THERAPEUTIC ADVANCES in
Medical Oncology

Review

Immune checkpoint inhibitors in the treatment of oesophageal squamous cell carcinoma: where are we and where are we going?

Ther Adv Med Oncol

2023, Vol. 15: 1–18

DOI: 10.1177/17588359231189420

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***Abstract*:** Oesophageal squamous cell carcinoma (ESCC) is a kind of malignant tumour with high invasiveness and a poor prognosis. Immunotherapy, especially immune checkpoint inhibitors (ICIs), is a rapidly growing therapeutic method that activates and enhances anti-tumour immunity to treat patients with malignancy. Several clinical trials have confirmed the efficacy of ICIs in the treatment of ESCC. ICIs have been approved for the treatment of patients with ESCC. However, only a subset of patients can obtain excellent benefits from ICI therapy. In recent years, there has been a growing interest in exploring predictive biomarkers of immunotherapy response. In this review, we highlighted the predictive biomarkers for the prognosis of ESCC patients treated with ICIs and pointed out the existing problems and the direction of future research in this field.

Keywords: biomarker, immune checkpoint inhibitor, immunotherapy, oesophageal squamous cell carcinoma, PD-L1

Received: 14 February 2023; revised manuscript accepted: 5 July 2023.

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Introduction

Based on the latest global cancer burden data published by the World Health Organization in 2020, oesophageal cancer (EC) ranked seventh in incidence and sixth in mortality worldwide.[1] EC has two predominant histological subtypes: oesophageal adenocarcinoma (EAC) and oesophageal squamous cell carcinoma (ESCC). In Western developed countries, EAC is the predominant histological type of EC.[2] In East Asian countries, such as China and Japan, ESCC accounts for ⩾90% of patients with EC.[1] The symptoms of early ESCC are insidious. In China, 60–70% of patients with ESCC are at an advanced stage at diagnosis.[3] The 5-year overall survival (OS) rate of ESCC is less than 20%.[4] Hence, the treatment of ESCC remains a severe healthcare challenge.

Tumour immunotherapy has rapidly grown over the past few decades, particularly for immune checkpoint inhibitors (ICIs). ICIs are monoclonal antibodies that can inhibit tumour progression by relieving the immunosuppressive effects generated by immune checkpoint-related molecules and can enhance the body's anti-tumour response.[5] The emergence of ICIs has expanded treatment options for patients with ESCC. The Food and Drug Administration (FDA) has formally recommended ICIs for the treatment of advanced EC.[6]

Surgery remains one of the cornerstones of current therapy for ESCC. However, recurrence occurs in approximately 50% of the patients within 5 years after surgery.[7,8] The CROSS[9] and JCOG9907[10] trials reported that patients with resectable ESCC could benefit from neoadjuvant chemoradiotherapy (nCRT) or adjuvant chemotherapy. The NICE,[11] ESONICT-1[12] and PALACE-1[13] trials demonstrated that ICIs plus chemotherapy as neoadjuvant therapy are conducive to achieve a pathological

complete response (pCR) after surgery in patients with resectable ESCC. Additionally, the CHECKMATE-577 trial[6] reported that nivolumab adjuvant treatment significantly prolonged disease-free survival (DFS) compared to that of placebo in patients with ESCC (Table 1).

For metastatic, unresectable ESCC, systemic chemotherapy is the standard treatment. However, the median overall survival (mOS) of standard first-line chemotherapy for ESCC, containing platinum and 5-fluorouracil (5-FU), was mostly below 9 months.[5] A series of trials on advanced ESCC, such as KEYNOTE-590,[14] CHECKMATE-648[15] and ESCORT-1st,[16] have demonstrated that the addition of ICIs to first-line therapy improves survival outcomes. Moreover, the KEYNOTE-590 trial was the first to report that the effectiveness of pembrolizumab combined with chemotherapy as the first-line treatment for metastatic unresectable advanced ESCC is better than that of chemotherapy alone. Compared with KEYNOTE-590 and CheckMate-648, the ESCORT-1st trial showed better survival outcomes. This is likely reflective of the biological differences in ESCC between Western and Asian patients and possibly the differential activity of various chemotherapeutic agents[17] (Table 2).

Table 1. ICIs in neoadjuvant/adjuvant therapy of ESCC.

Trial	Line	Phase	*N*	*N* (ESCC)	Intervention	Primary endpoints	pCR of ESCC group (%)	MPR of ESCC group (%)	Median follow-up (months)	DFS of ESCC group	Severe AEs incidence of ESCC group (%)
CHECKMATE-577[6]	Adjuvant	III	794	230	Nivolumab *versus* placebo	DFS	/	/	24.4	22.4 *versus* 11.0	34 *versus* 32
NICE[11]	Neoadjuvant	II	107	100	Camrelizumab + chemoradiotherapy *versus* chemoradiotherapy	eCR	47.2* *versus* 33.3*	/	14.7	/	/
ESONICT-1[12]	Neoadjuvant	II	30	30	Sintilimab + chemotherapy	pCR	22	52	6	/	3
PALACE-1[13]	Neoadjuvant	I	20	20	Pembrolizumab + chemoradiotherapy	Safety	55.6	89	6.6	/	65

AE, adverse event; ICI, immune checkpoint inhibitor; DFS, disease-free survival; eCR, endoscopic complete response; ESCC, oesophageal squamous cell carcinoma; MPR, major pathological response; pCR, pathological complete response; PFS, progression-free survival.
*eCR.

Table 2. ICIs in First-Line treatment of ESCC.

