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RESEARCH

Clinical efficacy and biomarkers of neoadjuvant chemoimmunotherapy in locally advanced esophageal squamous cell carcinoma

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Abstract

Background Neoadjuvant immunotherapy has emerged as a promising strategy for treating esophageal squamous cell carcinoma (ESCC). This study evaluates the therapeutic efficacy and safety of neoadjuvant immunochemotherapy (nICT) in ESCC and explores potential biomarkers associated with treatment outcomes.

Methods Patients with locally advanced ESCC were enrolled and received two cycles of nICT followed by surgical resection. The primary endpoint was the pathological complete response rate, while secondary endpoints included overall survival (OS), event-free survival (EFS), safety, and the identification of predictive biomarkers.

Results A total of 47 patients were enrolled in the study, with 42 undergoing surgical resection, all of whom achieved R0 resection. The rates of complete and partial pathological responses were 28.5% and 16.7%, respectively. The 1-year and 2-year EFS rates were 82% and 37.3%, while OS rates were 100% and 71.4%, respectively. The majority of treatment-related adverse events were Grade 1–2, and no surgical delays were observed. RNA sequencing analysis identified epithelial-mesenchymal transition as the most significantly enriched pathway in non-responders. Notably, higher infiltration of normal fibroblasts was associated with improved pathological response and enhanced long-term survival, while myofibroblastic cancer-associated fibroblasts (myCAF) negatively impacted treatment efficacy and clinical outcomes.

Conclusions Neoadjuvant PD-1 inhibitors combined with chemotherapy show promising potential for patients with locally advanced ESCC, inducing a robust immune response that correlates with clinical outcomes. The infiltration of myCAF emerges as a potential predictive biomarker for treatment response and disease progression, underscoring the need for further mechanistic exploration and validation in larger cohorts.

Keywords Neoadjuvant therapy · PD-1 blockade · Immunochemotherapy · Biomarkers · ESCC

Abbreviations

AEs	Adverse events
AJCC	American Joint Committee on Cancer
AUC	Area under the curve
CAFs	Cancer-associated fibroblasts
CIs	Confidence intervals
CT	Computed tomography
DEGs	Differentially expressed genes
EC	Esophageal cancer

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ECM	Extracellular matrix
ECOG	Eastern Cooperative Oncology Group
EFS	Event-free survival
EMT	Epithelial-mesenchymal transition
ESCC	Esophageal squamous cell carcinoma
EUS	Endoscopic ultrasonography
GEO	Gene expression omnibus
H&E	Hematoxylin and eosin
ICIs	Immune checkpoint inhibitors
KEGG	Kyoto Encyclopedia of Genes and Genomes
mIF	Multiplex immunofluorescence
myCAF	Myofibroblastic cancer-associated fibroblasts
NCI-CTCAE	National Cancer Institute Common Terminology Criteria for Adverse Events
NF	Normal fibroblasts
nCT	Neoadjuvant chemotherapy
nCRT	Neoadjuvant chemoradiotherapy
nICT	Neoadjuvant immunochemotherapy
nICRT	Neoadjuvant immunochemoradiotherapy
OS	Overall survival
pCR	Pathological complete response
pPR	Pathological partial response
pNR	Pathological non-response
RECIST	Response Evaluation Criteria in Solid Tumors
scRNA-seq	Single-cell RNA sequencing
TIME	Tumor immune microenvironment
TME	Tumor microenvironment
TNM	Tumor-node-metastasis
TRAEs	Treatment-related adverse events

Introduction

Esophageal cancer (EC) is the sixth leading cause of cancer-related mortality and the seventh most commonly diagnosed malignancy worldwide [1]. Among its histological subtypes, esophageal squamous cell carcinoma (ESCC) predominates, particularly in high-incidence regions such as East Asia [2]. Despite significant advancements in treatment, the prognosis for locally advanced ESCC remains poor, underscoring the urgent need for more effective treatment strategies to improve clinical outcomes.

Neoadjuvant chemoradiotherapy (nCRT) followed by surgical resection has been established as the standard treatment for patients with locally advanced ESCC. The CROSS and NEOCRTEC5010 trials have demonstrated improved 5- and 10-year overall survival (OS) rates with preoperative chemoradiotherapy compared to surgery alone [3, 4]. However, despite achieving high rates of R0 resection, approximately 15% of patients experience locoregional recurrence within 5 years, while the incidence of distant metastasis remains as high as 30% [4, 5]. Furthermore, the use of radiotherapy is associated with increased perioperative complications and mortality and does not offer a survival advantage over neoadjuvant chemotherapy (nCT), primarily due to the severe toxicity associated with radiotherapy [6, 7]. These limitations underscore the urgent need for novel therapeutic approaches that improve both safety and long-term clinical outcomes.

The advent of immune checkpoint inhibitors (ICIs) has revolutionized the treatment landscape of multiple malignancies, offering survival benefits across various cancer types [8–11]. Encouraged by the promising efficacy of immunotherapy combined with chemotherapy in advanced ESCC, there is growing interest in extending its application to the neoadjuvant setting. Emerging clinical evidence suggests that neoadjuvant immunochemotherapy (nICT) can induce favorable pathological responses with a manageable safety profile in patients with locally advanced ESCC [12–16]. However, limited data are available on long-term clinical outcomes.

Notably, not all patients exhibit a favorable response to immune checkpoint blockade, highlighting the urgent need for reliable pre-treatment biomarkers that can predict immune response and guide patient selection for nICT. In addition, emerging evidence suggests that postoperative adjuvant therapy may improve outcomes in patients at high risk of recurrence following neoadjuvant therapy [17]. Therefore, identifying post-treatment biomarkers to assess recurrence risk and inform adjuvant therapeutic decisions is equally important.

The tumor immune microenvironment (TIME) plays a critical role in tumor progression, immune escape, and therapeutic efficacy [18]. The crosstalk between the tumor and the TIME, both pre- and post-treatment, not only drives treatment resistance but also contributes to tumor progression [19]. A deeper understanding of these dynamic interactions has the potential to refine patient stratification and optimize personalized treatment strategies in the neoadjuvant setting for early-stage ESCC.

In this study, we conducted a single-arm, phase II prospective clinical trial to evaluate the efficacy and safety of nICT. Additionally, we explored biomarkers that could predict the therapeutic response of this regimen in resectable ESCC.

Materials and methods

Participants

This investigator-initiated, single-arm, phase II prospective clinical trial was conducted at Tongji Hospital, Tongji

Medical College, Huazhong University of Science and Technology. The trial was registered at ClinicalTrials.gov (NCT05028231) and adhered to the principles of the Declaration of Helsinki.

Inclusion criteria: Eligible patients were aged 18–75 years and diagnosed with locally advanced, resectable ESCC classified as stage II–IVA according to the 8th edition of the AJCC staging system. Resectability was determined by a thoracic surgeon. Additional eligibility requirements included an Eastern Cooperative Oncology Group (ECOG) performance status of 0–1 and adequate organ function.

Exclusion criteria: Patients were excluded if they had concurrent malignancies, were pregnant or breastfeeding, had autoimmune disorders, documented allergies to the study drugs, or were unable to tolerate esophagectomy based on preoperative pulmonary and cardiovascular assessments.

