Neoadjuvant programmed cell death 1 blockade combined with chemotherapy for resectable esophageal squamous cell carcinoma

Weixiong Yang,1 Xiangbin Xing,2 Sai-Ching Jim Yeung,3 Siyu Wang,4,5 Wenfang Chen,6 Yong Bao,7 Fang Wang,8 Shiting Feng,9 Fang Peng,7 Xiaoyan Wang,10 Shuling Chen,11 Minghui He,12 Ning Zhang,2 Honglei Wang,6 Bo Zeng,1 Zhenguo Liu,1 Biniam Kidane,13 Christopher W Seder,14 Kazuo Koyanagi,15 Yaron Shargall,16 Honghe Luo,1 Sui Peng,17 Chao Cheng1

ABSTRACT

Background Programmed cell death 1 (PD-1) blockade induces tumor regression in patients with advanced esophageal squamous cell carcinoma (ESCC); however, little is known about the efficacy of PD-1 blockade as neoadjuvant therapy in resectable ESCC. We aim to assess the safety and feasibility of using the combination of neoadjuvant PD-1 blockade with chemotherapy in patients with ESCC.

Methods Patients with previously untreated, resectable (stage II or III) ESCC were enrolled. Each patient received two 21-day cycles of neoadjuvant treatment with camrelizumab, nab-paclitaxel, and carboplatin before undergoing surgical resection approximately 6–9 weeks after the first cycle.

Results Between January 2020 and September 2020, 37 patients were screened, of whom 23 were enrolled. The neoadjuvant therapeutic regimen had an acceptable side effect profile, and no delays in surgery were observed. Severe (grade 3–4) treatment-related adverse events included neutropenia (9 of 23, 39.1%) and leukenemia (2 of 23, 8.7%). The objective response and disease control rates were 90.5% and 100%, respectively. Twenty patients received surgery, and R0 resection was achieved in all cases. Five (25%) patients had a pathological complete response (pCR) and 10 (50%) patients had a major pathological response. The proportion of patients with a high tumor mutation burden and a high expression of programmed death-ligand 1 (PD-L1) in primary tumors was significantly higher in the pCR group than in the non-pCR group (p=0.044). The number of infiltrating PD-L1+ CD163+ cells was significantly lower in the pCR group than in the non-pCR group after treatment (p=0.017).

Conclusions Neoadjuvant camrelizumab plus carboplatin and nab-paclitaxel had manageable treatment-related adverse effects and induced an objective response in 90.5% of patients, demonstrating its antitumor efficacy in resectable ESCC.

Trial registration number ChiCTR200028900.

INTRODUCTION

Esophageal cancer (EC) is the sixth leading cause of cancer-related mortality in the world.1 Esophageal squamous cell carcinoma (ESCC) is the predominant subtype of EC in the Asian populations.2 China has a high prevalence of EC and is home to more than half of patients with EC in the world. Majority of EC cases are initially diagnosed at an advanced stage of the disease.3 Despite the use of multidisciplinary/multimodal therapies, the 5-year survival rate of patients with EC is only 15%–25%.4

Surgery is still the cornerstone of treatment for potentially resectable ESCC. However, among patients with locally advanced EC, the R0 resection rate is low (around 50%), resulting in early recurrence after surgery.5,6 The combination of chemotherapy or chemoradiotherapy in the neoadjuvant setting can considerably improve the R0 resection rate and, subsequently, survival.7 Although moderately high incidence of pathologic response after chemoradiotherapy is reported, the clinical benefit of neoadjuvant therapy in EC is still suboptimal and unsatisfactory. Neoadjuvant chemotherapy increases the R0 resection rate by only 6% and the 5-year survival rate by only 5.9% at most.6,8 Studies have shown that, although neoadjuvant chemoradiotherapy can further increase the R0 resection rate, it is associated with more postoperative complications and higher postoperative mortality.9,10 A more effective and less toxic neoadjuvant treatment regimen is therefore needed to improve the clinical outcomes of patients with ESCC without increasing the burden of treatment-related adverse events (AEs).

Pembrolizumab and camrelizumab have already shown survival benefit over chemotherapy in the second-line treatment of patients with advanced or metastatic EC.11,12
In the KEYNOTE-590 study, pembrolizumab combined with chemotherapy significantly extended overall survival (OS) compared with placebo combined with chemotherapy in the first-line treatment of patients with advanced ESCC (median survival: 12.6 months vs 9.8 months; HR 0.72, 95% CI 0.60 to 0.88), with manageable toxicity.\textsuperscript{15} Immunotherapy has been recommended for treatment of advanced EC by the National Comprehensive Cancer Network guidelines.\textsuperscript{14}

Preclinical studies have confirmed that programmed cell death 1 (PD-1) inhibitors combined with chemotherapy can further enhance the host’s immune response and inhibit cancer cell immune escape.\textsuperscript{15} Neoadjuvant treatments combining PD-1 inhibitors with chemotherapy have been shown to induce tumor regression and achieve major pathological response in 83% of patients with lung cancer in the NADIM study.\textsuperscript{16} However, to date, there has been no conclusive evidence to support the effectiveness of neoadjuvant immunotherapy in patients with ESCC.

To lay the foundation for a future randomized clinical trial to demonstrate the clinical efficacy of neoadjuvant PD-1 blockade, we conducted a pilot study to examine the safety and feasibility of using the combination of neoadjuvant PD-1 blockade with chemotherapy in a small group of patients with resectable ESCC. The primary outcomes were safety and feasibility; and the secondary outcomes were objective response rate (ORR), disease control rate (DCR), R0 resection rate, and pathological response rate.

**Methods**

**Study design and participants**

This investigator-initiated, single-arm, prospective trial of neoadjuvant PD-1 blockade in combination with nab-paclitaxel and carboplatin for resectable ESCC was performed at the First Affiliated Hospital of Sun Yat-sen University. Patient eligibility criteria included the following: (1) aged 18–75 years; (2) clinical stage II–III ESCC as defined by the American Joint Committee on Cancer (AJCC Eighth Edition)\textsuperscript{17} considered to be surgically resectable by a thoracic surgeon; (3) an Eastern Cooperative Oncology Group (ECOG) performance status score of 0–1; (4) adequate organ function; and (5) no prior chemotherapy or radiotherapy. Exclusion criteria included the following: (1) a diagnosis of other malignant tumors within the previous 5 years; (2) history of anti-PD-1 or anti-programmed death-ligand 1 (PD-L1) therapy; (3) history of interstitial lung disease or active non-infectious pneumonia with corticosteroid treatment; and (4) treatment with corticosteroids or other immunosuppressants within the previous 2 weeks.

This Guangdong Association Study of Thoracic Oncology 1056 (GASTO1056) study is registered at http://www.chictr.org.cn/. Written informed consent to participate in the study was obtained from all patients.

**Procedures**

Patients received two cycles of drug treatment before surgical resection; in each 21-day cycle, the following were administered intravenously: camrelizumab (200 mg) on day 1, nab-paclitaxel (260 mg/m\textsuperscript{2}) on day 1, and carboplatin (area under the curve 5; 5 mg/mL/min) on day 1. At staging and after the first two neoadjuvant treatment cycles, enhanced CT of the neck, chest, and upper abdomen and/or positron emission tomography-CT and ultrasound endoscopy were carried out. Tumor response was assessed by two senior radiologists after two cycles of neoadjuvant treatment and before surgery according to Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1.

Surgery was scheduled for 21–42 days after the first day of the second treatment cycle. Resection of the primary tumor and lymph nodes was performed in line with standard procedures for minimally invasive esophagectomy.\textsuperscript{18} Pathological response was assessed by local pathologists through measurement of the percentage of residual viable tumor after primary tumor resection using previously reported methods.\textsuperscript{16–22} After evaluation, all pathological assessments for response were confirmed by consensus of two blinded pathologists. Pathological complete response (PCR) was defined as the absence of viable tumor cells in the resected cancer specimen; major pathological response (MPR) was defined as the presence of ≤10% viable tumor cells in the resected cancer specimen; pathological partial response (PR) was defined as the presence of >10% but ≤50% viable tumor cells in the resected cancer specimen; pathological stable disease (SD) was defined as the presence of >50% viable tumor cells in the resected cancer specimen; and incomplete pathological response was defined as the presence of >10% viable tumor cells in the resected cancer specimen.

At each visit, patients underwent physical examinations and laboratory tests. AEs and abnormal laboratory findings were assessed according to the National Cancer Institute’s Common Terminology Criteria for Adverse Events (NCI-CTCAE) version 5.0. Treatment was interrupted or delayed if a severe (grade 3–4) AE occurred and would be resumed if protocol-defined criteria for treatment resumption were met. As specified in the trial protocol, (online supplemental file 2), in the event of neutropenic fever, prolonged neutropenia, or thrombocytopenia (platelet count of less than 50×10\textsuperscript{9}/L), dose reductions for nab-paclitaxel and carboplatin were permitted. Patients had the right to withdraw from the study at any time and for any reason. The investigator had the authority to withdraw patients from the study for unacceptable toxicity, protocol violation, or other reasons.

The detailed methodology for follow-up, assessing quality of life, immunohistochemistry, multiplex immunofluorescence staining, and next generation sequencing, including analysis of PD-L1 expression, and CD4\textsuperscript{+}, CD8\textsuperscript{+}, CD56\textsuperscript{+}, PD-1\textsuperscript{+}, granzyme B (GRB\textsuperscript{+}), T-cell intracellular antigen-1 (TIA-1\textsuperscript{+}), and CD163\textsuperscript{+} tumor-infiltrating...
lymphocytes or macrophages, is described in the Methods section of the online supplemental file 1.

**Outcomes**

The primary endpoints of this study were safety and feasibility. Toxicity profiles were assessed according to the NCI-CTCAE (version 5.0) guidelines. Surgical outcomes were the operative time (the duration between skin incision and wound closure), intraoperative blood loss, perioperative mortality, and postsurgical complications. The secondary endpoints included MPR, R0 resection rate, ORR, DCR, disease-free survival (calculated from the date of enrollment), and OS. Pretreatment biopsy samples and post-treatment surgical samples were collected to identify immunological and genomic predictors of therapeutic response and to gain a mechanistic insight into the treatment’s efficacy.

**Statistical analysis**

Categorical variables were presented as absolute and relative frequencies and numerical variables as mean and SD. Safety data were presented as frequency and percentage of patients affected. Paired Student’s t-test was used for pre–post comparisons and Mann-Whitney U test was used for normally distributed continuous variables and non-normally distributed variables, respectively. The χ² test or Fisher’s exact test was used to analyze the associations between categorical measures and pathological response arms, as appropriate. SAS V.9.4 was used for all statistical analyses, with p<0.05 being considered statistically significant.

**RESULTS**

**Overview of patient cohort**

Between January 19, 2020 and September 12, 2020, 37 patients were screened for eligibility; eventually, 23 eligible patients were enrolled after signing informed consent documents (figure 1). All 23 patients finished the two cycles of neoadjuvant therapy, but 3 patients withdrew from the study after refusing surgery. Among the three withdrawn patients, one completed the post-treatment radiological examination before withdrawal. As shown in table 1, the enrolled patients were aged 58.6±10.1 years. Most of the cohort (16 of 23, 69.6%) were smokers, and most patients (22 of 23, 95.7%) were male. The tumor was located in the lower, middle, and upper segment of the esophagus in 13 (56.5%) patients, 9 (39.1%) patients, and 1 (4.3%) patient, respectively. At baseline, 15 (65.2%) patients had AJCC Eighth Edition-defined stage III disease, while the other 8 (34.8%) patients were defined as stage II. Regarding ECOG status, 21 (91.3%) patients had a performance score of 0, and 2 patients had a performance score of 1. PD-L1 expression and tumor mutation burden (TMB) were assessed on pretreatment biopsy samples. A commercially available PD-L1 immunohistochemistry assay (clone 22C3; DAKO Autostainer

![Figure 1](https://jitc.bmj.com/content/10/1/e003497.full)

*Figure 1* Study flow chart. AUC, area under the curve.
Link 48; ready to use (RTU)) was used to assess the PD-L1 combined positive score (CPS) according to the manufacturer’s instructions and international guidelines.23 24 Samples were considered to be PD-L1-positive if the CPS ≥1. The threshold used to define high TMB (TMB-H) depended on the top 25% of this cohort and the cut-off was 7 Muts/Mb. Due to issues with tissue sample quality, four patients were not evaluated for TMB or PD-L1.

Among the 19 samples with available biomarkers, 12 were positive for PD-L1 expression (PD-L1+, CPS ≥1) and 5 had TMB-H (≥7 Muts/Mb).

**Surgery outcomes**

Surgery was performed on 20 patients, all of whom achieved R0 surgical resection (Table 2). Minimally invasive esophagectomy (Mckeown) and open esophagectomy were received by 18 (90.0%) and 2 (10.0%) patients, respectively. Two patients converted to open surgery due to difficulty in esophageal dissection caused by fibrosis and suspected trachea involvement. The intraoperative blood loss and operative time were 120.0±37.7 mL (mean±SD) and 292.5±53.1 min, respectively. The number of resected lymph nodes and lymph node stations was 29.6±8.8 and 11±1.9, respectively. No treatment-related surgical delays were recorded, and the median interval between the last administration of neoadjuvant therapy and surgery was 31 days (IQR: 24–42). No esophageal fistula attributable to neoadjuvant treatment occurred before surgery.

**Table 1** Baseline characteristics of all enrolled patients

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>n (%) or mean±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>58.6±10.1</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>22 (95.7)</td>
</tr>
<tr>
<td>Female</td>
<td>1 (4.3)</td>
</tr>
<tr>
<td>Smoking status</td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>7 (30.4)</td>
</tr>
<tr>
<td>Former or current</td>
<td>16 (69.6)</td>
</tr>
<tr>
<td>Alcohol consumption</td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>11 (47.8)</td>
</tr>
<tr>
<td>Former or current</td>
<td>12 (52.2)</td>
</tr>
<tr>
<td>Tumor location</td>
<td></td>
</tr>
<tr>
<td>Upper segment</td>
<td>1 (4.3)</td>
</tr>
<tr>
<td>Middle segment</td>
<td>9 (39.1)</td>
</tr>
<tr>
<td>Lower segment</td>
<td>13 (56.5)</td>
</tr>
<tr>
<td>Clinical TNM stage*</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>8 (34.8)</td>
</tr>
<tr>
<td>III</td>
<td>15 (65.2)</td>
</tr>
<tr>
<td>Performance score</td>
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</tr>
<tr>
<td>0</td>
<td>21 (91.3)</td>
</tr>
<tr>
<td>1</td>
<td>2 (8.7)</td>
</tr>
<tr>
<td>PD-L1, CPS</td>
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</tr>
<tr>
<td>&lt;1</td>
<td>7 (30.4)</td>
</tr>
<tr>
<td>≥1</td>
<td>12 (52.2)</td>
</tr>
<tr>
<td>NE</td>
<td>4 (17.4)</td>
</tr>
<tr>
<td>TMB status</td>
<td></td>
</tr>
<tr>
<td>TMB-H (≥7 Muts/Mb)</td>
<td>5 (21.7)</td>
</tr>
<tr>
<td>TMB-L (&lt;7 Muts/Mb)</td>
<td>14 (60.9)</td>
</tr>
<tr>
<td>NE</td>
<td>4 (17.4)</td>
</tr>
</tbody>
</table>

*Clinical disease stage was assessed according to the criteria of the American Joint Committee on Cancer, Eighth Edition. CPS, combined positive score; NE, not evaluable; PD-L1, programmed death-ligand 1; TMB, tumor mutation burden; TMB-H, tumor mutation burden-high; TMB-L, tumor mutation burden-low; TNM, tumor node metastasis.

