Combination of checkpoint inhibitors with radiotherapy in esophageal squamous cell carcinoma treatment: A novel strategy (Review)

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Abstract. Despite the rapid development of numerous types of treatment, including radiotherapy (RT) as the main strategy, esophageal squamous cell carcinoma (ESCC) has a poor prognosis. Recent studies demonstrated that immunotherapy can improve the survival of patients with locally advanced and metastatic ESCC. Furthermore, previous studies reported that the expression of programmed death-ligand 1 is significantly associated with esophageal cancer prognosis. At present, several ongoing clinical trials have extended the use of immunotherapy from palliative and salvage treatments to neoadjuvant treatment with concurrent chemoradiation. The first- or second-line treatments were used to explore antitumor efficacy with reduced adverse events. The combination of RT and immunotherapy can exert a local therapeutic effect and improve the function of the immune system, enhancing antitumor efficacy. This review investigated the role of immunotherapy and radiotherapy in ESCC and described the potential efficacy of combining immunotherapy with radiotherapy in ESCC.

Contents

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1. Introduction

Esophageal cancer (EC) is the sixth leading cause of cancer-associated mortality worldwide due to its highly aggressive nature and poor prognosis (1). Although the incidence of esophageal adenocarcinoma and esophagogastric junctional carcinoma has increased in the United States and Europe, esophageal squamous cell carcinoma (ESCC) still accounts for ~78% of EC cases (2,3). At present, the standard therapy for ESCC includes surgery, radiotherapy and chemotherapy (4). Despite the use of multidisciplinary therapies, the prognosis of patients with ESCC remains poor. The overall survival (OS) rate at 5 years is only 30-40% for ESCC cases due to primary site tumor recurrence, metastasis development and treatment complications (5). It is therefore crucial to determine novel and effective treatment strategies for ESCC.

Recently, the application of next-generation sequencing in ESCC allowed for the identification of several processes that may contribute to carcinogenesis and disease prognosis, including driver gene mutations, changes in molecular and protein dynamics, dysregulation of cellular signaling pathways and alterations of the tumor microenvironment (6-8). Furthermore, it has been demonstrated that molecular targeted therapy can provide effective treatment in several types of cancer, including lung cancer and colon cancer (9,10). However, the benefits from this type of therapy on the development of locally advanced and metastatic ECs is lower than expected (11-14). The successful use of immune-checkpoint inhibitors (ICI), including monoclonal antibodies against programmed cell death 1 (PD-1), has considerably improved the prognosis of various types of malignancy, including melanoma and non-small cell lung cancer (15,16). Previous clinical trials reported promising antitumor activity of anti-PD-1-mAb in the treatment of ESCC (17-19). In addition, numerous phase II/III clinical trials examined whether combining immunotherapy with radiotherapy (RT) could enhance anti-tumor effects (20,21). However, the application of combined therapy in cancer requires further investigation.

2. RT in ESCC

At present, RT remains the main treatment for ESCC. RT results in a significant reduction in local tumor growth and simultaneously relieves dysphagia (22,23). The use of ionizing radiation on local tumor cells leads to direct or indirect DNA damage and induces a series of molecular events associated with cell death (24). It has been suggested that the combination of RT and immunotherapy can enhance treatment efficacy in non-small cell lung cancer and melanoma brain metastases (25). Tumor cells release tumor-associated antigens and cytokines, including interferon-γ and tumor necrosis factor-α, which modulate the tumor immune microenvironment and subsequently target dendritic cells (DC) (26,27). This phenomenon increases the expression of molecules of the major histocompatibility complex I and causes the upregulation of programmed death ligand 1 (PD-L1) in dendritic cells (26,27). However, RT also accelerates the production of regulatory T cells (Tregs) in systemic and intratumoral sites where Tregs acquire subsequently a highly suppressive phenotype (28). Subsequently, reduced radiation-induced tumor death can contribute to tumor escape from the host immune surveillance, and can suppress the antitumor immune response (29,30) (Fig. 1).

