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

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Abstract

Background

The effectiveness of chemo-immunotherapy for advanced esophageal squamous cell carcinoma (ESCC) remains limited. Therefore, we evaluated the safety and efficacy of radiotherapy plus chemo-immunotherapy as a first-line therapy for advanced ESCC.

Methods

In this single-arm clinical trial, individuals aged 18–75 years with previously untreated stage IV ESCC received chemotherapy comprising four cycles of 135–175 mg/m² paclitaxel with carboplatin every three weeks. Toripalimab (240 mg) was intravenously infused every three weeks for 12 months or until disease progression or intolerable toxicity. Radiotherapy commenced in the third cycle, encompassing radiation (50–50.4 Gy in 25–28 fractions) to primary lesions and metastases (30–40 Gy in 3–5 fractions). The primary outcome was progression-free survival (PFS), and secondary outcomes were objective response rate (ORR), disease control rate (DCR), duration of remission (DoR), one- and two-year overall survival rates, and adverse events.

Results

In this study, 33 participants (29 men; median age 59 years) were enrolled. Ultimately, 26 patients (78.8%) completed the entire radio-chemotherapy course, achieving an ORR, DCR, and DoR of 57.7% (95% CI: 37.3–78.0), 73.1% (95% CI: 54.8–91.3), and 11.5 months (IQR, 6.4–15.0 months), respectively. Within a median follow-up of 22.2 months, the median PFS was 12.8 months. Lymphopenia was the most frequent grade ≥ 3 adverse event (82%), and esophageal fistula occurred in three patients (9.1%). No treatment-related deaths occurred.

Conclusion

Radiotherapy supplementation to first-line chemo-immunotherapy for treatment-naive advanced ESCC demonstrated substantial antitumor activity and manageable safety, warranting further randomized controlled trials.

Trial Registration:

Esophageal cancer is associated with significant mortality, particularly in Asia, where esophageal squamous cell carcinoma (ESCC) is the most common histological subtype, accounting for approximately 90% of all cases. At the point of diagnosis, over two-thirds of patients already exhibit metastatic or locally advanced disease [1]. The National Comprehensive Cancer Network (NCCN) guidelines specify the use of chemo-immunotherapy as the first-line of treatment for advanced esophageal cancer [2]. This recommendation was based on several large-scale phase III trials that reported substantial prolongation of progression-free survival (PFS) to 5.7–7.3 months and OS to 12.6–17.2 months compared to those using chemotherapy alone [3–9]. However, despite progress in therapeutic outcomes, the average 6-month PFS remains unsatisfactory, suggesting the development of drug resistance and disease progression during maintenance immunotherapy.

For cases with advanced esophageal cancer who have not received previous treatment, radiotherapy targeting both the primary and metastatic lesions is a vital therapeutic option. This intervention significantly improves dysphagia and nutritional status, when directed at the primary lesion, and alleviates pain and other symptoms, while inducing an abscopal effect in metastatic cases. Two retrospective studies indicated that compared to the use of chemotherapy alone, the combination of chemotherapy with radiotherapy modestly enhances the objective response rate and prognosis [10, 11]. However, in the current landscape where chemo-immunotherapy is the standard for advanced esophageal cancer, radiotherapy is predominantly considered a salvage second-line treatment. Notably, reports on the use of radiotherapy with chemo-immunotherapy in cases of local recurrence and/or distant metastasis after first-line treatment failure have demonstrated clinical benefits and acceptable safety [12, 13]. Furthermore, some studies have primarily focused on patients with metachronous oligometastatic esophageal cancer, considering radical radiotherapy exclusively for metastatic lesions [14, 15]. In a recent prospective trial involving patients with a controlled esophageal primary lesion, the group receiving local treatment — mainly consisting of stereotactic body radiotherapy (SBRT) for metastatic lesions together with systemic therapy (chemotherapy or chemo-immunotherapy) — exhibited a median PFS of 15.3 months, compared to 6.4 months for systemic therapy alone.15 Nonetheless, the safety and efficacy of this approach as a primary treatment strategy in treatment-naive advanced ESCC have yet to be substantiated by extensive research data, although multiple ongoing prospective clinical studies are anticipated to publish their findings soon [16–18]. Consequently, the optimal treatment approach for treatment-naive advanced ESCC and the role of radiotherapy within this regimen remains a subject of debate. This encompasses the decision on when to integrate radiotherapy with chemo-immunotherapy and the consideration of the simultaneous irradiation of both primary and metastatic lesions.

In the present study, we performed a single-arm trial to determine the safety and efficacy of radiotherapy together with chemo-immunotherapy as a first-line treatment in advanced ESCC.

**Methods**

**Study design and patients**
This was a single-arm trial undertaken at Sichuan Cancer Hospital (Chengdu, Sichuan Province, China). Patients (18–75 years of age) with histologically diagnosed unresectable, treatment-naive, stage IV ESCC based on the eighth Edition of the American Joint Committee on Cancer (AJCC) tumor, node, metastasis (TNM) staging system, with multiple lymph node metastases (N3) or distant oligometastases (M1) were eligible. Oligometastasis was defined as the occurrence of ≤ 5 metastatic lesions in ≤ 3 metastatic organs. Detailed definition of oligometastases is shown in Appendix 2 S1. Other inclusion criteria included having a minimum of one measurable lesion, in terms of the Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1, Eastern Cooperative Oncology Group performance status (ECOG PS) score of 0–1, a life expectancy ≥ 6 months, and sufficient bone marrow and organ function. The exclusion criteria included a history of any other malignancy, metastases to the central nervous system, a previous history of immunotherapy, a history of autoimmune or interstitial lung disease, or serious comorbidities, such as congestive heart failure or uncontrolled diabetes (Appendix 1).

