# Long-term survival and post-hoc analysis of toripalimab plus definitive chemoradiotherapy for oesophageal squamous cell carcinoma: insights from the EC-CRT-001 phase II trial

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## Summary

**Background** In the EC-CRT-001 phase II study, the combination of toripalimab (an anti-programmed death-1 antibody) and definitive chemoradiotherapy (CRT) has shown promising efficacy in patients with locally advanced oesophageal squamous cell carcinoma (ESCC). Here, we reported the long-term outcomes and post-hoc exploratory analyses.

**Methods** This single-arm, phase II trial enrolled 42 patients diagnosed with unresectable stage I–IVA ESCC was conducted at Sun Yat-sen University Cancer Center between November 2019 and January 2021. Treatment consisted of chemotherapy (weekly 50 mg/m$^2$ of paclitaxel and 25 mg/m$^2$ of cisplatin for five cycles), concurrent radiotherapy (50.4 Gy in 28 fractions), and toripalimab (240 mg every 3 weeks for up to 1 year). The primary endpoint was clinical complete response (CR) rate at 3 months after CRT completion. The 3-year overall survival (OS) and progression-free survival (PFS) rates were evaluated. Additionally, the exploratory objectives included analysing recurrence patterns, assessing the associations between immune-related adverse events (irAEs) and efficacy, and identifying potential predictors for irAEs. The trial was registered with ClinicalTrials.gov (NCT04005170).

**Findings** With a median follow-up of 44.3 months (IQR 40.8–46.1), the 3-year OS and PFS rates were 44.8% (95% CI 31.9–62.8) and 35.7% (95% CI 23.8–53.6), respectively. Patients who failed to achieve a clinical complete response (CR) demonstrated significantly worse OS (hazard ratio [HR] = 13.73, 95% CI 4.43–42.54, $P < 0.0001$) and PFS (HR = 32.08, 95% CI 8.57–120.10, $P < 0.0001$). Disease recurrence occurred in 23 of 42 patients (55%), with recurrences being earlier and more frequent in the non-CR group compared to the CR group. Patients experiencing irAEs showed a significantly higher CR rate (72% vs. 39%, $P = 0.082$) and better PFS (HR = 0.43, 95% CI 0.19–0.93, $P = 0.027$) than those without irAEs. *GON4L* mutation was associated with a lower incidence of irAEs ($P = 0.036$).

**Interpretation** The updated survival outcomes confirmed the efficacy of toripalimab plus definitive CRT in locally advanced ESCC. Moreover, the development of irAEs may predict a more favourable prognosis.

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**Keywords:** Oesophageal squamous cell carcinoma; Definitive chemoradiotherapy; Toripalimab; Immune-related adverse event; Survival

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## Research in context

### Evidence before this study

We conducted a comprehensive search on PubMed for articles published until May 5, 2024, with the search terms (["esophageal squamous cell carcinoma" OR "oesophageal squamous cell cancer" OR "esophageal cancer"] AND ["chemoradiotherapy" OR "chemoradiation"] AND ["PD-L1" OR "PD-1" OR "pembrolizumab" OR "nivolumab" OR "atezolizumab" OR "durvalumab" OR "avelumab" OR "camrelizumab" OR "sintilimab" OR "toripalimab" OR "tislelizumab"]), and we identified 11 citations that included four ongoing trials with a study protocol and seven trials with published results. Of these seven published trials, three assessed neoadjuvant chemoradiotherapy (CRT) combined with immunotherapy for resectable oesophageal squamous cell carcinoma (ESCC), one reported a phase Ib/II study of sintilimab as maintenance after concurrent CRT for recurrent ESCC, and a phase Ib study of camrelizumab combined with definitive CRT and apatinib for locally advanced ESCC. The sixth trial was a phase II study evaluating the addition of induction sintilimab followed by concurrent CRT for locally advanced ESCC, and the seventh trial was our previous report of EC-CRT-001.

### Added value of this study

To the best of our knowledge, this is the first long-term analysis with more than 3 years of follow-up reported for immunotherapy plus definitive CRT in patients with locally advanced ESCC. The updated survival outcomes from the EC-CRT-001 trial confirmed our previous findings and further demonstrated the therapeutic advantage of ICIs in the treatment of locally advanced stages. Our analysis also revealed that the onset of irAEs might derive more of a clinical benefit and identified mutated-GON4L as a potential predictive biomarker for irAE status.

### Implications of all the available evidence

Extended follow-up of combination regimens with toripalimab plus definitive CRT in patients with locally advanced ESCC continues to show encouraging antitumor activity. Our study explored the role of irAE presence in predicting clinical benefits and underscored the need for further research to validate reproducible biomarkers. The identification of recurrence patterns also facilitates improved risk stratification and optimises surveillance strategies for ESCC.

## Introduction

Oesophageal cancer ranks among the leading causes of cancer-related morbidity and mortality worldwide.[1] Definitive chemoradiotherapy (CRT) is the standard regimen for unresectable locally advanced oesophageal squamous cell carcinoma (ESCC), although significant advancements in long-term survival have been lacking in recent years.[2,3] Results from previous studies on the antitumor activity of programmed death-1 (PD-1) or programmed death-ligand 1 (PD-L1) antibodies suggest an impending shift in therapeutic approaches.[4] The combination of immune checkpoint inhibitors (ICIs) built upon PD-1 or PD-L1 antibodies and chemotherapy has shown remarkable improvements in survival, emerging as a primary treatment modality for advanced ESCC.[5–8] However, the role of ICIs in locally advanced stages remains to be established.

