ESMO GOOD SCIENCE BETTER MEDICINE BEST PRACTICE

ESMO GASTROINTESTINAL ONCOLOGY

ORIGINAL ARTICLE

PD-L1 expression as a negative predictive biomarker in advanced esophageal squamous-cell cancer treated with chemotherapy alone

M. Sachdeva[1†], J. J. Zhao[1,2†], K. X. Zhu[3], D. T. W. Yap[2], N. Z. H. Wong[2], N. B. Kumarakulasinghe[2], J. Tey[4] & R. Sundar[1,2,5,6,7*]

[1]Department of Haematology-Oncology, National University Cancer Institute, National University Hospital, Singapore; [2]Yong Loo Lin School of Medicine, National University of Singapore, Singapore; [3]Department of Biomedical Informatics, Yong Loo Lin School of Medicine, National University of Singapore, Singapore; [4]Department of Radiation Oncology, National University Cancer Institute, National University Hospital, Singapore; [5]Cancer and Stem Cell Biology Program, Duke-NUS Medical School, Singapore; [6]The N.1 Institute for Health, National University of Singapore, Singapore; [7]Singapore Gastric Cancer Consortium, Singapore, Singapore

Available online 18 December 2024

Background: Programmed death-ligand 1 (PD-L1) expression is a well-established positive predictive biomarker for response to immunotherapy in advanced esophageal squamous-cell carcinoma (aESCC). However, the association between PD-L1 and response to chemotherapy alone remains unclear. This study aims to determine the prognostic significance of PD-L1 expression in patients treated with first-line chemotherapy alone in aESCC.

Materials and methods: First-line phase III randomized trials that included PD-L1 expression as a biomarker in aESCC were extracted after a systematic search. A graphical reconstructive algorithm was used to estimate time-to-event outcomes from reported Kaplan—Meier (KM) plots and, where unavailable, KMSubtraction was utilized to derive KM plots of unreported PD-L1 subgroups. Thereafter, an individual patient data meta-analysis was conducted. Survival analyses for overall survival (OS) and progression-free survival (PFS) were conducted with Cox proportional hazards models with a shared-frailty term incorporated to account for interstudy differences.

Results: Chemotherapy arms from five randomized phase III trials—CheckMate-648, ESCORT-1st, KEYNOTE-590, RATIONALE-306 and ORIENT-15—comprising 1517 patients were included in the OS analysis. Compared with PD-L1-low-expressing tumors, patients with PD-L1-high-expressing tumors were at a significantly higher risk of mortality [hazard ratio (HR) 1.153, 95% confidence interval (CI) 1.018-1.305, $P = 0.025$]. Three trials—CheckMate-648, ESCORT-1st and ORIENT-15—comprising 949 patients treated with chemotherapy alone were included in the PFS analysis. Patients with PD-L1-high-expressing tumors had a non-significant increased risk of tumor progression (HR 1.076, 95% CI 0.923 −1.253, $P = 0.349$).

Conclusions: Our study found PD-L1 expression is a negative predictor of OS in aESCC treated with first-line chemotherapy.

INTRODUCTION

Despite recent advances in modern medicine, the prognosis for advanced esophageal squamous-cell carcinoma (aESCC) remains poor.[1] Historically, ESCC was studied in trials alongside esophageal adenocarcinoma and gastric carcinoma, rather than being investigated as a separate disease subtype. However, with the advent of immunotherapy the treatment landscape has evolved significantly with landmark trials recruiting exclusively advanced ESCC patients. At the outset, immune checkpoint inhibitors (ICIs) targeting the programmed cell death protein 1 (PD-1)/programmed death-ligand 1 (PD-L1) were shown to improve survival compared with chemotherapy in the second-line metastatic ESCC setting, with KEYNOTE-181[2] leading to the approval of pembrolizumab in PD-L1 combined positive score (CPS) $\geq$ 10 and ATTRACTION-3[3] leading to the approval of nivolumab irrespective of PD-L1 status. Thereafter, ICIs were brought into the frontline setting for metastatic ESCC with the results of the landmark KEYNOTE-590 trial[4] leading to the approval of pembrolizumab in combination with chemotherapy, and CheckMate-648[5] resulting in the approval of nivolumab in combination with chemotherapy. Immunotherapy is now the standard of care in first-line ESCC with many studies demonstrating that ESCC with higher levels of PD-L1 expression tend to derive greater

*Correspondence to: Prof. Raghav Sundar, Department of Haematology-Oncology, National University Cancer Institute, National University Hospital, Singapore; Cancer and Stem Cell Biology Program, Duke-NUS Medical School, Singapore; The N.1 Institute for Health, National University of Singapore, Singapore; Singapore Gastric Cancer Consortium, Yong Loo Lin School of Medicine, National University of Singapore, Singapore, 1E Kent Ridge Road, Singapore 119228, Singapore. Tel: +65-67795555

E-mail: raghav.sundar@yale.edu (R. Sundar).

†Equal contributions.

benefit from treatment with anti-PD-1 blockade.[6] Thus, evaluation of PD-L1 expression via immunohistochemistry (IHC) has become an essential aspect to determining optimal treatment for patients with metastatic ESCC. However, different IHC PD-L1 antibody assays (e.g. Dako 22C3, Dako 28-8 and Ventana SP263)[7] were used in different clinical trials, with uncertainty regarding the concordance of CPSs among different PD-L1 assays. Moreover, while CheckMate-648 demonstrated OS benefit in the entire population and in those with PD-L1 tumor proportion score (TPS) ≥ 1, whether the addition of immunotherapy to chemotherapy benefits individuals with low PD-L1 expression remains a controversial issue. This led to different regulatory approvals, with the United States Food and Drug Administration (FDA) approving nivolumab regardless of PD-L1 expression[8] and the European Medicines Agency restricting approval of nivolumab to PD-L1 expression with TPS ≥ 1.[9]

PD-L1 expression is a well-established positive predictive biomarker for response to immunotherapy in aESCC. However, the association between PD-L1 and response to chemotherapy alone, without the concurrent use of immunotherapy, remains unclear. Intuitively, given that PD-L1 was developed as a biomarker to select patients deriving most benefit from immunotherapy, one would not expect it to affect response to chemotherapy. However, this has not been studied before and hence we seek to provide a more nuanced exploration of PD-L1 as a biomarker, as its significance in the context of traditional chemotherapy remains unknown. This study aims to determine the prognostic significance of PD-L1 expression in patients treated with first-line chemotherapy alone in aESCC.

