Neoadjuvant chemoradiotherapy combined with sequential perioperative toripalimab in locally advanced esophageal squamous cell cancer

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ABSTRACT

Background Programmed death 1 (PD-1) inhibitor demonstrated durable antitumor activity in advanced esophageal squamous cell carcinoma (ESCC), but the clinical benefit of perioperative immunotherapy in ESCC remains unclear. This study evaluated the efficacy and safety of neoadjuvant chemoradiotherapy (nCRT) combined with the PD-1 inhibitor toripalimab in patients with resectable ESCC.

Methods From July 2020 to July 2022, 21 patients with histopathologically confirmed thoracic ESCC and clinical staged as CT1-4aN1-2M0/cT3-4aNOM0 were enrolled. Eligible patients received radiotherapy (23 fractions of 1.8 Gy, 5 fractions a week) with concurrent chemotherapy of paclitaxel/cisplatin (paclitaxel 45 mg/m² and cisplatin 25 mg/m²) on days 1, 8, 15, 22, 29 and two cycles of toripalimab 240 mg every 3 weeks after nCRT for neoadjuvant therapy before surgery, four cycles of toripalimab 240 mg every 3 weeks for adjuvant therapy after surgery. The primary endpoint was the major pathological response (MPR) rate. The secondary endpoints were safety and survival outcomes.

Results A total of 21 patients were included, of whom 20 patients underwent surgery. One patient refused surgery and another patient was confirmed adenocarcinoma after surgery. The MPR and pathological complete response (pCR) rates were 78.9% (15/19) and 47.4% (9/19) for surgery ESCC patients. 21 patients (100.0%) had any-grade treatment-related adverse events, with the most common being lymphopenia (100.0%), leukopenia (85.7%), neutropenia (52.4%), and adverse events of grade 3 with the most common being lymphopenia (66.7%). The maximum standardized uptake value and total lesion glycolysis of positron emission tomography/CT after neoadjuvant therapy well predicted the pathological response. The peripheral CD4+, CD3+CD4, CD3+HLA-DR+, CD3+CD8+, CD6+HLA-DR+, CD8+, and IL-6 were significant differences between pCR and non-pCR groups at different times during neoadjuvant therapy. Three patients had tumor relapse after surgery with no evidence of disease in the entire body. The conclusion is that nCRT combined with perioperative toripalimab is effective and safe for locally advanced resectable ESCC. Long-term survival outcomes remain to be determined.

Trial registration number NCT04437212.

INTRODUCTION

Esophageal cancer (EC) ranks as the ninth most prevalent cancer globally and the sixth-leading cause of cancer-related mortality. The prevalence of EC is particularly significant in China, accounting for almost half of all cases worldwide. Recent statistics reveal approximately 346,633 new cases and 323,600 deaths from EC in China annually, placing it fourth in terms of major tumor-related deaths in the country. Esophageal squamous cell carcinoma (ESCC), a more malignant pathological type, accounts for over 85% of EC cases in China. Approximately 70% of EC cases are diagnosed at advanced stages, resulting in a poor prognosis. The standard treatment for locally advanced operable EC, particularly ESCC, involves surgery following neoadjuvant chemoradiotherapy (nCRT), which results in a pathological complete response (pCR) rate of approximately 30%–40%. Nevertheless,
there is a low 5-year overall survival (OS) rate, with nearly half of the patients experiencing disease progression. Additionally, 15% of pCR patients still experience disease progression postsurgery. Therefore, it is crucial to establish novel treatment strategies for locally advanced operable ESCC that can enhance the pCR rate of nCRT and reduce the risk of recurrence.

Toripalimab is an immune checkpoint inhibitor (ICI) targeting programmed cell death protein 1 (PD-1). The safety and efficacy of toripalimab in the treatment of ESCC have been previously demonstrated. In a recent phase III study (JUPITER-06), the combination of first-line toripalimab and chemotherapy was found to significantly enhance progression-free survival and OS in the treatment of advanced and metastatic ESCCs. While previous findings have established the safety and efficacy of anti-PD-1 immunotherapy for unresectable advanced EC, its applicability to patients with resectable EC remains uncertain. PD-1 antibodies have been evaluated in several small sample trials as part of neoadjuvant therapy. However, in most of these trials, PD-1 antibodies were used in combination with chemotherapy rather than CRT as a neoadjuvant strategy. Moreover, the CheckMate 577 trial indicated that nivolumab in adjuvant therapy improves disease-free survival (DFS) after operation. However, to date, there has been no conclusive evidence of perioperative immunotherapy combined with nCRT in ESCC patients.

Therefore, this study aims to examine the efficacy and safety of the combination of nCRT and perioperative toripalimab in patients with resectable ESCC.

METHODS

Study design and participants
This was a single-center, single-arm phase Ib study of perioperative toripalimab in combination with nCRT and esophagectomy for patients with ESCC. This study was exploratory and adopted a fixed sample size of 20 patients as required by the ethics committee. Written informed consents were provided by all patients. The study protocol could be checked in online supplemental file 2. Eligible patients were 18–75 years old and had histologically or cytologically confirmed locally advanced resectable thoracic ESCC, stageT1-4aN1-2M0 or T3-4aN0M0 (stages II–IVA) according to the American Joint Committee on Cancer staging manual eighth edition. Patients were required to have an Eastern Cooperative Oncology Group performance status of 0 or 1 and adequate organ and bone marrow function. Exclusion criteria were the presence of active infection; interstitial lung disease or non-infectious pneumonia; a history of autoimmune diseases or abnormal immune system; severe cardiac, lung dysfunction cannot tolerate CRT or surgery; any other malignant tumor within 5 years before enrolment; allergy to any study drug components; a history of immunodeficiency; and women during pregnancy or lactation.

Pretreatment staging
All patients underwent medical history taking, physical examination, routine laboratory tests, echocardiography, cardiac ultrasound and pulmonary function test. Pretreatment tumor staging was performed through contrast-enhanced chest CT, esophagogastroduodenoscopy diagnostic biopsy with endoscopic ultrasound, esophageal barium imaging, MRI of the esophagus, and positron emission tomography-computed tomography (PET-CT).

Neoadjuvant treatment and outcome measurements
Neoadjuvant radiotherapy was given by means of intensity-modulated radiotherapy. A total dose of 41.4 Gy radiation was given by 23 fractions with 1.8 Gy per fraction and five fractions per week. The chemotherapy regimen included paclitaxel (45 mg/m²) and cisplatin (25 mg/m²), which were administrated weekly on days 1, 8, 15, 22 and 29. Two cycles of toripalimab 240 mg were given intravenously every 3 weeks after completion of CRT. The first cycle of toripalimab was administrated within 1–3 days after completion of radiotherapy. 3 weeks after the administration of the second cycle of immunotherapy, disease reevaluation was performed, including physical examination, routine laboratory test, echocardiography; pulmonary function test, contrast-enhanced chest CT; MRI, and PET-CT, to restage and exclude cases not suitable for surgery.

Surgery and adjuvant treatment
Surgery was scheduled for 4–6 weeks after the second treatment cycle of toripalimab. A minimal invasive McKeown esophagectomy was performed, including two-field lymphadenectomy or three-field lymphadenectomy in individual patients by a single experienced surgeon. Adjunct treatment should be started 4–12 weeks after surgery. Toripalimab 240 mg was given every 3 weeks in four cycles for adjuvant treatment.

Outcomes
The primary endpoint was the major pathological response (MPR) rate in the primary tumor, and the secondary endpoints were DFS, OS, the incidence of treatment-related adverse events (TRAEs) as assessed by CTCAE V.5.0, and postsurgical complications. The pathological examination should report the tumor extension, lymph node status, resection margins, and tumor regression grade (TRG). The TRGs of the primary tumor were described using a previously reported method as follows: grade 1, no evidence of vital residual tumor cells; grade 2, 10% or fewer vital residual tumor cells; grade 3, 11%–50%; and grade 4, more than 50%. pCR was defined as the absence of viable tumor cells in the resected cancer specimen. MPR was defined as less than 10% vital residual tumor cells (TRG1 and TRG2). The DFS was calculated from the date of surgery and the OS was dated from the day of enrolment. The recurrence, survival outcomes, and TRAEs were followed up for 5 years after the end of surgery.
treatment. The prespecified exploratory endpoint was the investigation of response biomarkers.