Trail	Line	Phase	*N*	No. of patients with ESCC	PD-L1 status	Intervention	Group	mOS (months)	mPFS (months)	DOR (months)	ORR (%)
JUPITER-06[18]	First	III	514	514	Regardless of PD-L1 status	Toripalimab + chemotherapy *versus* placebo + chemotherapy	Overall Population	17.0 *versus* 11.0	5.7 *versus* 5.5	/	69.3 *versus* 52.1
KEYNOTE-590[19]	First	III	749	548	PD-L1 CPS ⩾ 10	Pembrolizumab + chemotherapy *versus* placebo + chemotherapy	Overall population	12.4 *versus* 9.8	6.3 *versus* 5.8	8.3 *versus* 6.0	45 *versus* 29.3
							ESCC + PD-L1positive group	13.9 *versus* 8.8			
							ESCC group:	12.6 *versus* 9.8	6.3 *versus* 5.8	/	/

(Continued)

Table 2. (Continued)

Trail	Line	Phase	*N*	No. of patients with ESCC	PD-L1 status	Intervention	Group	mOS (months)	mPFS (months)	DOR (months)	ORR (%)
CHECKMATE-648[15]	First	III	970	468	PD-L1 CPS ⩾ 10	Nivolumab + chemotherapy *versus* chemotherapy	Overall population	13.2 *versus* 10.7/12.7 *versus* 10.7	/	8.2 *versus* 7.1/11.7 *versus* 7.1	47 *versus* 20/28 *versus* 28
							Tumour-cell PD-L1 CPS ⩾ 10	15.4 *versus* 9.1/13.7 *versus* 9.1	/	8.4 *versus* 5.7/11.8 *versus* 5.7	53 *versus* 20/35 *versus* 20
						Nivolumab + ipulizumab *versus* chemotherapy	Overall population	12.7 *versus* 10.7	/	11.7 *versus* 7.1	28 *versus* 28
							Tumour-cell PD-L1 CPS ⩾ 10	13.7 *versus* 9.1	/	11.8 versus 5.7	35 versus 20
ORIENT-15[20]	First	III	659	659	PD-L1 CPS ⩾ 10	Sintilimab + chemotherapy *versus* placebo + chemotherapy	Overall population	16.7 *versus* 12.5	7.2 *versus* 5.7	9.7 *versus* 6.9	66 *versus* 45
							Tumour-cell PD-L1 CPS ⩾ 10	17.2 *versus* 13.6	8.3 *versus* 6.4	12.4 *versus* 5.7	68 *versus* 49
ESCORT-first[16]	First	III	596	596	Regardless of PD-L1 status	Camrelizumab + chemotherapy *versus* chemotherapy	Overall population	15.3 *versus* 12.0	6.9 *versus* 5.6	7.0 *versus* 4.6	72.1 *versus* 62.1

CPS, combined positive score; DCR, disease control rate; DOR, duration of response; ESCC, oesophageal squamous cell carcinoma; ICI, immune checkpoint inhibitor; mOS: median overall survival; mPFS: median progression-free survival; ORR: objective response rate; PD-L1, programmed death-ligand 1.

ICIs combined with chemotherapy have shown favourable clinical benefits for pre-treated patients with unresectable ESCC in multiple phase III trials, such as the KEYNOTE-181, ATTRACTION-3 and ESCORT trials.[21–23] Hence, ICIs have been approved for second-line therapy and beyond for advanced ESCC and have changed the therapeutic mode of ESCC. The key data are summarised in Table 3.

ICIs are expected to lead to further breakthroughs and improve the survival of patients with ESCC. However, not all patients with ESCC can benefit from ICI therapy; rare adverse events (AEs) caused by ICIs are dangerous and even fatal. Therefore, it is necessary to achieve an accurate ICI therapy. In this review, we highlighted biomarkers to predict the prognosis of patients with ESCC who received ICI therapy and pointed out existing problems and future research directions.

Predictive biomarkers for ICIs-based treatment of ESCC

ICIs have been recommended for the treatment of ESCC because of their excellent effects and improved prognosis. However, ICIs remain unable to achieve satisfactory clinical benefits in all patients with ESCC. It is important to select patients who can benefit from ICI therapy through precise and accurate predictive biomarkers. In recent decades, our interest in identifying reliable biomarkers for predicting the prognosis of ICI therapy has rapidly improved. Several valuable data sets on predictive biomarkers have emerged in recent years (Figures 1 and 2).

Clinical biomarkers

Clinical characteristics

Sex. Sex is a variable closely related to the immune response and has been reported in other tumours as an indicator related to the

Table 3. ICIs in second-line and beyond treatment of ESCC.

Trail	Line	Phase	Intervention	*N*	No. of ESCC	PD-L1 status	Group	mOS (months)	mPFS (months)	ORR (%)	DCR (%)	DOR (months)
KEYNOTE-28[24]	Third or later	IB	Pembrolizumab	477	18	PD-L1 positive	EC group	7	1.8	30	/	15
ORIENT-2[25]	Second or later	II	Sintilimab *versus* chemotherapy	190	190	Regardless of PD-L1 expression	Overall population	7.2 *versus* 6.2	1.6 *versus* 2.9	12.6 *versus* 6.3	44.2 *versus* 43.2	8.3 *versus* 6.2
KEYNOTE-180[26]	Third or later	II	Pembrolizumab	121	63	Regardless of PD-L1 expression	Overall population	5.8	2.0	9.9	30.6	NA
ATTRACTION-01[27]	Third or later	II	Nivolumab	65	65	Regardless of PD-L1 expression	Overall population	10.8	1.5	7.2	25	NA
ATTRACTION-03[22]	Second or later	III	Nivolumab *versus* chemotherapy	419	419	Regardless of PD-L1 expression	Overall population	10.9 *versus* 8.4	1.7 *versus* 3.4	33 *versus* 34	64 *versus* 99	6.9 *versus* 3.9
ESCORT[23]	Second or later	III	Camrelizumab *versus* chemotherapy	457	457	Regardless of PD-L1 expression	Overall population	8.3 *versus* 6.2	1.9 *versus* 1.9	46 *versus* 14	44.7 *versus* 34.5	7.4 *versus* 3.4
RATIONALE-302[28]	Second or later	III	Tislelizumab versus chemotherapy	512	512	TAP score of ⩾10%	Overall population	8.6 *versus* 6.3	1.6 *versus* 2.1	20.3 *versus* 9.8	/	7.1 *versus* 4.0
							TAP score of ⩾10%	10.3 *versus* 6.8	/	/	/	/
KEYNOTE-181[21]	Second or later	III	Pembrolizumab *versus* chemotherapy	628	411	PD-L1 CPS ⩾10	Overall population	7.1 *versus* 7.1	2.1 *versus* 3.4	13.1 *versus* 6.7	/	/
							PD-L1 CPS ⩾10 group	9.3 *versus* 6.7	2.6 *versus* 3.0	21.5 *versus* 6.1	/	9.3 *versus* 7.7
							PD-L1 CPS ⩾10 + ESCC group	10.3 *versus* 6.7	/	/	/	/
							ESCC group	8.2 *versus* 7.7	2.2 *versus* 3.1	16.7 *versus* 7.4	/	/

CPS, combined positive score; DCR, disease control rate; DOR, duration of response; ESCC, oesophageal squamous cell carcinoma; EC, oesophageal cancer; ICI, immune checkpoint inhibitor; Mos, median overall survival; mPFS, median progression-free survival; ORR, objective response rate; PD-L1, programmed death-ligand 1; TAP, tumour area positivity.