MATERIALS AND METHODS

This study was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses and Cochrane Guidelines (PRISMA) for individual patient data.[10]

Study selection

An electronic literature search was conducted on PubMed for randomized controlled trials (RCTs) from inception until 24 September 2022. Eligibility criteria included the following: (i) phase III RCTs, which included PD-L1 expression as a biomarker and reported the use of chemotherapy alone or in combination with immunotherapy regimens for the first-line treatment of aESCC, (ii) trials with manuscripts published in peer-reviewed journals, (iii) trials that reported PD-L1-stratified Kaplan–Meier (KM) curves of chemotherapy arms. All relevant publications and the data supplements will be screened to capture any KM plots, which might have been published in abstract form, but subsequently removed from the primary trial manuscript. If multiple publications of the same trial were found, the latest and most complete publication as of 24 September 2022 will be used. We excluded trials with data only presented in conference abstract form. The full search string is detailed in Table 1. Articles were reviewed by JJZ and DTWY and any differences were resolved by MS.

Reconstruction of time-to-event outcomes

A graphical reconstructive algorithm was used to estimate time-to-event outcomes from reported KM plots in all overall and reported subgroup cohorts. Where unavailable, KMSubtraction was utilized to derive KM plots of unreported PD-L1 subgroups.[11] Thereafter, an individual patient data meta-analysis was conducted. Survival analyses for overall survival (OS) and progression-free survival (PFS) were conducted with Cox proportional hazards models with a shared-frailty term incorporated to account for interstudy differences, with OS as our primary endpoint and PFS as our secondary endpoint. As part of our sensitivity analysis, we employed stratified Cox models. These models account for differences between studies by allowing patients within each study to have a unique baseline hazard specific to that study while constraining partial likelihood estimates of the Cox coefficients to be equal across strata.[12] $\tau 2$ values were retrieved using the DerSimonian-Laird estimation to evaluate for between-study heterogeneity.[13]

Matching of patients and derivation of unreported subgroups—KMSubtraction

All analyses were conducted in R-4.1.0 using packages KMSubtraction, IPDfromKM, MatchIt, RcppHungarian, blandr, powerSurvEpi, survRM2 and survival, and a two-sided $P < 0.05$ was regarded to indicate statistical significance.

RESULTS

Study selection

The electronic search identified 279 articles. After deduplication and screening, five studies comprising 1517 patients were eligible and included in the final analysis (Figure 1).

Baseline characteristics of trials included

Five trials, CheckMate-648,[5] ESCORT-1st,[14] KEYNOTE-590,[4] RATIONALE-306[15] and ORIENT-15,[16] were included in this analysis. The characteristics of the five studies are summarized in Table 2. Importantly, all trials were conducted in the first-line metastatic or unresectable esophageal cancer setting, with all specifically focused on squamous-cell histology apart from KEYNOTE-590,[4] which recruited both adenocarcinoma and squamous histology. We only analyzed the data for squamous histology from KEYNOTE-590. RATIONALE-306,[15] ORIENT-15[16] and ESCORT-1st[14] predominantly recruited patients from China while KEYNOTE-590[4] and CheckMate-648[5] recruited a more diverse patient population. All trials compared chemotherapy as the standard arm versus chemoimmunotherapy as the intervention