Procedures for biomarker assessments
PD-L1 expression was assessed on pretreatment formalin-fixed paraffin-embedded sections using an immunohistochemistry (IHC) assay (PD-L1 IHC 22C3). PD-L1 expression was evaluated by tumor proportion score (TPS) and PD-L1 expression positive was defined as TPS≥1%. Pretreatment biopsy and surgically resected specimens were used to assess tumour-infiltrating CD8+ lymphocytes by IHC. Flow cytometry was used to measure peripheral lymphocyte subsets consisting of T cells (CD3+ T cells, CD3+CD4+ T cells, CD3+CD8+ T cells), regulatory T cells (CD3+CD25+CD127-Treg cells), natural killer (NK) cells (CD16+CD56+ NK cells), B cells (CD19+B cells), and activation antigens (CD3+HLA-DR+/CD3+(%),CD8+CD38+/ CD8+(%), CD8+HLA-DR+/CD8+(%), CD4+HLA-DR+/ CD4+(%), CD4+CD38+/CD4+(%)), before and after nCRT, after one cycle of toripalimab, and before surgery. The cytokines including IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12P70, IL-17A, TNF-a, IFN-α, and IFN-γ were also measured by flow cytometry at the same times.

Statistical analysis
Statistical analyses were performed by using SPSS V.22.0 software (SPSS) and R V.3.4.4 software (Institute for Statistics and Mathematics, Vienna, Austria). Categorical variables were presented as the frequency with percentage and compared using Pearson’s χ² or Fisher’s exact tests. Continuous variables were presented as median with range or mean with SD and compared by t-test or Mann-Whitney U test. Survival analysis was performed by the Kaplan-Meier method, followed by log-rank tests. DFS is defined as survival time without disease progression from surgery till the date of last follow-up July 31, 2023. P values were two sided, with a significance level of 0.05 for all analyses.

RESULTS
Patients’ characteristics and disposition
Twenty-one patients were enrolled from July 2020 to July 2022 in the study. The demographic and baseline characteristics of the patients are listed in table 1. The included patients had a median age of 62 years old (range, 31–74 years old), and the majority of patients were male (95.2%). Most of the patients were cT3 (90.5%), cN2 (57.1%) stage, and located in the middle or lower esophagus (81.0%). Most of the tumors were cT3 (90.5%), cN2 (57.1%) stage, and located in the middle or lower esophagus (81.0%). Most of the patients were classified as having clinical stage III tumors (95.2%).

All 21 patients completed a total dose of 41.4 Gy/23 Fx neoadjuvant radiation. Sixteen patients (76.2%) completed the planned five cycles of weekly intravenous chemotherapy, whereas four patients completed four cycles and one patient completed three cycles. The most common reason for the insufficiency of chemotherapy cycles is neutropenia. All the patients received neoadjuvant toripalimab, 19 patients completed 2 cycles while 2 patients received only 1 cycle. Twenty out of 21 patients underwent surgery and 1 patient refused surgery. Although all the patients were confirmed ESCC by endoscopy biopsy at baseline, one patient was confirmed adenocarcinoma by surgery and was excluded from the efficacy analysis. Sixteen out of 19 patients received adjuvant toripalimab, 2 patients refused adjuvant treatment and 1 patient did not receive adjuvant treatment because of an anastomotic fistula. Twelve patients completed four cycles of adjuvant toripalimab. For the reason of the COVID-19 epidemic, two patients received three cycles, one patient received two cycles and one patient received only one cycle of toripalimab (online supplemental figure S1).

Efficacy
R0 resection was achieved in all the patients, and the mean number of lymph node resections was 26 (range: 14–52). MPR of primary tumors was observed in 15 patients (15/19, 78.9%) (figure 1A). 9 patients (9/19, 47.4%)
achieved pCR in primary tumors and lymph nodes, while 2 patients (2/19, 10.5%) were pCR in the esophagus but had residual tumor cells in lymph nodes. The pCR rate of the primary lesion was 57.9% (11/19). The pathological downstaging of the TNM stage occurred in 15 (78.9%) patients, 16 patients downstaged in the T stage, and 16 patients downstaged in the N stage (figure 1B).

As of the data cut-off, the median follow-up time was 23.4 months (range: 12.1–37.0 months) from enrolment, and 3 patients (15.8%) experienced progression of disease (DFS after surgery were 7, 11, and 13.5 months). Their pathological responses were relatively poor, with 70%, 80%, and more than 90% residual tumor cells, respectively. Two of these three patients did not receive adjuvant therapy after surgery (figure 2A). One patient had a mediastinal recurrence, one patient experienced supraclavicular and axillary lymph node recurrence, and one patient had lung metastases. The 2-year DFS rate and median DFS had not been reached yet. The non-MPR patients had much lower DFS than MPR patients, which was 12.2 months (p = 0.0002, figure 2B). The non-pCR patients also had lower DFS than pCR patients but the difference was not statistically significant (p = 0.08, figure 2C). A patient died of disease progression and the OS was 23.6 months.

Safety

During the treatment period, all 21 patients developed TRAEs of any grade. The TRAEs are summarized in table 2. The most common TRAEs were lymphopenia (21/21, 100%), leukopenia (18/21, 85.7%), neutropenia (11/21, 52.4%), esophagitis (8/21, 38.1%), and nausea (5/21, 23.8%). Most TRAEs were grades 1–2 and grade 3 TRAEs were observed in 15 of the 21 patients (71.4%). The most frequent grade 3 TRAE was lymphopenia (14/21, 66.7%). None of the patients experienced grades 4 and 5 TRAEs. Six patients (28.6%) experienced immune-related adverse events (irAEs). The most common irAEs included hypothyroidism (9.5%), diarrhea (9.5%), aminotransferase increased (9.5%), pneumonia (4.8%), nausea (4.8%), and fever (4.8%). All the irAEs were grades 1–2, except one patient developed grade 3 amino transaminase elevation. They all recovered without severe sequelae. All 20 patients received McKeown minimal invasive esophagectomy and no intraoperative conversion to open surgery. The median interval between the completion of nCRT and surgery was 56 days (range 48–104 days) and the median interval between the second administration of neoadjuvant toripalimab and surgery was 35 days (range 27–89 days). There were no treatment-related surgeries delay, but two patients’ surgeries were postponed because of the COVID-19 epidemic. The post-operative complications are summarized in table 3. The most common surgical complications were anastomotic leakage (15.0%), arrhythmia (15.0%), and pneumonia (10.0%). No treatment-related mortality occurred.

Positron emission tomography-CT

All the patients received PET-CT scans at baseline and after neoadjuvant therapy. No patient had a new lesion on the PET-CT scan after neoadjuvant therapy. One patient did not receive the PET-CT examination in our hospital, so the analysis of PET-CT parameters was performed on 18 patients. The method of evaluation of PET-CT parameters were described in online supplemental method. We found that the maximum standardized uptake value (SUVmax) and total lesion glycolysis (TLG) of the PET/CT after neoadjuvant therapy could well detect residual disease in the esophagus, the sensitivity and specificity of SUVmax for predicting pCR of esophagus were 71.4% and 100%, and those of TLG were 71.4% and 90.2%. We also found that the MTV and TLG in baseline PET/CT were significantly higher than in non-MPR patients compared with MPR patients (online supplemental table S1 and figure S2).

