Short-course neoadjuvant radiotherapy combined with chemotherapy and toripalimab for locally advanced esophageal squamous cell carcinoma (SCALE-1): a single-arm phase Ib clinical trial

Clinical research protocol

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1 Schema

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<tr>
<th>Research Topic</th>
<th>A single-arm phase Ib trial of short-course neoadjuvant radiotherapy combined with chemotherapy and toripalimab for locally advanced esophageal squamous cell carcinoma</th>
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<tbody>
<tr>
<td>Research Purpose</td>
<td>To determine the safety and efficacy of short-course neoadjuvant radiotherapy combined with chemotherapy and toripalimab in patients with resectable locally advanced esophageal squamous cell carcinoma (RLaESCC)</td>
</tr>
<tr>
<td>Research Design</td>
<td>Prospective, single-center, single-arm, phase Ib trial</td>
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<tr>
<td>Principal Investigator</td>
<td>Dr. Binhui Ren, Xiangzhi Zhu and Ming Jiang</td>
</tr>
<tr>
<td>Research Object</td>
<td>Resectable thoracic esophageal squamous cell carcinoma with clinical stage of cT3-4aN0M0/T1-4aN+M0 by the AJCC 8th edition</td>
</tr>
</tbody>
</table>
| Research Endpoints | 1) Primary endpoints: Safety  
2) Secondary endpoints: pathological response rate (pCR), radiological response rate, postoperative complications, progression-free survival (PFS) and overall survival (OS)  
3) PD-L1 expression, assessed through immunohistochemistry, and gene expression profiles (GEPs) via the nCounter platform, were employed for conducting exploratory biomarker analysis. |
| Inclusion Criteria | 1. Be able to provide written informed consent and understand and agree to follow the research requirements and evaluation schedule.  
2. Endoscopic biopsy of thoracic esophageal primary lesion histologically diagnosed as squamous cell carcinoma.  
3. Clinical stage T1-4aN+M0 or T3-4aN0M0 in the UICC-TNM classification 8th edition.  
4. The age is over 20 years old and under 75 on the enrollment date (including 20 and 75), including both female and male.  
5. PS 0-1.  
6. According to RECIST version 1.1, there were measurable or evaluable lesions.  
7. No medical history of treatment for cancer (No medical history of cancer treatment). |
chemotherapy, radiotherapy and endocrine therapy, immune-therapy or other study drugs including treatment for other types of cancer).

8. The results of laboratory tests within 14 days before enrollment meet the inclusion criteria (patients should not receive blood transfusion or growth factor support because neutrophil count, platelet or hemoglobin are lower than the research requirements within 14 days before blood sample collection).

(1) Bone marrow function: hemoglobin (Hb) ≥ 90g/L; white blood cell count (WBC) ≥ lower limit of normal value; absolute neutrophil value (ANC) ≥ 1.5 x 10^9 /L; platelet count ≥ 100 x 10^9 /L;

(2) Renal function: Cr ≤ 1.5 UNL, endogenous creatinine clearance rate (Ccr) ≥ 60 ml/min (Cockcroft-Gault);

(3) Liver function: total bilirubin ≤ 1.5 ULN; ALT and AST ≤ 2.5 ULN (patients with liver metastases can be relaxed to ≤5 ULN);

(4) Blood coagulation function: the international standardized ratio of prothrombin time ≤ 1.5 ULN, and the partial thromboplastin time is within the normal range.

9. Patients with hepatitis B virus (HBV) infection, inactive / asymptomatic HBV carriers, or patients with chronic or active HBV will be allowed to be enrolled if HBV DNA < 500 IU / ml (or 2500 copies / ml) at screening. Patients with positive hepatitis C antibody will be allowed to be enrolled if HCV-RNA is negative during screening. Note: patients who can detect hepatitis B surface antigen (HBsAg) or HBV DNA should be treated with antiviral drugs for more than 2 weeks before enrollment, and the treatment should be continued for 6 months after the study drug treatment.

10. Women of childbearing age should take the urine or serum pregnancy test, and the result of which should be negative within 72 hours before treatment. For females, who have agreed with contraception from start of investigational drug administration to 5 months after last dose of investigational drug. For males who have agreed with contraception from start of investigational drug administration to 7 months after last dose of investigational drug.

### Exclusion Criteria

1. Have received any treatment for esophageal squamous cell carcinoma in the past;
2. Patients with evidence or high risk of gastrointestinal hemorrhage or fistula (esophagus / bronchus or esophagus / aorta);
3. Patients with severe malnutrition, with body mass index lower than 18.5kg/m2, or PG-SGA score ≥ 9
4. Any active autoimmune disease or history of autoimmune disease (as follows, but not limited to: interstitial pneumonia, uveitis, enteritis,
autoimmune hepatitis, hypophysitis, vasculitis, nephritis, hyperthyroidism, thyroid dysfunction); Subjects with vitiligo or who have had complete remission from childhood asthma without any intervention after adulthood may be included; Asthma requiring medical intervention with bronchodilators was not included.  
5. Has a previous radiotherapy, chemotherapy, hormone therapy, surgery, molecular targeted therapy or immune therapy for this malignancy or for any other past malignancy; 
6. Any condition requiring systemic corticosteroid therapy (prednisone with a dose higher than 10 mg / day or equivalent dose of similar drugs) or other immunosuppressants within 14 days before treatment. (Excluding the following steroid regimensLocal, ophthalmic, intra-articular, nasal and inhaled corticosteroids with minimal systemic absorptionProphylactic short-term (<= 7 days) use of corticosteroids (e.g., prevention of contrast media allergy) or for the treatment of non-autoimmune disorders (e.g., delayed hypersensitivity caused by exposure to allergens). 
7. Live vaccine injection was received in <= 4 weeks before treatment. 
8. A history of immunodeficiency, including HIV infection, other acquired or congenital immunodeficiency, or a history of organ or bone marrow implantation that need immunosuppressive medications. 
9. There are clinical symptoms or diseases of the heart that are not well controlled, such as:
   (1) Heart failure above grade 2 by the Criteria of NYHA; 
   (2) Unstable angina pectoris; 
   (3) Myocardial infarction occurred within 1 year; 
   (4) Clinically meaningful supraventricular or ventricular arrhythmias require treatment or intervention; 
10. Has severe infections (CTCAE > 2 grade) within 4 weeks before treatment; basal thoracic imaging indicating active pneumonia, or other infectious situation that need oral or intravenous antibiotic treatment (excluding Prophylactic medication for antibiotics). 
11. A history of interstitial lung disease, non-infectious pneumonia or uncontrolled disease, including pulmonary fibrosis, acute lung disease, etc. 
12. Has active pulmonary tuberculosis found by CT imaging; or has active pulmonary tuberculosis less than 1 year before inclusion; or has active pulmonary tuberculosis but without standard treatment over 1 year before inclusion; 
13. Allergic to any drug used in this study. 
14. Pregnant or lactating women participants who unwilling to take contraception. 
15. Other factors that could lead to the termination of this study.
<table>
<thead>
<tr>
<th>Duration of Trial</th>
<th>Estimated enrollment time of the first subject: September 2020</th>
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<tr>
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<td>Estimated enrollment time of the last subject: December 2021</td>
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<td></td>
<td>Estimated end time of the study: December 2022</td>
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<tr>
<td><strong>Therapeutic Regimen</strong></td>
<td><strong>Neoadjuvant Chemotherapy</strong>: Paclitaxel (135 mg/m2) + Carboplatin (AUC=5), ivdrip, Days 1, 22</td>
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<td></td>
<td><strong>Neoadjuvant Immunotherapy</strong>: Toripalimab 240mg, ivdrip, Days 1, 22</td>
</tr>
<tr>
<td></td>
<td><strong>Neoadjuvant Radiotherapy</strong>: Total dose: 30.0 Gy, Dose / Fraction: 2.5 Gy, Fraction / week: 5, From Days 3 to 18</td>
</tr>
<tr>
<td></td>
<td><strong>Surgery</strong>: The Ivor Lewis operation (right transthoracic esophagectomy with reconstruction and laparoscopic dissection) Or the McKeown operation (right thoracotomy, laparoscopy dissection, and left cervical esophagectomy with reconstruction)</td>
</tr>
<tr>
<td><strong>Sample Size</strong></td>
<td>As an exploratory study, the study sample size will consist of 20 patients who underwent tumor resection.</td>
</tr>
<tr>
<td><strong>Statistical Analysis</strong></td>
<td>• The intent-to-treat (ITT) population included all eligible patients, regardless of the treatment they received. Analyses exploring the relationship between neoadjuvant treatment and safety were performed using the safety set (all patients who received neoadjuvant radiotherapy and at least one dose of neoadjuvant chemotherapy or immunotherapy). The modified ITT population included all patients who underwent surgery and had surgery results available for the end point analysis.</td>
</tr>
</tbody>
</table>
|                   | • Continuous variables were presented as the median with the range or the mean with the standard deviation. Survival was estimated using the Kaplan–Meier method, and the log-rank test was used for comparisons between groups. P-values were two sided, with a significance level of
0.05 for all analyses.
2 Summary

Neoadjuvant chemoradiotherapy (nCRT) followed by surgery was the standard care for patients with resectable, locally advanced ESCC (RLaESCC) for a long time. It has shown great superiority in terms of tumor regression and improved R0 resection rates.(1, 2) However, tumor recurrence still occurs in 40–50% of patients after surgery.(3-5) Systemic micro-metastasis is one of the main reasons for local regional relapse and metastasis, even in patients who have a pathological complete response (pCR).(4, 5) Moreover, only 20.1% of resectable ESCC patients receive nCRT in China, with nCRT-related postoperative complications and mortality being the major concerns.(3, 6) Therefore, more effective and less toxic neoadjuvant regimens and strategies for RLaESCC need to be explored.

Increasing evidence have shown that immune checkpoint inhibitors (ICIs) may help to eliminate radiographically occult metastatic diseases by enhancing systemic immunity against tumor antigens.(7) ICIs have shown promising results in treatment for metastatic esophageal carcinoma (8-14) and in resectable EC patients with residual pathological disease after nCRT and surgery (15). Hence, it is reasonable to move ICIs to an upfront setting, as a part of neoadjuvant treatment, to achieve better clinical outcomes. However, treatment related acute toxicity should not be neglected as reported by several phase I/II studies(16-18). Results from the recently released JCOG1109 NExT study also revealed that cancer-unrelated death was much higher in nCRT group than in neoadjuvant chemotherapy group(19). Late cardiopulmonary toxicities following thoracic radiation would be a rational explanation(20-22). These results suggest that the incorporation of immune checkpoint inhibitors (ICIs) into nCRT offers benefits beyond the mere combination of these three components. De-intensifying radiotherapy and chemotherapy could emerge as a viable approach for reducing both long-term and short-term toxicities.

Recent studies have shown that shorter treatment course and hypofractionated...
radiotherapy could induce better synergistic effect in combination with ICIs \(^{(23-26)}\). In this phase Ib SCALE study, de-intensified short-course radiotherapy with chemotherapy plus toripalimab, a humanized programmed cell death protein 1 (PD-1) monoclonal antibody,\(^{(11)}\) was administered to patients with RLaESCC. Neoadjuvant toripalimab and chemotherapy are performed simultaneously on days 1 and 22. Intensity-modulated radiation therapy (IMRT) technique with increased fractionated dosage and shorter treatment course was administered as “sandwich therapy” from day 3 to day 18. An esophagectomy was planned four to six weeks after completing neoadjuvant therapy. The patients were offered adjuvant treatment (ICI or ICI in combination with chemotherapy) at the investigators’ discretion, depending on the efficacy (i.e., pathological responses), tolerance of treatment, and general postoperative condition, and were followed up for progression-free and overall survival (OS).

3 Background

Esophagus cancer is the seventh most common cancer worldwide, and the fourth leading cause of cancer death in China\(^{(27, 28)}\). Squamous cell carcinoma (ESCC) accounts for almost 90% of esophagus cancer. Neoadjuvant chemoradiotherapy (nCRT) followed by surgery is the standard care for patients with resectable, locally advanced ESCC (RLaESCC). It has shown great superiority in terms of tumor regression and improved R0 resection rates, as well as improved overall survival and disease free survival.\(^{(1, 2)}\) However, tumor recurrence still occurs in 40–50% of patients after surgery.\(^{(3-5)}\) Thus, new treatment regimens are need to be explored for patients with RLaESCC.

Accumulating clinical evidence has shown that immune checkpoint inhibitors (ICIs) are promising treatment for esophagus cancer. ICIs alone or in combination with chemotherapy have achieved favorable objective response rate and prolonged overall survival in advanced esophagus cancer patients which have been proven by series
studies(8-14, 29). In an adjuvant setting, patients with residual disease after receiving neoadjuvant chemoradiotherapy and surgery, adjuvant nivolumab also reduced distant recurrence and death risk(15). Moreover, adding ICIs to standard neoadjuvant chemoradiotherapy induced promising pathologic complete response (pCR) rates which may present better survival(16, 18).

However, the intensity and regimens of conventional nCRT with integration of immunotherapy may have to be re-evaluated. Conventional nCRT has been reported to have a higher rate of therapy-related noncancer deaths than neoadjuvant chemotherapy (nCT)(30, 31). In addition, multiple phase I/II studies exploring ICIs in combination with nCRT as neoadjuvant treatment for RLaESCC also have reported treatment-related deaths(16-18). Opting for de-intensified radiotherapy and chemotherapy could be considered as a strategy to mitigate potential risks associated with both long-term and short-term toxicity.

Up to now, the ideal intensity of nCRT in combination with ICIs have not been determined yet. Recently, more and more evidence indicated that shorter treatment courses and hypofractionated radiotherapy could induce a better synergistic effect in combination with ICIs.(23-26) In this phase Ib SCALE study, a novel neoadjuvant regimen was proposed: de-intensified short-course radiotherapy in combination with chemotherapy and toripalimab, a humanized programmed cell death protein 1 (PD-1) monoclonal antibody,(11) following by esophagectomy. The primary goal was to assess the safety of this novel neoadjuvant immuno-chemoradiotherapy (nICRT) approach to treat RLaESCC.

4 Objective

To evaluated the safety and efficacy of short course neoadjuvant radiotherapy combined with chemotherapy and anti-PD-1 antibody (toripalimab) in patients with locally advanced squamous cell carcinoma of esophagus.
5 Study plan

5.1 Study design

This study is a prospective, single-center, single-arm phase Ib clinical trial. The study recruited patients with AJCC/UICC stage T3-4aN0M0, T1-4aN+M0 resectable locally advanced esophageal cancer. To evaluate the efficacy and safety of short-course neoadjuvant radiotherapy combined with chemotherapy and toripalimab in patients with RLaeSCC. This study was an exploratory study with an expected sample size of 20 patients who underwent tumor resection.

Subjects who met the inclusion criteria were screened for entry into this study. Primary end point: safety (The rates of grade 3 and higher-grade treatment-related adverse events according to CTCAE 5.0). The secondary end point including: pathological complete remission rate (pCR), defined as pT0N0M0 or pTisN0M0; radiographic response (evaluated according to RECIST 1.1 criteria); postoperative complications (evaluated by clavien-Dindo criteria); progression free survival (PFS, the date of enrollment until disease progression, recurrence, death, or the last day of follow-up); overall Survival (OS, the time from the date of enrollment to the date of death from any cause or the last date of follow-up).

5.2 Patient selection

1) Inclusion criteria

a. Be able to provide written informed consent and understand and agree to follow the research requirements and evaluation schedule.

b. Endoscopic biopsy of thoracic esophageal primary lesion histologically diagnosed as squamous cell carcinoma.

c. Clinical stage T1-4aN+M0 or T3-4aN0M0 in the UICC-TNM classification 8th edition.
d. The age is over 20 years old and under 75 on the enrollment date (including 20 and 75), including both female and male.

e. PS 0-1.

f. According to RECIST version 1.1, there were measurable or evaluable lesions.

g. No medical history of treatment for cancer (No medical history of chemotherapy, radiotherapy and endocrine therapy, immune-therapy or other study drugs including treatment for other types of cancer).

h. The results of laboratory tests within 14 days before enrollment meet the inclusion criteria (patients should not receive blood transfusion or growth factor support because neutrophil count, platelet or hemoglobin are lower than the research requirements within 14 days before blood sample collection) : (1) Bone marrow function: hemoglobin (Hb) >= 90 g/L; white blood cell count (WBC) >= lower limit of normal value; absolute neutrophil count (ANC) >= 1.5 x 10^9 /L; platelet count >= 100 x 10^9 / L; (2) Renal function: Cr <= 1.5 UNL, endogenous creatinine clearance rate (Ccr) >= 60 ml/min\(\text{Cockcroft-Gault}\); (3) Liver function: total bilirubin <= 1.5 UNL; ALT and AST <= 2.5 UNL (patients with liver metastases can be relaxed to <=5 UNL); (4) Blood coagulation function: the international standardized ratio of prothrombin time <= 1.5 UNL, and the partial thromboplastin time is within the normal range.

i. Patients with hepatitis B virus (HBV) infection, inactive / asymptomatic HBV carriers, or patients with chronic or active HBV will be allowed to be enrolled if HBV DNA < 500 IU / ml (or 2500 copies / ml) at screening. Patients with positive hepatitis C antibody will be allowed to be enrolled if HCV-RNA is negative during screening. Note: patients who can detect hepatitis B surface antigen (HBsAg) or HBV DNA should be treated with antiviral drugs for more than 2 weeks before enrollment, and the treatment should be continued for 6 months after the study drug treatment.

j. Women of childbearing age (wocbp) should take the urine or serum pregnancy test, and the result of which should be negative within <= 72 hours before treatment.
females, who have agreed with contraception from start of investigational drug administration to 5 months after last dose of investigational drug. For males who have agreed with contraception from start of investigational drug administration to 7 months after last dose of investigational drug.

2) Exclusion criteria

a. Have received any treatment for esophageal squamous cell carcinoma in the past;
b. Patients with evidence or high risk of gastrointestinal hemorrhage or fistula (esophagus / bronchus or esophagus / aorta);
c. Patients with severe malnutrition, with body mass index lower than 18.5kg/m2, or PG-SGA score >= 9
d. Any active autoimmune disease or history of autoimmune disease (as follows, but not limited to: interstitial pneumonia, uveitis, enteritis, autoimmune hepatitis, pituitritis, vasculitis, nephritis, hyperthyroidism, thyroid dysfunction); Subjects with vitiligo or who have had complete remission from childhood asthma without any intervention after adulthood may be included; Asthma requiring medical intervention with bronchodilators was not included.
e. Has a previous radiotherapy, chemotherapy, hormone therapy, surgery, molecular targeted therapy or immune therapy for this malignancy or for any other past malignancy;
f. Any condition requiring systemic corticosteroid therapy (prednisone with a dose higher than 10 mg / day or equivalent dose of similar drugs) or other immunosuppressants within 14 days before treatment. (Excluding the following steroid regimens local, ophthalmic, intra-articular, nasal and inhaled corticosteroids with minimal systemic absorption prophylactic short-term (<= 7 days) use of corticosteroids (e.g., prevention of contrast media allergy) or for the treatment of non-autoimmune disorders (e.g., delayed hypersensitivity caused by exposure to allergens).
g. Live vaccine injection was received in <= 4 weeks before treatment.
h. A history of immunodeficiency, including HIV infection, other acquired or congenital immunodeficiency, or a history of organ or bone marrow implantation that need immunosuppressive medications.

i. There are clinical symptoms or diseases of the heart that are not well controlled, such as:
   - Heart failure above grade 2 by the Criteria of NYHA;
   - Unstable angina pectoris;
   - Myocardial infarction occurred within 1 year;
   - Clinically meaningful supraventricular or ventricular arrhythmias require treatment or intervention;

j. Has severe infections (CTCAE > 2 grade) within 4 weeks before treatment; basal thoracic imaging indicating active pneumonia, or other infectious situation that need oral or intravenous antibiotic treatment (excluding Prophylactic medication for antibiotics).

k. A history of interstitial lung disease, non-infectious pneumonia or uncontrolled disease, including pulmonary fibrosis, acute lung disease, etc.

l. Has active pulmonary tuberculosis found by CT imaging; or has active pulmonary tuberculosis less than 1 year before inclusion; or has active pulmonary tuberculosis but without standard treatment over 1 year before inclusion;

m. Allergic to any drug used in this study.

n. Pregnant or lactating women participants who unwilling to take contraception.

o. Other factors that could lead to the termination of this study.

3) Withdrawal criteria

a. Patients themselves or their legal representatives requested withdraw from the study;

b. Continuation of the treatment protocol detrimental to patients’ health;

c. Esophageal perforation, severe lung/mediastinal infection, bleeding, myocardial infarction, heart failure, severe arrhythmia and other complications occurred
d. Patients with distant metastasis during nICRT;
e. Pregnancy;
f. All patients who dropped out should be followed up according to the study protocol, and the follow-up results should be recorded, unless the patients withdrew the informed consent and refused to accept the study follow-up.

4) Eliminate criteria

a. Violation of the requirements of the research protocol;
b. Poor quality of data recording, incomplete and inaccurate data.

5.3 Examinations and screening of patients

1) Examinations

a. Complete medical history and systemic physical examinations (symptoms, signs, body weight loss and function score). It is generally required to be completed within 7-10 days before recruitment.
b. Pre-treatment examinations:
   - Blood routine, blood type, urine routine, stool routine, biochemical routine, thyroid function, plasma cortisol;
   - Hepatitis virus examination. If HBsAg is positive, HBV-DNA should be tested; If HCV-Ab is positive, HCV-RNA should be tested;
   - Electrocardiogram;
   - Ultrasonic cardiogram (UCG);
   - Lung function tests;
   - Histopathological/cytological diagnosis: pathological examination will be done based on the tissues from endoscopic biopsy;
   - Esophageal barium swallowing;
   - Chest and abdominal CT (with contrast);
◆ High-resolution 3.0-T magnetic resonance imaging for the chest and brain;
◆ Esophagogastroduodenoscopy (EGD), with endoscopic ultrasound (EUS) (optional);
◆ Cervical ultrasonography (optional);
◆ Electronic bronchoscopy or endobronchial ultrasound, if necessary, to confirm the involvement of trachea and/or bronchus;
◆ Positron emission tomography–computed tomography (PET-CT) is optional;
◆ Nutritional risk screening.

2) Screening

Patients will be fully informed about the nature of the study before conducting research related tests. All potential patients will be screened according to the above-mentioned criteria, and those who meet the inclusion criteria and agree to sign informed consent forms will be recruited into the study.

5.4 Treatment plan

1) Neoadjuvant immune-chemotherapy (nICT)

Neoadjuvant toripalimab and chemotherapy are administered on the first day of treatment, and are administered every 21 days for totally two cycles (table 1).

The dosing window is ±3 days from the planned dosing date (based on the first dosing date). If the dosing window period is exceeded, the dosing will be regarded as delayed. During the combination administration, if the delay is expected to exceed 2 weeks due to toxicity of chemotherapy, only toripalimab will be given until the toxicity is restored to the chemotherapy administration standard, and then the combination administration will be resumed. The maximum allowed continuous suspension of chemotherapy is 2 weeks, and the chemotherapy will be terminated after 2 weeks. If the delay is expected to exceed 2 weeks due to toxicity of toripalimab, only chemotherapy will be given until the
toxicity is restored to the toripalimab administration standard, and then the combination administration will be resumed. The maximum allowed continuous suspension of toripalimab is 12 weeks, and the toripalimab will be terminated after 12 weeks. If a delay is required for toxicity reasons (not clear which drug is involved), all three drugs will need to be delayed at the same time if it is expected to return to re-dosing criteria within 2 weeks.

Table 1 nIChT drugs dosage and schedule

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dosage</th>
<th>Schedule</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paclitaxel</td>
<td>135 mg/m²</td>
<td>Day 1 and 22</td>
</tr>
<tr>
<td>Carboplatin</td>
<td>AUC=5</td>
<td>Day 1 and 22</td>
</tr>
<tr>
<td>Toripalimab</td>
<td>240mg</td>
<td>Day 1 and 22</td>
</tr>
</tbody>
</table>

Pretreatment before chemotherapy

- Prophylactic antiemetic therapy:

Acute and delayed vomiting induced by chemotherapy must be prevented.

Aprepitant + 5-HT3 antagonist + dexamethasone is recommended within 1 hour prior to chemotherapy.

- Allergy prevention:

Paclitaxel is pretreated with adrenocortical hormones (e.g., Dexamethasone), diphenhydramine, and H2-receptor antagonists (e.g., Cimetidine or Ranitidine).

- Adjustment for allergy:

Table 2 Adjustment for allergy

<table>
<thead>
<tr>
<th>Allergic symptoms Treatment</th>
<th>Treatment</th>
</tr>
</thead>
</table>
Grade 1: Local skin reactions such as mild itching, flushing and rashes

- Reduce the infusion rate until the symptoms disappear.
- Observe and monitor patients at the wards.
- Then continue dripping all paclitaxel at the original speed.

Grade 2: Any symptoms not listed above (mild symptoms) or below severe symptoms, (eg. systemic pruritus, flushing, rash, dyspnea and hypotension with systolic blood pressure >80 mm Hg)

- Stop dripping paclitaxel.
- Administer DPH 50 mg IV with or without DXM 10 mg IV until the symptoms disappear.
- Then continue dripping paclitaxel at a lower speed and gradually to the original speed.

Grade 3/4: Any severe symptoms such as bronchospasm, systemic rubella, systolic blood pressure ≤80 mm Hg and vascular edema

- Stop dripping paclitaxel.
- Administer DPH 50 mg IV with or without DXM 10 mg IV, and administer adrenaline, if necessary, until the symptoms disappear.
- Report as an adverse event, the patient will go off protocol therapy.

2) Neoadjuvant short course radiotherapy scheme

- **CT simulation:**

  The patient can be placed in the supine position on the fixed frame of the CT scanning bed, and esophageal cancer can be fixed using a head-neck-and-shoulder integrated thermoplastic mask, with the arms parallel to the sides of the body, and the whole body relaxed. The scanning condition can be set as axial scanning with a layer thickness of 3 mm, and the scanning range can be set according to the lesion location and range.
Definition of the targeted area:

Gross tumor volume (GTV): This includes primary tumors (GTVp) and metastatic lymph nodes (GTVn). GTVp is a visible esophageal lesion that can be determined using a combination of imaging techniques (e.g., esophagography, upper gastrointestinal tract radiography, contrast-enhanced CT, MRI, and/or PET/CT) and endoscopy. GTVn refers to metastatic lymph nodes with a diameter of \( \geq 10 \text{ mm} \) (paraesophageal, tracheoesophageal groove \( \geq 5 \text{ mm} \)) as observed on CT and/or MRI or a high SUV (except inflammatory lymph nodes) as observed on PET/CT. Even if the lymph node characteristics are under these standards, those with evident necrosis, circular enhancement, enhancement to a similar degree as that of the primary lesion, and eccentric calcification are also considered as GTVn.

Clinical target volume (CTV): No CTV was delineated in this study.

Planning target volume (PTV): The PTV of the primary tumor (PTVp) included the GTVp with an expansion of 0.8 cm radially for tumors in the upper or middle esophagus or 1.0 cm radially for tumors in the lower esophagus, and 2.0 cm crano-caudally along the esophageal wall. The PTVn was delineated following the principle of involved lesion radiation therapy(32) instead of elective nodal irradiation, including the GTVn with an expansion of 1.0 cm in all directions. PTVtotal is the sum of PTVp and PTVn, which was expanded to include potential gaps between the PTVs.

Target volume delineation

The target volume was firstly proposed by radiation oncologists, followed by consensus meetings held for discussion by both radiation oncologists and surgeons (Figure 1). Further delineation was performed and eventually submitted after consensus was reached.
Radiation oncologist delineate target volumes

Thoracic surgeons review target volumes

Discuss and revise on disagreements

Radiation oncologist modify the target volumes

Thoracic surgeons re-review

Submit to radiotherapy physicists

Figure 1 Steps in the consensus process for the development of target volumes delineation.

* Treatment dosage and course *

Sequential short-course neoadjuvant radiotherapy (30 Gy in 12 fractions, 2.5Gy per fraction, 5 days per week) was administered as “sandwich therapy” from day 3 to day 18.

* Organs at risk (OARs) *

Organs at risk (OARs) include both lungs, heart, spinal cord, and liver. Dose-Volume-Histograms (DVHs) will be used to select the most appropriate treatment plan and to evaluate the normal tissue damage. Standard dose constraints are applied for treatment plan: mean lung dose <14 Gy, total lung volume receiving greater than 20 Gy (V20) of < 28%, V30 < 18%, V5 < 65%, heart V30 < 40%, liver V30 < 30%, and maximum spinal cord dose <45 Gy. Every effort should be made to keep the total lung dose to a minimum.

* Criteria for radiation-related toxicity *
Continue radiotherapy if grade 3 toxicity is unrelated to radiotherapy. Radiotherapy will be withheld if any grade 4 toxicity is observed.

When grade 3 radiation-related toxicity is observed, active symptomatic treatment will be administered and radiotherapy will be withheld until the toxicity has recovered to grade 2.

If any of the following toxicity is present, patients will be excluded from the treatment protocol: heavy hemorrhage, non-healing esophageal tracheal leakage, myocardial infarction, heart failure, severe arrhythmias, and severe radiation pneumonia with dyspnea.

3) Principles for the adjustment of dosage of drugs

- **Toripalimab**

Adverse effect (AE) related to toripalimab may be associated to the immune system, which may occur at the first administration or a few months after the last administration. When symptoms listed below occur (table 3), the administration of toripalimab should be suspended or terminated if necessary. The resumption of regime is no longer than 12 weeks, otherwise it should be terminated.

Table 3 Recommended treatment modifications for Toripalimab.

<table>
<thead>
<tr>
<th>Immune related adverse reactions</th>
<th>Severity*</th>
<th>Treatment modification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pneumonitis</td>
<td>Grade 2</td>
<td>Suspend until adverse reactions improve to grade 0-1</td>
</tr>
<tr>
<td></td>
<td>Grade 3-4 or recurrent grade 2</td>
<td>Permanently discontinue</td>
</tr>
<tr>
<td>Diarrhea and Colitis</td>
<td>Grade 2-3</td>
<td>Suspend until adverse reactions improve to grade 0-1</td>
</tr>
<tr>
<td></td>
<td>Grade 4</td>
<td>Permanently discontinue</td>
</tr>
<tr>
<td>Condition</td>
<td>Criteria</td>
<td>Action</td>
</tr>
<tr>
<td>-------------------</td>
<td>---------------------------------------------------------------------------</td>
<td>---------------------------------------------</td>
</tr>
<tr>
<td><strong>Hepatitis</strong></td>
<td>Grade 2 with aspartate aminotransferase (AST) or alanine aminotransferase (ALT) of 3-5 x Upper Limit of Normal (ULN) or total bilirubin of 1.5-3 x ULN</td>
<td>Suspend until adverse reactions improve to grade 0-1</td>
</tr>
<tr>
<td></td>
<td>Grade 3-4 with AST or ALT &gt; 5 x ULN, or total bilirubin &gt; 3 x ULN</td>
<td>Permanently discontinue</td>
</tr>
<tr>
<td><strong>Nephritis</strong></td>
<td>Grade 2-3 with blood creatinine increased</td>
<td>Suspend until adverse reactions improve to grade 0-1</td>
</tr>
<tr>
<td></td>
<td>Grade 4 with blood creatinine increased</td>
<td>Permanently discontinue</td>
</tr>
<tr>
<td><strong>Endocrine disorders</strong></td>
<td>Symptomatic grade 2-3 hypothyroidism, grade 2-3 hyperthyroidism, grade 2-3 hypophysitis, grade 2 adrenal insufficiency Grade 3 hyperglycaemia or type I diabetes mellitus</td>
<td>Suspend until adverse reactions improve to grade 0-1</td>
</tr>
<tr>
<td></td>
<td>Grade 4 hypothyroidism</td>
<td>Permanently discontinue</td>
</tr>
<tr>
<td></td>
<td>Grade 4 hyperthyroidism</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Grade 4 hypophysitis Grade 3-4 adrenal insufficiency</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Grade 4 hyperglycaemia or type I diabetes mellitus</td>
<td></td>
</tr>
<tr>
<td><strong>Skin adverse reactions</strong></td>
<td>Grade 3 rash</td>
<td>Suspend until adverse reactions improve to grade 0-1</td>
</tr>
<tr>
<td></td>
<td>Grade 4 rash, Stevens-Johnson syndrome (SJS) or toxic epidermal necrolysis (TEN)</td>
<td>Permanently discontinue</td>
</tr>
<tr>
<td><strong>Thrombocytopenia</strong></td>
<td>Grade 3</td>
<td>Suspend until adverse reactions improve to grade 0-1</td>
</tr>
<tr>
<td></td>
<td>Grade 4</td>
<td>Permanently discontinue</td>
</tr>
<tr>
<td><strong>Others</strong></td>
<td>Grade 2-3 blood amylase increased or lipase increased</td>
<td>Suspend until adverse reactions improve to grade 0-1</td>
</tr>
<tr>
<td>Grade 2 pancreatitis</td>
<td>Grade 2 myocarditis a</td>
<td></td>
</tr>
<tr>
<td>----------------------</td>
<td>----------------------</td>
<td></td>
</tr>
<tr>
<td>Grade 2-3 other immune-associated adverse reactions of first occurrence</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Grade 4 blood amylase increased or lipase increased</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade 3-4 pancreatitis</td>
</tr>
<tr>
<td>Grade 3-4 myocarditis</td>
</tr>
<tr>
<td>Grade 3-4 encephalitis</td>
</tr>
<tr>
<td>Grade 4 other immune-associated adverse reactions of first occurrence</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Permanently discontinue</th>
</tr>
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</table>

**Recurrent or persistent adverse reaction**

<table>
<thead>
<tr>
<th>Recurrent grade 3-4 (except endocrine disorders)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade 2-3 adverse reaction not improved to grade 0-1 within 12 weeks after the last dose (except endocrine disorders)</td>
</tr>
<tr>
<td>Corticosteroid unable to be reduced to ≤10 mg/day prednisone equivalent dose within 12 weeks after the last dose</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Permanently discontinue</th>
</tr>
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</table>

**Infusion related reaction**

<table>
<thead>
<tr>
<th>Grade 2</th>
</tr>
</thead>
</table>

Reduction of infusion rate or suspension of administration, if the symptom is relieved, re-administration may be considered and patients be under close observation.

<table>
<thead>
<tr>
<th>Grade 3-4</th>
</tr>
</thead>
</table>

Immediately and permanently discontinue the dose and give symptomatic treatment.
Chemotherapy

Highest dose of chemotherapy is given and adjusted according to the most severe toxicity. Patients experience febrile neutropenia, grade 4 neutropenia or thrombocytopenia, grade 2/3 peripheral nerve toxicity and grade ≥3 non-hematological toxicity with receive 20% dose reduction of both paclitaxel and carboplatin. Other severe AEs such as grade 4 peripheral nerve toxicity will lead to a termination of the treatment. The minimum dose should be selected if multiple toxicities occur. Chemotherapy can only be delayed for up to 2 weeks, otherwise it should be terminated.

4) Surgery

The Ivor Lewis operation (right transthoracic esophagectomy with reconstruction and laparoscopic dissection) and the McKeown operation (right thoracotomy, laparoscopy dissection, and left cervical esophagectomy with reconstruction) are the usual procedures used for esophagectomy at our institution, which are widely used in China. Circular stapler anastomosis was performed. The definition of the two-field lymph node dissection was resection of the mediastinal and abdominal lymph node stations; in addition, the right recurrent laryngeal nerve chain was fully dissected, but the left recurrent laryngeal nerve chain was only dissected in select patients with suspected metastatic lymph nodes.

An esophagectomy was planned four to six weeks after the completion of neoadjuvant therapy according to the CROSS and 5010 study(1, 2). Among the first eight patients who received surgery within six weeks after the neoadjuvant treatment, two of them had Grade IIIb surgical complications: one with anastomotic leakage and one with anastomotic leakage and pleural cavity hematocele. Based on the experiences of our thoracic surgery team, a longer interval appears to facilitate patients' recovery from neoadjuvant treatment-related acute toxicity, without concomitant escalation in tissue fibrosis, which could complicate surgical procedures. Therefore, following a discussion meeting and
obtaining approval from all members of the research team, the time interval was extended to over eight weeks.

5.5 Endpoints evaluation

1) Toxicity evaluation

Adverse effects were collected from date of treatment allocation until surgery was applied during study period or up to at least 90 days after last dose. Therapeutic toxicity is evaluated according to CTCAE 5.0 criteria.

2) Radiographic evaluation

All radiographic images were independently analyzed by two experienced radiologists.

◆ Evaluation time: Before neoadjuvant treatment, at the end of nCRT and before surgery.

◆ Evaluation measures: chest and abdomen enhanced CT, high-resolution 3.0-T magnetic resonance imaging, and esophageal barium x-ray.

◆ Evaluation criteria: Radiological responses were recorded based on the Response Evaluation Criteria in Solid Tumors (RECIST), version 1.1.(33).

Complete response (CR) is defined as the disappearance of lesion in esophagus by utilizing comprehensive methods: 1) primary tumor: a normalized esophageal wall or only a thin area of hypointense signal without distortion of the wall on T2W images and the absence of hyperintense signal on DWI(34); showed no thickening of esophageal wall and smooth surface of esophageal outer membrane on CT images. 2) lymph-node: no radiographic evidence of disease on thin-slice computed tomography images (1 mm).

Partial response (PR) is defined as at least a 30% decrease in the sum of
diameters of target lesions, taking as reference the baseline sum diameters.

Progressive disease (PD) is defined as at least a 20% increase in the sum of diameters of target lesions (an absolute increase of at least 5 mm), or the appearance of one or more new lesions.

Stable disease (SD) is defined as neither sufficient decrease to qualify for partial response nor sufficient increase to qualify for progressive disease.

Note: According to CT evaluation, the longest diameter of esophageal lesions is defined as the sum of the maximum diameters of the longitudinal axis and the horizontal axis, in which the maximum diameters of the horizontal axis are defined as the length of the maximum cross-section of the tumor minus the length of the central cavity on the same measurement line.

3 ) Pathological evaluation

The surgical specimens were staged according to the criteria of the American Joint Committee on Cancer (eighth edition) by two expert onco-pathologists independently. Routine hematoxylin and eosin staining of primary tumors was assessed for pathological regression according to the criteria of the College of American Pathologists/National Comprehensive Cancer Network.(35) Since there is no consensus about carcinoma in situ (CIS) classification, we considered CIS as a pCR as stated in the Miller and Payne system for breast cancer.(36)

Scanned slides containing lymph node slices were identified, reviewed, and classified according to Martin-Romano et al (37) as: TRG-A: ‘true-negative’ LN without evidence of tumour or tumour regression; TRG-B: LN with viable tumour without evidence of tumour regression (no fibrosis, no mucin pools); TRG-C: LN with viable tumour and evidence of tumour regression (fibrosis or mucin pools or both); TRGD: LN without viable tumour and evidence of tumour regression (fibrosis or mucin pools or both) interpreted as ‘complete tumour regression’. Patients with only TRG-A LNs were classified as ‘true_ypN0’; while,
patients with only TRG-D LNs were classified as ‘complete responders’. All other patients were classified as ‘incomplete responders’.

Programmed death ligand 1 (PD-L1) expression was determined using the 22C3 pharmDx kit (Dako North America Inc., Carpinteria, CA, USA), according to the manufacturer’s instructions, and the combined positive score (CPS) was defined as reported previously.\(^{(15)}\)

4 ) Post-operative complications evaluation

Post-operative complications were carefully recorded within 30 days after surgery and evaluated according to the Clavien-Dindo classification of surgical complications (38).

Table 4 Post-operative complications evaluation according to the Clavien-Dindo classification

<table>
<thead>
<tr>
<th>Grades</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Grade I</strong></td>
<td>Any deviation from the normal postoperative course without the need for pharmacological treatment or surgical, endoscopic and radiological interventions. Allowed therapeutic regimens are: drugs as antiemetics, antipyretics, analgetics, diuretics and electrolytes and physiotherapy. This grade also includes wound infections opened at the bedside.</td>
</tr>
<tr>
<td><strong>Grade II</strong></td>
<td>Requiring pharmacological treatment with drugs other than such allowed for grade I complications. Blood transfusions and total parenteral nutrition are also included.</td>
</tr>
<tr>
<td>- IIIa</td>
<td>Intervention not under general anesthesia</td>
</tr>
<tr>
<td>- IIIb</td>
<td>Intervention under general anesthesia</td>
</tr>
<tr>
<td><strong>Grade III</strong></td>
<td>Requiring surgical, endoscopic or radiological intervention</td>
</tr>
<tr>
<td><strong>Grade IV</strong></td>
<td>Life-threatening complication (including CNS complications)* requiring IC/ICU-management</td>
</tr>
<tr>
<td>- IVa</td>
<td>single organ dysfunction (including dialysis)</td>
</tr>
<tr>
<td>- IVb</td>
<td>Multi-organ dysfunction</td>
</tr>
<tr>
<td><strong>Grade V</strong></td>
<td>Death of a patient</td>
</tr>
</tbody>
</table>

*Brain hemorrhage, ischemic stroke, subarachnoidal bleeding, but excluding transient ischemic attacks (TIA); IC: Intermediate care; ICU: Intensive care unit.
5.6 Statistical analysis

As an exploratory study, a sample size of 20 patients who underwent tumor resection was determined.

The intent-to-treat (ITT) population included all eligible patients, regardless of the treatment they received. Analyses exploring the relationship between nICRT and safety were performed using the safety set (all patients who received neoadjuvant radiotherapy and at least one dose of neoadjuvant chemotherapy or immunotherapy). The modified ITT population included all patients who underwent surgery and had surgery results available for the end point analysis.

Continuous variables were presented as the median with the range or the mean with the standard deviation. Categorical variables were presented as a frequency with percentage. Continuous variables were compared by the t-test. Survival was estimated using the Kaplan–Meier method, and the log-rank test was used for comparisons between groups.

Progression-free survival (PFS) was defined as the time from the date of enrollment until disease progression, recurrence, death, or the last day of follow-up. OS was defined as the time from the date of enrollment to the date of death from any cause or the last date of follow-up.

The Wilcoxon rank-sum test was used to compare the scores of immune cell infiltration and the immune signature between groups (pCR vs. non-pCR, pre- and post-treatment). All statistical analyses were performed using SPSS 20.0 and R version 4.1.1 (https://www.r-project.org). P-values were two sided, with a significance level of 0.05 for all analyses.

5.7 Follow-up

After the treatment has ended, patients will be re-examined in the hospital clinics once every 3 months within 2 years. Thereafter they will have regular follow-up visits once every 6 months until their deaths or the study ends. Physical
examination, tumor markers, contrast-enhanced chest and upper abdominal computed tomography, esophageal barium x-ray will be performed at follow-up visits. Cervical/abdominal ultrasonography and esophagogastroduodenoscopy were optional during the follow-up.

5.8 Ethics

1) Informed consent

Before patients’ recruitment, investigator should completely and comprehensively explain the objective of this study, the characteristics of drugs, and the potential toxicity and risk in the treatment, and allow the patients to be aware of their rights, risks and benefits. Informed consent form should be signed before recruitment and preserved in files as paper documentation.

2) Ethics and policy

This study will be conducted according to the Declaration of Helsinki (2000), Good Clinical Practice (GCP) published by CFDA and other relevant regulations. The study must be approved by the Ethics Committee from leading center and each participating institution. Any amendments of the study protocol should be re-approved by the Ethics Committee during the study.

6 References


chemotherapy regimen and chemoradiotherapy regimen as neoadjuvant treatment for locally advanced esophageal cancer, JCOG1109 NExT study. *Journal of Clinical Oncology* 2022;40(4_suppl).