Table 1. Search details

Date of search	24 September 2022
Databases	PubMed
Search string	(esophageal) AND (checkpoint inhibitors OR ICI OR pembrolizumab OR ipilimumab OR nivolumab OR avelumab OR camrelizumab OR durvalumab OR sintilimab OR programmed death ligand OR "PD-L1" OR "PD-1" OR immunotherapy OR Tislelizumab) AND (random* AND trial)
Search string exploded	("esophageal"[All Fields] OR "esophagic"[All Fields] OR "esophagitis"[MeSH Terms] OR "esophagitis"[All Fields] OR "esophagitides"[All Fields] OR "oesophagal"[All Fields] OR "oesophageal"[All Fields] OR "oesophagic"[All Fields] OR "oesophagitis"[All Fields]) AND ((("cell cycle checkpoints"[MeSH Terms] OR ("cell"[All Fields] AND "cycle"[All Fields] AND "checkpoints"[All Fields]) OR "cell cycle checkpoints"[All Fields] OR "checkpoint"[All Fields] OR "checkpoints"[All Fields]) AND ("antagonists and inhibitors"[MeSH Subheading] OR ("antagonists"[All Fields] AND "inhibitors"[All Fields]) OR "antagonists and inhibitors"[All Fields] OR "inhibitors"[All Fields] OR "inhibitor"[All Fields] OR "inhibitor s"[All Fields])) OR "ICI"[All Fields] OR ("pembrolizumab"[Supplementary Concept] OR "pembrolizumab"[All Fields]) OR ("ipilimumab"[MeSH Terms] OR "ipilimumab"[All Fields]) OR ("nivolumab"[MeSH Terms] OR "nivolumab"[All Fields] OR "nivolumab s"[All Fields]) OR ("avelumab"[Supplementary Concept] OR "avelumab"[All Fields]) OR ("camrelizumab"[Supplementary Concept] OR "camrelizumab"[All Fields]) OR ("durvalumab"[Supplementary Concept] OR "durvalumab"[All Fields]) OR ("sintilimab"[Supplementary Concept] OR "sintilimab"[All Fields]) OR (("program"[All Fields] OR "program s"[All Fields] OR "programe"[All Fields] OR "programmed"[All Fields] OR "programes"[All Fields] OR "programing"[All Fields] OR "programmability"[All Fields] OR "programmable"[All Fields] OR "programmably"[All Fields] OR "programme"[All Fields] OR "programme s"[All Fields] OR "programmed"[All Fields] OR "programmer"[All Fields] OR "programmer s"[All Fields] OR "programmers"[All Fields] OR "programmes"[All Fields] OR "programming"[All Fields] OR "programmings"[All Fields] OR "programs"[All Fields]) AND ("death"[MeSH Terms] OR "death"[All Fields] OR "deaths"[All Fields]) AND ("ligand s"[All Fields] OR "liganded"[All Fields] OR "liganding"[All Fields] OR "ligands"[MeSH Terms] OR "ligands"[All Fields] OR "ligand"[All Fields])) OR "PD-L1"[All Fields] OR "PD-1"[All Fields] OR ("immunotherapy"[MeSH Terms] OR "immunotherapy"[All Fields] OR "immunotherapies"[All Fields] OR "immunotherapy s"[All Fields]) OR ("tislelizumab"[Supplementary Concept] OR "tislelizumab"[All Fields])) AND ("random*"[All Fields] AND ("clinical trials as topic"[MeSH Terms] OR ("clinical"[All Fields] AND "trials"[All Fields] AND "topic"[All Fields]) OR "clinical trials as topic"[All Fields] OR "trial"[All Fields] OR "trial s"[All Fields] OR "trialed"[All Fields] OR "trialing"[All Fields] OR "trials"[All Fields])) Translations esophageal: "esophageal"[All Fields] OR "esophagic"[All Fields] OR "esophagitis"[MeSH Terms] OR "esophagitis"[All Fields] OR "esophagitides"[All Fields] OR "oesophagal"[All Fields] OR "oesophageal"[All Fields] OR "oesophagic"[All Fields] OR "oesophagitis"[All Fields] checkpoint: "cell cycle checkpoints"[MeSH Terms] OR ("cell"[All Fields] AND "cycle"[All Fields] AND "checkpoints"[All Fields]) OR "cell cycle checkpoints"[All Fields] OR "checkpoint"[All Fields] OR "checkpoints"[All Fields] inhibitors: "antagonists and inhibitors"[Subheading] OR ("antagonists"[All Fields] AND "inhibitors"[All Fields]) OR "antagonists and inhibitors"[All Fields] OR "inhibitors"[All Fields] OR "inhibitor"[All Fields] OR "inhibitor's"[All Fields] pembrolizumab: "pembrolizumab"[Supplementary Concept] OR "pembrolizumab"[All Fields] ipilimumab: "ipilimumab"[MeSH Terms] OR "ipilimumab"[All Fields] nivolumab: "nivolumab"[MeSH Terms] OR "nivolumab"[All Fields] OR "nivolumab's"[All Fields] avelumab: "avelumab"[Supplementary Concept] OR "avelumab"[All Fields] camrelizumab: "camrelizumab"[Supplementary Concept] OR "camrelizumab"[All Fields] durvalumab: "durvalumab"[Supplementary Concept] OR "durvalumab"[All Fields] sintilimab: "sintilimab"[Supplementary Concept] OR "sintilimab"[All Fields] programmed: "program"[All Fields] OR "program's"[All Fields] OR "programe"[All Fields] OR "programmed"[All Fields] OR "programes"[All Fields] OR "programing"[All Fields] OR "programmability"[All Fields] OR "programmable"[All Fields] OR "programmably"[All Fields] OR "programme"[All Fields] OR "programme's"[All Fields] OR "programmed"[All Fields] OR "programmer"[All Fields] OR "programmer's"[All Fields] OR "programmers"[All Fields] OR "programmes"[All Fields] OR "programming"[All Fields] OR "programmings"[All Fields] OR "programs"[All Fields] death: "death"[MeSH Terms] OR "death"[All Fields] OR "deaths"[All Fields] ligand: "ligand's"[All Fields] OR "liganded"[All Fields] OR "liganding"[All Fields] OR "ligands"[MeSH Terms] OR "ligands"[All Fields] OR "ligand"[All Fields] immunotherapy: "immunotherapy"[MeSH Terms] OR "immunotherapy"[All Fields] OR "immunotherapies"[All Fields] OR "immunotherapy's"[All Fields] Tislelizumab: "tislelizumab"[Supplementary Concept] OR "tislelizumab"[All Fields] trial: "clinical trials as topic"[MeSH Terms] OR ("clinical"[All Fields] AND "trials"[All Fields] AND "topic"[All Fields]) OR "clinical trials as topic"[All Fields] OR "trial"[All Fields] OR "trial's"[All Fields] OR "trialed"[All Fields] OR "trialing"[All Fields] OR "trials"[All Fields]

arm; CheckMate-648[5] had an additional dual immunotherapy intervention arm. PD-L1-low was defined as: TPS $<$ 1 for CheckMate-648 and ESCORT-first; TPS $<$ 10 for RATIONALE-306; and CPS $<$ 10 for KEYNOTE-590 and ORIENT-15. We used different thresholds as the different trials reported PD-L1-stratified KM curves of chemotherapy arms with different PD-L1 cut-offs. We also assessed publication bias qualitatively through visual inspection of funnel plot asymmetry which were both grossly symmetrical, as shown in Supplementary Figure S1, available at https://doi.org/10.1016/j.esmogo.2024.100109. Egger's test was not conducted as the number of studies included was $<$10.

OS

Chemotherapy arms from five randomized phase III trials comprising 1517 patients were included in the OS analysis. Compared with PD-L1-low-expressing tumors, patients with PD-L1-high-expressing tumors were at a significantly higher risk of mortality [PD-L1-high (n = 769) versus PD-L1-low (n = 748), HR 1.153, 95% CI 1.018-1.305, P = 0.025; stratified HR 1.158, 95% CI 1.021-1.313, P = 0.020; $\tau 2$ = 0.0350] (Figure 2). This result was consistent in all of the included RCTs when analyzed individually (Supplementary Figures S2-S6, available at https://doi.org/10.1016/j.esmogo.2024.100109), with the exception of ORIENT-15,