**Table 2** Surgical and pathological outcomes of patients who underwent surgery

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>n (%) or mean±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Successful R0 resection with curative intent</td>
<td>20 (100)</td>
</tr>
<tr>
<td>Surgical approach</td>
<td></td>
</tr>
<tr>
<td>MIE</td>
<td>18 (90.0)</td>
</tr>
<tr>
<td>OE</td>
<td>2 (10.0)</td>
</tr>
<tr>
<td>Pathological response</td>
<td></td>
</tr>
<tr>
<td>PCR</td>
<td>5 (25.0)</td>
</tr>
<tr>
<td>MPR</td>
<td>10 (50.0)</td>
</tr>
<tr>
<td>PR</td>
<td>3 (15.0)</td>
</tr>
<tr>
<td>SD</td>
<td>2 (10.0)</td>
</tr>
<tr>
<td>Downstaging of T stage</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>16 (80.0)</td>
</tr>
<tr>
<td>No</td>
<td>4 (20.0)</td>
</tr>
<tr>
<td>Downstaging of N stage</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>10 (50.0)</td>
</tr>
<tr>
<td>No</td>
<td>10 (50.0)</td>
</tr>
<tr>
<td>Downstaging of TNM stage</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>13 (65.0)</td>
</tr>
<tr>
<td>No</td>
<td>7 (35.0)</td>
</tr>
<tr>
<td>Blood loss (mL)</td>
<td>120.0±37.7</td>
</tr>
<tr>
<td>Cumulative operative time (min)</td>
<td>292.5±53.1</td>
</tr>
<tr>
<td>Number of resected lymph nodes</td>
<td>29.6±8.8</td>
</tr>
<tr>
<td>Number of resected lymph node stations</td>
<td>11±1.9</td>
</tr>
<tr>
<td>ICU stay</td>
<td>2 (10.0)</td>
</tr>
<tr>
<td>Surgical complications</td>
<td></td>
</tr>
<tr>
<td>Anastomotic leakage</td>
<td>2 (10.0)</td>
</tr>
<tr>
<td>Pulmonary infection</td>
<td>1 (5.0)</td>
</tr>
<tr>
<td>Postoperative bleeding</td>
<td>1 (5.0)</td>
</tr>
<tr>
<td>Postoperative hoarseness</td>
<td>1 (5.0)</td>
</tr>
<tr>
<td>90-day mortality</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

ICU, intensive care unit; MIE, minimally invasive esophagectomy; MPR, major pathological response; OE, open esophagectomy; PCR, pathological complete response; PR, partial response; SD, stable disease; TNM, Tumor Node Metastasis.
Postoperative complications are summarized in table 2. There were two (10%) cases of anastomotic leakage and one (5%) case each of pulmonary infection, postoperative bleeding, and postoperative hoarseness. No other severe complications such as respiratory failure, heart failure, deep vein thrombosis, or acute respiratory distress syndrome occurred. None of the patients died within 90 days after surgery.

Radiological and pathological response
According to the RECIST 1.1 criteria, 19 patients who underwent preneoadjuvant and postneoadjuvant therapy imaging attained an objective response: 1 (4.8%) patient had a complete response, 18 (85.7%) patients had PR, while the other 2 (9.5%) patients had SD. No patients had progressive disease during neoadjuvant therapy (figure 2A, online supplemental tables S1 and S2). The ORR and DCR were 90.5% (19 of 21) and 100% (21 of 21), respectively. Of the 20 patients who underwent surgery, 5 (25%) had PCR, 10 (50%) had MPR, 3 (15%) had partial pathological response, and 2 (10%) had SD (figure 2B–D, table 2). Sixteen (80%) patients achieved pathological downstaging of clinical T stage; 10 (50%) patients achieved pathological downstaging of clinical N stage; and 13 (65%) patients achieved pathological downstaging of overall clinical stage (table 2, online supplemental table S3). No significant association was identified between pathological response and smoking status, clinical TNM stage, clinical T stage, or lymph node metastases (online supplemental table S4).

Treatment-related AEs
All 23 enrolled patients received two cycles of neoadjuvant treatment of camrelizumab plus carboplatin and nab-paclitaxel. Treatment-related AEs are summarized in table 3. The most frequently occurring treatment-related AE of any grade was alopecia, which occurred in 19 (82.6%) of the 23 patients. Asthenia (15 of 23, 65.2%), neutropenia (14 of 23, 60.9%), leukopenia (14 of 23, 60.9%), rash (14 of 23, 60.9%), anemia (12 of 23, 56.5%)

Figure 2  Radiographic and pathological responses to neoadjuvant camrelizumab combined with chemotherapy. (A) Waterfall plots of best radiographic response by RECIST 1.1. (B) Pathological responses of the enrolled patients (n=20) who received surgery. (C) Pretreatment and post-treatment CT and H&E images of a representative patient with a PCR. The esophageal tumor showed significant shrinkage after treatment (red circles). There is no tumor visible in the resected esophagus. (D) Pretreatment and post-treatment CT and H&E images of a representative patient with a pathological response of SD. The esophageal tumor remained stable in size after treatment (red circles). The tumor is still visible in the resected esophagus. CR, complete response; MPR, major pathological response; PCR, pathological complete response; PR, partial response; RECIST, Response Evaluation Criteria in Solid Tumors; SD, stable disease.
and increased alanine aminotransferase (10 of 23, 43.5%) were also common among the patients. Despite the high incidence of reactive cutaneous capillary endothelial proliferation, which is commonly associated with camrelizumab, only cases of grade 1 or 2 were recorded (9 patients, 39.1%). The most common grade 3–4 AEs were neutropenia (9 of 23, 39.1%) and leukopenia (2 of 23, 8.7%). None of the AEs reported during neoadjuvant treatment led to discontinuation of treatment, dose reduction, or surgical delay. No treatment-related mortality occurred.

Quality of life
Health-related quality of life was assessed and compared between baseline and postneoadjuvant therapy using the European Organization for Research and Treatment of Cancer’s Quality of Life Questionnaire-Core 30 and the Quality of Life Questionnaire- Esophageal Cancer Module-18. Overall quality of life increased significantly (p=0.0001) from baseline to postneoadjuvant therapy. Patients’ physical (p=0.0244), emotional (p=0.0200), and cognitive (p=0.0158) functioning increased at post-treatment assessment compared with baseline. After the neoadjuvant therapy, fatigue (p=0.008), nausea and vomiting (p=0.0018), pain (p=0.0001), appetite loss (p=0.0153), and financial difficulties (p=0.0237) were alleviated, but there was no significant difference in the other aspects assessed by the questionnaires. Compared with those at baseline, symptoms of dysphagia (p=0.0002), difficulty swallowing saliva (p=0.0493), choking when swallowing (p=0.0001), eating (p=0.0001), and pain (p=0.0014) were significantly alleviated after neoadjuvant therapy (online supplemental table S5).

Follow-up
Up to June 30, 2021, the median follow-up was 13.77 months (IQR: 9.7–17.6) from the first day of treatment. During follow-up, 5 (25%) of the 20 patients who received surgery experienced disease recurrence or metastasis ranging from 4 to 12 months after surgery. None of them was found to have recurrence or metastasis on routine CT scan at 3 months after surgery. The pathological response of three patients was MPR and that of the other two patients was PR. Among these five patients, one had recurrence in the supraclavicular lymph nodes and liver metastasis at 6 months after surgery, one had disease recurrence in the mediastinal lymph nodes and liver metastasis at 4 months after surgery, and the other three had disease recurrence in the mediastinal lymph nodes.

Table 3  Neoadjuvant treatment-related adverse events

<table>
<thead>
<tr>
<th>Adverse Event</th>
<th>Any grade</th>
<th>Grades 1–2</th>
<th>Grade 3</th>
<th>Grade 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutropenia</td>
<td>14 (60.9)</td>
<td>5 (21.7)</td>
<td>5 (21.7)</td>
<td>4 (17.4)</td>
</tr>
<tr>
<td>Leukopenia</td>
<td>14 (60.9)</td>
<td>12 (52.2)</td>
<td>1 (4.3)</td>
<td>1 (4.3)</td>
</tr>
<tr>
<td>Alopecia</td>
<td>19 (82.6)</td>
<td>19 (82.6)</td>
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<td>0</td>
</tr>
<tr>
<td>Asthenia</td>
<td>15 (65.2)</td>
<td>15 (65.2)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Rash</td>
<td>14 (60.9)</td>
<td>14 (60.9)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Anemia</td>
<td>13 (56.5)</td>
<td>13 (56.5)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Alanine aminotransferase increased</td>
<td>10 (43.5)</td>
<td>10 (43.5)</td>
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<td>0</td>
</tr>
<tr>
<td>Aspartate aminotransferase increased</td>
<td>8 (34.8)</td>
<td>8 (34.8)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Reactive cutaneous capillary endothelial proliferation</td>
<td>9 (39.1)</td>
<td>9 (39.1)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Hyperbilirubinemia</td>
<td>8 (34.8)</td>
<td>8 (34.8)</td>
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<td>0</td>
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<tr>
<td>Decreased appetite</td>
<td>8 (34.8)</td>
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<td>0</td>
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<tr>
<td>Thrombocytopenia</td>
<td>7 (30.4)</td>
<td>7 (30.4)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Vomiting</td>
<td>5 (21.7)</td>
<td>5 (21.7)</td>
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<td>0</td>
</tr>
<tr>
<td>Oral mucositis</td>
<td>4 (17.4)</td>
<td>4 (17.4)</td>
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<tr>
<td>Nausea</td>
<td>3 (13.0)</td>
<td>3 (13.0)</td>
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<tr>
<td>Diarrhea</td>
<td>3 (13.0)</td>
<td>3 (13.0)</td>
<td>0</td>
<td>0</td>
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<tr>
<td>Constipation</td>
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<td>3 (13.0)</td>
<td>0</td>
<td>0</td>
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<tr>
<td>Edema</td>
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<tr>
<td>Fever</td>
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<td>Arthralgia</td>
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<td>1 (4.3)</td>
<td>1 (4.3)</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Data are presented as n (%). Adverse events were graded according to the National Cancer Institute’s Common Terminology Criteria for Adverse Events, version 5.0.
nodes at 8, 6, and 12 months after surgery, respectively (online supplemental table S3).

In the entire cohort of patients who received surgery, the median disease-free survival was not reached (figure 3A). For patients who achieved PCR, there was no significantly improved disease-free survival over those without PCR (figure 3B). Of note, there may be some potential confounders in the survival analysis such as comorbidity and concurrent medications that cannot be minimized due to the small sample size. Among the 10 patients with MPR, those with a pathological stage of T2/T3 had a higher risk of tumor recurrence or metastases than those with T1/Tis disease (p=0.033; figure 3C).

**Immunohistochemistry and multiplex immunofluorescence staining**

To examine the immune microenvironment and its potential association with pathological response, we performed immunohistochemistry to detect PD-L1 and other immune biomarkers in paired pretreatment tumor biopsies and post-treatment surgical resections obtained from 19 of the patients. The calculation of immune cells was performed both in the stromal region and in the tumor region in non-PCR patients. For cases with PCR after therapy, only stromal regions were scored due to no residual viable tumor cells. No significant difference was observed in the expression of PD-L1 determined by CPS between patients with PCR and those without PCR (online supplemental figure S7C). Significant increases in the number of infiltrating CD4+, CD8+, CD56+, PD-1+, GRB+, and TIA-1+ cells were observed after neoadjuvant chemoimmunotherapy, but there was no significant change in the number of infiltrating PD-L1+ and CD163+ cells (online supplemental figure S1). Increases in the number of infiltrating CD4+, CD8+, CD56+, PD-1+, GRB+, and TIA-1+ cells were observed in both the PCR and the non-PCR groups after treatment. There was no significant difference in the number of infiltrating CD4+, CD8+, CD56+, PD-1+, GRB+, or TIA-1+ cells in the pretreatment and post-treatment samples between the PCR and non-PCR groups (figure 4A,B, online supplemental figure S2). Similar trends of the infiltrating CD4+, CD8+, CD56+, PD-1+, GRB+, and TIA-1+ cells were observed between the PCR+MPR and PR+SD groups (online supplemental figure S3). Of note, after treatment, there were far more infiltrating PD-L1+ and CD163+ cells in the non-PCR group than in the PCR group (figure 4C,D); moreover, in the non-PCR group, the number of infiltrating PD-L1+ and CD163+ cells was significantly increased after treatment compared with before treatment (figure 4C,D). There was no significant difference in infiltrating CD163+ cells in the pretreatment and post-treatment samples between the PCR+MPR and PR+SD groups (online supplemental figure S4).

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**Figure 3** DFS curves of patients who received surgery (n=20). (A) DFS curve of all patients who received surgery (n=20). (B) DFS curves of the PCR group (n=5) and the non-PCR group (n=15). (C) Comparison of pathological T stage and recurrence among MPR patients (n=10) in a 2×2 contingency table. DFS, disease-free survival; MPR, major pathological response; PCR, pathological complete response.
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Figure 4 The immune microenvironment is correlated with the response to neoadjuvant camrelizumab combined with chemotherapy. (A) Comparison of infiltrating CD4+ cells between the PCR group (n=5) and the non-PCR group (n=14) before and after treatment. (B) Comparison of infiltrating CD8+ cells between the PCR group (n=5) and the non-PCR group (n=14) before and after treatment. (C) Comparison of infiltrating PD-L1+ cells between the PCR group (n=5) and the non-PCR group (n=14) before and after treatment. (D) Comparison of infiltrating CD163+ cells between the PCR group (n=5) and the non-PCR group (n=14) before and after treatment. (E) Correlation between infiltrating PD-L1+ and CD163+ cells in post-treatment samples based on multiplex immunofluorescence staining (n=18). (F) Comparison of change in PD-L1+ CD163+ cells between the PCR group (n=5) and the non-PCR group (n=13) before and after treatment based on multiplex immunofluorescence staining. A significant increase in PD-L1+ CD163+ cells (white arrows) is observed after neoadjuvant chemoimmunotherapy in the non-PCR group. Antibody panel: CD8 (magenta), PD-1 (red), PD-L1 (green), CD163 (orange), cytokeratin (CK, yellow), and 2-(4-amidinophenyl)-6-indolecarbamidine dihydrochloride (DAPI, blue). (G) The percentage of patients with both TMB-H and PD-L1+ was significantly higher in the PCR group (n=5) than those in the non-PCR group (n=14). mIF, multiplex immunofluorescence; PCR, pathological complete response; PD-L1, programmed death-ligand 1; TMB-H, tumor mutation burden-high.

