SUPPLEMENTARY INFORMATION

Toripalimab plus chemotherapy and radiotherapy for treatment-naive advanced esophageal squamous cell carcinoma: A single-arm phase 2 trial

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Supplementary Table 1. Treatment compliance (N=33)

Variable	No. (%)
Chemotherapy	
Completing four cycles of chemotherapy	26 (79)
Dose reduction	6 (18)
Radiotherapy	
Completing radiotherapy of 50–50.4 Gy for the primary tumor	27 (82)
Completing radiotherapy for all the metastasis	26 (79)
Median duration of primary tumor radiotherapy (IQR)	42 days (35–45)
Median duration of metastatic lesions radiotherapy (IQR)	14 days (8–21)
Reason for premature cessation	
Adverse events	3 (9)
Esophageal fistula	2 (6)
Arrhythmia	1 (3)
Progressive disease	1 (3)
Patient refusal	3 (9)
Toripalimab (one year planned)	
Median cycles (IQR)	10 (5–14)
One year (14–17 cycles)	14 (42)
<8 cycles	16 (49)
Reason for premature cessation	
Progressive disease	10 (3)
Adverse events	4 (13)
Esophageal fistula	2 (6)
Arrhythmia	1 (3)
Patient refusal	5 (15)

Abbreviations: IQR, interquartile range.

Supplementary Table 2. Tumor response to treatment

Variables	Three months after radiotherapy (n = 26)	Three months after radiotherapy (n = 33)	Best overall response (n = 33)
Complete response	0 (0 %)	0 (0 %)	0 (0 %)
Partial response	15 (57.7 %)	15 (45.5 %)	20 (60.6 %)
Stable disease	4 (15.4 %)	4 (12.1%)	10 (30.3 %)
Progressive disease	7 (26.9 %)	7 (21.2 %)	1 (3.0 %)
Not evaluable	0 (0 %)	7 (21.2 %)[a]	2 (6.1 %)[b]
ORR	15 (57.7%, 95%CI: 36.9–76.7%)	15 (45.5%, 95%CI: 28.1–63.7%)	
DCR	19 (73.1%, 95%CI: 52.2–88.4%)	19 (57.6%, 95%CI: 39.2–74.5%)	

Data are presented as n (%). [a]One patient withdrew informed consent after one cycle of chemo-immunotherapy. One patient experienced severe cardiac adverse events, two patients experienced esophageal fistulae, and one patient had disease progression after two cycles of chemo-immunotherapy. One patient withdrew consent after completing three sessions of radiotherapy, and another refused radiotherapy for liver metastasis after completing radiotherapy for the primary lesion. A total of seven patients did not complete radiotherapy, and their efficacy could not be evaluated. [b]One patient withdrew informed consent after one cycle of chemo-immunotherapy. One patient experienced severe cardiac adverse events after two cycles of chemo-immunotherapy. Both patients refused further assessment. Abbreviations: CI, confidence interval; DCR, disease control rate; ORR, objective response rate.

Supplementary Table 3. Tumor response to treatment after 2 cycles of C+I

Variables	Response after 2 cycles of C+I (ITT population, n=33)
Complete response	0 (0%)
Partial response	14 (42.4%)
Stable disease	16 (48.5%)
Progressive disease	1 (3.0%)
Not evaluable	2 (6.1%)
ORR	14 (42.4%, 95%CI: 24.6–60.2%)
DCR	30 (91%, 95%CI: 80.6–100%)

Data are presented as n (%). Abbreviations: C, chemotherapy; CI, confidence interval; DCR, disease control rate; I, immunotherapy; ITT, intention-to-treat population, ORR, objective response rate.

Supplementary Table 4. Recurrence pattern, sites, and reason for death (N=33)

Variables	No. (%)
Recurrence pattern	
No recurrence	10 (30)
LRR only	10 (30)
Esophagus only	5 (15)
Regional lymph nodes only	3 (9)
Both	2 (6)
DM only	6 (18)
Bone	2 (6)
Lung	1 (3)
Liver	3 (9)
LRR and DM	5 (15)
Regional and non-regional lymph nodes only	1 (3)
Esophagus, non-regional lymph nodes and other organs	4 (12)
Not available	2 (6)
Death reason	16 (49)
Cancer-specific	12 (36)
Non-cancer specific	2 (6)
Gastrointestinal bleeding	1 (3)
Pneumonitis	1 (3)

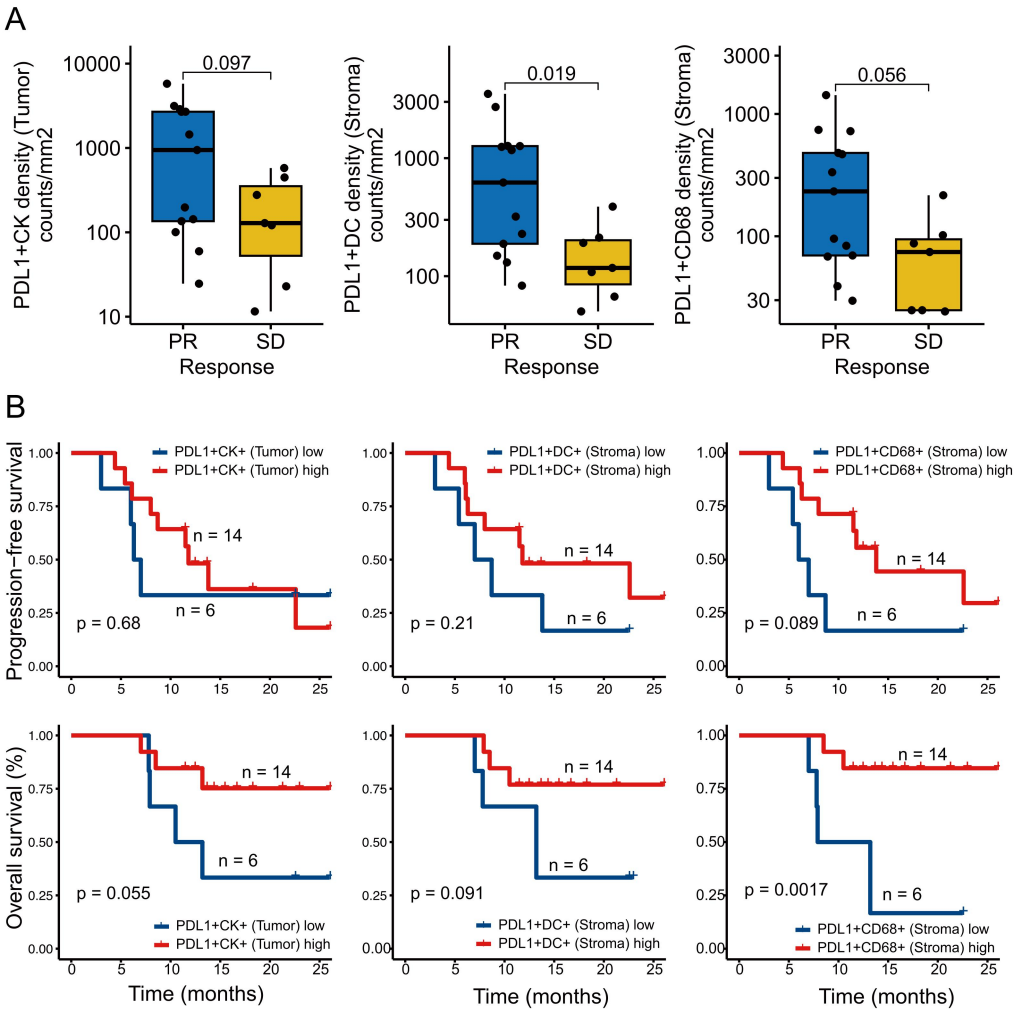
Abbreviations: LRR, locoregional recurrence; DM, distant metastases.

Supplementary Table 5. Second-line treatments for patients with recurrence

Treatment	No. (%)
Chemotherapy	7 (33)
Chemoradiotherapy	4 (19)
Chemotherapy combined with immunotherapy	5 (24)
Chemoradiotherapy combined with immunotherapy	2 (10)
Supportive care only	3 (14)

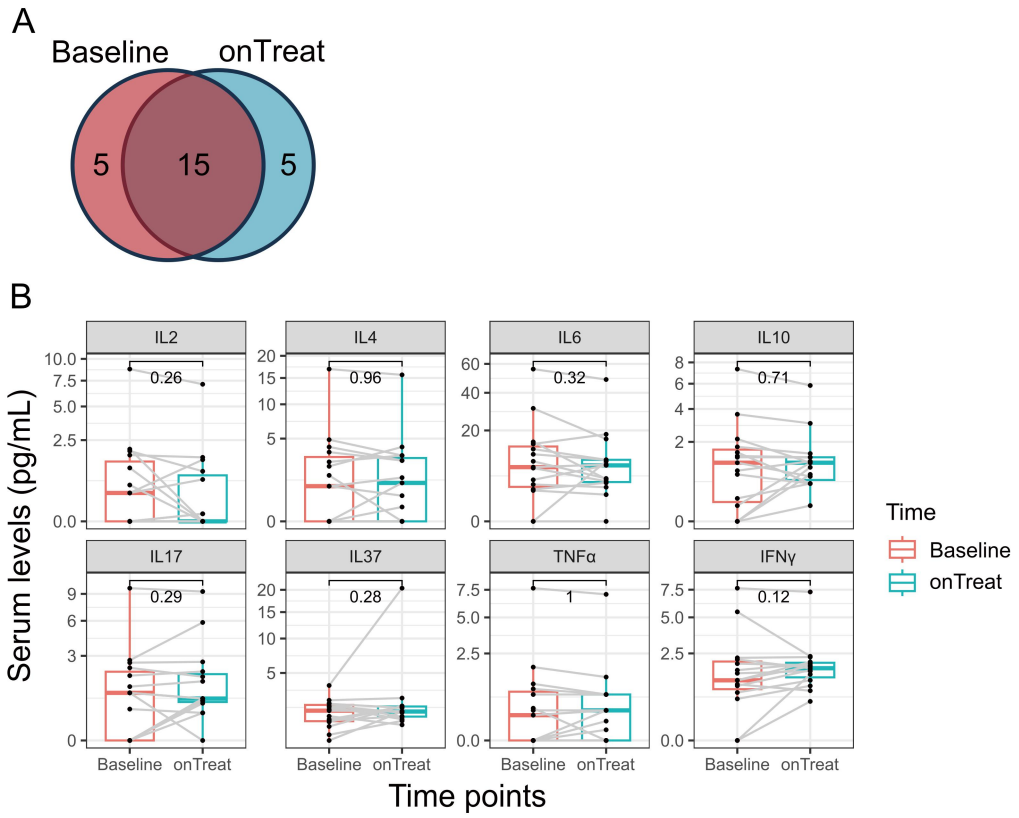
Supplementary Fig. 1. Extending biomarkers in the tumor microenvironment

(A) Infiltration levels of specific immune cells in the tumor tissues of patients achieving PR (n = 13) and stable disease (n = 7), as assessed by multiplex immunofluorescence. Immune cell densities were compared using the Mann–Whitney U test. (B) PFS and OS analyses of specific immune cell tumor infiltration levels (n = 20). The patients were divided into low and high immune cell infiltration groups based on the 30% quantile value of each variable. P-values were calculated using the two-sided log-rank test. Abbreviations: OS, overall survival; PFS, progression -free survival; PR, partial response; SD, stable disease.