This trial was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines. The trial protocol was approved by the Institutional Review Board of Sichuan Cancer Hospital, Sichuan, China (SCCHEC-02-2021-021). All patients provided written informed consent prior to enrollment. All authors had access to the study data and reviewed and approved the final manuscript.

**Treatment**

Each chemo-immunotherapy cycle lasted for 3 weeks and consisted of 240 mg toripalimab and 135–175 mg/m² paclitaxel plus carboplatin (area under the curve [AUC, 4–6]) on day 1. Concurrent radiotherapy was initiated on the third chemo-immunotherapy cycle. Primary lesions were treated with intensity-modulated radiotherapy (IMRT) at 45–50.4 Gy in 25–28 fractions 5 d a week. The gross tumor volume (GTV) included the primary tumor (GTV-P), metastatic lymph nodes (GTV-N), and metastatic lesions (GTV-M). The planning target volume (PTV) was defined as GTV with an additional 1–2 cm at the proximal and distal margins and a radical margin of 0.5–1.0 cm. For distant lymph nodes, such as supraclavicular and retroperitoneal lymph nodes, conventionally fractionated radiotherapy (45–50.4 Gy in 25–28 fractions) was administered at the physician's discretion, in consideration of adjacent organs at risk, such as the trachea, stomach, and intestines. SBRT was recommended in cases with suitable oligometastatic lesions in the liver, lungs, or bones, with consideration for the same factors. SBRT was administered to all metastatic lesions at doses of 30–40 Gy in 3–5 fractions. Delineation of the target area is shown in Appendix 2 S3.

Upon completion of four chemo-immunotherapy cycles, chemotherapy was discontinued; toripalimab was continued at 240 mg every three weeks for a maximum of 1 year or until the patient exhibited disease progression or evidence of intolerable toxicity. Dose reduction was permitted for paclitaxel and carboplatin but not toripalimab. Chemotherapy was suspended or deferred if grade 3 or the above adverse effects occurred.

**Follow-up and outcomes**
Baseline computed tomography (CT) examination was performed within 14 d before treatment initiation. Tumor evaluations were conducted at 6-weekly intervals (± 7 d) during chemotherapy. Meanwhile, during chemoradiotherapy, tumor evaluations were conducted once every 12 weeks (± 7 d) to the end of year 2, at 6-monthly intervals during the third and fourth years, and annually thereafter. Efficacy assessments were performed in accordance with the RECIST 1.1 criteria. Laboratory analyses, including complete blood count, blood chemical tests, electrocardiography (ECG), routine urine analysis and stool examination, coagulation testing, and thyroid function testing were conducted once every 3 weeks. Adverse events were identified and monitored using the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE) version 5.0.

The primary endpoint was progression-free survival (PFS), defined as the time between beginning treatment and tumor progression, patient death, or the last follow-up. Secondary endpoints included the objective response rate (ORR), disease control rate (DCR), duration of remission (DoR), 1- and 2-year OS rates, patient-reported health-related quality of life (HRQoL), and adverse events. Objective responses included complete remission (CR) and partial response (PR). Disease control represented CR, PR, and stable disease (SD) while DoR was determined as the interval between the first objective response to the first documentation of progression or all-cause death. OS was assessed from the start of therapy to all-cause death. Exploratory outcomes included the relationship between clinical outcomes with immune cell types in the tumor microenvironment, and biomarkers in peripheral blood (e.g., soluble PD-L1 and cytokines).

**Multiplex Immunohistochemistry/Immunofluorescence**

Tumor biopsy sections were analyzed via multiplex immunohistochemistry/Immunofluorescence (mIHC/mIF) analysis using the Opal 7-color kit (NEL811001KT; Akoya Biosciences, Marlborough, MA, USA), in accordance with the manufacturer’s instructions. Following antigen retrieval in EDTA buffer (pH 9.0; 3 min, 125°C) and cooling to room temperature (RT), the sections were washed with ddH$_2$O followed by TBST/0.5% Tween (repeated three times for two minutes each time). The slides were then blocked with a blocking buffer at RT for 10 min and treated with primary anti-PDL1 (ZA-0629, 1:50; ZSGB Biotech, Beijing, China) at 37°C for 60 min followed by rinsing in TBS. The slides were incubated with an HRP-conjugated secondary antibody (10 min, 37°C) followed by TSA dye 620 (1:100) for 5 min after further TBST washes. The same procedure was repeated for the other primary antibodies; namely, anti-CD68 (ZM-0060, 1:100, dye480; ZSGB Biotech), CD8 (ZA-0508, 1:100, dye570; ZSGB Biotech), CD11c (45581, 1:400, dye520; Abcam, Cambridge, UK), CD4 (ZA-0509, 1:100, dye690; ZSGB Biotech), and pan-cytokeratin (ZM-0067, 1:100, dye780; ZSGB Biotech). Additional rounds of antigen retrieval were undertaken in EDTA (pH 6.0) buffer using a pressure cooker for 2 min. DAPI was used for nuclear staining (100 µL DAPI, 5 min, RT).