3. Immunotherapy in ESCC

PD-L1 expression in tumor cells is associated with patients' prognosis. The development of immunotherapy has revolutionized cancer treatment (31). T lymphocytes are activated by the adaptive immune response during malignant progression. However, tumor cells have the capacity to frequently escape immune surveillance by controlling the checkpoint pathways, which can result in T cell function suppression, and ultimately leads to local invasion of the tumor and metastasis (32). PD-1 is expressed in various types of immune cell, including T cells, B cells, dendritic cells and tumor-infiltrating lymphocytes (33). PD-L1 binds to PD-1 and is expressed in tumor cells and antigen presenting cells (APCs). The interaction of PD-L1/PD-1 can usually inhibit the efficiency of T-cell activation and the induction of cell apoptosis (34). The use of anti-PD-1 and anti-PD-L1 monoclonal antibodies (mAbs) is therefore an effective way to maintain the activation of the effector function of $CD8^+$ T cells, and to improve the clinical outcome of patients with ESCC (35).

Numerous studies have demonstrated that PD-L1 tumor expression is associated with disease prognosis in patients with ESCC. The majority of these studies reported that high expression of PD-L1 is associated with poor prognosis in these patients (36-40). Wang *et al* (38) recruited 180 patients with ESCC and reported that patients with high expression of PD-L1 exhibited worse clinical outcome compared with patients with low expression (P=0.0010). In addition, this study by Wang *et al* (38) reported that the number of $CD8^+$ T cells is lower in ESCC tissues compared with normal tissues (P=0.0346), and the results suggested that PD-L1 expression may be considered as a predictive factor for OS (P=0.0114). A meta-analysis came to a similar conclusion, with the results suggesting that PD-L1 overexpression is associated with unfavorable outcomes and lower OS in patients with ESCC, notably in Eastern Asian countries such as China, Japan and South Korea [hazard ratio=1.43; 95% confidence interval (CI)=1.10-1.88] However, a limited number of studies reported that increased PD-L1 expression is associated with improved disease-free survival and OS (41,42). This controversy may be attributed to numerous factors, including different methodological approaches, different assessment criteria to define high PD-L1 expression and heterogeneity of PD-L1 expression. These factors may result in differing detection of infiltrating lymphocytes in tumor from the biopsy or the postoperative pathological specimens. However, staining cut-off values tumor proportion score (TPS) of 1 or 5% are frequently used to define the positive rate of PD-L1 expression. Various studies have defined the cut-off values differently. Borghaei *et al* (43) and Katsuya *et al* (44) defined a positive tumor PD-L1 protein expression as an incidence of TPS ≥1%, whereas other studies used TPS ≥5% as the threshold (45-47).

Anti-PD-1 and anti-PD-L1 mAbs in ESCC. At present, anti-PD-1 agents are approved by the Food and Drug Administration (FDA) for the treatment of melanoma and non-squamous cell lung cancer (48). Numerous anti-PD-1 antibodies, including pembrolizumab and nivolumab, anti-PD-L1 antibodies, including durvalumab, have demonstrated promising antitumor activity in advanced ESCC (Table I). The multicohort KEYNOTE-028 study investigated the use of pembrolizumab monotherapy for the treatment of advanced esophageal carcinoma (49). Preliminary results reported that 41% of patients had PD-L1 upregulation in tumors, and among these patients, the objective response rate (ORR) was 23% (49). Updated versions of this study reported promising antitumor effects of pembrolizumab monotherapy, with a response rate of 28% (5/18) for tumors exhibiting squamous histology, and a partial response of 30% for the samples (50,51). In addition, the median duration of response was 15 months (range, 6 to ≥26 months), the OS was 7 months, and the median progression free survival (PFS) was 1.8 months. In a phase II study using nivolumab administration for refractory ESCC, the median follow-up was 10.8 months (18). Another ongoing clinical trial, KEYNOTE 181, is currently evaluating the efficacy of an anti-PD-1 mAb in disease progression of patients with advanced ESCC following chemotherapy as first-line therapy (52). The preliminary results demonstrated that immune-related adverse events included rash (13%), decreased appetite (9%) and decreased lymphocyte count (9%). No treatment-associated mortality was reported (52). The KEYNOTE-180 study evaluated the efficacy of pembrolizumab in patients with metastatic EC, including ESCC (53). A total of 121 patients were enrolled in this trial, including 63 patients (52%) with ESCC and 58 patients (48%) with ESCS and PD-L1 overexpression. The results revealed that the median PFS was 2 months (95% CI, 1.9-2.1) and the ORR was 14% (95% CI, 5-17%) in patients with high PD-L1 expression. The comparison of the two trials revealed that the number of adverse events reported in the KEYNOTE-180 study was higher than in the KEYNOTE-028 study. In the latter trial, treatment-related grade 3-5 adverse events were observed in 15 patients (12%). Among these patients, 5 patients (4%) discontinued treatment and 1 patient died due to treatment-associated pneumonitis.