Our team published the first phase II trial of an anti-PD-1 antibody (toripalimab) combined with definitive CRT in unresectable locally advanced ESCC, which showed encouraging antitumor activity with a complete response (CR) rate of 62% and acceptable toxicity (EC-CRT-001 trial).[9] Moreover, a 1-year overall survival (OS) rate of 78.4% and a 1-year progression-free survival (PFS) rate of 54.5% were reported. Studies focusing on the efficacy of ICIs in combination with CRT for locally advanced ESCC are ongoing. However, long-term survival data are lacking. Additionally, understanding the relapse pattern of a novel treatment may provide key insights into the optimization of therapy and refinement of surveillance strategy. Therefore, patterns of recurrence after immunotherapy plus definitive CRT are of particular concern.

Although ICIs have achieved long-term durable responses and significantly improved survival outcomes in various malignancies, the vast majority of patients experience immune-related adverse events (irAEs) after treatment.[10,11] Accumulating evidence suggests a strong correlation between mild-to-moderate irAE occurrence and survival benefits, particularly in lung cancer and melanoma.[12,13] However, the relationship between irAE status and efficacy in patients with ESCC remains unclear, with limited data on the efficacy of CRT plus immunotherapy. Moreover, biomarkers from genomics and transcriptomics predicting irAE development remain poorly understood, posing challenges in clinical decision-making.

Here, we report updated 3-year OS and PFS data from the EC-CRT-001 trial, representing the longest follow-up data available for immunotherapy combined with CRT in ESCC. Additionally, a post-hoc exploratory analysis was performed to discern recurrence patterns, the association between irAEs and efficacy, and potential predictive biomarkers for irAEs.

## Methods

### Study design, population, and oversight

The open-label, single-arm, phase II trial conducted at the Sun Yat-sen University Cancer Center enrolled

patients aged 18–70 years with unresectable stage I–IVA (8th edition of the American Joint Committee on Cancer) histologically proven ESCC. Detailed eligibility criteria and pre-treatment evaluation have been described previously.[9] Enrolled patients received weekly paclitaxel (50 mg/m$^2$) and cisplatin (25 mg/m$^2$) for five cycles, concurrent radiotherapy of 50.4 Gy in 28 fractions, and toripalimab (240 mg every three weeks for up to 1 year, or until disease progression or unacceptable toxicity). The primary endpoint was clinical CR rate at 3 months after CRT completion. OS, PFS, and safety profiles were secondary endpoints.

### Ethics statement

The trial was conducted in accordance with the principles of the Declaration of Helsinki and the Good Clinical Practice guidelines. Written informed consent was obtained from all participants before enrolment, and the study protocol was approved by the Institutional Review Board of the Sun Yat-sen University Cancer Center (B2019-065-01).

### Procedures

Follow-up visits were scheduled every 9 weeks for the first year, every 3 months for the second year, and semi-annually thereafter. Throughout the follow-up period, physical examinations, laboratory tests, oesophageal barium swallow, contrast-enhanced chest/abdominal computed tomography (CT), and positron emission tomography (PET)/CT were performed as required. Oesophagoscopy with biopsies was performed at 6-month intervals for up to 3 years. Time to recurrence was defined as the date when relapse was first observed on imaging or histopathological reports, and the initial sites of recurrence were recorded. Locoregional recurrence (LRR) was defined as a confirmed recurrence within the oesophagus or regional lymph nodes, whereas distant recurrence was defined as non-regional lymph node recurrence (cervical, supraclavicular, or para-aortic) or systemic metastasis.

AEs were graded according to the Common Terminology Criteria for Adverse Events (version 4.0). The definition of irAEs was based on pathologic evidence of irAE, multidisciplinary adjudication, or clinical improvement with irAE-based treatment, including topical corticosteroid therapy, systemic corticosteroid therapy, or hormone replacement.[14] Multiorgan irAEs were defined as irAEs involving more than one organ system.

### DNA extraction, whole-exome sequencing, and mutation calling

For the 19 patients with pre-treatment tumour biopsies available for sequencing analysis, total DNA was extracted from tumour tissues and patient-matched normal blood using the TIANamp DNA Mini Kit (Tiangen). The extracted DNA samples underwent DNA quality testing in degradation and impurities. Nanodrop spectrophotometer was applied for detecting sample purity and the DNA sample concentration was detected by Qubit® 3.0 Flurometer. Libraries were prepared using the VAHTS Universal Plus DNA Library Prep Kit for Illumina V2. Briefly, genomic DNA was processed fragmenting, purifying, DNA Ends repairing, and purifying. Subsequently, the library was subjected to hybridization-based capture for specific targets using the SureSelect XT (Agilent SureSelect XT Reagent kit) target enrichment system. Library quantification was performed using Qubit 3.0, and the length of library was detected using Agilent 2100. Sequencing was performed using a paired-end 150 bp (PE150) approach on a high-throughput sequencing platform. Somatic mutations of ESCC tumours were detected using normal blood as a control, followed by filtering under specific criteria and annotation utilizing ANNOVAR.

