

Tumor immune microenvironment and immune checkpoint inhibitors in esophageal squamous cell carcinoma

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Abstract

Esophageal squamous cell carcinoma (ESCC) is the main prevalent histological type of esophageal cancer, predominantly constituting 90% of cases worldwide. Despite the development of multidisciplinary therapeutic approaches, its prognosis remains unfavorable. Recently, the development of monoclonal antibodies inhibiting programmed death 1 (PD-1) or programmed death-ligand 1 (PD-L1) has led to marked therapeutic responses among multiple malignancies including ESCC. However, only a few patients achieved clinical benefits due to resistance. Therefore, precise and accurate predictive biomarkers should be identified for personalized immunotherapy in clinical settings. Because the tumor immune microenvironment can potentially influence the patient's response to immune checkpoint inhibitors, tumor immunity, such as PD-L1 expression on tumors, tumor-infiltrating lymphocytes, tumor-associated macrophages, and myeloid-derived suppressor cells, in ESCC should be further investigated. In this review, accumulated evidence regarding the tumor immune microenvironment and immune checkpoint inhibitors in ESCC are summarized.

KEYWORDS

esophageal cancer, immune checkpoint inhibitors, immune microenvironment, squamous cell carcinoma, tumor immunity

1 | INTRODUCTION

Esophageal cancer is the 7th most common cause of cancer-related deaths worldwide.¹ Esophageal squamous cell carcinoma (ESCC) accounts for approximately 90% of all esophageal cancers, which are always in advanced stages upon 1st diagnosis.² Despite the development of multidisciplinary treatments, including surgery, chemotherapy, radiotherapy, and chemoradiotherapy, the prognosis of patients with esophageal cancer remains unfavorable.³ The limited improvement of the treatment outcome with conventional therapies resulted in the search for revolutionary

treatment strategies for ESCC, especially immunotherapeutic-targeted therapies.⁴

In recent years, immunotherapy has been considered an exciting therapeutic strategy for various types of cancers.⁵ It uses the patients' own immune system to fight malignant cells by suppressing the immune checkpoint pathway.⁶ Specifically, the development of monoclonal antibodies inhibiting programmed death 1 (PD-1) or programmed death-ligand 1 (PD-L1) resulted in compelling responses and clinical benefits across multiple malignancies including ESCC.^{7,8} However, the efficacy of these drugs is limited due to refractiveness. Thus, innovative biomarkers should be urgently identified to

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select patients who are likely to respond to these drugs as well as to determine combination therapy to overcome drug resistance. Accumulated evidence revealed that tumor cell-intrinsic factors (eg, PD-L1 expression, tumor mutation load, and microsatellite instability-high status) are relatively correlated with the efficacy of immune checkpoint inhibitors.⁹ In addition, cancer resistance to immune therapy can be caused by extrinsic factors including tumor-infiltrating lymphocytes (TIL), tumor-associated macrophages (TAM), and myeloid-derived suppressor cells (MDSC).¹⁰⁻¹³ Thus, a better understanding on the tumor immune microenvironment (TIME), such as tumor PD-L1 expression, TILs, TAMs, and MDSCs, is increasingly important (Figure 1). In this review, current knowledge on tumor immunity and immune checkpoint inhibitors in ESCC is summarized.

2 | IMMUNE CHECKPOINT INHIBITORS IN ESCC

An escape or evasion of the immune system is now established as one of the hallmarks of cancer.¹⁴ Malignant cells can escape immune destruction by developing mechanisms regularly employed by the immune system to regulate itself. PD-L1 engages PD-1 receptor and induces PD-1 signaling, resulting in T-cell-mediated immune response suppression.¹⁵ Tumor cells can co-opt the PD-1 pathway to evade immune responses by expressing PD-1 ligands on the cell surface and engaging immune effector cells with PD-1 receptor expression. Therefore, the PD-1/PD-L1 pathway has attracted much attention for its roles in tumor immunology and as immune-based therapeutic targets. To date, many clinical trials have focused on immune checkpoint inhibitors including PD-1/PD-L1 inhibitors in ESCC (Table 1).¹⁶

Nivolumab is a high-affinity, humanized IgG4 monoclonal PD-1 antibody.¹⁷ The ATTRACTION-01 trial is a multicenter phase II study assessing the safety and antitumor efficacy of nivolumab in esophageal cancer.⁸ This study included 65 patients with advanced ESCC who were refractory or could not tolerate standard chemotherapy. Treatment-related death was not observed, and only 17 (26%) patients had grade 3-4 adverse events (AEs). The objective response rate was 17%: 8 patients with partial response (PR) and 3 with complete response. The 1- and 2-y overall survival (OS) rates were 45% and 17%, respectively. Currently, results of phase III clinical trial