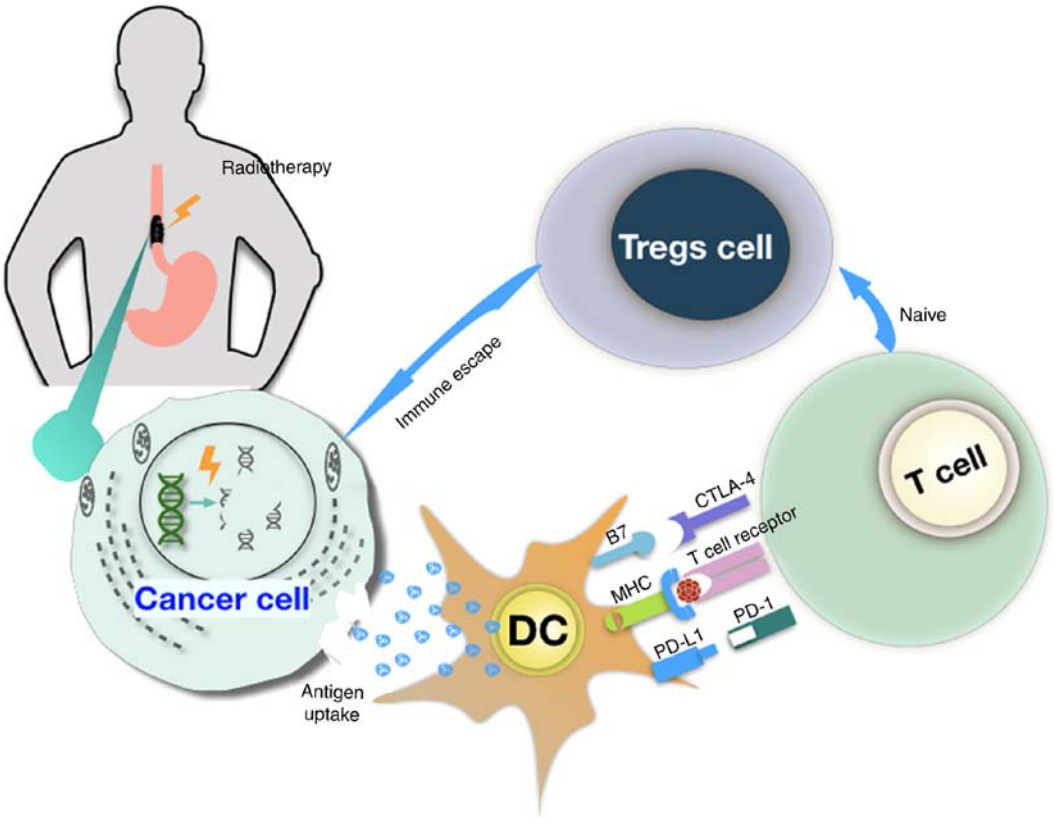

Figure 1. Radiotherapy accelerates the production of Tregs, reduces radiation-induced tumor death and contributes to tumor escape from immune surveillance. These events suppress the antitumor immune response. B7 is a peripheral membrane protein found on activated antigen presenting cells which interaction with CTLA-4 on T cells promotes antitumor immunity. Tregs, regulatory T cells; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; DC, dendritic cell; MHC, major histocompatibility complex; PD-1, programmed cell death 1; PD-L1, programmed death-ligand 1.

A previous similar study (ONO-4538-07) reported that nivolumab therapy had promising activity in patients with ESCC who were refractory or intolerant to chemotherapy (54). The results, based on a 2 year follow-up, reported that the ORR was 17.2% (95% CI, 9.9-28.2) and that 3 patients had a complete response (54). The toxicity observed with nivolumab was higher than perbrolizumab, and the majority of the patients exhibited grade 3-4 adverse events following nivolumab treatment. A total of 7 patients (10.8%) discontinued the treatment due to drug-associated adverse events. A recent study, CHECKMATE-032 (ClinicalTrials.gov Identifier code, NCT01928394), demonstrated that the combination of nivolumab and ipilimumab significantly improved the median OS and ORR of patients with advanced EC (55). However, this combined treatment resulted in higher toxicity compared with PD1 or PD-L1 blockade as monotherapy, with adverse effects including diarrhea and increased levels of alanine aminotransferase and aspartate transaminase in the serum (55).