### RNA library preparation, sequencing, and functional profiling

Pre-treatment tissues from 19 patients underwent a series of standardized procedures, including collection, snap-frozen storage, and homogenization. Total RNA was isolated from tissue samples using TRIzol reagent (Invitrogen, Carlsbad, CA, USA), and followed by an assessment of purity using a NanoPhotometer (IMPLEN, CA, USA). Total RNA concentrations and integrity were determined using an Agilent 2100 RNA Nano 6000 assay kit (Agilent Technologies, CA, USA). After extraction and quantification, transcriptome sequencing libraries were generated using the VAHTS Universal V6 RNA-seq Library Prep Kit for Illumina (NR604-01/02) following the manufacturer's instruction. Generally, mRNA was purified from total RNA using poly-T oligo-attached magnetic beads. First strand cDNA was synthesized using random hexamer primer and RNase H. Second strand cDNA synthesis was subsequently performed using buffer, dNTPs, DNA polymerase I and RNase H. The double stranded cDNA was purified by AMPure P beads and was repaired at the end. Library examination was then conducted. RNA concentration was measured by Bio-RAD CFX 96 fluorescence quantitative PCR instrument using Bio-RAD KIT iQ SYBRGREN. Finally, cluster generation and sequencing were performed on the Illumina NovaSeq 6000 platform.

Immune cellular profiling of samples was performed using immune-deconvolution tools, including xCell, ESTIMATE, and single-sample Gene Set Enrichment Analysis (ssGSEA).[15–17] Pathways and biological functions were enriched using the Kyoto Encyclopedia of Genes and Genomes (KEGG) and Gene Set Variation Analysis (GSVA).

### Statistical analysis

All enrolled patients were included in the analysis, with the data collection cutoff date set to March 12, 2024.

Continuous variables were summarised as medians with interquartile ranges (IQRs), and categorical variables were reported as counts and frequencies (n%). The Mann–Whitney U test was used for continuous variables, and the one-tailed Fisher's exact test was used for categorical variables, with reporting the *P* value as double the exact one-tailed probability. The median follow-up period was calculated using the reverse Kaplan–Meier method. Kaplan–Meier curves were generated for survival analysis using log-rank statistics for comparison. Stepwise backward selection was employed for cohort stratification to build multivariable Cox regression models. Hazard ratios (HRs) and 95% confidence intervals (CIs) were calculated using univariate and multivariate Cox proportional hazard models. The effects of tumour response on survival outcomes and recurrence patterns, the relationship between irAE status and efficacy, and potential predictive biomarkers for the development of irAEs were explored using post-hoc analyses. All statistical analyses were performed using R software 4.3.0 (R Core Team, 2023). Statistical significance was set at $P < 0.05$ (two-sided). The trial was registered at ClinicalTrials.gov (NCT04005170).

### Role of the funding source

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. Dr. Mian Xi had full access to all data in the study and had final responsibility for the decision to submit for publication.

## Results

### Clinicopathologic characteristics and survival

Between November 12, 2019, and January 25, 2021, 42 enrolled patients with locally advanced ESCC received toripalimab in combination with definitive CRT (Supplementary Fig. S1). In this phase II trial, 26 (62%; 95% CI 46–76) of 42 patients achieved a CR as previously described.[8] Patient characteristics at baseline of the CR and non-CR groups are presented in Table 1. Earlier cTNM stage ($P = 0.00034$), N0-2 stage ($P = 0.017$), and shorter primary tumour length ($P = 0.026$) were significantly associated with an increased likelihood of achieving a CR.

With a median follow-up of 44.3 months (IQR 40.8–46.1), 23 (55%) of 42 patients experienced recurrences, and 23 (55%) died in the entire cohort. The 3-year OS rate was 44.8% (95% CI 31.9–62.8), with a median OS of 26.9 months (95% CI 8.7–45.2). Median PFS was 12.2 months (95% CI 8.3–16.1), with a 3-year PFS rate of 35.7% (95% CI 23.8–53.6). Additionally, the 3-year locoregional failure-free survival (LRFFS) and 3-year distant metastasis-free survival (DMFS) were 46.3% (95% CI 32.9–65.1) and 67.8% (95% CI 54.1–85.0), respectively (Fig. 1).

| Characteristic | Overall (n = 42), % | CR (n = 26), % | Non-CR (n = 16), % | *P* |
|---|---|---|---|---|
| Age (year) | | | | 0.70 |
| <56 | 16 (38) | 11 (42) | 5 (31) | |
| ≥56 | 26 (62) | 15 (58) | 11 (69) | |
| Sex | | | | 0.33 |
| Male | 32 (76) | 18 (69) | 14 (88) | |
| Female | 10 (24) | 8 (31) | 2 (13) | |
| ECOG-PS | | | | 0.097 |
| 0 | 31 (74) | 22 (85) | 9 (56) | |
| 1–2 | 11 (26) | 4 (15) | 7 (44) | |
| Weight loss | | | | 1.00 |
| <10% | 37 (88) | 23 (89) | 14 (88) | |
| ≥10% | 5 (12) | 3 (12) | 2 (13) | |
| Tumour location | | | | 1.00 |
| Upper | 21 (50) | 13 (50) | 8 (50) | |
| Middle | 15 (36) | 9 (35) | 6 (38) | |
| Distal | 6 (14) | 4 (15) | 2 (13) | |
| Tumour length (cm) | | | | 0.026 |
| ≤5 | 26 (62) | 20 (77) | 6 (38) | |
| >5 | 16 (38) | 6 (23) | 10 (63) | |
| Clinical T stage | | | | 0.075 |
| T1–T2 | 10 (24) | 9 (35) | 1 (6) | |
| T3–T4 | 32 (76) | 17 (65) | 15 (94) | |
| Clinical N stage | | | | 0.017 |
| N0–N2 | 31 (74) | 23 (89) | 8 (50) | |
| N3 | 11 (26) | 3 (12) | 8 (50) | |
| Clinical TNM stage | | | | 0.00034 |
| I–III | 26 (62) | 22 (85) | 4 (25) | |
| IVA | 16 (38) | 4 (15) | 12 (75) | |
| PD-L1 status | | | | 0.52 |
| CPS < 10 | 29 (69) | 16 (62) | 13 (81) | |
| CPS ≥ 10 | 11 (26) | 8 (31) | 3 (19) | |
| Unknown | 2 (5) | 2 (8) | 0 | |

IQR, interquartile range; ECOG-PS, Eastern Cooperative Oncology Group performance status; PD-L1, programmed death-ligand 1; CPS, combined positivity score; CR, complete response.