Whole slide images were scanned using the TissueFAXS SL system (7.1.120). Digital images were analyzed using the HALO™ software (v 3.5). Immune cell densities were assessed as positively stained cell counts per mm$^2$. The cell density was calculated in total, tumor, and stromal areas respectively.
Periphery Cytokines

Sera were obtained at baseline and during treatment (after two cycles of chemo-immunotherapy and before radiotherapy). Inflammation-related cytokines, namely interleukin (IL)-2, IL-4, IL-6, IL-10, IL-17, tumor necrosis factor-alpha (TNF-α), and interferon-gamma (IFN-λ), were assessed using a magnetic beads kit (281004; Wellgrow, Beijing, China). Briefly, 50 µL of the serum sample or reference standards were added to 50 µL of capture beads suspension, mixed thoroughly, and incubated at RT in the dark for 1 h. The supernatant was removed after magnetic precipitation, and the beads were treated with 100 µL of a fluorescent-labeled antibody (1 h, RT). After washing, the beads were assessed using flow cytometry (BD FACSCanto™; BD Biosciences, San Jose, CA, USA). IL-37 levels were assessed using the human IL-37 ELISA Kit (ab213798, Abcam), following the manufacturer’s instructions.

Statistical Analyses

The necessary sample sizes were assessed based on an increase of median PFS from 6.3 months to 12 months with chemo-immunotherapy, as previously described, and a type I error of 0.05, a statistical power of 80%, and a 20% dropout rate. The follow-up duration was calculated from enrolment to the date of the last follow-up. To observe 16 PFS events, we calculated that 32 patients were needed. This was based on an assumption of a uniform accrual accomplished over a period of about 12 months, with an additional 12 months of follow-up subsequent to the enrolment of the last patient. The data collection cut-off date was 12 October 2023. The ORR, DCR, and DoR were assessed in the following two populations: firstly, intention-to-treat (ITT), which included all participants, and secondly, efficacy-evaluable, which included patients who actually received radiotherapy of all lesions and underwent at least one post-baseline disease assessment. Safety was assessed in all cases where a minimum of one dose of the study drug had been administered. OS, PFS, and the corresponding 95% confidence intervals (CIs) were determined using the Kaplan–Meier method. Survival outcomes were analyzed using log-rank tests. Clinical and demographic features, together with treatment-related adverse events (TRAEs), were analyzed using descriptive statistical methods. Associations between biomarkers and clinical outcomes were analyzed using the Mann-Whitney test, and between biomarkers and PFS and OS using the Log-rank. Analyses were conducted using R software (v4.3.1), SPSS 22.0, and SAS 9.4. Differences were considered statistically significant at \( p < 0.05 \).

Results

Participants and Treatment

From 30 June 2021 to 30 September 2022, we assessed 56 patients for eligibility; among whom, 33 (median age: 59 years, range: 43–74; 29 men) were enrolled (Fig. 1). The most common sites for oligometastasis were distant lymph nodes (63.6%), followed by the lungs (15.2%), and bones (9.1%) (Table 1). We observed that 27 (81.8%) patients had a total of 32 oligometastatic lesions, of which 12 were in distant organs and 15 in non-regional lymph nodes, whereas 6 patients (18.2%) had only regional
lymph node metastases (cTanyN3M0). Treatment was permanently stopped prior to the commencement of radiotherapy in five patients for the following reasons: informed consent withdrawal (n = 1), supraventricular arrhythmia (n = 1), esophageal fistulae (n = 2), and disease progression (liver metastasis; n = 1). Among the 28 patients who started radiotherapy, one 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 26 patients (78.8% of the enrolled individuals) completed radiotherapy of all lesions (both esophageal and metastatic) and 4 chemotherapy cycles. Among these patients, 14 (42.4%) completed the planned 1-year treatment with toripalimab, with a median of 10 treatment cycles (interquartile range (IQR): 4.5–14). The primary reasons for the early discontinuation of toripalimab included disease progression (n = 10), AEs (n = 4), and informed consent withdrawal (n = 5). Detailed information is available in Appendix 2 (Table S1). As this trial is ongoing, evaluation of the quality of life will be reported in subsequent studies.
Table 1
Participant characteristics