Biomarker analyses

The tumor tissues of all 19 patients before treatment were tested the PD-L1 expression. The TPS of PD-L1 ranges from 0 to 80%. There were 9 patients with PD-L1 negative or TPS<1%, 8 patients with TPS 1%–49%, and 2 patients with TPS≥50%. Baseline PD-L1 TPS did not have an obvious correlation with pathologic response...
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IHC was performed on 19 paired samples to test the CD8+T cells. The proportion of CD8+ cells increased in MPR patients (p=0.003) but was not different in non-MPR patients between baseline and after neoadjuvant treatment (p=0.71) (figure 3C). And the similar difference was observed in pCR patients versus those without pCR (online supplemental figure S3). The densities of CD8 cells did not differ between pCR and non-pCR or between MPR and non-MPR in both tumorous and stromal areas of the baseline specimens. On the basis of the cut-off value of 75 cells/mm², a high tumorous CD8 cell density was correlated with a relatively high pCR, 80% (4/5) vs 50% (7/14), but the difference was also not statistically significant (p=0.24). The CD8+ cells density increased in MPR patients but there was no difference in non-MPR patients. When stratified by pCR, the difference in CD8 cell density before and after neoadjuvant treatment was not statistically significant in both pCR and non-pCR groups (online supplemental figure S3).

To investigate the effect of systemic immune status on the efficacy of neoadjuvant therapy in combination with immunotherapy, we examined immune cells and cytokines in peripheral blood. After nCRT, the total number of lymphocytes decreased dramatically with the number...
of every subset decreased, and after the neoadjuvant ICI, the number of lymphocytes and all the subsets increased (online supplemental figure S4). However, the proportion of subpopulations changed differently. After CRT, the proportion of B lymphocytes decreased significantly, while due to the increase of CD4+T cells, the percentage of T cells increased (online supplemental figure S5). The changes in proportions of CD8+T cells, NK cells and regulatory T cells (Treg) were not significant. Compared with non-pCR patients, the pCR patients had higher CD4+% both before and after CR (figure 4A). The proportion of HLA-DR+activated T cells (CD3+HLA-DR+/CD3+) was decreased after CR, while the percentage of CD38+activated CD8+ T cell (CD8+CD38+/CD8+) was significantly increased (online supplemental figure S5). The ratios of CD3+HLA-DR+/CD3+ and CD8+ HLA-DR+/CD8+ were much lower in PCR patients compared with non-pCR patients and the CD8+CD38+/CD8+ was not different between these two groups both before and after CRT (figure 4B,C). After subsequent immunotherapy, these changes mostly exhibit opposite trends. The numbers of all lymphocyte subpopulations significantly increased compared with the levels after CRT, especially for CD8+cells, the number exceeded the levels before nCRT (online supplemental figure S4). The CD4+cell proportion dropped notably, on the contrary, the proportion of CD8+cells significantly increased, resulting in a remarkable decrease in the proportion of CD4+/CD8+ (online supplemental figure S5). Both CD3+HLA-DR+/CD3+, CD8+CD38+/CD8+ and CD8+HLA-DR+/CD8+ substantially rose and were significantly higher than the baseline level (online supplemental figure S5) but were similar between patients with different responses. After nCRT, we observed the levels of IL-1β, IL-4 and IL-17A exerted a decline and the levels of IL-2 and IL-6 increased (online supplemental figure S6). The IL-6 level suffered a dramatic decline after subsequent immunotherapy which is more substantial in pCR patients (figure 4D,E). Other cytokines did not change statistically significantly after immunotherapy.

**DISCUSSION**

To our knowledge, this is the first prospective clinical trial to assess the feasibility and efficacy of sequential perioperative PD-1 inhibitor toripalimab combined with nCRT in patients with resectable thoracic ESCC. The primary

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**Table 2** Treatment-related adverse events (TRAEs) (n=21)

<table>
<thead>
<tr>
<th>Events</th>
<th>Grades 1–2 (no (%)</th>
<th>Grade 3 (no %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRAE during nCRT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymphopenia</td>
<td>7 (33.3%)</td>
<td>14 (66.7%)</td>
</tr>
<tr>
<td>Leukopenia</td>
<td>13 (61.9%)</td>
<td>5 (23.8%)</td>
</tr>
<tr>
<td>Neutropenia</td>
<td>8 (38.1%)</td>
<td>3 (14.3%)</td>
</tr>
<tr>
<td>Esophagitis</td>
<td>8 (38.1%)</td>
<td>0</td>
</tr>
<tr>
<td>Anemia</td>
<td>4 (19.0%)</td>
<td>0</td>
</tr>
<tr>
<td>Nausea</td>
<td>5 (23.8%)</td>
<td>0</td>
</tr>
<tr>
<td>Dermatitis</td>
<td>2 (9.6%)</td>
<td>0</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>1 (4.8%)</td>
<td>0</td>
</tr>
</tbody>
</table>

**Table 3** Surgical and pathological outcomes of patients who underwent surgery

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>No (%) or median (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>R0 resection</td>
<td>20 (100%)</td>
</tr>
<tr>
<td>Surgical approach</td>
<td></td>
</tr>
<tr>
<td>MIE</td>
<td>20 (100%)</td>
</tr>
<tr>
<td>OE</td>
<td>0</td>
</tr>
<tr>
<td>Pathological response</td>
<td></td>
</tr>
<tr>
<td>MPR</td>
<td>15 (78.9%)</td>
</tr>
<tr>
<td>PCR</td>
<td>9 (47.4%)</td>
</tr>
<tr>
<td>yp T0</td>
<td>11 (57.9%)</td>
</tr>
<tr>
<td>yp N0</td>
<td>13 (68.4%)</td>
</tr>
<tr>
<td>Downstating of T stage</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>16 (84.2%)</td>
</tr>
<tr>
<td>No</td>
<td>0</td>
</tr>
<tr>
<td>Downstating of N stage</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>16 (84.2%)</td>
</tr>
<tr>
<td>No</td>
<td>0</td>
</tr>
<tr>
<td>Downstating of TNM stage</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>15 (78.9%)</td>
</tr>
<tr>
<td>No</td>
<td>4 (21.1%)</td>
</tr>
<tr>
<td>No of resected lymph nodes</td>
<td>26 (14–52)</td>
</tr>
<tr>
<td>No of positive lymph nodes</td>
<td>2 (1–6)</td>
</tr>
<tr>
<td>Surgical complications</td>
<td></td>
</tr>
<tr>
<td>Anastomotic leakage</td>
<td>3 (15.0%)</td>
</tr>
<tr>
<td>Arrhythmia</td>
<td>3 (15.0%)</td>
</tr>
<tr>
<td>Pneumonia</td>
<td>2 (10.0%)</td>
</tr>
<tr>
<td>Vocal cord paralysis</td>
<td>2 (10.0%)</td>
</tr>
<tr>
<td>Cervical chylous leakage</td>
<td>1 (5.0%)</td>
</tr>
</tbody>
</table>

The surgical outcome and complications were evaluated in 20 patients and pathological outcomes were evaluated in 19 patients. MIE, minimal invasive esophagectomy; MPR, major pathological response; OE, open esophagectomy; PCR, pathological completed response; TNM, tumor, node and metastasis.
endpoint MPR was observed in 15 of 19 (78.9%) patients and 47.4% (9/19) patients achieved pCR. Moreover, no new safety signals were identified in this regimen and the safety profile was well tolerated, indicating that this triplet neoadjuvant pattern was safe and effective.

In our study, immunotherapy was administered sequentially rather than concurrently with the nCRT. The sequential administration of ICIs after CRT was proven to improve the effect of CRT in non-small cell lung cancer by the PACIFIC study. Consequently, the PACIFIC mode was attempted in various tumors when combining immunotherapy with radiotherapy since it appears to be safer than the concurrent administration of radiotherapy and ICIs. A systematic review of the toxicity of combined ICs and thoracic radiotherapy in EC revealed that concurrent treatment might lead to a higher incidence of any-grade TRAEs compared with sequential treatment. Both the PALACE-1 study and the NEOCRTEC1901 study reported...