***Table 1:*** **Patient demographics and clinical characteristics.**

Regarding the effect of tumour response on long-term survival as a post-hoc analysis, patients achieving a CR had superior OS (HR = 13.73, 95% CI 4.43–42.54, $P < 0.0001$), PFS (HR = 32.08, 95% CI 8.57–120.10, $P < 0.0001$), LRFFS (HR = 43.60, 95% CI 8.89–214.40, $P < 0.0001$), and DMFS (HR = 5.35, 95% CI 1.52–18.81, $P = 0.0038$) than those without achieving a CR (Fig. 2).

### Prognostic factors for survival

Univariate and multivariate analyses of prognostic factors identified that tumour response (non-CR vs. CR) was the only independent prognostic indicator for OS (HR = 9.90, 95% CI 2.56–38.25, $P = 0.00089$). Similarly, tumour response was also the only independent

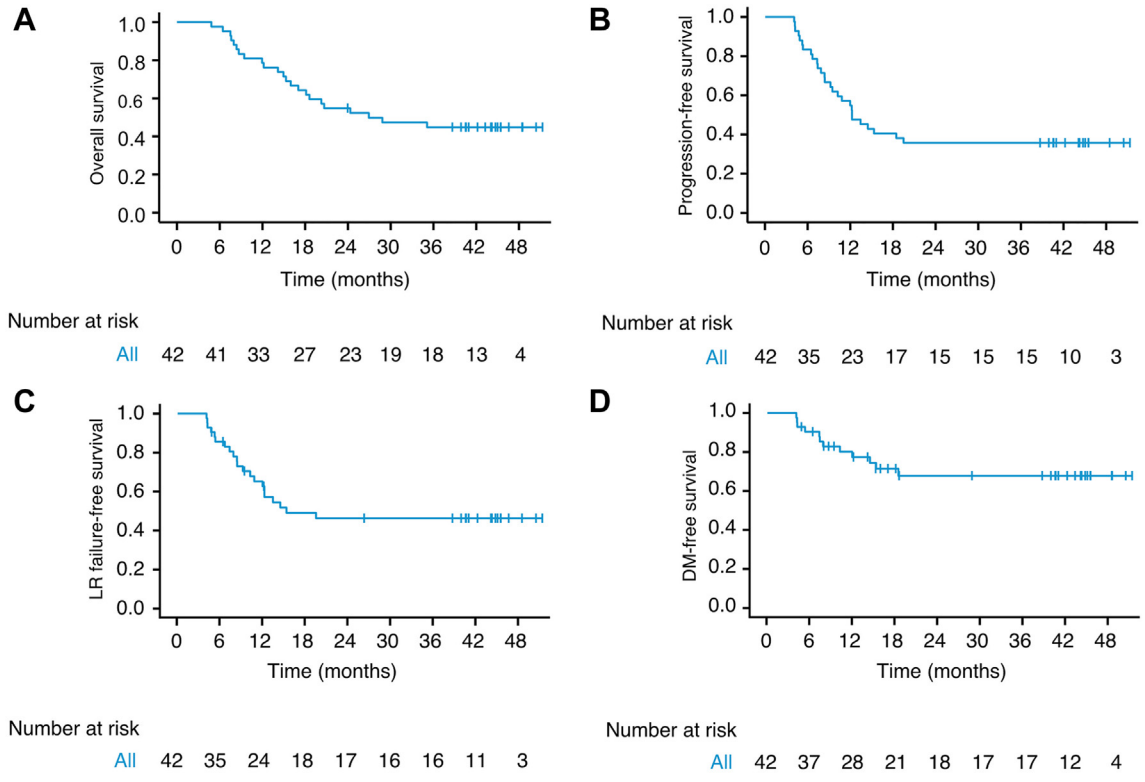


***Fig. 1:*** Overall survival (A), progression-free survival (B), locoregional failure-free survival (C), and distant metastasis-free survival (D) of the whole cohort.

prognostic factor for PFS (HR = 24.85, 95% CI 5.69–108.47, $P < 0.0001$; Supplementary Table S1).

### Recurrence pattern, timing, and site

Among the entire cohort of 42 patients, 11 (26%) had LRR, 2 (4.8%) experienced distant metastasis, and 10 (24%) experienced concurrent LRR and distant failure. Of 21 patients with LRRs in the cohort, in-field failure occurred in 20 patients and out-of-field failure occurred in 1 patient only. As expected, a lower LRR rate (31% [8 of 26] vs. 81% [13 of 16], $P = 0.0036$) was observed in the CR group than in the non-CR group; however, this difference was not statistically significant for distant recurrence (19% [5 of 26] vs. 44% [7 of 16], $P = 0.16$).

For patients experiencing recurrences, the median time to the first recurrence was 8.4 months (IQR 5.3–12.2). The median time to recurrence for the non-CR group (6.6 months, IQR 4.7–8.3) was significantly shorter than that for the CR group (12.8 months, IQR 11.1–15.1; $P < 0.0001$). The detailed timing and frequency of recurrences for the CR and non-CR groups are shown in Table 2. Notably, 100% (13 of 13) of the recurrences occurred in the non-CR group compared to only 30% (3 of 10) in the CR group within 1 year of follow-up ($P = 0.00097$). Another 70% (7 of 10) of recurrences occurred in the second year of follow-up for the CR group.