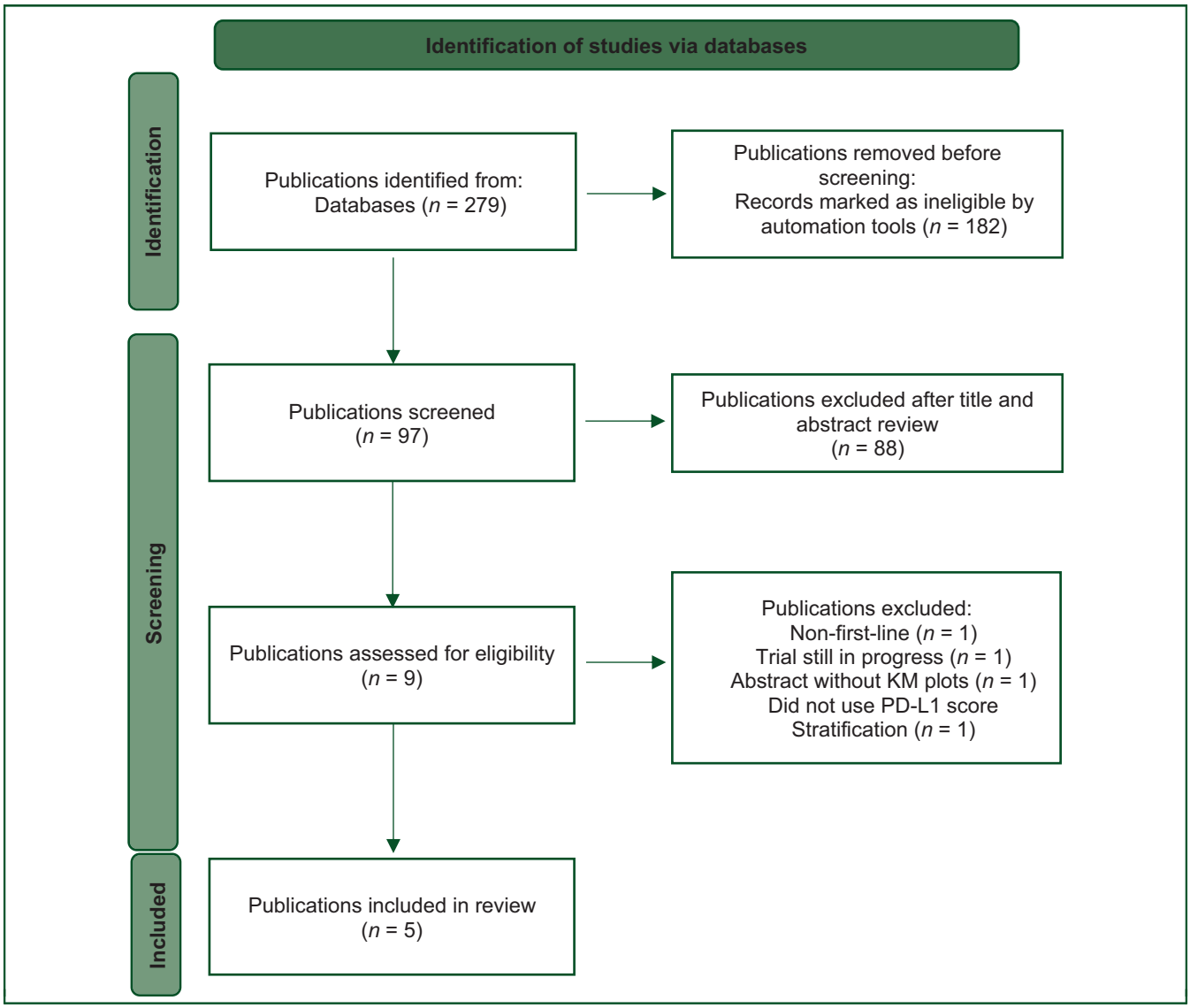

Figure 1. PRISMA flowchart.
KM, Kaplan–Meier; PD-L1, programmed death-ligand 1.

which found that PD-L1-low patients had a non-significant increase in the risk of mortality (HR 1.251, 95% CI 0.946−1.653, $P = 0.115$). Cohort analyses of the three trials that reported PD-L1 measurement as TPS (CheckMate-648, ESCORT-1st and RATIONALE-306) reiterated these findings [PD-L1-high ($n = 433$) versus PD-L1-low ($n = 478$), HR 1.243, 95% CI 1.057-1.462, $P = 0.009$] with PD-L1 TPS-high-expressing tumors at significantly higher risk of mortality. However, cohort analyses of the studies that reported PD-L1 measurement as CPS (KEYNOTE-590 and ORIENT-158) did not find an increased risk of mortality in PD-L1-CPS-high tumors [PD-L1-high ($n = 336$) versus PD-L1-low ($n = 270$), HR 1.037, 95% CI 0.855-1.258, $P = 0.711$].

PFS

Three trials—CheckMate-648,[5] ESCORT-1st[14] and ORIENT-15.[16]—comprising 949 patients treated with chemotherapy alone were included in the PFS analysis. KEYNOTE-590 and RATIONALE-306 did not report PD-L1 subgroup analysis for PFS and hence were not included in our analysis. Patients with PD-L1-high-expressing tumors were at a higher risk of tumor progression, although this was not significant [PD-L1-high ($n = 513$) versus PD-L1-low ($n = 436$), HR 1.076, 95% CI 0.923-1.253, $P = 0.349$; stratified HR 1.082, 95% CI 0.928-1.262, $P = 0.300$; $\tau 2 = 0.0455$] (Figure 3). Again, similar to the OS data, this result was consistent in all of the included RCTs when analyzed individually with the exception of ORIENT-15 (Supplementary Figures S7-S9, available at https://doi.org/10.1016/j.esmogo.2024.100109), which found that PD-L1-low patients had a non-significant increase in the risk of tumor progression (HR 1.200, 95% CI 0.932-1.547, $P = 0.157$).

DISCUSSION

Our study found PD-L1 expression is a negative predictor of OS in aESCC treated with first-line chemotherapy. There are

