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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 Jiang [6]

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For numbered affiliations see end of article.

Correspondence to
Dr Ming Jiang;
Mingjiang2023@hotmail.com

Dr Xiangzhi Zhu;
13182948068@163.com

Dr Binhui Ren;
robbish_ren@163.com

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 (RLaESCC). 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 RLaESCC patients with clinical stages cT3-4aN0M0/cT1-4aN+M0 received neoadjuvant paclitaxel (135 mg/m^2), 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 (nICT) 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 nICT 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 occured. Twenty patients underwent surgery, and 11 achieved pCR (55%). Two patients (10%) experienced grade IIIb 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 RLaESCC patients.

Trial registration number ChiCTR2100045104.

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 (RLaESCC). 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 RLaESCC patients.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ The SCALE regimen offers a promising neoadjuvant treatment option for patients with RLaESCC. 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.[1] China has a high prevalence of EC, accounting for 50% of the global morbidity and mortality, with 90% being esophageal squamous cell

carcinoma (ESCC).[2 3] Neoadjuvant chemoradiotherapy (nCRT) followed by surgery constitutes the established standard of care for patients with resectable, locally advanced ESCC (RLaESCC).[4 5] Nonetheless, tumor recurrence persists in 40%–50% of patients postsurgery.[6–8] 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.[6 9] Therefore, 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.[10] 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.[11] In metastatic EC, ICIs alone or in combination with chemotherapy have been proven to benefit patient survival.[12–17] 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.[18 19] Furthermore, several phase I/II studies investigating the combination of ICIs with nCRT have reported treatment-related deaths in RLaESCC.[20–22] 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.[23–26] 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.[15] The primary goal was to assess the safety of this novel neoadjuvant immune-chemoradiotherapy (nICRT) 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).[27] 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.[28] 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 3.0 T 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,[29] following Radiological the Response Evaluation Criteria in Solid Tumors version 1.1,[30] 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/m^2) 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.[4 5] 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 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.

Pathological evaluation

The surgical specimens were staged according to the criteria of the American Joint Committee on Cancer (eighth edition)[31] 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.[32] 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.[33] Scanned slides containing lymph node slices were identified, reviewed, and classified, as described previously.[34] 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.[11]

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 RNeasy 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 $\leq 1/2$). 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 65 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 cT4aN0-2 (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 γ-glutamyltransferase (n=2, 9%) (table 2). Notably, no grade 3 or higher radiation esophagitis or pneumonitis occured. 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.

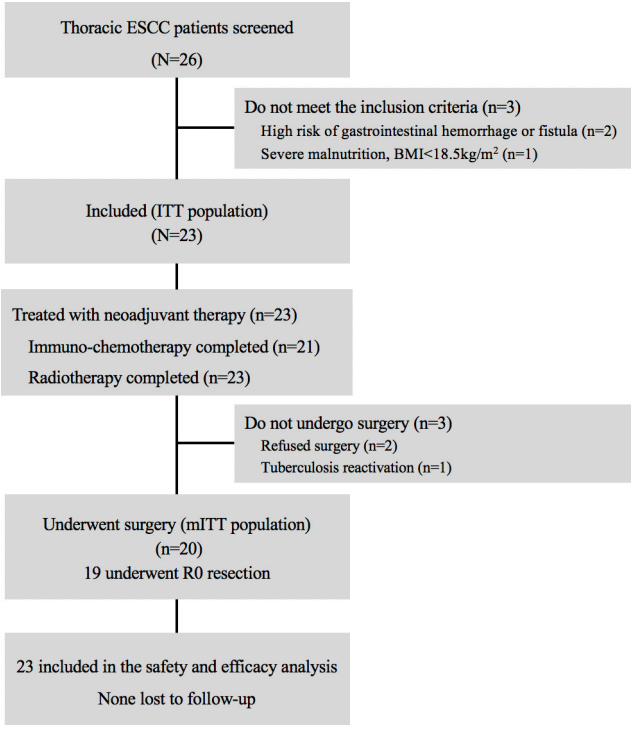

Figure 1 Study flow chart. BMI, body mass index; ESCC, esophageal squamous cell carcinoma; ITT, intention-to-treat; mITT, modified intentionto-treat

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 pertained 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 hematocele 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