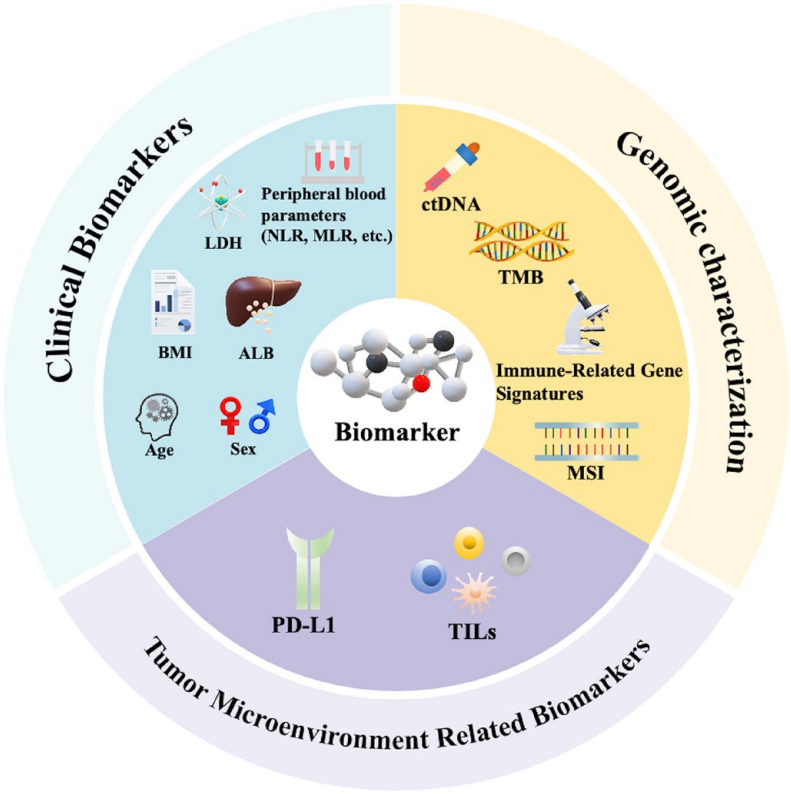

Figure 1. The predictive biomarkers for therapeutic effectiveness of ICIs in ESCC can be classified by clinical biomarkers, genomic characterisation and tumour microenvironment related biomarkers. Clinical biomarkers mainly include clinical characteristics (age, sex), biomarkers of nutrition (BMI, ALB) and biomarkers in peripheral blood (peripheral blood parameters, such as NLR, MLR, etc.). Genomic characterisation mainly includes TMB, MSI, ctDNA and immune-related genes. Tumour microenvironment-related biomarkers mainly include PD-L1, TILs.

ALB, serum albumin; BMI, body mass index; ctDNA, circulating tumour DNA; ESCC, oesophageal squamous cell carcinoma; MLR, monocyte-to-lymphocyte ratio; ICI, immune checkpoint inhibitor; MSI, microsatellite instability; NLR, neutrophil-to-lymphocyte ratio; TMB, tumour mutational burden; PD-L1, programmed death-ligand 1; TIL, tumour-infiltrating lymphocyte.

effectiveness of immunotherapy.[29–31] Women generally harbour stronger innate and adaptive immune responses than men, which contributes to stronger pathogen removal capacity and greater vaccine efficacy.[32] Tumours in women have a greater capacity to evade immune surveillance; thus, they are less immunogenic and more immunotherapy-resistant.[33] Also, women are more likely to develop ICI-related AEs owing to their increased susceptibility to autoimmune disorders. The subgroup analysis of ESCC in the KEYNOTE-181 trial[21] indicated that female patients who received pembrolizumab were at a higher risk of mortality than male patients. However, the reverse result was reported in the ESCORT trial,[23] where the female patients had a more favourable response to camrelizumab treatment than the male patients. This difference may be attributed to the different effects of camrelizumab and pembrolizumab on sex. In summary, male patients with cancer may have the advantage of enhanced efficacy in ICI treatment compared to female patients.

However, the potential sex-based differential responses to ICIs in ESCC remain controversial, as only a few studies, investigating the efficacy of ICIs for ESCC take sex into account as an influential factor. Furthermore, most of the patients with ESCC are male; thus, current results about the efficacy of ICIs are obtained mainly in male patients. More trials exploring the difference in the effectiveness of ICI treatment among male and female patients with ESCC need to be urgently carried out. It seems improper to extend the results from male patients to female patients if the potential sex-based differential responses to ICIs in ESCC are not verified.