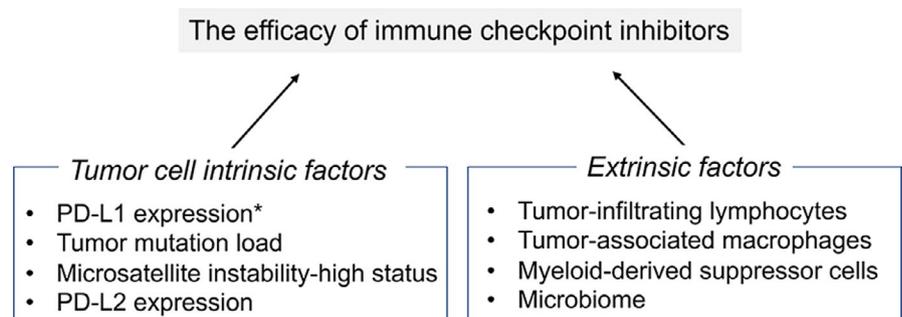
(ATTRACTION-03) comparing nivolumab with docetaxel or paclitaxel in patients with ESCC refractory to fluoropyrimidine and platinum have been released.¹⁸ At a minimum follow-up of 18 mo, the median OS improved from 8 mo in patients randomized to chemotherapy to 11 mo in those randomized to nivolumab, corresponding to a significant 23% reduction in the risk of death (hazard ratio [HR], 0.77; $P = .019$). Collectively, nivolumab might be considered as a new standard 2nd-line treatment strategy to address the high unmet needs of patients with advanced ESCC.

Pembrolizumab is a potent, highly selective, fully humanized IgG4-k monoclonal antibody against PD-1.¹⁷ The KEYNOTE-028 trial is a multicohort phase IB study, including 83 patients with esophageal cancer with standard chemotherapy failure who were PD-L1 positive, and 78% of these patients had ESCC. With a median follow-up of 7 mo, the overall response (OR) rate was 30%, and that in the subgroup of ESCC patients was 28%.¹⁹ Consecutively, the KEYNOTE-180 trial (phase II) further assessed the safety and antitumor activity of pembrolizumab in advanced/metastatic esophageal cancer. Among 63 patients with ESCC, the OR rate was 14%, and the median OS was 7 mo. Only 12% of patients experienced grade ≥ 3 treatment-related AEs, and 1 died of pneumonitis.²⁰ Following the KEYNOTE-180 trial, the KEYNOTE-181 trial evaluated pembrolizumab vs. the investigator's choice of chemotherapy as the 2nd-line therapy for patients with advanced/metastatic esophageal cancers. In the ESCC subgroup, the median OS was 8 mo with pembrolizumab and 7 mo with chemotherapy (HR = 0.78, $P = .0095$).²¹ These trials might indicate that pembrolizumab can be considered as a new 2nd-line treatment for patients with ESCC.

Clinical trials of other anti-PD-L1 antibodies (eg, durvalumab) for esophageal cancer are ongoing. In a phase I trial of durvalumab monotherapy for advanced solid tumors, 7 of 22 patients experienced grade 2 treatment-related AEs, and 1 patient experienced grade 3 AE. One patient had a PR, and the disease control rate at 12 weeks was 36%.²² Currently, several trials investigated the efficacy of durvalumab as a monotherapy for esophageal cancer, or in combination with chemotherapy, chemoradiotherapy, or immunotherapy.²³

In recent years, dual immune checkpoint inhibition and immunotherapy combined with cytotoxic agents are investigated to increase the therapeutic response to immune checkpoint inhibitors for patients with ESCC.²⁴ A randomized phase III study (CheckMate 648)

FIGURE 1 Cancer resistance to immune checkpoint inhibitors can be driven both by tumor cell-intrinsic and tumor cell-extrinsic factors



* promising predictive marker for esophageal cancer patients in KEYNOTE-180 and -181

TABLE 1 Key clinical trials using immune checkpoint inhibitors for esophageal squamous cell carcinoma

Drugs	Trial	Phase	Line	Histology	n	Regimen	Response rate	Median PFS	Median OS	References
Nivolumab	ATTRACTION-01 (ONO-4538-07)	II	≥2	SCC	65	Nivolumab	17%	1.5 mo	10.8 mo	8
Nivolumab	ATTRACTION-03 (ONO-4538-24)	III	2	SCC	419	Nivolumab vs. paclitaxel or docetaxel	19%	1.7 mo	10.9 mo (vs. 8.4 mo; HR 0.77)	18
Pembrolizumab	KEYNOTE-028	IB	≥2	SCC/Adeno (PD-L1-positive tumors only)	23	Pembrolizumab	All: 30% SCC: 28%	1.8 mo	7.0 mo	19
Pembrolizumab	KEYNOTE-180	II	≥3	SCC/Adeno	121	Pembrolizumab	All: 9.9% SCC: 14.3%	All: 2.0 mo SCC: 2.1 mo	All: 5.8 mo SCC: 6.8 mo	20
Pembrolizumab	KEYNOTE-181	III	2	SCC/Adeno	628	Pembrolizumab vs. paclitaxel, docetaxel, or irinotecan	All: 22% ^a SCC: 22% ^a	All: 2.6 mo ^a SCC: 2.1 mo ^a	All: 9.3 mo ^a (vs. 6.7 mo; HR 0.69) SCC: 10.3 mo ^a (vs. 6.7 mo)	21

Abbreviations: Adeno, adenocarcinoma; HR, hazard ratio; OS, overall survival; PFS, progression-free survival; SCC, squamous cell carcinoma.

