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*Review Article*

# CTLA-4 and its inhibitors in esophageal cancer: efficacy of therapy and potential mechanisms of adverse events

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**Abstract:** Esophageal cancer is one of the most prevalent diseases in the world, and its prognosis remains poor. Surgery, chemotherapy, and radiotherapy are the most common treatment strategies for esophageal cancer. Although these conventional treatment methods are sometimes beneficial, patients with esophageal cancer still have a high risk of local relapse and metastasis. Thus, novel and effective therapies are needed. Immune checkpoint inhibitors are a type of immunotherapy being studied as a treatment for patients with advanced cancers, and strategies using such inhibitors have rapidly progressed to be recognized as transformative treatments for various cancers in recent years. Immune checkpoint inhibitors combined with chemotherapy or radiotherapy have become the first-line and second-line treatment strategies for advanced esophageal cancer. In addition, immune checkpoint inhibitors have also been recognized as another option for patients with terminal esophageal cancer who cannot benefit from chemotherapy, and they even have potential benefits as a novel neoadjuvant treatment option for locally advanced esophageal cancer. Currently, there are two types of immune checkpoint inhibitors commonly applied in clinical practice: immune checkpoint inhibitors targeting programmed death 1/programmed cell death ligand 1 and immune checkpoint inhibitors targeting cytotoxic T-lymphocyte-associated protein 4. However, cytotoxic T-lymphocyte-associated protein 4 immune checkpoint inhibitors are rarely used compared with programmed death 1/programmed cell death ligand 1 inhibitors in esophageal cancer and other cancers, and the clinical benefit is unclear. We analyzed and summarized the efficacy and safety of cytotoxic T-lymphocyte-associated protein 4 immune checkpoint inhibitors in the treatment of esophageal cancer. Due to the lack of clinical applications, it is expected that cytotoxic T-lymphocyte-associated protein 4 immune checkpoint inhibitors in combination with other treatments may provide superior benefits and improve the prognosis of patients with esophageal cancer.

**Keywords:** Esophageal cancer, CTLA-4, immune checkpoint, immune suppressive, clinical prognosis, immunotherapy

## Introduction

With an estimated 604,100 new cancer cases and 544,000 deaths, esophageal cancer (EC) was the seventh most common cancer and the sixth leading cause of cancer death worldwide in 2020 [1]. Smoking, heavy alcohol use, and Barrett's esophagus can increase the risk of EC. China had more than 50% of all new cases in 2020 [2]. A meta-analysis revealed that neoadjuvant immunotherapy in patients with locally advanced EC was safe and effective [3]. Therefore, it is essential to explore the mechanism of immune checkpoint inhibitors (ICIs) for the management of EC. The immune checkpoint cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4/CD152) has recently been actively investigated. This molecule is expressed on the surface of T cells, where it induces inhibitory signaling. When EC cells have high expression of CTLA-4, they will not be attacked and killed by T cells [4]. Moreover, EC cells can evade immune surveillance by inducing the activation of diverse immunosuppressive cell subsets via other mechanisms. CTLA-4 was identified by Pierre Goldstein and colleagues as a second receptor for the T-cell costimulatory ligand B7 [5]. In 1995, it was confirmed to be an inhibitor of T-cell responses [6]. James P. Allison et al. found that CTLA-4 blockade can potenti-

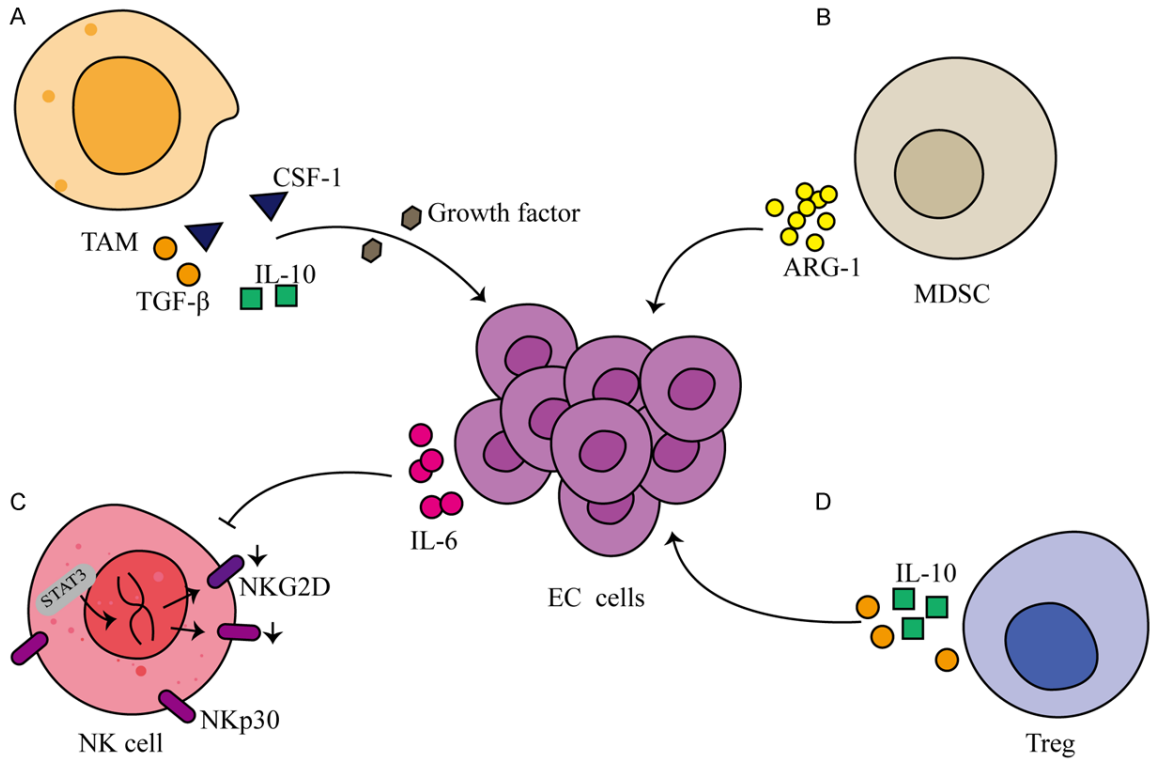


**Figure 1.** Major immunosuppressive cell subsets in the tumor microenvironment of EC. A. TAMs can produce various tumor-associated factors, such as TGF-β, IL-10, and colony-stimulating factor-1 (CSF-1). B. MDSCs inhibit T-cell effector function via increased ARG1 levels. C. STAT3 plays a crucial role in IL-6 and IL-8 secretion, leading to downregulation of the expression of natural cytotoxicity triggering receptor 3 (NKp30) and NK cell lectin-like receptor subfamily K (NKG2D) receptors on the surface of NK cells, ultimately impairing their function. D. Tregs are a heterogeneous subset of immunosuppressive T cells that produce inhibitory cytokines such as IL-10 and TGF-β to enhance immune tolerance and cell contact-based growth suppression.

ate effective immune responses against tumor cells in 1996 [7]. After years of study, the first anti-CTLA-4 monoclonal antibody, ipilimumab, was approved for melanoma treatment by the Food and Drug Administration (FDA). In this review, we focus on the correlation between CTLA-4 and clinical prognosis and further explore the possible mechanisms of anti-CTLA-4 antibodies in EC.