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>n = 33</th>
</tr>
</thead>
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<td>Age, median (range)</td>
<td>59 (43–74) years</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>29 (87.9%)</td>
</tr>
<tr>
<td>Women</td>
<td>4 (12.1%)</td>
</tr>
<tr>
<td>Smoking history</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>25 (75.8%)</td>
</tr>
<tr>
<td>No</td>
<td>8 (24.2%)</td>
</tr>
<tr>
<td>Alcohol history</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>24 (72.7%)</td>
</tr>
<tr>
<td>No</td>
<td>9 (27.3%)</td>
</tr>
<tr>
<td>Bodyweight loss</td>
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</tr>
<tr>
<td>&lt;10%</td>
<td>30 (90.9%)</td>
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<tr>
<td>≥10%</td>
<td>3 (9.1%)</td>
</tr>
<tr>
<td>ECOG performance status</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>15 (45.5%)</td>
</tr>
<tr>
<td>1</td>
<td>18 (54.5%)</td>
</tr>
<tr>
<td>Tumor location</td>
<td></td>
</tr>
<tr>
<td>Upper</td>
<td>6 (18.2%)</td>
</tr>
<tr>
<td>Middle</td>
<td>12 (36.4%)</td>
</tr>
<tr>
<td>Lower</td>
<td>15 (45.5%)</td>
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<tr>
<td>Primary tumor length (cm)</td>
<td></td>
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<tr>
<td>≤5</td>
<td>7 (21.2%)</td>
</tr>
<tr>
<td>&gt;5</td>
<td>26 (78.8%)</td>
</tr>
<tr>
<td>Clinical T stage</td>
<td></td>
</tr>
<tr>
<td>T3</td>
<td>26 (78.8%)</td>
</tr>
<tr>
<td>T4</td>
<td>7 (21.2%)</td>
</tr>
<tr>
<td>Data are n (%)</td>
<td></td>
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<tr>
<td>Characteristics</td>
<td>n = 33</td>
</tr>
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<td>--------------------------</td>
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<tr>
<td><strong>Clinical N stage</strong></td>
<td></td>
</tr>
<tr>
<td>N2</td>
<td>14 (42.4%)</td>
</tr>
<tr>
<td>N3</td>
<td>19 (57.6%)</td>
</tr>
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<td><strong>Clinical M stage</strong></td>
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</tr>
<tr>
<td>M0</td>
<td>6 (18.2%)</td>
</tr>
<tr>
<td>M1</td>
<td>27 (81.8%)</td>
</tr>
<tr>
<td><strong>Clinical TNM stage</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>33 (100%)</td>
</tr>
<tr>
<td><strong>Site of metastases</strong></td>
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<tr>
<td>Distant lymph nodes</td>
<td>22 (66.7%)</td>
</tr>
<tr>
<td>Lung</td>
<td>6 (18.2%)</td>
</tr>
<tr>
<td>Liver</td>
<td>1 (3.0%)</td>
</tr>
<tr>
<td>Bone</td>
<td>3 (9.1%)</td>
</tr>
<tr>
<td>Spleen</td>
<td>1 (3.0%)</td>
</tr>
<tr>
<td>Adrenal gland</td>
<td>1 (3.0%)</td>
</tr>
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</table>

**Efficacy Outcomes in Patients Who Completed Radiotherapy of All Lesions**

We evaluated the efficacy of the treatment regimen in the 26 patients who completed radiotherapy of all lesions (esophageal and metastatic) and 4 chemotherapy cycles. Assessment at three months after completing the radiotherapy revealed PR in 15 patients (57.7%), SD in 4 patients (15.4%), and disease progression in 7 patients (26.9%). Of note, the ORR was 57.7% (15/26, 95% CI: 37.3–78.0%), whereas the DCR was 73.1% (19/26, 95% CI: 54.8–91.3%) (Table S2). The 1-year PFS and OS rate was 50.0% (95% CI: 34–73.4%) and 76.9% (95% CI: 62.3–94.9%), respectively. The median PFS was 12.8 months (95% CI: 8.0 months–not estimable) and the median OS was not attained (Fig. 4).

**Efficacy Outcomes in Intent-to-Treatment Patients**

Analysis of the best overall response (BOR) showed decreased sizes of target lesions after treatment versus the baseline in 27 out of the 33 enrolled patients (Fig. 2). Responses included CR in 7 cases, PR in 13, and SD in 7. Four patients exhibited increases in the target lesion size, including one patient with
disease progression, while two patients could not be evaluated. A summary of the tumor responses observed following two cycles of chemo-immunotherapy is provided in Appendix 2 (Table S3).

Overall, the median follow-up was 22.2 months (range: 16.3–28.1 months) and the median DoR was 11.5 months (IQR, 6.4–15.0 months). Among the 33 patients, 21 experienced recurrences (64%), and 16 died as a result of the disease (48%). A summary of the information on recurrence (pattern, site, and reasons for death) is provided in Appendix 2 (Table S4). Responses and outcomes are summarized in Fig. 3. The 1-year PFS and OS rates were 41.9% (95% CI: 27.7–63.5%) and 69.7% (95% CI: 55.7–87.3%), respectively. The median PFS was 9.8 months (95% CI: 6.83 months–not estimable), while the median OS was 16.5 months (95% CI: 13.2 months–not estimable) (Fig. 4). Subsequent treatments after recurrence are provided in Appendix 2 (Table S5).