A recent ambitious trial investigated pembrolizumab as an alternative treatment for the second-line strategy reported in the KEYNOTE-181 study (52). A phase III clinical trial (KEYNOTE-590/MK-3475-590) will assess the efficacy of two different groups [pembrolizumab+cisplatin+5-fluorouracil (5-FU) vs. placebo+cisplatin+5-FU] in patients with esophageal neoplasms. The two treatments will be compared with pembrolizumab, which is used as the first-line treatment for locally advanced or metastatic EC. Early results of PFS and OS of patients are expected in 2021. Furthermore, preliminary results from clinical trials demonstrated that other anti-PD-L1 mAbs, including avelumab, durvalumab (56) and atezolizumab, have some potential antitumor activity in patients with previously-treated advanced gastric/gastroesophageal junction/esophageal (G/GEJ/E) cancers (57-59). In addition, avelumab treatment resulted in a similar ORR (15%) compared with findings from Taieb *et al* (60).

4. Combination of RT and immunotherapy in ESCC

The association between RT efficacy and the immunomodulatory effects on metastatic carcinoma cells was initially described by Mole in 1953 as the 'abscopal' effect (61). In this phenomenon, local tumor irradiation can cause metastasis regression in sites distant from the irradiated area, this rare abscopal effect has only been reported for a few metastatic solid tumors following radiotherapy treatment (62,63). Previous studies reported that the combination of immunotherapy with RT has additional efficacy in solid tumors (25,64-66). Retrospective studies, including 23 case reports of lymphoma and solid malignancy, revealed that the combination of RT and immunotherapy can enhance treatment efficacy (67,68). Furthermore, clinical studies reported an abscopal effect from primary tumor cell irradiation in metastatic carcinoma (69-71). Tumor-associated antigens are released from tumor cells following exposure to radiation, and are taken up by APCs, which cause priming and activation of cytotoxic T cells.

Table I. Clinical trials using immune checkpoint blockade in esophageal cancer.

Clinical trial registration number	Target	Agent	Treatment	Histological types/PD-L1 status	Number of patients	RR, %	PFS		OS		Common Terminology Criteria for Adverse Events [version 5.0 (120)], %	Condition or disease	(Refs.)
							Median time, months	Survival rate by time point, %	Median time, months	Survival rate by time point, %			
ATTRACTION-01/ONO-4538-07	PD-1	Nivolumab	3 mg, iv, Q2W	ESCC	64	17.2	NS	12-mo, 10.3 18-mo, 6 24-mo, 6	NS	12-mo, 45.3 18-mo, 25 24-mo, 7.2	Grade 3-4, 29.2% Discontinued, 10.8% No treatment-related death	Refractory, intolerant to standard chemotherapy ESCC	(54)
KEYNOTE-180	PD-1	Pembrolizumab	200 mg, iv, Q3W	ESCC PD-L1$^+$ PD-L1$^-$	121	10 14 6	2	6-mo, 16	5.8	12-mo, 28	Grade 3-5, 12%	At least two lines of prior therapy or metastatic EC	(53)
KEYNOTE-028	PD-1	Pembrolizumab	10 mg, iv, Q2W	PD-L1$^+$	23	30	1.8	6-mo, 30 12-mo, 22	7.0	6-mo, 60 12-mo, 40	Grade 3, 17%	Failure of standard therapy EC	(50)
CheckMate 032	PD-1	N3	N3, Q2W	GA/EC/GEJ	59	12			6.2	12-mo, 39 18-mo, 25 24-mo, 22	Grade 3-4, ≥10%; diarrhea (N3, 2%; N1 + I3, 14%; N3 + I1, 2%); ALT increased (N3, 3%; N1 + I3, 14%; N3 + I1, 4%); AST increased (N3, 5%; N1 + I3, 10%; N3 + I1, 2%)	Advanced/metastatic Chemotherapy refractory GA/EC/GEJ	(55)
				PD-L1$^+$		19			6.2	12-mo, 34 18-mo, 13			
				PD-L1$^-$		12				NA			
	PD-1	N1 + I3	N1 + I3, Q3W	GA/EC/GEJ	49	24			6.9	12-mo, 35 18-mo, 28 24-mo, 22			
				PD-L1$^+$		40			NA	12-mo, 50 18-mo, 50			
				PD-L1$^-$		22				NA			
	PD-1	N3 + I1	N3 + I1, Q3W	GA/EC/GE	52	8			4.8	12-mo, 24 18-mo, 13 24-mo, NA			
				PD-L1$^+$		23			5.6	12-mo, 23 18-mo, 15			
				PD-L1$^-$		0							
NEJM 2012; 366:2074	PD-L1	Durvalumab	1,500 mg	EC	6	60 (PR in 2 pts)					Grade 3-4, 17%	Pre-operative chemoradiotherapy for locally advanced EC	(56)