Procedure

Participants received two cycles of nICT, administered at 3-week intervals. Each cycle consisted of an intravenous infusion of either Pembrolizumab (240 mg, Day 1; Merck, USA) or Tislelizumab (240 mg, Day 1; BeiGene, China), in combination with Paclitaxel (75 mg/m^2, Day 1; Hengrui, China) and either Carboplatin (AUC 5 mg/mL/min, Day 1; Qilu Pharma, China) or Cisplatin (25 mg/m^2, Days 1–3; Haosun Pharma, China). The choice of PD-1 inhibitor was made by the treating physician, rather than through random allocation.

Baseline assessments, including contrast-enhanced thoracic and abdominal computed tomography (CT), endoscopic ultrasonography (EUS), and cervical/subclavicular ultrasonography, were performed prior to the initiation of neoadjuvant therapy. These imaging evaluations were repeated after each of the two neoadjuvant therapy cycles to assess tumor response and resectability. Tumor response was assessed using the Response Evaluation Criteria in Solid Tumors version 1.1 (RECIST 1.1) [20]. Treatment-related adverse events (TRAEs) were assessed and documented at each visit, following the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE) version 5.0 [21]. TRAEs occurring within 30 days post-surgery or within 90 days of the first neoadjuvant dose were also documented.

Approximately 6–8 weeks after the final cycle of nICT, patients who remained surgically eligible underwent esophagectomy. The choice of surgical approach (McKeown or Ivor Lewis) was based on tumor location and resectability. All patients underwent two-field (abdominal and thoracic) lymph node dissection, with cervical lymph node dissection performed selectively for those with suspected cervical metastasis. Pathological response was independently evaluated by two pathologists and categorized as follows: Grade 1 (pathological complete response, pCR; no residual tumor), Grade 2 (pathological partial response, pPR; $\leq 50\%$ residual tumor), and Grade 3 (pathological non-response, pNR; $> 50\%$ residual tumor or new lesions).

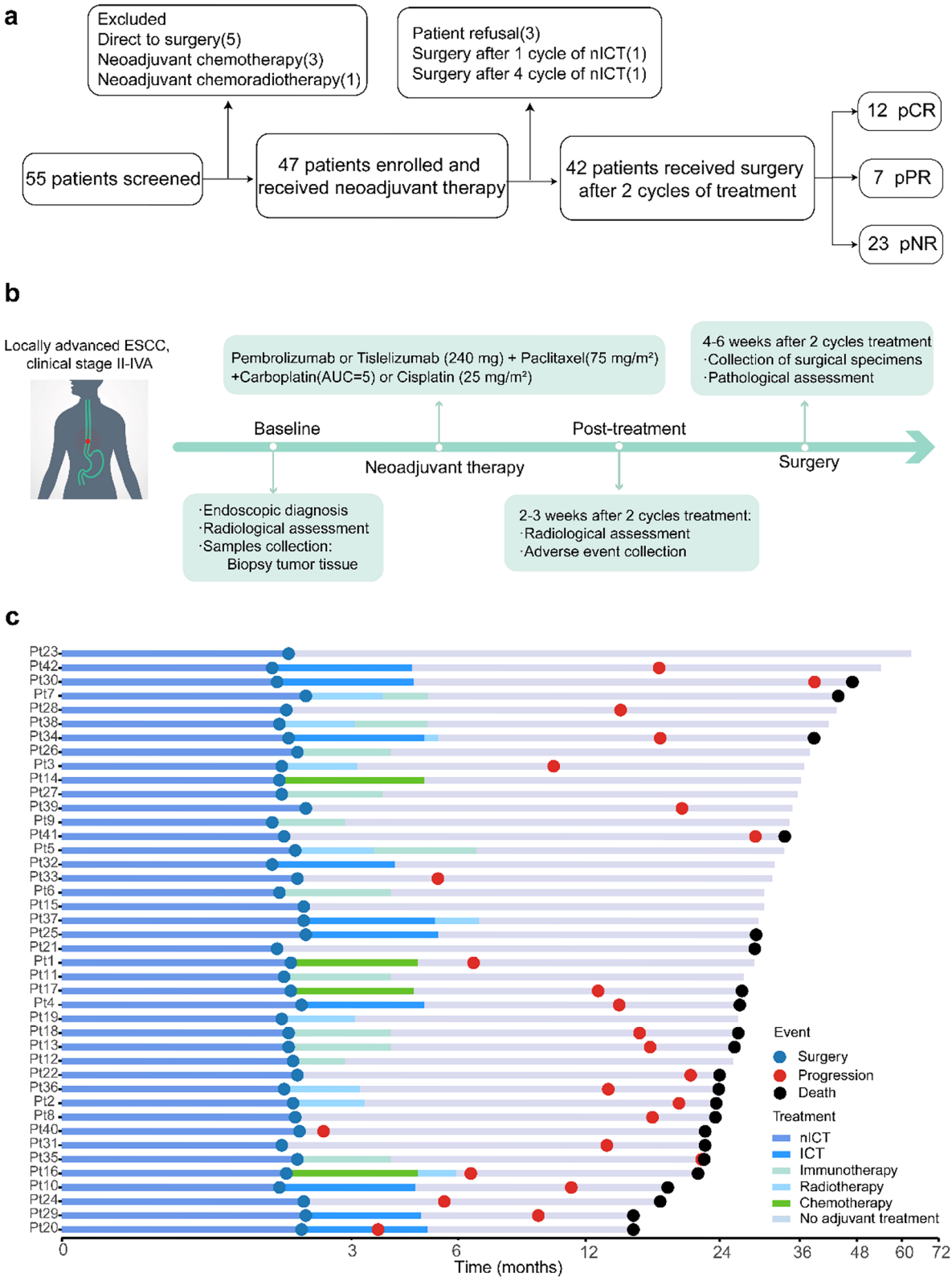
Following surgical resection, patients underwent routine follow-up assessments every 3 months during the first year and every 6 months in the second and third years. OS was defined as the time from surgery to death from any cause or the last follow-up. Event-free survival (EFS) was measured from the date of surgery to the first occurrence of tumor recurrence, disease progression, or death. Adjuvant therapy administered after surgery was documented accordingly.

Outcome

The primary endpoint of the study was the pCR rate. Secondary endpoints included OS, EFS, treatment safety, and the identification of biomarkers associated with treatment response.