Safety

All 33 cases experienced TRAEs of varying grades. No unexpected AEs or treatment-related deaths occurred (Table 2). Commonly observed TRAEs included myelosuppression, weight loss, and anorexia, and commonly observed grade 3 or higher AEs were lymphopenia (27/33, 82%), neutropenia (9/33, 27%), and leukopenia (8/33, 24%). More specifically, radiotherapy-related AEs mainly included radiation esophagitis (24/28, 86%), radiation dermatitis (21/28, 75%), esophageal/epigastric pain (18/28, 64%), and radiation pneumonitis (6/28, 22%). The most common immune-related AEs were hypertriglyceridemia (21/33, 64%), hypothyroidism (18/33, 54%), and rash (4/33, 12%). Three patients (9.1%) developed grade 3 esophageal fistula; two immediately after two cycles of chemo-immunotherapy, and one four months after the completion of radiotherapy. All three patients who developed esophageal fistula had T4 tumors (tumor length > 5 cm).
<table>
<thead>
<tr>
<th>Event</th>
<th>Grade 1</th>
<th>Grade 2</th>
<th>Grade 3</th>
<th>Grade 4</th>
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</thead>
<tbody>
<tr>
<td>Lymphopenia</td>
<td>0</td>
<td>2 (6%)</td>
<td>18 (55%)</td>
<td>9 (27%)</td>
</tr>
<tr>
<td>Leukopenia</td>
<td>1 (3%)</td>
<td>14 (42%)</td>
<td>7 (21%)</td>
<td>1 (3%)</td>
</tr>
<tr>
<td>Neutropenia</td>
<td>6 (18%)</td>
<td>7 (21%)</td>
<td>8 (24%)</td>
<td>1 (3%)</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>14 (42%)</td>
<td>6 (18%)</td>
<td>1 (3%)</td>
<td>0</td>
</tr>
<tr>
<td>Anemia</td>
<td>22 (67%)</td>
<td>5 (15%)</td>
<td>3 (9%)</td>
<td>1 (3%)</td>
</tr>
<tr>
<td>Elevated triglyceride</td>
<td>17 (52%)</td>
<td>2 (6%)</td>
<td>2 (6%)</td>
<td>0</td>
</tr>
<tr>
<td>Bilirubin elevation</td>
<td>6 (18%)</td>
<td>2 (6%)</td>
<td>0</td>
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</tr>
<tr>
<td>Hypoproteinemia</td>
<td>31 (94%)</td>
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<tr>
<td>Creatinine increased</td>
<td>3 (9%)</td>
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<tr>
<td>AST elevation</td>
<td>17 (52%)</td>
<td>1 (3%)</td>
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<td>ALP elevation</td>
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<td>Gamma-glutamyl transferase elevation</td>
<td>9 (27%)</td>
<td>1 (3%)</td>
<td>2 (6%)</td>
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</tr>
<tr>
<td>ALP elevation</td>
<td>7 (21%)</td>
<td>0</td>
<td>1 (3%)</td>
<td>0</td>
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<tr>
<td>Hypothyroidism</td>
<td>14 (42%)</td>
<td>4 (12%)</td>
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<tr>
<td>Anorexia</td>
<td>3 (9%)</td>
<td>8 (24%)</td>
<td>0</td>
<td>0</td>
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<tr>
<td>Fatigue</td>
<td>2 (6%)</td>
<td>1 (3%)</td>
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<td>Alopecia</td>
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<td>0</td>
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<tr>
<td>Nausea or vomiting</td>
<td>3 (9%)</td>
<td>5 (15%)</td>
<td>2 (6%)</td>
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<tr>
<td>Constipation</td>
<td>4 (12%)</td>
<td>3 (9%)</td>
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<tr>
<td>Rash</td>
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<td>1 (3%)</td>
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<tr>
<td>Weight loss</td>
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<tr>
<td>Fever</td>
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<tr>
<td>Diarrhea</td>
<td>1 (3%)</td>
<td>1 (3%)</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Data are n (%). Abbreviations: AST, aspartate aminotransferase; ALT alanine aminotransferase; GGT, gamma-glutamyl transferase, ALP, alkaline phosphatase.
### Biomarkers for Treatment Response and Outcomes

Baseline tumor biopsies from 76.9% (20/26) of patients were available for multiplex immunohistochemistry/Immunofluorescence (mIF) analysis. Typical mIF images showed higher infiltration of immune cells in three patients achieving PR (Fig. 4A, upper panel) than that in three patients achieving SD (Fig. 4A, lower panel). Patients achieving clinical PR exhibited higher densities of CD8+ T cells \( (p = 0.067) \), CD11c+ dendritic cells (DCs) \( (p = 0.067) \), and CD68+ macrophages \( (p = 0.019) \) than those in patients achieving SD (Fig. 4B). The density of overall PDL1+ cells, PDL1+ tumor cells, PDL1+ DCs, and PDL1+ macrophages was greater in the PR than in the SD group, and a significant difference was observed in PDL1+ DCs \( (p = 0.037) \) (Fig. 4B). Notably, the density of PDL1+ tumor cells, PDL1+ DCs, and PDL1+ macrophages in the stromal compartment were positively correlated with a better treatment response (supplementary Fig. S1 A). Higher infiltration of CD8+ T cells \( (\text{PFS}, p = 0.014; \text{OS}, p = 0.009) \), CD11c+ DCs \( (\text{PFS}, p = 0.055; \text{OS}, p = 0.006) \), and CD68+ macrophages \( (\text{PFS}, p = 0.009; \text{OS}, p = 0.061) \) were correlated with longer PFS and OS of patients (Fig. 4C). Elevated numbers of PDL1+ cells were correlated with better OS \( (p = 0.081) \) but not PFS \( (0.64) \) (Fig. 4C). Moreover, higher densities of PDL1+ DCs and PDL1+ macrophages (stroma) were associated with higher PFS and OS, respectively (Supplementary Fig. S1 B).