ALT, aspartate transaminase; AST, alanine transaminase; EC, esophageal cancer; ESCC, esophageal squamous cell carcinoma; GA, gastric cancer; GEJ, gastroesophageal junction; I1, ipilimumab (1 mg/kg); iv, intravenous; mo, months; OS, overall survival; N3, nivolumab (3 mg/kg); PD-1, programmed cell death 1; PD-L1, programmed death ligand 1; PFS, progression-free survival; pts, patients; Q2W, every 2 weeks; Q3W, every 3 weeks; RR, response rate; PR, pathological response; NA, not available.

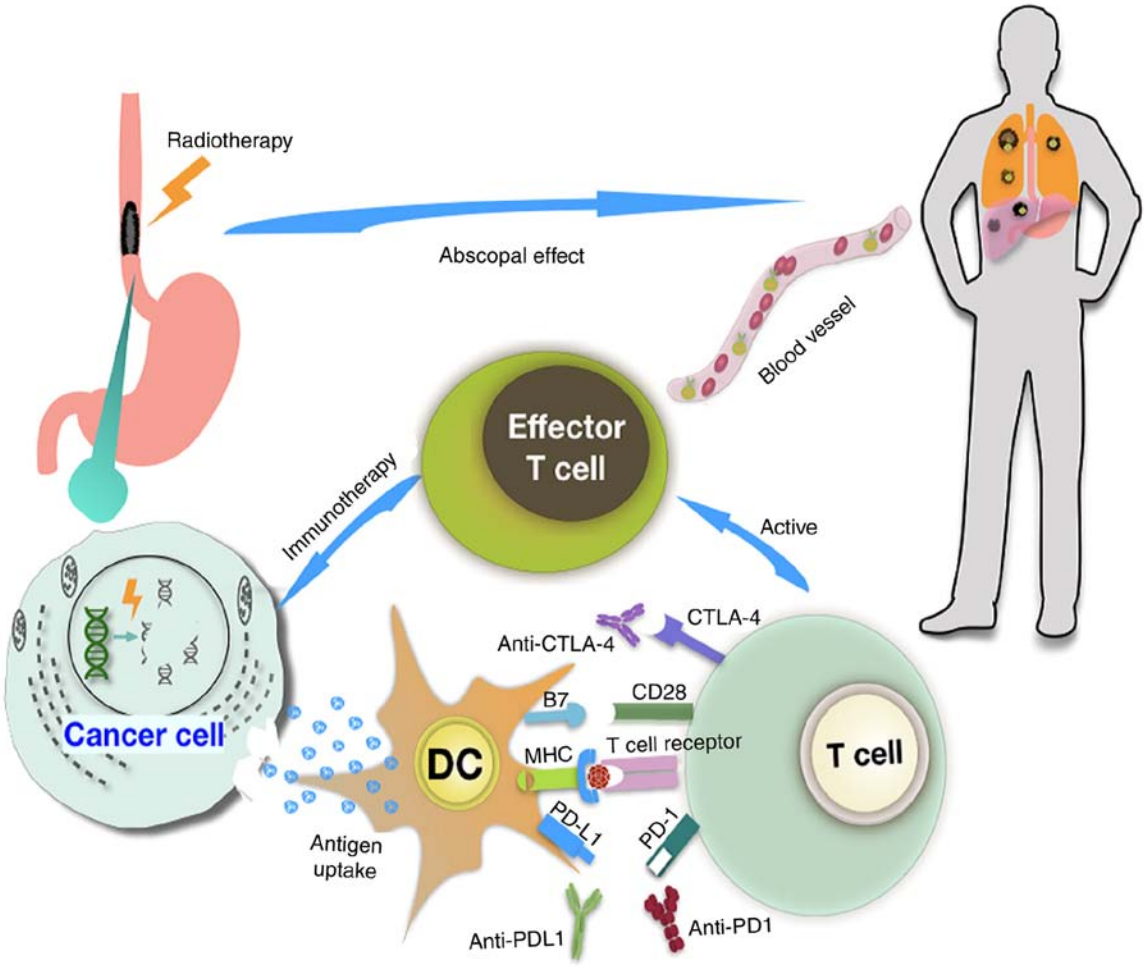

Figure 2. Combining radiation with anti-PD-L1, anti-PD-1 and anti-CTLA-4 activates effector T cells and promotes the recruitment and infiltration of immune cells, enhancing the abscopal effect. This ultimately increases the recognition and killing of tumor cells by the immune system. B7 is a peripheral membrane protein found on activated antigen presenting cells which interaction with CTLA-4 on T cells promotes antitumor immunity. CTLA-4, cytotoxic T-lymphocyte-associated protein 4; DC, dendritic cell; MHC, major histocompatibility complex; PD-1, programmed cell death 1; PD-L1, programmed death-ligand 1.