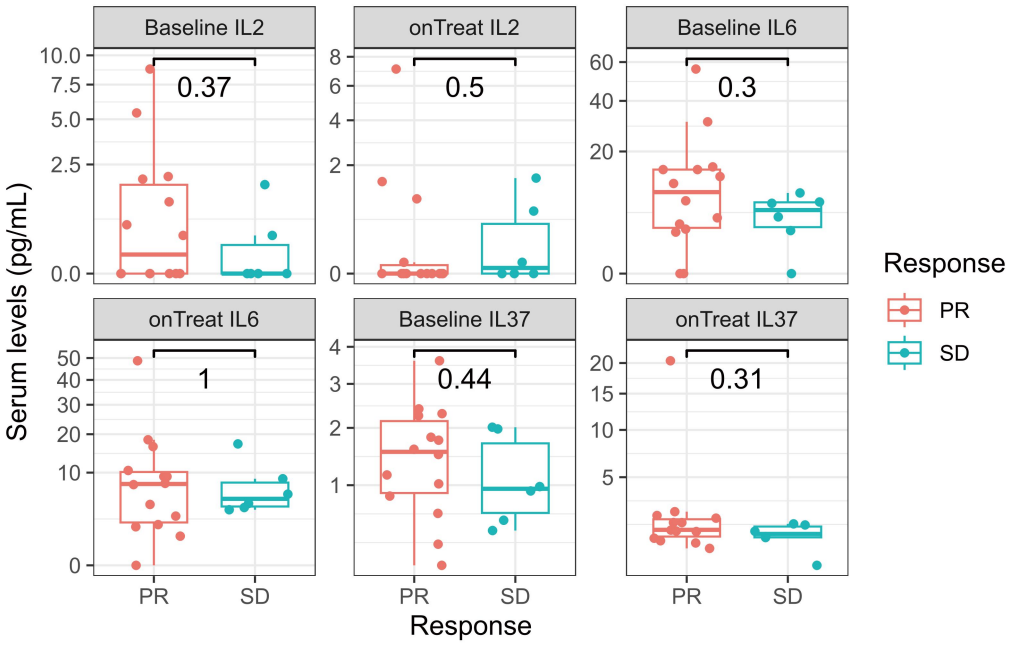
Supplementary Fig. 2. Assessment of peripheral cytokines

(A) A Venn diagram showing the distribution of serum samples assessed at baseline and during treatment time points. Paired samples were collected from fifteen patients. (B) Serum levels of eight cytokines were measured at baseline and during treatment (n = 15 samples). P values were calculated using the Wilcoxon signed-rank test.



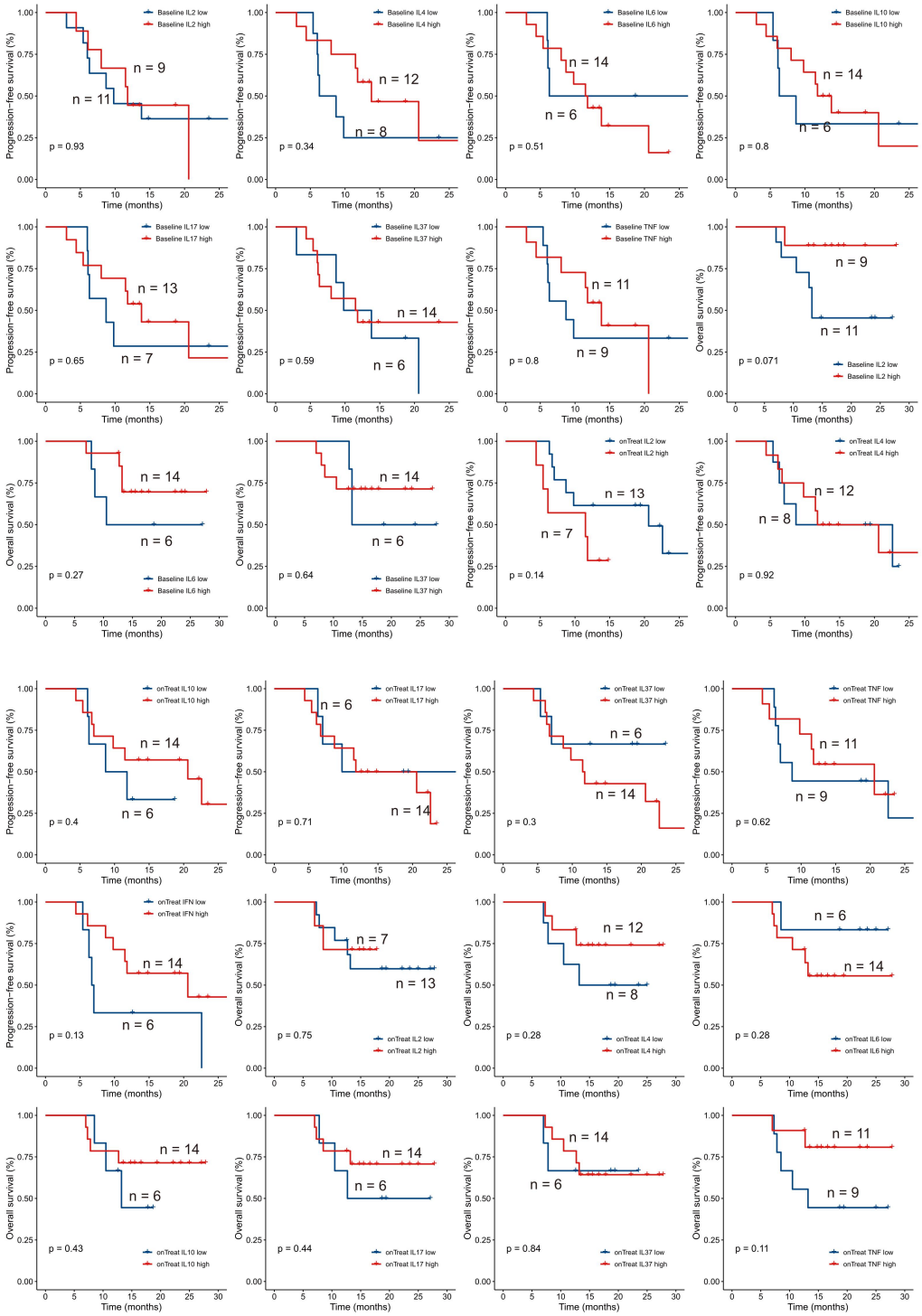
Supplementary Fig. 3. Association of specific peripheral cytokines with treatment response

The boxplots display the differences between clinical partial response (PR, n = 14) and stable disease (SD, n = 6). P-values were calculated using the Mann–Whitney U test. Abbreviations: PR, partial response; SD, stable disease.



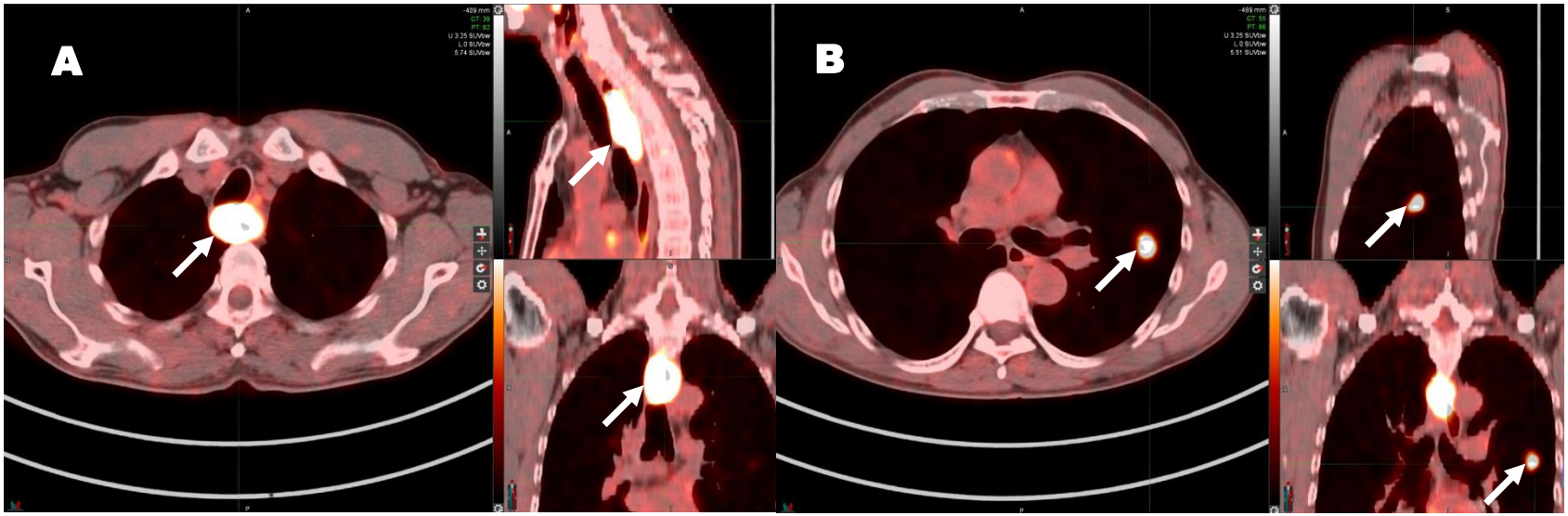
Supplementary Fig. 4. Prognostic value of peripheral cytokines

Kaplan–Meier curves showing PFS and OS for various peripheral cytokines at baseline and after treatment (n = 20). Patients were categorized based on low and high levels of various cytokines using the 30th quantile value of each variable. P-values were calculated using the two-sided log-rank test. PFS, progression-free survival; OS, overall survival.