To further assess whether different peripheral cytokines could predict treatment response and patient outcome, sera from 25 patients were analyzed both at baseline and during therapy (20 baseline samples and 20 treatment samples, including 15 paired samples, Supplementary Fig. S2 A). The results indicated that the levels of the eight tested cytokines were similar between the baseline and treatment groups (Supplementary Fig. S2 B). The patients with clinical PR exhibited higher levels of baseline IL-10 \( (p = 0.076) \), baseline IFN-\( \lambda \) \( (p = 0.063) \), and on-treatment IFN \( (p = 0.026) \) than those with SD (Fig. 5A). The levels of other cytokines, such as IL-2, IL-4, IL-6, IL-17, IL-37, and TNF-\( \alpha \) were also similar between the two groups (Fig. 5A, Supplementary Fig. S3). Notably, the PFS of patients with greater concentrations of
baseline IFN-λ \( (p < 0.001) \) and on-treatment IL6 \( (p = 0.029) \) was higher than in those with lower levels (Fig. 5B). Moreover, higher levels of baseline IL-4 \( (p = 0.001) \), baseline IL-10 \( (p = 0.047) \), baseline IFN-λ \( (p < 0.001) \), on-treatment IFN-λ \( (p < 0.001) \), on-treatment TNF-α \( (p = 0.007) \), and on-treatment IL-17 \( (p = 0.015) \) were significantly associated with improved OS (Fig. 5B). Neither PFS nor OS were associated with the levels of other serum cytokines (Supplementary Fig. S4).

**Discussion**

To the best of our knowledge, the present study is the first phase 2 trial reporting the safety, efficacy, and identification of candidate biomarkers for the outcomes of radiotherapy combined with chemo-immunotherapy in treatment-naive advanced ESCC. Our results reveal that radiotherapy targeting both the primary esophageal and metastatic lesions shows promising effectiveness and manageable toxicity when combined with with chemo-immunotherapy.

In clinical practice, chemo-immunotherapy remains the preferred approach for advanced esophageal cancer [3, 19]. However, adding radiotherapy to primary and metastatic lesions has been shown, in certain retrospective studies, to enhance local tumor control, potentially delaying disease progression and prolonging survival [5, 6, 20–22]. Despite this, chemo-immunotherapy remains the standard treatment, and evidence supporting the safety and therapeutic benefits of adding radiotherapy is currently lacking in prospective clinical research, especially regarding the potential additive toxicity of combined radio- and immunotherapy. In the present study, we studied participants with treatment-naive stage IV ESCC and observed a median PFS of 12.8 months and a 1-year PFS rate of 50% in patients who completed the radiotherapy regimen. Compared to the median PFS reported in previous studies, such as the KEYNOTE-590 (6.3 months), JUPITER-06 (5.7 months), and Checkmate-648 trials (5.8 months) [3, 4, 7], our findings are promising. Moreover, our Kaplan–Meier analysis of PFS indicated a plateau after 1 year, whereas the curves for OS appeared to plateau after approximately 16 months, suggesting long-term survival benefits in cases of advanced ESCC following treatment with radiotherapy plus chemo-immunotherapy. Notably, most of our studied patients had synchronous oligometastatic disease, with some patients exhibiting locally advanced N3 disease. Increasing evidence suggests that higher numbers of individuals with oligometastatic disease can attain long-term survival using local treatment together with systemic therapy compared to those with multiple metastases [23–25]. Additionally, our analysis involved a comparison between the study participants and an internal historical control cohort receiving standard chemo-immunotherapy, revealing a positive but non-significant correlation with survival. However, longer-term follow-up studies together with prospective randomized controlled trials is required to verify these findings.

Patients with advanced esophageal cancer include those with synchronous metastasis, metachronous metastasis, and locoregional recurrence. Thus, in studies on advanced esophageal cancer combining radiotherapy with chemo-immunotherapy, the enrolled patients exhibit substantial heterogeneity. In the ESO-SHANGHAI 13 study [15], approximately 90% of the patients developed metachronous oligometastatic disease after curative treatment, with radiotherapy limited to metastatic lesions. Given
these results, the efficacy and safety of simultaneously combining radiotherapy with systemic treatment for the primary lesion remains unclear. The ongoing trial EC-CRT-003 is enrolling patients with treatment-naive stage IVb ESCC [17], and is considering adding thoracic radiotherapy (45-50Gy/25-28f) after 4–6 cycles of standard chemo-immunotherapy, without irradiating metastatic lesions. Similar clinical studies are in progress [26–31]. Collectively, these study designs reveal considerable debate over the optimal timing and target area of radiotherapy intervention in chemo-immunotherapy for advanced esophageal cancer. This debate centres on two main issues: first, whether radiotherapy is added during first-line systemic treatment in treatment-naive patients, after completing primary lesion treatment, or as a second-line treatment for tumor recurrence and metastasis; and second, whether radiotherapy targets only the primary lesion, metastatic lesions, or both. Our study enrolled treatment-naive patients with advanced esophageal cancer, with 81% of the patients being oligometastatic, indicating a higher tumor burden at initial treatment compared to the ESO-SHANGHAI 13 study [15]. Therefore, in our study, radiotherapy was conducted concurrently after 2 cycles of systemic treatment, in an attempt to reduce the tumor burden and shrink the radiotherapy target volume to mitigate radiotherapy toxicity. During the 3rd cycle of chemo-immunotherapy, concurrent radiotherapy was performed because of the apparent synergistic mechanism of radiotherapy and chemo-immunotherapy. Radiotherapy of the primary lesion can increase the production of tumor antigens, thus enhancing antigen presentation via DCs [32]. Moreover, SBRT for metastatic lesions may achieve systemic antitumor immunity through local activation [33]. Notably, our radiotherapy covered both primary and metastatic lesions. An opinion piece reported that targeting all lesions could enhance the likelihood of successfully initiating an antitumor immune response, overcome the problem of tumor heterogeneity, and enhance the destruction of drug-resistant subclones [34]. However, further research is required to investigate whether these effects occur.