## Major immunosuppressive cell subsets in the tumor microenvironment of EC

The tumor microenvironment (TME) comprises many different components, including proliferating cancer cells, a wide variety of immune and stromal cells, and connective tissue that provides physical support within the TME. Tumors can induce the activation of diverse immunosuppressive cell subsets through multiple mechanisms. For example, esophageal squamous cell carcinoma (ESCC) can have a lower number of infiltrated B cells compared with adjacent TME tissues. Moreover, LAMP3+ dendritic cells (DCs) have higher activity and migration ability than other DC subsets in the TME can inhibit the immune response by expressing many regulatory molecules, such as indoleamine 2,3-dioxygenase 1 (IDO1) and IL10 [8]. Furthermore, tumor-associated neutrophils (TANs), as the most abundant circulating leukocytes, usually transform into type 2 neutrophils (N2s) during tumor progression. This subset can promote tumor progression by regulating the proliferation of cancer cells [9]. We briefly describe the impact of primary immune-related cells on tumor tissues (**Figure 1**).

### *Tumor-associated macrophages*

Tumor-associated macrophages (TAMs) are in tumor tissues. They can produce various tumor-associated factors, such as transforming

growth factor beta (TGF-β), interleukin 10 (IL-10), and colony-stimulating factor-1 (CSF-1). For example, in ESCC, CSF-1 can support tumorigenesis and recruit M2 macrophages to inhibit the acquired immune response [10].

*Myeloid-derived suppressor cells*

Myeloid-derived suppressor cells (MDSCs) are some of the most crucial immunosuppressive cells in the TME, including immature macrophages, neutrophils, and dendritic cells. They can produce IL-6 or mediate signaling pathways to influence the immune response. Moreover, MDSCs inhibit T-cell effector function via increased arginase 1 (ARG1) levels [11]. A study showed that high infiltration of MDSCs is associated with poor prognosis in patients with EC [12].

*Natural killer cells*

Natural killer (NK) cells are innate immune cells with potent cytolytic activity against EC tumor cells [13]. However, in ESCC, signal transducer and activator of transcription 3 (STAT3) plays a crucial role in IL-6 and IL-8 secretion, leading to the downregulation of the expression of natural cytotoxicity triggering receptor 3 (NKp30) and NK cell lectin-like receptor subfamily K (NKG2D) receptors on the surface of NK cells, ultimately impairing their function [14].

*Regulatory T cells*

Regulatory T (Treg) cells serve as a heterogeneous subset of immunosuppressive T cells that produce inhibitory cytokines such as IL-10 and TGF-β to enhance immune tolerance and cell contact-based growth suppression [15, 16]. CTLA-4 expressed on Treg cells can downregulate costimulatory signaling (CD28-CD80/CD86), which ultimately leads to failure of T-cell activation [17]. Furthermore, the interaction of Tregs and other cell subsets also have an influence on the immune response in the TME. For example, Tregs and MDSCs can secrete immunosuppressive factors such as vascular endothelial growth factor and TGF-β. These components inhibit the functions of DCs and transfer macrophages to the M2 phenotype to further promote cancer development [18]. Moreover, Tregs suppress polyclonal T-cell activation in vitro by inhibiting IL-2 production [19]. Infiltration of Tregs limits recruitment of NK cells and CD8+ T cells in tumor tissues, which also leads to tumor cells evading surveillance by the immune system [20]. In this review, we focus on the correlation between CTLA-4 and clinical prognosis and further explore the possible mechanisms of anti-CTLA-4 antibodies in EC.

## CTLA-4 expression in tumor-infiltrating lymphocytes targeting tumor cells

Treg cells are important for tumor immune tolerance in the TME. Tumor-infiltrating lymphocytes (TILs) can generate host immunity against cancer and affect the prognosis of cancer, mainly consisting of tumor-specific T cells, including CD4+ and CD8+ T cells. CD8+ T cells induce cytotoxicity or apoptosis of tumor cells, while CD4+ T cells can differentiate into T helper 2 (Th2) cells, Th17 cells, and Treg cells, which secrete cytokines to participate in the antitumor immune response [21]. However, CTLA-4 can weaken the capacity of cytotoxic T cells to kill tumor cells, especially CD8+ T cells. A pan-cancer study indicated that high CTLA-4 levels significantly reduced the degree of infiltration of CD8+ T cells in cancer [22]. In addition, CTLA-4 can promote the proliferation and growth of tumor cells according to a study by Andreas Herrmann et al. [23]. Additionally, the interactions of CTLA-4 and CD86 can promote Ki-67 antigen expression, resulting in the proliferation and growth of tumor cells. Recently, Chen et al. further demonstrated that IL1 receptor 2 (IL1R2) deficiency in Treg cells leads to decreased Treg cell numbers and increased CD8+ T-cell numbers in the TME. Treg cells and cancer-associated fibroblasts (CAFs) are known to interact and influence the differentiation of each other in the TME [24].

