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Neoadjuvant sintilimab and chemotherapy in patients with potentially resectable esophageal squamous cell carcinoma (KEEP-G 03): an open-label, single-arm, phase 2 trial

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ABSTRACT

Background The standard neoadjuvant treatments in patients with esophageal squamous cell carcinoma (ESCC) still have either poor safety or efficacy. Better therapies are needed in China.

Methods This was an open-label, single-arm, phase 2 trial. Patients with potentially resectable ESCC (cT1b-3, Nany, M0 or T4a, N0-1, or M0) received preoperative intravenous sintilimab plus triplet chemotherapy (liposomal paclitaxel, cisplatin, and S-1) every 3 weeks for two cycles. The primary endpoints were safety and surgical feasibility; the secondary endpoint was major pathological response (MPR) rate. Genomic biomarkers (genetic mutations, tumor mutational burden (TMB), circulating tumor DNA status and immune microenvironment) in baseline tumor samples were investigated.

Results All 30 patients completed two cycles of neoadjuvant treatment and underwent surgical resection. Grade 3–4 treatment-related adverse events (TRAEs) occurred in 36.7% (11/30) of patients. The most frequent TRAEs were decreased white cell count (76.7%), anemia (76.7%), and decreased neutrophil count (73.3%). All TRAEs were hematological toxicities; none caused ≥30 days surgical delay. The MPR and pathological complete response (pCR) rates were 50.0% (15/30; 95% CI 33.2 to 66.9) and 20.0% (6/30; 95% CI 9.5 to 37.3), respectively. Patients with higher TMB and more clonal mutations were more likely to respond. ERBB2 alterations and ctDNA high-releaser status have a negative correlation with neoadjuvant ICI response. No significant difference was observed between therapeutic response and tumor immune microenvironment.

Conclusions Neoadjuvant sintilimab plus platinum-based triplet chemotherapy appeared safe and feasible, did not delay surgery and induced a pCR rate of 20.0% in patients with potentially resectable ESCC.

Trial registration number NCT03946969.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ PD-1 inhibitor plus chemotherapy has shown promising antitumor activity and survival benefit over chemotherapy as first-line therapy in patients with advanced or metastatic esophageal squamous cell carcinoma (ESCC).

WHAT THIS STUDY ADDS

⇒ The neoadjuvant regimen of sintilimab combined with triplet chemotherapy is safe, feasible, and showed favorable antitumor efficacy with a promising pathological complete response rate.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ This study provided evidence for a further randomized trial investigating neoadjuvant immunotherapy plus triplet chemotherapy for ESCC in China and indicated which patients can benefit more from this regimen.

INTRODUCTION

Esophageal cancer (EC) is the sixth most common cause of cancer-related death worldwide. It is the sixth most common cancer in China, where approximately 90% of patients with EC have esophageal squamous cell carcinoma (ESCC), and most ESCC patients have locally advanced disease at the time of diagnosis.[1]

The standard treatment for resectable EC is neoadjuvant chemotherapy or chemoradiotherapy followed by radical oesophagectomy.[2] However, neoadjuvant chemotherapy for ESCC has poor efficacy with pathological complete response (pCR) rates as low as 4%, and it does not improve prognosis compared with surgery alone.[3 4] Neoadjuvant

chemoradiotherapy has better efficacy than chemotherapy, but safety and feasibility are poor,[4] indicating a need for more effective and safer therapy.

The combination of a programmed cell death protein 1 (PD-1) inhibitor, such as pembrolizumab or sintilimab, and chemotherapy has shown promising antitumor activity and significant survival benefit over chemotherapy as first-line therapy in patients with advanced or metastatic ESCC.[5 6] In the ORIENT-15 study, sintilimab combined with chemotherapy (paclitaxel+cisplatin or cisplatin+5-fluorouracil) significantly improved overall survival (OS) compared with placebo combined with chemotherapy in the first-line treatment of patients with advanced or metastatic ESCC (median 16.7 vs 12.5 months, HR 0.63, 95% CI 0.51 to 0.78) and had a manageable safety profile.[6] These findings have sparked great interest in exploring this combination therapy for non-metastatic ESCC. A neoadjuvant therapy regimen of a PD-1 inhibitor combined with chemotherapy showed positive results in CheckMate 816, a phase 3 randomized controlled study of lung cancer[7] ; treatment-related toxicity was manageable and surgical resection feasibility was not affected. However, there is little evidence for this combination as neoadjuvant therapy in patients with ESCC.

In our hospital, 52 patients with locally advanced ESCC received triplet neoadjuvant therapy of liposomal paclitaxel, cisplatin, and tegafur-gimeracil-oteracil (S-1). After two cycles, the ORR was 46.1%. Thirty-two patients underwent subsequent surgery, including 28 patients with R0 resection. The treatment was well tolerated, with most adverse events (AEs) being stages I–II bone marrow suppression and digestive tract AEs (unpublished data).

Not all patients respond to immune checkpoint blockade, however, and there is a need to identify biomarkers that can predict which patients are most likely to benefit from these treatments. Predictive biomarkers of ICI response, including PD-L1 expression, tumor mutational burden (TMB) and immune cell infiltrations, have been extensively explored in patients with advanced cancers. The predictive values of such markers in the neoadjuvant setting, particularly in early ESCC, remain to be characterized.

Accordingly, we hypothesized that combining sintilimab and liposomal paclitaxel, cisplatin, and S-1 would increase the proportion of patients achieving pCR without compromising surgical resection. We evaluated the safety, feasibility, and efficacy of sintilimab plus platinum-based triplet chemotherapy in patients with potentially resectable ESCC and explored the associations between this treatment regimen and biomarkers.