Supplementary Table S2 summarises the site-specific disease recurrence in this cohort. The most common sites of recurrence were the oesophagus (14 of 42, 33%) and regional lymph nodes (14 of 42, 33%), followed by non-regional lymph nodes (10 of 42, 24%). Specifically, the non-CR group had higher oesophageal and lymph node recurrence rates. However, no significant differences were observed between CR and non-CR groups in hematogenous metastasis rates ($P = 0.44$).

### Salvage treatment

Among patients with recurrences (n = 23), three underwent salvage surgery, thirteen received systemic therapy, two received second-course radiotherapy, and five received supportive care only. Patients who underwent salvage treatment demonstrated improved OS after recurrence compared to those who received supportive care alone (HR = 0.26, 95% CI 0.08–0.81, $P = 0.013$; Supplementary Fig. S2). Of 13 patients who underwent systemic therapy after relapse, 10 received re-treatment with immunotherapy in combination with chemotherapy. Three of them demonstrated at least 1-year survival time after recurrence. Specifically, of 11 patients with LRRs only, 2 patients received successful salvage esophagectomy, 2 underwent salvage CRT, 5 received systemic treatment, and 2 received supportive care. The two patients who underwent salvage surgery remained disease-free for at least 1 year after recurrence.

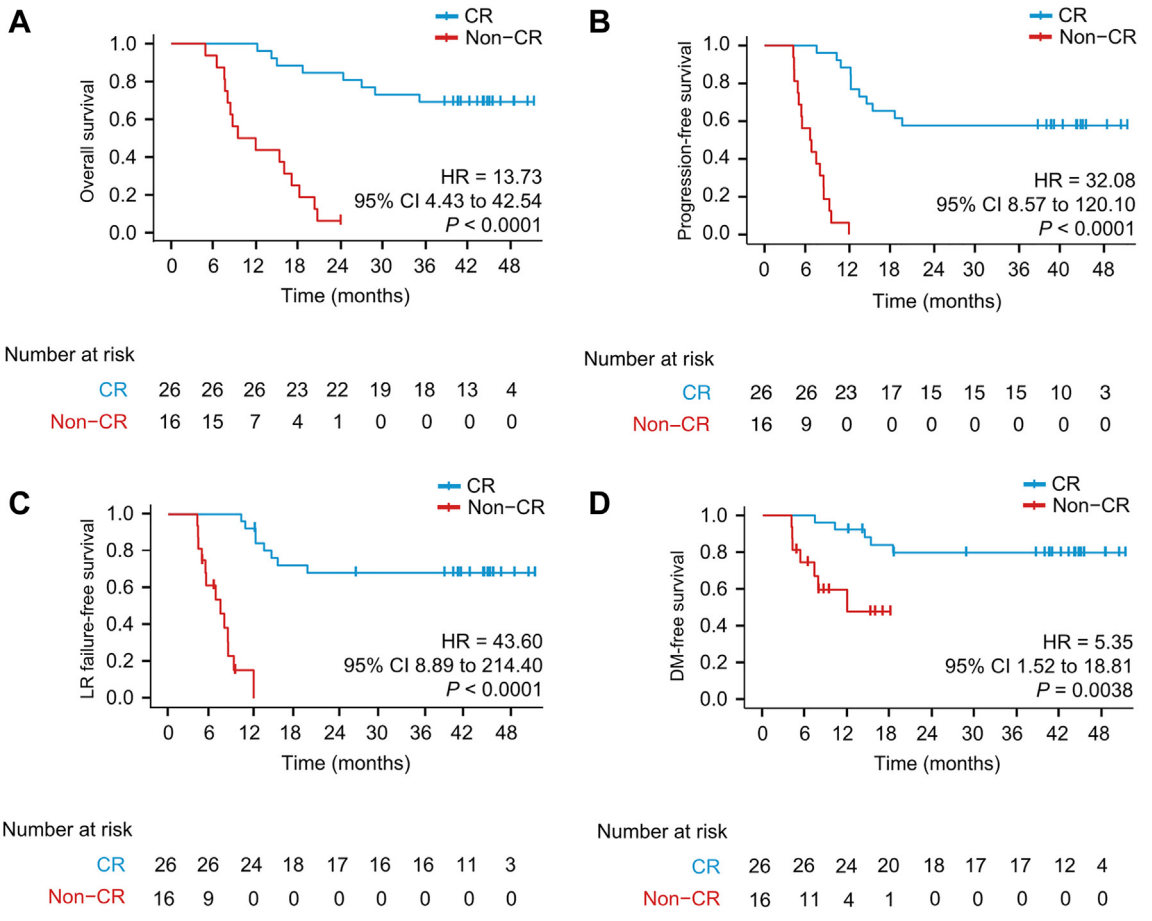


***Fig. 2:*** Overall survival (A), progression-free survival (B), locoregional failure-free survival (C), and distant metastasis-free survival (D) for CR vs. non-CR groups. CR, complete response.

### Tumour response and survival by irAE status

All patients experienced treatment-related AEs of any grade during treatment (Supplementary Table S3). Twenty-nine of 42 patients (69%) experienced any-grade irAEs (Table 3), with the most common being hypothyroidism (13 of 42, 31%), rash (12 of 42, 29%), and hyperlipidaemia (10 of 42, 24%). Grade 1–2 irAEs occurred in 67% of patients (28 of 42), whereas grade 3 events were observed in only one patient (1 of 42, 2%), with no occurrences of life-threatening irAEs. The clinical spectrum of irAEs across organ systems is described in Supplementary Table S4, involving skin, endocrine, neurologic, gastrointestinal tract, and others (e.g., fatigue). Nine of 42 patients (21%) experienced multi-organ irAEs, and 20 of 42 patients (48%) experienced single-organ irAEs.