^aPD-L1 combined positive score (CPS).

of nivolumab plus ipilimumab (anti-CTLA-4 antibody) or nivolumab combined with fluorouracil plus cisplatin vs. fluorouracil plus cisplatin in patients with advanced or metastatic ESCC is ongoing.²⁵ In addition, a randomized phase III trial (KEYNOTE-590) compared fluorouracil plus cisplatin plus pembrolizumab with fluorouracil plus cisplatin as the 1st-line treatment for patients with locally advanced/metastatic esophageal cancer.²⁶

Currently, only anti-PD-1 inhibitors (pembrolizumab and nivolumab) can be used for patients with ESCC in a clinical setting in Japan. Pembrolizumab was granted approval in December 2018 for the treatment of patients with unresectable/metastatic solid tumors harboring high microsatellite instability (MSI) or DNA mismatch repair (MMR) gene deficiency. Nivolumab has been approved in February 2020 for the treatment of patients with unresectable advanced or recurrent esophageal cancer that has progressed following chemotherapy.

3 | TIME

Although immune checkpoint inhibitors have manifested dramatic clinical effectiveness in human malignancies, the majority of patients still showed de novo or adaptive resistance.²⁷ Therefore, appropriate biomarkers to select patients who are likely to respond to these drugs as well as combination therapy to overcome resistance should be found. Cancer resistance to immune checkpoint inhibitors can be driven both by tumor cell-intrinsic (eg, PD-L1 expression, tumor mutation load, microsatellite instability-high status) and extrinsic factors (eg, TILs, TAMs, MDSCs) that contribute to immune evasion.¹⁰ Thus, the concept of TIME has attracted increasing interests for developing and optimizing immunotherapeutic approaches, identifying predictive biomarkers, and selecting the most appropriate treatment approach for a given ESCC patient (Table 2). The schematic view of TIME is shown in Figure 2. Recently, as will be described later, 4 different types of TIME have been proposed based on the presence or absence of TILs and PD-L1 expression on tumor cells.²⁸ This stratification may shed light on novel therapeutic approaches for rationally designing idealized combination therapies based on tumor immunology.²⁹

4 | PD-L1 EXPRESSION ON CANCER CELLS

PD-L1 expression on tumor cells is one of the most reasonable predictive biomarkers for the therapeutic efficacy of immune checkpoint inhibitors.³⁰ In fact, the predictive role of PD-L1 expression for PD-1/PD-L1 inhibitors has been reported in many types of cancers.³¹ Regarding esophageal cancer, in the KEYNOTE-180 trial, PD-L1 expression was evaluated using the combined positive score (CPS), defined as the number of PD-L1-positive cells (tumor cells, macrophages, and lymphocytes) divided by the total number of tumor cells.²⁰ In this trial, participants with high PD-L1 expression had a higher 1-y OS rate (35%) than those with PD-L1 low expression

TABLE 2 Characteristics of TIME in esophageal squamous cell carcinoma

TIME factor		Characteristics
Tumor cell-intrinsic	PD-L1	Probable predictive marker for PD-1 inhibitors in esophageal cancer (KEYNOTE-180 ²⁰ and -181 ²¹) Controversial as prognostic marker. In our 305 ESCC cohorts, PD-L1 expression was associated with an unfavorable prognosis ³²
	PD-L2	PD-L2 expression is associated with an unfavorable clinical outcome ³⁷
	MSI	Pembrolizumab can be used for MSI-high ESCC tumors. The frequency of MSI-H tumors in esophageal cancer is less than 2%. ⁶⁷
	TMB	A large proportion of esophageal cancer patients have tumors with high TMB. ⁶⁸ Predictive role for immune checkpoint inhibitors is unknown in ESCC
Extrinsic	TILs	Predictive role for immune checkpoint inhibitors is unknown in ESCC Peritumoral TILs is associated with favorable prognosis in ESCC. ⁴⁴ CD8 ⁺ , FOXP3 ⁺ TILs are associated with patient outcomes, ^{46,47} while CD3 ⁺ or CD45RO ⁺ TILs are associated with patient survival ⁴⁷ A stratification based on PD-L1 expression and TIL status is associated with overall survival ³²
	TAM	High TAM density is associated with shorter survival ⁵² TAMs elevate the PD-L1 expression in ESCC cells ⁵²
	MDSC	High infiltration of MDSCs is associated with poor prognosis in patients with esophageal cancer ⁵⁴
	DC	Mature LAMP3 ⁺ DCs is associated with increasing CD8 ⁺ TILs in ESCC ⁵⁸
	NK cells	Intratumoral NK cell infiltration is associated with a favorable outcome ⁶²