Table 1 Baseline characteristics for the ITT population

Characteristic	No of patients (n=23)
Median age, years (range)	65 (37–72)
Sex	
Male	18 (78)
Female	5 (22)
ECOG PS at baseline	
0	15 (65)
1	8 (35)
BMI, kg/m^2	
Median	22.4
Range	18.7–32.5
Median tumor length, cm (range)	5.1 (3.0–9.6)
Tumor location	
Proximal third	4 (17)
Middle third	13 (57)
Distal third	6 (26)
Clinical T stage	
cT2	1 (4)
cT3	13 (57)
cT4a	9 (39)
Clinical N stage	
cN0	6 (26)
cN1	14 (61)
cN2	3 (13)
Clinical stage*	
II	5 (22)
III	9 (39)
IVA	9 (39)
Smoking status	
Former or current	8 (35)
Never	15 (65)
Drinking history	
Former or current	8 (35)
Never	15 (65)
Family history	
Yes	3 (13)
No	20 (87)

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.

Table 2 TRAEs during neoadjuvant treatment in the ITT population

TRAEs*	All patients (n=23)	
	Any grade no (%)	Grades 3–4 no (%)
Leukopenia	21 (91)	9 (39)
Neutropenia	20 (87)	13 (57)
Anemia	18 (78)	0
Thrombocytopenia	12 (52)	0
Anorexia	23 (100)	0
Nausea	21 (91)	0
Alopecia	16 (70)	0
Constipation	9 (39)	0
Fever	8 (35)	0
Skin rash	7 (30)	7 (30)
Fatigue	4 (17)	0
Vomiting	2 (9)	0
Elevated γ-glutamyltransferase	8 (35)	2 (9)
Elevated aspartate aminotransferase	6 (26)	0
Elevated alanine aminotransferase	7 (30)	0
Elevated alkaline phosphatase	3 (13)	0
Blood creatinine increased	3 (13)	0
Elevated bilirubin	2 (9)	0
Abnormal myocardial enzyme	2 (9)	0
Esophagitis	3 (13)	0
Pneumonitis	1 (4)	0
Hypokalemia	4 (17)	0
Hyperthyroidism	3 (13)	0
Hypothyroidism	2 (9)	0
Hyponatremia	2 (9)	0
Hyperglycemia	2 (9)	0

Data are no (%).
*TRAEs were assessed during treatment and for up to 30 days after the last dose of neoadjuvant treatment according to the National Cancer Institute Common Terminology Criteria for Adverse Events, version 5.0.
ITT, intention-to-treat; TRAE, treatment-related adverse event.

Table 3 Type of surgery and overall surgery morbidity in the mITT population

Type of surgery*	Population with surgery (mITT) (n=20)†, no (%)			
1+3+5	10 (50)			
1+3+4	8 (40)			
1+2+4	1(5)			
1+2+5	1 (5)			
No of nodes examined				
Mean	20			
Min–max	10–29			
Overall postoperative complications‡	Total	4–6 weeks (n=8)	7 weeks (n=4)	≥8 weeks (n=8)
Anastomotic leakage	2	2	0	0
Tachycardias	3	2	1	0
Hypotension	1	0	1	0
Anastomotic hemorrhage	1	1	0	0
Postoperative intrathoracic hemorrhage	1	1	0	0
Airway sputum obstruction	1	0	1	0
Clavein–Dindo classification	Total	4–6 weeks (n=8)	7 weeks (n=4)	≥8 weeks (n=8)
II	4	2	2	0
IIIa	2	1	1	0
IIIb	2	2	0	0

*Surgery information: 1. right thoracotomy; 2. median laparotomy; 3. laparoscopic dissection; 4. right transthoracic esophagectomy with reconstruction; 5. left cervical esophagectomy with reconstruction.
†Three patients did not undergo surgery and two patients rejected surgery while the other one experienced tuberculosis reactivation.
‡One patient had anastomotic leakage and hemorrhage at the same time.
mITT, modified intention-to-treat.