## CTLA-4 expression in T cells

CTLA-4 (CD152) is an immune checkpoint molecule mainly expressed on the surface of Treg cells and activated T cells [25-27]. CTLA-4 is homologous to CD28 and has high affinity for CD80/CD86, which conveys an inhibitory signal to T cells. The underlying mechanism is as follows:

① The CD28-CD80/CD86 interaction serves as a costimulatory signal for T-cell activation and proliferation, while CTLA-4-CD80/CD86 binding acts as a coinhibitory signal to thwart T-cell activation [28]. Furthermore, this coin-

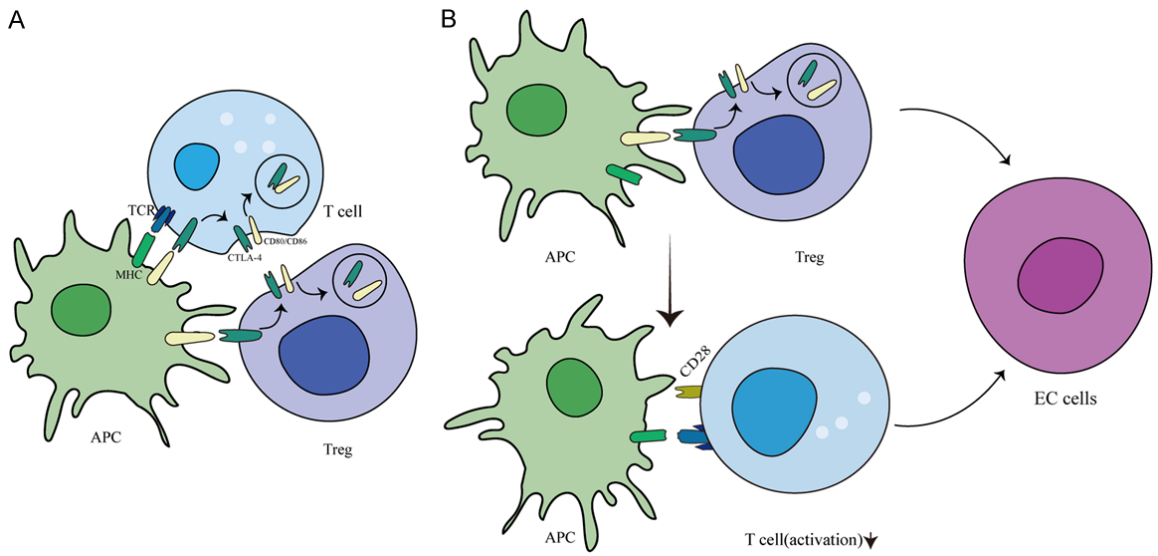


**Figure 2.** The expression and function of CTLA-4. A. CTLA-4 is expressed on the surface of conventional T cells. Tregs interact with the ligands CD80 and CD86 to enhance adhesion and deplete CD80 and CD86 by removing them from antigen-presenting cells (APCs) through transendocytosis, thereby reducing their availability for CD28 engagement. B. CTLA-4 expressed on the surface of Tregs interacts with its ligands CD80 and CD86 to enhance adhesion and deplete CD80 and CD86 by removing them from the surface of antigen-presenting cells (APCs) through transendocytosis, thereby reducing their availability for CD28 engagement, which inhibits T-cell activation and further impacts the ability of T cells to kill tumor cells.

hibitory signal may counteract the first and second signals for T-cell activation (T-cell receptor-major histocompatibility complex (TCR-MHC) binding and CD28-CD80/CD86) [29, 30].

② CTLA-4 expressed on the surface of conventional T cells interacts with its ligands CD80 and CD86 to enhance adhesion and deplete CD80 and CD86 by removing them from the surface of antigen-presenting cells (APCs) through transendocytosis, thereby reducing their availability for CD28 engagement [31]. This phenomenon can also be seen in CTLA-4+ Treg cells, which inhibit T-cell activation and further impact the ability of T cells to kill tumor cells [17] (**Figure 2**).

③ CTLA-4 is an intracellular protein, and its immunoreceptor tyrosine-based inhibitory motif (ITIM) domain inhibits protein phosphatase 2A (PP2A) and phosphatidylinositol 3-kinase (PI3K) or recruits src homology two domain-containing protein tyrosine phosphatase-2 (SHP-2) to deactivate Akt to inhibit further RAS/MEK/ERK signaling in T cells [32, 33] (**Figure 3**).

④ In addition, CTLA-4 also inhibits T-cell activation by extrinsic mechanisms, including induction of indoleamine 2,3-dioxygenase (IDO) through its ligands, stimulation of the production of regulatory cytokines such as TGF-β [31], interference with the production of IL-2 [34], and effects on cyclin D3, cyclin-dependent kinase (CDK)4, and CDK6 [35].

In summary, CTLA-4 serves as the immune system's "off" switch, negatively regulating the proliferation of T cells through the above pathways, which limits the efficacy of immunotherapy in cancer.

## Correlation between CTLA-4 and clinical prognosis in EC

A retrospective study showed that positive expression of CTLA-4 in tumor tissues was significantly correlated with lymph node metastasis of ESCC (P=0.002). Patients who develop lymph node metastases tend to have a shorter overall survival (OS). In contrast, the median overall survival (mOS) of patients with positive expression of CTLA-4 was higher than that of patients with negative expression (35 months vs. 28 months, P=0.162) in 161 patients with ESCC, but the difference was not significant. The results of this study may not be representative due to the small sample of patients with

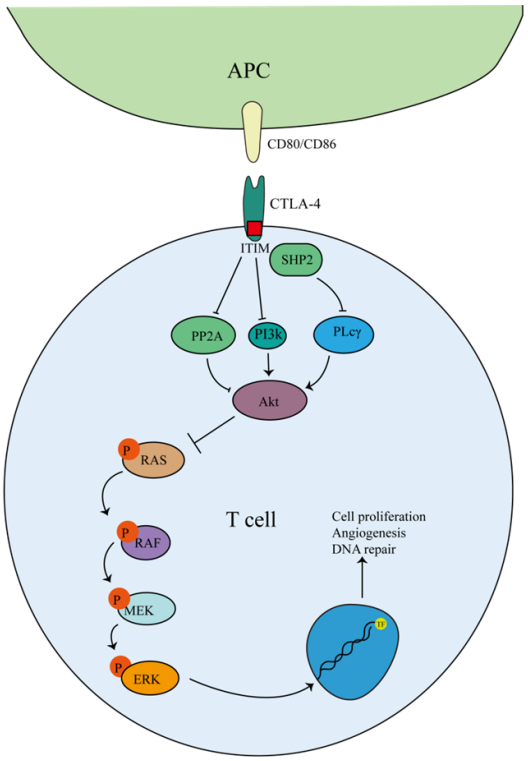


**Figure 3.** Signaling pathway of CTLA-4 in T cells. The ITIM domain inhibits PP2A and PI3K or recruits SHP-2 to deactivate Akt to inhibit further RAS/MEK/ERK signaling in T cells.