The change in infiltrating immune cells after treatment in each patient was calculated by the density of infiltrating immune cells in the post-treatment samples divided by the infiltrating immune cells in the pretreatment samples. After treatment, PD-L1+ and CD163+ cells tended to show an increased number from the pretreatment in the non-PCR group, but the opposite tendency was seen in the PCR group (online supplemental figure S4). There was no significant difference between changes in the pretreatment and post-treatment number of infiltrating CD4+, CD8+, CD56+, PD-1+, GRB+, and TIA-1+ cells between the PCR and non-PCR groups (online supplemental figure S4). Based on these findings, we further examined multiplexed immunofluorescence using the antibodies for CD8, CD163, PD-1, PD-L1, and cytokeratin (CK) to characterize the immune microenvironment of the tumor. CK was used to define the tumor region, and the density of markers in the CK-positive tumor region and stromal region was evaluated separately. The results showed that the number of PD-L1+ cells was positively correlated with CD163+ cells and PD-L1+ CD163+ cells at pretreatment and post-treatment, respectively (figure 4E, online supplemental figure S5). However, there was no correlation between PD-L1+ cells and infiltrating CD8+, PD-1+, or CD8+ PD-1+ cells (online supplemental figure S5). Furthermore, we found that the number of infiltrating PD-L1+ CD163+ cells in the non-PCR group was significantly higher than in the PCR group after neoadjuvant treatment, and the number of PD-L1+ CD163+ cells was significantly increased after treatment compared with before treatment in the non-PCR group (figure 4F, online supplemental figure S6). Additionally, there was no significant difference in the number of infiltrating CD8+ PD-1+ cells in the pretreatment and post-treatment samples between the PCR +MPR and Pr+SD groups (online supplemental figure S3) or the PCR and non-PCR groups (online supplemental figure S6).
Genomic analyses

We further performed next generation sequencing of pretreatment tumor specimens obtained from 19 patients who had adequate amounts of tissues available. A median of 10 somatic mutations (range: 2–32) per tumor was noted, and specific driver mutations identified included TP53, CDKN2A, CDKN2B, CCND1, and MYC (online supplemental figure S7A). Patients with PCR demonstrated a higher TMB compared with patients without PCR, but it was not statistically significant (p=0.083; online supplemental figure S7B). The percentage of patients with both TMB-H and PD-L1 was significantly higher in the PCR group (p=0.044; figure 4G). No significant difference in the immune-related pathways was found between the PCR and non-PCR groups (online supplemental figure S7D). No significant difference was found in disease-free survival based on different TMB and PD-L1 status (online supplemental figure S8). There was also no significant difference in PD-L1 and TMB-H status between downstaged and non-downstaged patients (online supplemental table S6).

DISCUSSION

Our study reported the application of neoadjuvant PD-1 blockade in combination with chemotherapy in patients with resectable (stage II or III) ESCC. Neoadjuvant camrelizumab plus carboplatin and nab-paclitaxel had manageable treatment-related toxic effects and did not delay surgery. This regimen induced PCR or MPR in 75.0% of resected tumors, demonstrating its antitumor efficacy in resectable ESCC.

Overall, the neoadjuvant combination therapy of camrelizumab with carboplatin and nab-paclitaxel had favorable safety and feasibility. In terms of toxicity, the main treatment-related AE of grade 3–4 was neutropenia (39.1%), the incidence of which was lower than those reported in the MRC OE02 neoadjuvant chemotherapy group (61.3%) and the NEOCRT5010 neoadjuvant chemoradiotherapy group (48.8%). In our study, the incidence of reactive cutaneous capillary endothelial proliferation, an AE commonly associated with camrelizumab, was 39.1%, which was much lower than the incidence previously reported for neoadjuvant chemoradiotherapy (23.1%). Moreover, no perioperative deaths occurred in this study. Collectively, these results suggest that the toxicity of neoadjuvant immunotherapy combined with camrelizumab, carboplatin, and nab-paclitaxel is acceptable.

Encouragingly, in this study, the PCR rate of neoadjuvant therapy with camrelizumab combined with carboplatin and nab-paclitaxel reached 25%, which was higher than that previously reported for neoadjuvant chemotherapy (10.2%) and similar to that previously reported for neoadjuvant PD-1 blockade in combination with chemotherapy (33%). Previous studies have shown that achieving an MPR after neoadjuvant therapy is associated with a better survival outcome in other cancers, such as lung cancer. In patients who achieved an MPR, we found that those with a pathological stage of T2/T3 had a higher risk of tumor recurrence or metastasis than those with T1/Tis, indicating that different pathological T stages may lead to different prognoses among patients with MPR after neoadjuvant immunochemotherapy. Among the 20 surgical patients, 13 (65%) achieved downstaging after treatment, which was higher than reported in the previous literature (40%). Previous studies have suggested that patients with EC who achieve downstaging after neoadjuvant therapy may have a better survival outcome. Our data also showed that patients’ quality of life was significantly improved after neoadjuvant therapy and their symptoms of dysphagia were significantly relieved, which might be related to the high PCR and downstaging rates. These encouraging results provide clinical evidence for the application of immunotherapy combined with chemotherapy in the neoadjuvant setting.

The tumor immune microenvironment of ESCC has been reported to be in an immunosuppressive state dominated by exhausted T and natural killer (NK) cells. In the present study, there were few tumor-infiltrating immune cells before treatment; however, a significant increase in tumor-infiltrating CD4+, CD8+, and CD56+ lymphocytes was observed after therapy. The priming of CD4+ and CD8+ T cells helps signals to cytotoxic T lymphocytes and further establishes efficient and durable anti-tumor immunity. CD56+ cells are a major cell subset of NK cells, which provide protection against infectious pathogens and cancer. Our findings suggest that neoadjuvant PD-1 blockade might enhance the systemic priming of antitumor T cells and natural killer cells in the ESCC microenvironment. However, the number of infiltrating PD-L1+ CD163+ cells significantly increased in the non-PCR group after therapy. It is well-known that CD163 is a specific biomarker of M2-like macrophages, and it was reported that M2-like macrophages with increased expression of PD-L1 could promote immunosuppression. Our findings suggest that the induction of M2-like macrophages with increased expression...
of PD-L1 may be associated with ineffective immuno-
therapy. The association between changes in the tumor
immune microenvironment and the efficacy of neoadju-
vant chemoimmunotherapy in ESCC needs to be further
verified in full-stage studies. The PD-L1 expression
level and TMB are the most studied predictive markers
of the efficacy of immune checkpoint inhibitors in the
ESCC clinical trial of pembrolizumab (KEYNOTE-181
and KEYNOTE-590).11 13 In our study, the percentage
of patients with both TMB-H and PD-L1+ was significantly
higher in the PCR group, suggesting that neoadjuvant
chemoimmunotherapy may favor patients with both high
 genomic instability and PD-L1 expression. The pro-
gnostic value of TMB and PD-L1 in patients receiving this
regimen should be further verified by larger-scale clinical
studies.

There are some limitations to this study. First, due to
this study being an exploratory pilot study, the number of
enrolled patients was small. Therefore, our findings
and the survival data need to be interpreted with caution
since some potential confounders may significantly influ-
ence the results, and full-scale randomized controlled
trials are required to further verify our findings. Second,
the follow-up time was short and the median survival was
not reached. Longer follow-ups are needed to examine
whether neoadjuvant immunochemotherapy can deliver
duration survival benefits for patients. Further inves-
tigation into the optimal duration of treatment and biomarkers to predict response should be a focus of future research.

In summary, we report that neoadjuvant camrelizumab
plus carboplatin and nab-paclitaxel has good safety and
feasibility and does not delay surgery. This regimen has
favorable antitumor efficacy. Neoadjuvant camrelizumab
combined with carboplatin and nab-paclitaxel is a poten-
tial treatment strategy for ESCC. However, the impact of
adjuvant anti-PD-1 therapy remains to be examined.

Author affiliations
1Department of Thoracic Surgery, Sun Yat-sen University First Affiliated Hospital,
Guangzhou, Guangdong, China
2Department of Gastroenterology, Sun Yat-sen University First Affiliated Hospital,
Guangzhou, Guangdong, China
3Department of Emergency Medicine, The University of Texas MD Anderson Cancer
Center, Houston, Texas, USA
4Department of Thoracic Surgery, Sun Yat-sen University Cancer Center, Guangzhou,
Guangdong, China
5State Key Laboratory of Oncology in South China, Collaborative Innovation Center
for Cancer Medicine, Guangzhou, Guangdong, China
6Department of Pathology, Sun Yat-sen University First Affiliated Hospital,
Guangzhou, Guangdong, China
7Department of Radiotherapy, Sun Yat-sen University First Affiliated Hospital,
Guangzhou, Guangdong, China
8Institute of Precision Medicine, Sun Yat-sen University First Affiliated Hospital,
Guangzhou, Guangdong, China
9Department of Radiology, Sun Yat-sen University First Affiliated Hospital,
Guangzhou, Guangdong, China
10Department of Nuclear Medicine, Sun Yat-sen University First Affiliated Hospital,
Guangzhou, Guangdong, China
11Division of Interventional Ultrasound, Sun Yat-sen University First Affiliated
Hospital, Guangzhou, Guangdong, China
12Department of Liver Surgery, Sun Yat-sen University First Affiliated Hospital,
Guangzhou, Guangdong, China
13Department of Surgery, Max Rady College of Medicine, University of Manitoba,
Winnipeg, Manitoba, Canada
14Department of Cardiovascular and Thoracic Surgery, Rush University Medical
Center, Chicago, Illinois, USA
15Department of Gastroenterological Surgery, Tokai University School of Medicine,
Isehara, Japan
16Division of Thoracic Surgery, McMaster University/St. Joseph's Healthcare
Hamilton, Hamilton, Ontario, Canada
17Clinical Trials Unit and Institute of Precision Medicine, Sun Yat-sen University First
Affiliated Hospital, Guangzhou, Guangdong, China

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Data acquisition: WY, XX, WC, YB, FW, SF, FP, XW, NZ, HW, BZ, and ZL. Data analysis:
WY, FW, SC, MH, and HW. Data interpretation: XX, WC, YB, SF, FP, XW, NZ, HW, BZ, and
ZL. Drafting of the article: WY, S-CJY, SC, SP, and CC. Critical revisions of the manu:
script: S-CJY, BK, CWS, KK, YS, SP, and CC. Responsible for the overall content as
the guarantor: CC.

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design, data collection, data analysis, data interpretation, or writing of the report.

Competing interests None declared.

Patient consent for publication Obtained.

Ethics approval The study protocol was approved by an independent ethics
committee at the Guangdong Association Study of Thoracic Oncology, and the trial
was conducted in accordance with the International Conference on Harmonization
Good Clinical Practice guidelines and the Declaration of Helsinki.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available upon reasonable request. The data
sets generated in the current study are available from the corresponding author on
reasonable request.

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ORCID iD
Chao Cheng http://orcid.org/0000-0003-3571-8154

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Lancet 2013;381:400–12.
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SUPPLEMENTARY MATERIALS

1. SUPPLEMENTARY METHODS

1.1 Quality of life

Health-related quality of life was assessed using the European Organization for Research and Treatment of Cancer (EORTC) Quality of Life Questionnaire-Core 30 (QLQ-C30) and EORTC Quality of Life Questionnaire-Esophageal Cancer Module-18 (QLQ-OES18) scales\(^1\), at the start of neoadjuvant therapy and again before the operation. The EORTC QLQ-C30 scale comprises 30 items that are combined to form 5 functioning scales (physical, role, cognitive, emotional, and social), 3 symptom scales (fatigue, pain, and nausea or vomiting), a global health status quality-of-life scale, and 6 single-item scales (dyspnea, insomnia, appetite loss, constipation, diarrhea, and financial problems). The EORTC QLQ-OES18 scale contains 18 items for patients with esophageal cancer.

1.2 Follow up

The patients were scheduled to commence adjuvant treatment with camrelizumab 4–8 weeks following surgery, once they had made a full recovery. Camrelizumab at a dose of 200 mg was given as adjuvant treatment intravenously every 3 weeks for one year after the operation. Restaging and surveillance CT imaging were performed every 3 months during the 1\(^{st}\) year of follow-up, every 6 months during the 2\(^{nd}\) and 3\(^{rd}\) years, and every year thereafter. The endoscopies would be performed when clinically indicated development of dysphagia or worrisome findings on surveillance CT.

1.3 Immunohistochemical and multiplex immunofluorescence staining

1.3.1 PD-L1 immunohistochemistry

A commercially available PD-L1 immunohistochemistry assay (clone 22C3; DAKO Autostainer Link48; RTU) was used to assess the PD-L1 combined positive score in formalin-fixed tumor diagnostic samples in line with the manufacturer’s instructions and international guidelines\(^3\). PD-L1 CPS was assessed in 19/23 patients (82.6%). Samples were considered to be PD-L1-positive if CPS ≥1 of tumor cells showed membranous PD-L1 expression. When multiple pre-treatment specimens were available for PD-L1 testing, the patient was considered PD-L1-positive if any of the pre-treatment specimens were positive, and the highest percentage of PD-L1 positive tumor cells is reported here\(^5\).
1.3.2 CD4, CD8, CD56, CD163, PD-1, GRB, and TIA-1 immunohistochemistry

Selected specimens were assessed by immunohistochemistry as previously described, with minor modifications. Tumor tissues were stained for CD4, CD8, CD56, CD163, PD-1, GRB, and TIA-1, as outlined in the table below.

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<th>Antibody</th>
<th>Clone/Company</th>
<th>Dilution</th>
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</thead>
<tbody>
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<td>CD4</td>
<td>clone 4B12/ Leica</td>
<td>RTU</td>
</tr>
<tr>
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<td>clone 4B11/ Leica</td>
<td>RTU</td>
</tr>
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<td>clone CD564/ Leica</td>
<td>RTU</td>
</tr>
<tr>
<td>CD163</td>
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<td>RTU</td>
</tr>
<tr>
<td>PD-1</td>
<td>clone UMAB199/ ZSGB-Bio</td>
<td>RTU</td>
</tr>
<tr>
<td>GRB</td>
<td>clone 11F1/ Leica</td>
<td>RTU</td>
</tr>
<tr>
<td>TIA-1</td>
<td>clone 2G9A10F5/ Gene tech</td>
<td>RTU</td>
</tr>
</tbody>
</table>

1.3.3 Multiplex Immunofluorescence Staining

36 samples were adequate to perform the multiplex immunofluorescence staining from 18 patients. Paired samples (pre and post-treatment) were available for the 18 patients (5 PCR patients and 13 Non-PCR patients). The 4μm-thick slides cut from the Formalin-fixed paraffin embedded (FFPE) blocks were dewaxed in xylene, rehydrated through a decreasing ethanol series and fixed in NBF (10% neutral buffered formalin) for 10 min. Slides were stained to enable the simultaneous visualization of six markers: Abs anti-CD8 (Cat# ab93278, Abcam), anti-PD-1 (Cat# 84651S, CST), anti-PD-L1 (Cat# 13684S, CST), anti-CD163 (Cat# ab182422, Abcam), anti-CK (Cat# Kit-0009, MXB Biotechnologies), on the same slide using PANO 7-plex IHC kit, cat 0004100100 (Panovue, Beijing, China). At the beginning of each staining cycle, microwave-heated treatment in EDTA solution was applied to perform antigen retrieval. After blocking proteins for 10 minutes, these five primary antibodies were sequentially incubated for 30, 30, 30, 60, 60 minutes at 37°C, respectively. Then the incubation of HRP-conjugated secondary antibody and tyramide signal amplification (TSA) with Opal was
followed. Five staining cycles were performed for the following antibodies/fluorescent dyes combinations: anti-CD8/Opal-690, anti-PD-1/Opal-620, anti-PD-L1/Opal-520, anti-CD163/Opal-570, anti-CK/Opal-650. Microwave treatment was performed at each cycle of staining to remove the Ab TSA complex. Finally, all slides were stained with 4′-6′-diamidino-2-phenylindole (DAPI, SIGMA-ALDRICH) for 8 min and enclosed with Mounting Medium 0022001010 (Panovue, Beijing, China).