Radiation can therefore stimulate antitumor immunity (72). The combination of radiation and ICI can thus have a direct cytotoxic effect on tumor cells and further activate effector T cells, enhancing the immune surveillance of tumor cells. This process also promotes the recruitment and infiltration of immune cells in the tumor, and stimulates the recognition and killing of tumor cells by the immune system (Fig. 2) (27).

The combination of irradiation and anti-PD-L1 treatment synergistically promotes antitumor activity *in vitro* (73,74). In mouse models, RT induces upregulation of PD-L1 expression in DCs and promotes antigen cross-presentation in tumor-draining lymph nodes (75,76). Pre-clinical results demonstrated that irradiated effector T cells induce a decrease in the number of PD-L1-pexpressing tumor cells, which suggests that combinating RT with anti-PD-L1 mAbs may enhance the antitumor effects of RT as monotherapy (77). Accumulating clinical evidence has demonstrated that 2-3 courses of combination therapy (checkpoint inhibitors with RT) has promising potential and is a well-tolerated treatment in patients with various types of locally advanced or metastatic malignancy, including non-small cell lung cancer (NSCLC), melanoma and renal cell carcinoma (78,79). A previous study reported that administration of nivolumab in 26 patients with metastatic brain melanoma (BM) during or after RT resulted in an increased 1-year OS rate of 55% and a median OS of 11.8 months (80). A previous study investigated 75 patients with BM who were treated concurrently or at different time points with RT and pembrolizumab, nivolumab or ipilimumab (81). The results demonstrated that concurrent treatment improved the volume reduction of the lesion compared with non-concurrent treatment after 3 and/or 6 months treatment. In addition, the median percentage of the lesion volume was reduced to a greater extent following anti-PD-L1 treatment compared with anti-cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) treatment (81). A retrospective study reviewed consecutive patients with metastatic NSCLC and melanoma. Among the 59 patients who received radiation and anti-PD-1 therapy, 25 patients continued to receive PD-1 inhibition treatment for a median of 238 additional days (82).

Irradiation can cause an upregulation of PD-L1 expression in human EC cells (83). Therefore, a number clinical trials are underway to study the effect of RT combined with immune checkpoint blockade (ICB) in patients with ESCC (Table II). A phase II trial is scheduled to evaluate the benefit of neoadjuvant

Table II. Ongoing clinical trials involving immune checkpoint inhibitors with radiotherapy for ESCC.