METHODS

Study design and patients

KEEP-G 03 was an open-label, single-arm, phase 2, exploratory clinical trial undertaken at the First Affiliated Hospital of Nanjing Medical University (Nanjing, China). The study comprised safety run-in, efficacy pilot, and efficacy confirmation stages (figure 1 and online supplemental text).

Patients in this study were aged 18–70 years; with histologically or cytologically confirmed ESCC that was potentially resectable (clinical stage T1b-3, Nany, M0 or T4a, N0-1, M0; American Joint Committee on Cancer eighth edition[8]); ESCC treatment-naïve; with an Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1; and with adequate organ function. Key exclusion criteria included history of, or active, autoimmune disease or undergoing treatment for autoimmune diseases; requiring immunosuppressive therapy within 7 days before the neoadjuvant therapy; and having received

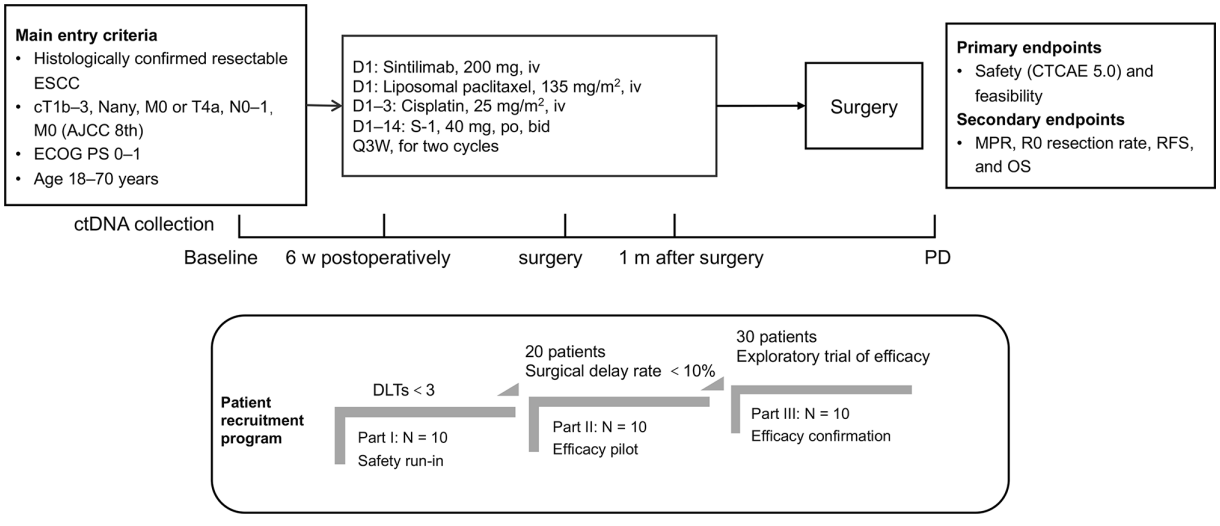

Figure 1 Study design. bid, two times a day; CTCAE, Common Terminology Criteria for Adverse Events; DLTs, dose-limiting toxicity; ECOG PS, Eastern Cooperative Oncology Group performance status; ESCC, esophageal squamous carcinoma; iv, intravenous; MPR, major pathological response; OS, overall survival; PD, progressive disease; PO, orally; Q3W, every 3 weeks; RFS, recurrence-free survival.

any antitumor therapy. Detailed inclusion and exclusion criteria are provided in online supplemental text.

Procedures

Pretreatment workup and staging are described in online supplemental text. Patients received the following neoadjuvant treatments before surgery: sintilimab (200 mg) on day 1 administered intravenously; liposomal paclitaxel (135 mg/m^2) on day 1 intravenously; cisplatin (25 mg/m^2) on days 1–3 intravenously; and S-1 (40 mg two times per day) on days 1–14 orally, and every 21 days for a total of two cycles. Surgical feasibility and postsurgery evaluations were planned within 6 weeks after the second cycle of neoadjuvant therapy.

Surgical procedures included esophagectomy and lymph node dissection assisted by thoracoscope or laparoscope, or conventional thoracotomy.[9] Patients could proceed to surgery early if they could not tolerate two cycles of neoadjuvant treatment. Any patient evaluated as infeasible for surgery could opt for radical chemoradiotherapy or other treatments at the investigators' discretion. Postoperative adjuvant treatment was determined based on the investigator's choice and could include radiotherapy, chemotherapy, or chemoradiotherapy. See online supplemental text for information regarding dose adjustment and modification.

AEs and dose-limiting toxicities (DLTs; see online supplemental text) in the safety run-in stage were monitored and recorded from the day of neoadjuvant therapy until 90 days after the last dose or postoperative day 30 and were graded according to Common Terminology Criteria for Adverse Events (version 5.0). Pathological response was assessed by pathologists at The First Affiliated Hospital of Nanjing Medical University as detailed in online supplemental text. To prevent the onset of postchemotherapy myelosuppression in patients who could not attend hospital visits during the COVID-19 pandemic, long-acting granulocyte colony-stimulating factor (G-CSF) was pre-emptively administered. Details of the postoperative follow-up period can be found in online supplemental text.

Pathological response was assessed by pathologists at Jiangsu Provincial People's Hospital by measuring the percentage of residual viable tumor in resected primary tumors. Major pathological response (MPR) was defined as the presence of ≤10% viable cancer cells in the primary tumor. Complete pathological response (pCR) was defined as tumors without viable cells in the resected primary tumor sample and all sampled regional lymph nodes. Depth of tumor invasion, lymph node metastasis, and resection margin evaluation were based on the AJCC eighth edition.[8]

PD-L1 immunohistochemistry assay was used to evaluate the PD-L1 CPS score of the tumor. TMB, clonal mutations, genetic alterations, and circulating tumor DNA (ctDNA) were investigated via next-generation sequencing-based gene panel tests conducted by Nanjing Geneseeq Technology, Nanjing, China (see online supplemental text).