The relationship between irAE status and clinical outcomes were analysed as an exploratory post-hoc analysis. The occurrence of irAEs positively correlated with treatment efficacy, including a higher CR rate (39% vs. 72%, $P = 0.082$) and better PFS (HR = 0.43, 95% CI 0.19–0.93, $P = 0.027$). Nevertheless, irAEs status did not significantly predict OS (HR = 0.59, 95% CI 0.25–1.40, $P = 0.23$) (Fig. 3A–C). No significant differences in OS or PFS were noted among patients who developed multi-organ, single-organ, or no irAE status (Supplementary Fig. S3).

| Category | Total | Recurrences | ≤6.0 months | 6.1–12.0 months | 12.1–18.0 months | 18.1–24.0 months | >24.0 months |
|---|---|---|---|---|---|---|---|
| | No. | No. (%) | No. (%) | No. (%) | No. (%) | No. (%) | No. (%) |
| Locoregional recurrences | | | | | | | |
| CR | 26 | 8 (31) | 0 | 2 (25) | 5 (63) | 1 (13) | 0 |
| Non-CR | 16 | 13 (81) | 6 (46) | 7 (54) | 0 | 0 | 0 |
| Distant recurrences | | | | | | | |
| CR | 26 | 5 (19) | 0 | 2 (40) | 2 (40) | 1 (20) | 0 |
| Non-CR | 16 | 7 (44) | 4 (57) | 3 (43) | 0 | 0 | 0 |
| Total recurrences | | | | | | | |
| CR | 26 | 10 (39) | 0 | 3 (30) | 5 (50) | 2 (20) | 0 |
| Non-CR | 16 | 13 (81) | 6 (46) | 7 (54) | 0 | 0 | 0 |

CR, complete response.

***Table 2:*** **Recurrence timing and frequency in CR group vs. non-CR group.**

### Integrated analysis of whole-exome and RNA sequencing with irAE status

A total of 4701 nonsynonymous somatic single nucleotide variants (SNVs) were identified in the 19 patients with available tumour samples for sequencing, with the most frequently mutated genes being *TP53* and *TTN*, observed in 89% (17 of 19) and 53% (10 of 19) of patients, respectively (Fig. 3D). Among these, the *GON4L* mutation, which ranked foremost for the disparity between patients with or without irAEs, appeared to deactivate the occurrence of irAEs (Fig. 3E). Furthermore, we found a positive association between high levels of immunological components, including CD8+ naive T-cells, class-switched memory B-cells, M2 macrophages, and wild-type *GON4L* in the post-hoc analysis (Supplementary Fig. S4A). Additionally, our study conducted exploratory univariable analyses of PFS and OS according to gene mutation prevalence, and the results indicated that patients with *TP53* mutations had a marginally prolonged OS compared to those with wild-type (Supplementary Table S5).

Although the difference in immune cellular fraction between patients with or without irAEs was somewhat diminished due to sample size limitations, a consistent trend persisted, with patients experiencing irAEs consistently demonstrating enhanced immune activation, including B cells, CD4+ T cells, and CD8+ T cells (Supplementary Fig. S4B and C). KEGG and GSVA functional classifications revealed enriched pathway patterns related to irAE status, such as the Wnt signalling pathway, Hedgehog signalling pathway, cell cycle, and tumour necrosis factor (TNF) targets (Supplementary Fig. S4D and E).

| Variable | No. (%) | Grade 1 | Grade 2 | Grade 3 | Grade 4 | Grade 5 |
|---|---|---|---|---|---|---|
| Any irAE | 29 (69) | 19 (45) | 9 (21) | 1 (2) | 0 | 0 |
| Hypothyroidism | 13 (31) | 12 (29) | 1 (2) | 0 | 0 | 0 |
| Rash | 12 (29) | 8 (19) | 4 (10) | 0 | 0 | 0 |
| Hyperlipidaemia | 10 (24) | 8 (19) | 1 (2) | 1 (2) | 0 | 0 |
| Fatigue | 6 (14) | 6 (14) | 0 | 0 | 0 | 0 |
| Oral mucositis | 5 (12) | 2 (5) | 3 (7) | 0 | 0 | 0 |
| Hyperthyroidism | 2 (5) | 2 (5) | 0 | 0 | 0 | 0 |
| Hyperglycaemia | 2 (5) | 2 (5) | 0 | 0 | 0 | 0 |
| Neurotoxicity | 2 (5) | 2 (5) | 0 | 0 | 0 | 0 |
| Adrenal insufficiency | 1 (2) | 1 (2) | 0 | 0 | 0 | 0 |
| Diarrhoea | 1 (2) | 0 | 1 (2) | 0 | 0 | 0 |

*Table 3:* **Immune-related adverse events (irAEs).**

## Discussion

In this phase II trial of locally advanced ESCC, with more than 3 years of follow-up, the combination of toripalimab and definitive CRT resulted in a long-term survival benefit. To the best of our knowledge, this study represents the first long-term analysis that confirms our previous findings and further highlights the role of ICIs when combined with traditional regimens. The study's strengths encompass detailed data on recurrence patterns, standardized documentation of irAEs, and insights into the potential biological modulation of irAEs.