negative expression of CTLA-4 (eighteen cases) [36]. CTLA-4 expression is also significantly increased in esophageal adenocarcinoma (EAC) according to a study by Wagener-Ryczek et al. However, the study did not indicate a correlation between CTLA-4 expression and the survival of patients with EAC [37]. Similarly, another study also revealed no significant correlation between the expression level of CTLA-4 and the OS of patients with ESCC (P=0.453). However, patients with both low expression of CTLA-4 and a low platelet lymphocyte ratio (PLR) had superior OS (P=0.023) [38]. Notably, a study by Zhang et al. showed a different result: the OS of CTLA-4-positive patients with ESCC was significantly shorter than that of CTLA-4-negative patients, and there was a statistically significant difference (36 months vs. 65 months, P<0.001). In addition, CTLA-4 expression in tumor specimens was positive (+) in 87% (137/158) of patients with ESCC. Elevated expression of CTLA-4 (++/+++) was observed in 52.6% (72/137) of samples expressing CTLA-4, and the study further indicated that elevated expression of CTLA-4 was associated with poor prognosis in ESCC [39]. Similarly, other studies have indicated that CTLA-4 expression is increased in tumors and peritumoral specimens of EAC or ESCC patients, and this increased or high expression was found to be significantly associated with worse OS [40-42] (**Table 1**). In addition, a meta-analysis indicated that there is no significant correlation between CTLA-4 and OS in patients with cancer [43], but many studies have shown that CTLA-4 is correlated with OS. For example, CTLA-4 is negatively correlated with OS and PFS in colorectal cancer, breast cancer, kidney renal clear cell carcinoma, glioma, thymoma, nasopharyngeal carcinoma, pancreatic adenocarcinoma, and spinal chordoma [22, 42, 44-50]. In contrast, CTLA-4 is positively correlated with prognosis in colon adenocarcinoma, skin cutaneous melanoma, uterine corpus endometrial carcinoma, head-neck squamous cell carcinoma, and mesothelioma [22, 42, 51]. However, there is not enough evidence from the above studies suggest that the expression level of CTLA-4 in EC tumor tissues can influence the OS of patients. The lack of evidence may be related to the source of specimens, the clinical EC classification and histologic subtyping schemes used, and the employed patient treatment strategies. Based on the effect of CTLA-4 on immune cells, we speculate that the expression level of CTLA-4 in tumor tissues is negatively correlated with the prognosis of patients with EC, and patients with increased CTLA-4 expression may benefit from an immune checkpoint inhibitor targeting CTLA-4. However, this correlation needs to be further confirmed by more clinical studies.

## Clinical application of anti-CTLA-4 monoclonal antibodies in EC

### *Efficacy and safety of anti-CTLA-4 antibody monotherapy*

Currently, there are three types of anti-CTLA-4 monoclonal antibodies: ipilimumab, tremelimumab, and quavonlimab. Ipilimumab is a recombinant human immunoglobulin G1 (IgG1) monoclonal antibody approved for use in the treatment of melanoma. Quavonlimab is also a humanized IgG1 monoclonal antibody and has been investigated in clinical trials, while tremelimumab is a human IgG2 immunoglobulin

**Table 1.** Correlation between CTLA-4 expression and overall survival

| Author | Cancer type | Sex | Age (years) | Level of CTLA-4 expression in tumor tissue | Overall survival (OS)/median overall survival (mOS*) | *P* values |
|---|---|---|---|---|---|---|
| Zhijun Chen et al., 2020 (36) | ESCC | Males: 119<br>Females: 42 | >60: 116<br><60: 45 | Proportion of cells with positive expression of CTLA-4: 88.2% | CTLA-4 positive patients: 35 months*<br>CTLA-4 negative patients: 28 months* | 0.162 |
| Svenja Wagener-Ryczek et al., 2020 (37) | EAC | Males: 41<br>Females: 6 | >50: 43<br><50: 4 | CTLA-4 expression was 3.5-4-fold increased in esophageal tumor specimens compared to normal tissues | / | / |
| Cui-Ying Zhang et al., 2019 (38) | ESCC | Males: 74<br>Females: 10 | Average: 60 | Proportion of cells with positive expression of CTLA-4: 48.8% | / | 0.453 |
| Xiao-Fei Zhang et al., 2016 (39) | ESCC | Males: 126<br>Females: 32 | <56: 97<br>>56: 61 | Proportion of cells with positive expression of CTLA-4: 87% | CTLA-4-positive patients: 36 months;<br>CTLA-4-negative patients: 65 months | <0.001* |
| Wenjia Wang et al., 2019 (40) | ESCC | Males: 147<br>Females: 36 | ≤65: 106<br>>65: 77 | Higher in ESCC than normal tissues | Patients with high expression < patients with low expression | <0.001* |
| Karl-Frederick Karstens et al., 2020 (41) | EAC | Males: 36<br>Females: 3 | Average: 61.9 | Higher in EAC than normal tissues | Patients with high expression < patients with low expression | / |

EAC, esophageal adenocarcinoma; ESCC, esophageal squamous cell carcinoma; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; OS, overall survival; mOS*, median overall survival; **P*<0.05.

monoclonal antibody that has been investigated in phase III clinical trials [52, 53].

*Ipilimumab:* Bang et al. evaluated the efficacy of ipilimumab (Y group) and best supportive care (BSC group, with continuation of fluoropyrimidine) among 143 patients with esophagogastric junction (EGJ) cancer who had previously received first-line chemotherapy with fluorouracil. Patients treated with ipilimumab achieved a shorter progression-free survival (PFS) than those treated with BSC, 2.9 months and 4.9 months, respectively. The mOS was 12.7 and 12.1 months in the Y and BSC groups, respectively. The rates of immune-related adverse events (irAEs) were 72% and 56% in the Y group and BSC group, respectively, and irAEs included itching (31.6%), diarrhea (24.6%), fatigue (22.8%), rash (17.5%), etc. [54, 55].

*Tremelimumab:* Similarly, tremelimumab monotherapy is not as good as a second-line treatment in EAC. In one study, eighteen patients who had failed at least one platinum-based chemotherapy received tremelimumab (15 mg/kg) every 90 days until disease progression. One patient achieved partial response (PR), four patients achieved stable disease (SD), the mOS was 4.8 months, the objective response rate (ORR) was 5%, and most patients had mild adverse reactions (pruritus 50%, erythema 33% and eosinophilia 33%) [56] (**Table 2**).

In addition, quavonlimab has not been studied in clinical trials for EC. In summary, there are few studies on CTLA-4 inhibitor monotherapy in EC, and thus far, these agents as monotherapy have not produced superior outcomes. Most studies have focused on CTLA-4 inhibitors combined with PD-1/PD-L1 inhibitors or chemotherapy rather than CTLA-4 inhibitor monotherapy.