Abbreviations: DC, dendritic cells; ESCC, esophageal squamous cell carcinoma; MDSC, myeloid-derived suppressor cells; MSI, microsatellite instability; NK cells, natural killer cells; TAM, tumor-associated macrophages; TILs, tumor-infiltrating lymphocytes; TMB, tumor mutation burden.

(22%). The phase III KEYNOTE-181 trial demonstrated that patients with PD-L1 high expression (ie, CPS \geq 10) had a median OS of 9.3 mo with pembrolizumab vs. 6.7 mo with chemotherapy (HR = 0.69), supporting that pembrolizumab as a new 2nd-line standard therapeutic option for esophageal cancers with high expression of PD-L1.²¹ Therefore, in the United States, the Food and Drug Administration (FDA) has approved pembrolizumab monotherapy for patients with recurrent, locally advanced or metastatic ESCC expressing PD-L1 (CPS \geq 10), as determined by an FDA-approved test, experiencing disease progression after 1 or more prior lines of systemic therapy in July 2019.

PD-L1 expression can not only be a predictive marker but also a prognostic marker in human cancers. Several studies focusing on the relationship between PD-L1 expression and clinical outcome in esophageal cancer yielded inconsistent results. In majority of these studies, PD-L1 overexpression was associated with poor clinical outcomes.³² However, 2 other studies demonstrated that PD-L1 overexpression was related to favorable prognosis.^{33,34} In a previous study, using a non-biased database of 305 curatively resected esophageal cancers, PD-L1

expression was found to be associated with an unfavorable clinical outcome in esophageal cancer, supporting its role as a prognostic biomarker.³²

5 | PROGRAMMED DEATH-LIGAND 2 EXPRESSION ON CANCER CELLS

Programmed death-ligand 2 (PD-L2) also engages the PD-1 receptor to induce PD-1 signaling and associated T-cell exhaustion as well as reversible inhibition of T-cell activation and proliferation. Given that PD-L2 demonstrates higher affinity for PD-1 compared with PD-L1,³⁵ the expression levels of PD-L1 as well as PD-L2 in ESCC may serve as predictive biomarkers for the utility of immune checkpoint inhibitors. In head and neck squamous cell carcinoma, PD-L2 status has been reported to be a significant predictor of progression-free survival with pembrolizumab independently of PD-L1.³⁶ We have recently reported that the expression of PD-L2 as well as PD-L1 is associated with unfavorable clinical outcomes in esophageal cancer.³⁷ We have also found that PD-L2 and PD-L1 exhibit

