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

Xin Xu, Zhiyong Sun, Qiang Liu, Yao Zhang, Lei Shen, Chenpeng Zhang, Haiping Lin, Bin Hu, Ling Rong, Haiyan Chen, Xiaohang Wang, Xiaojing Zhao, Yong-Rui Bai, Qing Ye, Xiumei Ma

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/cT3-4aN0M0 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. 1 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%). 14 patients (66.7%) had 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+HLA-DR+, CD3+%, CD8+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 and patients with MPR have longer disease-free survival than non-MPR patients.

Conclusions 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.¹ 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 malignant tumor-related deaths in the country.² Esophageal squamous cell carcinoma (ESCC), a more malignant pathological type, accounts for over 85% of EC cases in China.³ Approximately 70% of EC cases are diagnosed at advanced stages, resulting in a poor prognosis.³ 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%.⁴ 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, stage T1-4aN1-2M0 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 enrollment; allergy to any study drug components; a history of immuno-deficiency; 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. Adjuvant 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

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 HIC 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 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 (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-α, IFN-γ, 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 or numbers (percentages). Continuous variables were presented as the 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 classified as having clinical stage III (95.2%). 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 was 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 postoperative 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.
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 $75 \text{ cells/mm}^2$, 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
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 ICSs 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.\textsuperscript{7,17} Another trial, the EC-CRT-001 trial, reported esophageal fistula in 14% of locally advanced ESCC patients who received concurrent anti-PD-1 antibody and definitive CRT.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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 postoperatively, a rate similar to that reported in some previous studies.\textsuperscript{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.\textsuperscript{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,\textsuperscript{21} 61% and 29% in CROSSE,\textsuperscript{14} 63.4% and 27.7% in CMISG1701,\textsuperscript{22} 43.2% pCR in NEOCRTEC 5010\textsuperscript{20}) and the combination of chemotherapy and immunotherapy (68.6% and 39.2% in NIC\textsuperscript{10} 58.8% and 31.4% in NIC-ESCC2019,\textsuperscript{19} 50% and 25% in GAST01056,\textsuperscript{11} 50% and 20% in KEEP-G03).\textsuperscript{23} Combining our results with the PALACE-1\textsuperscript{17} and NEOCRTEC 1901\textsuperscript{17} 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.\textsuperscript{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 rates, and both were superior to NICRT.\textsuperscript{24} Further randomized controlled studies are required to validate whether the trcombination 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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{29, 30} However, other studies did not show this association.\textsuperscript{31, 32} 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.\textsuperscript{7, 9, 10, 23} 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+CD58+/CD8+, and CD8+HLA-DR+/CD8+. Higher CD4+/% and 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.\textsuperscript{33–35} 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 peroperative 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.

### 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 esophagogastrectomy diagnosis and endoscopic ultrasound evaluation. CZ contributed to PET/CT evaluation. XX, ZS, HL, BH, LR, HC, WX, 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.

### Funding

This was supported by the Science and Technology Commission of Shanghai Municipality (232R143890).

### Competing interests

None declared.

### 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 (KY2019-174). Participants gave informed consent to participate in the study before taking part.

### 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.

### Supplemental material

This content has been supplied by the author(s). It has not been vetted by BMJ Publishing Group Limited (BMJ) and may not have been peer-reviewed. Any opinions or recommendations discussed are solely those of the author(s) and are not endorsed by BMJ. BMJ disclaims all liability and responsibility arising from any reliance placed on the content. Where the content includes any translated material, BMJ does not warrant the accuracy and reliability of the translations (including but not limited to local regulations, clinical guidelines, terminology, drug names and drug dosages), and is not responsible for any error and/or omissions arising from translation and adaptation or otherwise.

### Open access

This is an open access article distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited, appropriate credit is given, any changes made indicated, and the use is non-commercial. See http://creativecommons.org/licenses/by-nc/4.0/.

### ORCID iD

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

### REFERENCES


27 Uboha NV, Maloney JD, McCarthy D, et al. Safety of neoadjuvant chemotherapy or chemoradiotherapy for advanced esophageal or gastroesophageal junction cancer. *Int J Radiation Oncology Biol Phys* 2022;106(5):e107163.