Exploratory analysis

Pre-treatment endoscopic biopsy specimens and post-treatment surgical samples were collected for biomarker analysis, including RNA sequencing, cell-type composition analysis, and tumor microenvironment profiling. RNA sequencing was performed using the Illumina Novaseq 6000 platform (Shanghai Biotechnology Corp., China). Differential gene expression was analyzed using the DESeq2 package (v1.44.0) in R, with a significance threshold set at |fold change (FC)|> 1.0 and $p < 0.05$. Enrichment analysis of differentially expressed genes (DEGs) was conducted using the clusterProfiler R package (v4.12.6), focusing on Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways and Hallmark gene sets. Data visualization was performed using ggplot2 in R. To assess the cellular composition of tumors, single-cell RNA sequencing (scRNA-seq) data from 60 ESCC tumor samples were obtained from the gene expression omnibus (GEO) database (accession number GSE160269) [22]. Deconvolution of bulk RNA sequencing data was performed using Scaden, a machine-learning algorithm trained on scRNA-seq datasets to estimate the relative abundance of distinct cell types [23]. Multiplex immunofluorescence (mIF) analysis was performed to assess the distribution and composition of post-treatment tumor tissues. To quantify the abundance of myofibroblastic cancer-associated fibroblasts (myCAF) and map their localization within the tumor microenvironment (TME), co-localization analysis was conducted using four myCAF-related markers—*MMP11*, *COL3A1*, *α-SMA*, and *FAP*.


a
Excluded
Direct to surgery(5)
Neoadjuvant chemotherapy(3)
Neoadjuvant chemoradiotherapy(1)
Patient refusal(3)
Surgery after 1 cycle of nICT(1)
Surgery after 4 cycle of nICT(1)
55 patients screened
47 patients enrolled and received neoadjuvant therapy
42 patients received surgery after 2 cycles of treatment
12 pCR
7 pPR
23 pNR
b
Locally advanced ESCC, clinical stage II-IVA
Pembrolizumab or Tislelizumab (240 mg) + Paclitaxel(75 mg/m²) +Carboplatin(AUC=5) or Cisplatin (25 mg/m²)
4-6 weeks after 2 cycles treatment
·Collection of surgical specimens
·Pathological assessment
Baseline
Post-treatment
Neoadjuvant therapy
Surgery
·Endoscopic diagnosis
·Radiological assessment
·Samples collection:
Biopsy tumor tissue
2-3 weeks after 2 cycles treatment:
·Radiological assessment
·Adverse event collection
c
Pt23
Pt42
Pt30
Pt7
Pt28
Pt38
Pt34
Pt26
Pt3
Pt14
Pt27
Pt39
Pt9
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Pt8
Pt40
Pt31
Pt35
Pt16
Pt10
Pt24
Pt29
Pt20
0
3
6
12
24
36
48
60
72
Time (months)
Event
Surgery
Progression
Death
Treatment
nICT
ICT
Immunotherapy
Radiotherapy
Chemotherapy
No adjuvant treatment

◂**Fig. 1** Study design. **a** Flowchart of the patient screening process. pCR, pathological complete response (no viable tumor cells in the resected specimens, including primary tumors, tumor thrombosis, and lymph nodes); pPR, pathological partial response (≤50% viable tumor cells in the primary tumor); pNR, pathological non-response (>50% viable tumor cells in the primary tumor or emergence of new lesions.). **b** Trial schema. Eligible patients received two cycles of neoadjuvant therapy, consisting of Pembrolizumab (240 mg, Day 1; Merck, USA) or Tislelizumab (240 mg, Day 1; BeiGene, China), combined with Paclitaxel (75 mg/m^2, Day 1; Hengrui, China) and either Carboplatin (AUC 5 mg/mL/min, Day 1; Qilu Pharma, China) or Cisplatin (25 mg/m^2, Days 1–3; Haosun Pharma, China), followed by surgical resection. Radiological assessments were conducted at baseline, 2 weeks after completion of the two neoadjuvant therapy cycles, and prior to surgery. Tumor samples were collected at both baseline and post-treatment for further analysis. ESCC: esophageal squamous cell carcinoma; AUC: area under the curve. **c** Overview of the treatment regimen in the neoadjuvant and adjuvant settings, along with the follow-up status of each patient ($n=42$). nICT, neoadjuvant immunochemotherapy, ICT, immunochemotherapy

Statistical analysis

This study was designed as a single-arm phase II trial with pCR as the primary endpoint. To demonstrate superiority, a pCR rate of 25% was anticipated with nICT. Considering an anticipated dropout rate of 10%, a total of 47 patients were required to achieve 90% power at a one-sided alpha level of 10%. For continuous variables, the median and range were reported, while for categorical variables, the frequency and percentage were reported. Intergroup comparisons were performed using the Wilcoxon test to assess statistical differences. Kaplan–Meier survival curves were constructed to estimate OS and EFS, with intergroup differences assessed using the log-rank test. The 95% confidence intervals (CIs) were calculated using the Clopper–Pearson method. A P value <0.05 was considered statistically significant. Quantitative analysis of mIF was performed using ImageJ software (NIH, Bethesda, MD, USA). All statistical analyses and data visualization were conducted using SPSS version 23.0 and R version 4.3.1.

Results

Patient characteristics

Between September 2021 and May 2023, 55 patients were screened for eligibility. Of these, 47 met the inclusion criteria and were enrolled in the study. Ultimately, 42 patients (89.3%) who successfully completed two cycles of neoadjuvant therapy followed by surgical resection were included in the final analysis (Fig. 1a, b). The treatment course and follow-up status are shown in the swimmer's plot for each patient (Fig. 1c). The median age of the cohort was 59.5 years, with a predominance of male patients (81.0%). Most patients (73.8%) presented with T3-stage tumors, and lymph node involvement (N+) was identified in 83.3% of cases. According to the AJCC 8th edition staging system, 59.5% of patients were classified as stage III. Tumors were predominantly located in the middle (59.5%) and lower (28.6%) esophagus, collectively accounting for 88.1% of cases. Additional baseline characteristics are detailed in Table 1.

Surgical outcomes

Among the 42 patients who completed two cycles of nICT followed by surgical resection, the mean interval from the first treatment dose to surgery was 2.02 ± 0.16 months. All surgeries achieved R0 resection. The mean operative time was 346.75 ± 76.13 min, and a median of 18.65 ± 10.45 lymph nodes were dissected per patient. Pathological lymph node involvement was observed in 13 patients (30.9%). The median intraoperative blood loss was 121.25 ± 275.28 mL. Postoperative complications included anastomotic leakage in 2 patients (4.76%) and pulmonary infection in 3 patients (7.14%). The median postoperative length of stay was 14 days (range 7–30 days). Notably, no immune-related complications or deaths occurred within 90 days of surgery (Table 2).

Clinical efficacy

Following two cycles of nICT, 12 patients achieved a pCR, 7 achieved a pPR, and 23 exhibited pNR, as assessed according to RECIST 1.1. The distribution of primary tumor pathological responses and corresponding clinical characteristics are shown in Fig. 2a. Notably, no cases of disease progression were observed during the neoadjuvant therapy phase.

As of the data cutoff on December 1, 2024, and with a median follow-up of 29.95 months (range 15.83–44.9 months), the 1-year and 2-year EFS rates were 82% (95% CI 70.7–90.5) and 37.3% (95% CI 23.0–60.3), respectively (Fig. 2b). The corresponding OS rates were 100% (95% CI 100.0–100.0) at 1 year and 71.4% (95% CI 59.0–86.5) at 2 years as shown in Fig. 2c. Among the 25 patients who experienced recurrence, 12 (48%) had regional lymph node recurrence, 7 (28%) had both lymph node and distant recurrence, and 6 (24%) had distant recurrence only. We further compared the 24-month relapse rates between responders (pCR/pPR) and non-responders (pNR), which were 32.6% and 82.6%, respectively.

Radiological and pathological assessments, including CT imaging, endoscopy, and histopathological reports, confirmed significant tumor regression in the responders, further supporting the efficacy of the neoadjuvant therapy regimen (Fig. 2d). Compared to non-responders, responders demonstrated a clear trend toward improved EFS and OS (Fig. 2e).