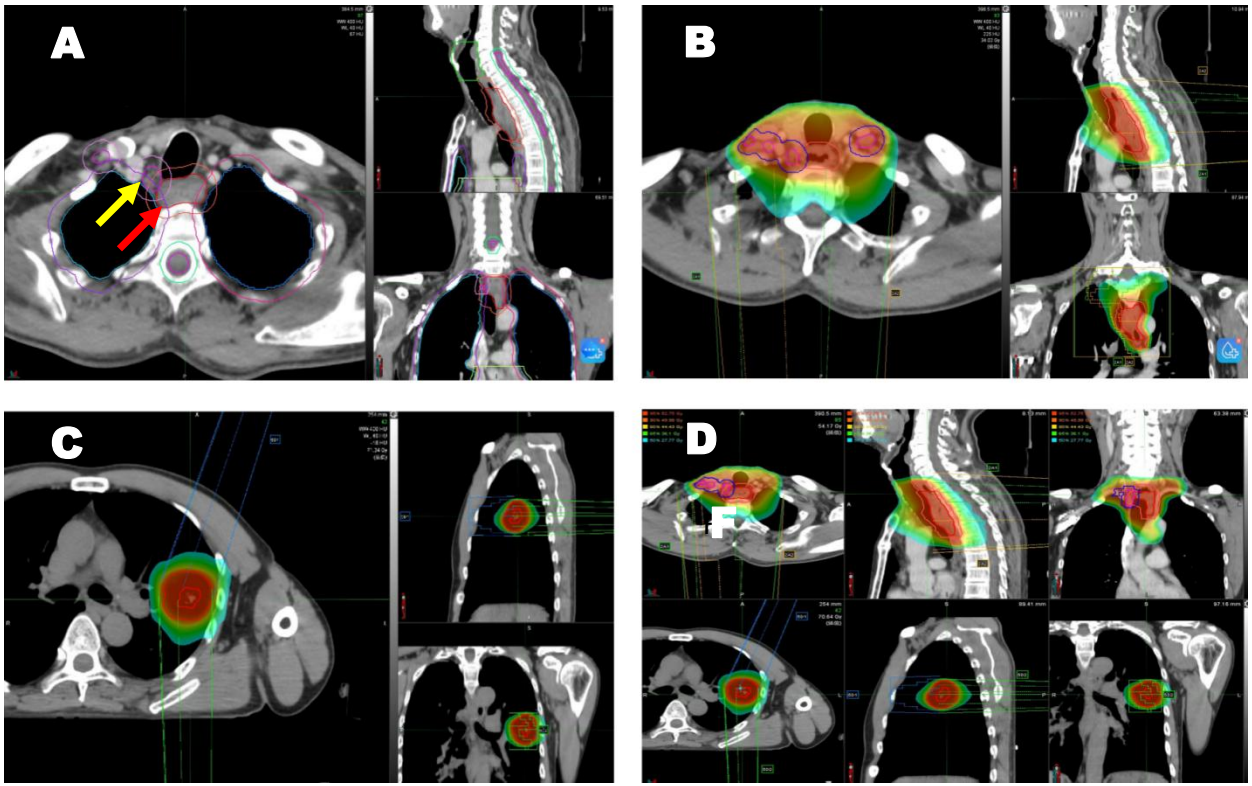
Supplementary Fig. 5. Tumor location in a patient with cT3N2M1 (AJCC 8th) advanced cervical esophageal squamous cell carcinoma

(A) The white arrow points to the primary esophageal lesion identified by PET-CT. (B) The white arrow indicates the location of the metastatic lesion in the left lung, as indicated by PET-CT. Abbreviations: PET-CT, positron emission tomography-computed tomography.



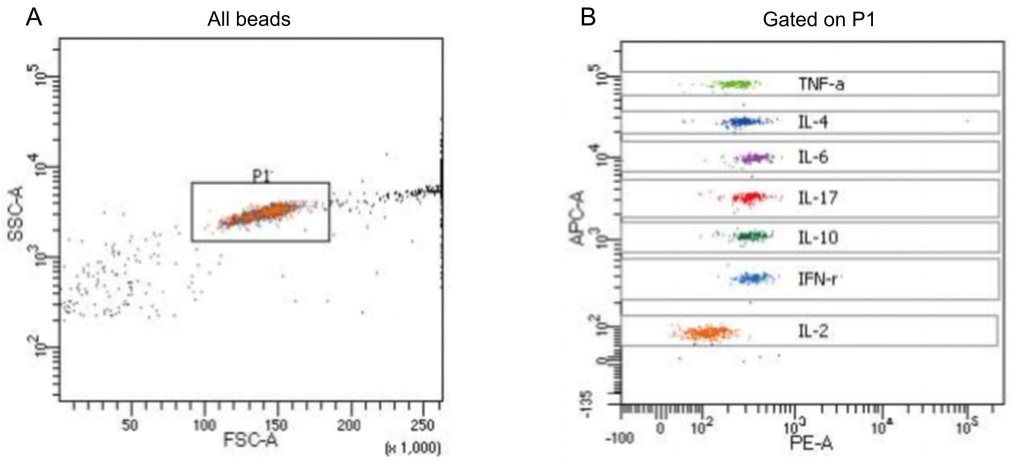
Supplementary Fig. 6. Target area delineation and radiation plan example

(A) Delineation of the primary tumor and metastatic lymph nodes, where the red arrow indicates gross target volume of the primary lesion and the yellow arrow indicates gross target volume of the metastatic lymph node. (B) A representative example of a conventional radiation plan for a primary tumor. (C) A representative example of a stereotactic body radiation plan for left lung metastases. (D) A combination of radiation plans for primary and metastatic lesions. The 95%, 80%, and 50% isodose lines are indicated in red, yellow, and green, respectively.



Supplementary Fig. 7. Gating strategy for cytokine detection.

(A) Target populations were gated in all beads as instructed by the manufacturer (P1). (B) Within the P1 subpopulation, seven different cytokines were labeled with distinct fluorescence intensities in the APC channel. The corresponding fluorescence intensity of each cytokine was detected in the PE channel. The concentration of each cytokine was subsequently calculated using standard curves created from established standards. Abbreviations: APC, allophycocyanin; PE, phycoerythrin.



Supplementary Note

Clinical Research Protocol

TORIPALIMAB IN COMBINATION WITH INDUCTION CHEMOTHERAPY AND SUBSEQUENT CHEMORADIATION AS FIRST-LINE TREATMENT IN PATIENTS WITH ADVANCED ESOPHAGEAL CARCINOMA: A SINGLE-ARM, PROSPECTIVE, OPEN-LABEL, PHASE II CLINICAL TRIAL (TR-EAT)

Clinical Research Protocol

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1. Schema

Research Topic	Toripalimab in combination with induction chemotherapy and subsequent chemoradiation as first-line treatment in patients with advanced esophageal carcinoma: a single-arm, prospective, open-label, phase II clinical trial (TR-EAT)
Research Purpose	To evaluate the efficacy, safety, and potential biomarkers of triple therapy involving toripalimab (an anti-PD-1 antibody) in combination with induction chemotherapy followed by chemoradiation in patients with unresectable, treatment-naive, primary stage IV esophageal squamous cell carcinoma (ESCC).
Research Design	Prospective, single-center, single-arm, phase II trial
Principal Investigator	Dr. Qifeng Wang
Research Object	Unresectable, treatment-naive, primary stage IV esophageal squamous cell carcinoma
Research Endpoints	1) Primary endpoints: progression-free survival (PFS) 2) Secondary endpoints: objective response rate (ORR), disease control rate (DCR), duration of response (DOR), 1 - and 2-year OS rates, toxicity, and quality of life. 3) Exploratory endpoints: To investigate the potential predictive and prognostic biomarkers, including programmed death-ligand 1 (PD-L1) expression in archived and/or fresh tumor tissue and blood samples obtained before and/or after the completion of the study treatment and/or at the time of PD via next-generation sequencing and multicolor immunohistochemical assays. Thereafter, we will assess the relationships between biomarkers, including PD-L1, circulating tumor DNA (ctDNA), and cytokines as well as the therapeutic effect of combination treatment. Furthermore, we aim to investigate the immune microenvironment, immune-related gene expression, and immune-related factors, as well as their associations with disease status and treatment response.
Inclusion Criteria	1) Pathologically confirmed unresectable, treatment-naive, stage IV esophageal squamous cell carcinoma, suitable for cCRT (T1b-4b, N3, M0, or TanyNanyM1 with oligometastases as per AJCC 8th edition). Oligometastases are defined as ≤5 metastatic lesions and ≤3 metastatic organs; 2) No prior cancer therapy; 3) Estimated life expectancy >6 months;

	4) Age at diagnosis being 18-75 years; 5) At least 1 measurable lesion according to RECIST v1.1; 6) The function of important organs meets the following requirements:a.white blood cell count (WBC) ≥ 4.0×109/L, absolute neutrophil count (ANC) ≥ 1.5×109/L; b. platelets ≥ 100×109/L; c. hemoglobin ≥ 9g/dL; d. serum albumin ≥ 2.8g/dL; e. total bilirubin ≤ 1.5×ULN, ALT, AST and/or AKP ≤ 2.5×ULN; f. serum creatinine ≤ 1.5×ULN or creatinine clearance rate >60 mL/min; g. normal thyroid function; 7) PS score 0-1; 8) Ability to understand the study and sign informed consent.
Exclusion Criteria	1) Patients who have been treated previously with anti-tumor therapy (including chemotherapy, radiotherapy, surgery, immunotherapy, etc.); 2) Active or untreated CNS metastases, as determined using CT or MRI during screening and prior radiographic assessments; 3) Uncontrolled cancer-related pain; 4) Uncontrolled pleural effusion, pericardial effusion, or ascites requiring recurrent drainage procedures (once monthly or more frequently); 5) Uncontrolled or symptomatic hypercalcemia; 6) Bone metastases of the multi-segmental vertebral body, ilium, and other sites; 7) Patients with the tendency to exhibit a complete obstruction under endoscopy that requires interventional therapy or surgery for relief of the obstruction; 8) Stent implanted in the esophagus or trachea; 9) High risk of hemorrhage or perforations due to tumor invasion in adjacent organs (aorta or trachea), or the presence of a fistula; 10) Known or suspected allergy or hypersensitivity to monoclonal antibodies, any ingredients of toripalimab, and the chemotherapeutic drugs paclitaxel or carboplatin; 11) History of or comorbid bleeding disease; 12) Dsease of the hematopoietic or immune system, or cachexia; 13) Participation in another interventional clinical study at the same time;