*The efficacy and safety of anti-CTLA-4 antibody combination therapy*

*Ipilimumab plus nivolumab:* Nine hundred seventy patients with ESCC were randomly enrolled at a 1:1:1 ratio to receive nivolumab (PD-1 inhibitor) plus chemotherapy, nivolumab plus ipilimumab, or chemotherapy as described above in the phase III CheckMate648 clinical trial. The mOS was 13.2 months, 12.7 months, and 10.7 months ($P<0.05$) and the ORRs were 47%, 28%, and 27% in the nivolumab plus chemotherapy, nivolumab plus ipilimumab, and chemotherapy groups, respectively. However, the difference in PFS between the groups was not statistically significant. Interestingly, the incidence of grade 3 or 4 irAEs in the nivolumab plus ipilimumab group (32%) was lower than that in the chemotherapy group (36%) and that in the nivolumab plus chemotherapy group (47%). In addition, the nivolumab plus ipilimumab group had the longest median duration of response (DOR) (11.1 months) compared with the nivolumab plus chemotherapy group (8.2 months) and chemotherapy group (7.1 months) [57, 58]. A single-arm, phase II study conducted by Park et al. suggested that the application of dual ICIs (durvalumab, a PD-L1 inhibitor, and tremelimumab, a CTLA-4 inhibitor) with concurrent chemoradiotherapy (CCRT) achieved promising efficacy in locally advanced ESCC. Forty patients were enrolled in the study group. In comparison, 75 patients were matched in the historical control group, and the 24-month PFS and OS of the study group were significantly higher than those of the historical control group (57.5% vs. 44.6%, $P=0.4$; 75% vs. 59.2%, $P=0.43$, respectively) [59]. Other trials have assessed the efficacy and safety of dual ICIs in EGJ cancer. A study by Shitara et al. suggested that the mOS (11.7 months vs. 11.8 months), PFS (2.8 months vs. 7.1 months), and ORR (23% vs. 47%) were not better or even worse in the nivolumab plus ipilimumab group than in the chemotherapy group in EC. In contrast, the incidence of grade 3/4 irAEs was lower (38% vs. 46%), and the median DOR was longer with nivolumab plus ipilimumab versus chemotherapy (13.8 vs. 6.8 months) [60]. The phase I/II CheckMate-032 trial indicated that nivolumab plus ipilimumab has potential as a therapeutic strategy for metastatic esophagogastric cancer (EGC). In that clinical trial, 160 patients were randomly assigned to three groups to receive nivolumab 3 mg/kg (N3 group); nivolumab 1 mg/kg plus ipilimumab 3 mg/kg (N1+I3 group); or nivolumab 3 mg/kg plus ipilimumab 1 mg/kg (N3+I1 group). In the N3 group, the N1+I3 group, and the N3+I1 group, the mOS was 6.2 months, 6.9 months, and 4.8 months; the ORR was 12%, 24%, and 8%; and the median PFS was 1.4 months, 1.6 months, and 1.4 months, respectively. It is encouraging that patients in the N1+I3 group had the highest ORR compared with those in the other two groups. However, the dual ICI groups had a higher incidence of

**Table 2.** Results of anti-CTLA-4 antibodies and other treatments in esophageal, esophagogastric junction, and esophagogastric cancer

| Study | Patient population | Cancer type | Treatment arms | Primary end point | Immune-related adverse events | |
|---|---|---|---|---|---|---|
| | | | | | Any grade | Grade 3/4 |
| Yung-Jue Bang et al., 2017 (54) | 143 | EGJ | Y group:<br>Ipilimumab 10 mg/kg every 3 weeks for four doses, then 10 mg/kg every 12 weeks for up to 3 years<br>BSC group:<br>fluoropyrimidine | Ipilimumab<br>PFS 2.9 months (18.3%)<br>mOS 12.7 months<br>BSC<br>PFS 4.9 months (38.5%)<br>mOS 12.1 months | Ipilimumab:<br>Pruritus (31.6%)<br>Diarrhea (24.6%)<br>Fatigue (22.8%)<br>Rash (17.5%)<br>BSC:<br>Fatigue (17.8%)<br>Asthenia (17.8%)<br>Palmar-plantar erythrodysesthesia (15.6%) | Ipilimumab:<br>Fatigue (8.8%)<br>Rash (5.3%)<br>BSC:<br>Palmar-plantar erythrodysesthesia (4.4%) |
| Christy Ralph et al., 2010 (56) | 18 | EAC | Tremelimumab (15 mg/kg/90 days) | Tremelimumab<br>mOS 4.8 months<br>ORR 5% | Pruritus (50%)<br>Rash (33%)<br>Diarrhea and/or fatigue (28%) | / |
| Yuichiro Doki et al., 2022 (57) | 970 | ESCC | Nivolumab<br>plus ipilimumab (322): 3 mg/kg every 2 weeks plus 1 mg/kg every 6 weeks<br>Chemotherapy (304)<br>Nivolumab plus chemotherapy (310): 240 mg every 2 weeks plus chemotherapy consisting of a 4-week cycle of intravenous fluorouracil at a dose of 800 mg per square meter of body-surface area on days 1 through 5 and intravenous cisplatin at a dose of 80 mg per square meter on day | Nivolumab plus ipilimumab<br>mOS 12.7 months<br>ORR 28%<br>DOR 11.1 months<br>Chemotherapy<br>mOS 10.7 months<br>ORR 27%<br>DOR 7.1 months<br>Nivolumab plus chemotherapy<br>mOS 13.2 months<br>ORR 43%<br>DOR 8.2 months | Nivolumab plus Ipilimumab:<br>Diarrhea (10%)<br>Fatigue (9)<br>Rash (17%)<br>Chemotherapy:<br>Diarrhea (15%), fatigue (16%), rash (2%)<br>Nivolumab plus chemotherapy:<br>Diarrhea (19%)<br>Fatigue (20%)<br>Rash (8%) | Nivolumab plus ipilimumab: (32%)<br>Chemotherapy: (36%)<br>Nivolumab plus chemotherapy: (47%) |
| Sehhoon Park et al., 2022 (59) | 40 | ESCC | CCRT plus immunotherapy:<br>after completing CCRT plus immunotherapy, patients received 2 cycles of consolidative durvalumab and tremelimumab followed by durvalumab monotherapy every 4 weeks for 2 years | CCRT plus immunotherapy:<br>PFS (rate) 57.5%<br>OS (rate) 75% | Rash (42.5%)<br>Pruritus (57.5%)<br>Diarrhea (5%)<br>Fatigue | 20% |
| Kohei Shitara et al., 2022 (60) | 3,185 | EGC | Nivolumab plus ipilimumab<br>Chemotherapy<br>Nivolumab<br>plus chemotherapy | Nivolumab plus ipilimumab<br>PFS (rate) 20%<br>mOS 11.7 months<br>Chemotherapy<br>PFS (rate) 24%<br>mOS 11.8 months<br>Nivolumab plus chemotherapy<br>PFS (rate) 20%<br>mOS 13.8 months | Nivolumab plus ipilimumab: (12.8%)<br>Chemotherapy: (24.7%)<br>Nivolumab plus chemotherapy: (38.24%) | Nivolumab plus ipilimumab: (38%)<br>Increased lipase (7%)<br>Chemotherapy: (46%)<br>Neutropenia (13%)<br>Nivolumab plus chemotherapy:<br>60%<br>Neutropenia (15%) |