Table 1 Baseline characteristics of the patients

Characteristic	Patients (n = 42)
Age, years[a]	59 (41, 75)
Sex	
Male	34 (81.0)
Female	8 (19.0)
ECOG performance status	
0	32 (76.2)
1	10 (23.8)
Smoking status	
Never	19 (45.2)
Former or current	23 (54.8)
Alcohol consumption	
Never	14 (33.3)
Former or current	28 (66.7)
Tumor location	
Upper	5 (11.9)
Middle	25 (59.5)
Lower	12 (28.6)
Tumor length(cm)	6 (1, 16)
Clinical T stage[b]	
T2	7 (16.7)
T3	31 (73.8)
T4	4 (9.5)
Clinical N stage	
N0	7 (16.7)
N1	28 (66.7)
N2	7 (16.7)
Clinical stage	
II	13 (31.0)
III	25 (59.5)
IVa	4 (9.5)

Data are presented as *n* (%), unless otherwise specified

Abbreviations: ECOG, Eastern Cooperative Oncology Group; TNM, tumor-node-metastasis

[a]Continuous variables are presented as median (range)

[b]Clinical disease stage was assessed according to the criteria of the American Joint Committee on Cancer, Eighth Edition

Safety

In our study, nICT was generally well tolerated, with no previously unreported TRAEs observed (Table 3). Adverse events (AEs) of any grade occurred in 95.2% (40/42) of patients, with the majority (88.8%) experiencing Grades 1–2 AEs. The most common Grade 3–4 AEs were leukopenia and thrombocytopenia, which occurred in 11.9% of patients. Other common TRAEs included alopecia (n = 25), hepatotoxicity ($n = 23$), decreased appetite ($n = 17$), constipation ($n = 17$), and elevated BNP levels ($n = 16$), all of which were mild and manageable. The overall incidence of AEs was comparable between groups (89.5% in responders vs. 95.6% in non-responders), however, Grade 3–4 TRAEs were observed exclusively in the non-responder group. Notably, no treatment-related deaths occurred, all Grade 3–4 TRAEs resolved with appropriate medical intervention, and no Grade 5 TRAEs or surgical delays were reported, indicating that the treatment was generally well tolerated.

Table 2 Summary of surgery-related adverse events

Complication	Patients ($n = 42$)
Anastomotic leakage	2 (4.76)
Pulmonary infection	3 (7.14)
Bleeding	0
Immune-related myocarditis	0
Immune-related nephritis	0
Immune-related hepatitis	0
Immune-related pneumonia	0
Immune-related hyperthyroidism	0
Immune-related hypothyroidism	0
Death within 90 days	0

Data are presented as *n* (%), unless otherwise specified

RNA-seq analysis of tumor samples

RNA sequencing was performed on tumor samples collected before and after nICT. Patients were categorized as responders (pCR/pPR) and non-responders (pNR) based on their pathological response (Fig. 3a). In the non-responder group, pathways associated with epithelial-mesenchymal transition (EMT) and focal adhesion were significantly enriched, while in the responder group, immune-related pathways—such as cytokine signaling, interferon-gamma response, and antigen presentation—were notably activated (Fig. 3b). Additionally, 10 fibroblast-related genes exhibited differential expression between responders and non-responders, including genes involved in fibroblast activation and TME modulation (*CTHRC1, MFAP2, POSTN*), extracellular matrix (ECM) synthesis and remodeling (*COL1A1, COL1A2, COL3A1, SPARC*), and ECM degradation (*MMP1, MMP11, SERPINH1*) (Fig. 3c).

Based on the 2-year relapse outcomes, the patients were categorized into relapse (early relapse within 2 years) and non-relapse groups (Fig. 3d). In the relapse group, pathways related to cell cycle progression, metabolic reprogramming, genomic instability and carcinogenesis were enriched, while in the non-relapse group, immune-related pathways were upregulated (Fig. 3d). Additionally, differential expression trends of fibroblast-related genes between the relapse and non-relapse groups were observed, although the differences did not reach statistical significance (Fig. 3f).

Fibroblast subpopulation dynamics

A deep learning-based model was employed for cell-type deconvolution and composition analysis, revealing dynamic shifts in TME composition. The analysis showed the relative proportions of epithelial cells, fibroblasts, endothelial cells, T cells, B cells, and myeloid cells in both the responder and non-responder groups at baseline and post-treatment (Table S1). Post-treatment samples demonstrated a decrease in epithelial cell dominance, accompanied by increased immune cell infiltration, particularly in the responder group (Fig. 4a).

Further analysis of fibroblast subpopulations revealed changes in the proportions of normal fibroblasts (NF) and myCAF between pre- and post-treatment samples (Fig. 4b and Table S2). A significant correlation was observed between the proportions of NF and myCAF and pathological response in both pre- and post-treatment TME (Fig. 4c). Moreover, the proportions of NF and myCAF in post-treatment samples were significantly different between the relapse (early relapse within 2 years) and non-relapse groups, suggesting a potential role for fibroblast subpopulations in tumor recurrence (Fig. 4d).

Additionally, the proportion of NF and myCAF were strongly correlated with both EFS and OS (Fig. 4e). Patients with a higher NF proportion had significantly longer EFS (log-rank $P = 0.0077$) and a trend toward improved OS (log-rank $P = 0.07$). In contrast, patients with a higher myCAF proportion exhibited significantly worse EFS (log-rank $P = 0.03$) and OS (log-rank $P = 0.035$).

To further validate these findings, mIF analysis was performed on operative tumor samples. MyCAF subpopulations were identified through the co-expression of *COL3A1*, *FAP*, *MMP11*, and *α-SMA*. The mIF analysis revealed a higher proportion of myCAF in the non-responders compared to responders. Notably, the myCAF proportion was also higher in patients in the relapse group.