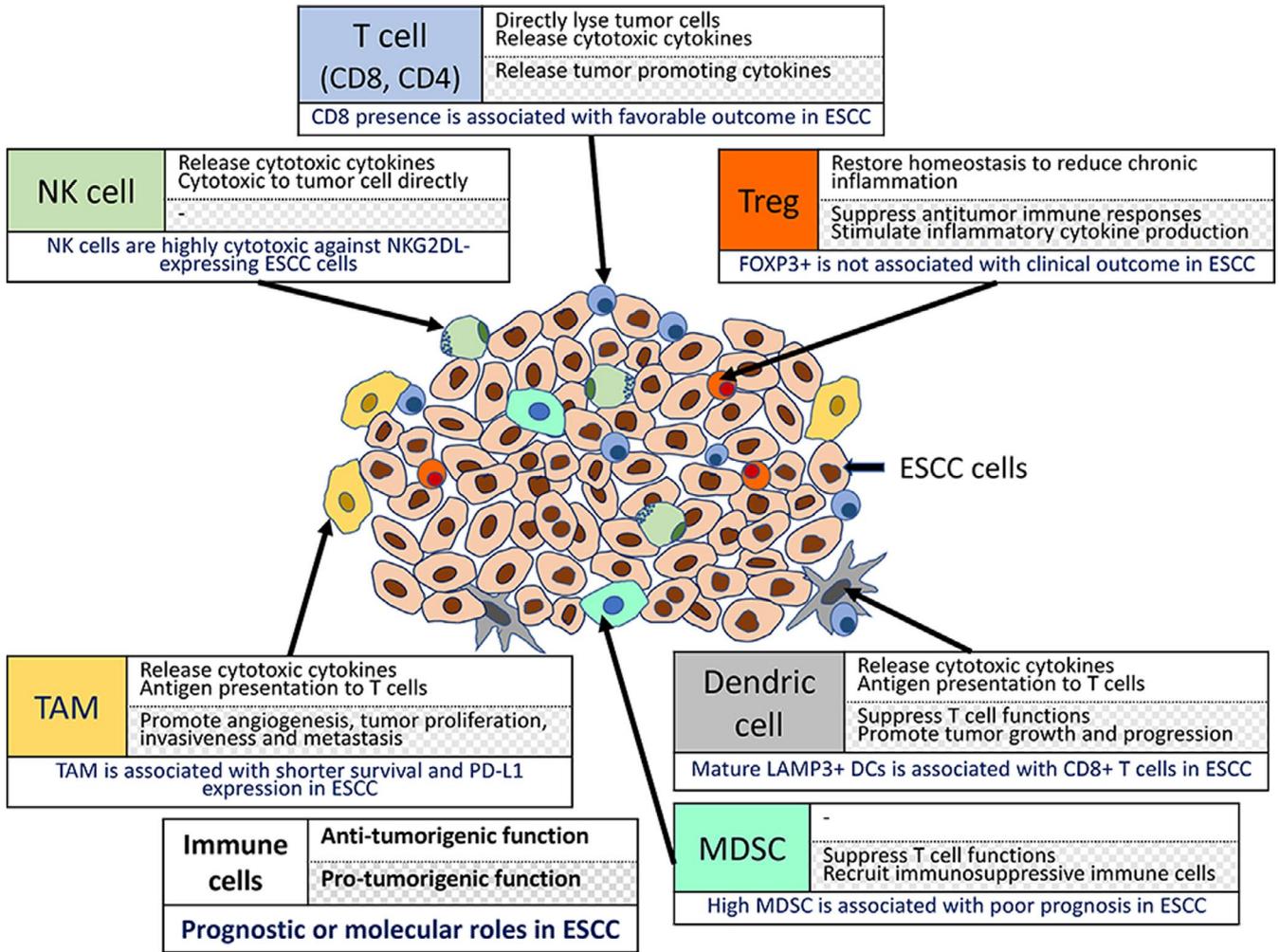
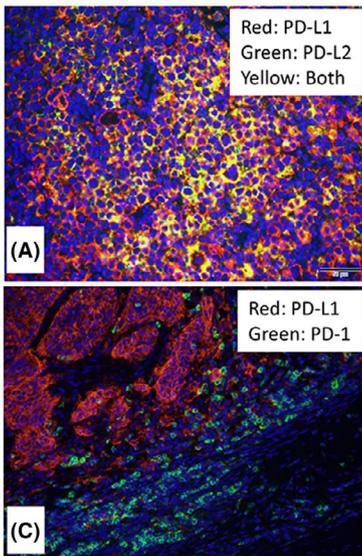
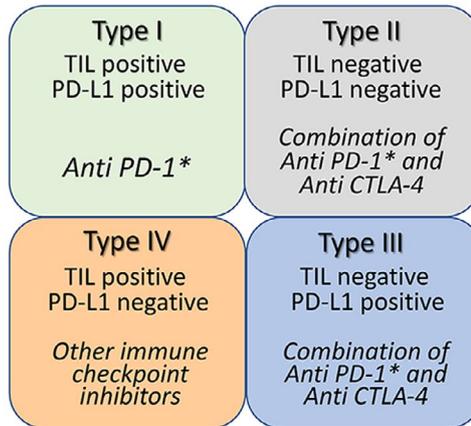


FIGURE 2 Schematic view of the tumor immune microenvironment. MDSC, myeloid-derived suppressor cell; TAM, tumor-associated macrophages

Multiple immunofluorescence using ESCC tissues



Subtypes based on TIL and PD-L1 status



*approved for ESCC in Japan (as of May 2020)
Pembrolizumab (MSI-high tumors)
Nivolumab

FIGURE 3 A, Multiplex immunofluorescence for PD-1 and PD-L2 expression on tumor cells B, Four subtypes of tumor immune microenvironments based on the presence or absence of tumor-infiltrating lymphocytes (TILs) and PD-L1 expression. C, Multiplex immunofluorescence for PD-1 expression on TILs and PD-L1 status on tumor cells

distinct expression patterns during tumor development and variations in responses to chemotherapeutic agents. Simultaneous evaluation of PD-L2 along with PD-L1 may result in an increased number of patients indicated for inhibitors of PD-1/PD-L1 signaling; these therapeutic approaches may yield favorable outcomes in patients negative for PD-L1 and positive for PD-L2 (Figure 3A).