Multiplexed immunofluorescence (mIF) staining and multispectral imaging analysis were used to investigate the correlation of baseline tumor immune microenvironment (TIME) with pathological response (see online supplemental text).

Outcomes

The primary endpoints were safety and surgical feasibility. Safety was defined as the incidence of grade 3–4 treatment-related AEs (TRAEs) from the day of neoadjuvant therapy to 30 days postoperatively or within 90 days after the last neoadjuvant treatment, and surgical feasibility as the incidence of TRAEs causing surgery delays of ≥30 days and/or inoperable patients.

The secondary endpoints were MPR rate; R0 resection rate (defined as no cancer cells seen microscopically at the resection margin following surgery); recurrence-free survival (RFS), calculated from surgery until recurrence or death; and OS, calculated from the beginning of neoadjuvant treatment until death from any cause.

Exploratory endpoints included genomic biomarkers (genetic mutations, TMB, ctDNA status, and immune microenvironment) in baseline tumor samples that may correlate with therapeutic response to neoadjuvant sintilimab plus chemotherapy.

Statistical analysis

For this exploratory, hypothesis-generating, proof-of-concept study, the primary endpoints were safety and feasibility and there were no data on neoadjuvant immunotherapy and the expected effect size in ESCC; thus, no formal hypothesis and sample size calculation was performed for efficacy. Efficacy was described by pCR rate (95% CI). Although a fixed sample size of 40 patients was planned for this study, the final sample size was 30, with 10 patients enrolled in each stage, and this protocol amendment was approved by the Ethics Commission of Jiangsu Province Hospital (2019-SR-159.A1). The sample size was reduced because the primary endpoint of the study was achieved. Furthermore, efficacy of neoadjuvant sintilimab combined with chemotherapy was demonstrated in 30 patients as 20.0% (95% CI 9.5 to 37.3) and the lower boundary of the pCR rate was >4.0%. The choice of a 4.0% pCR rate was based on historical data of neoadjuvant chemotherapy for EC.[3]

The safety, feasibility, and survival analyses were based on the intention-to-treat population (all patients who received at least one neoadjuvant treatment), and the pathological response analysis was carried out in patients who completed neoadjuvant treatment and surgery. Continuous data are expressed as median and range, and categorical data as numbers and percentages. Descriptive statistics were used for baseline demographic characteristics, safety data, and pathological response data. Proportions of patients with an MPR and pCR were estimated along with 95% CIs. The χ^2 test (Pearson's χ^2 test) was used to analyze the correlation between baseline characteristics, prophylactic use of long-acting G-CSF, and MPR

and pCR. The Kaplan-Meier method and corresponding 95% CIs were used to analyze RFS and OS. Fisher's exact test, t-test, Wilcoxon rank sum test, or Mann-Whitney test was used for intergroup comparison as needed.

The statistical analysis of biomarker testing was conducted by Nanjing Geneseeq Technology, Nanjing, China. Genetic mutations and ctDNA status at baseline between pathological response groups (a responder was defined as any patient who achieved MPR) were compared using Fisher's exact test. TMB differences between responders and non-responders were compared using the Wilcoxon rank sum test. All tests were two sided, and a $p<0.05$ was considered significant; all analyses were performed using R software (V.3.4.3).

RESULTS

Between May 2019 and Jan 2022, 67 patients were screened and 30 were enrolled (online supplemental figure 1). Two patients experienced DLTs in the safety run-in stage, leading to the second study stage. All patients underwent surgery without delay during the second stage, allowing entry into the efficacy confirmation stage.

Patients had a median age of 64 years (range 42–70), and 46.7% (14/30) were aged ≥65 years. Most patients had ECOG performance status 1 (22/30, 73.3%) and stage II disease 83.3% (25/30) (table 1). All patients completed two cycles of neoadjuvant treatment; 15 received adjuvant treatment postoperatively, and the rest were under observation.

The incidence of grade 3–4 TRAEs was 36.7% (11/30), all of which were hematological toxicities. No TRAEs caused ≥30 day delays in surgery. One patient had surgery delayed for >30 days, attributable to the family's initial refusal of surgery (table 2). The incidence of any TRAE was 100%; the most frequent (≥20%) were decreased white cell count (76.7%), anemia (76.7%), decreased neutrophil count (73.3%), alopecia (56.7%), decreased platelet count (56.7%), nausea (50.0%), loss of appetite (33.3%), and vomiting (30.0%); most TRAEs were grade 1 or 2. Three patients (10.0%) presented Grade 1 post-operative complications: recurrent laryngeal nerve injury, esophageal stenosis, and pneumothorax (table 3). No deaths were reported from neoadjuvant therapy up to 30 days postoperatively.

All patients underwent surgery and achieved R0 resection. The median interval was 20 days from the end of neoadjuvant therapy to surgery (IQR 15.0–22.5 days). Twenty-five patients (83.3%) received right thorax-epigastric two-incision esophagectomy (Ivor-Lewis method), and five (16.7%) received left cervical-right thorax-epigastric midline three-incision esophagectomy (McKeown method). The median number of resected lymph nodes was 30 (range 3–43).