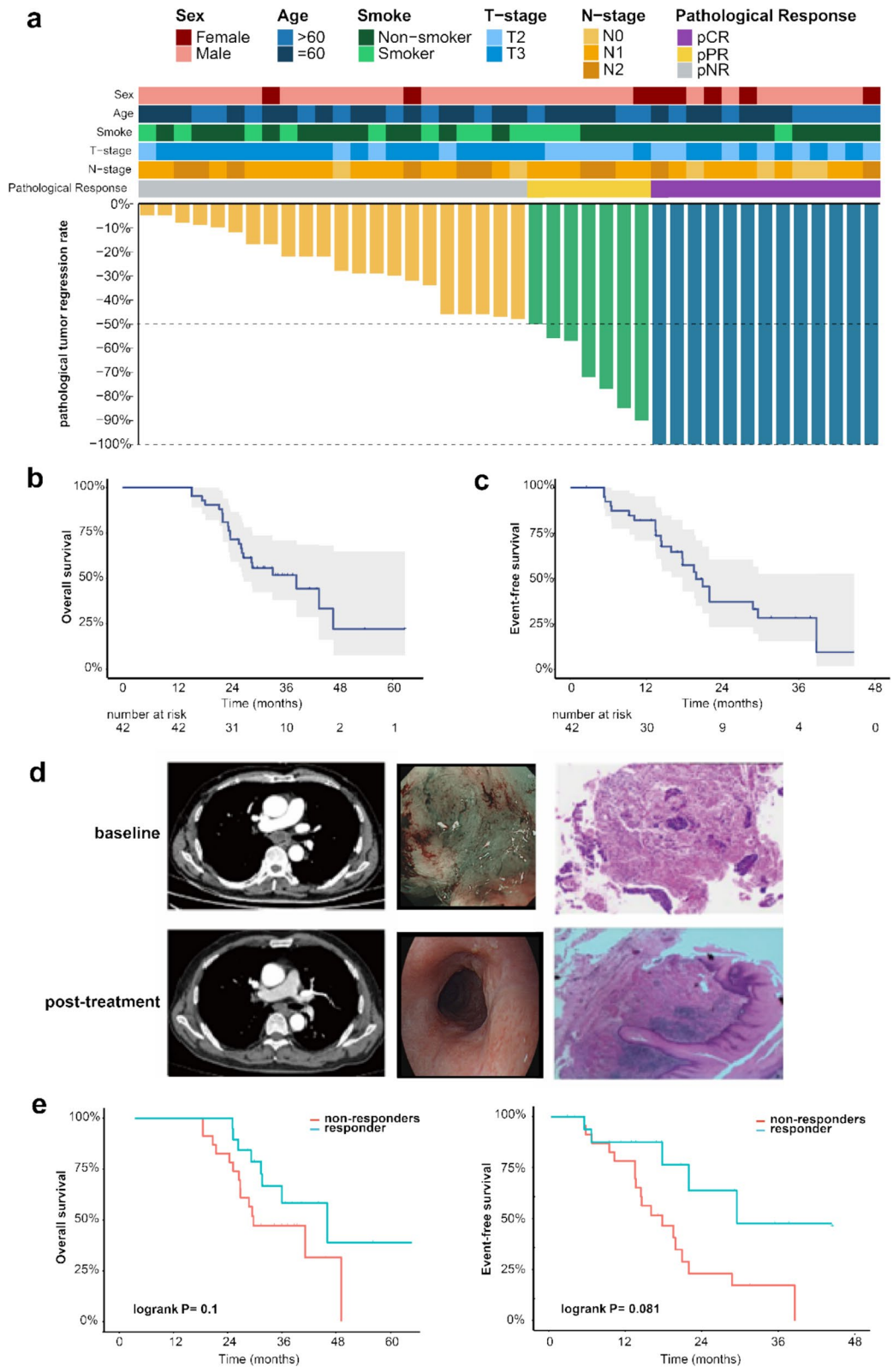
Discussion

This study demonstrates that nICT is a feasible and effective approach for treating locally advanced ESCC, yielding promising pathological and survival outcomes while maintaining a favorable safety profile. The trial met its primary endpoint, with 12 of 42 (28.5%) surgically treated patients achieving pCR. Notably, there were no instances of postoperative mortality or an increased risk of surgical complications, confirming the manageable safety profile of nICT. Given the importance of predicting immune response in neoadjuvant therapy and organ preservation strategies, the observed association between myCAF proportion and pNR is particularly noteworthy.

The pCR rate (28.5%) and pPR rate (16.7%) observed in our study represent a substantial improvement over the 3.8–4% pCR rates reported in trials of neoadjuvant chemotherapy alone [24, 25]. In addition, survival analysis showed 1-year and 2-year EFS rates of 82% and 37.3%, respectively, and OS rates of 100% and 71.4% at the same time points. These results underscore the therapeutic potential of nICT and align with findings from prospective trials evaluating neoadjuvant chemoimmunotherapy in resectable ESCC.

Notably, the treatment demonstrated a manageable safety profile, with TRAEs occurring in only 11.9% of patients, significantly lower than the 32–49% incidence reported for traditional neoadjuvant regimens. These results suggest that nICT offers a promising approach for tumor regression while maintaining acceptable tolerability. Unlike nCRT, nICT omits radiation, sparing patients from radiation-associated complications such as esophagitis, pneumonitis, and long-term tissue fibrosis. Radiation-induced esophagitis often leads to dysphagia, reduced oral intake, and subsequent nutritional decline, which can impair immune function and delay hematologic recovery. By preserving swallowing function and nutritional status, nICT may enhance patients' systemic resilience and tolerance to chemotherapy. In our cohort, patients receiving nICT experienced few severe cytopenias and gastrointestinal toxicities, indicating that the addition of ICIs did not exacerbate, and may have alleviated, the overall treatment burden. ICIs have a distinct toxicity profile and typically do not cause overlapping myelosuppressive or mucosal damage, which may explain the lower incidence of hematological AEs compared to radiation-based regimens. Furthermore, their immunomodulatory properties may help mitigate chemotherapy-induced inflammatory damage, though further mechanistic studies are warranted [26]. From a surgical perspective, avoiding radiation may also reduce perioperative risks. Radiotherapy can cause local fibrosis and microvascular damage, increasing surgical difficulty and impairing anastomotic healing, which raises the risk of complications such as esophageal fistulas [27]. In contrast, patients in our study underwent surgery without increased operative difficulty, and no treatment-emergent esophageal fistulas were observed. Taken together, these observations suggest that nICT not only achieves comparable oncologic efficacy to existing regimens but also offers a more favorable safety and recovery profile. Overall, the favorable risk–benefit ratio of nICT supports its broader adoption as a neoadjuvant strategy in locally advanced ESCC, particularly for patients who may not tolerate radiation-based protocols.

It is also important to contextualize our findings within the evolving landscape of multimodal strategies for ESCC. nCRT has long been a standard of care, notably supported by the CROSS trial, which demonstrated an improvement in 5-year survival from approximately 50% with surgery



alone to 67% with the addition of nCRT. Building on this foundation, current research is exploring the incorporation of immunotherapy into nCRT regimens—referred to as neoadjuvant immunochemoradiotherapy (nICRT). Several trials are investigating the addition of PD-1/PD-L1 inhibitors to the standard CROSS regimen or other nCRT protocols.

◂**Fig. 2** Clinical efficacy. **a** Waterfall plot illustrating pathological tumor regression in the surgical cohort ($n=42$). Each bar represents an individual patient, with clinical response categories indicated above: pCR (pathological complete response), pPR (pathological partial response), and pNR (pathological non-response). **b** Overall survival of the surgical cohort ($n=42$). **c** Event-free survival of the surgical cohort ($n=42$). **d** Representative computed tomography (CT) and endoscopy images, along with hematoxylin and eosin (H&E)-stained tumor sections, obtained pre-neoadjuvant therapy and post-surgery from a representative responder. **e** Survival analysis of responders versus non-responders based on clinical efficacy evaluation (pCR/pPR vs. pNR)

A study found that adding immunotherapy to nCRT did not significantly improve pCR rates or decrease complications [28]. However, the long-term survival benefits of nICRT remain to be confirmed. Whether the addition of immunotherapy can effectively mitigate radiotherapy-induced toxicities and reduce distant metastases requires further validation in prospective studies.

Although PD-1 blockade has not yet been established as a standard neoadjuvant strategy, its emerging therapeutic potential warrants further investigation. Several ongoing clinical trials, including KEYNOTE-002, are evaluating the role of postoperative immunotherapy in reducing recurrence and metastasis [29]. Prospective studies will be critical to determining the optimal sequencing, patient selection, and integration of these strategies to maximize long-term survival outcomes.