1.3.4 Multispectral Imaging Analysis

The stained slides were scanned using the TissueFAXS platform (TissueGnostics, Vienna, Austria) at 20× magnification, which captures the fluorescent spectra at 20-nm wavelength intervals from 420 to 720 nm with identical exposure time; and the scans were combined to build a single stack image. Spectral libraries were established from the extracted images in which images of unstained and single-stained slides were applied to extract the spectrum of autofluorescence of tissues and each fluorescein, respectively. The library was then used to unmix the multispectral images (seven colors staining) with the StrataQuest software (TissueGnostics, Vienna, Austria). Using this spectral library, reconstructed images of slides with the autofluorescence removed were acquired for imaging analysis. For each primary antibody, the cut-off value for positivity was determined according to the staining pattern and intensities of all images.

1.4 Next-generation sequencing

1.4.1 DNA Extraction and Quantification

Tumor tissue DNA (tDNA) and whole blood control samples were extracted using the QIAamp DNA FFPE Tissue kit and DNeasy Blood and tissue kit (Qiagen, USA), respectively. Purified DNA was qualified by Nanodrop2000 (Thermo Fisher Scientific, Waltham, MA) and quantified by Qubit 2.0 using a dsDNA HS Assay Kit (Life Technologies, Waltham, MA).

1.4.2 Library Preparation
Sequencing libraries were prepared using the KAPA Hyper Prep kit (KAPA Biosystems, Wilmington, MA) with an optimized manufacturer’s protocol. Briefly, ~1 μg of fragmented genomic DNA was subjected to end-repairing, A-tailing, and ligation with indexed adapters sequentially; these steps were followed by size selection using Agencourt AMPure XP beads (Beckman Coulter, Mississauga, Canada) and PCR amplification using the KAPA Hyper DNA Library Prep Kit (KAPA Biosystems, Wilmington, MA).

1.4.3 Hybridization Capture and Sequencing

A customized next-generation sequencing panel targeting exons of 425 cancer-relevant genes (exons and selected introns) was used for hybridization enrichment. Briefly, indexed DNA libraries were pooled together to a total amount of 2 μg and subjected to probe-based hybridization using IDT xGen Lockdown reagents (Integrated DNA Technologies, Coralville, IA) and Dynabeads M-270 (Thermo Fisher Scientific, Waltham, MA) with an optimized manufacturer’s protocol. Libraries captured on-beads were amplified with Illumina p5 and p7 primers in KAPA HiFi HotStart ReadyMix (KAPA Biosystems, Wilmington, MA). The final library was quantified using the KAPA Library Quantification kit (KAPA Biosystems, Wilmington, MA) per the manufacturer’s instructions. The Bioanalyzer 2100 (Agilent, Santa Clara, CA) was used to determine the fragment size distribution of the final library. The target-enriched library was then sequenced on HiSeq4000 NGS platforms (Illumina) according to the manufacturer’s instructions.

1.4.4 Sequencing Data Processing

Sequencing was performed on the Illumina HiSeq4000 platform followed by data analysis as previously described. The medium depth of coverage after the removal of PCR duplicates is 1000X for tumor tissue specimen and 100X for the whole blood control samples, respectively. Specifically, sequencing data were analyzed by Trimmomatic to remove low-quality (quality <15) or N bases, and then mapped to the human reference genome hg19 using the Burrows-Wheeler Aligner (https://github.com/lh3/bwa/tree/master/bwakit). PCR duplicates were removed with Picard (available at: https://broadinstitute.github.io/picard/). The Genome Analysis Toolkit (GATK) (https://software.broadinstitute.org/gatk/) was used to perform local realignment around indels and base quality reassurance. SNPs and indels were analyzed using VarScan2 and the Haplotype Caller/Unified Genotyper in GATK, with the mutant allele frequency (MAF) cutoff as 0.5% for tissue samples, and a minimum of 3 unique mutant reads. Common SNPs were excluded if they had a population frequency of >1% in the 1000 Genomes Project or the Exome Aggregation Consortium.
(ExAC) 65000 exomes database. The resulting mutation list was further filtered using an in-house list of recurrent artifacts based on a normal pool of whole blood samples.

Tumor mutational burden was defined as the number of coding somatic base substitutions, and short insertions and deletions (indels) per megabase of genome. Copy number variations were detected using ADTEx (http://adtex.sourceforge.net) with default parameters. Identified genetic alterations, including missense, nonsense, indel splicing, and fusion, were also grouped into different Kyoto Encyclopedia of Genes and Genomes pathways, and we focused on immune-related pathways. Comparisons of proportion between groups were done using Fisher's exact test. A 2-sided P value of <0.05 was considered statistically significant for all tests unless indicated otherwise. All statistical analyses and oncoprint were done in R (v.3.5.3).

2. SUPPLEMENTARY TABLES

Supplemental table S1. Clinical response in the population assessed by RECIST 1.1

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<th>Clinical response</th>
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<tr>
<td>Partial response</td>
<td>18 (85.7%)</td>
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<tr>
<td>Stable disease</td>
<td>2 (9.5%)</td>
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<td>Progressive disease</td>
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Supplemental table S2. Clinical response versus pathologic response in the 20 patients who underwent surgery

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<tr>
<td>Clinical response</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Complete (n=1)</td>
<td>1 (100.0%)</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
</tr>
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<td>Partial (n=17)</td>
<td>4 (23.5%)</td>
<td>10 (58.8%)</td>
<td>3 (17.7%)</td>
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<tr>
<td>Stable disease (n=2)</td>
<td>0 (0.0%)</td>
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### Supplemental table S3. Pathologic downstaging and recurrence status in 20 patients who underwent tumor resection.

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<th>Stage</th>
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<td>N0</td>
<td>M0</td>
<td>I</td>
</tr>
</tbody>
</table>

Abbreviations: PCR, pathological complete response; MPR, major pathological response; CR, complete response; PR, partial response; SD, stable disease; yp, pathologic stage after neoadjuvant treatment.
### Supplemental table S4. Association of patient characteristics with pathological complete response.

<table>
<thead>
<tr>
<th></th>
<th>PCR (n=5)</th>
<th>Non-PCR (n=15)</th>
<th>P value</th>
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<tbody>
<tr>
<td><strong>Smoking status</strong></td>
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<tr>
<td>Never</td>
<td>1</td>
<td>5</td>
<td>1.000</td>
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<tr>
<td>Former or current</td>
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<td>10</td>
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</tr>
<tr>
<td><strong>Clinical TNM stage</strong></td>
<td></td>
<td></td>
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<tr>
<td>II</td>
<td>3</td>
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</tr>
<tr>
<td>III</td>
<td>2</td>
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<tr>
<td><strong>Clinical T stage</strong></td>
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<td></td>
<td>0.447</td>
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<tr>
<td>2</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td><strong>Lymph node metastasis</strong></td>
<td></td>
<td></td>
<td>0.612</td>
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<td>Yes</td>
<td>3</td>
<td>11</td>
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</tr>
<tr>
<td>No</td>
<td>2</td>
<td>4</td>
<td></td>
</tr>
</tbody>
</table>

### Supplemental table S5. Summary of quality of life for all enrolled patients (n=20) before and after chemoimmunotherapy.

<table>
<thead>
<tr>
<th>Outcome variables</th>
<th>Before neoadjuvant therapy</th>
<th>After neoadjuvant therapy</th>
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<tbody>
<tr>
<td><strong>Quality of Life</strong></td>
<td></td>
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<tr>
<td>EORTC-QLQ-C30</td>
<td></td>
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<tr>
<td>Overall quality of life scalea</td>
<td>57.92±14.78</td>
<td>81.25±9.82**</td>
</tr>
<tr>
<td>Functioning scaleb</td>
<td></td>
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<tr>
<td>Physical functioning</td>
<td>92.33±10.39</td>
<td>97.33±7.72*</td>
</tr>
<tr>
<td>Role functioning</td>
<td>92.50±14.41</td>
<td>99.17±3.63</td>
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<tr>
<td>Emotional functioning</td>
<td>82.92±13.30</td>
<td>91.67±8.33*</td>
</tr>
<tr>
<td>Cognitive functioning</td>
<td>83.33±23.57</td>
<td>98.33±7.26*</td>
</tr>
<tr>
<td>Social functioning</td>
<td>81.67±25.22</td>
<td>92.50±12.33</td>
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<tr>
<td><strong>General symptom scales</strong></td>
<td></td>
<td></td>
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<tr>
<td>Fatigue</td>
<td>18.89±16.14</td>
<td>7.22±11.26**</td>
</tr>
<tr>
<td>Nausea and vomiting scale</td>
<td>12.50±14.79</td>
<td>0.83±3.63**</td>
</tr>
<tr>
<td>Pain</td>
<td>24.17±17.85</td>
<td>1.67±5.00**</td>
</tr>
<tr>
<td>Symptom</td>
<td>Score ± Standard Deviation</td>
<td>Score ± Standard Deviation</td>
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<tr>
<td>------------------------------</td>
<td>----------------------------</td>
<td>----------------------------</td>
</tr>
<tr>
<td>Dyspnea</td>
<td>10.00 ± 15.27</td>
<td>3.33 ± 10.00</td>
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<tr>
<td>Insomnia</td>
<td>10.00 ± 15.28</td>
<td>8.33 ± 17.87</td>
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<tr>
<td>Appetite loss</td>
<td>11.67 ± 19.07</td>
<td>0.00 ± 0.00*</td>
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<tr>
<td>Constipation</td>
<td>15.00 ± 26.82</td>
<td>3.33 ± 10.00</td>
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<tr>
<td>Diarrhea</td>
<td>11.67 ± 15.90</td>
<td>3.33 ± 10.00</td>
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<tr>
<td>Financial difficulties</td>
<td>38.33 ± 30.32</td>
<td>16.67 ± 26.87*</td>
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</table>

EORTC-QLQ-OES18

**General functional scales**

- Dysphagia: 30.55 ± 21.33, 6.10 ± 13.37**

**General symptom scales**

- Difficulty swallowing saliva: 11.67 ± 24.21, 0.00 ± 0.00*
- Choking when swallowing: 46.67 ± 30.55, 8.33 ± 17.87**
- Eating: 24.58 ± 18.35, 3.75 ± 8.53**
- Dry mouth: 13.33 ± 19.44, 13.33 ± 16.33
- Difficulty tasting: 11.67 ± 21.79, 1.67 ± 7.26
- Coughing: 1.67 ± 7.26, 0.00 ± 0.00
- Difficulty talking: 0.00 ± 0.00, 0.00 ± 0.00
- Reflux: 18.33 ± 21.67, 7.50 ± 13.41
- Pain: 17.22 ± 15.51, 2.78 ± 5.96**

*P < 0.05; **P < 0.01.

- a Higher scores mean better health;
- b,d Higher scores mean better function;
- c,e Higher scores mean worse symptoms.

Abbreviations: EORTC-QLQ-C30, European Organization for Research and Treatment of Cancer Quality of life Question-Core-30; OES-18, Esophageal Cancer Module-18.
### Supplemental table S6. Association between PD-L1 and/or TMB and downstaging of patients (n=19).

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<td>TMB status</td>
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<tr>
<td>TMB-H</td>
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<td>TMB-L</td>
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<tr>
<td>TMB-L</td>
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3. SUPPLEMENTARY FIGURES

Supplemental figure 1. Number of cells per mm² in tissue before and after treatment among all patients (n=19) who received surgery based on immunohistochemistry.

(A) The number of CD4⁺ cells was significantly increased after treatment. (B) The number of CD8⁺ cells was significantly increased after treatment. (C) The number of CD56⁺ cells was significantly increased after treatment. (D) The number of PD-1⁺ cells was significantly increased after treatment. (E) The number of GRB⁺ cells was significantly increased after treatment. (F) The number of TIA-1⁺ cells was significantly increased after treatment. (G) No significant change in the number of PD-L1⁺ cells was observed after chemoimmunotherapy. (H) No significant change in the number of CD163⁺ cells was observed after chemoimmunotherapy.

**: P<0.01; NS: not significant
Supplemental figure 2. Comparison of infiltrating immune cells between the PCR group (n=5) and the non-PCR (n=14) group before and after treatment based on immunohistochemistry.

(A) Comparison of infiltrating CD56+ cells between the PCR group (n=5) and the non-PCR group (n=14) before and after treatment.

(B) Comparison of infiltrating PD-1+ cells between the PCR group (n=5) and the non-PCR group (n=14) before and after treatment.

(C) Comparison of infiltrating GRB+ cells between the PCR group (n=5) and the non-PCR group (n=14) before and after treatment.

(D) Comparison of infiltrating TIA-1+ cells between the PCR group (n=5) and the non-PCR group (n=14) before and after treatment.
Supplemental figure 3. Comparison of infiltrating immune cells between the PCR + MPR group (n=14) and the PR+SD (n=5) group before and after treatment based on immunohistochemistry and multiplex immunofluorescence staining.

Comparison of infiltrating CD4\(^+\) (A), CD8\(^+\) (B), CD56\(^+\) (C), PD-1\(^+\) (D), GRB\(^+\) (E), TIA-1\(^+\) (F), PD-L1\(^+\) (G), CD163\(^+\) (H) and CD8\(^+\) PD-1\(^+\) (I) cells between the PCR + MPR group (n=14) and the PR+SD (n=5) group before and after treatment.
Supplemental figure 4. Comparison of the change in infiltrating immune cells between the PCR group (n=5) and the non-PCR (n=14) group in pre-treatment and post-treatment samples based on immunohistochemistry.

There was no significant difference in the change in infiltrating CD4⁺ (A), CD8⁺ (B), CD56⁺ (C), PD-1⁺ (D), GRB⁺ (E), TIA-1⁺ (F), PD-L1⁺ (G) and CD163⁺ (H) between the PCR group (n=5) and the non-PCR group (n=14) before and after treatment. The ratios represented by the Y axis were calculated by the densities of infiltrating immune cells in post-treatment samples divided by the infiltrating immune cells in pre-treatment samples. The ratio >1 means the infiltrating immune cells increase after treatment, while the ratio <1 means the infiltrating immune cells decrease after treatment.
Supplemental figure 5. The correlation analysis between PD-L1 and infiltrating immune cells in pre-treatment and post-treatment samples (n=18) based on multiplex immunofluorescence staining.

(A, B) The number of PD-L1$^+$ cells were positively correlated with CD163$^+$ cells and PD-L1$^+$ CD163$^+$ cells in pre-treatment samples. (C-E) There was no correlation between PD-L1$^+$ cells and infiltrating PD-1$, CD8^+$ and CD8$^+$ PD-1$^+$ cells in pre-treatment samples. (F) The number of PD-L1$^+$ cells were positively correlated with PD-L1$^+$ CD163$^+$ cells in post-treatment samples. (G-I) There was no correlation between PD-L1$^+$ cells and infiltrating PD-1$, CD8^+$ and CD8$^+$ PD-1$^+$ cells in post-treatment samples.
Supplemental figure 6. Comparison of the infiltrating immune cells between the PCR group (n=5) and the non-PCR group (n=13) before and after treatment based on multiplex immunofluorescence staining.

