Laboratories.

### 13. Case report form (CRF) and statistical report

- 1) The CRF in duplicate should be filled out by the physician in charge during clinical observation.

After verification, the physician should cross out the contents to be corrected in pen, clearly write the correct data, sign and indicate the date of correction in a responsible manner. The test reports should be attached to the CRF, and the CRF should be signed by the doctor in charge, verified and signed by the principal investigator of the institution.

- 2) The statistical analysis plan should be formulated by the statistician according to the trial protocol, and the statistical analysis report should be completed by the statistician according to the Biostatistics Guidelines for Clinical Trials.

#### **14. Summary of reports**

After the end of the trial, the data statistics center should conduct data analysis in accordance with the Statistics Guidelines for Clinical Trials. The clinical trial sponsor and participating institutions should write the clinical trial summary and sub-center summary reports based on the statistical analysis reports and the guidelines for clinical trials.

#### **15. Investigator responsibilities**

The principal investigator and participating investigators should conduct the trial according to the clinical study protocol and in accordance to the Declaration of Helsinki, relevant Chinese laws and regulations and current GCP guidelines.

#### **16. Trial Funding**

A separate contract is signed for the trial to state the specific funding provision.

#### **17. Document Archival**

The CRF should be filled in with black pen in duplicate, one for the sponsor and one for the participating institution. All original data, statistical data and summary reports should be archived by the clinical trial institution for at least 5 years after the end of the trial.