The MPR rate was 50.0% (15/30) and 6/30 patients (20.0%; 95% CI 9.5% to 37.3%) achieved pCR in the primary tumor and lymph nodes (table 3). One patient (3.3%) achieved a pCR of the primary lesion (T0) despite the presence of tumor cell residue in the resected lymph node (N1). The median pathological tumor regression ratio was 88.0% (range: 10.0%–100%) (figure 2A). No significant association was identified between pathological response and baseline characteristics (online supplemental tables 1 and 2).

During the COVID-19 pandemic, 10 patients were pre-emptively administered long-acting G-CSF. In a post hoc analysis, the pCR rate of these patients was significantly higher than those not treated with G-CSF (50.0% vs 5.0%; p=0.0088, figure 2B).

At data cut-off (January 18, 2022), the median (range) follow-up was 17.3 (2.7–28.4) months from the first treatment and 12.9 (0.3–24.9) months postoperatively. Four patients had a primary recurrence, and two died without recurrence. The median RFS was not reached, and the 12-month RFS rate was 78.9% (95% CI 56.4% to 90.6%)

Table 1 Patient baseline characteristics

Characteristic	N=30
Age, years, n (%)	
Median (range)	64 (42–70)
≥65	14 (46.7)
Sex, n (%)	
Male	22 (73.3)
Female	8 (26.7)
ECOG performance status, n (%)	
0	8 (26.7)
1	22 (73.3)
Tumor location at initial diagnosis, n (%)	
Middle	24 (80.0)
Lower	6 (20.0)
Clinical T stage, n (%)	
cT2	7 (23.3)
cT3	21 (70.0)
cT4a	2 (6.7)
Clinical N stage, n (%)	
cN0	23 (76.7)
cN1	7 (23.3)
Initial clinical staging	
II	25 (83.3)
III	3 (10.0)
IVA	2 (6.7)
Smoking history, n (%)	
Yes	20 (66.7)
No	10 (33.3)
History of alcohol consumption, n (%)	
Yes	19 (63.3)
No	11 (36.7)

ECOG, Eastern Cooperative Oncology Group.

Table 2 List of treatment-related adverse events in the intention-to-treat population

TRAEs (N=30)					
Adverse events, n (%)	**Any grade**	**Grade 1**	**Grade 2**	**Grade 3**	**Grade 4**
All adverse events	30 (100.0)	6 (20.0)	13 (43.3)	6 (20.0)	5 (16.7)
Hematological toxicity					
Decreased WCC	23 (76.7)	6 (20.0)	15 (50.0)	2 (6.7)	0
Anemia	23 (76.7)	21 (70.0)	1 (3.3)	1 (3.3)	0
Decreased neutrophil count	22 (73.3)	3 (10.0)	9 (30.0)	5 (16.7)	5 (16.7)
Decreased platelet count	17 (56.7)	12 (40.0)	5 (16.7)	0	0
Granulocyte deficiency with fever	1 (3.3)	0	0	1 (3.3)	0
Non-hematological toxicity					
Alopecia	17 (56.7)	8 (26.7)	9 (30.0)	0	0
Nausea	15 (50.0)	12 (40.0)	3 (10.0)	0	0
Loss of appetite	10 (33.3)	10 (33.3)	0	0	0
Vomiting	9 (30.0)	7 (23.3)	2 (6.7)	0	0
Elevated glutamate transaminase	5 (16.7)	5 (16.7)	0	0	0
Lethargy	4 (13.3)	3 (10.0)	1 (3.3)	0	0
Elevated glutathione aminotransferase	4 (13.3)	4 (13.3)	0	0	0
Elevated creatinine	4 (13.3)	4 (13.3)	0	0	0
Decreased albumin	1 (3.3)	1 (3.3)	0	0	0
Diarrhea	1 (3.3)	1 (3.3)	0	0	0
Dry cough	1 (3.3)	1 (3.3)	0	0	0
Itchy skin	1 (3.3)	1 (3.3)	0	0	0
Rash	1 (3.3)	1 (3.3)	0	0	0
Blood clots in the upper extremities	1 (3.3)	0	1 (3.3)	0	0
Decreased fibrinogen	1 (3.3)	1 (3.3)	0	0	0
Elevated blood glucose	1 (3.3)	1 (3.3)	0	0	0

Data are n (%). Some patients experienced more than one adverse event and at different grades. There were no treatment-related deaths.
TRAEs, treatment-related adverse events; WCC, white cell count.

(figure 2C). Twenty-six patients (86.7%) remained alive, and four died. The median OS was not reached (figure 2D). Of note, all patients with pCR survived, without evidence of recurrence.

Biomarker analyses were conducted in 25 patients with sufficient tissue biopsy material available. In the 25 patients with evaluable PD-L1 status, we analyzed the correlation of PD-L1 expression with the response to neoadjuvant immunotherapy. The result showed a trend toward higher rates of MPR in patients with PD-L1 CPS ≥1 (66.7% vs 37.5%; p=0.23, figure 2E), although patient survival, such as RFS and OS, were not stratified by PD-L1 expression (online supplemental figure 2), likely related to the immaturity of the data. A trend toward higher TMB was observed in responders compared with non-responders (median TMB 12.69 vs 8.46 mutations/Mb, p=0.08; figure 3A). At a higher TMB cut-off of 14.8 (top 1/3 of the cohort), patients with high TMB had significantly higher response rates compared with patients with low TMB (87.5% vs 41.2%, p=0.05; figure 3B). There was also a trend toward more clonal mutations in responders than non-responders (median 6.0 vs 3.0, p=0.08; figure 3C). Receiver operating characteristic (ROC) analyses suggested a higher predictive value of clonal mutations than TMB for pathological response to neoadjuvant immunotherapy, with an area under the ROC curve of 0.76 (p=0.03 figure 3D).