(A) Comparison of the infiltrating immune cells between the PCR group (n=5) and the non-PCR group (n=13) before treatment.

(B) Comparison of the infiltrating immune cells between the PCR group (n=5) and the non-PCR group (n=13) after treatment.
Supplemental figure 7. Genomic and PD-L1 analyses in the PCR group (n=5) and the non-PCR group (n=14).
(A) The landscape of genomic alterations in the tumors of patients who received surgery before treatment (n=19).
Comparison of TMB (B), PD-L1-CPS (C), and the immune-related pathway (D) between the PCR (n=5) and non-PCR groups (n=14).
Supplemental figure 8. Disease-free survival (DFS) by different TMB and PD-L1 status (n=19).

(A) DFS by TMB-H (n=5) and TMB-L (n=14).
(B) DFS by PD-L1+ (n=12) and PD-L1- (n=7).
(C) DFS by both TMB-H and PD-L1+ (n=4) and others (n=15).
4. SUPPLEMENTARY REFERENCES


A prospective, single-arm clinical study on the safety and efficacy of camrelizumab combined with carboplatin and nab-paclitaxel in the neoadjuvant therapy of potentially resectable stage II-III esophageal squamous cell carcinoma

Protocol

Project No.: GASTO-1056  
Version number: Version 1.0  
Version date: December 1, 2019  
Center: The First Affiliated Hospital of Sun Yat-sen University  
Principal Investigator: Chao Cheng  
58 Zhongshan 2nd Road, Guangzhou 510080, P. R. China  
Phone: +86-20-87755766-8782  
E-mail: chengch3@mail.sysu.edu.cn
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## Protocol Synopsis

<table>
<thead>
<tr>
<th>Objective</th>
<th>Primary objective:</th>
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<td>(1) To evaluate the safety and feasibility of camrelizumab combined with carboplatin and nab-paclitaxel in the neoadjuvant therapy of potentially resectable stage II-III esophageal squamous cell carcinoma.</td>
</tr>
</tbody>
</table>

### Secondary objectives:

1. To evaluate the efficacy of camrelizumab combined with carboplatin and nab-paclitaxel in neoadjuvant therapy of potentially resectable stage II-III esophageal squamous cell carcinoma (objective response rate, ORR; disease control rate DCR; major pathological response rate MPR, R0 resection rate, etc.).

2. To evaluate the effect of camrelizumab combined with carboplatin and nab-paclitaxel on quality of life of patients undergoing neoadjuvant therapy for stage II-III potentially resectable esophageal squamous cell carcinoma.

3. To evaluate the disease-free survival (DFS) of patients receiving neoadjuvant therapy of camrelizumab combined with carboplatin and nab-paclitaxel for stage II-III potentially resectable esophageal squamous cell carcinoma.

4. To evaluate the overall survival (OS) of patients receiving neoadjuvant therapy of camrelizumab combined with carboplatin and nab-paclitaxel for stage II-III potentially resectable esophageal squamous cell carcinoma.

5. To evaluate the correlation between immune-related markers (PD-L1, tumor mutation burden TMB, tumor neoantigen burden TNB,
microsatellite instability, MSI, tumor-infiltrating lymphocyte TIL, DDR pathway, etc.) and the efficacy of camrelizumab in neoadjuvant therapy for stage II-III potentially resectable esophageal squamous cell carcinoma.

<table>
<thead>
<tr>
<th>Study design</th>
<th>Single-center, prospective, single-arm clinical study</th>
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<tbody>
<tr>
<td>Sample size</td>
<td>20 patients</td>
</tr>
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<td>Patients</td>
<td>Patients with clinical TNM stage II-III potentially resectable esophageal squamous cell carcinoma</td>
</tr>
<tr>
<td>Inclusion criteria</td>
<td>1. The patient's pathological biopsy results must be confirmed to be esophageal squamous cell carcinoma (not including mixed adenosquamous carcinoma or other pathological types) by pathologists.</td>
</tr>
<tr>
<td></td>
<td>2. Age ≥18 years old and ≤75 years old.</td>
</tr>
<tr>
<td></td>
<td>3. ECOG or PS score is 0 or 1.</td>
</tr>
<tr>
<td></td>
<td>4. Patients did not previously receive cancer related chemotherapy, radiotherapy, or surgery.</td>
</tr>
<tr>
<td></td>
<td>5. Endoscopically diagnosed cervical esophageal cancer and gastroesophageal junction tumors were not included in this study.</td>
</tr>
<tr>
<td></td>
<td>6. Clinical stage should be T2-3/N0-2/M0 (II-III), with potential of radical surgical treatment.</td>
</tr>
<tr>
<td></td>
<td>7. Patients should have PET-CT examination or neck/chest and upper abdomen CT scan, ECT, brain MR/CT, etc. to clarify staging and exclude distant metastasis.</td>
</tr>
<tr>
<td></td>
<td>8. Adequate organ and marrow function as defined below:</td>
</tr>
<tr>
<td></td>
<td>1) Blood routine examination: Absolute neutrophil count (ANC)</td>
</tr>
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</table>
≥1.5×10⁹/L; Platelet count (PLT) ≥100×10⁹/L; Hemoglobin content (HGB) ≥ 9.0g/dL.

2) Liver function: serum total bilirubin (TBIL) ≤1.5×Upper Limit of Normal (ULN); Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) ≤2.5×ULN.

3) Renal function: Creatinine clearance (Ccr) ≥60 mL/min (calculated by Cockcroft/Gault formula):

   female: \[
   Ccr = \frac{(140 - \text{age}) \times \text{weight (kg)} \times 0.85}{72 \times \text{Scr (mg/dL)}}
   \]

   male: \[
   Ccr = \frac{(140 - \text{age}) \times \text{weight (kg)} \times 1.00}{72 \times \text{Scr (mg/dL)}}
   \]

4) Adequate coagulation function, defined as international normalized ratio (INR) or prothrombin time (PT) ≤1.5 ULN; If patient is receiving anticoagulant therapy, it is acceptable that PT is in the prescribed anticoagulant range.

9. Female patients of reproductive age or male patients whose sexual partner is female patients of reproductive age shall use effective contraceptive measures throughout the treatment period until 6 months after the treatment period.

10. Sign written informed consent and be able to comply with the visits and related procedures specified in the program.

11. Can provide archived pathological tissues or fresh pathological tissues within 6 months for the detection of PD-L1 and other markers.

<table>
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<th>Exclusion criteria</th>
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<tr>
<td>1. Patients were taking other investigational drugs at the same time.</td>
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<td>2. Those who have not recovered from recent major surgical procedure.</td>
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3. Any treatment with anti-PD-1, anti-PD-L1, anti-PD-L2, anti-CD137, or anti-CTLA-4 antibodies, or any other antibody or drug that specifically targets at T-cell costimulation or checkpoint pathways prior to study.

4. History of allergic reactions attributed to any monoclonal antibody or chemotherapy drug (paclitaxel, carboplatin) preparations or excipients.

5. Patients were also taking rifampicin, phenytoin sodium, carbamazepine, or barbiturates (these drugs induce CYP3A and may reduce plasma content of paclitaxel).

6. Received systemic therapy of Chinese herbal medicine with anti-tumor indications or immunomodulatory drugs (including thymosin, interferon, interleukin, etc.) within 2 weeks prior to first administration.

7. Administration of a live, attenuated vaccine within 4 weeks prior to the first dose of treatment or planned for it during the study.

   Note: Administration of inactivated virus vaccine for seasonal influenza is permitted within 4 weeks prior to the first dose of treatment, while live attenuated flu vaccines are not allowed.

8. Toxicity from prior antineoplastic therapy that did not return to grade 0 or 1 defined by National Cancer Institute General Adverse Event Terminology version 5.0 (NCI CTCAE version 5.0, excluding alopecia, non-clinically significant or asymptomatic laboratory abnormalities).

9. Known autoimmune disease that needs symptomatic treatment or history of disease within 2 years (patients with vitiligo, psoriasis, hair loss, or Graves disease that doesn’t need systemic treatment, hypothyroidism that only needs thyroid hormone replacement therapy and type 1 diabetes
which only need insulin replacement therapy can be enrolled).

10. Known history of primary immunodeficiency.

11. Known to have active infection of tuberculosis.

12. Known history of allogeneic organ transplantation and allogeneic hematopoietic stem cell transplantation.

13. HIV infection and carriers are known to exist (HIV antibody positive).

14. Severe infections that are in active period or clinically poorly controlled.

15. Symptomatic congestive heart failure (NYHA II-IV) or symptomatic or poorly controlled arrhythmia.

16. Uncontrolled arterial hypertension (systolic blood pressure ≥160mmHg or diastolic blood pressure ≥100mmHg) even with standard therapy.

17. Any arterial thromboembolic event, including myocardial infarction, unstable angina, cardiocerebral events or transient ischemic attack (TIA), occurred within 6 months prior to enrollment.

18. Significant malnutrition which need intravenous supplements while malnutrition corrected for over 4 weeks prior to first dose of the study was excluded.

19. A history of deep vein thrombosis, pulmonary embolism, or any other severe thromboembolism within 3 months prior to enrollment (Implantable Venous Access Port or duct-derived thrombosis, or superficial venous thrombosis is not considered as "severe" thromboembolism).
20. Uncontrolled metabolic disorders or other non-malignant organ or systemic diseases or secondary reactions to cancer that may result in higher medical risk and/or uncertainty in the assessment of survival.

21. Hepatic encephalopathy, hepatorenal syndrome, Child-Pugh B liver cirrhosis or worse.

22. History of intestinal obstruction or following diseases: inflammatory bowel disease (IBD) or extensive bowel resection (partial resection of the colon or extensive resection of the small intestine with chronic diarrhea), Crohn's disease, ulcerative colitis (UC).

23. Known to have acute or chronic active hepatitis B (HBsAg positive with HBV DNA viral load $\geq 10^3$ IU/ml/mL or $>200$ IU/mL) or acute or chronic active hepatitis C (HCV antibody positive and HCV RNA positive).

24. History of gastrointestinal perforation and/or fistula within 6 months prior to enrollment.

25. Interstitial lung disease (ILD) requiring steroid therapy.

26. History of other primary malignancies, excluding:

1) Complete remission (CR) of malignant tumors for at least 2 years before enrollment and no other treatment was required during the study;

2) Non-melanoma skin cancer or Lentigo maligna (LM) that has been adequately treated and has no evidence of disease recurrence;

3) Adequately treated carcinoma in situ (CIS) with no evidence of disease recurrence.
27. Pregnant or lactating female patients.

28. Other acute or chronic diseases, psychiatric disorders, or abnormal laboratory test values that may cause: increase related risk of study participation or drug administration, interfere with the interpretation of study results or ineligible patients for study participation from researcher's judgment.

29. Patients with difficulty in obtaining satisfactory biopsy specimens through endoscopy for relevant detection of the study.

<table>
<thead>
<tr>
<th>Withdraw criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. The patient is found to be ineligible for the inclusion/exclusion criteria and is deemed unsuitable for further study by the investigator.</td>
</tr>
<tr>
<td>2. The patient violates the study protocol, and it is considered inappropriate to continue to participate in further study by the investigator.</td>
</tr>
<tr>
<td>3. Any other type of pharmaceutical research that is considered scientifically or medically incompatible with this study.</td>
</tr>
<tr>
<td>4. The patient fails to finish the defined follow-up evaluations (Research center staff should contact the patient who has lost follow-up in order to determine the reason and try to reschedule the visit. The date the patient was contacted, and the contact details should be recorded in the study file).</td>
</tr>
<tr>
<td>5. Evaluated by investigator</td>
</tr>
<tr>
<td>1) If, for any reason, the patient needs to be treated with another drug that has been shown to be effective in treating the indication of the study, the patient should withdraw from the study before using the new drug.</td>
</tr>
<tr>
<td>2) Disease progression or further treatment is deemed unsuitable by the investigator.</td>
</tr>
</tbody>
</table>
3) Any treatment-related event considered life-threatening has occurred, regardless of the severity of the event.

4) Study drugs meet withdrawal criteria due to toxicity.

5) Patient or client (such as parent or legal guardian) requests to withdraw from the study or discontinuation of study drugs (if patient withdraws informed consent for treatment but not for follow-up, long-term follow-up is still available).

6) The Investigator or co-sponsors may terminate the study or discontinue the patient's participation for medical, safety, regulatory, or other reasons related to GCP.

Note: Reasons for termination and termination dates for all patients will be collected.

<table>
<thead>
<tr>
<th>Drugs, Dose, Route, Regimen</th>
<th>1) Camrelizumab</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specification: 200 mg/bottle</td>
<td>Administration method: 200mg IV D1 Q3W</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Drugs, Dose, Route, Regimen</th>
<th>2) Carboplatin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specification: 150mg/piece</td>
<td>Administration method: AUC (mg/mL/min) set 5, total carboplatin (mg)= Set AUC× (Ccr +25) IV D1 Q3W</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Drugs, Dose, Route, Regimen</th>
<th>3) Nab-paclitaxel</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specification: 100 mg/piece</td>
<td>Administration method: 260mg/m² IV D1 Q3W</td>
</tr>
<tr>
<td>Evaluation Criteria</td>
<td>(1) Safety evaluation:</td>
</tr>
<tr>
<td>---------------------</td>
<td>------------------------</td>
</tr>
<tr>
<td></td>
<td>The incidence and severity of all Adverse Events (AE), Treatment Emergent Adverse Event (TEAE), Adverse Events of Special Interest (AESI), and Serious Adverse Events (SAE) were evaluated according to CTCAE version 5.0.</td>
</tr>
<tr>
<td></td>
<td>The changes of vital signs, physical examination results, and laboratory results before, during, and after treatment.</td>
</tr>
<tr>
<td></td>
<td>Assessment of surgical safety: surgical R0 resection rate, operation duration, blood loss, chest drainage fluid volume, length of hospital stay, incidence of complications, perioperative mortality, etc.</td>
</tr>
<tr>
<td>(2) Effectiveness evaluation:</td>
<td>The ORR and DCR were assessed according to RECIST1.1 criteria.</td>
</tr>
<tr>
<td>Pathological evaluation:</td>
<td>MPR rate, PCR rate, R0 resection rate, tumor infiltrating lymphocytes (TIL), tumor cell death rate, etc.</td>
</tr>
<tr>
<td>Follow-up of survival:</td>
<td>Disease-Free Survival (DFS) and Overall Survival (OS).</td>
</tr>
<tr>
<td>(3) Biomarkers:</td>
<td>Tumor tissue samples were collected for tumor biomarker analysis, including but not limited to the changes of the following indicators in tumor specimens (PD-L1, TMB, TNB, MSI, dMMR, TIL, DDR pathway, etc.).</td>
</tr>
<tr>
<td></td>
<td>Blood samples were collected for circulating tumor cell (CTC) and circulating tumor DNA (ctDNA) analysis, including but not limited to PD-</td>
</tr>
</tbody>
</table>
L1 dynamic changes.

(4) Evaluation of Quality of life:

Changes in quality of life and health status of enrolled patients during treatment were assessed according to EORTC QLQ-C30 and EORTC QLQ-OES18.