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

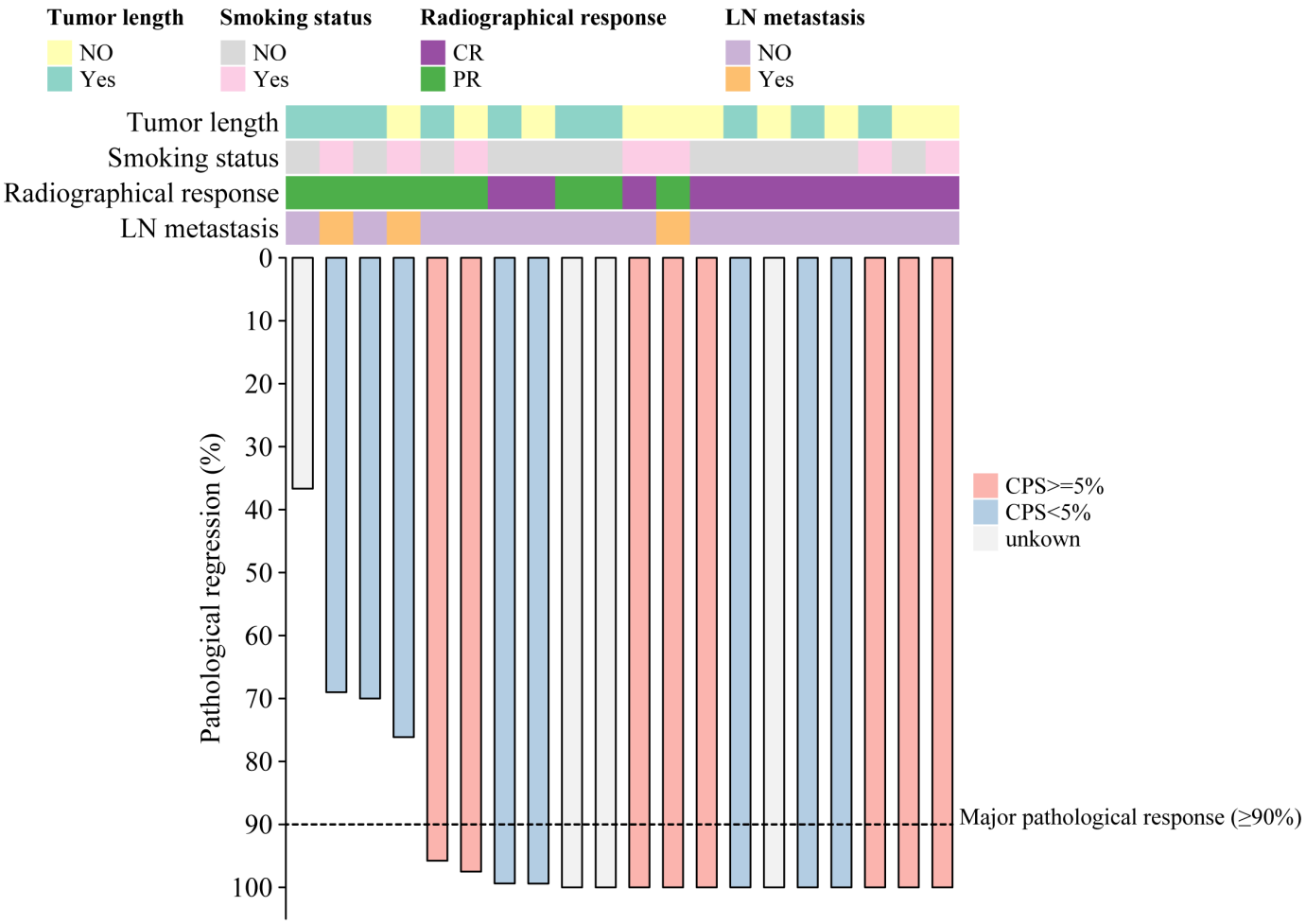

Figure 2 Pathological assessment of the response to neoadjuvant treatment in the primary tumor. Pathological regression in the resected primary esophageal tumor after neoadjuvant treatment, according to the percentage of remaining viable tumor cells, for each of the 20 patients who underwent surgical resection. The dashed horizontal line indicates the threshold for a major pathological response (90% regression). Clinical and pathological features that include programmed death ligand 1 (PD-L1) expression (CPS≥5 or <5), tumor length (≥5 cm or <5 cm), smoking status, preoperative radiological response (according to Response Evaluation Criteria in Solid Tumors (RECIST)), and presence or absence of lymph-node (LN) metastases in the surgical specimen are annotated for each patient. CPS, Combined Positive Score of PD-L1; CR, complete response; PR, partial response.