The most frequently mutated genes in 25 ESCC patients were *TP53* (96%), *NOTCH1* (60%), *CCND1* (52%), and *FGF19* (48%) (online supplemental figure 3A). Association analyses of genetic alterations (occurring in ≥3 patients) with pathological response revealed enrichment of Erb-B2 receptor tyrosine kinase 2 (*ERBB2*) alterations in non-responders, which was absent in responders (63.6% vs 0%, p=0.07; online supplemental figure 3B). All three *ERBB2*-altered non-responders carried mutations for tumorigenesis. A mutual exclusivity or co-occurrence analysis showed *ERBB2* alterations occurred independently of other genetic alterations, with no co-occurring or mutually exclusive genetic events (online supplemental figure 3C). We further analyzed the correlation of ERBB2 alterations with TIME. M2 macrophages

Table 3 Surgical and pathological outcomes of patients who underwent surgery

Characteristics	n (%) or median (IQR) or median (range)
R0 resection	30 (100.0)
Interval from the end of neoadjuvant therapy to surgery	20 (IQR 15.0–22.5)
Surgical methods	
Ivor Lewis	25 (83.3)
McKeown	5 (16.7)
Pathological response	
pCR (no residual tumor cells)	6 (20.0)
MPR (residual tumor cells≤10%)	15 (50.0)
Non-MPR (residual tumor cells>10%)	15 (50.0)
No of resected lymph nodes	30 (range 3.0–43.0)
Postoperative complications	
Recurrent laryngeal nerve injury	1 (3.3)
Esophageal stenosis	1 (3.3)
Pneumothorax	1 (3.3)
30-day mortality	0 (0)

MPR, major pathologic response; pCR, pathological complete response.

were enriched in the tumor parenchyma in patients with ERBB2 alterations than those with wide-type ERBB2 (p=0.05). No differences in the distribution of other infiltrated immune cells, including CD8, CD56 cells and M1 macrophages, were observed between ERBB2 mutant and wide-type patients (online supplemental figure 3D). Notably, two patients with high TMB (≥10 mutations/Mb[10]) and ERBB2 alterations were non-responders (online supplemental figure 3E).

Analysis of plasma-derived ctDNA before the neoadjuvant treatment (n=30) showed no difference in the proportions of ctDNA low-releasers (ie, no detectable ctDNA (0% ctDNA maximum somatic allele frequency)) between responders and non-responders (33.3% vs 13.3%, p=0.39), but a higher proportion of patients who achieved pCR were ctDNA low-releasers compared with those without pCR (83.3% vs 8.3%, p=0.008) (online supplemental figure 4).

To examine the immune microenvironment and its potential association with pathological response, mIF was performed on baseline biopsy samples from 26 patients of which 3 samples failed to pass quality control. Of 23 patients, 11 patients were responders (patients who achieved MPR) and 12 patients were non-responders. Overall, $CD8^+$ T cells and M2-like macrophages were the predominant cell types in the TIME of ESCC patients especially in tumor region (online supplemental figure 5). No significant difference was observed between responders and non-responders in the composition of all the assessed immune cells, including $CD8^+$ T cells,

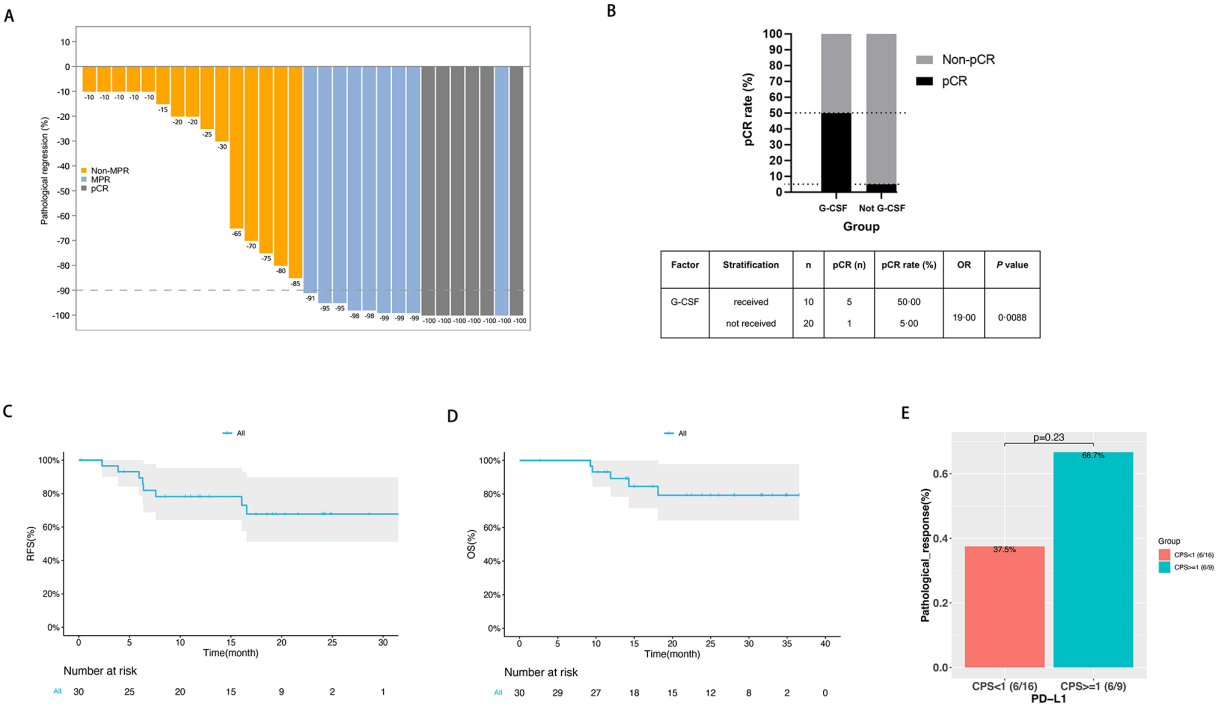

Figure 2 (A) Proportion of pathological tumor remnants (N=30) (B) correlation between prophylactic use of long-acting G-CSF and pCR G-CSF (C) Kaplan-Meier analysis for recurrence-free survival (N=30) (D) Kaplan-Meier analysis for overall survival (n=30) (E). Correlation between baseline PD-L1 CPS expression and MPR. CPS, combined positive score; G-CSF, granulocyte colony-stimulating factor; MPR, major pathological response; pCR, pathological complete response.