Figure 3  IHC analysis of baseline PD-L1 expression and CD8+ cell before and after neoadjuvant therapy. (A) Correlation between baseline PD-L1 TPS expression and pCR. (B) Correlation between baseline PD-L1 TPS expression and MPR. (C) Percentages of CD8+ cells before and after neoadjuvant therapy in MPR patients and non-MPR patients. (D) Representative IHC images of two patients illustrating the higher number of CD8+T cell infiltrates after neoadjuvant therapy in MPR patients and no change in non-MPR patients. IHC, immunohistochemistry; MPR, major pathological response; pCR, pathological complete response; TPS, tumor proportion score.

Figure 4  Lymphocyte subsets and cytokines between different pathological responses. (A–C) CD4+, CD3+HLA-DR+/CD3+ and CD8+ HLA-DR+/CD8+ between pCR and non-pCR patients before nCRT, after nCRT and after neoadjuvant immunotherapy. (D, E) Changes of IL-6 level after nCRT and neoadjuvant immunotherapy in pCR patients (D) and non-pCR patients (E). postCRT, after neoadjuvant chemoradiotherapy; postICI, after immune checkpoint inhibitor; preCRT, before neoadjuvant CRT.
one patient developed a grade 5 esophageal hemorrhage after neoadjuvant treatment.7,17 Another trial, the EC-CRT-001 trial, reported esophageal fistulae in 14% of locally advanced ESCC patients who received concurrent anti-PD-1 antibody and definitive CRT.18 However, in our present study, we observed no TRAEs above grade 3, with lymphopenia being the most common grade 3 adverse event. This adverse event did not raise concerns and was not reported in most studies. A similar incidence of grade 3 AEs, especially lymphopenia, was observed in the PALACE-1 study, where all the patients experienced some degree of lymphopenia.7 Given the potential impact of lymphopenia on the efficacy of immunotherapy, we specifically focused on this TRAE during nCRT. However, we found no difference in lymphocyte counts between pCR and non-pCR patients, which aligns with previous findings in our nCRT cohort.19 The immune-related AEs were manageable, with 28.6% of patients experiencing irAEs during neoadjuvant and adjuvant immunotherapy, predominantly grades 1–2. These irAEs were consistent with those reported in previous studies.

We have not observed any increase in postoperative complications with this neoadjuvant therapy mode in our study. The main postoperative complications include anastomotic fistula, pneumonia, and arrhythmia. Anastomotic leakage occurred in 15% (3/20) of patients postoperations, a rate similar to that reported in some previous studies.10,11,17 Two patients were successfully treated with fasting and parenteral nutrition therapy, while one patient required esophageal stenting. Postoperative arrhythmia was observed in three patients, which was the only distinguishing postoperative complication between the nCRT group and the surgery group in the NEOCRTEC 5010 study.20 However, the arrhythmia in these patients was temporary and did not necessitate long-term medication. The incidence of postoperative pulmonary complications is also similar to previous studies. This demonstrates that this treatment mode has good short-term safety, while long-term safety requires further follow-up.

Our study reported an encouraging efficacy, with 78.9% of patients achieving MPR and 47.4% of patients achieving pCR. The MPR and pCR rates in our study were higher than that reported in some previous nCRT studies (69.1% and 33.3% in FFCD 9901,21 61% and 29% in CROSS,14 63.4% and 27.7% in CMISG1701,22 43.2% pCR in NEOCRTEC 501020) and the combination of chemotherapy and immunotherapy (68.6% and 39.2% in NICE,10 58.8% and 31.4% in NIC-ESCC2019,19 50% and 25% in GASTO1056,11 50% and 20% in KEEPGO3).23 Combining our results with the PALACE-1 and NEOCRTEC 190117 studies, it appears that nCRT combined with immunotherapy has a superior pathological response. This could be attributed to the synergistic effect of CRT and immunotherapy, whereby the release of tumor neoantigens induced by CRT enhances adaptive immunity. Additionally, radiotherapy, as a local treatment, contributes to the reduction of primary tumors. Nevertheless, in two studies comparing this triple neoadjuvant therapy with propensity score-matched nCRT cohort, although the combination therapy showed a higher pCR rate, it did not reach statistical significance compared with the nCRT group.8,17 Another systematic review and network meta-analysis demonstrated that there were no significant differences between NICRT and NCRT with regard to pCR or mPR, and both were superior to NICRT.24 Further randomized controlled studies are required to validate whether the tricombination neoadjuvant therapy approach yields a superior pathological response compared with standard nCRT. The ultimate objective of improving pCR is to provide survival benefits to patients. While pCR patients have demonstrated significantly better survival outcomes than non-pCR patients in nCRT studies, several studies comparing nCRT with neoadjuvant chemotherapy have shown that although nCRT significantly improves pCR, it does not improve survival.22,25,26 Consequently, further investigation is necessary to evaluate the survival outcomes of novel neoadjuvant approaches.

Based on current evidence and guidelines, it appears that four cycles of adjuvant immunotherapy may not be adequate for patients who have not achieved pCR after neoadjuvant therapy. However, our study was initiated prior to the publication of results from the Checkmate 577 study. At that time, the NCCN guideline recommended postoperative observation following R0 resection for patients who underwent nCRT. Our research design was based on a study presented at the 2019 American Society of Clinical Oncology annual meeting, NCT03490292, where avelumab was administered at a dose of 10 mg/kg every 2 weeks for three cycles after nCRT before surgery and for six cycles after surgery.27 The tolerability of this treatment regimen was favorable, with no grade 3 or higher irAEs reported. In our study, three non-pCR patients experienced disease progression after surgery, two of them without adjuvant treatment, which indicated that adjuvant therapy was very necessary for non-pCR patients. However, despite the use of only four cycles of adjuvant toripalimab, among the seven non-pCR patients who received adjuvant treatment, with median follow-up time 24.3 months, no patients experienced recurrence within 1 year after surgery, and only one patient experienced recurrence within 2 years after surgery. Although the sample size is small and the follow-up time is short, the recurrence and survival outcomes we observed do not appear inferior to those of the previous. Currently, there is a lack of consistent regimen and treatment cycles for adjuvant therapy in various studies on neoadjuvant immunotherapy for EC. Even for adjuvant immunotherapy after nCRT and surgery, there are studies with inconsistent conclusions.28 Therefore, further research is needed to explore the optimal adjuvant therapy for patients who have undergone neoadjuvant immunotherapy and surgery.

To date, some studies have suggested a correlation between PD-L1 expression and the efficacy of PD-1 inhibitors combined with chemotherapy in advanced and
metastatic EC. However, other studies did not show this association. In previous studies involving neoadjuvant chemotherapy or nCRT plus ICIs, PD-L1 status did not show a correlation with the pathological response in ESCC patients. Our recent study also demonstrated that baseline PD-L1 TPS did not exhibit a significant correlation with the pathologic response. Additionally, we assessed the CD8+ T cell in tumor microenvironment as an indicator of the response of immunotherapy. We observed a significant increase in CD8+ T cells after nCRT and two doses of toripalimab, particularly in the patients who achieved MPR. To identify other indicators of response, we monitored the dynamic changes of peripheral blood immunocytes during neoadjuvant therapy. We found that both nCRT and immunotherapy induced significant changes in peripheral blood immunocytes. Interestingly, the effects of these treatments were opposite or complementary. The number of various lymphocyte subsets decreased significantly after nCRT but increased after immunotherapy. These increases were influenced by the cessation of nCRT effects on one hand, and the impact of immunotherapy on the other hand, particularly leading to a substantial increase in the number of CD8+ T cells. While nCRT caused an increase in the proportion of CD4+ T cells, immunotherapy induced an increase in the percentage of CD8+ T cells but a decrease in the proportion of CD4+ T cells. Both nCRT and the immunotherapy activated lymphocytes, with nCRT contributing to the elevation of CD8+CD38+/CD8+ and the ICI multiplying the CD3+HLA-DR+/CD3+, CD8+CD38+/CD8+, and CD8+HLA-DR+/CD8+. Higher CD4+/CD8+ ratios, along with lower CD3+HLA-DR+/CD3+ and CD8+HLA-DR+/CD8+, were favorable for the pathological response. These findings were consistent with that of some previous studies. Nevertheless, these preliminary results still require confirmation in a larger population. Furthermore, it is important to thoroughly analyze the consistency of the immune cell subset compositions between peripheral blood and tumor microenvironment, as well as the specific status and function of T cell subsets.