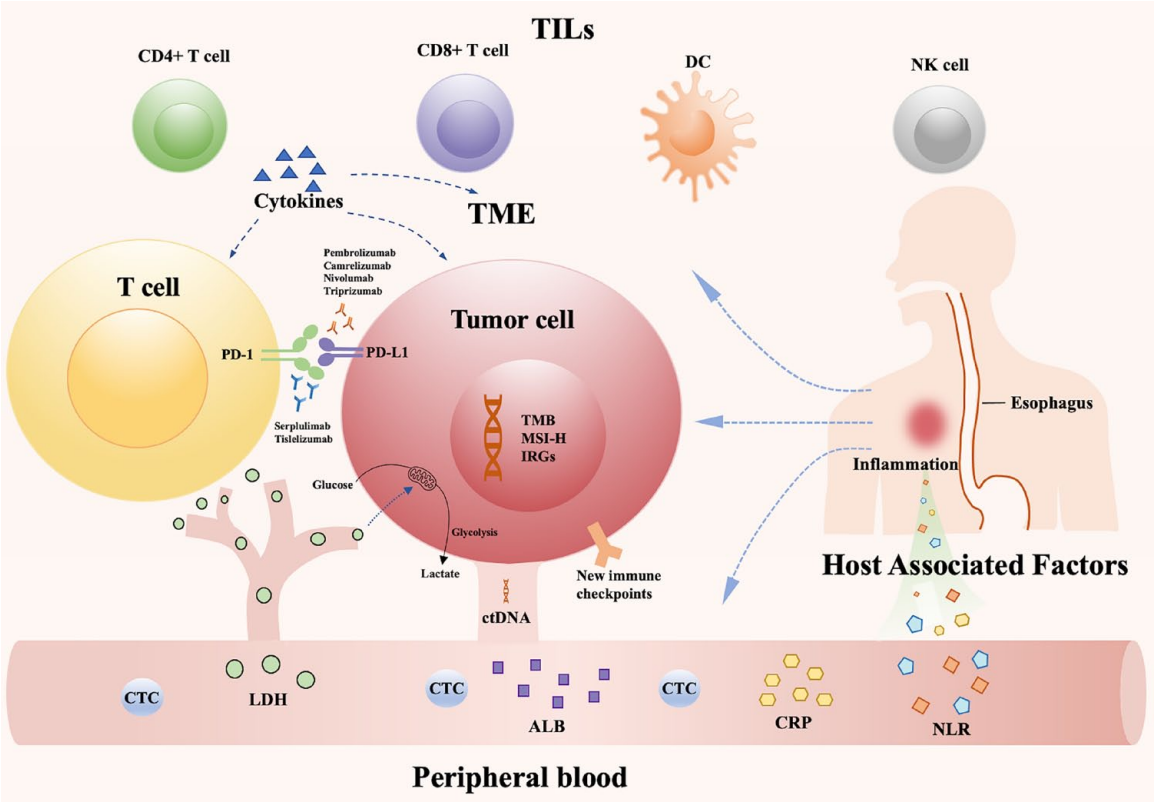

Figure 2. Biomarkers that affect tumour development and ICIs therapeutic effectiveness. Predictive biomarkers from host, peripheral blood and TME can influence anti-tumour immune response and tumour develop process in various ways. PD-L1 can inhibit immune response by binding to programmed cell death 1 (PD-1) on the surface of T cells, finally causes immune escape. PD-1 inhibitors, such as serplulimab and tislelizumab, can combine with PD-1 to relive the immune system depression. PD-L1 inhibitors, such as pembrolizumab and camrelizumab, which can inhibit the PD-1/PD-L1 signal pathway by binding to PD-L1. TILs, including CD8 + T cells, CD4 + T cells, DC cells, natural killer (NK) cells, can affect the development of tumours and anti-tumour effects. The inflammatory of the body contributed to the increased CRP and NLR in the peripheral blood, which affects the tumour immune effect. Serum albumin (ALB) level reflects the nutritional status of the body and is further related to the efficacy of anti-immunotherapy. LDH affects the growth of tumour cells by promoting the glycolysis process of tumour cells, which is related to adverse anti-tumour immune response. TMB is usually positively correlated with new antigens produced by tumours. MSI-H is mostly related to good anti-tumour immune response. Tumour cells can induce immunosuppression by overexpressing IRGs.
CRP, C-reactive protein; ICI, immune checkpoint inhibitor; IRG, immune-related gene; LDH, lactate dehydrogenase; MSI-H, microsatellite instability-high; NLR, neutrophil-to-lymphocyte ratio; PD-L1, programmed death-ligand 1; TMB, tumour mutational burden; TIL, tumour-infiltrating lymphocyte.

Age. The immune function decreases with age. The immune system experiences major changes with ageing when substantial immune cells become altered and adaptive immunity becomes less functional. Older patients usually have a low prognostic nutritional index, attributed to poor nutritional status and immune function, which results in enhanced tumour invasion and an increase in the number of lymph node metastases. In a retrospective study of ESCC,[34] favourable outcomes in progression-free survival (PFS) from ICI treatment were obtained in older patients (⩾65 years) compared to that with younger patients (<65 years) (4.1 month *versus* 1.6 month, $p = 0.025$). However, in the subgroup analysis of several prospective trials on ESCC,[18,22] participants aged <65 years showed better survival outcomes. Thus, more studies are warranted to confirm the association between age and prognosis in patients with ESCC treated with ICIs.

Biomarkers of nourishment. Patients with EC usually have a higher risk of malnutrition, possibly caused by eating disorders and tumour

cachexia. Impaired nutritional status affects the tumour microenvironment (TME) or upsets the intestinal microbiome[35] and is thus closely related to a reduced anti-tumour immune response.

Body mass index. Body mass index (BMI) is an acknowledged indicator to evaluate the nutritional status of the body. It is generally assumed that patients with a high BMI have sufficient energy reserves to resist nutrient consumption and maintain an immunomodulatory response.[36] BMI has been confirmed to be related to the outcomes of checkpoint blockade immunotherapy in various tumours.[37] BMI $< 18.5\,kg/m^2$ was regarded as a diagnostic criterion of cancer cachexia in the past. Cancer cachexia is a complex multifactorial syndrome with ongoing skeletal muscle loss. As our understanding of cachexia improves, the diagnosis of cancer cachexia is becoming more complicated, including physical function, energy expenditure, body composition and quality of life.[38] However, BMI remains crucial for the diagnosis of cachexia. Cancer cachexia is an important negative predictor of the efficacy of programmed cell death 1 (PD-1)/programmed death-ligand 1 (PD-L1) inhibitors because of its desensitising effect on PD-1/PD-L1 inhibitors.[39] Unfortunately, the predictive effect of BMI in EC is still unverified.

Serum albumin. Serum albumin (ALB) is the primary indicator of the nutritional status and inflammatory pressure in patients with cancer. Hypoalbuminaemia (ALB < 30) is associated with impaired immune responses and a poor prognosis in patients with malignancy.[40,41] Low levels of ALB aid in the development of cancer cachexia.[42] Yoo *et al.*[43] reported the benefits of high ALB levels in patients with cancer receiving ICI therapy. In the EC subgroup, patients with high ALB levels who received ICI treatment had better OS [hazard ratio (HR), 0.35, $p < 0.01$] and PFS (HR, 0.38, $p < 0.01$) than patients with low ALB levels. A real-world study[41] demonstrated that low baseline ALB levels may predict the poor prognosis of patients with ESCC treated with anti-PD-1 inhibitors. Other indicators commonly used to evaluate nutritional statuses, such as haemoglobin and globulin, may be associated with the therapeutic effectiveness of ICIs, thus deserving further study as predictive biomarkers. Furthermore, new prognostic biomarkers, such as the Haemoglobin, Albumin, Lymphocyte, Platelet Score (HALP) and the Glasgow Prognostic Score, which indicate ALB levels, also deserve further investigation.