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

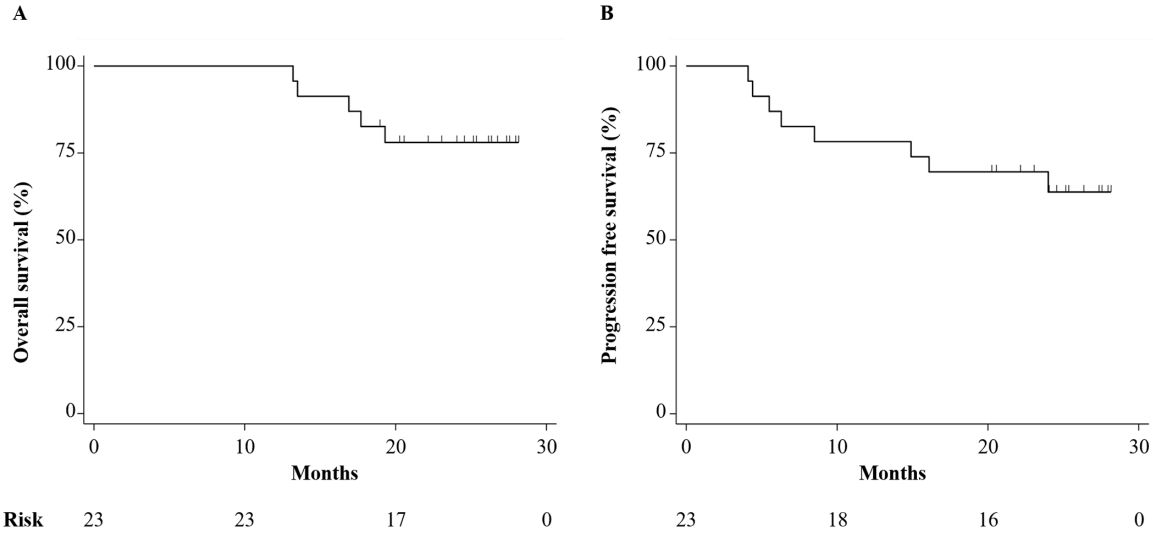

Figure 3 Survival outcomes for patients in the ITT population (n=23). (A) Overall survival; (B) Progression-free survival. ITT, intention-to-treat.

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 furter 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.[4 5] 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.