The present trial has several limitations. First, it was a small sample size, single-center and single-arm study, which may limit the generalizability of our findings. And due to the small sample, the statistic power of p value between subgroups is limited. Second, the completion rate of the adjuvant treatment in the study was low, with approximately one-third of patients not completing the adjuvant ICIs. Additionally, a longer follow-up period is necessary to confirm the long-term safety and efficacy of the treatment.

In conclusion, nCRT combined with sequential perioperative toripalimab is both safe and feasible. It has also demonstrated favorable antitumor efficacy, as indicated by promising rates of MPR and pCR. Larger studies are needed to validate the efficacy and the safety of the novel neoadjuvant therapy regimen, and to determine the best predictive biomarkers of response, and long-term outcome.

**Outcomes**

**Acknowledgements** The authors wish to thank Shanghai Junshi Biosciences for providing toripalimab.

**Contributors** XM and QY contributed to study design and enrolled patients. ZS and XX enrolled patients, collected the data and analyzed the data. QL contributed to pathological evaluation. YZ and LS contributed to esophagogastroduodenoscopy diagnosis and endoscopic ultrasound evaluation. CZ contributed to PET/CT evaluation. XX, ZS, HL, BR, HC, XW, XZ and YB contributed to patients’ treatment management. All authors participated in data interpretation. XX, ZS, XM and QY drafted the manuscript and all authors reviewed. The final version was approved to be submitted by all authors. XM, QY and YB are responsible for the overall content as the guarantor.

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**Patient consent for publication** Not applicable.

**Ethics approval** This study involves human participants and this study conducted in accordance with the provisions of the Declaration of Helsinki and Good Clinical Practice guidelines and was approved by the Ethic Commission of Renji Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China. All authors participated in data interpretation. XX, ZS, HL, BR, HC, XW, XZ and YB contributed to patients’ treatment management. All authors participated in data interpretation. XX, ZS, XM and QY drafted the manuscript and all authors reviewed. The final version was approved to be submitted by all authors. XM, QY and YB are responsible for the overall content as the guarantor.

**Provenance and peer review** Not commissioned; externally peer reviewed.

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

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Xin Xu http://orcid.org/0000-0001-6392-804X

**REFERENCES**


Supplementary Method

**PET-CT parameters**

The Philips IntelliSpace Portal software was used for image processing, quantifying the maximum standardized uptake value (SUVmax), the metabolic tumor volume (MTV) and total lesion glycolysis (TLG) in the pre- and post-treatment PET/CTs. SUVmax was defined as the point of maximal radiotracer uptake value within the delineated tumor volume (g/mL). MTV represents the metabolically active volume of the main tumor (cm³), whereas TLG was defined as SUVmean \times MTV. A radiologist first identified the approximate center of the tumor then the software automatically drew a region of interest (ROI) and manual modifications to avoid surrounding high metabolism normal tissues being delineated into ROI. For tumor delineation we used a 40% threshold, as it is the most commonly used in the literature. The software automatically calculated SUVmax, SUVmean, MTV and TLG. The primary lesion of the esophagus(T) and the regional lymph nodes (LN) were contoured separately. In this study, we only evaluated these parameters of primary lesion.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>pCR-T (n=11)</th>
<th>non-pCR-T (n=8)</th>
<th>P value</th>
<th>MPR</th>
<th>nonMPR</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>preSUVmax-T</td>
<td>14.9 (6.8,27.8)</td>
<td>23.8 (10.2,32.6)</td>
<td>0.15</td>
<td>14.9 (6.8,29.5)</td>
<td>23.8 (17.3,32.6)</td>
<td>0.11</td>
</tr>
<tr>
<td>preMTV-T</td>
<td>10.2 (1.2,37.1)</td>
<td>13.7 (7.2,43.9)</td>
<td>0.19</td>
<td>10.2 (1.2,37.1)</td>
<td>38.7 (37.1,43.9)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>preTLG-T</td>
<td>58.7 (4.9,296.8)</td>
<td>151.9 (32.5,447.4)</td>
<td>0.10</td>
<td>61.6 (4.9,296.8)</td>
<td>406.9 (296.8,447.4)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>postSUVmax-T</td>
<td>3.0 (2.0,4.7)</td>
<td>8.0 (3.5,12.6)</td>
<td>&lt;0.01</td>
<td>3.5 (2.0,7.4)</td>
<td>8.6 (7.1,12.6)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>postMTV-T</td>
<td>1.4 (0.4,3.2)</td>
<td>2.9 (1.0,10.6)</td>
<td>0.06</td>
<td>1.4 (0.4,3.2)</td>
<td>3.6 (3.1,10.6)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>postTLG-T</td>
<td>3.5 (1.2,7.1)</td>
<td>11.2 (2.8,61.4)</td>
<td>0.04</td>
<td>3.5 (1.2,11.2)</td>
<td>20.0 (14.3,61.4)</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

\(^{18}\text{F-}\text{FDG-}\text{PET/CT};\) \(^{18}\text{F-}\text{Fluorodeoxyglucose-}\text{positron emission tomography/computed tomography};\) pCR-T: complete response of; \(\text{SUVmax: maximum standard uptake value; MTV: metabolic tumor volume; TLG: total lesion glycolysis; pre: before neoadjuvant treatment; post: after neoadjuvant treatment.}\)
21 patients enrolled and received nCRT and neoadjuvant toripalimab

20 patients underwent surgery

16 patients received adjuvant toripalimab

1 patient refused surgery

1 patient was adenocarcinoma

21 patients included in the safety analysis
20 patients included in the surgical complications analysis
19 patients included in the pathologic evaluation

Figure S1 Trial profile
Figure S2. PET/CT for predicting pathological response. (A) PET/CT images of one pCR case before (upper row) and after (lower row) neoadjuvant therapy. (B) ROC of SUVmax and TLG of PET/CT post neoadjuvant therapy for predicting pCR of primary tumor. (C) Pathological response of different SUVmax and TLG in PET/CT after neoadjuvant therapy.
Figure S3. CD8+ TILs before and after neoadjuvant therapy. (A-B) Percentages of CD8+ cells before and after neoadjuvant therapy in pCR patients and non-pCR patients. (C-D) The density of CD8+ cells before and after neoadjuvant therapy in MPR patients and non-MPR patients. (E-F) The density of CD8+ cells before and after neoadjuvant therapy in pCR patients and non-pCR patients.
Figure S4 Changes in numbers of lymphocytes and subpopulations after CRT and immunotherapy. preCRT: before neoadjuvant chemoradiotherapy; postCRT: after neoadjuvant chemoradiotherapy; postICI: after neoadjuvant immunotherapy. ***: p<0.001

Figure S5 Changes of different lymphocyte subpopulation proportions after CRT and immunotherapy. preCRT: before neoadjuvant chemoradiotherapy; postCRT: after neoadjuvant chemoradiotherapy; postICI: after neoadjuvant immunotherapy.
Figure S6 Changes of different cytokines after nCRT and immunotherapy.

preCRT: before neoadjuvant chemoradiotherapy; postCRT: after neoadjuvant chemoradiotherapy; postICI: after neoadjuvant immunotherapy.
Neoadjuvant Chemoradiotherapy Combined with Sequential Perioperative Toripalimab in Locally Advanced Esophageal Squamous Cell Cancer

<table>
<thead>
<tr>
<th>Institution</th>
<th>Ren Ji Hospital, School of Medicine, Shanghai Jiao Tong University</th>
</tr>
</thead>
<tbody>
<tr>
<td>Principal Investigator</td>
<td>Xiumei Ma</td>
</tr>
<tr>
<td>Version No.</td>
<td>V2.1</td>
</tr>
<tr>
<td>Version Date</td>
<td>April 10, 2020</td>
</tr>
</tbody>
</table>
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# Protocol summary

**Study title**  
Neoadjuvant Chemoradiotherapy Combined with Sequential Perioperative Toripalimab in Locally Advanced Esophageal Squamous Cell Cancer

**Study phase**  
Phase II

**Version (date)**  
Version 2.1 (April 10, 2020)

**Institution**  
Ren Ji Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai

**Study objectives**  
To evaluate the efficacy and safety of preoperative PD-1 inhibitor toripalimab combined with chemoradiotherapy in the treatment for locally advanced esophageal squamous cell carcinoma (ESCC).