<table>
<thead>
<tr>
<th>Study Duration</th>
<th>The duration of enrollment in this study lasts about 2 years, and patients were followed up for long-term survival after the intervention.</th>
</tr>
</thead>
</table>
| Trial Progress | (1) Patients need to complete relevant examinations and evaluations. If the patients meet all inclusion and exclusion criteria, they will be enrolled and sign the informed consent.  
(2) Enrolled patients will receive 2 cycles of camrelizumab (200mg IV D1 Q3W) combined with carboplatin (AUC set 5, IV D1 Q3W) and nab-paclitaxel (260mg/m2, IV D1 Q3W, 30 min each infusion), and its safety was evaluated during the treatment.  
(3) After completing 2 cycles of camrelizumab combined with carboplatin and albumin-paclitaxel, the patient needs to return to the hospital for re-examination of physical examination, blood biochemical examination, liver and kidney function examination. PET/CT or neck, chest and upper abdomen CT scan needs to be performed again. Patients suspected of distant metastasis should be performed examination of the specific site to confirm the diagnosis. At the same time, relevant preoperative examination should be performed to exclude surgical contraindications. Efficacy was evaluated according to RECIST 1.1 criteria.  
(4) Surgical treatment: the surgical method adopted in this study was... |
radical resection of esophageal cancer through three incisions of right chest, neck and upper abdomen (McKeown). Surgery is performed within 3-6 weeks after completion of the second chemotherapy. The scope of lymph node dissection: lymph nodes in the thoracic and upper abdominal surgical fields. Surgical margin: the proximal and distal margin should be over 5cm beyond the edge of tumor. Pathology was used to determine the depth of tumor invasion, whether the resection margin contained tumor cells, and the proportion of tumor cells. This procedure is used to assess R0 resection, pathological response rate, and subsequent treatment decisions.

(5) Camrelizumab maintenance therapy (200mg IV D1 Q3W) was started 4-8 weeks after surgery, and the maintenance therapy lasts a year after surgery. (Note: Maintenance therapy will not be carried out for serious infection or other conditions that are not suitable for maintenance therapy after operation.)

(6) After treatment, regular follow-up of survival was performed every 3 months in the first year after surgery, and every 6 months in 2-5 years after surgery, including recurrence and related treatment.

Safety

According to the mechanism of camrelizumab and clinical safety information of products with the same mechanism, adverse events may occur in the process of this clinical trial are primarily reactive capillary hyperplasia and immunity inflammation caused by the immune system activation such as pneumonia, colitis, hepatitis, nephritis, renal insufficiency and inflammation of the endocrine system, etc. According to the clinical data of existing anti-PD-1 monoclonal antibody drugs, although the incidence of adverse events is high, however these are...
tolerated. Only a small part of patients will stop taking drugs due to adverse reactions, and most of the adverse reactions can be relieved after treatment. For early symptoms of immune related adverse reaction are varied, special attention should be paid to early signs and symptoms of various immune related reaction to make correct judgement in time and perform dose adjustment according to the plan and give the specific treatment. Reduce the risk of patients who use the drugs. At the same time, attention should be paid to exclude patients with autoimmune diseases to avoid exacerbation of the original disease caused by activation of the immune system.

Phase I-III clinical pharmacology and safety data of camrelizumab indicate that camrelizumab has definite pharmacological activity and is well tolerated in patients with advanced tumors.

The data above preliminarily indicate that SHR-1210 (camrelizumab) has satisfying safety and pharmacological activity. And similar drugs have significant antitumor activity in advanced esophageal cancer patients that supports conducting clinical studies in esophageal cancer patients in China.

| Statistical Methodology | The sample size was 20 cases. The detailed statistical method is described in the study protocol. |
1. Background and rationale

1.1 Epidemiology of esophageal cancer.

Esophageal cancer (EC) is one of the malignant tumors that seriously threaten human health. The pathology of EC are mainly squamous cell carcinoma and adenocarcinoma. China has a high prevalence of EC and is home to more than half of the EC patients in the world. According to the latest cancer statistics in China, the incidence and mortality of esophageal cancer rank third and fourth among all kinds of malignant tumors. The main pathological type is squamous cell carcinoma in the Asian populations, while adenocarcinoma is the main type of esophageal cancer in western countries. There are many differences between esophageal squamous cell carcinoma (ESCC) and adenocarcinoma carcinoma in pathophysiology and pathogenesis. It is of great significance to study the prevention and treatment of ESCC.

Most EC cases are initially diagnosed at an advanced stage of the disease. In the past 30 years, although emphasis has been placed on multidisciplinary comprehensive treatment of ESCC, the overall survival of ESCC patients has improved little, with a 5-year survival rate of only 15-25%. Due to the limited efficacy of chemotherapy and radiotherapy in the treatment of ESCC, it is easy to recur and metastasize.

1.2 Efficacy and deficiency of neoadjuvant chemotherapy in the treatment of stage II-III esophageal cancer.

Surgical treatment is still the main therapy for the treatment of stage II-III esophageal cancer. However, clinical studies have shown that the pathological R0 resection rate of these patients was only 60% and resulted early esophageal recurrence in 58% patients, and the median survival time is only about 17 months. Therefore, for patients with stage II-III esophageal cancer, how to improve the R0 resection rate and reduce postoperative local recurrence is one of the important directions to improve the prognosis of esophageal cancer.

The purpose of neoadjuvant chemotherapy is to increase the R0 resection rate and improve the overall prognosis of patients. Some studies have been carried out in neoadjuvant chemotherapy for esophageal cancer. The early application of cisplatin and fluorouracil improved the curative resection rate. And the neoadjuvant chemotherapy had a better OS (5-year rate 38% vs 24%) and a better disease-free survival (5-year rate: 34% vs 19%). In recent years, paclitaxel drugs were added, and the efficiency and pathological complete response (pCR) rate were further improved. In a study of 209 patients with stage III esophageal cancer, traditional CF regimen combined with docetaxel (DCF) regimen was used in preoperative neoadjuvant chemotherapy. The results showed that the total effective rate of DCF regimen was significantly better than that of CF regimen. Another domestic study on locally advanced esophageal squamous cell carcinoma showed that in preoperative neoadjuvant chemotherapy for esophageal squamous cell carcinoma, docetaxel combined with cisplatin has better clinical tolerance.
than traditional CF regimen, and the postoperative pathological complete remission rate also had a significant advantage. However, the side effects of neoadjuvant chemotherapy such as severe neutropenia, fever, nausea and vomiting could not be ignored. If the neoadjuvant chemotherapy is ineffective, it will undoubtedly increase the financial burden of patients and may lead to the loss of surgical opportunities, and the worse prognosis.

1.3 Neoadjuvant chemotherapy and neoadjuvant chemoradiotherapy.

Neoadjuvant chemoradiotherapy and neoadjuvant chemotherapy are the main neoadjuvant therapy modes for esophageal cancer. NEOCRTEC 5010 and CROSS study both demonstrated that neoadjuvant chemoradiotherapy plus surgery improves survival over surgery alone among patients with locally advanced EC, extending median overall survival by 33.6 months and 60.5 months, respectively. Therefore, for patients with resectable esophageal cancer (clinical stage T1B-T2 N+ or T3-T4A with any N or suspected involvement of surrounding organs but no T4b is identified), the CSCO Guidelines recommended preoperative neoadjuvant chemoradiotherapy (class 1A evidence) or neoadjuvant chemotherapy (class 1B evidence).

However, due to the toxicity of neoadjuvant chemoradiotherapy, most of the patients are prone to intolerance and suffer poor nutritional status, decreased KPS score and quality of life. Moreover, preoperative chemoradiotherapy require close cooperation with multi-disciplinary such as pre-treatment assessment, radiotherapy, surgery, nutrition, perioperative care and pathology. The application of neoadjuvant chemoradiotherapy in clinical practice is limited.

In addition, neoadjuvant chemoradiotherapy has not been able to improve the status of locally advanced esophageal cancer with tumor recurrence. In NEOCRTEC 5010, distant recurrence accounted in 71.0% of all patients with recurrence, and distant recurrence accounted for 80.5% of all patients in CROSS study. It is suggested that the recurrence mode after neoadjuvant chemoradiotherapy is mainly distant metastasis, and there is still no effective systemic treatment for locally advanced esophageal cancer. A more effective and less toxic neoadjuvant treatment regimen is therefore needed to improve the clinical outcomes of ESCC patients without increasing the burden of treatment-related adverse events.

1.4 Immunotherapy for esophageal cancer

PD-1 inhibitors have achieved encouraging antitumor efficacy in a variety of tumors. In recent years, some clinical studies have shown the efficacy of PD-1 inhibitors in advanced esophageal cancer. The KEYNOTE-028 study enrolled 23 PD-L1-positive patients who received pembrolizumab. 29% (5/17) ESCC patients and 40% (2/5) esophageal adenocarcinoma achieve partial response (PR) and the overall ORR was 30%. KEYNOTE-180 and KEYNOTE-181 is recruiting patients. A phase II study from Japan reported that the ORR of nivolumab for advanced ESCC was 17%, and the median OS was 23 months. In another phase II clinical study of nivolumab in advanced esophageal cancer in which at least first-line treatment failed or intolerable patients, the ORR was 17.2% and mOS was 12.1
months. The SHR-1210 study demonstrated the safety and efficacy of camrelizumab in the treatment of advanced esophageal cancer. The ORR was 33.3% in 30 enrolled patients with advanced ESCC. The ESCORT study was launched to evaluate the efficacy of camrelizumab in the treatment of advanced esophageal squamous cell carcinoma and announced that the study has reached the main end point. The results of the study will be published in the near future. At the 2018 CSCO meeting, Professor Shen Lin presented a phase 3 study of a new anti-PD-1 drug tislelizumab in the treatment of advanced esophageal squamous cell carcinoma and reported that tislelizumab achieve an overall ORR of 40%. Based on the above studies, PD-1 blockade induced tumor regression in patients with advanced esophageal squamous cell carcinoma and suggested that immunotherapy would become an effective therapy for the treatment of advanced esophageal cancer in the future.

1.5 Rationale for immunotherapy combined with chemotherapy for resectable esophageal squamous cell carcinoma

Rationale for carboplatin and nab-paclitaxel in the neoadjuvant therapy of esophageal cancer

Carboplatin combined with paclitaxel is also one of the recommended first-line regimens for neoadjuvant therapy in esophageal squamous cell carcinoma in the NCCN guideline. The efficacy of nab-paclitaxel and paclitaxel was comparable and nab-paclitaxel produced less severe adverse events such as neuropathy, neutropenia, myalgia, and arthralgia compared with paclitaxel in the first-line therapy of patients with advanced esophageal cancer and non-small-cell lung cancer. In addition, to our knowledge, although there is lack of head-to-head study to compare the efficacy of carboplatin/paclitaxel and cisplatin/FU for neoadjuvant therapy in ESCC, it was reported that the efficacy of carboplatin and paclitaxel (CROSS) was comparable with that of cisplatin and fluorouracil (CALGB 9781) in the neoadjuvant chemoradiotherapy setting (median overall survival: 49.4 months vs 53.76 month).

Rationale for neoadjuvant immunotherapy in neoadjuvant chemotherapy for esophageal cancer

According to the existing research results, immunotherapy shows good anti-tumor efficacy in patients with advanced esophageal cancer, which can improve the objective response rate and prolong survival. Moreover, the PD-1 blockade also showed good safety had a manageable treatment-related adverse effects. Can the immunotherapy also benefit patients with potential resectable tumors? In recent years, immunotherapy has made a breakthrough in the neoadjuvant therapy of lung cancer. In a small sample clinical study of nivolumab for neoadjuvant therapy of lung cancer, the pathological remission rate reached 45%, and the side effects were acceptable. Neoadjuvant immunotherapy did not lead to the delay of surgery. The research results of immunotherapy in neoadjuvant therapy of lung cancer suggested that the block of PD-1 pathway in patients with early lung cancer may increase host immune adaptability and reduce tumor heterogeneity, which may enhance the anti-tumor effect. At present, the role of immunotherapy in neoadjuvant therapy for esophageal cancer is not clear. It is of great significance to further clarify the safety and effectiveness of immunotherapy in neoadjuvant therapy for esophageal cancer. It is of great importance to explore a new treatment model and improve the effective rate of neoadjuvant therapy for esophageal cancer.
Rationale for immunotherapy combined with chemotherapy for resectable esophageal squamous cell carcinoma

Preclinical studies have confirmed that PD-1 inhibitors combined with chemotherapy can further enhance host's immune response and inhibit cancer cell immune escape\(^2\). Chemotherapy could disrupt the activity of immunosuppressive cells such as regulatory T cells (Treg), myeloid suppressor cells (MDSC), and tumor-associated macrophages (TAM). Moreover, chemotherapy can promote immune response by inducing tumor cell apoptosis, up-regulating of MHC-1 molecule expression and dendritic cell maturation\(^2\).

1.6 References:


2. Objectives

2.1 Primary

To evaluate the safety and feasibility of camrelizumab combined with carboplatin and nab-paclitaxel in the neoadjuvant therapy of potentially resectable stage II-III esophageal squamous cell carcinoma.

2.2 Secondary

1) To evaluate the efficacy of camrelizumab combined with carboplatin and nab-paclitaxel chemotherapy in neoadjuvant therapy for potentially resectable esophageal squamous cell carcinoma in stage II-III (ORR, DCR, MPR, R0 resection rate, etc.)
2) To evaluate the quality of life of patients diagnosed with stage II-III potentially resectable esophageal squamous cell carcinoma who received neoadjuvant therapy with carboplatin and albumin-paclitaxel chemotherapy;
3) To evaluate the disease-free survival (DFS) of camrelizumab combined with carboplatin and nab-paclitaxel in neoadjuvant therapy for stage II-III potentially resectable esophageal squamous cell carcinoma;
4) To evaluate the overall survival (OS) of neoadjuvant therapy with camrelizumab combined with carboplatin and nab-paclitaxel for stage II-III potentially resectable esophageal squamous cell carcinoma;
5) To evaluate the correlation between immune-related markers (PD-L1, TMB, TNB, MSI, dMMR, TIL, DDR pathway, etc.) and the efficacy of neoadjuvant therapy with camrelizumab for stage II-III potentially resectable esophageal squamous cell carcinoma.
3. Study design

20 patients with stage II-III potentially resectable esophageal squamous cell carcinoma were enrolled.

2 cycles of camrelizumab combined with carboplatin and nab-paclitaxel chemotherapy

- Camrelizumab 200mg IV D1 Q3W;
- Carboplatin AUC=5, IV D1 Q3W;
- Nab-paclitaxel 260mg/m² IV D1 Q3W

Evaluation of safety and efficacy
Evaluate the feasibility of surgical treatment

Operable
- Surgical treatment
  - Evaluation of the pathological remission rate and R0 excision rate
  - Camrelizumab maintenance monotherapy for a year (200mg IV D1 Q3W)
  - Survival follow-up

Inoperable
- Radical chemoradiotherapy/palliative care
4. Patient selection

4.1 Inclusion Criteria

1) The patient's pathological biopsy results must be confirmed to be esophageal squamous cell carcinoma (not including mixed adenosquamous carcinoma or other pathological types) by pathologists.

2) Age $\geq 18$ y and $\leq 75$ y.

3) ECOG or PS score is 0 or 1.

4) Patients did not previously receive cancer related chemotherapy, radiotherapy, or surgery.

5) Endoscopically diagnosed cervical esophageal cancer and gastroesophageal junction tumors were not included in this study.