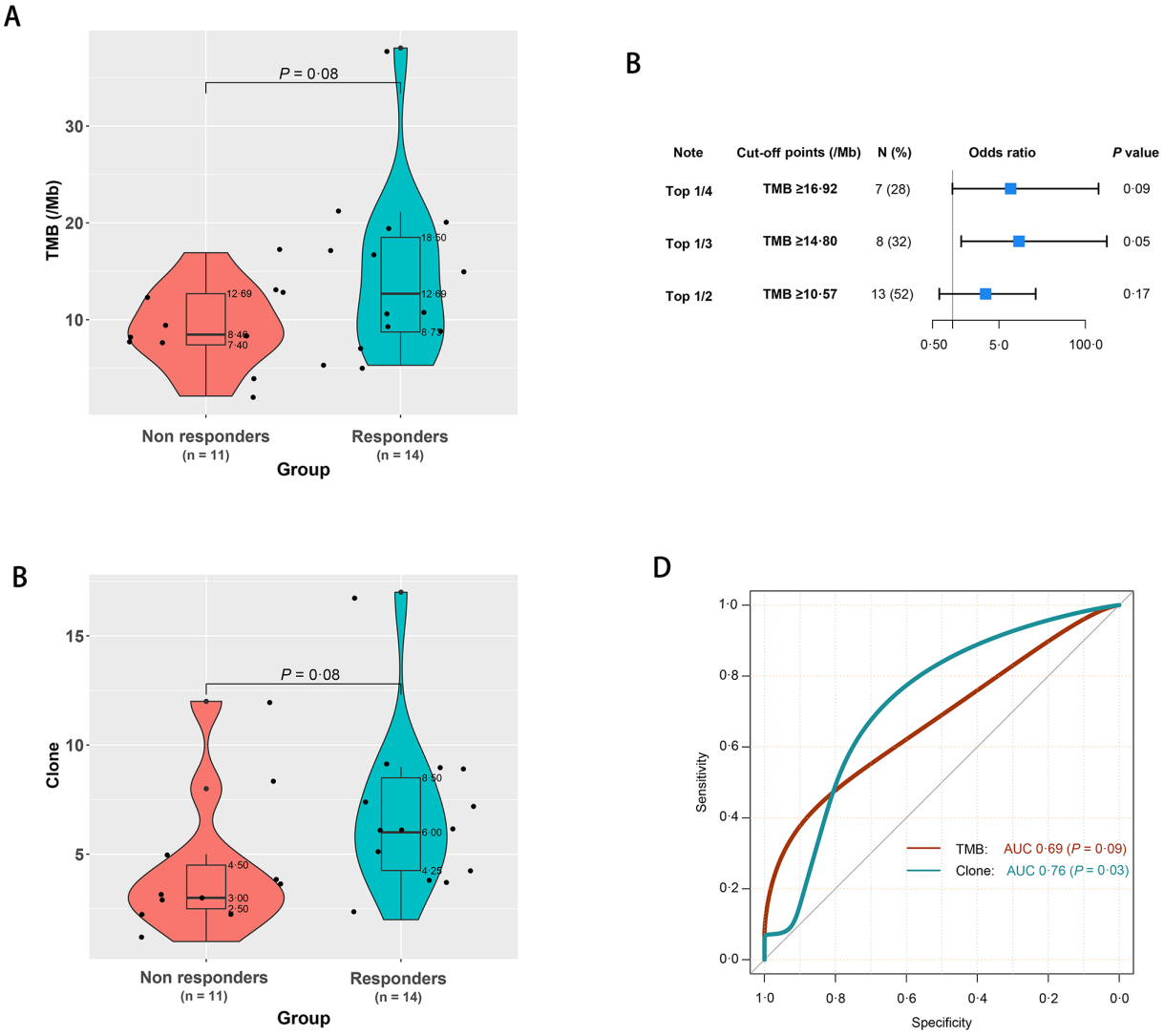

Figure 3 (A) TMB in non-responders versus responders (B) TMB forest plot (C) clonal number in non-responders versus responders (D) TMB versus clonal number. AUC, area under the curve; TMB, tumor mutational burden.

M1-like macrophages, M2-like macrophages, CD56^{dim} natural killer (NK) cells and CD56 ^{bright} NK cells both in the tumor (online supplemental figure 6A) and the stroma (online supplemental figure 6B). Similar results were obtained comparing patients with pCR and non-pCR (online supplemental figure 6C,D).