	14) Female patients who are pregnant or lactating; 15) Inability to provide informed consent due to psychological, familial, social and other factors; 16) A history of malignancies other than esophageal cancer before enrollment, excluding non-melanoma skin cancer, in situ cervical cancer, or cured early prostate cancer; 17) Patients who cannot tolerate chemoradiotherapy due to severe cardiac, lung, liver or kidney dysfunction, or hematopoietic disease or cachexia; 18) A history of diabetes for more than 10 years and poorly controlled blood glucose levels; 19) Active autoimmune diseases, a history of autoimmune diseases (including but not limited to these diseases or syndromes, such as colitis, hepatitis, hyperthyroidism), a history of immunodeficiency (including a positive HIV test result), or other acquired or congenital immunodeficiency diseases, a history of organ transplantation or allogeneic bone marrow transplantation; 20) A history of interstitial lung disease or non-infectious pneumonia 21) Presence of active hepatitis B (HBV DNA ≥ 2000 IU/mL or 104 copies/mL), hepatitis C (positive for hepatitis C antibody, and HCV-RNA levels higher than the lower limit of the assay); 22) Any unstable condition or condition that may compromise patients' safety and compliance.
Duration of Trial	Estimated enrollment time of the first subject: June 2021 Estimated enrollment time of the last subject: June 2022 Estimated end time of the study: June 2023
Therapeutic Regimen	**Induction chemotherapy + Immunotherapy:** ● Paclitaxel: 135-175 mg/m2, ivdrip, d1, + Carboplatin: area under the curve [AUC] = 4–6, ivdrip, d1, every 3 weeks, total 2 cycles ● Toripalimab: 240 mg, ivdrip, d1, every 3 weeks, total 2 cycles **Concurrent radiochemotherapy combined with immunotherapy:** ● Paclitaxel: 135-175 mg/m2, ivdrip, d1, + Carboplatin: area under the curve [AUC] = 4–6, ivdrip, d1, every 3 weeks, total 2 cycles

	● Toripalimab: 240 mg, ivdrip, d1, every 3 weeks, total 2 cycles ● Radiotherapy: IMRT and involved-field irradiation (IFl) ● Target region: primary esophageal lesion, (GTVp)-positive lymph nodes, (GTVnd), with a dose of 45-50.4 Gy, 1.8-2.0Gy/F, 5 times a week ● Oligometastatic lesion (GTV-M): with a dose of 30-40Gy, 4-8 Gy/f **Immunomaintenance therapy:** ● Toripalimab 240mg, ivdrip, repeated every 3 weeks for up to 1 year or until disease progression, illness, unacceptable toxicity, investigator's decision, or patient withdrawal of consent
Sample Size **and** **Statistical Methods**	● All enrolled patients will be included in the analysis regardless of whether they complete the treatment protocol. Data analyses will be performed according to the "Intention to treat" principle. ● The primary endpoint of this study is PFS. Based on the literature, the median PFS of Pembrolizumab combined with cisplatin and 5-fluorouracil as the first-line treatment of unresectable locally advanced or metastatic esophageal cancer was 6.3 months. Our preliminary work showed that the mPFS of toripalimab in combination with induction chemotherapy and subsequent chemoradiation in the treatment of primary stage IV ESCC was 12.0 months or more. We hypothesized that the median PFS of our trial can reach 12 months. The type I error rate is 5%, and the power is 80%. Follow-up duration was calculated from enrollment to the date of the last follow-up. Assuming a uniform accrual accomplished over a period of about 12 months, with an additional 12 months of follow-up subsequent to the enrollment of the last patient, to observe 16 PFS events, a calculation determined that 25 cases (or a minimum of 25 patients) were needed. In consideration of a 20% drop-out rate, the final sample size is set at 32 cases. ● ORR, DCR, DOR, and safety will be analyzed by descriptive methods. OS and PFS will be calculated by Kaplan-Meier method and log-rank test will be performed. $P<0.05$ is considered statistically significant.
Version Number	2.0

2. Summary

Immune checkpoint inhibitor therapy combined with chemotherapy is safe and effective in treating advanced esophageal carcinoma; however, some patients still experience tumor progression and/or metastasis. Whether the addition of radiotherapy to immunotherapy combined with chemotherapy improves the prognosis of patients with advanced/metastatic esophageal carcinoma needs to be investigated. In the present study, we developed a protocol for our clinical trial indicating that toripalimab combined with induction chemotherapy followed by chemoradiotherapy can safely prolong survival in patients with stage IV esophageal carcinoma. This open-label, single-arm, phase II trial will include patients with unresectable, treatment-naive stage IV esophageal squamous cell carcinoma who have not received prior systemic therapy. The patients will be treated with two cycles of toripalimab (240 mg, 1 day before chemotherapy, Q3W) combined with induction chemotherapy (paclitaxel, 135–175 mg/m2 + carboplatin, area under the curve = 4–6, day 1, intravenous, Q3W). Thereafter, they will undergo two cycles of the aforementioned treatment with concurrent radiotherapy (45–50.4 Gy in 25–28 fractions), followed by toripalimab (240 mg, day 1, Q3W) for 1 year. The primary outcome measure will be progression-free survival; the secondary outcome measures will include the objective response rate, disease control rate, duration of remission, 1- anad 2-year overall survival rates, safety and tolerability, and changes in health-related quality of life. The study protocol was approved by the Ethics Committee of Sichuan Cancer Hospital (SCCHEC-02-2021-021). The trial is underway in accordance with the Declaration of Helsinki.

3. Background

Esophageal carcinoma is a potentially life-threatening malignant disease with a poor prognosis (1). Among the malignant tumors in China, the prevalence of esophageal carcinoma ranks sixth, and its mortality ranks fourth (2). Squamous cell carcinomas form a majority of esophageal cancers in China; most patients with these carcinomas are diagnosed at an advanced stage.

For advanced esophageal cancer, immunotherapy combined with chemotherapy has become the standard treatment recommended by the guidelines. In the phase III trial of pabolizumab or placebo combined with first-line chemotherapy for advanced esophageal cancer (keynote-590) (3), the median overall survival (OS) in the immunotherapy combined with chemotherapy group was more than 12 months, and the efficacy exceeded that of the previous standard first-line chemotherapy. Another phase III clinical study (ESCORT-1ST) showed that carilizumab combined with chemotherapy can significantly prolong the median survival (mOS, 15.3 months vs. 12.0 months) and median progression free survival (mPFS, 6.9 months vs 5.6 months) of patients with advanced esophageal squamous cell carcinoma (ESCC) and has good safety (4).

Despite the combination of immunotherapy and chemotherapy, the prognosis of advanced esophageal cancer is still unsatisfactory. Radiotherapy is a crucial treatment method for patients with advanced esophageal cancer. Theoretically, radiotherapy has a good local tumor control effect, and the improvement of the local control rate is helpful in alleviating symptoms and prolonging survival. Therefore, some studies have tried to add radiotherapy to the first-line treatment of advanced esophageal cancer. Suzuki et al. (5) treated 32 patients with stage IVB esophageal cancer with palliative radiotherapy at an external dose of 30–60 Gy. After treatment, dysphagia in 73% of patients was relieved. Li et al. (6) conducted a retrospective study of 82 patients with heterochronic, oligometastatic esophageal cancer; patients were divided into radiotherapy and non-radiotherapy groups. The median OS of the radiotherapy group (RT) and non-radiotherapy group (NRT) were 14 months and 7 months, respectively. Multivariate analysis showed that treatment mode (RT vs NRT) was an independent prognostic factor for patients with oligometastatic esophageal cancer. In these studies, radiotherapy only was used for local palliative treatment. Whether radiotherapy can achieve a better therapeutic effect in combination with immunotherapy and chemotherapy is an urgent research topic (7-9) However, there are no reports on the combination of radiation with chemoimmotherapy in advanced esophageal cancer.

Although radiotherapy combined with chemotherapy may have benefits, its mechanism of action raises safety concerns because radiotherapy- and immunotherapy-related toxic and side effects might overlap. In addition, the optimal target range, dose fraction schemes, total radiation dose, and timing of incorporation of radiation into the treatment region are unknown. To address this question, we will conduct a single-arm phase II study involving 30 patients with unresectable advanced ESCC. Patients will initially receive a combination of programmed cell death protein 1 (PD-1) inhibitor (toripalimab) therapy and induction chemotherapy, followed by immunotherapy and concurrent chemoradiotherapy (cCRT); eventually, they will be treated with toripalimab as maintenance therapy. Through this trial, we aim to provide preliminary evidence regarding the feasibility of this combination regimen as a first-line treatment option for patients with advanced ESCC.

The main objective of this study is to evaluate the efficacy and safety of triple therapy involving toripalimab in combination with induction chemotherapy followed by chemoradiation in patients with unresectable, treatment-naive, primary stage IV ESCC.

4. Objective

To evaluate the efficacy, safety, and potential biomarkers of triple therapy involving toripalimab in combination with induction chemotherapy followed by chemoradiation in patients with unresectable, treatment-naive, primary stage IV ESCC.

5. Plan

5.1 Patient Selection

5.1.1 Inclusion criteria

1) Pathologically confirmed unresectable, treatment-naive stage IV esophageal squamous cell carcinoma, suitable for cCRT (T1b-4b, N3, M0, or TanyNanyM1 with oligometastases as per AJCC 8th edition). Oligometastases are defined as ≤5 metastatic lesions and ≤3 metastatic organs;
2) No prior cancer therapy;
3) Estimated life expectancy >6 months;
4) Age at diagnosis being 18-75 years;
5) At least 1 measurable lesion according to RECIST v1.1;
6) The function of important organs meets the following requirements:a.white blood cell count (WBC) ≥ 4.0×109/L, absolute neutrophil count (ANC) ≥ 1.5×109/L; b. platelets ≥ 100×109/L; c. hemoglobin ≥ 9g/dL; d. serum albumin ≥ 2.8g/dL; e. total bilirubin ≤ 1.5×ULN, ALT, AST and/or AKP ≤ 2.5×ULN; f. serum creatinine ≤ 1.5×ULN or creatinine clearance rate >60 mL/min; g. normal thyroid function;
7) PS score 0-1;
8) Ability to understand the study and sign informed consent.