Biomarkers in peripheral blood

Peripheral blood parameters. Peripheral blood parameters reflect the immune state and response to immunotherapy in the host. In various malignancies, the neutrophil-to-lymphocyte ratio (NLR) has become a potential predictor of the therapeutic effectiveness of ICIs.[44] A retrospective analysis[45] conducted on 119 patients with ESCC demonstrated that patients with a high NLR at 6 weeks after PD-1 inhibitor treatment obtained observable benefits in PFS compared with that of the low NLR group (12.80 months *versus* 9.23 months), thus indicating that a low NLR is positively related to the prognosis of ESCC patients treated with PD-1 inhibitors. However, the relevance of NLR to the outcomes of ESCC patients treated with ICIs requires further research. In addition, the monocyte-to-lymphocyte ratio (MLR), platelet-to-lymphocyte ratio (PLR) and systemic immune-inflammation index (SII) also play a predictive role in ICI treatment.[46,47] Compared with those in tumour-free patients, increased NLR, PLR, MLR and SII at baseline were observed in patients with ESCC, thus confirming the correlation between PLR, MLR, SII and MLR and the prognosis of ICI therapy in patients with ESCC.

Lactate dehydrogenase. Lactate dehydrogenase (LDH) is a key enzyme in glycolysis. The LDH level in tumour tissues is reported to be higher than that in normal tissues because tumour cells are dominated by anaerobic glycolysis.[48] Therefore, LDH may act as an indicator of tumour burden and aggressiveness. A multivariate trial in China first verified that a normal LDH level at baseline was associated with longer OS in patients treated with PD-1 inhibitors.[49] However, the sample size was too small to provide solid evidence supporting the predictive role of LDH in ESCC and thus requires further study in a large-sample trial.

Other potential predictive markers. Subgroup analysis of several trials of ESCC demonstrated that the Eastern Cooperative Oncology Group performance status, smoking history, region, sites of metastases and tumour-node-metastasis stage are related to the therapeutic outcomes of patients with ESCC treated with ICIs. However, there is an inconsistency between the specific relevance of different trials.

A previous study confirmed that microbial diversity was associated with improved effectiveness of ICIs[50] and reduced risk of immune-related AEs.[51]

Decreased microbial diversity is observed in patients with ESCC,[52] which may lead to the poor efficacy of ICI-based treatment. Furthermore, in patients with malignancy, antibiotic exposure is associated with resistance and worse effectiveness of ICIs, by decreasing the microbiome's diversity.[53–55]

Other biomarkers in peripheral blood, such as C-reactive protein (CRP),[56] cytokines, interleukins[57] and metabolites of metabolomics,[58] which are related to the prognosis of patients treated with ICIs, also have the potential to be predictors of ESCC.

TME-related biomarkers

TME refers to the internal and external environments in which the tumour occurs and develops. Various immune cells, endothelial cells and fibroblasts are present in the TME.[59] An imbalance in the TME is associated with tumour progression and prognosis.[60]

Programmed death-ligand 1. PD-L1 is a transmembrane protein expressed on the surface of tumour cells. PD-L1 can inhibit immune response by binding to PD-1 on the surface of T cells, thus resulting in immune evasion. About 45.5% of patients with ESCC have a positive expression of PD-L1 [combined positive score (CPS) $\geqslant$10].[61] The reactivity of tumours to PD-1/PD-L1 inhibitors can be reflected in the expression level of PD-L1 in tumour tissues. The expression level of PD-L1 is the most commonly used biomarker for predicting the efficacy of ICI therapy in multiple malignancies, including ESCC. The phase II trial KEY-NOTE-059[62] was conducted among 749 patients with EC, of whom 73.2% were pathologically diagnosed with ESCC. The results showed that patients who were PD-L1-positive (CPS $\geqslant$ 10) had better effectiveness from pembrolizumab therapy compared to patients who were PD-L1 negative [objective response rate (ORR), 22.7% *versus* 16.4%]. PD-L1 was the first proposed and most commonly used predictive biomarker for the therapeutic effectiveness of ICIs in ESCC.

Tumour-infiltrating lymphocytes. Tumour-infiltrating lymphocytes (TILs) can influence tumour development and the effectiveness of anti-tumour therapy.[63] The density of TILs at the invasive tumour margin has been proposed as a predictor for the prognosis of ICIs treatment.[64] It is found that tumours that are PD-L1-positive with the presence of TILs are most likely to benefit from ICIs treatment.[65] A large cohort study[66] conducted on 305 patients with EC, of whom 91.5% were pathologically diagnosed with ESCC, revealed that the TIL-positive group presented better OS and DFS from ICIs treatment than did the TIL-negative group ($p < 0.0001$). Therefore, the baseline TIL status could serve as a predictive biomarker for ICI therapy.

Other potential markers. Transforming growth factor-β (TRF-β) is a cytokine involved in immune evasion and resistance to ICIs.[67] An in vitro experiment showed that blocking the TGF-β signalling pathway could improve susceptibility to ICIs and contribute to overcoming resistance.[68] A high TGF-β level may be associated with the efficacy of ICI therapy. The potentiating effect of TGF-β inhibition on ICIs treatment has been confirmed in several tumours.[69]

Tumour-associated macrophages[70] and myeloid-derived suppressor cells[71–73] have been found to participate in anti-tumour immunity and are related to the prognosis of ICI therapy. However, their correction with the ESCC prognosis remains unproven.

In recent years, there have been numerous new attempts to develop other co-inhibitory receptors like lymphocyte activation gene-3 (LAG-3), T-cell immunoglobulin and mucin-domain-containing-3 (TIM-3) and T-cell immunoglobulin and immunoreceptor tyrosine-based inhibitory motif domain (TIGIT) as emerging ICI targets. TIM-3 and TIGIT were found to be upregulated in TILs in patients with ESCC[74]; thus, further studies to develop them as new targets for ICI therapy are required.