Clinical trial registration number	Target	Agents	Phase	Treatment groups	Condition	Primary endpoints	Number of patients
NCT 02642809	PD-1	Pembrolizumab	1	Pembrolizumab + brachytherapy	Metastatic EC	Tolerability, treatment related adverse events	18
NCT 02844075	PD-1	Pembrolizumab	2	Neoadjuvant: Pembrolizumab + taxol + carboplatin + RT (44.1 Gy, 21f, 2.1Gy/f) + surgery	ESCC received preoperative CRT followed by surgery	Complete pathological response rate	28
NCT03064490	PD-1	Pembrolizumab	2	Weekly neoadjuvant pembrolizumab + concurrent CRT (carboplatin/paclitaxel + radiation (45 Gy, 25f; 1.8 Gy/f)), followed by surgery	Locally advanced esophageal and GC	Complete pathological response rate	38
NCT 02830594	PD-1	Pembrolizumab	2	RT + pembrolizumab	ESCC, EAC, GEJ, GA	Biomarkers and outcome	14
NCT 03278626	PD-1	Nivolumab	1/2	Nivolumab + paclitaxel + carboplatin + RT	Locally advanced ESCC	Unacceptable toxicity grade 3,4 hematological toxicity	10
NCT 03544736	PD-1	Nivolumab	1/2	RT (30-50 Gy, 2 Gy/f) + nivolumab CRT (41.4 Gy, 1.8 G/f) + nivolumab Neoadjuvant CRT + nivolumab	EC	Incidence of treatment-emergent adverse events, safety and tolerability	54
NCT 03437200	PD-1	Nivolumab + ipilimumab	2	CRT (50 Gy, 2 Gy/f) + nivolumab CRT (50 Gy, 2 Gy/f) + nivolumab + ipilimumab	Inoperable EC	12-month progression-free survival	130
NCT 03044613	PD-1	Nivolumab + relatlimabb	1	Nivolumab + CRT Nivolumabab + relatlimab + CRT	II/III stage GC, EC, GEC	Treatment-related adverse events	32
NCT 03278626	PD-1	Nivolumab	1/2	Nivolumab + carboplatin/paclitaxel + CRT	ESCC	Unacceptable toxicity grade 3,4	10
NCT 03490292	PD-L1	Avelumab	1/2	Avelumab + CRT	Resectable EC	Dose limiting measures, pathological response rate, pathological complete response rate	24
NCT 02520453	PD-L1	Durvalumab	2	Neoadjuvant concurrent CRT + surgery + durvalumab Neoadjuvant concurrent CRT + surgery + placebo	ESCC	Disease-free survival	84
NCT 03377400	PD-L1	Durvalumab	2	Concurrent CRT and ICI (durvalumab/tremelimumab)	ESCC	Disease-free survival	40
NCT 03087864	PD-L1	Ateolizumab	2	Ateolizumab + CRT (50.4 Gy, 1.8Gy/f)	EC	Feasibility	40

PD-1, programmed cell death 1.

chemoradiotherapy (CRT) with pembrolizumab followed by surgery in patients with ESCC, and the completion date is estimated to be in the year 2022 (ClinicalTrials.gov Identifier code, NCT02844075). A multicenter phase I/II trial of CRT combined with nivolumab as a treatment for locally advanced ESCC is currently ongoing (NCT03278626). Three parallel cohort clinical trials are analyzing the safety and feasibility of different doses of irradiation and nivolumab administration in the treatment of EC (NCT03544736). The feasibility of combining definitive CRT (84) with anti-PD-1 and anti-CTLA-4 mAbs in inoperable EC is being evaluated (NCT03437200). At present, studies on the evaluation of concurrent treatment with pembrolizumab and chemoradiation as a neoadjuvant therapeutic strategy for locally advanced EC are ongoing (NCT03064490). Stereotactic body radiation therapy (SBRT) is a novel methodology that delivers a very intense dose of RT over a short-course of treatment (85). This treatment may stimulate immunity and induce an immunotherapeutic response, and is recommended for the control of local lesions in recurrent or metastatic EC with doses of 30 and 50 Gy in 5 daily fractions (85). The combination of multisite SBRT with pembrolizumab treatment has shown potential activity and acceptable toxicity in metastatic solid tumors (86). Additional clinical trials are required to verify this treatment strategy. Although the optimization of radiation techniques, dose and treatment duration remains unclear, it may be possible to validate the efficacy of combination of RT and immunotherapy by continuously collecting and analyzing clinical data from the aforementioned trials (NCT 03278626, NCT 02520453, NCT 03377400, NCT 03278626 and NCT 02844075).

5. Outlook and conclusion

The majority of studies have reported that the high expression of PD-L1 in EC is associated with poor treatment outcome (87). Patients with high PD-L1 expression tend to respond well to anti-PD1/PD-L1 mAbs and exhibit a significant increase in OS rate (88). These results suggest that PD-L1 expression may be used as a predictive biomarker for suitablility of anti-PD1/PD-L1 treatment. Recently, PD-L1 status has been used to evaluate the number of circulating tumor cells (CTCs) in breast cancer (89). Furthermore, previous studies have demonstrated that PD-L1 is associated with the number of CTCs present in advanced NSCLC, and that the combination of PD-L1 status and CTC number can be used as a potential noninvasive biopsy to evaluate disease progression (90-92). A previous study revealed that the abundance of CTCs with high expression of PD-L1 at baseline could be used as a predictor of immunotherapy response in advanced solid tumors, including EC (93). It is therefore crucial to develop a highly sensitive, accessible and reliable assay for the evaluation of PD-L1 expression, and for the detection of PD-L1 status in CTCs (94). PD-L1 expression is a potential biomarker for determining the feasibility of immunotherapy. Further investigation is required to confirm the correlation of PD-L1 expression in CTCs.