5.1.2 Exclusion criteria

1) Patients who have been treated previously with anti-tumor therapy (including chemotherapy, radiotherapy, surgery, immunotherapy, etc.);
2) Active or untreated CNS metastases, as determined using CT or MRI during screening and prior radiographic assessments;
3) Uncontrolled cancer-related pain;
4) Uncontrolled pleural effusion, pericardial effusion, or ascites requiring recurrent drainage procedures (once monthly or more frequently);
5) Uncontrolled or symptomatic hypercalcemia;
6) Bone metastases of the multi-segmental vertebral body, ilium, and other sites;

7) Patients with the tendency to exhibit a complete obstruction under endoscopy that requires interventional therapy or surgery for relief of the obstruction;

8) Stent implanted in the esophagus or trachea;

9) High risk of hemorrhage or perforations due to tumor invasion in adjacent organs (aorta or trachea), or the presence of a fistula;

10) Known or suspected allergy or hypersensitivity to monoclonal antibodies, any ingredients of toripalimab, and the chemotherapeutic drugs paclitaxel or carboplatin;

11) History of or comorbid bleeding disease;

12) Dsease of the hematopoietic or immune system, or cachexia;

13) Severe malnutrition (PG-SGA ≥ 9), and the nutritional status fails to improve despite 1 to 2 weeks of intensive nutritional intervention.

14) Participation in another interventional clinical study at the same time;

15) Female patients who are pregnant or lactating;

16) Inability to provide informed consent due to psychological, familial, social and other factors;

17) A history of malignancies other than esophageal cancer before enrollment, excluding non-melanoma skin cancer, in situ cervical cancer, or cured early prostate cancer;

18) Patients who cannot tolerate chemoradiotherapy due to severe cardiac, lung, liver or kidney dysfunction, or hematopoietic disease or cachexia;

19) A history of diabetes for more than 10 years and poorly controlled blood glucose levels;

20) Active autoimmune diseases, a history of autoimmune diseases (including but not limited to these diseases or syndromes, such as colitis, hepatitis, hyperthyroidism), a history of immunodeficiency (including a positive HIV test result), or other acquired or congenital immunodeficiency diseases, a history of organ transplantation or allogeneic bone marrow transplantation;

21) A history of interstitial lung disease or non-infectious pneumonia

22) Presence of active hepatitis B (HBV DNA ≥ 2000 IU/mL or 104 copies/mL), hepatitis C (positive for hepatitis C antibody, and HCV-RNA levels higher than the lower limit of the assay);

23) Any unstable condition or condition that may compromise patients' safety and compliance.

5.1.3 Withdrawal criteria

1) patients with disease progression, according to Response Evaluation Criteria In Solid Tumors (RECIST) 1.1;

2) patients who experience any unacceptable treatment-related adverse events and cannot continue the study after being evaluated by the study physician;

3) patients that may significantly affect the evaluation of clinical status, for example if they are non-compliant with the research plan, received other treatment, etc.;

4) patients with diseases requiring interruption of treprizumab treatment, such as an allergy, sudden onset of other diseases, and accidents and injuries not related to the disease;

5) patients exercising their right to withdraw from the trial at any time and for any reason.

5.1.4 Eliminate criteria

1) Violation of the requirements of the research protocol;

2) Poor quality of data recording, incomplete and inaccurate data.

5.2 Examinations and screening of patients

5.2.1 Examinations

1) Complete medical history and systemic physical examinations (symptoms, signs, body weight loss and function score). It is generally required to be completed within 7-14 days before recruitment.

2) Pre-treatment examinations:

. Blood routine, blood type, urine routine, stool routine, biochemical routine, CB4, thyroid function, plasma cortisol;

. Hepatitis virus examination. If HBsAg is positive, HBV-RNA should be tested; If HCV-Ab is positive, HCV-RNA should be tested;

. Electrocardiogram;

. Ultrasonic cardiogram (UCG);

. Lung function tests;

. Histopathological/cytological diagnosis: pathological examination will be done based on the tissues from endoscopic biopsy;

. Esophageal barium swallowing;

. Chest and abdominal CT (with contrast);

. Esophagogastroduodenoscopy (EGD), with endoscopic ultrasound (EUS);

. Cervical ultrasonography;

. Electronic bronchoscopy or endobronchial ultrasound if necessary, to confirm the involvement of trachea and/or bronchus;

. Positron emission tomography–computed tomography (PET-CT);

. Quality of life questionnaire;

. Nutritional risk screening.

5.2.2 Screening of patients

Patients will be screened within 2 weeks prior to treatment commencement to assess their tolerance to the treatment. Comprehensive information on potentially eligible patients will be collected and recorded during this period. The screening process will include obtaining written informed consent, collection of demographic information and medical history, physical examination, evaluation

of ECOG PS score and vital signs, clinical testing (chemistry, hematology, and coagulation), examination of liver and kidney function, and cardiac analyses. Tumor information will be obtained via imaging evaluation [computed tomography (CT) or magnetic resonance imaging (MRI)], fibroscopy, esophagoscopy, or positron emission tomography/CT. Eventually, the inclusion and exclusion criteria will be reviewed to make a final judgment regarding each patient's eligibility.

5.3 Treatment Plan

5.3.1 Overall Design

This study, a prospective, single-center, single-arm, phase-II clinical trial, intends to enroll at least 32 patients with unresectable, treatment-naive, primary stage IV esophageal squamous cell carcinoma (ESCC). Patients with primary N3 and M1 oligometastases are eligible. PD-1 antibody (toripalimab) and paclitaxel/carboplatin chemotherapy will be administered concurrently with radiotherapy. After the completion of chemoradiotherapy (CRT), PD-1 antibody monotherapy will continue until disease progression or the absence of clinical benefits, with the maximum duration of treatment being one year.

5.3.2 Therapeutic Regimen

1) After completing two cycles of induction Chemoimmunotherapy, radiotherapy is initiated at the start of the third cycle of systemic treatment.

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

3) Protocols of drugs delivery

	Toripalimab	Paclitaxel	Carboplatin
Dosage and route of administration	240 mg ivdrip	135-175 mg/m2 ivdrip	area under the curve [AUC] = 4–6 ivdrip
Transfusion speed	≥30 min , ≤60 min , including saline flush	180 min	60 min
Pretreatment	Not required	Required	Required

Administration time	D1, 22, 43, 64	D1, 22, 43, 64	D1, 22, 43, 64
Administration sequence	After toripalimab infusion, paclitaxel and carboplatin will be given at least 60 minutes interval.		
Maintain dosing frequency	Toripalimab is administered every 3 weeks after completing 4 cycles of chemoimmunotherapy, with a maximum duration of 1 year.		

5.3.3 Management of toripalimab infusion reaction

CTCAE	Symptoms	Treatment	Toripalimab
Grade 1	Mild	Bedside observation and close monitoring until recovery. Prophylaxis is recommended for future infusion: diphenhydramine 50mg, or equivalent and/or acetaminophen 325-1000mg, at least 30 minutes before administration of pd-1 antibody.	Continue
Grade 2	Moderate Requiring treatment or suspension. Rapid remission after treatment (e.g., antihistamines, nonsteroidal anti-inflammatory drugs, anesthetics, bronchodilators, intravenous fluids, etc.)	Intravenous saline, diphenhydramine 50mg or equivalent and/or paracetamol 325-1000mg. Bedside observation and close monitoring until recovery. Corticosteroids or bronchodilators may be considered according to clinical needs. Original medical records to study drug infusion volume. Prophylaxis is recommended for future infusion: diphenhydramine 50mg, or equivalent and/or acetaminophen 325-1000mg, at least 30 minutes before administration of pd-1 antibody. When necessary, cortisol (equivalent to a 25mg dose of hydrocortisone).	Pause. 50% initial infusion rate when retaking after symptom resolution. If there are no complications within 30 minutes, the infusion rate can be increased to 100%. Monitor closely, and if symptoms recur, no PD-1 antibody is administered
Grade ≥ 3	Severe No immediate response to treatment and/or suspension; Or recurrence of symptoms after	Stop the infusion of PD-1 antibody immediately; Start an i.v. drip of saline. Bronchodilators are recommended by subcutaneous injection of 0.2-1mg of 1:1000 epinephrine solution, or 0.1-0.25mg of 1:10,000 epinephrine solution intravenously, if necessary, diphenhydramine 50mg slowly and/or plus	Terminate

	remission; Sequelae that require	methylprednisolone 100mg or equivalent intravenously. Follow institute guidelines for treating	
	hospitalization.	anaphylaxis. Bedside observation and close monitoring until recovery.	

5.3.4 Pretreatment before chemotherapy

1) Prophylactic antiemetic therapy:

Acute and delayed vomiting induced by cisplatin must be prevented. Aprepitant + 5-HT3 antagonist + dexamethasone is recommended within 1 hour prior to chemotherapy.

2) Allergy prevention:

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

3) Adjustment for allergy:

Allergic symptoms	Treatment
Grade1: Local skin reactions such as mild itching, flushing and rashes	-Reduce the infusion rate until the symptoms disappear. -Observe and monitor patients at the wards. -Then continue dripping all paclitaxel at the original speed.
Grade2: Any symptoms not listed above (mild symptoms) or below (severe symptoms), such as systemic pruritus, flushing, rash, dyspnea and hypotension with systolic blood pressure >80 mm Hg	-Stop dripping paclitaxel. -Administer DPH 50 mg IV with or without DXM 10 mg IV until the symptoms disappear. -Then continue dripping paclitaxel at a lower speed and gradually to the original speed.
Grade3/4: Any severe symptoms such as bronchospasm, systemic rubella, systolic blood pressure ≤80mmHg and	-Stop dripping paclitaxel. -Administer DPH 50 mg IV with or without DXM 10 mg IV, and administer adrenaline, if necessary, until the symptoms disappear.

vascular edema	-Patients will withdraw from the study

Notes:Patients who has severe allergic symptoms will withdraw from the study, and the followed treatment will be decided by researchers.