| | | | | | | |
|---|---|---|---|---|---|---|
| Yelena Y Janjigian et al., 2018 (62) | 160 | EGC | Nivolumab 3 mg/kg (NIVO3): intravenously every 2 weeks<br>Nivolumab 3 mg/kg plus ipilimumab 1 mg/kg (NIVO3+IPI1): every 3 weeks for four cycles<br>Nivolumab 1 mg/kg plus ipilimumab 3 mg/kg (NIVO1+IPI3): every 3 weeks for four cycles | Nivolumab<br>PFS 1.4 months<br>OS 6.2 months<br>ORR 12%<br>Nivolumab (3 mg/kg) plus ipilimumab (1 mg/kg):<br>PFS 1.4 months<br>OS 4.8 months<br>ORR 8%<br>Nivolumab (1 mg/kg) plus ipilimumab (3 mg/kg):<br>PFS 1.6 months<br>OS 6.9 months<br>ORR 24% | Nivolumab:<br>Pruritus (17%)<br>Diarrhea (15%)<br>Fatigue (34%)<br>Rash (8%)<br>Nivolumab (3 mg/kg) plus ipilimumab (1 mg/kg):<br>Pruritus (23%)<br>Diarrhea (10%)<br>Fatigue (19%)<br>Rash (15%)<br>Nivolumab (1 mg/kg) plus ipilimumab (3 mg/kg):<br>Pruritus (18%)<br>Diarrhea (31%)<br>Fatigue (29%)<br>Rash (20%) | Nivolumab:<br>Pruritus (0%)<br>Diarrhea (2%)<br>Fatigue (2%)<br>Rash (0%)<br>Nivolumab (3 mg/kg) plus ipilimumab (1 mg/kg):<br>Pruritus (0%)<br>Diarrhea (2%)<br>Fatigue (0%)<br>Rash (0%)<br>Nivolumab (1 mg/kg) plus ipilimumab (3 mg/kg):<br>Pruritus (2%)<br>Diarrhea (14%)<br>Fatigue (6%)<br>Rash (0%) |
| Ronan J. Kelly et al., 2019 (63) | 63 | EGJ | Arm A:<br>durvalumab 20 mg/kg plus tremelimumab 1 mg/kg Q4W for four cycles, followed by durvalumab 10 mg/kg Q2W<br>Arm B:<br>durvalumab monotherapy (10 mg/kg) Q2W<br>Arm C:<br>tremelimumab monotherapy (10 mg/kg) Q4W for seven doses and then every 12 weeks for two doses for a total of up to 9 doses | Tremelimumab:<br>PFS (rate) 20%<br>OS (rate) 8.3%<br>mOS 7.7 months<br>ORR 8.3%<br>Durvalumab:<br>PFS (rate) 0%<br>OS (rate) 0%<br>ORR 0%<br>mOS 3.4 months<br>Durvalumab plus tremelimumab:<br>PFS (rate) 6.1%<br>OS (rate) 7.4%<br>mOS 9.2 months<br>ORR 7.4% | Rash, Pruritus, Diarrhea, Fatigue<br>Tremelimumab: 66.7%<br>Durvalumab: 33.3%<br>Durvalumab plus tremelimumab: 70.4% | Tremelimumab: 58.3%<br>Durvalumab: 0%<br>Durvalumab plus tremelimumab: / |
| Megan Greally et al., 2019 (64) | 161 | EGC | Anti-CTLA4 mAb+anti-PD-1/PD-L1 mAb<br>Anti-PD-1/PD-L1 mAb | Anti-CTLA4+anti-PD-1/PD-L1 mAb:<br>PFS 1.9 months<br>OS 8.8 months<br>Anti-PD-1/PD-L1 mAb:<br>PFS 1.6 months<br>OS 4.3 months | Skin toxicity (47%)<br>hypothyroidism (19%) | Hepatitis (47%)<br>Colitis (47%) |

Ongoing trials:
NCT02872116 EGJ Experimental: Arm A: Nivolumab + Ipilimumab; Arm B: Chemotherapy
NCT03437200 EC Experimental: Arm A: Chemoradiation + Nivolumab; Arm B: Chemoradiation + Nivolumab + Ipilimumab
NCT03647969 EGC Experimental: Arm A: Chemotherapy/Nivolumab/Ipilimumab A/A1; Arm B: Chemotherapy/Nivolumab/Ipilimumab sequential A2; Arm C: Chemotherapy/Nivolumab C
NCT03416244 ESCC Experimental: Arm A: Nivolumab/Ipilimumab combination treatment; Arm B: Nivolumab monotherapy

EAC, esophageal adenocarcinoma; EGC, esophagogastric cancer; ESCC, esophageal squamous cell carcinoma; EGJ, esophagogastric junction; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; PD-1, programmed death receptor 1; PD-L1, programmed cell death-ligand 1; CCRT, concurrent chemoradiotherapy; PFS, progression-free survival; OS, overall survival; mOS, median overall survival; ORR, objective response rate; BSC, best supportive care.

irAEs (84% in the N1+I3 group, 75% in the N3+I1 group) than the nivolumab group (69%). The N1+I3 group had the highest incidence of grade 3 or 4 irAEs (47%), which might be the result of a dose-dependent nivolumab-mediated immune response [61, 62].