In 2016, the FDA approved the use of checkpoint inhibitors, including pembrolizumab and nivolumab, in the treatment of recurrent or metastatic head and neck squamous cell carcinoma (HNSCC) due to their antitumor efficacy and safety (95). Patients with HNSCC may also develop EC, as these tumors share a common origin and clonal expansion (96). ESCC gene expression was similar to the classical subtype described in The Cancer Genome Atlas studies of HNSCC, which possess similar somatic alterations (97). Furthermore, numerous *in vitro* studies reported a significant antitumor effect of checkpoint inhibitors in esophageal cell lines (98). Additional clinical trials are therefore required to fully examine the OS rate, tumor response and toxicity caused by immunotherapy in ESCC. At present, ICB is used as a salvage therapy following treatment failure, disease relapse or metastasis in ESCC due to ineffective chemoradiation and/or unsuccessful surgery. The combination of immunotherapy and radiation has been reported to enhance the antitumor effect compared to treatment with only one of the two (27,99,100). Immunotherapy can influence the tumor microenvironment (101,102) and the tumor-associated blood and lymphatic vasculature (103,104), and can further improve local oxygen and nutritional conditions (105). These elements and changes in the surrounding stromal cells can markedly influence the efficacy of radiation (106). Immunotherapy can therefore be a potential sensitizing agent for RT.

At present, the evaluation of clinical response following CRT is determined by Response Evaluation Criteria in Solid Tumors (RECIST) (107). Additional criteria that have not been included in RECIST are used for immunotherapy evaluation, and are designated as immune-Related Response Criteria (108). When T cells are recruited to tumors, they may increase the tumor volume as a result of immunotherapy, a process termed 'pseudoprogression' (109,110). Therefore, to accurately evaluate the tumor response to the combination of RT and immunotherapy, additional evidence is required from *in vitro* functional studies and clinical trials.

The majority of common immune-related adverse events occur in the gastrointestinal tract, endocrine glands, skin and liver (111). A treatment-related patient death occurred due to pneumonia in the KEYNOTE-180 trial (53). Furthermore, the grade 3-4 adverse events in the KEYNOTE-180 trial were significantly higher than in the KEYNOTE-028 trial (49), which may be due to the different inclusion criteria. The KEYNOTE-180 trial included disease progression of patients treated with chemotherapy and/or RT. The results demonstrated that patient organs, including heart, lung, liver, bone marrow and gastrointestinal tract, suffered considerable tissue damage following immunosuppression. The patients further exhibited reduced healing abilities, although results from blood tests and radiographs were normal. It has been demonstrated that radiation can lead to T lymphocyte inactivation at a dose of 2 Gy/fraction (112). A previous study reported that the incidence of grade 4 absolute lymphocyte count was 27% in patients with EC treated with chemoradiation therapy (113). Ongoing clinical trials include concurrent CRT combined with immunotherapy as neoadjuvant treatment for ESCC. The radiation-related adverse events of concurrent CRT including early radiation-induced esophagitis, cardiac toxicity, radiation-associated pneumonia and whole blood cell reduction (114). Furthermore, the extent of adverse events can be increased if the chemotherapy is provided concomitantly with RT. Esophageal perforation is one of these adverse events, which is a rare and life-threatening event (115). Additional mechanistic studies and clinical trials are therefore required.

The delivery of the radiation optimal dose is unclear when administered in combination with immunotherapy. Definitive CRT is the established treatment of choice in advanced ESCC, and the maximum dose of 60 Gy is considered feasible to limit side effects in patients (116). The combination of neoadjuvant CRT with immunotherapy can be used following surgery, with a radiation dose of 44.1 Gy in 21 fractions (117). A previous study reported a radiation treatment at 50.4 Gy in 28 fractions (118). Concurrent RT (41.4 Gy in 23 fractions, 5 days per week) followed by surgery is also used as a protocol in EC treatment strategies (119). Optimizing the RT parameters and dose, clinical methodology, fraction number and duration with the course of immunotherapy in order to maximize antitumor effects and minimize the adverse events is therefore very challenging. The insights highlighted in this review suggest that immunotherapy can be applied to patients with ESCC, and that a combination of multiple strategies may be the future direction of treatment.

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TZ and LSW participated in the conception and design of the manuscript. XYL wrote the manuscript. CYL, TZ and JFH critically reviewed the manuscript for important intellectual content. All authors have read and approved the final version of the manuscript.

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Patient consent for publication

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Competing interests

The authors declare that they have no competing interests.

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