6) Clinical stage should be T2-3/N0-2/M0 (II-III), with potential of radical surgical treatment.

7) Patients should have PET-CT examination or neck/chest + upper abdomen CT scan, ECT, brain MR/CT, etc. to clarify staging and exclude distant metastasis.

8) Adequate organ and marrow function as defined below:

   i. Blood routine examination: Absolute neutrophil count (ANC) $\geq 1.5 \times 10^9$/L; Platelet count (PLT) $\geq 100 \times 10^9$/L; Hemoglobin content (HGB) $\geq 9.0$ g/dL.

   ii. Liver function: serum total bilirubin (TBIL) $\leq 1.5 \times$ Upper Limit Of Normal (ULN); Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) $\leq 2.5 \times$ ULN.

   iii. Renal function: Creatinine clearance (Ccr) $\geq 60$ mL/min (calculated by Cockcroft/Gault formula):

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\text{female: } Ccr = \frac{(140 - \text{age}) \times \text{weight}(kg) \times 0.85}{72 \times \text{Scr}(mg/dL)}
\]

\[
\text{male: } Ccr = \frac{(140 - \text{age}) \times \text{weight}(kg) \times 1.00}{72 \times \text{Scr}(mg/dL)}
\]
iv. Adequate coagulation function, defined as international normalized ratio (INR) or prothrombin time (PT) ≤1.5 ULN; If patient is receiving anticoagulant therapy, it is acceptable that PT is in the prescribed anticoagulant range.

9) Female patients of reproductive age or male patients whose sexual partner is female patients of reproductive age shall use effective contraceptive measures throughout the treatment period until 6 months after the treatment period.

10) Sign written informed consent and be able to comply with the visits and related procedures specified in the program.

11) Can provide archived pathological tissues or fresh pathological tissues within 6 months for the detection of PD-L1 and other markers.

4.2 Exclusion Criteria

1) Patients were taking other investigational drugs at the same time.

2) Those who have not recovered from recent major surgical procedure.

3) Any treatment with anti-PD-1, anti-PD-L1, anti-PD-L2, anti-CD137, or anti-CTLA-4 antibodies, or any other antibody or drug that specifically targets at T-cell costimulation or checkpoint pathways prior to study.

4) History of allergic reactions attributed to any monoclonal antibody or chemotherapy drug (paclitaxel, carboplatin) preparations or excipients.

5) Patients were also taking rifampicin, phenytoin sodium, carbamazepine, or barbiturates (these drugs induce CYP3A and may reduce plasma content of paclitaxel).

6) Received systemic therapy of Chinese herbal medicine with anti-tumor indications or immunomodulatory drugs (including thymosin, interferon, interleukin, etc.) within 2 weeks prior to first administration.

7) Administration of a live, attenuated vaccine within 4 weeks prior to the first dose of treatment or planned for it during the study.
Note: Administration of inactivated virus vaccine for seasonal influenza is permitted within 4 weeks prior to the first dose of treatment, while live attenuated flu vaccines are not allowed.

8) Toxicity from prior antineoplastic therapy that did not return to grade 0 or 1 defined by National Cancer Institute General Adverse Event Terminology version 5.0 (NCI CTCAE version 5.0, excluding alopecia, non-clinically significant or asymptomatic laboratory abnormalities).

9) Known autoimmune disease that needs symptomatic treatment or history of disease within 2 years (patients with vitiligo, psoriasis, hair loss, or grave disease that doesn’t need systemic treatment, hypothyroidism that only needs thyroid hormone replacement therapy and type 1 diabetes which only need insulin replacement therapy can be enrolled).

10) Known history of primary immunodeficiency.

11) Known to have active infection of tuberculosis.

12) Known history of allogeneic organ transplantation and allogeneic hematopoietic stem cell transplantation.

13) HIV infection and carriers are known to exist (HIV antibody positive).

14) Severe infections that are in active period or clinically poorly controlled.

15) Symptomatic congestive heart failure (NYHA II-IV) or symptomatic or poorly controlled arrhythmia.

16) Uncontrolled arterial hypertension (systolic blood pressure $\geq 160\text{mmHg}$ or diastolic blood pressure $\geq 100\text{mmHg}$) even with standard therapy.

17) Any arterial thromboembolic event, including myocardial infarction, unstable angina, cardiocerebral events or transient ischemic attack (TIA), occurred within 6 months prior to enrollment.

18) Significant malnutrition which need intravenous supplements while malnutrition corrected for over 4 weeks prior to first dose of the study was excluded.
19) A history of deep vein thrombosis, pulmonary embolism, or any other severe thromboembolism within 3 months prior to enrollment (Implantable Venous Access Port or duct-derived thrombosis, or superficial venous thrombosis is not considered as "severe" thromboembolism).

20) Uncontrolled metabolic disorders or other non-malignant organ or systemic diseases or secondary reactions to cancer that may result in higher medical risk and/or uncertainty in the assessment of survival.

21) Hepatic encephalopathy, hepatorenal syndrome, Child-Pugh B liver cirrhosis or worse.

22) History of intestinal obstruction or following diseases: inflammatory bowel disease (IBD) or extensive bowel resection (partial resection of the colon or extensive resection of the small intestine with chronic diarrhea), Crohn's disease, ulcerative colitis (UC).

23) Known to have acute or chronic active hepatitis B (HBsAg positive with HBV DNA viral load $\geq 10^3$ IU/ml/mL or >200IU/mL) or acute or chronic active hepatitis C (HCV antibody positive and HCV RNA positive).

24) History of gastrointestinal perforation and/or fistula within 6 months prior to enrollment.

25) Interstitial lung disease (ILD) requiring steroid therapy.

26) History of other primary malignancies, excluding:

i Complete remission (CR) of malignant tumors for at least 2 years before enrollment and no other treatment was required during the study;

ii Non-melanoma skin cancer or Lentigo maligna (LM) that has been adequately treated and has no evidence of disease recurrence;

iii Adequately treated carcinoma in situ (CIS) with no evidence of disease recurrence.

27) Pregnant or lactating female patients.

28) Other acute or chronic diseases, psychiatric disorders, or abnormal laboratory test values that may cause: increase related risk of study participation or drug administration, interfere with the interpretation...
of study results or ineligible patients for study participation from researcher's judgment.

29) Patients with difficulty in obtaining satisfactory biopsy specimens through endoscopy for relevant detection of the study.

### 4.3 Withdraw criteria

1. The patient is found to be ineligible for the inclusion/exclusion criteria and is deemed unsuitable for further study by the investigator.

2. The patient violates the study protocol, and it is considered inappropriate to continue to participate in further study by the investigator.

3. Any other type of pharmaceutical research that is considered scientifically or medically incompatible with this study.

4. The patient fails to finish the defined follow-up evaluations (Research center staff should contact the patient who has lost follow-up in order to determine the reason and try to reschedule the visit. The date the patient was contacted, and the contact details should be recorded in the study file).

5. Evaluated by investigator

1) If, for any reason, the patient needs to be treated with another drug that has been shown to be effective in treating the indication of the study, the patient should withdraw from the study before using the new drug.

2) Disease progression or further treatment is deemed unsuitable by the investigator.

3) Any treatment-related event considered life-threatening has occurred, regardless of the severity of the event.

4) Study drugs meet withdrawal criteria due to toxicity.

5) Patient or client (such as parent or legal guardian) requests to withdraw from the study or discontinuation of study drugs (if patient withdraws informed consent for treatment but not for follow-
up, long-term follow-up is still available.

6) The Investigator or co-sponsors may terminate the study or discontinue the patient's participation for medical, safety, regulatory, or other reasons related to GCP. 

Note: Reasons for termination and termination dates for all patients will be collected.

5. Treatment Plan

5.1 Neoadjuvant administration Regimen

Camrelizumab, 200mg IV D1 Q3W, maintain 30 to 60 minutes per infusion; Carboplatin AUC=5, IV D1 Q3W; Nab-paclitaxel, 260 mg/m² IV D1Q3W, maintain 30 minutes per infusion. Chemotherapy should be started 1 hour after the end of camrelizumab infusion and finish monitoring of vital signs.

Camrelizumab 200 mg fixed dose (3mg/kg for patients weighing less than 40kg) is administered intravenously every 3 weeks. 200mg of each camrelizumab freeze-dried powder injection was first redissolved with 5mL of water, and then further diluted with 100mL 5% glucose or 0.9% sodium chloride, each infusion maintains 30-60 minutes.

For patients with normal renal function, the recommended dose of carboplatin is calculated by AUC set as 5, and a single dose of carboplatin is given intravenously for over 15-60 minutes every 3 weeks. Neutrophil count $\geq 2000/mm^3$; Platelet count $\geq 100000/mm^3$ is required for the next cycle of treatment. During configuration, the product should be dissolved with 5% glucose injection at a concentration of 10mg/ml, and then add it into 250-500ml 5% glucose for injection intravenously.

Nab-paclitaxel 260 mg/m² was given intravenously for over 30 minutes and observed for 30 minutes after infusion. The total dose per cycle was 260 mg/m².

5.2 Surgical plan and Procedure:

The operation was performed 3-6 weeks after the end of the second neoadjuvant chemotherapy, and the surgical method adopted in this study was radical resection of esophageal cancer through three incisions of right chest, neck and upper abdomen (Mckeown).

5.2.1 Preoperative preparation of patients
1) Psychological nursing: communication and reduce the apprehension.
2) Nutritional support: food with high nutrition, rich in protein and vitamin.
3) Preparation of respiratory tract: quit smoking, effective cough training.
4) Preparation of gastrointestinal tract: fasting for 8 hours and no drinking for 6 hours before surgery.
5) To control comorbidity: hypertension, coronary heart disease, diabetes, nutritional metabolism disorders, etc.

5.2.2 Surgical procedure

Consciousness, body temperature (T), electrocardiogram (ECG), heart rate (HR), blood pressure
(Bp), oxygen saturation (SpO2), respiratory rate (RR) were continuously monitored once patients get into the operating room. After the artificial airway was established, pressure of end-tidal CO2 (P_{e}CO2) and fraction of inspiration O₂ (FiO2) were monitored.

Thoracic surgery: thoracoscopic esophageal dissociation and lymph node dissection were performed on the chest, requiring complete dissection of lymph nodes in each regions including para-esophageal lymph nodes, para-laryngeal recurrent nerve lymph nodes and subcarinal lymph nodes. If complete resection could not be performed, metal clips should be used and fill in the form with explanation. Intraoperative transfer to open surgery should be recorded.

Abdominal and neck surgery: abdominal gastric dissociation and abdominal lymph node dissection were performed. Lymph nodes in each region were required to be completely cleaned. If complete resection could not be performed, metal clips were added and instructions were filled in. After the completion of dissociation, the tube stomach can be made through a small open incision in the middle of the abdomen, and the intraoperative transfer should be recorded. In principle, the esophageal bed route is preferred for the gastric ascending route. Special cases can be adjusted according to the experience of the center, which needs to be explained in the form. For neck anastomosis, stapler mechanical anastomosis or manual anastomosis can be selected. No special requirements are required and records should be made. It is necessary to indwelling nasoenteral nutrition tube or jejunal fistula during operation. Whether to indwelling neck drainage tube and gastric tube during the operation according to experience, no special requirements, need to record.

Surgical margin: the proximal and distal resection should be more than 5cm beyond the tumor margin.

5.2.3 Surgical indicators
1) Operation time: total operation duration, chest operation duration, abdominal + neck operation duration.
2) Intraoperative blood loss (ml).
3) Biological specimens will be saved for use, the time and number were recorded.
4) Pathological data, number of stations of dissected lymph nodes and positive number.

5.2.4 Postoperative evaluation
Postoperative adverse reactions and adverse events were recorded and evaluated by the investigator. The records content include:
1) Postoperative weight monitoring, postoperative blood loss, chest tube retain time, drainage flow and color.
2) Occurrence of postoperative complications, including total complications, respiratory complications, cardiovascular complications, recurrent laryngeal nerve injury, chylous leakage, anastomotic fistula, 30-day mortality, etc.
3) Postoperative hospital stay (from end of surgery to discharge), time of postoperative oral feeding, postoperative stitches removal time, duration of ICU stay, and re-admission to ICU.

5.2.5 Discharge and readmission
The patients were evaluated for discharge after reaching the standard. The duration of reaching the standard of discharge after the operation was recorded. Discharge criteria are as follows:
1) Axillary body temperature below 38°C;
2) Blood oxygen saturation >90% without oxygen inhalation,
3) No complications that need hospitalization,
4) For other cases, the investigator will evaluate whether the patient can be discharged.

6. Dose adjustment

6.1 Dose modification of camrelizumab

<table>
<thead>
<tr>
<th>ADR</th>
<th>Description</th>
<th>Management</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pneumonia</td>
<td>Grade 2</td>
<td>Discontinue for short</td>
</tr>
<tr>
<td></td>
<td>Grade 3 or 4</td>
<td>Discontinue Permanently</td>
</tr>
</tbody>
</table>
| Diarrhea/enterocolitis   | Grade 2 or 3                                                                | Discontinue for short  
                          | Grade 4                                                                   |                   |
| Dermatitis               | Grade 3                                                                     | Discontinue for short  
                          | Grade 4                                                                   |                   |
| Hepatitis                | For patients with normal baseline ALT, AST, or TBIL, there appears Grade 2  | Discontinue for short  
                          | elevation of AST, ALT, or TBIL. For patients with baseline AST, ALT, or TBIL > ULN, AST, ALT, or TBIL elevates ≥50% and maintains < 7 days |                   |
                          | For patients with normal baseline ALT, AST, or TBIL, there appears Grade 3  | Discontinue Permanently |
                          | or 4 elevation of AST, ALT, or TBIL. For patients with baseline AST, ALT, or TBIL > ULN, AST, ALT, or TBIL elevates ≥50% and maintains ≥7 days |                   |
| Hypophysitis             | Grade 2                                                                     | Discontinue for short  
                          | Grade 3 or 4                                                              |                   |
| Adrenocortical           | Grade 2                                                                     | Discontinue for short  
                          | insufficiency                                                            |                   |
                          | Grade 3 or 4                                                               | Discontinue Permanently |

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Permanently

Hyperthyroidism
Grade 3 or 4
Discontinue
Permanently

Type 1 diabetes
Grade 3
Discontinue
for short \textsuperscript{b}
Grade 4
Discontinue
Permanently

Renal insufficiency
Grade 2 or 3 / elevation of Cr
Discontinue
for short \textsuperscript{a}
Grade 4 / elevation of Cr
Discontinue
Permanently

Neurotoxicity
Grade 2
Discontinue
for short \textsuperscript{a}
Grade 3 or 4
Discontinue
Permanently

Infusion reaction
Grade 3 or 4
Discontinue
Permanently

Other AE
Other Grade 3 AE appears for the first time
Discontinue
for short \textsuperscript{a}
The same grade 3 AE occurred for second time
Discontinue
Permanently
Grade 3 AE which cannot fall to level 0-2 / baseline within 7 days or recover to level 0-1 / baseline level within 14 days
Discontinue
Permanently
Grade 4 AE
Discontinue
Permanently \textsuperscript{c}

Note:
\textsuperscript{a}: Resuming dosing after symptom improvement to level 0-1 or baseline.
\textsuperscript{b}: Pituitaritis, adrenocortical insufficiency, and type 1 diabetes mellitus can be re-administered if they are adequately controlled and only physiologic hormone replacement therapy is required.
\textsuperscript{c}: In the case of abnormal grade 4 laboratory results, the decision to discontinue medication should be based on concomitant clinical symptoms/signs and the investigator's clinical judgment.