Next, patients were subgrouped based on unsupervised clustering of the TIME compositions in the tumor region into three different clusters (CT1, CT2 and CT3; figure 4A). The CT1 has more infiltrating CD8^{+} T cells, and CT3 has more infiltrating M2-like macrophages while immune cells infiltration was low in CT2. Patients

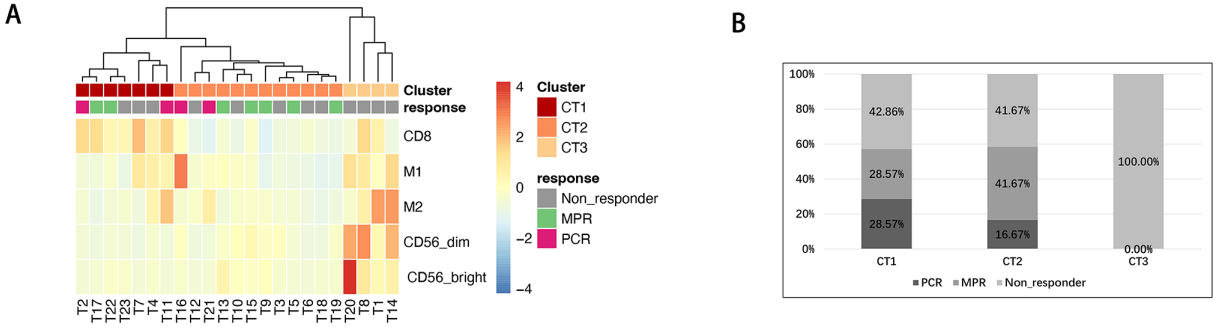

Figure 4 (A) Clustering based on the positive cells of infiltrating immune cells in tumor parenchyma identified cluster 1 (C1), cluster 2 (C2) and cluster 3 (C3) (B) comparisons of pathological response among the time clusters.

in either CT1 or CT2 clusters had higher proportions of responders (CT1, 57.14%; CT2, 58.34% and CT3, 0.0%, p=0.17) and pCR cases (CT1, 28.5%; CT2, 16.67% and CT3, 0.0%, p=0.62) compared with those in the CT3 cluster (figure 4B).

In 19 patients who also underwent NGS testing, we explored the association between TMB and TIME. Higher TMB showed modest correlation with an increased proportion of $CD8^+$ T cells (ρ=0.397, p=0.09). No significant correlation was observed between TMB and other tumor-infiltrating immune cells (online supplemental figure 7A,B).

DISCUSSION

To the best of our knowledge, this is the first prospective clinical trial to assess the safety, feasibility, and efficacy of neoadjuvant immunotherapy plus triplet chemotherapy in patients with potentially resectable ESCC. Sintilimab combined with platinum-based triplet chemotherapy as a neoadjuvant regimen was safe and feasible. The pCR rate was 20.0%, and interestingly, prophylactic use of long-acting G-CSF was found to be significantly associated with pCR.

The primary endpoint was met. The incidence of grade 3–4 TRAEs (36.7%) was acceptable and manageable compared with previous studies (40.0% in the UK MRC OE05 study on neoadjuvant chemotherapy,[11] 61.5% in the NEOCRTEC5010 study on neoadjuvant chemoradiotherapy,[12] and 65.0% in the PALACE-1 study on neoadjuvant chemoradiotherapy plus pembrolizumab[13]). Regarding feasibility, the median time from the end of neoadjuvant therapy to surgery was 20 days in this study, no TRAEs resulted in ≥30 days surgical delay, and the surgical feasibility was 100%. In OE02, the feasibility of surgery at the end of neoadjuvant chemotherapy was 92%,[3] while that in NEOCRTEC5010 at the end of neoadjuvant radiotherapy was 82.6%.[12] Additionally, only three patients (10%) in this study experienced postoperative complications; in contrast, the morbidity rates reported in NCT03001596 for neoadjuvant chemoradiotherapy and chemotherapy were 47.4% and 42.6%, respectively.[14] The present results could be attributed to the good safety profile of immunotherapy as reported in both ORIENT-15[6] and KEYNOTE-590,[5] which reported a manageable toxicity in first-line treatment of EC. A similar phenomenon was observed in the neoadjuvant lung cancer immunotherapy study (CheckMate-816),[7] where nivolumab combined with chemotherapy compared with chemotherapy neoadjuvant treatment of lung cancer did not increase the rate of surgical delay. In addition, liposomal paclitaxel, which has shown less toxicity in first-line therapy of lung cancer, may also contribute to a better safety profile.[15]

Additionally, this lower risk of toxicity did not seem to result in a decreased benefit. The MPR rate in this study was 50.0% (95% CI 33.2% to 66.9%), higher than that of 13.4% with prior neoadjuvant chemotherapy reported in NCT03001596.[14] The present pCR rate was 20.0% (95% CI 9.5% to 37.3%), which was higher than the rates of 4% and 3.8% reported for neoadjuvant chemotherapy in OE02[3] and NCT03001596,[14] respectively. The present pCR rate was similar to that previously reported for neoadjuvant PD-1 inhibitor combined with chemotherapy (25%–33%)[16 17] and may be due to the synergistic effect of chemotherapy and immunotherapy,[18] especially in the neoadjuvant phase, where tumor tissues release more antigens in response to chemotherapy and induce stronger adaptive immune responses. Further, triplet chemotherapy with liposomal paclitaxel, cisplatin, and S-1 may contribute to a higher pathological response rate, as confirmed in JCOG 1109.[19]

Correlative assessment showed that no significant associations were identified between any baseline characteristics and pathological responses, and a similar finding was reported recently.[16] Notably, long-acting G-CSF was significantly associated with pCR, consistent with previous preclinical tumor models and clinical studies.[20 21] The possible mechanism was that G-CSF altered the tumor microenvironment and enhanced the infiltration of effector T cells.[20 21] However, this interpretation requires further exploration. The predictive role of PD-L1 expression in tumor and immune cells remains uncertain in EC. In KEYNOTE-180, patients with PD-L1-positive tumors had better ORR.[22] Similarly, in KEYNOTE-181, pembrolizumab improves OS in EC patients with positive PD-L1 expression.[23] By contrast, in ESCC patients treated with camrelizumab, PD-L1 status exhibits no correlation with ORR or DCR.[24] Besides, PD-L1 status was also not found to be a predictive biomarker for ESCC patients in a trial of toripalimab.[25] In our study, we observed a trend toward better response to neoadjuvant immunotherapy in patients with PD-L1 CPS ≥1, while no differences in RFS and OS were revealed in correlation with PD-L1 status. Therefore, the relationship of PD-L1 status and efficacy of anti-PD-1/PD-L1 inhibitors in ESCC patients, especially in the neoadjuvant setting warrants provide more clinical evidence in the future.