**Sample size**  
20

**Study Design**  
Patients with histopathologically confirmed thoracic SCC and clinical staged as cT1-4aN1-2M0/cT3-4aN0M0 were enrolled. Eligible patients received radiotherapy (23 fractions of 1.8 Gy, 5 fractions a week) with concurrent chemotherapy of paclitaxel/cisplatin (paclitaxel 45mg/m2 and cisplatin 25 mg/m2) on days 1, 8, 15, 22, 29 and two cycles of toripalimab 240 mg every 3 weeks after nCRT for neoadjuvant therapy before surgery, four cycles of toripalimab 240 mg every 3 weeks for adjuvant therapy after surgery.

**Inclusion criteria**  
1. Age 18–75 years, male or female;
2. Histologically confirmed T1b-4aN2-3M0 ESCC (AJCC/UICC TNM staging, 8th Edition);
3. Without any previous treatment;
4. ECOG performance score is 0-1;
5. The indexes of hematology, biochemistry and organ function meet the following requirements: white blood cell count (WBC) $\geq 3.0 \times 10^9$/L; neutrophil count (ANC) $\geq 1.5 \times 10^9$/L; platelets $\geq 85 \times 10^9$/L; hemoglobin $\geq 9$g/dL; total bilirubin $\leq 14.4 \mu$mol/L; ALT $\leq 75$U/L; serum creatinine $\leq 104 \mu$mol/L and creatinine clearance rate $>60$ mL/min;
6. Women of childbearing age must undergo a pregnancy test within 7 days before enrolling in the treatment, and those who are negative can be enrolled. Patients of childbearing age and their sexual partners agree to use reliable methods of contraception before entering the study, during the study, and at least 180 days after the end of the study;
7. Ability to understand the study and sign informed consent.
<table>
<thead>
<tr>
<th>Exclusion criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Patients with active infection within 2 weeks before the first use of the study drug or need to be treated with oral or intravenous antibiotics;</td>
</tr>
<tr>
<td>2. A history of interstitial lung disease or non-infectious pneumonia;</td>
</tr>
<tr>
<td>3. Patients whose clinician judges surgery as the first choice for the best treatment;</td>
</tr>
<tr>
<td>4. A history of autoimmune diseases or abnormal immune system;</td>
</tr>
<tr>
<td>5. Patients who cannot tolerate chemoradiotherapy or surgery due to severe cardiac, lung dysfunction.</td>
</tr>
<tr>
<td>6. Patients diagnosed with any other malignant tumor within 5 years before enrollment, except for malignant tumors with low risk of recurrence and risk of death, such as fully treated basal cell or squamous cell skin and carcinoma in situ of the cervix;</td>
</tr>
<tr>
<td>7. Known or suspected allergy or hypersensitivity to monoclonal antibodies, any ingredients of Toripalimab, and the chemotherapeutic drugs paclitaxel or cisplatin;</td>
</tr>
<tr>
<td>8. A history of immunodeficiency, including a positive HIV test result or other acquired or congenital immunodeficiency diseases, a history of organ transplantation or allogeneic bone marrow transplantation;</td>
</tr>
<tr>
<td>9. Women during pregnancy or lactation;</td>
</tr>
<tr>
<td>10. Other situations not suitable for enrollment.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Endpoints</th>
<th>Primary endpoint: Major pathological response rate (MPR)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Secondary endpoints: Disease-free survival; Overall survival rate; Safety; Surgical complication rate</td>
</tr>
</tbody>
</table>

| Statistical analysis | Statistical analyses were conducted using SPSS 25.0 software. DFS and OS will be analyzed using the Kaplan-Meier method and log-rank test. The safety analysis was based on descriptive statistics. |
1. Study Background

Esophageal cancer is a common and highly lethal malignant tumor in China. In the past, the main treatment approach was comprehensive therapy based on surgery, but its effectiveness has been unsatisfactory. Specifically, locally advanced esophageal cancer has a poor prognosis, with a 5-year survival rate of only 20.6%-34% for stage II-III patients who receive surgery as the sole treatment. The CROSS study conducted in the Netherlands in 2012 identified the role of preoperative neoadjuvant chemoradiotherapy in the treatment of locally advanced esophageal cancer: the median postoperative survival of patients who received neoadjuvant chemoradiotherapy alone increased from 24 months to 49 months. Preoperative neoadjuvant therapy can improve survival in patients with locally advanced esophageal cancer and achieve a pathological complete response rate of about 30%. Patients who achieve a pathological complete response experience significantly prolonged survival, with a 5-year survival rate ranging from 40% to 60%. The NEOCRTEC5010 study also demonstrated that the overall survival after neoadjuvant chemoradiotherapy for esophageal squamous cell carcinoma patients reached 100 months. However, there is still a need to enhance the effectiveness of preoperative neoadjuvant therapy, for more than half of these patients can not reach major pathological response leading high recurrence rate, and also about 10% of patients experience disease progression and miss the chance for surgery.

PD-1 monoclonal antibodies have demonstrated therapeutic advantages in clinical studies of advanced or metastatic esophageal cancer, either as monotherapy or in combination with other drugs. However, there is still a lack of reliable clinical evidence on whether PD-1 can improve the efficacy of neoadjuvant therapy for esophageal cancer. Preliminary results from a small sample trial presented at the 2019 ASCO conference suggest that PD-1 monoclonal antibodies can improve the pathological response rate in neoadjuvant therapy for esophageal cancer. Toripalimab, a PD-1 monoclonal antibody, has been approved for treating melanoma and has brought significant survival benefits to melanoma patients. It has also shown effectiveness in applications for lung cancer, esophageal cancer, and other tumors. The JS001-CT5 study showed that toripalimab, used in patients with metastatic esophageal squamous cell carcinoma, has definite efficacy and controllable safety, indicating its potential application in the neoadjuvant treatment for locally advanced esophageal cancer.

2. Study Objectives

To investigate the feasibility and safety of perioperative toripalimab combined with neoadjuvant chemoradiotherapy (paclitaxel and cisplatin) in the treatment of locally advanced esophageal squamous cell carcinoma.

2.1 Primary study endpoints

Major Pathological Response Rate (MPR): No more than 10% of tumor cells were found in neoadjuvant surgical specimens.

2.2 Secondary study endpoints

Disease-free survival; overall survival rate; safety and surgical complication rate.

3. Study Design
This project is a prospective single-arm, exploratory study with a small sample size of 20 patients to initially observe the efficacy and safety.

4. Study Population

4.1 Inclusion criteria

1) Age 18-75 years, male or female;

2) Histologically confirmed T1b-4aN2-3M0 ESCC (AJCC/UICC TNM staging, 8th Edition)

3) Without any previous treatment;

4) ECOG performance score is 0-1;

5) The indexes of hematology, biochemistry and organ function meet the following requirements: white blood cell count (WBC) ≥ 3.0×10^9/L; neutrophil count (ANC) ≥ 1.5×10^9/L; platelets ≥ 85×10^9/L; hemoglobin ≥ 9g/dL; total bilirubin ≤ 14.4μmol/L; ALT ≤ 75U/L; serum creatinine ≤ 104μmol/L and creatinine clearance rate >60 mL/min;

6) Women of childbearing age must undergo a pregnancy test within 7 days before enrolling in the treatment, and those who are negative can be enrolled. Patients of childbearing age and their sexual partners agree to use reliable methods of contraception before entering the study, during the study, and at least 180 days after the end of the study;

7) Ability to understand the study and sign informed consent.