Table 2. Suggestions for the treatment of Camrelizumab infusion reaction

<table>
<thead>
<tr>
<th>CTCAE Grade</th>
<th>Dose Modification</th>
<th>Management</th>
</tr>
</thead>
</table>
| Any Grade   |                   | - Manage according to local clinical practice  
- Monitor patients’ infusion related reactions (fever or chills, flushing and/or itching, changes in heart rate and blood pressure, dyspnea, chest discomfort, rash, |
The infusion speed can be lowered by 50% or temporarily interrupted until the infusion reaction is relieved for Grade 1 or 2 AE:
- Acetaminophen and/or antihistamines will be administered at the investigator's discretion based on local clinical practice.
- Consider prophylactic administration prior to subsequent treatment according to local clinical practice.

For Grade 3 or 4:
- Manage severe infusion related reactions according to local clinical practice (epinephrine, diphenhydramine, ranitidine, and glucocorticoids).

### 6.2 Dose modification of nab-paclitaxel

If the patient's peripheral blood neutrophil count is lower than 1500/mm³ before treatment, this drug should not be given.

Dose reduction: patients with severe neutropenia (ANC<500/mm³ for 1 week or more) or severe sensory neurotoxicity during treatment should be treated with a dose reduction to 220mg/m² for subsequent courses. In the event mentioned above appears again, the subsequent treatment dose should be reduced to 180mg/m². For patients with Grade 3 sensory neurotoxicity, drug administration should be suspended, and the treatment can continue until the neurotoxicity recovered to ≤ Grade 2, and the dose should be reduced in subsequent treatment.

Table 3. Initial doses are recommended for patients with abnormal liver function

<table>
<thead>
<tr>
<th>level of AST(SGOT)</th>
<th>Level of Bilirubin</th>
<th>dose of Nab-paclitaxel</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤10×ULN</td>
<td>&gt;ULN to ≤1.5×ULN</td>
<td>260 mg/m²</td>
</tr>
<tr>
<td>≤10×ULN And ≥1.5×ULN to ≤3×ULN</td>
<td>220 mg/m²</td>
<td></td>
</tr>
<tr>
<td>≤10×ULN And ≥3×ULN to ≤5×ULN</td>
<td>180 mg/m²</td>
<td></td>
</tr>
<tr>
<td>&gt;10×ULN Or ≥5×ULN</td>
<td></td>
<td>not recommended</td>
</tr>
</tbody>
</table>

Note: The recommended dose is for the first course only. Dose adjustment requirements for subsequent courses should refer to individual tolerance.

If a patient can tolerate two courses of lower doses, an increased dose to 260 mg/m² may be considered in subsequent courses.
6.3 Dose modification of carboplatin

This product is for intravenous only in patients with normal renal function. According to Calvert formula in Martindale 35th edition: carboplatin dose (mg) = set AUC (mg/ mL /min) × [Ccr (mL /min) +25], the recommended dose is calculated according to AUC=5. Single dose intravenous infusion for over 15-60 minutes. 4 weeks between treatments and/or neutrophil count ≥ 2000/mm\(^3\); Platelet count ≥ 100000/mm\(^3\) is required for the next course of treatment. Patients with renal insufficiency had an increased risk of severe myelosuppression when Ccr was less than 60ml/min. The initial dose of carboplatin was adjusted according to Ccr (see Table 4), and the subsequent dose was adjusted according to patient tolerance and acceptable degree of bone marrow suppression.

<table>
<thead>
<tr>
<th>Baseline of CCr</th>
<th>Carboplatin initial Dose (Day 1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>41-59 ml/min</td>
<td>250mg/m(^2) iv</td>
</tr>
<tr>
<td>16-40 ml/min</td>
<td>Stop the medication, blood test once a week, continue the medication after CCr becomes normal</td>
</tr>
</tbody>
</table>

Detection of CCr is complex and is not frequently tested, but Ccr can be calculated through Scr.

Male:  
CCr (mL /min) = [(140 - age (year))× weight (Kg) ×1.23] ÷ SCr (μmol/ L);

Female:  
CCr (mL /min) = male (mL /min) ×0.85.

For patients with risk factors, such as a history of myelosuppression and poor general condition (ECOG 2-4), a reduction of 20% to 25% of the initial dose is recommended. Initial and subsequent doses should be adjusted for patients aged over 65 years according to their physical status. It is recommended that peripheral blood cell counts should be measured weekly during the first cycle of medication to determine the lowest point of cytopenia in order to adjust the dose for the next cycle.
Table 5. Dose modification for myelosuppression

<table>
<thead>
<tr>
<th>WBC</th>
<th>PLT</th>
<th>Dose of Carboplatin</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥4 × 10^9/L</td>
<td>120 × 10^9/L</td>
<td>100% of the recommended dose</td>
</tr>
<tr>
<td>(2.5-3.9) × 10^9/L</td>
<td>(75-119) × 10^9/L</td>
<td>50% of the recommended dose</td>
</tr>
<tr>
<td>&lt;2.5 × 10^9/L</td>
<td>&lt;75 × 10^9/L</td>
<td>Stop the medication, blood test once a week, continue the medication after it becomes normal</td>
</tr>
</tbody>
</table>

7. Concomitant medication

7.1 Drugs cannot be used during treatment

1) Other biotherapies (including but not limited to interferon, IL-2, thymosin, immunocell therapy, etc.) and other systemic chemotherapy are prohibited during treatment.
2) Immunotherapy not specified in this protocol is prohibited during treatment.
3) Live vaccine inoculation is prohibited within 28 days before and during drug administration.

7.2 Concomitant therapy during treatment

The conditions permit the use of concomitant drugs during the study are as follows:
1) When adverse reactions occur in the test, it should be strictly observed and treated. All concomitant drugs should be recorded with explanation on the CRF form.
2) When patients vomit due to chemotherapy, antiemetic agents can be given.
3) Neurotrophic agents, such as adenosine cobalamin and vitamin B, can be used when patients develop neurotoxicity.
4) When the patient has pain that affects quality of life, effective analgesic treatment should be given.
5) When the patient has constipation, diarrhea and other symptoms, symptomatic drugs can be given.
6) If severe myelosuppressive toxicity (Grade 3 or 4 toxicity) occurred during the medication, Granulocyte Colony-Stimulating Factor (G-CSF) and other treatment could be given.
7) Bisphosphonates should be used in patients with bone metastasis.
8) Calcium supplements are allowed for treatment.

8. Study process

8.1 Patients need to complete relevant examinations and evaluations. If the patient meets all inclusion
and exclusion criteria, they will be enrolled and sign the informed consent.

(1) Collection of demographic characteristic information.

(2) Information collection of present history, past history and physical examination. Present history and physical examination information should be collected within 28 days of the initial visit.

(4) Height, weight and score of ECOG status.

(5) Complete laboratory tests. All laboratory tests, including routine blood tests, blood biochemistry tests, liver and kidney function tests, and potential infection screening tests, should be completed within 14 days of the first chemotherapy to exclude contraindications related to chemotherapy and immunotherapy.

(6) Pre-treatment examination: Within 28 days of the initial treatment, patients should receive PET-CT examination or enhanced CT of neck, chest and upper abdomen, ECT, and brain MRI for accurate staging and set the baseline of treatment. This is part of standardized treatment. Before treatment, endoscopic ultrasound should be performed to evaluate the depth of invasion, and biopsy should be taken to confirm the pathological diagnosis. At the same time, peripheral blood, biopsy tissue, urine and feces specimens were collected for subsequent detection of immune therapy efficacy indicators. For women of childbearing age, \( \beta \)-HCG test should be performed to exclude pregnancy or early pregnancy.

8.2 Enrolled patients will receive 2 cycles of camrelizumab (200mg IV D1 Q3W) combined with carboplatin (AUC=5 IV D1 Q3W) and nab-paclitaxel (260mg/m2 IV D1 Q3W), and its safety was evaluated during the treatment.

8.3 After completing 2 cycles of camrelizumab combined with carboplatin and albumin-paclitaxel, the patient needs to return to the hospital for re-examination of physical examination, blood biochemical examination, liver and kidney function examination. PET/CT or neck, chest and upper abdomen CT scan needs to be performed again. Patients suspected of distant metastasis should be performed examination of the specific site to confirm the diagnosis. At the same time, relevant preoperative examination should be performed to exclude surgical contraindications. Efficacy was evaluated according to RECIST 1.1 criteria.
8.4 Surgical treatment: the surgical method adopted in this study was radical resection of esophageal cancer through three incisions of right chest, neck and upper abdomen (Mckeown). Surgery is performed within 3-6 weeks after completion of the second chemotherapy. The scope of lymph node dissection: lymph nodes in the thoracic and upper abdominal surgical fields. Surgical margin: the proximal and distal margin should be over 5cm beyond the edge of tumor. Pathology was used to determine the depth of tumor invasion, whether the resection margin contained tumor cells, and the proportion of tumor cells. This procedure is used to assess R0 resection, pathological response rate, and subsequent treatment decisions.

8.5 Camrelizumab maintenance therapy (200mg IV D1 Q3W) was started 4-8 weeks after surgery, and the maintenance therapy lasts a year after surgery. (Note: Maintenance therapy will not be carried out for serious infection or other conditions that are not suitable for maintenance therapy after operation.)

8.6 After treatment, regular follow-up of survival was performed every 3 months in the first year after surgery, and every 6 months in 2-5 years after surgery, including recurrence, survival and related treatment.

9. Adverse events

An adverse event refers to the occurrence or worsening of any clinical symptom, syndrome or disease that occurs during a clinical study and affects the health of the patient. Adverse events may be: new disease; worsening of symptoms or signs or worsening of concomitant disease; the influence of test methods or drugs; A combination of one or more factors.

Any adverse medical event that occurred between the time the patient signed the informed consent and was enrolled in the study and the last visit was considered an adverse event.

9.1 Adverse events include but are not limited to:
(1) All adverse drug reactions.
(2) Obviously unrelated diseases, including new diseases and exacerbations of pre-existing diseases.
(3) Injuries and accidents.
9.2 Criteria for the severity of adverse events

(1) Mild: tolerable to the patient, does not affect treatment or follow-up, does not require specific treatment, and has no impact on the rehabilitation of the patient.

(2) Moderate: unbearable to the patient, requiring specific treatment, which has a direct impact on the rehabilitation of the patient.

(3) Severe: life threatening, causing death or disability and requiring emergency treatment.

9.3 Recording and management of adverse events

(1) Record: If serious adverse events (SAE) occur during the trial, the investigator must report to the department responsible for clinical research and the ethics committee within 24 hours or no later than the second working day. The researcher must sign the signature and the date. When, how and to whom a serious adverse event was reported should be recorded in the original data. The main research institutes shall immediately notify all participating hospitals and ensure that all reporting procedures required by laws and regulations are implemented.

(3) Patient management: When adverse events are found, researchers can take necessary treatment according to the condition. All adverse events should be tracked and investigated, and the treatment process and results should be recorded in detail until they are properly resolved or the condition is stable. If the laboratory examination is abnormal, it should be tracked until returning to normal. Follow-up can be conducted in hospital, out-patient department, home visit, telephone, and other forms according to the severity of adverse events. Serious adverse events (including abnormal laboratory tests) that were not resolved at the end of the study or the time the patient dropped out early must be followed up to any of the following conditions: 1) event resolved 2) event stabilized 3) event return to baseline 4) it has been determined that the study treatment or participation is not the cause 5) When additional information is not available (patient refuses to provide additional information or remains lost to follow-up)

Some events requiring hospitalization or prolonged hospitalization may not be considered as
serious adverse events, including hospitalization for reasons other than adverse event, and hospitalization for surgical or other purposes scheduled prior to the study.

9.4 Serious adverse events

A serious adverse event is an unexpected medical event occurring during the study period that results in death, life-threatening, hospitalization or prolonged hospitalization, persistent or severe disability, congenital abnormalities/defects and other serious events.

After entering the study, if the patient has serious adverse events, in addition to treatment or rescue, the patient should inform the leader of clinical study, clinical supervisor and ethics committee by telephone/fax within 24 hours after being informed. For all serious adverse events, the investigator should immediately take adequate measure and draft a detailed report of the serious adverse event to be submitted to the relevant administrative authorities and the ethics committee. In case of death related to treatment, the clinical trial of this group should be stopped immediately and report to the ethics committee of the clinical research institution as soon as possible. Keep detailed records and proper storage of relevant information.

9.5 Safety Evaluation

It is mainly the observation and evaluation of adverse events during the study, and recorded in detail in the CRF in time.

9.6 Management of tumor recurrence and metastasis

Patients with tumor recurrence and metastasis during follow-up will be recorded in detail in the CRF and treated according to the current clinical pathway.
10. Others

10.1 Case Report

During the study, all patients were required to fill in the case report form according to the study schedule and requirements.

10.2 Ethical Requirements

Before the initiation of clinical trial, the protocol shall be signed by the investigator, and the trial protocol shall be reviewed and approved by the ethics committee before it can be implemented. During the period of the trial, if problems occur in the actual implementation of the clinical trial and the plan needs to be revised, the revised trial protocol shall be approved by the ethics committee again before it can be implemented. Any serious adverse events and deaths that occur during the trial should be reported by the investigator to the ethics committee. The ethics committee should be informed at the end of the study.

10.3 Quality control

Investigators should adopt standard operating procedures to ensure the quality control of clinical trials and the implementation of quality assurance systems. All observations and findings in clinical trials should be verified to ensure the reliability of data and to ensure that conclusions in clinical trials are derived from original data. Quality control must be applied at every stage of data processing to ensure that all data are reliable and processed correctly.

10.4 Training of researchers

Prior to the initiation of clinical trials, the investigator shall be trained by the trial protocol so that the investigator can understand and be familiar with the nature, procedure, function and safety of the trial.
10.5 Improve the compliance of patients

1) The researcher should carefully implement informed consent so that the patients can fully understand the requirements and cooperate with the investigator.

2) Follow up regularly to monitor the compliance of patients. Follow-up should be strengthened for those with poor compliance.

10.6 Storage and summary of data

10.6.1 Data Preservation

All data were stored for five years after the termination of the clinical trial.

10.6.2 Confidentiality

All information related to this study (including but not limited to the following documents: study protocol, Investigator's Brochure and summary report) must be kept strictly confidential. Information related to the study or conclusions drawn from the study may be published by the investigator only with the written consent of the project leader. The researcher should send the paper, abstract, or poster intended for publication or academic lecture to the project leader, who will give a reply within 1 month.
11. Attachments


Annex 2: ECOG PS Scoring Criteria

Annex 3: Response Evaluation Criteria in Solid Tumors-version 1.1 (RECIST V1.1)

Annex 4: Evaluation criteria for Common Terminology Criteria for Adverse Events 5.0 (CTCAE V 5.0)

Annex 5: NCCN Guidelines for the Management of Immunotherapy-associated Toxicities (2019 V1.0)

Annex 6: List of pre-inclusion autoimmune diseases

Annex 7: Guidelines for dose adjustment and toxicity management of camrelizumab

Annex 8: Quality of Life Scale of EORTC QLQ-C30 and EORTC QLQ-OES18

Signature of principal investigator:
Date: