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

Ning Jiang,1 Jingyuan Zhang,2 Zhen Guo,3 Yinan Wu,2 Lijun Zhao,1 Cheng Kong,1 Xue Song,1 Lingling Gu,3 Yang Zhao,4 Si Li,5 Xia He,1 Binhui Ren,6 Xiangzhi Zhu,1 Ming Jiang6

ABSTRACT
Background The optimal dosages, timing, and treatment sequencing for standard-of-care neoadjuvant chemoradiotherapy necessitate re-evaluation when used in conjunction with immune checkpoint inhibitors for patients with resectable, locally advanced esophageal squamous cell carcinoma (RLeSCC). The SCALE-1 phase Ib study aimed to evaluate the safety and efficacy of short-course neoadjuvant radiotherapy combined with chemotherapy and toripalimab in this patient population.

Methods RLeSCC patients with clinical stages cT3-4aN0M0 or cT1-4aN+M0 received neoadjuvant paclitaxel (135 mg/m²), carboplatin (area under the curve=5), and toripalimab (240 mg) every 3 weeks for two cycles. Short-course neoadjuvant radiotherapy (30 Gy in 12 fractions; 5 days per week) was administered between neoadjuvant immune-chemotherapy (nCT) doses. Esophagectomies were scheduled 4–6 weeks after completing neoadjuvant treatment. The primary endpoint was safety, with secondary endpoints including pathological complete response (pCR) rate, postoperative complications, progression-free survival (PFS), and overall survival (OS). Exploratory biomarker analysis used gene expression profiles via the nCounter platform.

Results Of the 23 patients enrolled, all completed neoadjuvant radiotherapy, while 21 cases finished full nCT doses and cycles. Common grade 3/4 adverse events included neutropenia (57%), leukopenia (39%), and skin rash (30%). No grade 3 or higher esophagitis or pneumonitis occurred. Twenty patients underwent surgery, and 11 achieved pCR (55%). Two patients (10%) experienced grade IIb surgical complications. At the database lock, a 2-year PFS rate of 63.8% (95% CI 43.4% to 84.2%) and 2-year OS rate was 78% (95% CI 64.9% to 91.1%) were achieved. Tumor immune microenvironment analysis indicated that tumors with pCR exhibited significantly higher pretreatment T-cell-inflamed score and post-treatment reshaping of antitumor immunity.

Conclusions Combining short-course neoadjuvant radiotherapy with chemotherapy and toripalimab demonstrated favorable safety and promising efficacy in RLeSCC patients.

WHAT IS ALREADY KNOWN ON THIS TOPIC
⇒ The combined approach of neoadjuvant chemoradiotherapy (nCRT) and immune checkpoint inhibitors holds promise for patients with resectable, locally advanced esophageal squamous cell carcinoma (RLeSCC). However, concerns regarding possible toxicity linked to the combination necessitate a reevaluation of the conventional intensity of nCRT in clinical practice.

WHAT THIS STUDY ADDS
⇒ This study demonstrated that short-course neoadjuvant radiotherapy with a reduced prescribed dose, in combination with chemotherapy and toripalimab, a humanized programmed cell death protein 1 (PD-1) monoclonal antibody (the SCALE regimen), exhibits promising efficacy and favorable toxicity profiles in RLeSCC patients.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY
⇒ The SCALE regimen offers a promising neoadjuvant treatment option for patients with RLeSCC. Further clinical trials are necessary to validate these findings, and the knowledge generated by this study may inform future research, clinical practice, or policy decisions.

BACKGROUND
Esophageal cancer (EC) is the sixth-leading cause of cancer deaths worldwide. China has a high prevalence of EC, accounting for 50% of the global morbidity and mortality, with 90% being esophageal squamous cell
carcinoma (ESCC). Neoadjuvant chemoradiotherapy (nCRT) followed by surgery constitutes the established standard of care for patients with resectable, locally advanced ESCC (RLaESCC). Nonetheless, tumor recurrence persists in 40%–50% of patients postsurgery. Adding to this, only 20.1% of resectable ESCC patients in China undergo nCRT, with nCRT-related postoperative complications and mortality being the major concerns. There is a pressing need to explore more effective and less toxic neoadjuvant regimens and strategies for RLaESCC.

Increasing evidence shows that immune checkpoint inhibitors (ICIs) may help to diminish tumor recurrence by eradicating radiographically occult diseases and enhancing systemic immunity. Among patients with resectable EC who retain residual pathological disease following nCRT, the use of adjuvant nivolumab has shown a correlation with diminished risks of distant recurrence and mortality. In metastatic EC, ICIs alone or in combination with chemotherapy have been proven to benefit patient survival. Hence, it is reasonable to move ICIs to an upfront setting, as a part of neoadjuvant treatment, to achieve better clinical outcomes.

Nonetheless, there might be a need to reassess the intensity and regimens of conventional nCRT when incorporating immunotherapy. Reports indicate that conventional nCRT may exhibit a greater incidence of therapy-related non-cancer fatalities compared with neoadjuvant chemotherapy. Furthermore, several phase I/II studies investigating the combination of ICIs with nCRT have reported treatment-related deaths in RLaESCC. Therefore, deintensified nCRT in combination with ICIs might be an option to reduce both long-term and short-term toxicities.

Recent studies have shown that shorter treatment courses and hypofractionated radiotherapy induced a better synergistic effect in combination with ICIs. Hence, in this phase Ib SCALE-1 study, patients with RLaESCC were administered a combination treatment involving short-course radiotherapy with higher fraction doses and reduced total dose, along with chemotherapy and toripalimab—a humanized programmed cell death protein 1 (PD-1) monoclonal antibody. The primary goal was to assess the safety of this novel neoadjuvant immune-chemoradiotherapy (nICR) approach.

**PATIENTS AND METHODS**

**Study design**

This prospective, single-arm phase Ib study was conducted at Jiangsu Cancer Hospital, China. The primary outcome was safety. Any grade of treatment-related adverse events (TRAEs) was closely monitored and recorded based on the National Cancer Institute Common Terminology Criteria for Adverse Events (version 5.0). The key secondary endpoints included the pathological complete response (pCR) rate, radiological response rate, postoperative complications, progression-free survival (PFS) and overall survival (OS). Complications within 30 days after surgery were rated according to the Clavien-Dindo system. The study protocol was included in online supplemental materials.

**Patients**

Eligible patients were those with histologically confirmed, resectable thoracic ESCC clinically staged as T1-4aN+M0 or T3-4aN0M0 before treatment; aged 20–75 years old; with an Eastern Cooperative Oncology Group Performance Status score of 0 or 1; normal hematological, renal, and hepatic function; and adequate pulmonary function. Key exclusion criteria included a high risk of gastrointestinal hemorrhage or fistula, immunodeficiency, ongoing systemic immunosuppressive therapy, active autoimmune or infectious disease, and clinically significant concurrent cancer.

**Pretreatment staging and radiological evaluation**

All patients underwent baseline tumor staging, including pretreatment upper gastrointestinal endoscopy and biopsy, contrast-enhanced chest and upper abdominal CT, high-resolution 3.0T MRI for the chest and brain, and upper gastrointestinal tract radiography. Ultrasonography of the neck with fine-needle aspiration was performed when cervical lymph node involvement was suspected. High-resolution MRI and thin-slice CT images (1 mm) were used for clinical tumor–lymph node–metastasis staging. The radiological responses of the primary tumor and lymph node were independently assessed by two experienced radiologists as described previously, following Radiological the Response Evaluation Criteria in Solid Tumors version 1.1, which was outlined in the protocol of this study.

**Neoadjuvant and adjuvant treatment**

The patients received two doses of intravenous toripalimab (240 mg) in combination with paclitaxel (135 mg/m²) and carboplatin (area under the curve=5) on day 1 and day 22. Sequential short-course neoadjuvant radiotherapy (30 Gy in 12 fractions, 5 days per week) was administered as “sandwich therapy” from day 3 to day 18. Target volumes were delineated following the principle of involved lesion radiation therapy through deliberations among radiation oncologists and surgeons (elaborated in online supplemental material protocol), with any differences in opinion being documented. Patients were offered adjuvant treatment (ICI or ICI in combination with chemotherapy) at the investigators’ discretion, depending on the efficacy (ie, pathological responses), tolerance of treatment, and general postoperative condition, and were followed up for PFS and OS.

**Surgery**

An esophagectomy was initially planned 4–6 weeks after completing neoadjuvant therapy. The time interval was extended to over 8 weeks due to perioperative complications observed. The Ivor Lewis operation (right transthoracic esophagectomy with reconstruction and
laparoscopic dissection) and the McKeown procedure (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.

Pathological evaluation

The surgical specimens were staged according to the criteria of the American Joint Committee on Cancer (eighth edition) by two expert oncopathologists independently. Routine H&E staining of primary tumors was assessed for pathological regression according to the criteria of the College of American Pathologists/National Comprehensive Cancer Network. Since there is no consensus about carcinoma in situ (CIS) classification, we considered CIS as a pCR as stated in the Miller and Payne consensus about carcinoma in situ (CIS) classification, as reported previously. Scanned slides containing lymph node slices were identified, reviewed, and classified, as described previously. Programmed death ligand 1 (PD-L1) expression was determined using the 22C3 pharmDx kit (Dako North America, Carpinteria, California, USA), according to the manufacturer’s instructions, and the combined positive score (CPS) was defined as reported previously.

Tumor immune microenvironment analysis based on transcriptional profiling

For tumor immune microenvironment (TIME) analysis, RNA was isolated from pretreatment biopsies and resected formalin-fixed paraffin-embedded FFPE samples using an RNAsafe FFPE kit (Qiagen, Valencia, California, USA) and was directly inserted into the nCounter platform (NanoString Technologies, Seattle, Washington, USA) to assess the expression of 289 immune-related genes (online supplemental appendix table 1). Gene counts were normalized using housekeeping genes. Differentially expressed genes (DEGs) between groups were selected with the DESeq2 package (p<0.01 and expression fold change (FC)≥2 or ≤1/2). Gene Ontology enrichment analysis was performed to examine the immune processes in which the DEGs were involved (p<0.05). Infiltration scores of 14 types of immune cells and 9 immune signatures were calculated based on the expression level of the marker genes (online supplemental appendix table 2) and were visualized by heatmap analysis without clustering.

Statistical analysis

As an exploratory study, a sample size of 20 patients who underwent tumor resection was determined. The intention-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 SD. 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. PFS was defined as the time from the date of enrolment 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, pretreatment and post-treatment). All statistical analyses were performed using SPSS V.20.0 and R V.4.1.1 (https://www.r-project.org). P values were two sided, with a significance level of 0.05 for all analyses.

RESULTS

Patient characteristics

Twenty-six patients with pathologically confirmed thoracic ESCC were screened from January 29, 2021 to November 3, 2021, and 23 were eligible for inclusion in this study (figure 1). The demographic and baseline characteristics of the patients are listed in table 1. The included patients had a median age of 66 years old (range: 37–72 years old) and a median tumor length of 5.1 cm (range: 3.0–9.6 cm). Approximately 57% of the tumors were located at the middle third of the thoracic esophageal. The clinical stages were cT2N1/cT3N0 (n=5), cT3N1 (n=9), and cT4A-T4N0 (n=9). Most of the patients were classified as having clinical stage III or IVA tumors (n=18, 78%).

Neoadjuvant treatments

Throughout the neoadjuvant treatment period, all patients experienced TRAEs of any grades (table 2). Grade 3/4 TRAEs included neutropenia (n=13, 57%), leukopenia (n=9, 39%), skin rash (n=7, 30%) and elevated yglutamyltransferase (n=2, 9%) (table 2). Notably, no grade 3 or higher radiation esophagitis or pneumonitis occurred. All the patients successfully completed neoadjuvant radiotherapy, with 21 (91.3%) patients fulfilling the regimen involving two planned doses of chemotherapy along with toripalimab. During the administration of the second dose of neoadjuvant immune-chemotherapy, one patient developed a grade 3 skin rash while receiving paclitaxel, leading to the discontinuation of the drug. Another patient experienced a grade 3 skin rash along with grade 4 leukopenia and neutropenia, prompting a reduced second dose of chemotherapy without toripalimab.

The evaluation of radiological responses was performed after neoadjuvant treatment and prior to surgery: 13 patients (57%) had a CR, and 10 (43%) patients had a partial response. No patients showed disease progression, resulting in an objective response rate and a disease control rate of 100%. Representative radiological images are shown in online supplemental appendix figure 1.

Disagreements concerning target volume delineation arose between radiation oncologists and thoracic surgeons in 11 out of 23 patients (48%). Among these, five disagreements pertain to the inclusion of small lymph nodes that approached diagnostic criteria, while the remaining six centered around reducing irradiating to potential anastomosis areas. Illustrative images can be found in online supplemental appendix figure 2.

Surgery and postoperative complications
Twenty out of 23 (87%) patients underwent surgery (table 3). Surgery cancelations were attributed to tuberculosis reactivation in one patient and personal decisions made by two patients. During surgery, one patient with clinical stage III disease was found to have extracapsular invasion of the lymph nodes, so a complete tumor resection could not be performed (R0 resection rate: 19/20, 95%). The mean duration of surgery was 345.9±45.9 min. The mean number of resected lymph nodes examined for pathology was 20 (range: 10–29) after the lymphadenectomy (table 3).

Eight out of 20 patients (40%) developed postoperative complications (table 3). Among these cases, one patient with a pleural cavity hematoma and another patient with anastomotic leakage plus hemorrhage received a second surgery (grade IIIb) (online supplemental appendix figure 3). One patient with anastomotic leakage and the other with airway sputum obstruction underwent surgery and postoperative complications.

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**Table 1** Baseline characteristics for the ITT population

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<th>Characteristic</th>
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Data are no (%) unless otherwise indicated.
*The clinical stages were evaluated according to the criteria of the American Joint Committee on Cancer, eighth edition.
BMI, body mass index; ITT, intention-to-treat population; N, node; ECOG PS, Eastern Cooperative Oncology Group Performance Status; T, tumor.
radiological or endoscopic interventions without general anesthesia (grade IIIa). In addition, four patients who exhibited tachycardias or hypotension postsurgery received vasoactive agents and/or blood transfusions (grade II).

Notably, all eight complications were observed in patients who underwent surgery within 8 weeks from the conclusion of neoadjuvant treatment (table 3). In the subsequent cases, the time interval was extended to over 8 weeks after neoadjuvant treatment, resulting in the absence of perioperative complications. The median interval between the conclusion of neoadjuvant treatment and surgery was 49.5 days (range: 30–70 days). In an effort to understand the underlying cause, a comparison of body weights was conducted between patients who underwent surgery within 8 weeks and those beyond this time frame. While the difference did not reach statistical significance, there was a trend toward increased body weight in patients undergoing surgery after 8 weeks (p=0.198) (online supplemental appendix figure 4).

**Pathological findings**

All 20 patients who underwent surgery showed pathological T or N stage downstaging (online supplemental...
appendix figure 5, table 3), with 11 (55%) achieving a pCR in both the primary tumor and lymph nodes (figure 2). One case showed residual tumor cells in one node, which was not considered as a pCR. A major pathological response (MPR) was observed in 16 (80%) patients (figure 2). Notably, CIS was observed in specimens from 9 out of 12 patients with a pCR in the primary tumors.

PD-L1 expression could be evaluated in pretreatment biopsy samples from 16 patients (figure 2, online supplemental appendix figure 6). There was no difference in the pCR rate between patients with CPS≥5% and <5% (p=0.315). Histological examination of the tumor bed tissue sections revealed inflammatory cell infiltration (20/20), vascular formation (16/20), fibrosis with hyalinosis (16/20), tertiary lymphoid structures (14/20), foamy cell aggregation (13/20), multinucleated cells (10/20), and necrosis (1/20) (online supplemental appendix figure 6).

**Survival**

At the time of analysis (cut-off date: May 28, 2023), with a median follow-up time of 24.5 months (range: 13.2–28.1), 18 out of 23 patients (78.3%) were alive, and 15 (65.2%) were recurrence-free. The median durations of PFS and OS had not been reached. The rate of PFS and OS in the ITT population at 24 months was 63.8% (95% CI 43.4% to 84.2%) and 78.0% (95% CI 64.9% to 91.1%), respectively (figure 3).

Eight patients showed tumor recurrence and five of them died from disease progression. Two patients showed brain metastasis at 13.6 months and 2.5 months after surgery. The first patient refused further treatment and died 49 days later. The second patient received stereotactic radiosurgery and ICI but died from cancer-related cachexia at 11.1 months after surgery. The third patient who underwent R1 resection, had pelvic lymph node metastasis 2 months after surgery. Despite receiving
chemotherapy and ICI, this patient showed abdominal and pelvic effusion and died 9 months later. Other two patients showed local regional recurrence 2.6 and 12.2 months after surgery and died from cancer progression 11.4 and 4.4 months later, respectively.

Three patients with cancer recurrence were still alive at the latest follow-up. One had left supraclavicular lymph node recurrence 6.5 months after surgery and received radiotherapy in combination with chemotherapy and ICI. This patient showed no further progression. The second patient had a solitary celiac lymph node metastasis 3.5 months after surgery and received radiotherapy to the recurrent site. The third patient received no surgery due to tuberculosis reactivation was diagnosed with peritoneal metastasis 23.3 months after the completion of neoadjuvant treatment. These two patients were still receiving anticancer treatment at the latest follow-up.

Among the seven patients who were pathologically evaluable and exhibited tumors recurrence, four cases showed residual tumors in lymph nodes (pN+). In one instance, residual tumors were identified within the primary tumor site, while the other two patients with brain metastasis achieved ypTisN0 after surgery.

**Molecular biomarker detection and analysis**

The pretreatment TIME was compared between the pCR (n=7) and non-pCR (n=6) groups. Among the scores of cell infiltration and immune signatures, the T-cell-inflamed score (TIS) was significantly higher in the pCR group (figure 4A,B, online supplemental appendix figure 7A). The DEGs among groups were mostly involved in the response to interferon gamma (online supplemental appendix figure 7B,C). After treatment, both groups revealed an increased infiltration score of dendritic cells, M2 macrophages, and mast cells (figure 4C). Tumors with a pCR showed additional higher levels of CD45+, T-cell and cytotoxic cell infiltration (p<0.05) (figure 4C), and an increasing trend of CD8+ T cells (p=0.078) (online supplemental appendix figure 8C). The immune signatures of effector T cells, T-cell markers, cytotoxic immune cells, cytotoxic T lymphocytes, and cytolytic activity also showed increased scores after treatment in the pCR group (figure 4D). Moreover, the signature of melanoma-associated antigens, a tumor-specific antigen, presented a significant decrease (figure 4D). A similar trend of immune signatures was observed in the non-pCR group, while none achieved a significant change (online supplemental appendix figure 8). In summary, the TIS and interferon-gamma response pathways might be associated with the antitumor effect and pathological remission, and tumors with a pCR showed a relatively greater reshaping of antitumor immunity after neoadjuvant treatment.

**DISCUSSION**

The SCALE-1 study introduced an innovative neoadjuvant regimen involving deintensified radiotherapy combined with immunotherapy and dose-reduced chemotherapy for RLaESCC. Our initial findings suggest that this approach is associated with manageable TRAEs while showing lower occurrences of esophagitis and pneumonitis. Additionally, it showcases a promising pCR rate of 55%, a notable outcome when compared with the standard nCRT approach, with rates of 49% in the ESCC cohort from the CROSS study and 43.2% in the NEOCRTEC5010 study, as reported in the literature. Importantly, there was no instances of postoperative mortality or an elevated risk of surgical complications associated with this treatment regimen. Implementing this neoadjuvant protocol also allowed for a reduction in the preoperative treatment duration from 5 weeks to 3 weeks, while maintaining high levels of locoregional control and tolerability. This could potentially lead to cost savings while offering substantial benefits.
Figure 4  Comparison of the pretreatment tumor immune microenvironment (TIME) between tumors that achieved pCR and non-pCR, and the change of TIME after treatment. (A) Boxplot representing the T-cell-inflamed score (TIS), which appears significantly higher in tumors that achieved a pathological complete response (pCR) compared with non-pCR tumors (Wilcoxon rank-sum test, p<0.05); (B) Heatmap of the expression levels of 18 genes constructing the TIS score (rows denote genes and columns represent samples; red indicates relatively upregulated, while blue indicates downregulated); (C) change of immune cell infiltration scores between pretreatment and post-treatment in the pCR and non-pCR groups (Wilcoxon rank-sum test, p<0.05); (D) change of immune signature scores between pretreatment and post-treatment in the pCR and non-pCR groups (Wilcoxon rank-sum test, p<0.05).
Safety was one of the major concerns of this study, prompted by the possible increase in both short-term and long-term toxicities associated with nCRT, whether used alone or in combination with ICIs. \(^4\) \(^5\) \(^20\) \(^21\) \(^35\) Compared with conventional nCRT, radiotherapy in our study had a lower biologically effective dose (BED), increased fraction dose, shorter treatment course, and smaller target volume. \(^24\) \(^25\) The dose of paclitaxel was also reduced to 135 mg/m\(^2\) in comparison to 175 mg/m\(^2\) in other studies. \(^19\) With a median follow-up time of 24.5 months, our results reported no treatment-related deaths, and the pattern of any grade AEs was similar to that of the nCRT. \(^4\) \(^5\) Notably, the incidence of grade 1/2 radiation esophagitis was 13.0%, and no grade 3 or higher esophagitis was observed, which was lower than that reported in the NEOCRT5010 and CROSS studies. \(^4\) These results indicated that the SCALE regimen had a manageable safety profile.

An esophagectomy is usually recommended within 4–6 weeks after the completion of nCRT. \(^13\) A few studies suggested that longer nCRT–surgery intervals may not negatively affect the treatment responses and postoperative outcomes. \(^36\) \(^37\) \(^43\) Moreover, when combined with PD-1 inhibitors, the most suitable timing for surgery has not been definitively established. In this study, perioperative complications occurred in eight patients received surgery within 8 weeks from the completion of preoperative treatment. Following our adjustment to extend this interval to more than 8 weeks, no postoperative complications were identified. A trend of increase in body weight was observed, implying that a longer interval might allow patients to recover from neoadjuvant treatment without introducing a rise in surgical complexity. Additionally, no progression was observed in the ITT population, indicating that the longer interval did not impair the treatment response. In an ongoing phase II study (NCT05424432), continuous recording of patients’ body weight and quality of life is being recorded to provide further evidence for the extended interval.

To date, there is no generally accepted standard regarding the definition of the neoadjuvant irradiation volume in EC, whereas elective nodal irradiation is recommended by European radiation oncologists. \(^38\) Recent studies have shown that elective nodal irradiation may impair the function of immune cells and increase the local and distant failure rates when used in combination with ICIs. \(^35\) \(^36\) \(^38\) \(^43\) Reduced-volume radiation could be an alternative solution. In this study, target volumes were delineated, discussed, and revised by the radiation oncologists and thoracic surgeons following the principle of involved lesion radiation therapy (online supplemental material protocol). Although two patients had an anastomotic fistula after surgery, none of the leakage was related to radiation of the anastomotic area. No patient had in-field recurrence during the follow-up. In China, locoregional recurrence including the cervical and upper mediastinal lymph nodes remains the main failure pattern following the widely applied radical two-field lymph node dissection. \(^10\) \(^42\) In our study, we observed two patients with relapse in the left supraclavicular lymph nodes, and an additional two with relapse in the celiac lymph nodes. These findings highlight the necessity for improvement in neoadjuvant target volume delineation.

We believe that in selected patients with locoregional site metastasis, such as in the mediastinum, the supraclavicular region, or the celiac trunk region, the PTvN should be properly expanded to include high-risk regions such as the cervical and para-aortic lymph nodes below the level of the pancreas, since these areas are difficult to be entirely resected during the radical two-field lymph node dissection.

Increasing evidence suggests that a synergistic antitumor effect can be achieved through combining immunotherapy and chemoradiotherapy, \(^10\) \(^22\) particularly when increasing the radiation fraction dose and shortening treatment courses. \(^24\) \(^25\) \(^43\) The initial efficacy of our study has confirmed this. We observed no progression after the neoadjuvant treatment and achieved an objective response rate of 100%. The pCR rate was 55% in this study, which was 43.2% in the NEOCRT5010 study and 49% in the ESCC cohort from the CROSS study. \(^13\) With a median follow-up time of 24.5 months, the study achieved promising 2-year PFS and OS rates of 63.8% and 78.0%, respectively. Despite the reduction in the dosage of radiation and chemotheraphy, the SCALE regimen has demonstrated promising therapeutic efficacy. Hence, there is a need for additional validation and extended long-term observation within a broader population.

Our study also verified the predictive role of TIS in ESCC. In ESCC, a suppressive immune microenvironment is dominated by exhausted CD8\(^+\) T and natural killer (NK) cells, regulatory T cells (Tregs), as well as alternatively activated macrophages and tolerogenic dendritic cells. \(^44\) In our treatment set, tumors with a pCR possessed a significantly higher TIS score, which comprises interferon gamma-responsive genes related to antigen presentation, chemokine expression, cytotoxic activity, and adaptive immune resistance. \(^45\) A higher TIS level might indicate superior antitumor immune function and lead to greater pathological remission after nCRT. The intervention simultaneously promoted antitumor immune cell infiltration after nCRT, particularly increasing the infiltration of CD4\(^+\) and CD8\(^+\) T cells, which was in accordance with previous findings. \(^20\) \(^46\) Here, in addition to the changes in the non-pCR group, the tumors in the pCR group showed additional greater changes in T cells, CD8\(^+\) T cells, and cytotoxic cell infiltration. The more widespread increase in immune cell infiltration in the pCR group could be attributed to a higher baseline TIS and more tumor antigens released during treatment. As in ESCC, chemoradiotherapy has shown preliminary reprogramming of the local immune environment by increasing immunogenicity, including increased infiltration of CD8\(^+\) T cells \(^37\) \(^48\) and neutrophils, \(^39\) and decreased resting T and NK cells and Tregs. \(^45\) Our results revealed enhanced antitumor immunity, including increased...
infiltration of dendritic cells and cytotoxic cells, as well as a signature of antitumor activities through cytotoxic T cells and NK cells. These effects might be induced by direct reinvigoration of CD8+ T cells by PD-1 inhibitors and activation of an immunosuppressive environment, suggesting that nICRT is a promising synergistic strategy for efficient antitumor inhibition in ESCC.

The limitations of our study include, but are not limited to, the small number of patients enrolled and the short postoperative follow-up period. Second, endoscopic ultrasound (EUS), EUS-guided fine-needle aspiration and positron emission tomography/CT (PET/CT) are recognized as valuable diagnostic modalities for precise N staging. It is important to note that in this study, these modalities were considered optional. The further use of EUS and PET/CT in future studies will be integral in identifying regional nodes at risk for metastasis. Additionally, tumor recurrence in the left supraclavicular and celiac lymph nodes suggests that the target volumes for neoadjuvant radiotherapy may need to be optimized in future studies. Furthermore, neoadjuvant immunotherapy combined with chemoradiotherapy is currently being investigated across a diverse range of tumor types and settings, including our ongoing SCALE-2 study (NCT05424432). We hold the belief that our extended follow-up study will provide additional evidence regarding the role of SCALE regimen in RLaESCC.

In conclusion, nICRT according to the SCALE regimen is associated with an acceptable safety and a promising efficacy for treating RLaESCC. However, additional research is imperative to substantiate the effectiveness and safety of the SCALE regimen. Furthermore, identifying the most accurate predictive biomarkers for treatment response and long-term outcomes remains a crucial aspect that requires further investigation.

Contributors Conception and design: MJ, XZ, and NJ. Administrative support: XH. Provision of study materials or patients: MJ, XZ, and BR. Collection and assembly of data: MJ, XZ, BR, NJ, JZ, ZG, YW, LZ, CK, and XS. Data analysis and interpretation: MJ, XZ, NJ, YZ, and SL. Manuscript writing: all authors. Final approval of manuscript: all authors. Accountable for all aspects of the work: all authors. MJ, as the guarantor, accepts full responsibility for the work and the conduct of the study.

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Patient consent for publication Not applicable.

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Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available on reasonable request. The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

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ORCID iD Ming Jiang http://orcid.org/0009-0005-7016-2384

REFERENCES


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

Appendix figures and tables
Contents

Appendix Table A1. Tumor immune micro-environment (TIME) 289 gene list...........3
Appendix Table A2. Key genes and corresponding references for immune cell infiltration scores and immune signature.................................................................5
Appendix Figure A1. Representative images for radiologic and pathological response to neoadjuvant therapy ...............................................................6
Appendix Figure A2. Representative images of disagreements on target volume delineation between radiation oncologists and thoracic surgeons. .........................8
Appendix Figure A3. Representative CT images of Grade IIIb surgical complications. .....................................................................................................9
Appendix Figure A4. Body weight changes in patients received surgery within and over 8 weeks. ..................................................................................10
Appendix Figure A5. Downstaging of T and N stage after neoadjuvant treatment....10
Appendix Table A3. Pathologic stages of patients who underwent surgery (the mITT population) ......................................................................................11
Appendix Figure A6: Representative images of PD-L1 IHC staining and pathological findings after tumor resection .............................................................12
Appendix Figure A7: TIME of tumors with pCR and non-pCR pre-treatment ..........13
Appendix Figure A8. Change of cell infiltration scores pre- and post-treatment in group of pCR and non-pCR. .................................................................14
### Appendix Table A1. Tumor immune micro-environment (TIME) 289 gene list

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**Appendix Table A2. Key genes and corresponding references for immune cell infiltration scores and immune signature**

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Appendix Figure A1. Representative images for radiologic and pathological response to neoadjuvant therapy.

Panel A shows images from patient 17 with stage IVA disease located in the middle third of thoracic esophagus before (upper row) and after (lower row) the administration of preoperative treatment. From left to right: MRI sagittal T1-weighted images; axial T1-weighted enhanced image; axial high-resolution T2-weighted image; CT images; H&E images. This patient achieved pathological complete regression with
no residual lymph node metastasis according to postoperative specimen. Panel B shows images from patient 20 with stage III disease located in the lower third of thoracic esophagus before (upper row) and after (lower row) preoperative treatment. This patient exhibited a remarkable 69% pathological regression of the esophageal lesion, accompanied by persistent lymph node metastasis after surgery. MRI, magnetic resonance images; CT, computed tomography; H&E, hematoxylin and eosin staining.

(A)

(B)
Appendix Figure A2. Representative images of disagreements on target volume delineation between radiation oncologists and thoracic surgeons.

Panel A: CT images of disagreement on the diagnosis of small lymph node. The indicated lymph node (red arrow) was included into PTV after discussion. Panel B: representative images of target volume modification on reducing potential anastomosis area irradiation. A and B upper row: before modification; lower row: after modification.
Appendix Figure A3. Representative CT images of Grade IIIb surgical complications.

Panel A: CT images of patient 3 who had initially experienced pleural cavity hematocoele following surgery (left) but subsequently showed recovery after treatment (right). Panel B: CT images of patient 9 who initially suffered from anastomotic leakage after surgery (left) but recovered after treatment (right).
Appendix Figure A4. Body weight changes in patients received surgery within and over 8 weeks.

A trend of increased body weight in patients who had surgery over 8 (including 8) weeks after neoadjuvant treatment was observed. Unpaired t test was used for the analysis of P value.

Appendix Figure A5. Downstaging of T and N stage after neoadjuvant treatment.

Panel (A): the distribution of clinical and pathological T stage; Panel (B): the distribution of clinical and pathological N stage.
### Appendix Table A3. Pathologic stages of patients who underwent surgery (the mITT population)

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Abbreviations: mITT, modified intent-to-treat; N, node; T, tumor; yp, pathological.
Appendix Figure A6: Representative images of PD-L1 IHC staining and pathological findings after tumor resection.

Panel A: from left to right: Negative PD-L1 expression of samples from non-pCR (I. Patient 19) and pCR (II. Patient 03) groups. Positive PD-L1 expression of samples from non-pCR (III. Patient 06) and pCR (IV. Patient 15) groups. Panel B: representative images of vascular formation (I), fibrosis with hyalinosis (II), tertiary lymphoid structures and inflammatory cell infiltration (III), foamy cell aggregation (IV), multinucleated cell (V), and necrosis (VI). pCR, pathologic complete response; PD-L1, programmed cell death ligand 1.
Appendix Figure A7: TIME of tumors with pCR and non-pCR pre-treatment.

Panel A: Differentially expressed genes (DEGs) (DESeq2, $P < 0.01$ and expression fold change (FC) $\geq 2$ or $\leq 1/2$), up-regulated genes included HLA-DRB1, HLA-DRA, CTAG1B, IFIT1, HERC6, CD79A, PNOC, CD27, GZMH, MRC1, CCL7, down-regulated genes were CCL21 and DLL4; Panel B: Gene Ontology (GO) enrichment analysis based on DEGs ($P < 0.05$); Panel C: Scores of pre-treatment cell infiltration and immune signatures in individual tumors.
Appendix Figure A8. Change of cell infiltration scores pre- and post- treatment in group of pCR and non-pCR.

Panel A: Tregs; Panel B: B cells; Panel C: CD8+ T cells, a trend of improved CD8+ T cell infiltration is observed in group of pCR ($P = 0.078$); Panel D: Exhausted CD8+ T cell; Panel E: Macrophage M1; Panel F: Neutrophils; Panel G: NK cells; Panel H: Th1 cells (Wilcoxon rank-sum test, $P < 0.05$).
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

Version 2.0/20210729

Principal investigators

Professor Ming Jiang
Department of Thoracic Surgery, Jiangsu Cancer Hospital, Jiangsu Institute of Cancer Research, The Affiliated Cancer Hospital of Nanjing Medical University, 42 Baiziting Rd., Xuanwu District, Nanjing 210009, Jiangsu Province, People's Republic of China; E-mail: Mingjiang2023@hotmail.com

Professor Xiangzhi Zhu
Department of Radiation Oncology, Jiangsu Cancer Hospital, Jiangsu Institute of Cancer Research, The Affiliated Cancer Hospital of Nanjing Medical University, 42 Baiziting Rd., Xuanwu District, Nanjing 210009, Jiangsu Province, People's Republic of China; E-mail: 13182948068@163.com

Professor Binhui Ren
Department of Thoracic Surgery, Jiangsu Cancer Hospital, Jiangsu Institute of Cancer Research, The Affiliated Cancer Hospital of Nanjing Medical University, 42 Baiziting Rd., Xuanwu District, Nanjing 210009, Jiangsu Province, People's Republic of China; E-mail: robbish_ren@163.com
Contents

1 Schema ................................................................................................................................... 4
2 Summary ................................................................................................................................ 9
3 Background .......................................................................................................................... 10
4 Objective ............................................................................................................................... 11
5 Study plan ............................................................................................................................. 12
   5.1 Study design ............................................................................................................... 12
   5.2 Patient selection ......................................................................................................... 12
      1) Inclusion criteria .................................................................................................. 12
      2) Exclusion criteria ................................................................................................. 14
      3) Withdrawal criteria ............................................................................................... 15
      4) Eliminate criteria .................................................................................................. 16
   5.3 Examinations and screening of patients ..................................................................... 16
      1) Examinations ....................................................................................................... 16
      2) Screening ............................................................................................................. 17
   5.4 Treatment plan ........................................................................................................... 17
      1) Neoadjuvant immune-chemotherapy (nICT) ....................................................... 17
      2) Neoadjuvant short course radiotherapy scheme ................................................... 19
      3) Principles for the adjustment of dosage of drugs ................................................. 22
      4) Surgery ................................................................................................................. 25
   5.5 Endpoints evaluation .................................................................................................. 26
      1 ) Toxicity evaluation ............................................................................................. 26
      2 ) Radiographic evaluation ..................................................................................... 26
      3 ) Pathological evaluation ...................................................................................... 27
      4 ) Post-operative complications evaluation ............................................................ 28
   5.6 Statistical analysis ...................................................................................................... 29
   5.7 Follow-up ................................................................................................................... 29
   5.8 Ethics ......................................................................................................................... 30
      1 ) Informed consent ............................................................................................... 30
      2 ) Ethics and policy ................................................................................................. 30
6 References ............................................................................................................................ 30

### 1 Schema

<table>
<thead>
<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>
</tr>
</thead>
<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>
</tr>
<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
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) \( \geq 90 \text{g/L} \); white blood cell count (WBC) \( \geq \) lower limit of normal value; absolute neutrophil value (ANC) \( \geq 1.5 \times 10^9 / \text{L} \); platelet count \( \geq 100 \times 10^9 / \text{L} \);

(2) Renal function: \( \text{Cr} \leq 1.5 \text{UNL} \), endogenous creatinine clearance rate \( \text{Ccr} \geq 60 \text{ml/min} \) (Cockcroft-Gault);

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

(4) Blood coagulation function: the international standardized ratio of prothrombin time \( \leq 1.5 \text{ULN} \), 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 \( < 500 \text{IU/ml} \) (or \( < 2500 \text{copies/ml} \)) 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 \( \geq 9 \)

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.
| **Duration of Trial**                          | Estimated enrollment time of the first subject: September 2020 |
|                                              | Estimated enrollment time of the last subject: December 2021   |
|                                              | Estimated end time of the study: December 2022                 |

| **Therapeutic Regimen**                      | **Neoadjuvant Chemotherapy**: Paclitaxel (135 mg/m2) + Carboplatin (AUC=5), ivdrip, Days 1, 22 |
|                                              | **Neoadjuvant Immunotherapy**: Toripalimab 240mg, ivdrip, Days 1, 22 |
|                                              | **Neoadjuvant Radiotherapy**: Total dose: 30.0 Gy, Dose / Fraction: 2.5 Gy, Fraction / week: 5 From Days 3 to 18 |
|                                              | **Surgery**: 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) |

| **Sample Size**                              | As an exploratory study, the study sample size will consist of 20 patients who underwent tumor resection. |

| **Statistical Analysis**                     | ◆ 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. |
|                                              | ◆ 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) >= 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.

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. 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.

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 18.5 kg/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:
   (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;

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
during neoadjuvant treatment;

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 nIiCT 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>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

18
### 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, (e.g. 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 ≥10 mm (paraesophageal, tracheoesophageal groove ≥5 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 cranio-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, 2.5 Gy 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**

21
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>
<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>Permanently discontinue</td>
<td></td>
</tr>
<tr>
<td>----------------------</td>
<td>-------------------------</td>
<td></td>
</tr>
<tr>
<td>Grade 2 myocarditis</td>
<td>Permanently discontinue</td>
<td></td>
</tr>
<tr>
<td>Grade 2-3 other immune-associated adverse reactions of first occurrence</td>
<td>Permanently discontinue</td>
<td></td>
</tr>
<tr>
<td>Grade 4 blood amylase increased or lipase increased</td>
<td>Permanently discontinue</td>
<td></td>
</tr>
<tr>
<td>Grade 3-4 pancreatitis</td>
<td>Permanently discontinue</td>
<td></td>
</tr>
<tr>
<td>Grade 3-4 myocarditis</td>
<td>Permanently discontinue</td>
<td></td>
</tr>
<tr>
<td>Grade 3-4 encephalitis</td>
<td>Permanently discontinue</td>
<td></td>
</tr>
<tr>
<td>Grade 4 other immune-associated adverse reactions of first occurrence</td>
<td>Permanently discontinue</td>
<td></td>
</tr>
</tbody>
</table>

**Recurrent or persistent adverse reaction**

| Recurrent grade 3-4 (except endocrine disorders) | Permanently discontinue |
| Grade 2-3 adverse reaction not improved to grade 0-1 within 12 weeks after the last dose (except endocrine disorders) | Permanently discontinue |
| Corticosteroid unable to be reduced to ≤10 mg/day prednisone equivalent dose within 12 weeks after the last dose | Permanently discontinue |

**Infusion related reaction**

| Grade 2 | Reduction of infusion rate or suspension of administration, if the symptom is relieved, re-administration may be considered and patients be under close observation. |
| Grade 3-4 | 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 experiences 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>Grade I</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>Grade II</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>Grade III</td>
<td>Requiring surgical, endoscopic or radiological intervention</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>Grade IV</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>Grade V</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).