Genomic characterisation

Tumour mutational burden. Tumour mutational burden (TMB) refers to the total number of somatic genes mutated per million bases detected in the genome of a single tumour. TMB is a burgeoning biomarker for the prediction of ICI efficacy. Theoretically, more TMB usually leads to more tumour-specific antigens called neoantigens, which are largely correlated with sensitivity to ICIs.[75] Tumours with high TMB usually show positive responses to ICI treatment. EC tumour cells have been found to harbour high TMB.[76,77] A phase I trial[78] assessing the activity and pharmacokinetics of camrelizumab treatment in

advanced EC demonstrated that the high TMB group showed better survival benefit than the low TMB group ($p = 0.0123$). Nevertheless, results will be different due to different detection methods; moreover, the TMB threshold varies greatly among studies, thereby challenging the accuracy of TMB as a predictive biomarker.

Microsatellite instability. Microsatellite instability (MSI) is a hypermutator phenotype caused by mismatch-repair deficiency (dMMR). MSI is characterised by the deletion or amplification of short and repetitive DNA sequences, leading to frameshift mutations.[79] Tumours with MSI-high (MSI-H) status have a high TMB count and increased neoantigens.[77] Reportedly, patients with dMMR cancers are sensitive to ICI treatment.[80] Hence, pembrolizumab has been recommended by the FDA for the treatment of unresectable or metastatic MSI-H/dMMR tumours. However, the frequency of MSI-H in EC was less than 2%. Therefore, its widespread application in clinical practise is limited.

Circulating tumour DNA. Circulating tumour DNA (ctDNA), a fragment of DNA in the blood released by tumour cells, carries the original tumour mutation characteristics and reflects the burden of tumours. In addition, ctDNA can assess the presence of minimal residual disease, which is associated with tumour recurrence.[81] A meta-analysis[82] has demonstrated that elevated ctDNA levels were related to worse OS (HR, 3.35, $p < 0.00001$) and poorer PFS (HR, 3.28, $p < 0.00001$) in ICI-based therapy. However, there is little evidence to support its predictive value in ESCC. Moreover, the standardisation and sensitivity of ctDNA detection are still lacking. Therefore, more studies are urgently needed to explore the predictive value of ctDNA in ICI therapy for ESCC, especially in postoperative adjuvant immunotherapy.

Immune-related gene signatures. Tumour cells can induce immunosuppression by overexpressing immune-related gene (IRG) signatures, thereby promoting tumour progression. Accumulating evidence has demonstrated that the expression of IRGs may reflect immune response, thus being a promising predictor of immunotherapy.[64] Multiple trials have confirmed the predictive role of IRG expression levels in predicting the effectiveness of ICIs in various solid tumours.[83] Ji *et al.*[84] suggested that high-immune-related gene prognostic index is related to a stronger response to ICI therapy but poorer clinical survival outcomes in ESCC.[84] IRGs, such as COL9A3, GFRA2, VSIG4 and METTL3, have been reported in ESCC. However, the predictive value of these genes for ICI therapy in patients with ESCC remains ambiguous.

Potential predictive markers. DNA methylation is the main epigenetic mechanism. Changes in DNA methylation levels are related to the degree of immune infiltration in the tumour. A multicentre retrospective analysis confirmed the predictive role of DNA methylation in the anti-PD-1 treatment of non-small-cell lung cancer.[85] Additionally, mutations in DNA demethylase ten-eleven translocation 1 (TET1) are associated with better survival outcomes in ICI treatment of multiple cancers, including EC.[86] Furthermore, alterations in T-cell-inflamed gene expression profiles and epithelial–mesenchymal transition-related genes have the potential to play a role in anti-tumour immunotherapy.

Challenges and future prospects

Immunotherapy, especially ICIs, has undergone phenomenal development over the last few decades and has been recommended as a therapeutic method for advanced ESCC. However, they come with some problems that require prompt solutions (Figure 3).

What is the optimal neoadjuvant therapeutic regimen for resectable ESCC?

Neoadjuvant therapy can improve survival and decrease disease recurrence in patients with resectable ESCC.[9] nCRT and neoadjuvant chemotherapy (nCT) have been recommended as standard therapeutic modalities for locally advanced ESCC. In recent years, several phase early trials[11] have investigated the effectiveness of nCRT plus ICIs as neoadjuvant therapy and shown promising results. However, larger studies need to be carried out to validate these findings.

Compared with adjuvant immunotherapy, neoadjuvant immunotherapy makes primary treatment less extensive, downstages cancer by destroying disseminated cancer cells and enhances the effectiveness of nCRT/nCT. Theoretically,