An increasing body of evidence now indicates that the interaction between the tumor microenvironment (TME) and cancer cells is a key factor in tumor progression [35]. Our analyses using multiple immunohistochemistry/immunofluorescence techniques indicate that higher densities of CD8+ T cells, CD11c+ DCs, and CD68+ macrophages correlate with improved tumor response and prognosis. This effect is due to the enhanced tumor immune response from the adequate infiltration of these immune cells in the immune microenvironment, a relationship well-established in prior studies [36–38]. However, there have been conflicting reports on the predictive value of PD-L1 status in esophageal cancer [3, 4, 7, 36]. This variation in findings may arise because the studies investigating this have typically used immunohistochemistry (IHC) to detect PD-L1 expression, with the combined positive score (CPS) being most commonly used. However, CPS only includes tumor-associated immune cells, such as macrophages and lymphocytes, that are in close proximity to the tumor cells. In contrast, our study examined PD-L1 expression in a broader range of cells, including tumor cells, macrophages, and dendritic cells. Our results show that a high expression of PD-L1 on both tumor and immune cells is associated with better tumor outcomes. This finding suggests that comprehensive PD-L1 detection across all relevant cells may more accurately predict a patient’s treatment response. Moreover, our findings in concordance with existing studies [4, 5, 6] that highlight the beneficial predictive value of PD-L1 in cancer therapy.
In addition, our findings demonstrate a significant correlation between elevated baseline serum IFN levels and reduced on-treatment IL-6 levels and an extended PFS in patients with advanced ESCC. A previous investigation has established a correlation between reduced IL-6 levels and improved prognoses in advanced melanoma [39], and attributed this to the role of IL-6 in accelerating tumor progression through the inhibition of cancer cell apoptosis and the promotion of angiogenesis. In contrast, IFN-λ activates antitumor immune cells and suppresses immunoregulatory cells in immune antigens [40]. Therefore, IFN-λ and IL-6 could serve as reliable predictors of response to combined immunotherapy and chemoradiotherapy in advanced esophageal cancer. IL-4, IL-10, and TNF-α play multifaceted roles in cancer immunity, both activating antitumor immune cells and facilitating tumor immune suppression and escape. IL-10, IL-17, and TNF-α, known for their immunosuppressive properties, promote tumor growth and are associated with poor prognoses, as corroborated by several clinical studies, whereas IL-4 is considered an enhancer of immune cell antitumor activity [41–44]. Our results showed that higher baseline levels of IL-4, IL-10, and IFN-λ, as well as elevated treatment levels of IFN-λ, TNF-α, and IL-17, were associated with improved OS. This result contrasts with previous findings and highlights the complexity and variability of the local tumor immune microenvironment. Given that different cancers harbor distinct immunosuppressive cells and cytokines within their tumor microenvironment, and that various immune checkpoint inhibitors (ICIs) employ unique mechanisms of action, the relationship between cytokines and immunotherapy efficacy warrants further exploration.

A key issue in combining first-line radiotherapy with chemo-immunotherapy is safety. The present findings corroborate those of earlier clinical trials combining ICIs with radiotherapy for esophageal cancer [36, 45]. In the present study, we did not observe any unexpected safety signals, and most TRAEs were of grades 1–2. Grade 3 or higher TRAEs included predominantly myelosuppression, with no treatment-related deaths. Of note, 17 (51.5%) patients in this trial were still alive at the time of cut-off. Importantly, among the three cases of esophageal fistula, two occurred after two cycles of chemoradiotherapy and prior to radiotherapy. A retrospective analysis indicated that the incidence of esophageal perforation or fistula in patients with the T4 stage was as high as 30.1%. Hence, there may be a link between the occurrence of esophageal fistula and the clinical stages of the primary tumors, as all three patients presented with cT4 stage with tumor length > 5 cm. In conclusion, the precise influence of the addition of PD-1 inhibitors with chemoradiotherapy on fistula risk requires further investigation.

This study had several limitations. First, it was a single-arm study conducted at a single center with a small sample size, which may have led to selection bias, thus limiting the generalization of the findings. Second, although all patients received the necessary imaging and multidisciplinary evaluation before enrollment, not all metastatic lesions were pathologically confirmed in this study. Finally, this study investigated biomarkers; however, the limited sample size precludes definitive conclusions. These results may inform the design of future large-scale trials. To enhance the reliability of our findings, we are currently participating in a multi-center, randomized, controlled, phase III clinical trial to examine the effect of first-line radiotherapy combined with chemo-immunotherapy in 100 patients (ClinicalTrials.gov identifier: ChiCTR2300070300).
Conclusions

In patients with treatment-naive, advanced ESCC, first-line radiotherapy to both primary and metastatic lesions in combination with chemo-immunotherapy demonstrated promising antitumor effects with a manageable safety profile. Furthermore, our findings provide new insights into potential biomarkers for assessing clinical effectiveness.