6 | HUMAN LEUCOCYTE ANTIGEN IN ESCC

Variations in the expression of human leucocyte antigens (HLA), which play a role in the presentation of tumor antigens to T cells, are involved in human cancers by influencing host defenses against tumor development. Class I HLA genes (eg, *HLA-A*, *HLA-B*, and *HLA-C*) encode proteins expressed on the surface of all nucleated cells, which present intracellular peptides to CD8⁺ T cells. Class II HLA genes (eg, *HLA-DR*, *HLA-DQ*, *HLA-DP*, *HLA-DM*, *HLA-DOA*, and *HLA-DOB*) encode proteins expressed only on the surface of antigen-presenting cells, which serve as crucial restriction elements for the induction and proliferation of CD4⁺ T cells. Several studies focusing on the prognostic significance of HLA expression patterns in patients with ESCC have reported that alterations in *HLA-A*, *HLA-B*, *HLA-C*, *HLA-F*, *HLA-DQA1*, and *HLA-G* are correlated with survival in patients with ESCC.³⁸⁻⁴⁰ Circulating CD14⁺, *HLA-DR*^(-/low) MDSCs are an indicator of poor prognosis in patients with ESCC.⁴¹ Interestingly, high PD-L1 expression was a significant independent prognostic factor in patients with ESCC and high HLA class I expression.⁴² In a preclinical study, MiR-148a was shown to modulate *HLA-G* expression and influence tumor cell apoptosis in an ESCC model.⁴³

7 | TILs

The density of TILs at the invasive tumor margin may predict the response to immune checkpoint inhibitors.¹⁰ Morphological lymphocytic reaction observed by pathological examination may be an indicator of host immune response to tumor cells. In a recent study, 4 morphological components of lymphocytic reactions (ie, peritumoral reaction, intra-nest reaction, lymphoid reaction, stromal reaction) to tumors were evaluated in patients with esophageal cancer.⁴⁴ Only peritumoral reaction among the 4 components was associated with patient prognosis (multivariate *P* for trend < .001): patients with higher peritumoral reaction experienced significantly longer OS than those with lower reaction (multivariate HR: 0.48; *P* < .001). We of course understand that the subtyping of TILs might provide any additional information beyond the morphological or histopathological evaluation of lymphocytic reaction patterns. Effector/cytotoxic (CD3⁺ and CD8⁺) and memory (CD45RO⁺) T cells play crucial roles in antitumor immune response. FOXP3⁺ T cells (regulatory T cells) have been shown to modulate antitumor immune response and suppress the activity of cytotoxic T cells. Thus, these specific

subsets of effector/cytotoxic (CD3⁺ and CD8⁺), memory (CD45RO⁺), and regulatory (FOXP3⁺) T cells are thought as indicators of host immune response to tumor cells and might be a target for immunotherapy.⁴⁵ Previous studies on esophageal cancer have reported that the presence of infiltrates or their localization by a specific subtype of TILs (eg, CD8⁺, FOXP3⁺ lymphocytes) is associated with patient outcomes.^{46,47} Conversely, other TIL subsets including CD3⁺ or CD45RO⁺ lymphocytes appear not to be associated with patient survival.⁴⁷

Recent research has described 4 different types of TIME based on the presence or absence of TILs and PD-L1 expression²⁸: type I (PD-L1-positive with the presence of TILs, driving adaptive immune resistance), type II (PD-L1-negative without TIL, indicating immune ignorance), type III (PD-L1-positive without TIL, indicating intrinsic induction), and type IV (PD-L1-negative with the presence of TIL, indicating the role of other suppressor(s) in promoting immune tolerance; Figure 3B). This proposed classification of TIME types may be important in designing optimal immunotherapeutic strategies.^{28,29} Type I tumors are most likely to benefit from single-agent PD-1/PD-L1 inhibitors, since such tumors possess preexisting TILs turned off by PD-L1 engagement. In type II group, a single-agent checkpoint blockade would most likely not be beneficial given the lack of preexisting TILs. For such a situation, to bring T cells into tumors and then prevent them being turned off, the combination of anti-CTLA-4 and anti-PD-1 may be promising. In the type III group, PD-L1 positivity alone cannot be considered as a predictive factor for response to anti-PD-1 or anti-PD-L1 therapies, because without TILs in the tumor, blocking PD-1 or PD-L1 will be unlikely to lead to a T-cell response to tumor cells. Therefore, a similar approach for type II patients might be employed to try to recruit lymphocytes into tumor cells. In the type IV group, considering that many tumors are heterogeneous with respect to the proportion of lymphoid and myeloid cells, other suppressive pathways might prevail. Although the classification of TIME types is expected to facilitate the development of optimal immunotherapeutic strategies for patients with ESCC, immunotherapeutic drugs available in a clinical setting are limited in Japan. The prompt availability of other immunotherapeutic drugs is therefore necessary for patients with ESCC.