Table 2. Study information and characteristics of trials

Trial, year, ClinicalTrials.gov identifier	No. of countries	Main eligibility criteria	Intervention groups, dosing	PD-L1 assay and reporting method	Reported outcomes (as KM plots)	Derived outcomes of interest unreported as KM plots in the original study	No of patients enrolled
CheckMate-648 Doki et al. (2022)[5]	26 (North America, South America, Europe, Asia and Oceania)	1. Age $\geq$18 years 2. Previously untreated, histologically or cytologically confirmed, locally advanced, unresectable or metastatic esophageal squamous-cell carcinoma 3. ECOG PS 0-1 4. Measurable disease (RECIST v1.1) 5. Adequate organ function	Chemotherapy FU 800 mg/m^2 once daily (days 1-5) plus cisplatin 80 mg/m^2 once daily (day 1) once every 4 weeks versus Immunochemotherapy Nivolumab 240 mg once every 2 weeks plus chemotherapy (as above) versus Dual immunotherapy Nivolumab 3 mg/kg once every 2 weeks plus ipilimumab 1 mg/kg every 6 weeks	IHC 28-8 pharmDx assay (Dako, Santa Clara, CA)	OS 1. All patients 2. PD-L1 TPS $\geq$ 1 PFS 1. All patients 2. PD-L1 TPS $\geq$ 1	OS 1. Tumor PD-L1 TPS $<$ 1 PFS 2. Tumor PD-L1 TPS $<$ 1	970
KEYNOTE-590 Sun et al. (2021)[21]	26 (North America, South America, Europe, Asia and Oceania)	1. Age $\geq$18 years 2. Previously untreated, histologically or cytologically confirmed, locally advanced, unresectable or metastatic adenocarcinoma or squamous-cell carcinoma of the esophagus or Siewart type 1 gastroesophageal junction adenocarcinoma 3. ECOG PS 0-1 4. Measurable disease (RECIST v1.1) 5. Adequate organ function	Chemotherapy FU 800 mg/m^2 once daily (days 1-5) plus cisplatin 80 mg/m^2 once daily (day 1) once every 3 weeks versus Immunochemotherapy Pembrolizumab 200 mg once every 3 weeks plus chemotherapy (as above)	IHC 22C3 (Agilent Technologies, Carpinteria, CA)	OS 1. All patients 2. PD-L1 CPS $\geq$ 10 3. ESCC tumor type 4. ESCC PD-L1 CPS $\geq$ 10 PFS 1. All patients 2. PD-L1 CPS $\geq$ 10 3. ESCC tumor typePD-L1 data indeterminate, not evaluable or missing from 19 patients	OS 1. Tumor PD-L1 CPS $<$ 10	749
ESCORT-1st Luo et al. (2021)[14]	1 (China)	1. Age 18-75 years 2. Previously untreated, histologically or cytologically confirmed, locally advanced, unresectable or metastatic esophageal squamous-cell carcinoma 3. ECOG PS 0-1 4. Measurable disease (RECIST v1.1) 5. Adequate organ function 6. Newly obtained or archival tumor sample for PD-L1 analysis	Chemotherapy Paclitaxel 175 mg/m^2 plus cisplatin 75 mg/m^2 versus Immunochemotherapy Camrelizumab 200 mg once every 3 weeks plus chemotherapy (as above)	6E8 antibody (Shuwen Biotech, Deqing, Zhejiang, China)	OS 1. All patients 2. PD-L1 TPS $\geq$ 1 3. PD-L1 TPS $<$ 1 PFS 1. All patients 2. PD-L1 TPS $\geq$ 1 3. PD-L1 TPS $<$ 1	—	596

Continued

https://doi.org/10.1016/j.esmogo.2024.100109

Table 2. Continued

Trial, year, ClinicalTrials.gov identifier	No. of countries	Main eligibility criteria	Intervention groups, dosing	PD-L1 assay and reporting method	Reported outcomes (as KM plots)	Derived outcomes of interest unreported as KM plots in the original study	No of patients enrolled
ORIENT-15 Lu (2022)[16]	5 (Asia, Europe, North America and Oceania)	1. Age $\geq$18 years 2. Previously untreated, histologically or cytologically confirmed, locally advanced, unresectable or metastatic esophageal squamous-cell carcinoma 3. ECOG PS 0-1 4. Measurable disease (RECIST v1.1) 5. Adequate organ function 6. Newly obtained or archival tumor sample for PD-L1 analysis	Chemotherapy Investigators choice of cisplatin 75 mg/m^2 once daily (day 1) plus paclitaxel 175 mg/m^2 once daily (day 1) or FU 800 mg/m^2 once daily (days 1-5) versus Immunochemotherapy Sintilimab 3 mg/kg in patients weighing $<$60 kg or 200 mg in patients weighing $\geq$60 kg once every 3 weeks plus chemotherapy (as above)	IHC 22C3 (Agilent Technologies, Carpinteria, CA)	OS 1. All patients 2. PD-L1 CPS $\geq$ 10 PFS 1. All patients 2. PD-L1 CPS $\geq$ 10	OS 1. PD-L1 CPS $<$ 10PFS 1. PD-L1 CPS $<$ 10	659
RATIONALE-306 Xu et al. (2023)[15]	16 (Asia, Europe, North America and Oceania)	1. Age $\geq$18 years 2. Previously untreated, histologically or cytologically confirmed, locally advanced, unresectable or metastatic esophageal squamous-cell carcinoma 3. ECOG PS 0-1 4. Measurable disease (RECIST v1.1) 5. Adequate organ function 6. Newly obtained or archival tumor sample for PD-L1 analysis	Chemotherapy Investigators choice of cisplatin 60-80 mg/m^2 once daily (day 1) or oxaliplatin 130 mg/m^2 once daily (day 1) plus paclitaxel 175 mg/m^2 once daily (day 1) or FU 750-800 mg/m^2 once daily (days 1-5) or capecitabine 1000 mg/m^2 orally twice daily (days 1-14) versus Immunochemotherapy Tislelizumab 200 mg once every 3 weeks plus chemotherapy (as above)	IHC SP263 (VENTANA, Tucson, AZ)	OS 1. All patients 2. PD-L1 TPS $\geq$ 10 3. PD-L1 TPS $<$ 10 PFS 1. All patients	—	649

CPS, combined positive score; ECOG PS, Eastern Cooperative Oncology Group performance status; ESCC, esophageal squamous-cell carcinoma; FOLFOX, folinic acid, fluorouracil, and oxaliplatin; FU, fluorouracil; HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry; KM, Kaplan—Meier; OS, overall survival; PD-L1, programmed death-ligand 1; PFS, progression-free survival; TC, tumor cells; XELOX, capecitabine and oxaliplatin.