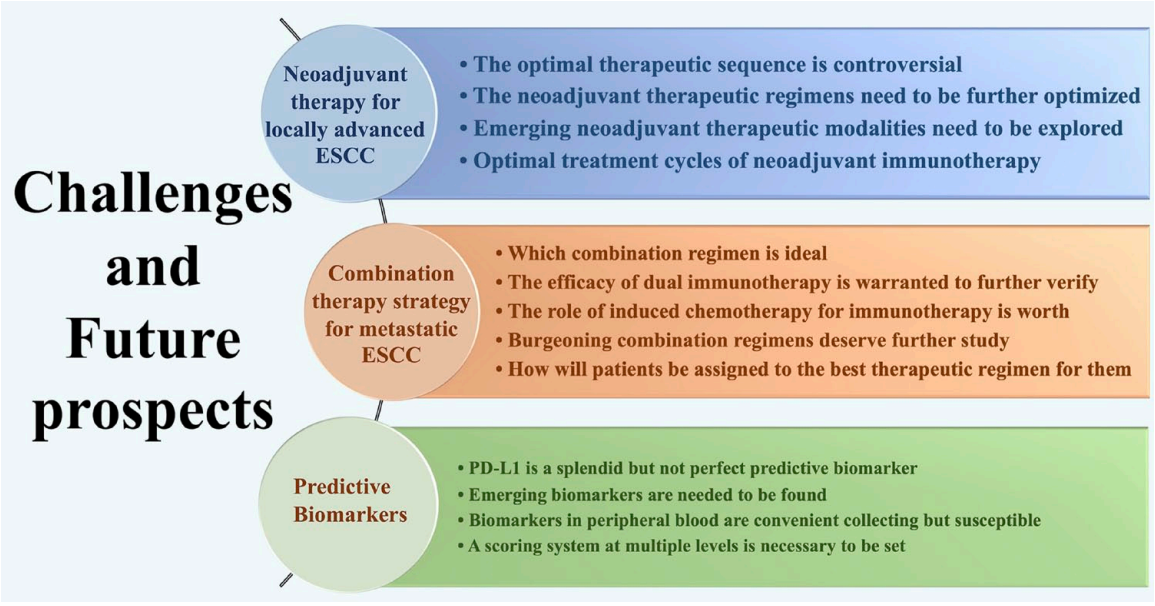

Figure 3. Challenges and future prospects of treatment of ESCC.
ESCC, oesophageal squamous cell carcinoma.

patients may have a higher TMB when receiving neoadjuvant therapy, resulting in a stronger anti-tumour response. Beyond that, neoadjuvant immunotherapy may induce immunosuppression caused by surgery. Also, patients benefitting from immunotherapy before surgery may be sensitive to immunotherapy after surgery, which can offer some elicitation for subsequent immunotherapy.

Accumulating evidence suggests that there are synergistic effects of immunotherapy, chemotherapy and radiotherapy. Preconditioning with a low dose of chemotherapeutic agents can induce inflammation in the TME, thereby favouring immunotherapy.[87] Immunotherapy could interfere with the ability of tumour cells to grow and spread and lead to the proliferation of anti-tumour immune cells. Besides, ICIs may increase the sensitivity of tumours to radiation, whereas radiotherapy could enhance the effectiveness of immunotherapy. In the future, the combination of nCRT with ICIs may become the new therapeutic standard for resectable ESCC. However, the optimal therapeutic sequence between immunotherapy, chemotherapy and radiotherapy remains controversial to date.

The National Comprehensive Cancer Network guidelines recommend 4–6 cycles of nCT, but the optimal course of the neoadjuvant immunotherapy cycle remains uncertain. On the one hand, the efficacy of immunotherapy cannot be fully exploited because there are only a few therapeutic cycles. On the other hand, delayed surgery may result in disease progression and decreased patient tolerance to surgery. Additionally, AEs caused by neoadjuvant therapy are also concerning. Severe therapeutic toxicity may lead to delayed surgery or even missed surgical opportunities. Several trials have attempted to determine the optimal number of therapeutic cycles.[88–90] Unfortunately, they failed to conclude. More prospective studies are required to verify the optimal number of treatment cycles.

The OS is usually used as the standard primary endpoint in oncology clinical trials; however, its use as the primary endpoint in neoadjuvant trials is unpractical, so optimal surrogate endpoints are still being investigated for neoadjuvant trials. Major pathological response (MPR) and pCR are widely used in practise as surrogate endpoints nowadays; however, they are not perfect to replace OS as standard primary endpoints. One of the reasons for that is that the association between MPR or pCR and improved survival is not completely determined. Moreover, in neoadjuvant immunotherapy trials, accurately determining whether the therapeutic response is from immunotherapy or not is not possible. Furthermore, MPR or pCR cannot timely capture the impact of treatment-related AEs. Thus, the association

between MPR or pCR and improved survival and between treatment-related AEs needs to be validated in prospective studies.

In addition, patients with resectable ESCC who received concurrent nCRT plus ICIs showed a higher incidence of AEs.[10] The neoadjuvant therapeutic regimen containing immunotherapy needs to be further optimised. Furthermore, the combination of nCT and ICIs, or dual immunotherapy combinations, has attracted the interest of researchers as a burgeoning combination strategy.[91] The data of the ongoing phase II/III EA2174 trial,[92] which investigates the effectiveness of treatment with nivolumab and ipilimumab plus nCRT in patients with EC and gastroesophageal junction cancer, are anticipated. Further studies are required to explore more emerging neoadjuvant therapeutic modalities for locally advanced ESCC.

What is the optimal combination therapy strategy for advanced EC?

A combination of chemotherapy and immunotherapy has been recommended as the first-line treatment for patients with advanced EC. To date, the combination regimens of pembrolizumab plus 5-FU and cisplatin, nivolumab plus 5-FU and cisplatin, and camrelizumab plus paclitaxel and cisplatin have shown encouraging results.[15] Chemotherapy combined with immunotherapy may replace chemotherapy alone as the future treatment for advanced EC. Nevertheless, which chemotherapy regimen is optimal as a combination therapy remains inconclusive. For patients with ESCC, platinum/paclitaxel is the most common combination chemotherapy scheme. For patients with EAC, investigators usually choose platinum/fluoropyrimidine-based combinations or capecitabine plus oxaliplatin (CAPOX) as the chemotherapy backbone. In addition, other chemotherapy like docetaxel plus nedaplatin are also combined with ICI in clinical trials.[21,28] However, few trials[93] have directly compared the efficacy of different chemotherapy regimens when combined with ICI.

The different immune checkpoints and pathways may result in differences in the effectiveness of different inhibitors. For instance, PD-L1 is mainly expressed on the surface of cancer cells, whereas PD-1 is mainly expressed on the T cells of the immune system. Other immune checkpoints, such as CTLA4,[15] TIM-3,[94] LAG-3[95] and TIGIT,[96] are also under investigation in various clinical trials. CheckMate-648[15] has reported that patients with metastatic ESCC receiving nivolumab plus ipilimumab as the first-line treatment showed a significantly longer mOS than did the chemotherapy-alone group (12.8 months *versus* 10.7 months; HR, 0.78; $p < 0.011$). However, the trial did not allow for a direct comparison of survival benefits between nivolumab plus ipilimumab and nivolumab plus chemotherapy or ipilimumab plus chemotherapy. Besides, TIM-3 can result in the death of Th1 cells by engaging with galectin-9 and triggering intracellular calcium flux.[94] I In an in vitro experiment, dual blockade of PD-1/PD-L1 and TIM-3 strengthened the anti-tumour immune response and postponed the growth of tumour cells.[97] More prospective research is warranted to verify the efficacy and improve the safety of dual immunotherapy combinations.