In contrast to the high response rates achieved with nICT, a subset of patients in our study were non-responders, highlighting the ongoing challenge of primary resistance. Our analysis showed a significant survival advantage among responders compared to non-responders. Patients achieving pCR or pPR had markedly better OS and EFS, with relapse rates of 32.6% in responders versus 82.6% in non-responders. These results emphasize the critical impact of immune-mediated tumor regression on long-term survival. Notably, all Grade 3–4 TRAEs occurred in non-responders, suggesting that immune activation may help mitigate treatment-related toxicity. Although the underlying mechanisms remain unclear, these findings highlight the need for predictive biomarkers for immune response to refine patient stratification and personalize neoadjuvant therapy.

To identify potential biomarkers, RNA sequencing was performed to explore molecular pathways associated with treatment response. Responders showed activation of pathways related to interferon-γ response and antigen presentation, both of which are essential for T cell activation and immune surveillance [30]. In contrast, non-responders exhibited upregulated EMT and hypoxia-related pathways, which are known to facilitate immune evasion and resistance to immunotherapy [31, 32]. Among the pathways, EMT was the most strongly correlated with treatment response, potentially contributing to tumor invasion, metastasis, and immune escape, thereby reducing treatment efficacy [33, 34]. Notably, fibroblast-related gene upregulation was predominantly observed in non-responders, implying that fibroblast-mediated ECM remodeling may hinder immune cell infiltration, a phenomenon previously reported in pancreatic cancer models [35].

Our study further investigated the role of cancer-associated fibroblasts (CAFs) in predicting treatment responses. We identified two distinct fibroblast subtypes, NF and myCAF, and found a strong correlation between their proportions and both treatment response and prognosis in ESCC. Responders and non-relapsing patients exhibited a higher proportion of NF, which may enhance CD8 + T cell infiltration and foster an immune-permissive TME. In contrast, myCAFs, associated with a fibrotic and immunosuppressive TME, were more prevalent in non-responders and relapsing patients. These findings highlight the importance of understanding fibroblast dynamics within the TME to optimize treatment strategies. Since myCAFs contribute to immune suppression and hinder drug delivery, while NFs promote an immune-permissive environment, evaluating fibroblast proportion may offer a novel approach to predict the efficacy of neoadjuvant immunotherapy. Furthermore, the myCAF-driven microenvironment resembles resistance mechanisms observed in pancreatic and breast cancers, reinforcing its potential as a therapeutic target [36, 37].

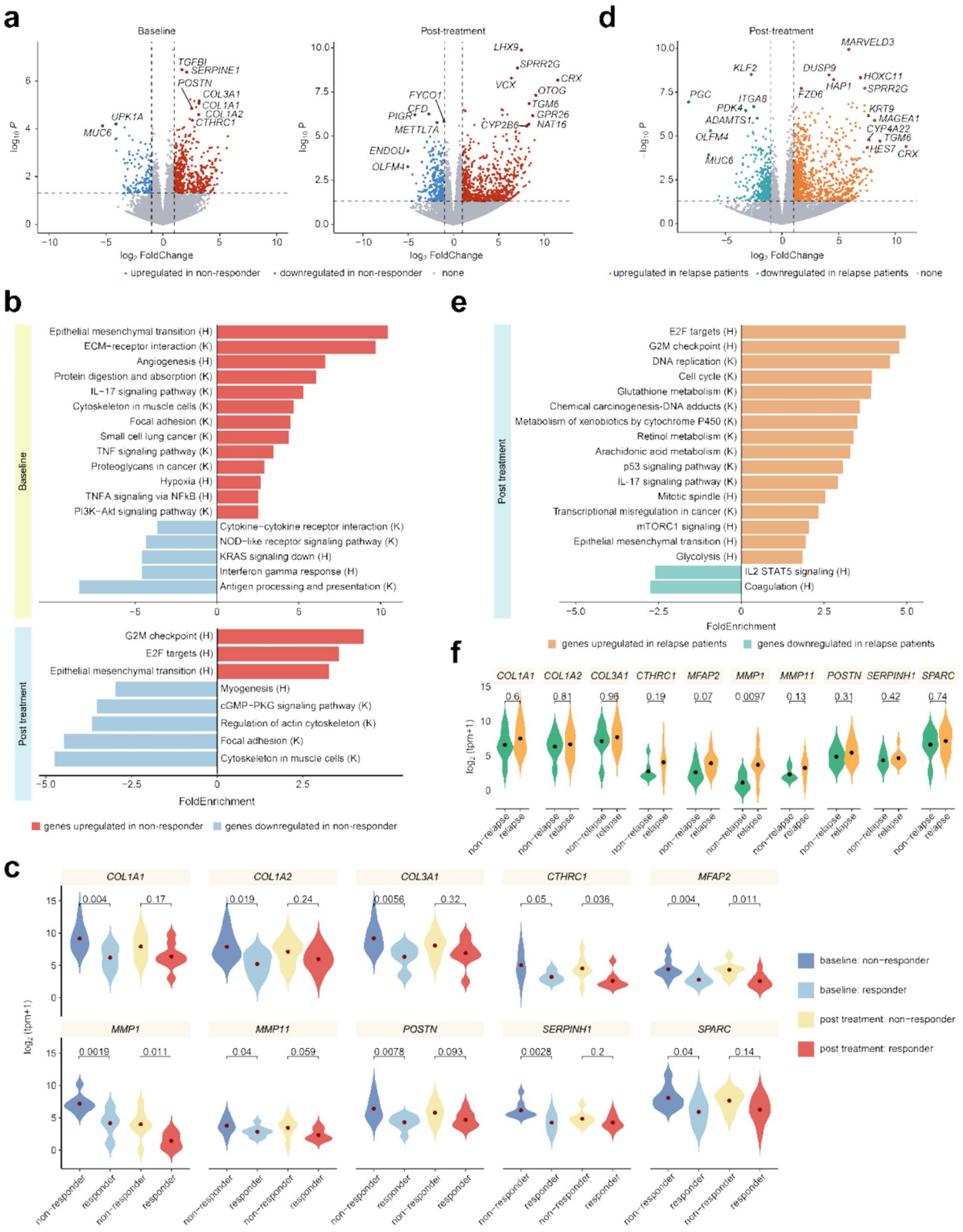
Table 3 Treatment-related adverse events in all patients ($n=42$)

TRAEs[a]	All patients ($n=42$)		
	Grade 1–2	Grade 3	Grade 4
Neutropenia	12 (28.5)	3 (7.1)	1 (2.4)
Thrombocytopenia	7 (16.7)	2 (4.8)	0
Decreased appetite	15 (35.7)	2 (4.8)	0
Nausea	15 (35.7)	0	0
Vomiting	11 (26.2)	0	0
Alopecia	24 (57.1)	1 (2.4)	0
Constipation	17 (40.4)	0	0
Hepatotoxicity	23 (54.8)	0	0
Dizziness	4 (9.5)	0	0
Fever	3 (7.1)	0	0
Cardiotoxicity	0	0	0
Elevated BNP levels	16 (38.1)	0	0
Rash	7 (16.7)	0	0
Pneumonitis	2 (4.7)	0	0