5.3.5 Principles for the adjustment of dosage of drugs

1) Toripalimab

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

Immune-associated AE for interruption	Conditions for resumption
Diarrhea / Colitis	
Grade 2/3	Recovery to grade 0-1 and corticosteroid reduced to less of prednisone 10 mg or its equivalent
Grade 4	Termination
Hepatic dysfunction	
Grade 2	Recovery to grade 0-1 and corticosteroid reduced to less of prednisone 10 mg or its equivalent
Grade 3/4	Termination
Hyperthyroidism	
Grade 3	Recovery to grade 0-1 and corticosteroid reduced to less of prednisone 10 mg or its equivalent
Grade 4	Termination
Hypothyroidism	The start of thyroid hormone replacement therapy
Pneumonia	
Grade 2	Recovery to grade 0-1 and corticosteroid reduced to less of prednisone 10 mg or its equivalent
Grade 3/4	Termination
hypophysis	

Grade 2/3	Recovery to grade 0-1 and the start of endocrine replacement therapy
Grade 4	Termination
Newly diagnosed type I diabetes or grade 3/4	Clinical and metabolic stabilization
Hyperglycemia withβ-cell failure	
Renal failure or nephritis	
Grade 2	Recovery to grade 0-1 and corticosteroid reduced to less of prednisone 10 mg or its equivalent
Grade 3/4	Termination
Transfusion reaction	
Grade 2	Disappearance of symptoms
Grade 3/4	Termination
Other AE related to toripalimab	
Grade 3	Recovery to grade 0-1 and corticosteroid reduced to less of prednisone 10 mg or its equivalent
Grade 4	Termination
Notes: Regime should be terminated for any recurrence of grade 3 AE or any life-threatening event. For patients with liver metastasis and grade 2 elevation of AST or ALT at baseline, regime should be terminated if AST or ALT is more than 50% of the baseline and lasts for at least 1 week. Toripalimab can be suspended or terminated by researchers for patients with intolerable or persistent grade 2 AE. If persistent grade 2 adverse events do not return to grade 0-1 within 12 weeks of the last administration, toripalimab should be terminated.	

2) Paclitaxel/Carboplatin

Highest dose of chemotherapy is given and adjusted according to the most severe toxicity. Patient will continue to receive reduced dose chemotherapy once dose is adjusted. The minimum dose should be selected if multiple toxicities occur. After two times of adjustment of dose, chemotherapy must be terminated if adjustment is necessary for the third time. Chemotherapy can only be delayed for up to 2 weeks, otherwise it should be terminated.

AE	Paclitaxel	Cisplatin

Febrile neutropenia Grade 4 neutropenia Grade 4 thrombocytopenia Grade 1 renal toxicity Grade 2/3 peripheral nerve toxicity Grade ≥3 non-hematological toxicity	20% reduction	20% reduction
Grade ≥2 renal toxicity Grade 4 peripheral nerve toxicity	Termination	Termination

Dose level of paclitaxel/Carboplatin

AE	Paclitaxel	Carboplatin	Restart treatment
Febrile neutro-enia	Two dose reductions are allowed, with each reduction being 20%.	Two dose reductions are allowed, with each reduction being 20%.	Dose reduction after recovery
Grade 4 neutr-penia			Reduce dose if recovered to ≤ Grade 1
Grade 4 thro-bocytopenia			
Grade 1 renal toxicity	R20% reduction	20% reduction	Dose reduction
Grade 2/3 peripheral neu-otoxicity			Reduce dose if recovered to ≤ Grade 1
Grade ≥3 non-hematological toxicities			
Grade ≥2 renal toxicity	Not applicable	Not applicable	Termination
Grade 4 peripheral neu-otoxicity	Not applicable	Not applicable	Termination

Notes: Evaluation criteria for acute toxicity of chemotherapy: CTCAE 4.0 toxicity evaluation criteria.

5.3.6 Radiotherapy scheme

1) During CT simulation, patients will be immobilized with a vacuum bag in the supine position with arms raised. Radiation will be delivered by intensity-modulated radiotherapy (IMRT) using 6-8 MV X-ray.

2） In accordance with the involved-field irradiation technique (IFI), radiotherapy is specifically targeted to the primary esophageal lesion and metastatic foci, excluding regional prophylactic irradiation of the lymphatic drainage areas. Gross tumor volume (GTV) is defined as the primary tumor and involved lymph nodes on CT or other imaging (MRI, PET-CT etc.). The planning target volume (PTV) was defined as GTV with an additional 1-2 cm proximal and distal margins and a radical margin of 0.5–1.0 cm.

3) Radiotherapy plan design: IMRT or Volumetric Modulated Arc Therapy (VMAT) technology, isocentric irradiation, and evaluation and optimization of treatment plan according to dose volume histogram, fault dose distribution, NTCP model. Planning evaluation criteria refer to QUANTEC standard (IJROPB, 2013).

4）Radiation dose: For the primary lesion and regional lymph nodes, the total dose is 45-50.4 Gy in 25 -28 fractions (1.8-2.0 Gy/F), administered 5 times a week. For distant (non-regional) lymph nodes, such as supraclavicular and retroperitoneal lymph nodes, conventionally fractionated radiotherapy (45–50.4 Gy in 25–28 fractions) may be administered at the investigator's discretion, taking into account the dose constraints of surrounding organs at risk, such as the trachea, stomach, and intestines. Stereotactic body radiation therapy (SBRT) is recommended for certain patients with suitable oligometastatic lesions in the bones, lungs, and liver, with consideration for the same factors. SBRT is administered to all metastatic lesions at doses of 30–40 Gy in 3–5 fractions (4-8 Gy/F).

5）Organs at risk (OARs)

Organs at risk	Dose limitation
Spinal cord	Dmax<45Gy
Lungs	V20<30%, V5<65%, Dmean<17 Gy
Stomach	D1cc<54 Gy, V40<60%
Liver	V20 <30%, V30<20%, Dmean<23 Gy
Heart	Dmean<26Gy, V30<40%. Every effort should be made to keep the total heart dose to a minimum

5.3.7 Criteria for radiation-related toxicity

1) Continue radiotherapy if grade 3 toxicity is unrelated to radiotherapy, and the adjustment of chemotherapy is according to Table 4. Radiotherapy will be withheld if any grade 4 toxicity is observed.

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

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

5.3.8 Regular evaluation during chemoimmunotherapy and CRT

Regular evaluation will consist of: routine blood tests, liver and kidney function once a week; All patients fill in the quality of life questionnaire-EORTC-QLQ-C30 version3 at the end of radiotherapy and chemotherapy. Adverse reactions, concomitant medication, and whether the study is terminated early need to be recorded.

5.3.9 Regular evaluation during maintenance

Routine blood tests and liver and kidney function are performed before each course of treatment. ECG and thyroid function are reviewed every 2 courses. Fill in quality of life questionnaire EORTC-QLQ-C30 version3 every 3 months. Adverse reactions, concomitant medication, and whether the study is terminated early need to be recorded.

5.3.10 Concomitant Therapy

1) During the administration period of this clinical study, the use of other anti-tumor drugs, immunotherapy and biotherapy that are not approved by this protocol shall be stopped.

2) Symptomatic medications can be administered, including prophylactic antiemetic drugs and G-CSF when blood drops. Prophylaxis with hematopoietic growth factor is permitted to avoid treatment interruption or delay. All symptomatic medications should be recorded and explained in detail on the CRF table.

3) Local use of corticosteroids is allowed, such as eye, nasal, intraarticular, inhaled, etc., and pretreatment of corticosteroids before chemotherapy is allowed.

4) Avoid alcoholic beverages during treatment.

5.4 Management of pseudo-progression

5.4.1 Definition

Some subjects may experience a temporary tumor outbreak in the first few months after initiation of immunotherapy, followed by a disease response, so subjects are allowed to continue with the original treatment regimen after the first onset of progressive disease (based on RECIST1.1 criteria).

A tumor outbreak can include any of the following: deterioration of the original target lesion; deterioration of the original non-target lesion; a new lesion appears.

5.4.2 Management

The investigator can decide whether to continue the study treatment based on the subjects' overall clinical condition, including physical condition, clinical symptoms and laboratory results. If the subjects are clinically stable, they can continue treatment and have their tumor evaluated again after an interval of at least 4 weeks (±7 days). According to iRECIST and RECIST 1.1 criteria, if PD is

unconfirmed, the treatment should be continued. If PD is confirmed, treatment will be discontinued unless the investigator determines that the subject will continue to benefit clinically and allows the subject to continue treatment after PD is confirmed. For clinically unstable subjects, treatment should be discontinued after the first evaluation of PD, without the need for repeated imaging examination to confirm PD. For subjects who are first evaluated for PD, whether or not they continue to study therapy after the progression, the initial progression date assessed by the investigator will be used for all statistical analyses that include progression information.

Definition of clinical stability: no significant decrease in physical status and no significant worsening of tumor-related symptoms; no rapid disease progression; no advanced tumors at critical anatomical sites requiring urgent medical intervention (e.g. spinal cord compression). The criteria for PD:

	Confirm PD （Any of the following）	Unconfirm PD （All of the following）
Target lesion	The tumor load increased by ≥5 mm in absolute terms compared to the first progression.	The tumor load increased by <5 mm in absolute terms compared to the first progression.
Non-target lesion	Continuous progression of compared with initial progression (qualitative).	No clear progress compared to initial progress (qualitative).
New lesion	(1) Compared with the first progression, new lesions appeared. (2) If a new lesion has appeared before, the new lesion increases, or other new lesions appear.	(1) Compared with the first progression, there were no other new lesions. (2) If a new lesion has appeared before, the new lesion is stable or shrunken.

5.5 Tumor and blood samples collection

5.5.1 Collection time

Baseline tumor biopsies are collected. Blood samples will be collected before the first dose, after 2 cycles of chemoimmunotherapy, and after completion of chemoradiotherapy.

5.5.2 Processing and storage

A total of 4-6 mL of venous blood is collected at each time point and placed in the serum separation tube, which is placed in a cryogenic refrigerator.

5.6 Observing targets

5.6.1 Toxicity evaluation

Therapeutic toxicity is evaluated according to CTCAE 5.0 criteria.

5.6.2 Evaluation of short-term clinical efficacy after CRT

1) Evaluation time: 3 months after CRT (approximately the 25th week of treatment).

2) Evaluation measures: neck, chest and abdomen enhanced CT.

3) Evaluation criteria: according to RECIST 1.1criteria. As a general rule, the primary esophageal lesion is not considered a target lesion, unless no other lesions can serve as the target lesion. In such cases, the primary esophageal lesion may be considered a target lesion, provided it meets the following criteria: 1) The primary esophageal lesion has clear boundaries and is easy to measure on CT; 2) The length of the esophageal longitudinal axis ≥5cm.