With the examination of these subtypes as a predictive marker of immunotherapeutic response, the prognostic impact of this classification has also been examined. Regarding esophageal cancer, 305 patients were placed into 4 groups based on PD-L1 expression and TIL status and it was found that these subgroups possessed diverse prognostic features. Among PD-L1-positive cases, the disease-free survival (DFS) in TIL-positive cases (ie, type I) was significantly better (log-rank *P* = .019) than that in TIL-negative cases (ie, type III). Similarly, among PD-L1-negative cases, DFS was significantly better in TIL-positive cases (ie, type IV) (log-rank *P* < .0001) than that in TIL-negative cases (ie, type II).³² Considering the role of PD-1 on TILs in the antitumor immune response, a better understanding of these subgroups defined by PD-1 expression on TILs and PD-L1 status on tumor cells may have clinical implications (Figure 3C).

8 | TAMS

Macrophages have a crucial role in phagocytosis, antigen presentation, and cytokine and/or growth factor production and are promising effectors of cancer immunotherapy. In response to microenvironmental stimulations, macrophages polarize into anti-tumorigenic M1 or pro-tumorigenic M2 phenotype. TAMs are defined as macrophages located in, or at the close vicinity of, the tumor. As TAMs can produce various pro-tumorigenic factors, including growth factors, cytokines, and proteases, they can be attractive targets for recalibrating the immune response within TIME.¹¹ For example, by secreting cytokines such as colony-stimulating factor-1 (CSF-1), tumor cells can recruit M2 macrophages and support tumorigenesis.¹¹ Recently, TAM-targeting therapies such as CSF-1/CSF-1R blockade have gained attention in cancer research.⁴⁸ An ongoing clinical trial is currently evaluating the combination of CSF-1R antagonists with PD-1/PD-L1 inhibitor (NCT02323191). In this respect, clarifying the relationship between TAMs and PD-1/PD-L1 expression is imperative.

The presence of TAMs has been correlated with poor prognosis in various types of human cancers.⁴⁹⁻⁵¹ Recently, high TAM density in esophageal cancer tissues is reportedly associated with shorter survival, suggesting a prognostic biomarker role of TAMs. In addition, our experimental studies showed that cell invasion and migration ability were significantly more upregulated in esophageal cancer cell lines co-cultured with activated macrophages than that in control cell lines. Furthermore, co-culture with activated macrophages elevated the PD-L1 expression in cancer cells.⁵² Given the significant interest in cancer immunotherapies targeting TAMs and PD-L1, our findings might have considerable clinical implications.

9 | MDSCs

MDSCs have been extensively investigated as one of the most crucial immunosuppressive cells in TIME.⁵³ They may drive tumor progression through cytokine and chemokine secretion with pro-tumorigenic functions, depending on TIME. High infiltration of MDSCs has been reportedly associated with poor prognosis in patients with esophageal cancer.⁵⁴ In ESCC, IL-6 or other signaling pathways mediated by aldehyde dehydrogenase can regulate the activation of MDSCs.⁵⁵ A previous study showed that MDSCs were heterogeneous, and CD38 could serve as a marker for MDSCs with increased immunosuppressive ability in esophageal cancer.⁵⁶ Various therapeutic approaches are designed targeting MDSCs in an attempt to eradicate cancer cells.⁵⁷ Some of these approaches are currently undergoing clinical trials to estimate the efficacy and safety of their application in cancer patients.

10 | DENDRITIC CELLS

Dendritic cells (DCs) play a crucial role in a forefront of an immune response due to their advanced ability to recognize foreign

antigens and mobilize naive T cells to effectors. Thus, DCs are recognized a promising target to activate the immune system in immunotherapeutic strategies against malignant cells. In the last decade, many researchers have tried to develop immunotherapeutic strategies against human cancers through vaccination. A cluster of LAMP3⁺ DCs appeared to be the mature form of conventional DCs and express diverse immune-relevant ligands and potentially regulate multiple subtypes of lymphocytes.³⁵ In ESCC, mature LAMP3⁺ DCs has been reportedly associated with increasing tumor-infiltrating CD8⁺ T cells.⁵⁸ Recently, promising findings of a phase I study on LV305, an engineered harmless virus targeting DCs, have been released.⁵⁹ LV305 upregulated the expression level of the New York ESCC-1 (NY-ESO-1) cancer testis antigen in DCs, promoting immune responses against NY-ESO-1-expressing tumors.⁶⁰ All treatment-related AEs were grade 1 or 2. The disease control rate was 56% in all patients.⁵⁹ Further investigation of LV305 is continuously explored, possibly in combination with a boosting vaccine and/or other agents, such as immune checkpoint inhibitors.