*Other combination therapy strategies:* In a phase Ib/II clinical trial in patients with advanced EGJ cancer, 63 second-line patients were randomly divided into three groups to receive durvalumab plus tremelimumab (arm A), durvalumab alone (arm B) or tremelimumab (arm C) alone. The mOS was higher in arm A (9.2 months) than in the other arms (3.4 months in arm B and 7.7 months in arm C), and the overall response rates were 7.4%, 0%, and 8.3% in arm A, arm B, and arm C, respectively. However, there were no significant differences among the treatment groups in ORR or PFS. In addition, irAEs were observed in 70.4%, 33.3%, and 66.7% of patients in arms A, B, and C, respectively. This study showed that response rates were low in patients with EGJ cancer regardless of therapy type (monotherapy or ICI combination strategies). However, the combination therapy (arm A) group had the highest OS rate at 12 months compared with arms B and C (37.0%, 4.6%, and 22.9%, respectively) [63] (**Table 2**). Greally et al. divided 161 patients with advanced EGC into two arms. One arm had 110 patients who were treated with PD-1/PD-L1 inhibitors, while the other had 51 patients who received anti-CTLA-4 and anti-PD-1/PD-L1 antibodies. Notably, the mOS was 8.8 months in the latter arm versus 4.3 months in the former arm (P=0.008). However, there was no significant difference in median PFS (1.6 vs. 1.9 months, P=0.208). Additionally, 43 patients had irAEs, which were positively associated with OS [64].

*Other ongoing clinical trials:* Other ongoing phase II/III clinical trials comparing survival time in patients with EC who received nivolumab plus ipilimumab, nivolumab plus chemotherapy, or chemotherapy alone should help to answer questions regarding the relative efficacy and safety of the two combination strategies [NCT02872116, NCT03437200, NCT03647969, NCT03416244]. Several studies in melanoma, colorectal cancer, and other types of cancer have demonstrated an improved long-term clinical benefit and manageable safety profile of anti-CTLA-4 antibodies in combination with anti-PD-1/PD-L1 antibodies, although there was a dose-dependent increase in toxicity [53, 65-68]. These studies suggest promising therapeutic strategies for patients with advanced EC. Moreover, there are some other novel treatments for ECs, such as chimeric antigen receptor T-cell therapy (CAR-T cell) and tumor vaccines. These treatments are commonly used and have been proven to be effective in patients with diffuse large B-cell lymphoma and leukemia [69, 70]. Shi and colleagues explored treatment for EC. They constructed erythropoietin-producing hepatocellular receptor A2 (EphA2)-targeting CAR-T cells that showed a better ability to kill ESCC cells and promote cytokine activity in vitro [71]. Similarly, in a study by Yu and associates, human epidermal growth factor receptor 2 (HER2) was found to be highly expressed in EC, and the researchers successfully developed CAR-T cells targeting the HER2 antigen [72]. Regarding tumor vaccines, Kageyama et al. conducted a clinical trial with 25 patients with advanced EC. The researchers administered a cholesteryl pullulan-NY-ESO-1 (CHP-NY-ESO-1) complex vaccine to the patients; no adverse events were observed during the treatment period, and the treatment provided a survival benefit [73]. In addition, chemoradiation therapy in combination with a multipeptide vaccine targeting kinase of the outer chloroplast membrane 1 (KOC1), upregulated lung cancer 10 (URLC10), TTK, VEGFR1, and VEGFR2 showed a superior effect and satisfactory safety in patients with unresectable ESCC [74]. H. Daiko et al. further noted that vaccination induces functional CD8+ and CD4+ TILs and PD-L1 expression in EC, which suggests that combinations of antibodies and tumor vaccines or traditional chemoradiation therapies can be used in EC in the future [75]. In terms of anti-CTLA-4 mAbs, combination therapy may be an option for treating EC in the future.

## Discussion

The OS of patients with positive or high expression of CTLA-4 is generally shorter than that of patients with negative or low expression, which is consistent with the fact that CTLA-4 inhibits immune cells and induces the proliferation of tumor cells. CTLA-4 plays a critical role in the maintenance of self-tolerance to self-antigens.

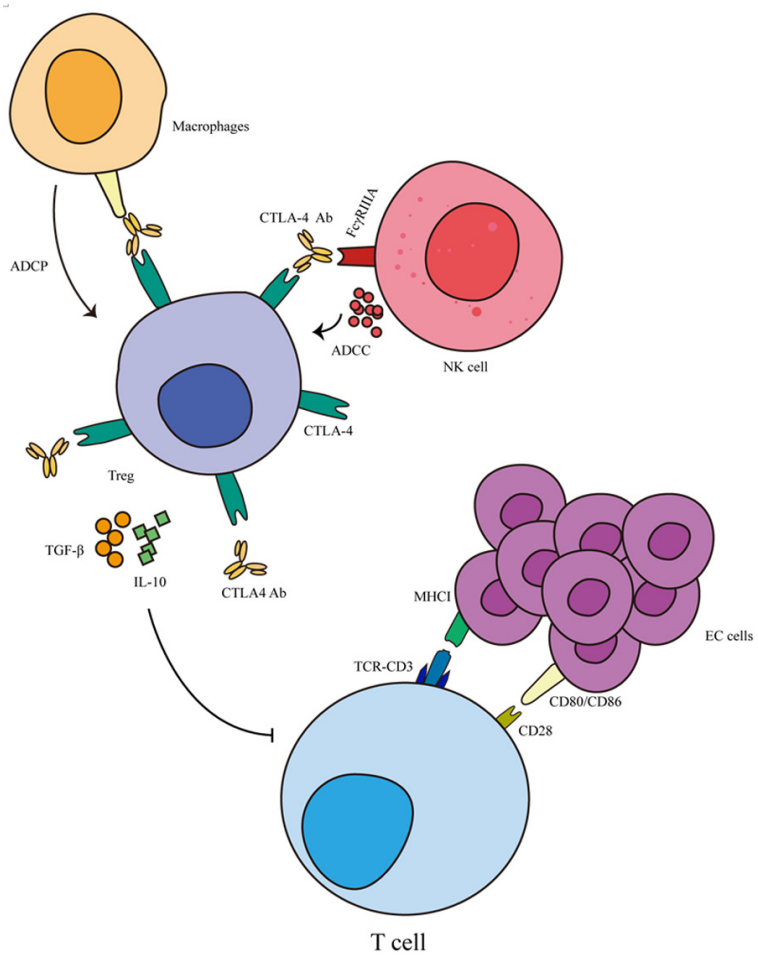


**Figure 4.** Anti-CTLA-4 antibodies meditate ADCC/ADCP. Anti-CTLA-4 antibodies mainly suppress the activities of CTLA-4+ Treg cells by mediating their depletion via antibody-dependent cellular cytotoxicity (ADCC) or antibody-dependent cellular phagocytosis (ADCP).