https://doi.org/10.1016/j.esmogo.2024.100109

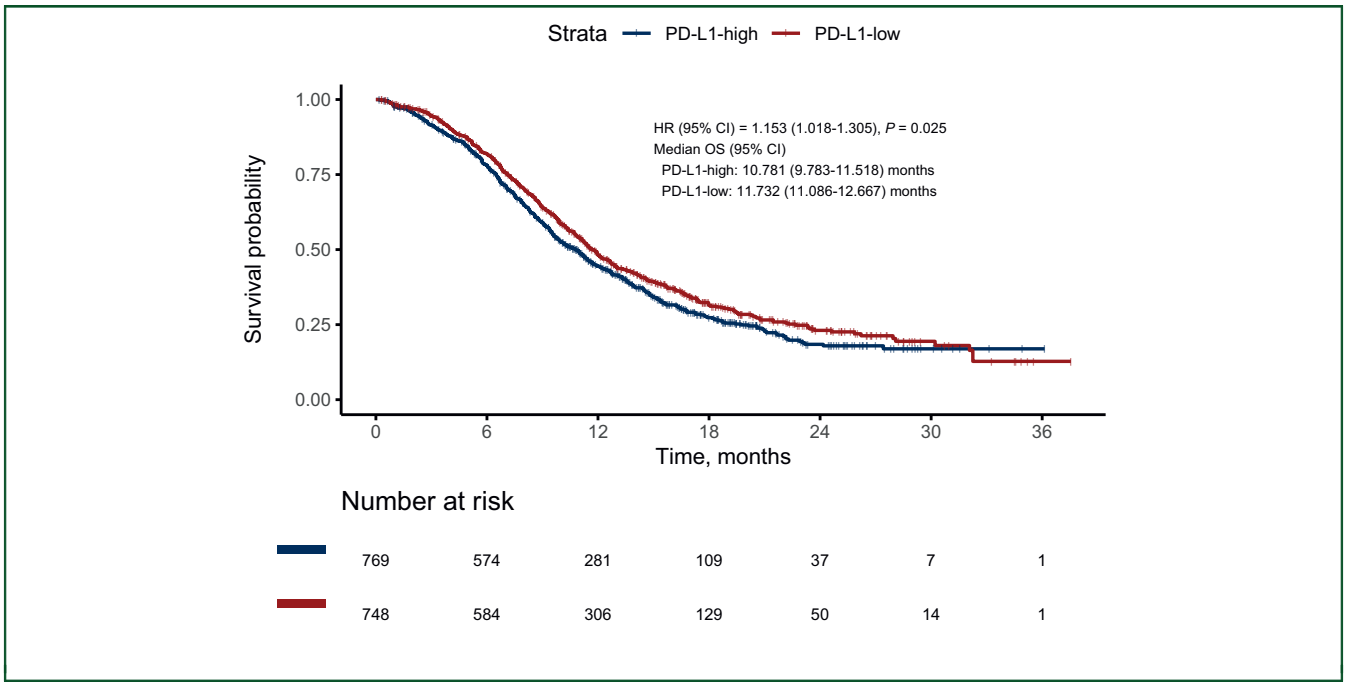

Figure 2. Kaplan–Meier curve for overall survival in PD-L1-high versus PD-L1-low subgroups derived with KMSubtraction: CheckMate-648, ESCORT-first, KEYNOTE-590, ORIENT-15, RATIONALE-306.
CI, confidence interval; OS, overall survival; PD-L1, programmed death-ligand 1.

several clinical implications of our study. Our findings strengthen the argument for combination chemo-immunotherapy in the first-line setting in patients with PD-L1-high status, as we have shown that with chemotherapy alone these patients have worse outcomes compared with patients with low PD-L1 status. Access to immunotherapy remains challenging in many parts of the world, and our findings highlight the importance of patients being able to receive immunotherapy in a timely fashion, especially so, for PD-L1-high status. For example, even within the United States, adoption of immunotherapy within 2 years after FDA approvals for new indications is significantly lower in rural, non-academic practices compared with urban, academic centers.[17] Moreover, this

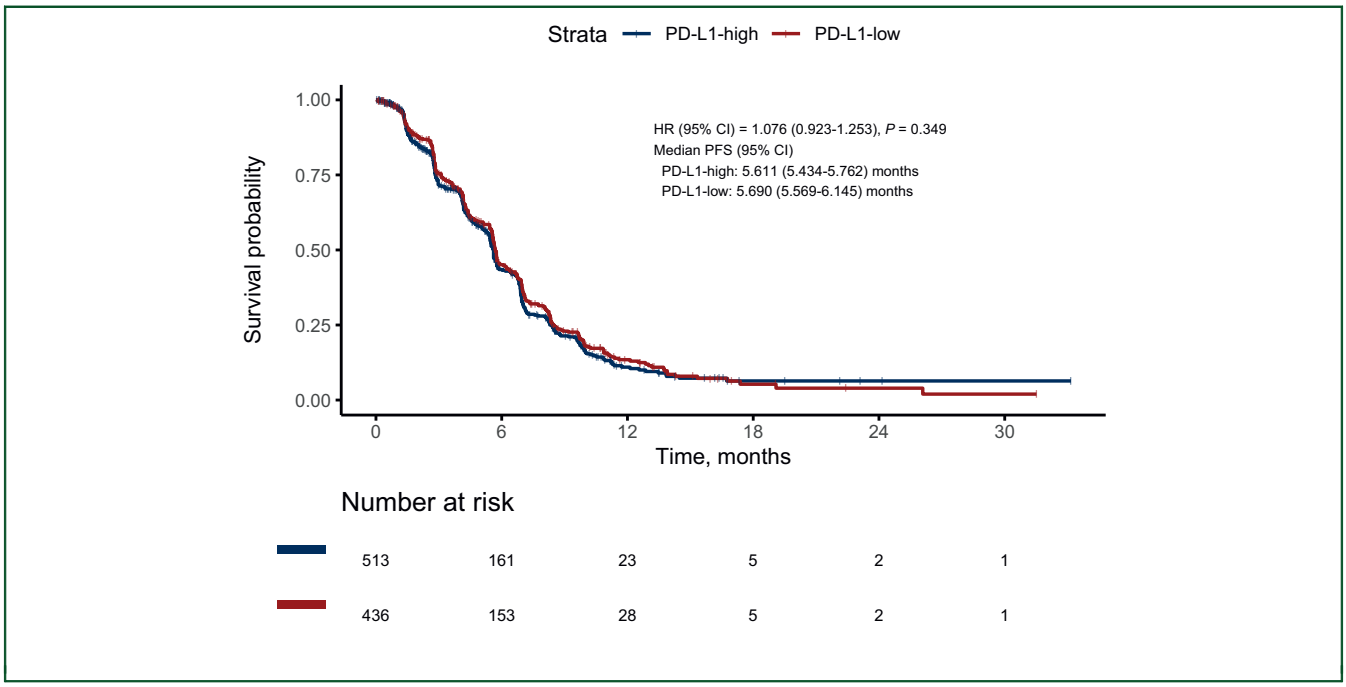

Figure 3. Kaplan–Meier curve for progression-free survival in PD-L1-high versus PD-L1-low subgroups derived with KMSubtraction: CheckMate-648, ESCORT-first, ORIENT-15.
CI, confidence interval; OS, overall survival; PD-L1, programmed death-ligand 1.

prognostic information can assist clinicians in counselling patients with known PD-L1 status who may have concerns about treatment toxicity or who may indeed develop low-grade immune-related adverse events, and hence opt to continue chemotherapy alone. Treatment-related adverse events caused by chemoimmunotherapy led to treatment discontinuation in 3%-15% of ESCC patients.[18] For example, in the ESCORT-1st trial[14] there was a higher rate of treatment discontinuation with camrelizumab-chemotherapy than with chemotherapy alone (45.3% versus 23.9%). Immune-related adverse events were also higher with chemoimmunotherapy compared with chemotherapy alone (84.6% versus 33%); however, severe immune-related adverse events remained rare. Hence, while it is important to counsel patients on ICI-related toxicity, it is also necessary to contextualize this information to ensure patients and clinicians understand that without ICI, PD-L1-high patients may have worse outcomes. Our findings further strengthen the argument for early and appropriate management of immune-related adverse events as we now demonstrate that without immunotherapy, PD-L1-high ESCC patients will have a poorer prognosis.