11 | NATURAL KILLER (NK) CELLS

NK cells are innate immune cells with potent cytolytic activity against tumors and in addition act as regulatory cells for the immune system. Based on the interesting concept of utilizing such innate immune system effectors, the adoptive NK cell therapy or the use of monoclonal antibodies targeting the main NK cell immune checkpoints is being investigated.⁶¹ The clinical efficacy of T-cell-based immunotherapy (eg, PD-1/PD-L1 inhibitors) presents some limitations, including its inability to recognize and kill HLA-I^{neg} tumor cells. Blockade with NK cell checkpoint inhibitors reversing their functional block may overcome such limitations of T-cell-based immunotherapy, mainly against HLA-I^{neg} tumor targets.⁶¹ Therefore, NK cells are attracting attention as a promising target for cancer immunotherapy. In ESCC, intratumoral NK cell infiltration is reportedly associated with a favorable outcome.⁶² Lim et al have shown that their expanded NK cells are highly cytotoxic against NKG2DL-expressing ESCC cells, suggesting a strong rationale for its clinical use in patients with ESCC.⁶³

12 | MICROSATELLITE INSTABILITY IN ESCC

MSI is a strong mutator phenotype due to genetic alterations caused by genetic and epigenetic inactivation of DNA MMR genes.⁶⁴ MMR-deficient tumors harbor a high mutational burden, which translates into the production of tumor neoantigens which evade immune response through the upregulation of immune checkpoint proteins.⁶⁴ MSI status is a promising predictive marker for treatment with immune checkpoint inhibitors. Le et al reported that patients with MMR-deficient tumors who were treated with pembrolizumab achieved

better outcomes across 6 cancer types.⁶⁵ The observed efficacy of pembrolizumab was further confirmed in patients with MMR-deficient tumors across 12 types of cancer, including esophageal cancer.⁶⁶ A functional study in a patient with complete response revealed the quick expansion of T-cell clones responsive to mutated neoantigens detected in the tumor. Based on these results, the FDA granted the 1st tissue/site-agnostic approval for the use of pembrolizumab in patients with unresectable/metastatic solid tumors harboring MSI-high or MMR-deficient tumors in 2017. However, the frequency of MSI-high tumors in esophageal cancer is less than 2%.⁶⁷

13 | TUMOR MUTATION BURDEN IN ESCC

Tumor mutation burden (TMB) is defined as the total number of mutations, including both base substitutions and short insertions/deletions, per coding area of a tumor genome. Although not all mutations generate neoantigens, more somatic mutations can lead to more neoantigens, more tumor-infiltrating T cells, and a stronger antitumor immune response. Thus, TMB can be used to predict immune checkpoint inhibitor efficacy and has become a useful biomarker across many cancer types for the identification of patients who will benefit from immunotherapy. Interestingly, a large proportion of patients with esophageal cancer harbor tumors with high a TMB.⁶⁸ Greally et al examined the relationship between TMB and survival in 89 patients with esophagogastric cancer treated with immunotherapy and found that TMB was associated with a significant improvement in OS in univariate analyses.⁶⁹ However, the observed association did not persist after adjusting for other risk factors and after the exclusion of MSI tumors. The merits of TMB as a clinically useful biomarker to guide treatment with immune checkpoint inhibitors in patients with ESCC require further prospective studies.

14 | FUTURE PERSPECTIVES

Predicting responses using a single biomarker is difficult due to the complexity of immune response in tumors. Thus, a comprehensive assessment of TIME as a dynamic spatiotemporal process is crucial for further development of immunotherapy in ESCC. Nomogram, a tool for integrating multiple variables based on mathematical models, may be useful to establish a model based on scores.⁷⁰ This hypothetical model for patients with ESCC may entirely or partly include the following variables: PD-L1 and PD-L2 expression, MSI, TMB, and immune cell status (ie, TILs, TAMs, MDSCs, DCs, and NK cells) as well as serum markers and clinical and pathological factors. Another potential approach is machine learning using artificial intelligence (AI). AI can digitize whole-slide images of tissue samples and enable an accurate and reproducible means for the unbiased assessment of regularities in the expression of immunohistochemical markers, tumor morphology, and TILs.⁷¹ The ability of machine learning tools to detect key features in complex immunophenotypic datasets underlines their potential importance for the development of novel

predictive models in cancer immunotherapy. Organ-specific cancers present as different diseases in terms of their pathogenesis, tumor biology, and TIME and therefore require different immunotherapeutic approaches and predictive biomarkers. Unfortunately, the above-mentioned approaches such as nomogram and AI have not yet been applied in clinical research aimed at ESCC, highlighting the need for further clinical studies.

15 | CONCLUSION

Over the last decade, our understanding on mechanisms underlying TIME in ESCC has rapidly improved, allowing the phenomenal development of cancer immunotherapy. Challenges in moving forward are to put much effort into the immunologic and biologic exploration in ESCC setting to, more precisely, tailor various available or emerging immunotherapeutic approaches. In the near future, large prospective trials should be designed and conducted to validate reliable predictive biomarkers, allowing the selection of patients with ESCC with the highest chance of benefiting from immunotherapy. Ongoing intensive efforts to establish biomarkers for immunotherapy response prediction hold great promise to maximize patient benefits from an immunotherapeutic approach including immune checkpoint inhibitor.

CONFLICT OF INTEREST

The authors have no conflict of interest.

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