Despite 38% (16 of 42) of patients enrolled had stage IVA disease in our study, the 3-year OS rate of 44.8% still exceeded those reported in previous studies involving definitive CRT in oesophageal cancer, such as RTOG 8501 (30%), PRODIGE5/ACCORD17 (27%), ARTDECO (39%), RTOG 9405 (40% for 2-year), RTOG 0436 (34%), and JCOG 0303 (26%) trials.[2,3,18–20] Although a prior report published the short-term clinical outcomes from this trial,[9] the current study extends these findings to reveal that achieving CR did translate into a long-term survival benefit. Our results showed prolonged OS and PFS in patients with CR, providing convincing evidence that the clinical benefits of toripalimab combined with CRT, in terms of short-term response, are maintained over a long follow-up period.

We observed a cumulative incidence of 50% (21 of 42) for LRR and 29% (12 of 42) for distant metastasis in this cohort. For comparison, a retrospective study involving 182 patients with stage I-III ESCC treated with definitive CRT reported an LRR rate of 43% (78 of 182) and a distant metastasis rate of 28% (50 of 182).[21] Overall, during the first 2-year follow-up period, 88% (160 of 182) of failures occurred in that study compared to a corresponding rate of 100% (23 of 23) in our trial. One possible explanation for this difference is the collective impact of PD-1 inhibitor resistance and the tumour immunosuppressive microenvironment, leading to accelerated failure of the initial therapy in a fraction of patients. Compared to the CR group, the occurrence of early recurrence (<12 months) served as a corollary for the non-CR group. Thus, close surveillance during the first year is crucial for patients who do not achieve CR, prompting clinicians to select timely salvage treatment. In contrast, the CR group still had a recurrence rate of 39%, and the majority of cases occurred in the second year of the follow-up period, suggesting that intensive follow-up within the first 2 years is necessary for patients with CR. However, intensive surveillance after 2 years may provide less value. Additionally, the moderate recurrence rate observed in the CR group implied the inaccuracy of conventional imaging-based assessment of tumour response. Liquid biopsy, especially for the detection of circulating tumour DNA, may be a promising supplementary approach for monitoring tumour response and microscopic progression in ESCC.[22,23]

Among patients who experienced disease relapse, our findings indicated that they might derive more benefit from adequate salvage treatment than supportive care alone. In this study, four patients attained promising long-term survival after salvage therapy, with three of them successfully undergoing salvage surgery. These