Complete response (CR) is defined as the disappearance of all target lesions, and all pathological lymph nodes (including target and non-target nodes) must have reduction in short axis to <10 mm. Considering that the esophagus is a hollow organ, the assessment of a clinical complete remission (cCR) of primary esophageal lesions depends not only on CT imaging examinations but also on the use of various methods such as endoscopy with biopsy, endoscopic ultrasound, and positron emission tomography-computed tomography (PET-CT) to confirm the status. In the current study, efficacy was assessed primarily by CT, warranting additional caution when evaluating CR of esophageal lesions.

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

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

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

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

4) If there is any ambiguous finding, re-evaluation will be conducted within 6 weeks to determine the final response.

5.6.3 Endpoint evaluation

1) Primary endpoint: PFS.

2) Secondary endpoint: ORR, DCR, DOR , OS, Toxicity, Quality of life.

3) Exploratory endpoint: To investigate the potential predictive and prognostic biomarkers, including programmed death-ligand 1 (PD-L1) expression in archived and/or fresh tumor tissue and blood samples obtained before and/or after the completion of the study treatment and/or at the time of PD via next-generation sequencing and multicolor immunohistochemical assays. Thereafter, we will assess the relationships between biomarkers, including PD-L1, circulating tumor DNA (ctDNA), and cytokines as well as the therapeutic effect of combination treatment. Furthermore, we aim to investigate the immune microenvironment, immune-related gene expression, and immune-related factors, as well as their associations with disease status and treatment response.

5.6.4 Follow-up

Tumor assessments were performed once every 6 weeks (±7 days) during chemotherapy, and once every 12 weeks (±7 days) from the end of chemoradiotherapy to the end of the second year, once every

6 months during the third and fourth years, and once yearly thereafter. The follow-up contents include physical examination, serological examination (blood routine, biochemical routine, thyroid function, cortisol, etc.), esophageal barium swallowing, neck, chest and abdomen enhanced CT, and PET-CT if necessary; The use of esophagoscopy is determined by the researcher, and pathological biopsy will be performed if necessary.

5.7 Ethics

5.7.1 Informed consent

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

5.7.2 Ethics and policy

This study will be conducted in accordance with the Declaration of Helsinki (2000), the Good Clinical Practice (GCP) guidelines published by the CFDA, and other relevant regulations. The study must be approved by the Ethics Committee of the study center. Any amendments to the study protocol should be re-approved by the Ethics Committee during the course of the study.

5.8 Quality guarantee

5.8.1 Requirements

In order to ensure that the trial can be carried out in strict accordance with the protocol, the clinical investigator should strictly follow the requirements of the GCP during the whole process of the clinical trial, and ensure that the trial procedure is standardized, the trial data is accurate and the study conclusions are reliable. Specific requirements are as follows:

1) Obtain informed consent signed by each subject or his/her agent;

2) Carefully fill in the case report form (CRF) as required;

3) Regularly follow-up;

4) Maintain complete laboratory examination records, clinical records, and subject's original medical records.

5.8.2 Data processing and preservation

1) Case Report Form (CRF): CRF should be filled in timely to assure information accuracy and prompt summary. CRF should generally not be altered. If there is indeed a mistake that need to be corrected, the investigator should sign at the site of alteration. CRF shall be filled out in duplicate and handed out to the sponsor and the investigator after the study. Data will be input into the database after being reviewed by the Clinical Research Associate (CRA), and all the content of CRF can no longer be amended afterwards.

2) Establishment of database: Once the CRF is received by the statisticians, the queries about the data should be answered by the investigator who filled in the CRF. The statisticians will then establish the

database which is then reviewed and locked by the major investigators, sponsors, statisticians and CRA. Irrelevant persons have no access to the database. The database should be backed up.

3) Preservation of materials: According to the GCP, the documentations should be properly preserved by the investigators for more than five years.

5.9 Security measures

Results of studies conducted through this program may be published in medical journals, but we will keep patient information confidential as required by law. When necessary, government administrative departments and hospital Ethics Committees and their relevant personnel may consult the patient's data according to regulations.

5.10 Safety assessment

The information below is based on the results of non-clinical and clinical studies, as well as published data on similar drugs.

5.10.1 Safety plan

Various measures will be taken to ensure the safety of patient participated in this study, including strict inclusion and exclusion criteria and close monitoring. Study drug administration will be conducted in the presence of emergency medical facilities and staff trained in emergency surveillance and management. All adverse event (AE) and serious adverse event (SAE) will be recorded during the study.

1) Risks associated with toripalimab: The PD-L1/PD-1 pathway is involved in peripheral immune tolerance. Thus, such treatments may increase the risk of immune-mediated AE, particularly inducing or exacerbating autoimmune diseases. Potential immune-mediated AE have been observed in ongoing clinical studies of the safety and efficacy of toripalimab injection (JS001) in solid tumors, including interstitial lung disease, hypothyroidism and hyperthyroidism, liver dysfunction, pancreatitis, hyperglycemia, and adrenal insufficiency. For more details on clinical safety, please see the toripalimab injection (JS001) investigator's manual.

2) AE monitoring: This study will assess safety by monitoring all serious and non-serious AE (defined and graded according to the NCI CTCAE version 5.0 standard). Patients will be assessed for safety (including laboratory values) based on the duration of their visit. Laboratory values must be reviewed before each infusion. General safety assessments include a series of interphase histories, physical examinations, and specific laboratory studies, including serum chemistry and blood count. Patients will be closely monitored for signs and symptoms of autoimmune diseases and infections during the study. Reporting of all SAE will be expedited. Patients will be followed for safety for 60 days after the last dose of the study drug. After completion of study or exit to remain study treatment-related AE in patients with follow-up, ease to baseline levels, or until the researchers reckon the event has been stable, or start a new anti-cancer treatment, patients are lost to follow-up, or withdraw consent, or have been confirmed in patients’ treatment or to participate in the study is not the cause of AE.

5.10.2 Safety parameters and definitions

The safety assessment includes monitoring and recording AE, including SAE, performing protocol -designated safety laboratory assessments, measuring protocol-designated vital signs, and performing other protocol-designated tests that are critical to the safety assessment of the study.

1) AE: An adverse event is any adverse medical event, regardless of causality, that occurs in a clinical study subject receiving a drug product, according to the ICH Guideline for The Quality Management of Clinical Trials. Therefore, adverse events can be any of the following:

- Any adverse and unexpected signs (including abnormal laboratory findings), symptoms or illnesses associated with the time of drug use, whether or not they are believed to be related to the drug;
- Any new disease or exacerbation of an existing disease (an increase in the characteristic, frequency or severity);
- The condition is intermittent and recurrent (e.g.headache) and does not exist at baseline;
- Deterioration of laboratory values or other clinical findings (e.g. ECG, X-ray);
- AE associated with protocol-required interventions, including those that occurred before the study treatment was assigned (e.g. invasive procedures during screening, such as tissue biopsies).

2) SAE: A serious adverse event is an adverse event that meets any of the following criteria:

- Results in death;
- Is life-threatening (defined as when a subject is in danger of death at the time of the event);
- Requires hospitalization or prolonged hospital stay;
- Results in persistent or significant disability/incapacity;
- Leads to congenital anomalies or birth defects;
- Other significant medical events requiring intervention to prevent permanent injuries or damages.

5.10.3 Collect AE information

AE information should be solicited using a consistent, non-guided inquiry method at all patient evaluation time points. Example of a non-leading question: "How are you feeling since your last clinic visit?" "Have you had any new health problems or changes in your health since your last departure?"

5.10.4 Severity judgement of AE

Refer to the CTCAE Version 5 classification criteria for adverse drug reactions. The following criteria can be used as references if unlisted adverse reactions occur:

Grade I: Mild, asymptomatic or mild symptoms; clinical or laboratory test abnormality only; treatment not indicated.

Grade II: Moderate; minimal, local or non-invasive intervention required; limited age-appropriate instrumental activities of daily living (e.g., cooking, shopping, using the telephone and counting money, etc.).

Grade III: Severe or medically significant but not immediately life-threatening; leading to hospitalization or prolongation of hospitalization; leading to disabling; limiting self-care activities of

daily living, but not bedridden. Self-care ADL: refers to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden.

Grade IV: Life-threatening consequences; urgent intervention indicated. Grade V: Death related to AEs.

5.10.5 Causality judgement of AE

The investigator should determine whether an adverse event is related to the investigational drug based on the knowledge of patient, the context in which the event occurred, and an assessment of any possible alternative causes, and state "related" or "unrelated" accordingly. The following guidance should be considered:

- Event occur in a plausible time relationship to drug administration;
- The course of events, with special consideration of the effects of dose reduction, discontinuation of the study drug, or re-use of the study drug, if applicable;
- Known associations of events with the study drug or similar treatment; . Known associations of events with study diseases;
- Patients with risk factors or use of concomitant medications known to increase event rates;
- Non-therapeutic factors known to be associated with the occurrence of the event;
- Patients receiving combination therapy are separately assessed for causality between adverse events and the treatment prescribed by the protocol.

5.10.6 AE recording procedure

The investigator should document AE on the AE page of the CRF using the correct medical terminology/concepts. Avoid colloquialisms and abbreviations. In the EVENT column of the AE page of CRF, only one AE term can be recorded for an event.

5.10.7 AE reporting

All SAE and AE of particular concern will be evaluated in a timely manner and reported promptly to the appropriate regulatory authorities and Ethics Committees in accordance with applicable legal requirements.

5.11 Statistical analysis

5.11.1

Professional statisticians will undertake the task of statistical analysis and participate in the whole process from experimental design, implementation to analysis and summary. After the completion of the test protocol and CRF, they will formulate the statistical analysis plan, make necessary modifications during the test, and provide the statistical analysis report after the completion of data analysis. Data analysis will be performed according to the “Intention to treat” (ITT) principle.

5.11.2 Sample size determination

The primary endpoint of this study is PFS. Based on the literature, the median PFS of Pembrolizumab combined with cisplatin and 5-fluorouracil as the first-line treatment of unresectable

locally advanced or metastatic esophageal cancer was 6.3 months. Our preliminary work showed that the mPFS of toripalimab in combination with induction chemotherapy and subsequent chemoradiation in the treatment of primary stage IV ESCC was 12.0 months or more. We hypothesized that the median PFS of our trial can reach 12 months. The type I error rate is 5%, and the power is 80%. Follow-up duration was calculated from enrollment to the date of the last follow-up. Assuming a uniform accrual accomplished over a period of about 12 months, with an additional 12 months of follow-up subsequent to the enrollment of the last patient, to observe 16 PFS events, a calculation determined that 25 cases (or a minimum of 25 patients) were needed. In consideration of a 20% drop-out rate, the final sample size is set at 32 cases.