Angiogenesis inhibitors enhance the effectiveness of immunotherapy by blocking some proangiogenic pathways and promoting T-cell infiltration and dendritic cell maturation.[98] The combination of immunotherapy with angiogenesis inhibitors has exhibited promising efficacy in various solid tumours[99–101]; this combination deserves further investigation in the future as a burgeoning combination regimen for advanced ESCC. Besides, dual inhibition of the epidermal growth factor receptor/human epidermal growth factor receptor 2 and insulin-like growth factor 1 receptor signalling pathways showed superior anti-tumour efficacy *in vitro* experiments of ESCC[102] and is expected to be confirmed in clinical trials.

Moreover, the differences in clinical characteristics between patients may result in different responses to anti-tumour agents. Assigning patients correctly to the therapeutic regimen best suited for them deserves further consideration.

Which is the ideal biomarker?

Precisely predictive biomarkers are conducive to increase[101] the percentage of patients that could benefit from ICIs and avoid the AEs, thus achieving individualised and accurate therapy.

Among the biomarkers mentioned above, the PD-L1 expression level is the most widely accepted biomarker to predict the effect of PD-1/PD-L1

inhibitors. It is usually thought that patients with high levels of PD-L1 expression are more likely to benefit from ICIs. But patients with low levels or even negative expression of PD-L1 were also observed to benefit from ICIs. Several standardised immunohistochemistry PD-L1 antibody assays, such as Dako 22C3, Dako 28–8 and Ventana SP-142, are used to predict treatment response to different ICIs in various tumours[103]; however, the concordance between each assay is uncertain. Different detection methods, antibodies and cut-offs may result in different frequencies of PD-L1 positivity. Several methodologies for PD-L1 immunostaining scoring have been developed, such as the ratio of PD-L1-positive tumour cells – the tumour proportion score (TPS) – and the ratio of PD-L1-stained tumour and immune cells – the CPS. The KEYNOTE-224 phase II trial[104] evaluated PD-L1 using both scoring methods, with the CPS turning out to be a more applicable biomarker. However, some trials choose TPS as the evaluation method for PD-L1 expression. Which methodology is better for evaluating PD-L1 expression is still uncertain. Kulangara *et al.*[105] report that CPS has a closer link with immunotherapy because it comprehensively assesses the expression of PD-L1 in both tumour cells and immune cells and is more applicable for digested tumours. In addition, sample acquisition for detecting the expression level of PD-L1 is inconvenient. Moreover, the expression of PD-L1 can be easily affected by many factors, for instance, the infiltrating density of Tregs, interferon-γ secreted by TILs, the time of biopsy and the therapeutic method.[106–108] Collectively, PD-L1 is a splendid, but not perfect, predictive biomarker.

In recent years, interest in the potential of biomarkers in peripheral blood to predict the prognosis of ICIs in anti-tumour therapy has gradually increased owing to their broad availability and relatively low cost. As mentioned in the previous section, epigenetic biomarkers, such as DNA methylation, have a close association with TME and can be measured in liquid biopsies and body fluids. However, the accuracy of DNA methylation to predict the therapeutic response for patients treated with ICIs is uncertain. Further research is needed to confirm the reliability of DNA methylation as a predictive biomarker and explore other prospective epigenetic biomarkers. Moreover, it is necessary to develop detection methods to improve the sensibility and precision of the biomarkers.

Serum metabolomics has been applied to the study of ESCC for early diagnosis, staging, prognostic prediction and improving understanding of its underlying mechanisms. ESCC is metabolically characterised by upregulation of the tryptophan pathway, including the accumulation of tryptophan, formylkynurenine and kynurenine, as well as increased expression of indoleamine 2,3-dioxygenase 1 (IDO1). IDO1 plays a key role in the formation of the tumour immunosuppressive microenvironment by suppressing natural killer (NK) and T-cell responses and promoting tumour immune tolerance.[51] IDO1 may influence the prognosis of ICIs by inhibiting the Trp–Kyn pathway. The relationship between IDO1 and the effect of ICIs in ESCC warrants further studies for validation.

Biomarkers in peripheral blood are susceptible to various factors that need to be considered. Further research is needed to confirm specific detection indicators, determine the best time for detection and determine the threshold of each index. Additionally, it is not sufficient to use one biomarker to rule in or out the use of ICI therapy. For this reason, it is necessary to set up a scoring system including various biomarkers to accurately assess the effect of ICI treatment at multiple levels. Accumulating evidence has indicated that tumour immune microenvironment subtypes, classified by PD-L1 expression and the presence of TILs, are closely associated with survival outcomes. Patients with high PD-L1 expression and high immune infiltration are more likely to benefit from chemotherapy plus immunotherapy.[109] Furthermore, the EGIC scheme proposed by Chen *et al.*,[110] which integrates ccTMB, specific HLA genotypes and four risk oncogenic alterations, has shown a promising ability to select ESCC patients who can benefit from chemotherapy plus PD-1 inhibitor treatment.[110]

In the future, large sample trials are warranted to provide strong evidence for potential predictive markers. Moreover, novel antibodies directed towards alternative immune checkpoints need to be further researched and developed. New technologies, such as single-cell sequencing, digital pathology and spatial transcriptomics, are expected to be used for exploring predictive biomarkers.

Conclusion

ESCC is a severe healthcare challenge, especially in East Asia, with high prevalence and mortality. Numerous trials have reported that ICIs

contribute to better survival outcomes in patients with ESCC.

Immunotherapy in advanced EC has been recommended as a standard treatment and even as an effective treatment in early EC. Precisely predictive biomarkers may make immunotherapy more effective and safer. However, only a few biomarkers have been proven to predict the prognosis of ICI therapy. More valuable predictive biomarkers need to be identified in the future to achieve individualised and accurate ICI therapy.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Author contributions
Ning Chen: Conceptualisation; Writing – original draft; Writing – review & editing.

Xiaoling Xu: Writing – review & editing.

Yun Fan: Conceptualisation; Writing – original draft; Writing – review & editing.

Acknowledgements
None.

Funding
The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was supported by the National Natural Science Foundation of China under Grant No. 81972718.

Competing interests
The authors declare that there is no conflict of interest.

Availability of data and materials
Not applicable.

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