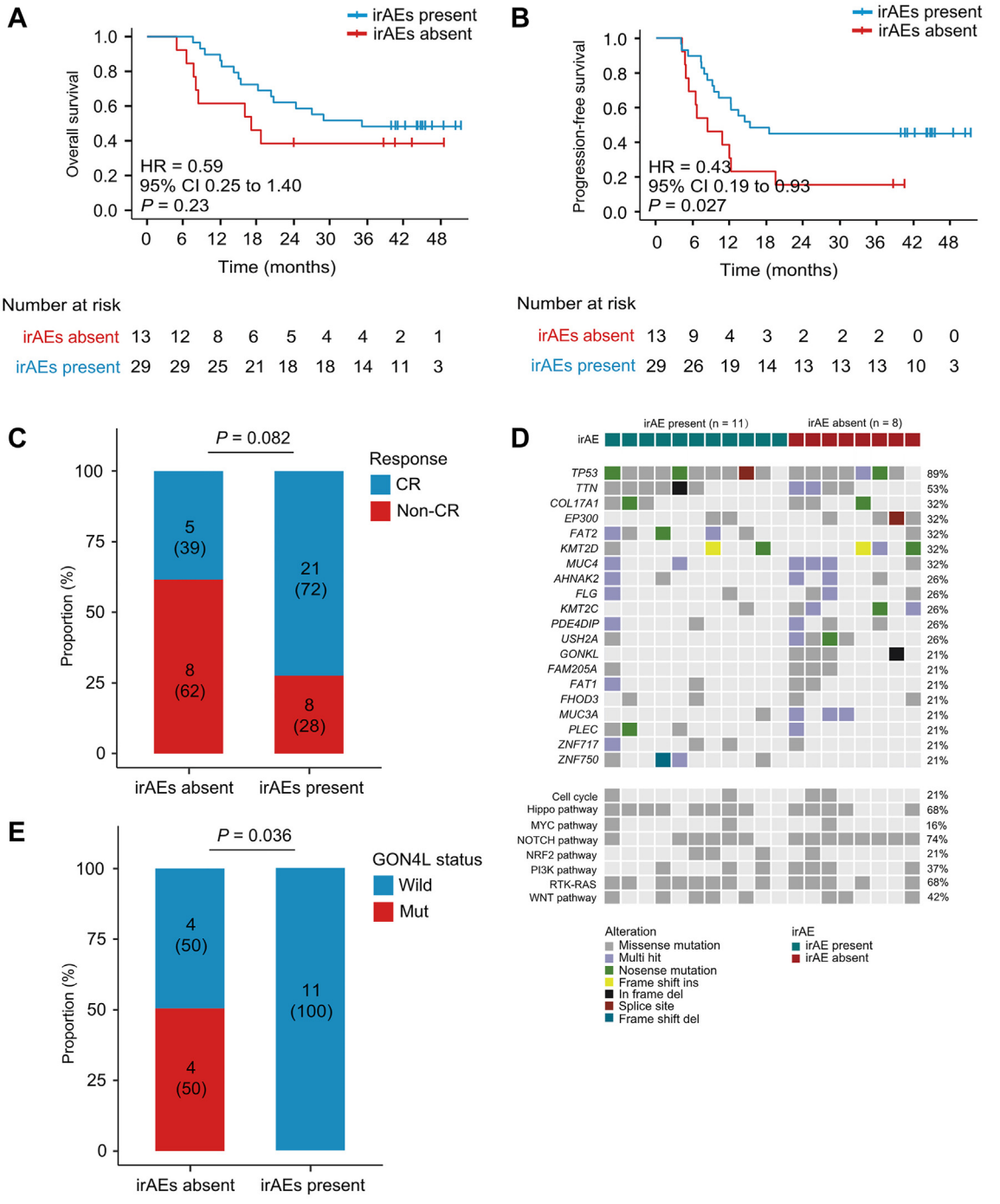


*Fig. 3:* Impact of irAE status on survival, tumour response, and genomic profiling. Overall survival (A), progression-free survival (B), and CR rates (C) among patients with or without irAEs. (D) Genomic landscape of 19 patients with available tumour tissue in the EC-CRT-001 cohort. Top track: irAE status per sample. Bottom heatmap: mutations included in cancer-related pathways. (E) CR rates among patients with different mutated-GON4L types. irAE, immune-related adverse event; CR, complete response.

results highlight the potential for active salvage treatment to translate into a survival benefit for patients with a stable performance status after careful restaging evaluation.

Our report supports the general assumption that the presence of irAEs is an early indicator of prolonged survival. Mild-to-moderate irAEs typically require close observation and supportive treatment.[24,25] The

documentation of irAEs in our trial emphasized the manageable safety profile of toripalimab, particularly as only one grade 3 irAE event existed. Previous literature suggests a long-term clinical benefit of multiorgan irAE occurrence compared to single-organ involvement.[14,26] However, due to the limited sample size in our study, no significant difference was observed. Another possible explanation is that the combination of immunotherapeutic agents with concurrent chemoradiotherapy conducted across the entire cohort in our study suggests that identifying the spectrum of immunotherapy toxicity may be more complex than that seen in other studies focusing solely on anti-PD-1 monotherapy. Given this complexity, investigators have theorized that standard assessment protocols may encounter challenges in recognizing all potential irAEs; thereby, limiting our exploratory analysis to specific symptoms typically associated with irAEs.

Ultimately, as the long-term survival of patients with ESCC who experience irAEs is promising, further investigation is required to identify predictive biomarkers that are enriched for irAE onset. This post-hoc exploratory subgroup analysis aimed to reveal potential modulatory effects using available genotyping and molecular phenotyping datasets. We observed the role of mutated-GON4L, previously reported as a cancer driver gene, in inhibiting the occurrence of irAEs.[27] In vitro studies have demonstrated that mutated-GON4L may result in the cessation of B lymphopoiesis and impaired proliferation.[28] Our findings also suggested an inherent association between GON4L alteration and a compromised immune microenvironment, such as CD8+ naive T-cells and class-switched memory B-cells, consistent with earlier research. Moreover, recent studies have suggested an increased risk of specific irAE onset in individuals with a pro-inflammatory microenvironment.[29,30] Although molecular omics data from our study presented a comparable trend, no significant association was established due to the limited sample size, thus highlighting the need for further large-scale studies to validate our findings.

This study had several limitations. Firstly, the single-centre and single-arm design of the trial, alongside the small cohort size, directly influenced the statistical power. Secondly, compared to ICI monotherapy administered in several previous studies, the combination regimens of immunotherapy and CRT in this study may have introduced complexities in accurately diagnosing irAEs. Finally, the analysis of the relationship between irAE severity and long-term survival was limited due to only one patient in our study experiencing a grade 3 irAE. Despite the above limitations, based on the encouraging long-term outcomes of this phase II trial, a randomized, controlled, phase III trial validating the efficacy of toripalimab plus definitive CRT for patients with locally advanced ESCC is necessary. Considering the significantly lower CR rate and worse survival of patients with stage IVA vs. stage I-III disease, the addition of induction immunochemotherapy prior to the current regimen might be a reasonable strategy for this setting, which is currently being investigated in a prospective randomized trial (NCT05520619). Moreover, the value of genomic alterations as biomarkers to predict patients who may benefit most from immunotherapy should be further investigated in the phase III study.

In conclusion, the updated 3-year survival outcomes from the EC-CRT-001 trial confirmed the efficacy of toripalimab plus definitive CRT in locally advanced ESCC. The development of irAEs might predict a more favourable prognosis. Additionally, considering the differences in recurrence patterns, timing, and frequency between the CR and non-CR groups, further investigation into individualized surveillance strategies is warranted.

#### Contributors

Conception and design: S.L.L., Q.Q.L., M.X.; Supply of study materials or patients: Y.H.L., B.Q.C., Y.J.Z, S.L.L., Q.Q.L., M.X.; Gathering and assembly of data: R.X.W., Y.H.L., B.Q.C., Y.H.H, M.Z.L, Y.J.Z., Y.D.Y, L.Z., Y.X.L., S.L.L. Q.Q.L., M.X.; Data analysis and interpretation: R.X.W., Y.H.L., B.Q.C., S.L.L., Q.Q.L., M.X.; Manuscript drafting and revision: all authors.

All authors had access to the data, participated in reviewing and editing of the manuscript, and approved the final version before submission. Three principal investigators (S.L.L., Q.Q.L., and M.X.) verified the raw data of the study and had final responsibility for the decision to submit for publication.

#### Data sharing statement

Data will be made available following publication upon reasonable request from Dr. Mian Xi via email.

#### Declaration of interests

All authors have no conflicts of interest to declare.

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#### Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.eclinm.2024.102806.

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