Patients with EC who have received anti-CTLA-4 therapy have not achieved ideal outcomes and have tended to have a higher incidence of autoimmune disease than those who have received chemotherapy or strategies targeting PD-1/PD-L1. There are some possible reasons for this phenomenon. ① CTLA-4 deficiency and LPS responsive beige-like anchor protein (LRBA) gene mutation may inhibit the binding of anti-CTLA-4 antibodies. ② CTLA-4 is synthesized normally but appears to be aberrantly trafficked in T cells with LRBA deficiency. This ultimately results in enhanced degradation in lysosomes and low levels of CTLA-4 on the surface of T cells [76, 77]. Low levels of CTLA-4 cause poor efficacy of CTLA-4 ICIs and lead to a higher incidence of irAEs. ③ Anti-CTLA-4 antibodies mainly suppress the activities of CTLA-4+ Treg cells by mediating their depletion via antibody-dependent cellular cytotoxicity (ADCC) or antibody-dependent cellular phagocytosis (ADCP) [78] (**Figure 4**). Liu et al. further demonstrated that irAE-prone and non-irAE-prone antibodies differentially affect intracellular CTLA-4 trafficking based on the pH sensitivity of their CTLA-4 binding. pH-sensitive anti-CTLA-4 antibodies dissociate from CTLA-4 and thus allow it to recycle to the cell surface.

In contrast, pH-insensitive antibodies continue to bind to CTLA-4 and prevent it from recycling to the cell surface. Thus, pH-insensitive antibodies cause downregulation of CTLA-4 through lysosomal degradation and thus reduce ADCC/ADCP, which causes autoimmune diseases. As such, it is crucial to control ADCC/ADCP by preserving the recycling of CTLA-4 molecules [79]. Sharma et al. highlighted that ADCC caused by ipilimumab is essentially mediated through FcγRIIIA expressed on NK cells or macrophages in patients [80]. Nevertheless, their research did not indicate which type of Fc-receptor tremelimumab binds. Ipilimumab is an unmodified, fully human IgG1 antibody, while tremelimumab is an unmodified, fully human IgG2 antibody. Both tremelimumab and ipilimumab induce ADCP and ADCC of CTLA-4-expressing cells in vitro, but ipilimumab shows a more significant effect, although it can lead to severe irAEs [81]. We speculate that the ratio of NK cells/macrophages may influence the outcome of anti-CTLA-4 antibody therapy by affecting ADCC/ADCP, and this mechanism can be exploited to improve outcomes in EC.

Furthermore, many studies have focused on the relationship between microsatellite instability (MSI)-high tumors and mismatch repair-deficient (dMMR) tumors, which may be related

to the outcome of anti-PD-1/PD-L1 therapy. Future studies should also consider the relationship between CTLA-4 and MSI/dMMR status and the roles of other molecules, such as CD3, granzyme A (GZMA), and lymphocyte-activation gene 3 (LAG-3), in EC because some of these factors may have value as biomarkers for anti-CTLA-4 therapy [82-86]. Moreover, it is essential to focus on the function of other molecules and cells in EC tissues.

## Conclusion

CTLA-4 ICIs (ipilimumab and tremelimumab) have been applied in patients with various tumors in the clinic. However, the overall efficacy rate of EC treatment remains low, while the incidence of irAEs is still high. Future research on CTLA-4 should further explore the relevant cell subtypes and mechanisms underlying its effects, with a focus on the impact of ICIs on the tumor microenvironment. With more research on the mechanism of CTLA-4 ICIs, immunotherapy will become a cornerstone of therapy for patients with EC.

## Disclosure of conflict of interest

None.

## Abbreviations

ADCC, antibody-dependent cell-mediated cytotoxicity; ADCP, antibody-dependent cellular phagocytosis; Akt, protein kinase B; APC, antigen-presenting cell; ARG1, arginase 1; BSC, best supportive care; CAFs, carcinoma-associated fibroblasts; CCRT, concurrent chemoradiotherapy; CAR-T, chimeric antigen receptor T; CSF-1, colony-stimulating factor-1; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; CHP-NY-ESO-1, cholesteryl pullulan-NY-ESO-1; dMMR, mismatch repair-deficient; DOR, duration of response; DC, dendritic cell; EAC, esophageal adenocarcinoma; EGC, esophagogastric cancer; EGJ, esophagogastric junction; ESCC, esophageal squamous cell carcinoma; EphA2, erythropoietin-producing hepatocellular receptor A2; FDA, Food and Drug Administration; GZMA, granzyme A; HER2, human epidermal growth factor receptor 2; ICIs, immune checkpoint inhibitors; IFN-γ, interferon-γ gamma; IL1R2, IL1 receptor 2; IDO, indoleamine 2,3-dioxygenase 1; irAE, immune-related adverse event; ITIM, immunoreceptor tyrosine-based inhibitory motif; KOC1, kinase of the outer chloroplast membrane 1; LAG-3, lymphocyte-activation gene 3; LRBA, LPS responsive beige-like anchor protein; MDSCs, myeloid-derived suppressor cells; mOS, median overall survival; MSI, microsatellite instability; NK cell, natural killer cell; NKG2D, NK cell lectin-like receptor subfamily K; NKp30, natural cytotoxicity triggering receptor 3; N2, type 2 neutrophil; ORR, objective response rate; OS, overall survival; PD-1, programmed death 1; PD-L1, programmed cell death ligand 1; PFS, progression-free survival; PI3K, phosphatidylinositol-4,5-bisphosphate 3-kinase; PLR, platelet lymphocyte ratio; PP2A, protein phosphatase 2; PR, partial response; SD, stable disease; SHP2, src homology 2 domain-containing protein tyrosine phosphatase-2; STAT3, signal transducer and activator of transcription 3; TIL, tumor-infiltrating lymphocyte; TME, tumor microenvironment; TNF-α, tumor necrosis factor-α; Treg, regulatory T cell; Tyk2, tyrosine kinase 2; TANs, tumor-associated neutrophils; TTK, TTK protein kinase; URLC10, upregulated lung cancer 10; VEGFR1, vascular endothelial growth factor receptor 1; VEGFR2, vascular endothelial growth factor receptor 2.

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