4.2 Exclusion criteria

1) Patients with active infection within 2 weeks before the first use of the study drug or need to be treated with oral or intravenous antibiotics;

2) A history of interstitial lung disease or non-infectious pneumonia;

3) Patients whose clinician judges surgery as the first choice for the best treatment;

4) A history of autoimmune diseases or abnormal immune system;

5) Patients who cannot tolerate chemoradiotherapy or surgery due to severe cardiac, lung dysfunction;

6) Patients diagnosed with any other malignant tumor within 5 years before enrollment, except for malignant tumors with low risk of recurrence and risk of death, such as fully treated basal cell or squamous cell skin and carcinoma in situ of the cervix;

7) Known or suspected allergy or hypersensitivity to monoclonal antibodies, any ingredients of Toripalimab, and the chemotherapeutic drugs paclitaxel or cisplatin;

8) A history of immunodeficiency, including a positive HIV test result or other acquired or congenital
immunodeficiency diseases, a history of organ transplantation or allogeneic bone marrow transplantation;

9) Women during pregnancy or lactation;

10) Other situations not suitable for enrollment.

4.3 Removal criteria
Subjects who are enrolled but violate the protocol should be removed, including: (1) mis-diagnosis; (2) not meeting the inclusion criteria but meeting the exclusion criteria; (3) having incomplete data for evaluation of efficacy and safety. The reason of rejection should be explained and recorded. However, patients who have received treatment and have safety record should be included in the safety analysis.

4.4 Drop-out/Withdrawal criteria
Subjects could withdraw from the study at any time and for any reason. The investigator could withdraw a subject from the study including the following reasons: serious adverse events, trial protocol violation, poor compliance, lack of efficacy, trial discontinuation is determined to be necessary for subjects by the investigator.

4.5 Discontinuation criteria
Serious adverse events, or other events that affect subject safety; Radiographic disease progression or metastasis; Patients receive other anti-tumor treatments that affect the results of the study; The subject withdrew his informed consent; Other conditions that the investigator considers necessary to terminate the study drug treatment.

5. Treatment Plan
1.1 Pre-treatment staging
All patients underwent medical history taking, physical examination, routine laboratory tests, echocardiography, cardiac ultrasound and pulmonary function test. Pre-treatment tumor staging was performed through contrast-enhanced chest CT, esophagogastroduodenoscopy diagnostic biopsy with endoscopic ultrasound (EUS), esophageal barium imaging, magnetic resonance imaging (MRI) of the esophagus, and positron emission tomography-computed tomography (PET-CT).

1.2 Neoadjuvant treatment and outcome measurements
Neoadjuvant radiotherapy was given by means of intensity-modulated radiotherapy (IMRT). A total dose of 41.4 Gy radiation was given by 23 fractions with 1.8 Gy per fraction and five fractions per week. The chemotherapy regimen included paclitaxel (45 mg/m2) and cisplatin (25 mg/m2) were administrated weekly on days 1, 8, 15, 22 and 29. Two cycles of toripalimab 240 mg were given intravenously every 3 weeks after completion of chemoradiotherapy. The first cycle of toripalimab was administrated within 1-3 days after completion of radiotherapy. 3 weeks after the administration of the second cycle of immunotherapy, disease reevaluation was performed, including physical examination, routine laboratory test, echocardiography,
pulmonary function test, contrast-enhanced chest CT, MRI, and PET-CT, to restage and exclude cases not suitable for surgery.

5.3 Surgery and adjuvant treatment
Surgery was scheduled for 4-6 weeks after the second treatment cycle of toripalimab. A minimal invasive McKeown esophagectomy was performed, including two-field lymphadenectomy or three-field lymphadenectomy in individual patients by a single experienced surgeon. Adjuvant treatment should be started within 12 weeks after surgery. Toripalimab 240 mg were given every 3 weeks in 4 cycles for adjuvant treatment.

6. Dose adjustment

6.1 Dose adjustment of chemotherapy drugs
Neutropenia is the dose-limiting toxicity in paclitaxel monotherapy. Other possible adverse events include hypersensitivity, skin reactions, gastrointestinal toxicity (nausea, vomiting, stomatitis, diarrhea), alopecia, muscle weakness, mild injection site reactions (phlebitis), peripheral neurotoxicity, and fluid retention/edema. Cisplatin monotherapy is associated with major adverse events such as ototoxicity, peripheral neuropathy, renal failure, and vomiting. When subjects experience multiple toxicities and there are differing dose adjustment principles, the minimum dose is chosen. If the drug dose is reduced due to drug-related toxicity, it cannot be increased back to the original dose and should continue at the reduced dose.

6.1.1 Dose adjustment grade of chemotherapeutic agents:

<table>
<thead>
<tr>
<th>Drug</th>
<th>Original dose (100%)</th>
<th>Dose level (90%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>cisplatin</td>
<td>25mg/m2</td>
<td>22.5mg/m2</td>
</tr>
<tr>
<td>paclitaxel</td>
<td>45mg/m2</td>
<td>40.5mg/m2</td>
</tr>
</tbody>
</table>

6.1.2 Adjustment for bone marrow toxicity:

<table>
<thead>
<tr>
<th>ANC≥1,000/mm³ and platelets≥100,000/mm³</th>
<th>Full doses</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANC &lt; 1,000/mm³ or platelets &lt;100,000/mm³</td>
<td>Postpone chemotherapy for one week, if ANC≥1,000/mm³ and platelets≥100,000/mm³ in the next week examination, the full dose chemotherapy with both drugs should be continued. If only partial recovery of bone marrow toxicity, both drugs should be administered at a dose level of -1.</td>
</tr>
<tr>
<td>Febrile neutropenia</td>
<td>Postpone both chemotherapy and radiotherapy until ANC ≥ 1,000/mm³ and platelet ≥ 100,000/mm³, then both drugs will be given at -1 dose level, the suspension time should not exceed 2 weeks.</td>
</tr>
</tbody>
</table>

6.1.3 Adjustment for nephrotoxicity:

<table>
<thead>
<tr>
<th>CCr≥50 ml/min</th>
<th>Full doses</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCr &lt;50 ml/min</td>
<td>Paclitaxel was used as scheduled, and cisplatin was postponed. If CCr ≥ 50 ml/min next week, resume the previous dose of cisplatin. If the CCr</td>
</tr>
</tbody>
</table>
remains <50 ml/min in the next week, continue to postpone cisplatin, but not beyond 2 weeks, otherwise withdraw from the trial.

### 6.1.4 Adjustment for neurotoxicity:

| Level 3 or 4 | Paclitaxel and cisplatin were postponed. |
| Level 2      | Reduce paclitaxel and cisplatin doses to dose levels of-1. |
| Level 1      | Full-dose administration of paclitaxel and cisplatin. |

### 6.1.5 Adjustment of the mucositis

| Level 3 or 4 | Paclitaxel was postponed but not beyond 2 weeks, otherwise withdrawn from the trial. When returning to grade 2 or lower, reduce to -1 dose level. No reduction in cisplatin dose is required. |