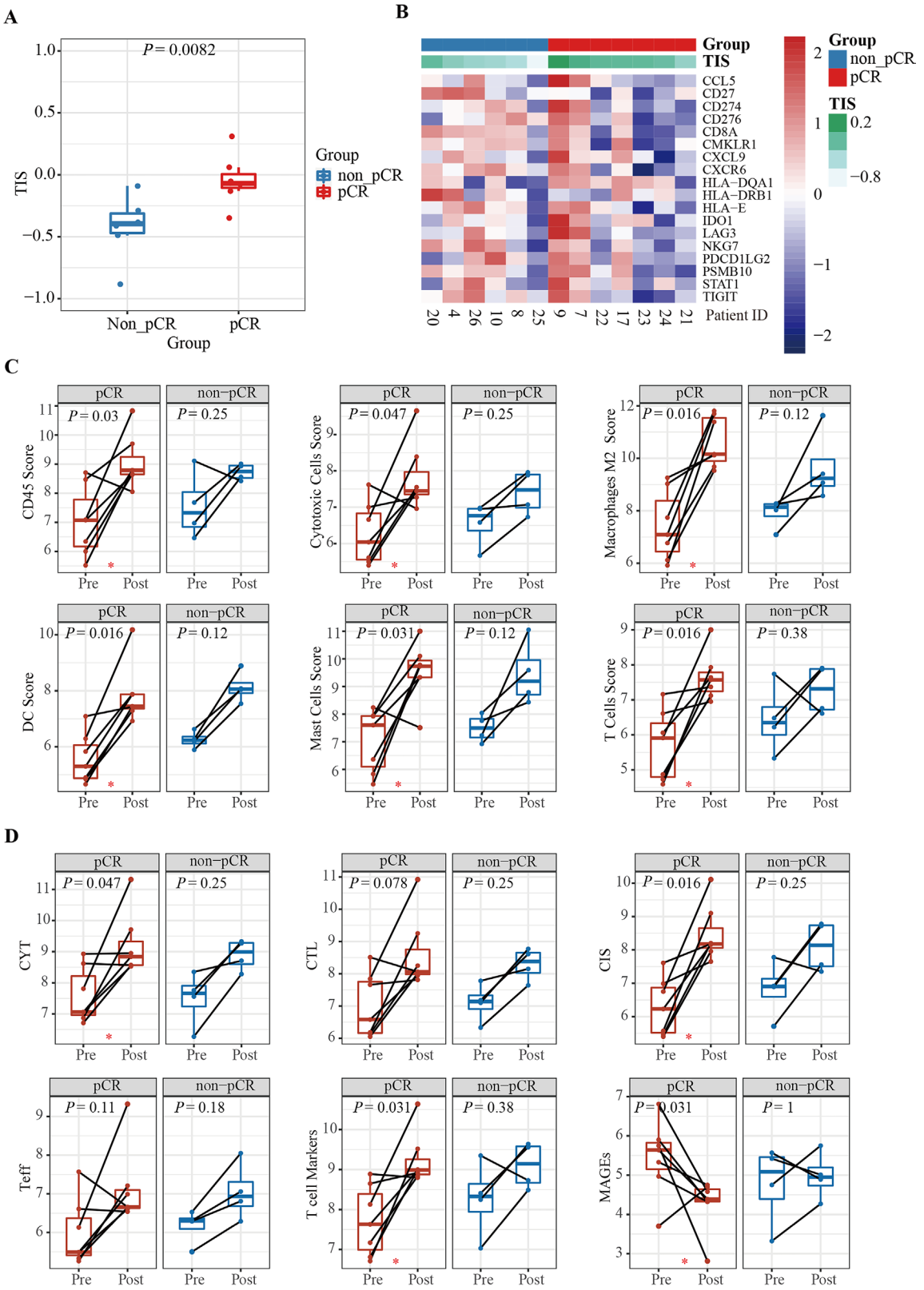

Figure 4 Comparison of the pretreatment tumor immune microenvironment (TIME) between tumors that achieved pCR and non-pCR, and the change of TIME after treatment. (A) Boxplot representing the T-cell-inflamed score (TIS), which appears significantly higher in tumors that achieved a pathological complete 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 NEOCRTEC5010 and CROSS studies.[4 5] 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.[4 5] A few studies suggested that longer nCRT–surgery intervals may not negatively affect the treatment responses and postoperative outcomes.[36 37] 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-related acute toxicity 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 provied 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.[25 39] 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.[40–42] 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 NEOCRTEC5010 study and 49% in the ESCC cohort from the CROSS study.[4 5] 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 chemotherapy, 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 nICRT. The intervention simultaneously promoted antitumor immune cell infiltration after nICRT, 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[47 48] and neutrophils,[49] and decreased resting T and NK cells and Tregs.[49] 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,[50] 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.

Author affiliations
[1]Department of Radiation Oncology, Jiangsu Cancer Hospital, Jiangsu Institute of Cancer Research, The Affiliated Cancer Hospital of Nanjing Medical University, Nanjing, Jiangsu, China
[2]Department of Pathology, Jiangsu Cancer Hospital, Jiangsu Institute of Cancer Research, The Affiliated Cancer Hospital of Nanjing Medical University, Nanjing, Jiangsu, China
[3]Department of Medical Imaging Center, Jiangsu Cancer Hospital, Jiangsu Institute of Cancer Research, The Affiliated Cancer Hospital of Nanjing Medical University, Nanjing, Jiangsu, China
[4]Department of Biostatistics, Nanjing Medical University, Nanjing, China
[5]Medical Department, Jiangsu Simcere Diagnostics Co. Ltd.; Nanjing Simcere Co. Ltd.; The State Key Laboratory of Translational Medicine and Innovative Drug Development, Nanjing, China
[6]Department of Thoracic Surgery, Jiangsu Cancer Hospital, Jiangsu Institute of Cancer Research, The Affiliated Cancer Hospital of Nanjing Medical University, Nanjing, Jiangsu, China

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

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