5.11.3 Statistical methods

All patients who receive the experimental drugs at least once and have had at least one safety evaluation will be included in the Safety Set (SS) analysis. According to the principle of intentionto-treat (ITT) analysis, the full analysis set (FAS) will include data from the last observation of all the cases that had used drugs at least once and were followed up at least once; the entire treatment process cannot be observed until the final results. The FAS data set will be used for fall-out analysis, equilibrium analysis of basic indicators, analysis of the main efficacy indicators, and analysis of safety indicators. The per protocol set (PPS) analysis is a statistical analysis of case data that can meet all the prescribed requirements in accordance with the protocol. This analysis method does not include cases that violate the trial protocol, such as cases lost on follow-up or where patients used prohibited drugs. In this study, the SPSS22.0 software will be used for statistical analysis. Quantitative data satisfies the requirements of normal distribution using the mean ± standard deviation and meets the requirements expressed by median (P25, P75). Qualitative data will be expressed as percentage (%), and a confidence level of 95% will be used for confidential intervals. The Kaplan–Meier method will be used to estimate survival rates and median survival time and to draw the survival curve. The LogRank test will be used to compare the survival rate. A two-sided test will be conducted for all statistical tests, and $P<0.05$ will indicate that the differences were significant.

6. Route

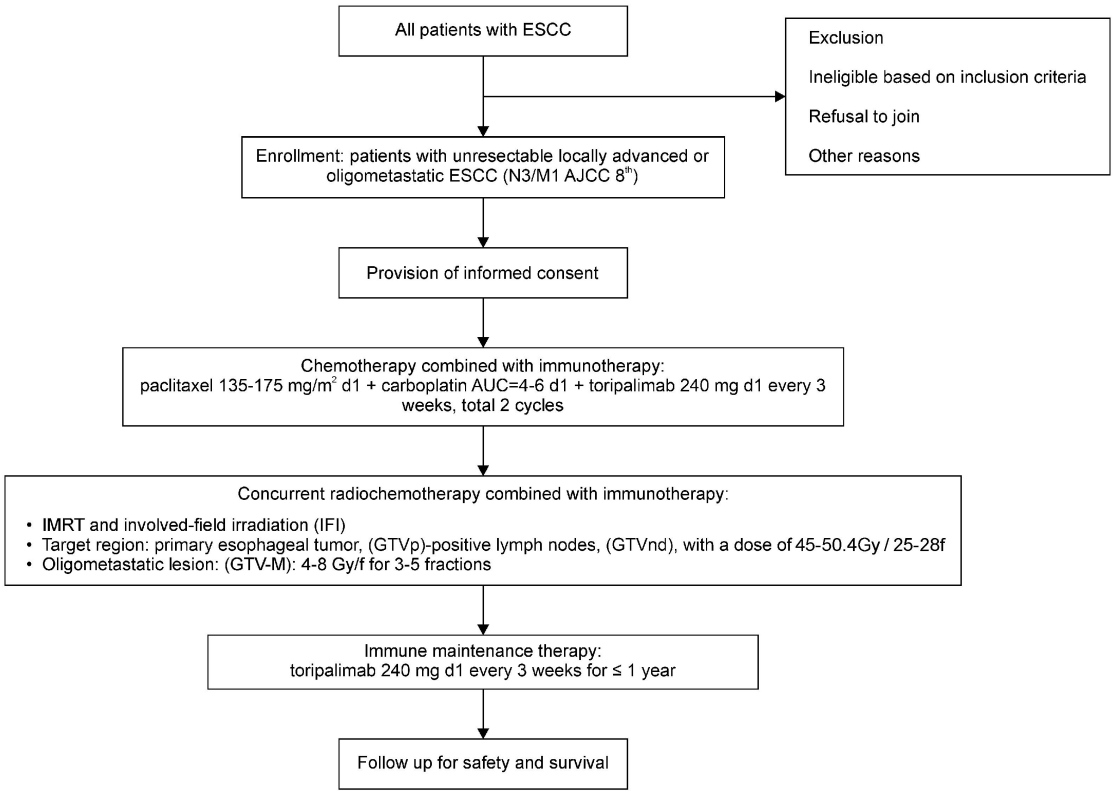

Figure1: Flowchart of the phase II study. ESCC, esophageal squamous cell carcinoma; AJCC, American Joint Committee on Cancer; d1, day 1; AUC, area under the curve, IMRT, intensity-modulated radiation therapy; GTV-M, gross tumor volume of the primary metastatic lesions; GTVnd, gross tumor volume of lymph nodes; GTVp, primary gross tumor volume.

7. Summary of Amendments to Protocol of Toripalimab in Combination with Induction Chemotherapy and Subsequent Chemoradiation as First-line Treatment in Patients with Advanced Esophageal Carcinoma: A Single-arm, Prospective, Open-label, Phase II Clinical Trial (TR-EAT)

(Version 1.0, Date: 5 Feb., 2021 - Version 2.0, Date: 07 May., 2022)

Page/title	Original content Version 1.0	Revision Version 2.0	Reason for modification
Page 1 and 7 "Inclusion criteria" and "5.1.1 Inclusion criteria"	"Pathologically confirmed unresectable esophageal squamous cell carcinoma Multiple lymph node metastases (N3) and/or distant oligometastasis (M1)"	"Pathologically confirmed unresectable, treatment-naive stage IV esophageal squamous cell carcinoma, suitable for cCRT (T1b-4b, N3, M0, or TanyNanyM1 with oligometastases as per AJCC 8th edition)"	A more detailed definition has been provided for the primary inclusion criteria.
Page 8 "5.1.2 Exclusion criteria"	"Severe malnutrition (PG-SGA ≥ 9)"	"Severe malnutrition (PG-SGA ≥ 9), and the nutritional status fails to improve despite 1 to 2 weeks of intensive nutritional intervention."	Malnutrition is frequently observed in patients with advanced esophageal cancer. Improvement in physical status through active nutritional support therapy can still lead to benefits from systemic and localized treatments for these patients.
Page 16 and 25 (Figure 1) "5.3.6 Radiotherapy scheme" and "6.Route"	"Primary lesions were treated with intensity modulated radiotherapy (IMRT) at 30–50 Gy in 15–25 fractions 5 d a week." and "SBRT was administered to all metastatic lesions at doses of 4-8 Gy for 3–5 fractions."	"Primary lesions were treated with intensity modulated radiotherapy (IMRT) at 45–50.4 Gy in 25–28 fractions 5 d a week." and "SBRT was administered to all metastatic lesions at doses of 30–40 Gy in 3–5 fractions."	In our study, we uniformly employed a higher radiation dose, with the primary lesion receiving a radiotherapy dose ranging from 50 to 50.4 Gy

Page 19-20 “5.6.4 Follow-up”	“Patients will undergo tumor assessments at baseline (screening period) and every 6 weeks (± 7 days) for the first 12 months after treatment initiation. Patients will be followed up once every 3 months in the first two years, once every 6 months in the third to fifth year, and once a year thereafter.”	“Tumour assessments were performed once every 6 weeks (±7 d) during chemotherapy, and once every 12 weeks (±7 d) from the end of chemo-radiotherapy to the end of the second year, once every 6 months during the third and fourth years, and once yearly thereafter.”	In the original protocol, the follow-up schedule was overly frequent, posing challenges for full compliance in practical research. Therefore, the follow-up timeline has been modified to ensure a more reasonable frequency and enhanced patient adherence
Page 23-24 “5.11.2 Sample size determination”	“The type I error rate is 5%, and the power is 80%. The sample size calculated by PASS 22.0 was 25 cases (or a minimum of 25 patients). In consideration of a 20% drop-out rate, the final sample size is set at 30 cases.”	“The type I error rate is 5%, and the power is 80%. Follow-up duration was calculated from enrollment to the date of the last follow -up. Assuming a uniform accrual accomplished over a period of about 12 months, with an additional 12 months of follow-up subsequent to the enrollment of the last patient, to observe 16 PFS events, a calculation determined that 25 cases (or a minimum of 25 patients) were needed. In consideration of a 20% drop -out rate, the final sample size is set at 32 cases.”	The original sample size calculation contained a minor error. Considering a dropout rate of 20%, the final sample size should be no less than 32 cases.

8. References

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2018. CA Cancer J Clin 2018;68:7–30.

2. Zheng RS, Sun KX, Zhang SW, et al. Report of cancer epidemiology in China, 2015. Zhonghua Zhong Liu Za Zhi (2019) 4:19–28.

3. Sun JM, Shen L, Shah MA, et al. Pembrolizumab plus chemotherapy versus chemotherapy alone for first-line treatment of advanced oesophageal cancer (KEYNOTE-590): a randomised, placebo-controlled, phase 3 study. Lancet (2021) 398:759–71.

4. Luo H, Lu J, Bai Y, et al. Effect of Camrelizumab vs Placebo Added to Chemotherapy on Survival and Progression-Free Survival in Patients With Advanced or Metastatic Esophageal Squamous Cell Carcinoma: The ESCORT-1st Randomized Clinical Trial. JAMA (2021) 326:916 –925.

5. Suzuki G, Yamazaki H, Aibe N, et al. Palliative radiotherapy in the local management of stage IVB esophageal cancer: factors affecting swallowing and survival. Anticancer Res 2017;37:3085–92.

6. Li J, Wen Y, Xiang Z, et al. Radical radiotherapy for metachronous oligometastasis after initial treatment of esophageal cancer. Radiother Oncol (2021) 154:201–6.

7. Lee S, Ahn BC, Park SY, et al. A phase II trial of preoperative chemoradiotherapy and pembrolizumab for locally advanced esophageal squamous cell carcinoma (ESCC). Ann Oncol (2019) 30 (Suppl 5):v754.

8. van den Ende T, de Clercq NC, van Berge Henegouwen MI, et al. Neoadjuvant chemoradiotherapy combined with atezolizumab for resectable esophageal adenocarcinoma: a single-arm phase II feasibility trial (PERFECT). Clin Cancer Res (2021) 27:3351–9.

9. Liao XY, Liu CY, He JF, et al. Combination of checkpoint inhibitors with radiotherapy in esophageal squamous cell carcinoma treatment: a novel strategy. Oncol Lett 2019;18:5011–21.