### 6.2 Dose adjustment of toripalimab

<table>
<thead>
<tr>
<th>Toxicities</th>
<th>Severity</th>
<th>Adjustment Scheme</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diarrhea/colitis</td>
<td>Grade 2-3</td>
<td>Postpone until recovery to Grade 0-1</td>
</tr>
<tr>
<td></td>
<td>Grade 4</td>
<td>Termination</td>
</tr>
<tr>
<td>Hepatic dysfunction</td>
<td>Grade 2</td>
<td>Postpone until recovery to Grade 0-1</td>
</tr>
<tr>
<td></td>
<td>Grade 3 or 4</td>
<td>Termination</td>
</tr>
<tr>
<td>Renal failure or nephritis</td>
<td>Grade 2</td>
<td>Postpone until recovery to Grade 0-1</td>
</tr>
<tr>
<td></td>
<td>Grade 3/4</td>
<td>Termination</td>
</tr>
<tr>
<td>Endocrine disorders</td>
<td>Grade 2-3 hyperthyroidism</td>
<td>Postpone until recovery to Grade 0-1</td>
</tr>
<tr>
<td></td>
<td>Grade 2-3 hypophysitis</td>
<td></td>
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<tr>
<td></td>
<td>Grade 2 adrenal insufficiency</td>
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</tr>
<tr>
<td></td>
<td>Grade 3 hyperglycemia or diabetes mellitus</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Grade 4 hypothyroidism</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Grade 4 hyperthyroidism</td>
<td>Termination</td>
</tr>
<tr>
<td></td>
<td>Grade 4 hypophysitis</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Grade 3-4 adrenal insufficiency</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Grade 4 hyperglycemia or diabetes mellitus</td>
<td></td>
</tr>
<tr>
<td>Pneumonia</td>
<td>Grade 2</td>
<td>Postpone until recovery to Grade 0-1</td>
</tr>
<tr>
<td></td>
<td>Grade 3-4 or recurrent Grade 2</td>
<td>Suspension</td>
</tr>
</tbody>
</table>
6.3 Dose adjustment of radiotherapy
The toxicity during radiotherapy will be assessed using (CTCAE v4.0). Radiotherapy should be continued if grade 3 toxicity is unrelated to radiotherapy. Radiotherapy should be discontinued if grade 4 toxicity is observed. If grade 3 radiation-related toxicity is observed, actively treat the symptoms. If the grade 3 reaction reduces or disappears, continue with the radiotherapy. If any of the following toxicities are present, subjects should be excluded from the study: esophageal perforation and heavy hemorrhage, non-healing esophageal tracheal leakage, myocardial infarction, heart failure, severe arrhythmias, and radiation pneumonia with dyspnea. If radiotherapy is temporarily interrupted or cumulatively interrupted for more than 2 weeks due to various reasons, the patient will be considered a major deviation, but follow-up will continue.

7. Outcomes evaluation
7.1 Efficacy evaluation
The primary endpoint is major pathological response (MPR) rate. The pathological examination should report the tumor extension, lymph node status, resection margins, and tumor regression grade (TRG). The TRGs of the primary tumor were described using a previously reported method as follows: grade 1, no evidence of vital residual tumor cells; grade 2, 10% or fewer vital residual tumor cells; grade 3, 11 to 50%; and grade 4, more than 50%. 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 less than 10% vital residual tumor cells (TRG1 and TRG2). MRI evaluation will be performed during nCRT in the third week to evaluate the early response. 3 weeks after the administration of the second cycle of immunotherapy and before surgery, disease reevaluation will be performed, including physical examination, routine laboratory test, echocardiography, pulmonary function test, contrast-enhanced chest CT, MRI, and PET-CT, to restage and exclude cases not suitable for surgery.
Secondary endpoints include DFS and OS. DFS is calculated from the date of surgery until the date of death from any cause or the date of first documented disease progression whichever came first, and OS is calculated...
from the date of randomization until the date of death from any cause or the date of last follow-up, whichever came first. During the adjuvant treatment radiological evaluation will be performed before the first and fourth cycle of immunotherapy, and during follow-up evaluation will be performed every 3 month in the first two years and then every 6 month until 5 years.

7.2 Safety evaluation
The toxicity of chemotherapy and radiotherapy will be evaluated according to the National Cancer Institute Common Terminology Criteria for Adverse Events Version 4.0 (CTCAE v 4.0). The post-operative complications will be evaluated according to Clavien-Dindo classification. All AEs occurring during the trial should be recorded in the CRF. Changes in patients’ subjective symptoms (nausea, vomiting, poor appetite, alopecia, etc.), PS scores, blood routine, hepatic and renal function should be recorded every week during nCRT and before every cycle of toripalimab and thyroid function, adrenal cortical function, myocardial enzymes, BNP, etc. should be recorded before every cycle of toripalimab. All postoperative complications occurring within 30 days after surgery were recorded according to Clavien-Dindo Classification and ECG definition, respectively.

7.3 Exploratory analysis
PD-L1 expression was assessed on pretreatment formalin-fixed paraffin-embedded (FFPE) sections using an immunohistochemistry (IHC) assay (PD-L1 IHC 22C3). PD-L1 expression was evaluated by tumor proportion score (TPS) and PD-L1 expression positive was defined as TPS≥1%. Pretreatment (baseline) biopsy and surgical resected specimen were used to assess tumor-infiltrating CD8+ lymphocytes by IHC. Flow cytometry was used to measure peripheral lymphocyte subsets consisting of T cells (CD3+ T cells, CD3+CD4+ T cells, CD3+CD8+ T cells), regulatory T cells (CD3+CD4+CD25+CD127- Treg cells), natural killer cells (CD16+CD56+ NK cells), B cells (CD19+ B cells), and activation antigens (CD3+HLA-DR+/CD3+(%), CD8+CD38+/CD8+(%), CD8+HLA-DR+/CD8+(%), CD4+HLA-DR+/CD4+(%), CD4+CD38+/CD4+(%)), before and after nCRT, after one cycle of toripalimab, before surgery and during adjuvant treatment. The cytokines including IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12P70, IL-17A, TNF-α, IFN-α, and IFN-γ were also measured by flow cytometry at the same times.

8. Data management
8.1 Case report form (CRF)
To ensure accuracy of information and facilitate efficient analysis, it is imperative that the data be promptly recorded in the CRF. Under normal circumstances, the CRF should not be modified. However, if there are genuine errors that need correction, the investigator must sign at the location of the alteration to document the change. After the study, duplicate copies of the completed CRF will be distributed to both the sponsor and investigator as per standard protocol. Once the data has been thoroughly reviewed by the Clinical Research Associate (CRA), it will be entered into a secure database for further analysis. At this stage, any further amendments to the CRF will no longer be possible, as the contents will have been finalized. According to GCP, documentation should be properly preserved by the investigators for more than 5 years.

8.2 Severe adverse event (SAEs) handling and reporting methods
Accurately complete the adverse event record, recording the occurrence time, severity, duration, measures taken, and outcome of the adverse events. Explain the criteria for judging the severity of adverse events and the 5-level classification criteria for determining the relationship between adverse events and investigational drugs (definitely related, probably related, possibly unrelated, unrelated, and cannot be determined). Any serious adverse events occurring during the clinical trial period must be reported to the principal investigator of the research institution, the ethics committee of the drug clinical research base, and the sponsor within 24 hours.

9. Ethics requirements

Before conducting this experiment, the protocol, proposed informed consent form, and other materials provided to patients must be submitted for review by the Institutional Ethics Committee (IEC). A written approval with the committee’s signature and date indicating their endorsement is required prior to the start of the experiment. Any amendments to the research plan, informed consent form, or materials provided to the patients must be approved by the committee.

The clinical trial can only proceed after obtaining the informed consent of the subjects and having them sign the informed consent form. During the study, the rights of the subjects should be ensured, and confidentiality principles should be followed regarding subject information.

10. Statistical analysis

SPSS version 22.0 will be used for statistical analysis. All statistical tests are two-tailed, and a P-value less than or equal to 0.05 is considered statistically significant for the tested differences (excluding those specifically stated otherwise). Descriptive statistics for quantitative variables will include mean, standard deviation, median, minimum value, maximum value, lower quartile (Q1), upper quartile (Q3). Categorical variables will be described with counts and percentages for each category. For comparisons between two groups, appropriate methods will be used based on the type of variable. Group comparisons for quantitative data will use either independent samples t-test (assuming homogeneity of variance and normal distribution) or Wilcoxon rank-sum test. Chi-square test or exact probability method (if chi-square test is not applicable) will be used for categorical data, and Wilcoxon rank-sum test or CMH test will be used for ordinal data. Tumor pathological remission rate, drug-related adverse events, perioperative data, and incidence of complications will be reported as descriptive statistics. The Kaplan-Meier method will be used for analysis of DFS and OS.