The association between high TMB and ICI therapy benefits in progressive advanced stage solid tumors has been well established,[26] but was unclear in the neoadjuvant setting. Higher TMB was shown to be associated with better MPR rates in CheckMate 159[27] but not in LCMC3.[28] In our study, higher levels of TMB were seen in responders compared with non-responders, along with stronger association with pathological responses with increasing cut-off points of TMB. Notably, patients with more clonal mutations were more likely to respond, with better predictive performance than TMB. Given our moderate sample size, the potential predictive value of TMB and/or clonal mutations in ESCC patients in the neoadjuvant setting requires further investigation.

Our study also identified a negative correlation between *ERBB2* alterations with neoadjuvant ICI response. Of the three *ERBB2*-mutated patients, none achieved MPR. In addition, two of the TMB-high patients without

pathological response could be attributed to the presence of *ERBB2* alterations.[10] In patients with lung cancer, the presence of activating mutations in driver oncogenes, such as *EGFR*, *ALK*, *ROS1*, *RET*, and *MET*, has been associated with poor ICI outcomes.[29] Increasing evidence points to the negative predictive role of *ERBB2* alterations in ICI response.[30] The association between ERBB2 alterations and resistance to immunotherapy might be related to the unique TIME of tumors with EGFR/ERBB2 alterations.[31] EGFR/ERBB2-mutated NSCLC has been shown to present with reduced levels of CD8+TILs[32–34] as well as diminished CD8+TIL function,[35] leading to impaired cytotoxicity and poor response to ICIs.[36] In addition, tumor infiltration of M2-like macrophages is associated with poor prognosis in several cancers, such as glioma,[37] renal cell carcinoma,[38] cholangiocarcinoma,[39] and esophageal carcinoma.[40 41] Similarly, our results showed that tumors with ERBB2 alterations harbored more M2-like macrophages compared with their wild-type counterparts, which may partially explain the relatively poor response to ICI in patients with ERBB2 alterations. However, given the small size of the dataset, the association of ERBB2 alterations with response to ICIs should be explored in future large-scale studies.

Additionally, ctDNA status is a reliable indicator of TMB and has enabled minimal residual disease detection in early-stage cancer patients at the molecular level.[42 43] ctDNA status at baseline has been shown to be closely related to the immune response in advanced lung cancer.[44] Our findings also suggest that ctDNA low-releaser status at baseline in ESCC patients might be a potential predictive biomarker for pCR to neoadjuvant immunotherapy.

Immunotherapy has been known to modulate the immunocomposition of the tumor microenvironment. In a previous clinical study (GASTO1056) on the efficacy of neoadjuvant camrelizumab plus carboplatin and nab-paclitaxel in resectable ESCC,[16] neoadjuvant immunotherapy has led an increase in the M2-like macrophages in the non-pCR patients and a simultaneous decrease in the pCR patients. However, whether baseline TIME plays a role in mediating immunotherapy response has been unclear. While a 'hot' TIME at baseline could more readily respond to immunotherapy,[45] no difference in baseline TIME was found between pCR and non-pCR patients in the GASTO1056 study. Similarly, we also detected no correlation between TIME composition and therapeutic response in our study, although a slight difference in the $CD8^+$ T cell infiltration. Tumor-infiltrating $CD8^+$ T cells play an important role in host immune defense against tumor progression in patients with ESCC.[46] In this study, there was a trend toward an increase in the positive rate of tumor-infiltrating $CD8^+$ T cells at baseline in patients who derived benefit from neoadjuvant immunotherapy. Of note, both this study and the GASTO1056 study were limited in sample size. Thus, further investigation into association between baseline TIME composition and pathological response should be warranted.

Notably, by clustering patients based on their TIME profile, we found that patients in CT3 cluster had an immune microenvironment that was enriched with M2-like macrophages and were 100% non-responder. It has been shown that the presence of *ERBB2* alteration is associated with poor ICI outcome.[47] Indeed, two of the four patients in the CT3 cluster harbored *ERBB2* alterations, suggesting that *ERBB2* alterations might be associated with a distinct immune microenvironment. The correlation of ICI outcome in ESCC with *ERBB2* alterations should be explored in future research. Finally, TMB is often used as a surrogate marker for neoantigens, which is associated with an increase in tumor-infiltrating T cells and a stronger antitumor immune response.[48] As expected, although limited by the sample size in this study, we did observe a modestly positive correlation between TMB and CD8+T cells.

Limitations include the fact that this was a non-randomized, single-arm study design with a small and fixed sample size and may not have been powered for correlation analysis of pathological response according to G-CSF and genetic biomarkers. Furthermore, the post-operative follow-up period was short; longer follow-up is needed to determine whether neoadjuvant therapy could improve RFS or OS.

In conclusion, the neoadjuvant regimen of sintilimab combined with triplet chemotherapy is safe, feasible, and showed favorable antitumor efficacy with a promising pCR rate; long-term survival benefit needs to be validated in future.

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