Data are presented as *n* (%), unless otherwise specified

Abbreviations: TRAE, treatment-related adverse event

[a]TRAEs were assessed during treatment and up to 30 days following the last dose of neoadjuvant therapy, in accordance with the National Cancer Institute Common Terminology Criteria for Adverse Events, version 5.0


a
Baseline
Post-treatment
TGFBI
SERPINE1
POSTN
COL3A1
COL1A1
COL1A2
CTHRC1
UPK1A
MUC6
LHX9
SPRR2G
CRX
VCX
OTOG
TGM6
GPR26
NAT16
CYP2B6
FYCO1
CFD
PIGR
METTL7A
ENDOU
OLFM4
log10 P
log2 FoldChange
upregulated in non-responder
downregulated in non-responder
none
d
Post-treatment
MARVELD3
KLF2
DUSP9
HOXC11
HAP1
SPRR2G
PGC
ITGA8
FZD6
PDK4
ADAMTS1
KRT9
MAGEA1
OLFM4
CYP4A22
TGM6
HES7
CRX
MUC6
upregulated in relapse patients
downregulated in relapse patients
none
b
Baseline
Epithelial mesenchymal transition (H)
ECM−receptor interaction (K)
Angiogenesis (H)
Protein digestion and absorption (K)
IL−17 signaling pathway (K)
Cytoskeleton in muscle cells (K)
Focal adhesion (K)
Small cell lung cancer (K)
TNF signaling pathway (K)
Proteoglycans in cancer (K)
Hypoxia (H)
TNFA signaling via NFkB (H)
PI3K−Akt signaling pathway (K)
Cytokine−cytokine receptor interaction (K)
NOD−like receptor signaling pathway (K)
KRAS signaling down (H)
Interferon gamma response (H)
Antigen processing and presentation (K)
Post treatment
G2M checkpoint (H)
E2F targets (H)
Epithelial mesenchymal transition (H)
Myogenesis (H)
cGMP−PKG signaling pathway (K)
Regulation of actin cytoskeleton (K)
Focal adhesion (K)
Cytoskeleton in muscle cells (K)
FoldEnrichment
genes upregulated in non-responder
genes downregulated in non-responder
e
Post treatment
E2F targets (H)
G2M checkpoint (H)
DNA replication (K)
Cell cycle (K)
Glutathione metabolism (K)
Chemical carcinogenesis−DNA adducts (K)
Metabolism of xenobiotics by cytochrome P450 (K)
Retinol metabolism (K)
Arachidonic acid metabolism (K)
p53 signaling pathway (K)
IL−17 signaling pathway (K)
Mitotic spindle (H)
Transcriptional misregulation in cancer (K)
mTORC1 signaling (H)
Epithelial mesenchymal transition (H)
Glycolysis (H)
IL2 STAT5 signaling (H)
Coagulation (H)
FoldEnrichment
genes upregulated in relapse patients
genes downregulated in relapse patients
f
COL1A1
COL1A2
COL3A1
CTHRC1
MFAP2
MMP1
MMP11
POSTN
SERPINH1
SPARC
0.6
0.81
0.96
0.19
0.07
0.0097
0.13
0.31
0.42
0.74
log2 (tpm+1)
non−relapse
relapse
c
COL1A1
COL1A2
COL3A1
CTHRC1
MFAP2
0.004
0.17
0.019
0.24
0.0056
0.32
0.05
0.036
0.004
0.011
MMP1
MMP11
POSTN
SERPINH1
SPARC
0.0019
0.011
0.04
0.059
0.0078
0.093
0.0028
0.2
0.04
0.14
log2 (tpm+1)
non−responder
responder
baseline: non−responder
baseline: responder
post treatment: non−responder
post treatment: responder

◂**Fig. 3** RNA-seq analysis of tumor specimens at baseline and post-treatment. **a** Differential gene expression between responders (pCR/pPR) and non-responders (pNR), with upregulated fibroblast-related genes highlighted. A cutoff of gene expression fold change ≥ 1.0 and $p < 0.05$ were used to select differentially expressed genes (DEGs). **b** Pathway enrichment analysis in responders and non-responders. **c** Representative fibroblast-related genes in responders and non-responders. **d** Differential gene expression between the relapse and non-relapse groups based on 2-year relapse outcomes. **e** Pathway enrichment analysis in the relapse and non-relapse groups. **f** Expression of representative fibroblast-related genes in the relapse and non-relapse groups. Definitions: Responders were defined as patients with complete or partial response based on RECIST 1.1, while non-responders were defined as those with stable disease or progressive disease. Intergroup statistical comparisons were performed using the Wilcoxon test

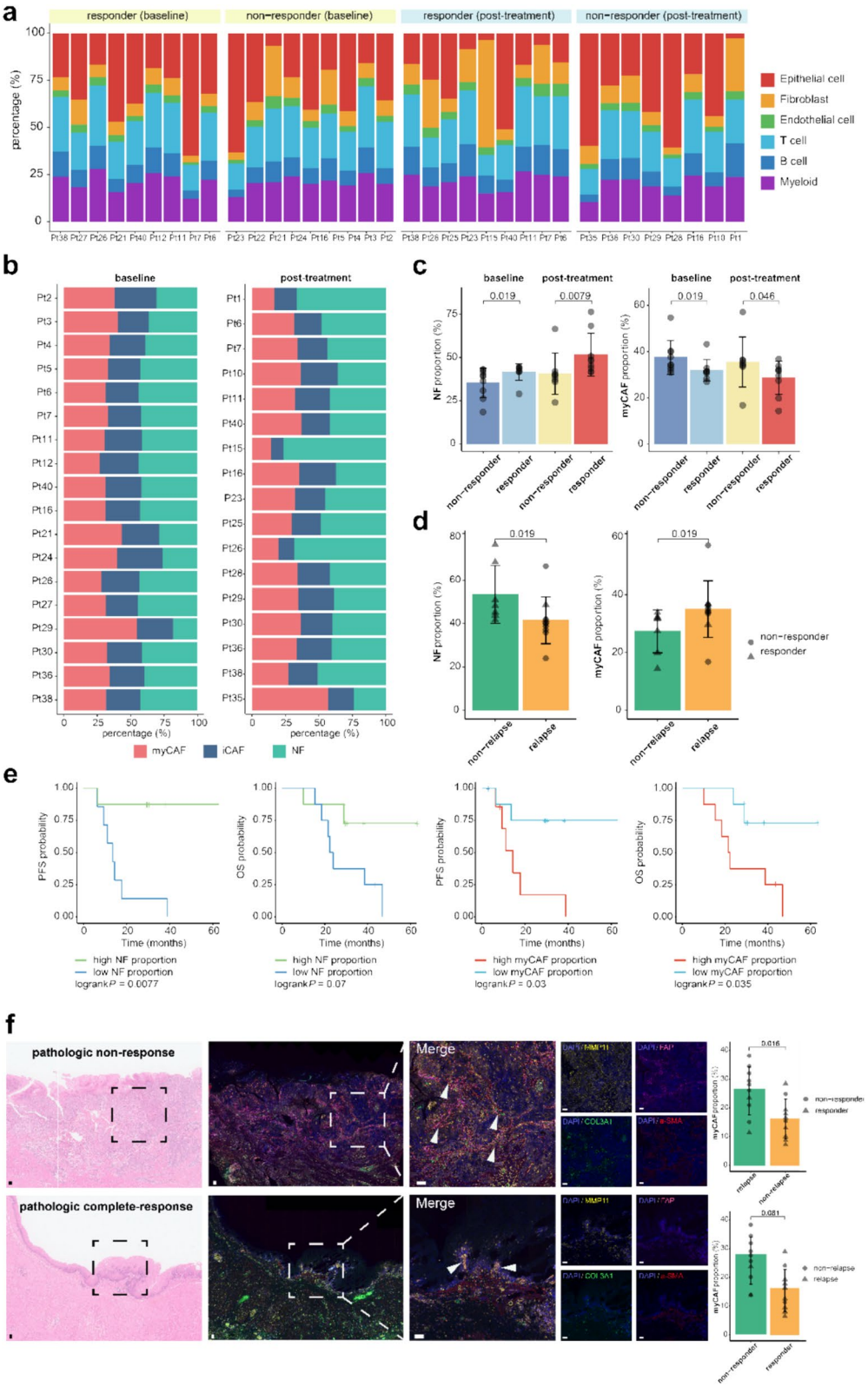
Mechanistically, myCAFs mediate ECM remodeling and activate EMT through the TGF-β signaling pathway, potentially creating physical barriers that hinder immune cell infiltration and promote T cell exhaustion, thereby reducing the efficacy of immunotherapy [38, 39]. Additionally, the presence of myCAF was associated with increased tumor cell proliferation, invasion, and metastatic potential, which may negatively affect the prognosis of patients undergoing nICT [40]. Despite their vital role in tumor progression and treatment resistance, CAFs exhibit considerable cellular and functional heterogeneity, and no definitive markers have been established to precisely identify or characterize myCAFs in ESCC [41]. To identify potential biomarkers, we selected four markers with specificity and high expression in myCAFs for further investigation. The co-localization of these markers in postoperative tumor samples revealed higher expression levels and a greater proportion of myCAFs in non-responders and relapsing patients, suggesting that myCAF abundance may serve as a prognostic indicator. These findings highlight the potential of myCAF as predictive biomarkers in ESCC, which may guide the rational use of adjuvant therapies to improve survival, particularly in patients at high risk of recurrence.