The observed association between PD-L1-high expression and an increased risk of mortality in aESCC patients undergoing chemotherapy alone adds a layer of complexity to the understanding of PD-L1 as a predictive biomarker. While PD-L1 has been extensively studied in the context of immunotherapy, our study underscores its potential relevance in the realm of traditional chemotherapy. This finding prompts a re-evaluation of the role of immune checkpoint molecules, such as PD-L1, beyond the scope of immunotherapeutic interventions. The inclusion of five randomized phase III trials in our analysis, encompassing a substantial cohort of 1517 patients, lends robustness to our results. While PD-L1-high expression was significantly associated with poorer OS on chemotherapy alone, our PFS results were not statistically significant (though trending towards poorer PFS as well). This phenomenon of discordance between PFS and OS is reported in many other trials, and may potentially indicate a link between higher PD-L1 expression and poorer post-progression survival.[19] Nonetheless, we would focus on our significant OS results as our gold-standard primary endpoint, as arguably PFS remains a poor surrogate for OS.[20]

While the exact mechanisms remain unclear, higher PD-L1 levels have been linked to poorer responses or survival following chemotherapy alone in prior studies. For example, a study using RNA sequencing data from The Cancer Genome Atlas along with a validation cohort of IHC tumor samples from the local population, also found that higher PD-L1 score correlated with poorer OS in a population treated with chemotherapy or radiotherapy alone.[21] Furthermore, in the locally advanced ESCC setting, patients who received neoadjuvant paclitaxel and cisplatin-based chemoradiotherapy in three prospective phase II trials were observed to have a poorer OS if PD-L1 expression on tumor cells was $\geq$1%.[22] Hence, our findings may extend across tumor stage, although small sample size in these studies remains a limitation.

The main limitations of the study include the retrospective nature of the analysis and heterogeneity across the included trials which may introduce confounding factors. The trials used different assays for determining PD-L1 status and used different thresholds for defining PD-L1-high expression. In particular, there are a lack of data regarding the unique antibody 6E8 used in the ESCORT-1st trial. While overall concordance rates of CPS scores of 22C3, SP263 and SP142 in a multicenter concordance study were reasonable with overall agreement rates of 0.78, 0.79 and 0.76, respectively,[23] they are still not considered completely interchangeable and guidelines for cross-platform and antibody validation are lacking. Additionally, the trials we have included in our study differ in how they measure PD-L1, with CheckMate-678,[5] ESCORT-1st[14] and RATIONALE-306[15] reporting PD-L1 as TPS while KEYNOTE-590[4] and ORIENT-15[16] used CPS, further complicating cross-study comparisons. Furthermore, our definition of PD-L1-low and -high had to use different thresholds for different trials as detailed in our results section, and hence collating results from different assays with different cut-offs may not be as robust.

Additionally, as described in Table 2, the various trials we included have differing characteristics, including study population and chemotherapy backbone. For example, ESCORT-1st and ORIENT-15 enrolled almost exclusively Chinese patients, while RATIONALE-306, CheckMate-658 and KEYNOTE-590 had a more diverse population enrolled. Of note, KEYNOTE-590, which enrolled an almost equal population from Asian and non-Asian regions, demonstrated more survival benefit from pembrolizumab in the Asian subgroup, though this is only hypothesis-generating subgroup analysis (HR of Asian versus non-Asian: 0.64 versus 0.83).[24] These differences may be related to the differing etiologies of ESCC between Asian and non-Asian populations (with pickled vegetables, hot drinks and betel nut as significant risk factors in Asia compared with alcohol and tobacco in Western countries[25]), as well as their distinct genetic features.[26] Chemotherapy backbones differed between our included studies, with KEYNOTE-590 and CheckMate-648 using a backbone of cisplatin and fluorouracil, ESCORT-1st using cisplatin and paclitaxel and ORIENT-15 and RATIONALE-306 allowing investigator's choice of chemotherapy (with options detailed in Table 2). There is retrospective evidence demonstrating that cisplatin and paclitaxel has superior PFS over cisplatin and 5-fluorouracil,[27] with paclitaxel able to promote greater synergy with ICI.[28] Hence, ESCORT-1st, which was the only trial to mandate use of this chemotherapy backbone, may be exerting a stronger influence on our overall results.

In conclusion, our study provides compelling evidence that PD-L1 expression serves as a negative predictor of OS in aESCC patients treated with first-line chemotherapy alone. This novel insight prompts a paradigm shift in considering the role of PD-L1 beyond immunotherapy, highlighting its potential as a biomarker in the context of

traditional chemotherapy too. It also strengthens the argument for patients with aESCC with PD-L1-high to have early access to combination chemotherapy and immunotherapy in the first-line setting, as they have poorer outcomes with chemotherapy alone.

FUNDING

This work was supported by the National University Health System Seed Fund [grant number NUHSRO/2024/008/RO5+6/Seed-Sep23/01 to JJZ]; National University Hospital Junior Research Award 2023 [grant number JRA/Sep23/002 to JJZ]; Dean's Research Development Award awarded by the Yong Loo Lin School of Medicine, National University of Singapore [no grant number, to JJZ]; the 2025 Conquer Cancer Merit Award [no grant number, to JJZ]; the Chan Heng Leong Education & Research Fund 2024 award by the National University Hospital Singapore [no grant number, to JJZ]; the National Medical Research Council (NMRC) [grant numbers NMRC/Fellowship/0059/2018 and NMRC/MOH/000627 to RS].