Abbreviations

AE, adverse events; AJCC, American Joint Committee on Cancer; AUC, area under the curve; BOR, best overall response; CI, confidence interval; CPS, combined positive score; CR, complete response; CT, computed tomography; DCR, disease control rate; DCs, dendritic cells, DoR, duration of remission; ECOG PS, Eastern Cooperative Oncology Group performance status; ESCC, esophageal squamous cell carcinoma; ECG, electrocardiography; GTV, gross tumor volume; HR, hazard ratio; HRQoL, health-related quality of life; ICIs, immune checkpoint inhibitors; IFN-λ, interferon-gamma; IL, interleukin; IMRT, intensity-modulated radiotherapy; IQR, interquartile range; ITT, intention-to-treat; mIHC/mIF, multiplex immunohistochemistry/immunofluorescence; NCCN, National Comprehensive Cancer Network; NCI-CTCAE, National Cancer Institute Common Terminology Criteria for Adverse Events; ORR, objective response rate; OS, overall survival; PD, progressive disease; PD-1, programmed cell death-1; PD-L1, programmed cell death-ligand 1; PFS, progression-free survival; PR, partial response; PTV, planning target volume; QoL, quality of life; RECIST, Response Evaluation Criteria in Solid Tumors; RT, room temperature; SABR, stereotactic ablative radiotherapy; SBRT, stereotactic body radiotherapy; SD, stable disease; TME, tumor microenvironment; TNF-α, tumor necrosis factor-alpha; TRAEs, treatment-related AEs

Declarations

Ethics approval and consent to participate

The clinical protocol was approved by the Institutional Review Board of Sichuan Cancer Hospital. All participants provided written informed consent.

Consent for publication

All authors have given their consent for publication.

Availability of data and materials

The study protocol is available in Appendix 1. In order to protect the privacy of study participants, the data from this study are not publicly available. However, with the permission of the corresponding author, these data can be made available from the authors upon reasonable request.

Competing interests
The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest. Shanghai Junshi Biosciences donated the investigational drugs. This study protocol was presented at the Journal for ImmunoTherapy of Cancer 2021 Annual Meeting as a poster presentation (Washington, D.C., November 10-14, 2021: abstract #413). This study was presented at the American Society for Therapeutic Radiology and Oncology 2022 Annual Meeting as a poster presentation (San Antonio, TX, October 23-26, 2022: abstract #2374).

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**Authors' contributions**

QW, BC, and LW were responsible for the conception and design of the study. All authors contributed to the collection of data. QW, WZ, QP, and WZ supervised the study. BL, GW, YW, and JZ performed the literature search. LY, BC, WZ, and SH conducted an analysis of the biological samples. LP, YH, and YL verified the underlying study data. All authors had access to the data and were responsible for the data interpretation and manuscript writing, as well as reviewing and approving the manuscript for submission. All authors accept responsibility for the decision to submit the manuscript for publication.

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Figures
Figure 1

Flow diagram of study enrollment, treatment, and outcomes.
Figure 2

Tumor responses. (A) Best percentage changes in target lesion sizes from baseline. Dashed lines at +20% and −30% represent thresholds for disease progression and partial response, respectively, according to the RECIST 1.1 criteria. (B) Onset of response, duration of response, and outcome. Abbreviations: RECIST, Response Evaluation Criteria in Solid Tumors.
Figure 3

Kaplan–Meier survival analysis. (A-B) PFS and OS in the ITT population (n = 33). (C-D) PFS and OS in the efficacy-evaluable population (n = 26). Abbreviations: PFS, progression-free survival; OS, overall survival; ITT, intention-to-treat.
Figure 4

Biomarkers in the tumor microenvironment. (A) Representative mIF of the tumor immune microenvironment. The upper panel shows elevated infiltration of immune cells including CD8, CD68, CD11c, and CD4 cells in three patients achieving clinical PR. The lower panel shows relatively depressed immune cell infiltration in three patients achieving stable SD (bar, 100 μm). (B) Immune cell infiltration levels in the tumor tissues between patients achieving PR (n = 13) and those achieving SD (n = 7)
assessed by mIF. The comparison of immune cell densities was performed using the Mann-Whitney test. (C) Progression-free and overall survival analyses for the tumor infiltration levels of diverse immune cells. Patients were grouped into low- and high-immune cell infiltration based on the quantile 30% value of each variable: CD8+ cells, 210 cells/mm²; CD11c+ cells, 1318 cells/mm²; CD68+ cells, 257 cells/mm²; PDL1+ cells, 190 cells/mm². Abbreviations: mIF, multiplex immunofluorescence; PR, partial response; SD, stable disease.

Figure 5
Peripheral cytokines predict treatment response and patient survival. (A) Serum levels of different cytokines at baseline or on-treatment between patients achieving partial response (PR, n = 14) and those achieving stable disease (SD, n = 6). Cytokine levels were compared using Mann-Whitney U tests. (B) Progression-free and overall survival analyses for different periphery cytokines. Patients were grouped into low- and high-level groups based on the expression of diverse cytokines with reference to the quantile 30% value of each variable: baseline IFN-λ, 0.92 pg/mL; on-treatment IL-6, 3.8 pg/mL; baseline IL-4, 0 pg/mL; baseline IL-10, 0.34 pg/mL; on-treatment IFN-λ, 1.18 pg/mL; baseline TNF, 0 pg/mL; and baseline IL-17, 0 pg/mL. Abbreviations: PR, partial response; IFN-λ, interferon-gamma; IL, interleukin; TNF, tumor necrosis factor.

**Supplementary Files**

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