These insights underscore the urgent need for adjunctive strategies to overcome immune resistance in myCAF-rich or EMT-activated tumors. A promising approach is the co-targeting of stromal compartment and immune checkpoints. In particular, inhibition of TGF-β, a key regulator of CAF activation and EMT, has shown potential to restore antitumor immunity. Preclinical studies have demonstrated that dual blockade of TGF-β and PD-1/PD-L1 can synergistically enhance T cell infiltration and overcome immune exclusion [42]. This concept is translationally relevant to ESCC, as early-phase clinical trials of bifunctional anti-PD-L1/TGF-β agents have reported manageable toxicity and early signs of efficacy [43]. Beyond TGF-β, other anti-stromal strategies, such as fibroblast activation protein (FAP) inhibitors and CAF-reprogramming agents, may help mitigate fibrotic immunosuppression. Similarly, experimental EMT inhibitors hold promise for reversing mesenchymal phenotypes and sensitizing tumors to immunotherapy and chemotherapy. Although still investigational, these combinatorial approaches provide a rational framework for improving outcomes in patients with limited response to nICT. Future clinical trials should stratify patients based on CAF or EMT signatures to evaluate whether personalized stromal targeting can convert resistant phenotypes into responders.

Despite these encouraging findings, our study has several limitations. As a single-arm trial, the absence of a control group limits direct comparisons between nICT and other treatment regimens. Moreover, the relatively small sample size constrains the generalizability of our results, highlighting the need for larger-scale clinical trials to validate the long-term efficacy and safety of this approach. Additionally, while we identified distinct fibroblast subpopulations as potential biomarkers, further studies are needed to substantiate these findings and explore fibroblast-targeted therapeutic strategies to enhance the efficacy of nICT.

Conclusions

In summary, neoadjuvant chemotherapy combined with a PD-1 inhibitor appears to be a promising treatment strategy for locally advanced ESCC, offering a favorable safety profile and inducing significant pathological responses and tumor downstaging. The abundance of myCAF in tumor samples may serve as a predictive biomarker for


a
responder (baseline)
non−responder (baseline)
responder (post-treatment)
non−responder (post-treatment)
percentage (%)
Epithelial cell
Fibroblast
Endothelial cell
T cell
B cell
Myeloid
b
baseline
post-treatment
percentage (%)
myCAF
iCAF
NF
c
baseline
post-treatment
NF proportion (%)
myCAF proportion (%)
0.019
0.0079
0.046
non−responder
responder
d
NF proportion (%)
myCAF proportion (%)
0.019
non−relapse
relapse
non−responder
responder
e
PFS probability
OS probability
Time (months)
high NF proportion
low NF proportion
logrank P = 0.0077
logrank P = 0.07
high myCAF proportion
low myCAF proportion
logrank P = 0.03
logrank P = 0.035
f
pathologic non-response
pathologic complete-response
Merge
DAPI / MMP11
DAPI / FAP
DAPI / COL3A1
DAPI / α-SMA
0.016
0.081
non−responder
responder
non−relapse
relapse

◂**Fig. 4** Fibroblast Subpopulation of TME at baseline and post-treatment. **a** Tumor microenvironment (TME) composition at baseline and post-treatment, showing the relative proportions of epithelial cells, fibroblasts, endothelial cells, and immune cells (T cells, B cells, myeloid cells) in the responder and non-responder groups. **b** Changes in fibroblast subpopulations pre- and post-treatment. **c** Proportions of normal fibroblasts (NF) and myofibroblastic cancer-associated fibroblasts (myCAF) in the responder and non-responder groups. **d** Proportions of NF and myCAF in the relapse and non-relapse groups. **e** Correlation of fibroblast subpopulation proportions with EFS and OS, analyzed using the log-rank test. **f** mIF analysis showing higher expression of myCAF markers (*COL3A1*, *FAP*, *MMP11*, *α-SMA*) in patients from the non-responder and relapse groups. *COL3A1* (green), *MMP11* (yellow), *α-SMA* (red) and *FAP* (rose-red). Intergroup statistical comparisons were performed using the Wilcoxon test

immunotherapy efficacy and help guide adjuvant therapy decisions.

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Author contributions All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by Siyou Deng, Qi Wang, Yueping Li, Ruijie Zhang, Jinjie Lin, Yujie Zhang, Yixin Cai, and Wei Sun. The first draft of the manuscript was written by Siyou Deng, with subsequent revisions contributed by Qi Wang, Yueping Li, and Li Zhang. Jiang Chang was responsible for methodology and supervision. Ni Zhang contributed to conceptualization, investigation, and supervision. Li Zhang was responsible for conceptualization, funding acquisition, validation, resource management, and overall supervision. All authors read and approved the final manuscript.

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Data availability Relevant data is provided within the manuscript or supplementary information files. Additional details and raw data can be made available upon reasonable request to the corresponding author.

Declarations

Conflict of interest The authors declare no competing interests.

Ethical approval This study was performed in accordance with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of Tongji Hospital, Huazhong University of Science and Technology (Approval No. 2019-S910). The trial was registered at ClinicalTrials.gov (Identifier: NCT05028231).

Consent to participate Written informed consent was obtained from all individual participants included in the study.

Consent to publication Not applicable. This manuscript does not contain any individual person's data in any form (including images or videos).

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