DISCLOSURE

RS has received honoraria from Astellas, Beigene, Daiichi-Sankyo, Ipsen, Bristol-Myers Squibb, Eli Lilly, Roche, Taiho, AstraZeneca, DKSH and MSD; has advisory activity with Astellas, Bristol-Myers Squibb, Beigene, Daiichi Sankyo, DKSH, Merck, Eisai, GSK, Pierre-Fabre, Sanofi, Bayer, Taiho, Novartis, Tavotek BioTherapeutics, MSD and AstraZeneca; received research funding from CytoMed, MSD, Natera, and Paxman Coolers; owns stock and has other ownership interests with Teladoc; patents with Auristone and Paxman Coolers; and has received travel grants from AstraZeneca, Cytomed, DKSH, Ipsen, Paxman Coolers, Eisai, Roche and Taiho Pharmaceutical. MS has received honorarium from Ipsen; travel grants from MSD, GSK and Pfizer. All other authors have declared no conflicts of interest.

REFERENCES

1. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. *CA Cancer J Clin*. 2011;61:69-90.
2. Kojima T, Shah MA, Muro K, et al. Randomized phase III KEYNOTE-181 study of pembrolizumab versus chemotherapy in advanced esophageal cancer. *J Clin Oncol*. 2020;38:4138-4148.
3. Kato K, Cho BC, Takahashi M, et al. Nivolumab versus chemotherapy in patients with advanced oesophageal squamous cell carcinoma refractory or intolerant to previous chemotherapy (ATTRACTION-3): a multicentre, randomised, open-label, phase 3 trial. *Lancet Oncol*. 2019;20:1506-1517.
4. Kato K, Shah MA, Enzinger P, et al. KEYNOTE-590: phase III study of first-line chemotherapy with or without pembrolizumab for advanced esophageal cancer. *Future Oncol*. 2019;15:1057-1066.
5. Doki Y, Ajani JA, Kato K, et al. Nivolumab combination therapy in advanced esophageal squamous-cell carcinoma. *N Engl J Med*. 2022;386:449-462.
6. Moehler M, Högner A, Wagner AD, et al. Recent progress and current challenges of immunotherapy in advanced/metastatic esophagogastric adenocarcinoma. *Eur J Cancer*. 2022;176:13-29.
7. Maule JG, Clinton LK, Graf RP, et al. Comparison of PD-L1 tumor cell expression with 22C3, 28-8, and SP142 IHC assays across multiple tumor types. *J Immunother Cancer*. 2022;10:e005573.
8. Squibb ER, Sons LL. *OPDIVO-nivolumab injection*. Bethesda, MD: National Library of Medicine; 2024.
9. EMEA/CHMP. *Annex I Summary of Product Characteristics*. Amsterdam: European Medicines Agency; 2022.
10. Stewart LA, Clarke M, Rovers M, et al. Preferred reporting items for a systematic review and meta-analysis of individual participant data. *JAMA*. 2015;313:1657.
11. Zhao JJ, Syn NL, Tan BKJ, et al. KMSubtraction: reconstruction of unreported subgroup survival data utilizing published Kaplan-Meier survival curves. *BMC Med Res Methodol*. 2022;22:93.
12. de Jong VMT, Moons KGM, Riley RD, et al. Individual participant data meta-analysis of intervention studies with time-to-event outcomes: a review of the methodology and an applied example. *Res Synth Methods*. 2020;11:148-168.
13. Higgins JPT, Thompson SG. Quantifying heterogeneity in a meta-analysis. *Stat Med*. 2002;21:1539-1558.
14. 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. *JAMA*. 2021;326:916.
15. Xu J, Kato K, Raymond E, et al. Tislelizumab plus chemotherapy versus placebo plus chemotherapy as first-line treatment for advanced or metastatic oesophageal squamous cell carcinoma (RATIONALE-306): a global, randomised, placebo-controlled, phase 3 study. *Lancet Oncol*. 2023;24:483-495.
16. Lu Z, Wang J, Shu Y, et al. Sintilimab versus placebo in combination with chemotherapy as first line treatment for locally advanced or metastatic oesophageal squamous cell carcinoma (ORIENT-15): multicentre, randomised, double blind, phase 3 trial. *BMJ*. 2022:e068714.
17. Carroll CE, Landrum MB, Wright AA, Keating NL. Adoption of innovative therapies across oncology practices—evidence from immunotherapy. *JAMA Oncol*. 2023;9:324.
18. Zhang Z, Huang J, Xu Y, Luo H. Chemoimmunotherapy for esophageal squamous cell carcinoma—summary and discussion of recent clinical trials. *MedComm Future Med*. 2023;2:e56.
19. Merino M, Kasamon Y, Theoret M, Pazdur R, Kluetz P, Gormley N. Irreconcilable differences: the divorce between response rates, progression-free survival, and overall survival. *J Clin Oncol*. 2023;41:2706-2712.
20. Booth CM, Eisenhauer EA, Gyawali B, Tannock IF. Progression-free survival should not be used as a primary end point for registration of anticancer drugs. *J Clin Oncol*. 2023;41:4968-4972.
21. Wang P, Chen Y, Long Q, et al. Increased coexpression of PD-L1 and TIM3/TIGIT is associated with poor overall survival of patients with esophageal squamous cell carcinoma. *J Immunother Cancer*. 2021;9:e002836.
22. Huang T-C, Liang C-W, Li Y-I, et al. Prognostic value of PD-L1 expression on immune cells or tumor cells for locally advanced esophageal squamous cell carcinoma in patients treated with neoadjuvant chemoradiotherapy. *J Cancer Res Clin Oncol*. 2022;148:1803-1811.
23. Wang X, He J, Li J, et al. Concordance of assessments of four PD-L1 immunohistochemical assays in esophageal squamous cell carcinoma (ESCC). *J Cancer Res Clin Oncol*. 2024;150:43.
24. Sun J-M, 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;398:759-771.
25. Ghosh NR, Jones LA. Dietary risk factors for esophageal cancer based on World Health Organization regions. *Nutrition*. 2022;95:111552.
26. The Cancer Genome Atlas Research Network. Integrated genomic characterization of oesophageal carcinoma. *Nature*. 2017;541:169-175.
27. Liu Y, Ren Z, Yuan L, et al. Paclitaxel plus cisplatin vs. 5-fluorouracil plus cisplatin as first-line treatment for patients with advanced squamous cell esophageal cancer. *Am J Cancer Res*. 2016;6(10):2345-2350.
28. Galluzzi L, Humeau J, Buqué A, Zitvogel L, Kroemer G. Immunostimulation with chemotherapy in the era of immune checkpoint inhibitors. *Nat Rev Clin Oncol*. 2020;17:725-741.