Immune checkpoint inhibitors in esophageal squamous cell carcinoma: progress and opportunities

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Abstract: Esophageal squamous cell carcinoma (ESCC) is one of the common malignant tumors in the world. More than half of patients with ESCC were detected in advanced or metastatic disease at the time of initial diagnosis and lost the opportunities of surgery. Currently, surgical resection, radiotherapy, and chemotherapy are most utilized in clinical practice, however, they are associated with limited survival benefits. Recognition of the limitation of traditional antitumor strategies prompt the development of new means to treat human cancer. In recent years, studies on immune checkpoint inhibitors (eg PD-1/PD-L1 inhibitors, CTLA-4 inhibitors, etc.) in ESCC have shown promising results. In addition, the combination of immune checkpoint inhibitor and traditional antitumor strategies for ESCC has caused extensive interest, and the results are encouraging. Previous analysis indicated that tumor cell PD-L1 expression, tumor mutation load (TMB), microsatellite instability-high status (MSI-H), and other biomarkers have relatively correlated with the efficacy of immunotherapy. This review explores the recent studies investigating checkpoint inhibitors in ESCC.

Keywords: esophageal squamous cell carcinoma (ESCC), immune checkpoint inhibitors, biomarkers, research progress

Background
Esophageal cancer is the eighth most common cancer in the world and is the sixth leading cause of cancer-related deaths. There were 572,034 cases of new diagnosed esophageal cancer worldwide and 508,585 deaths were reported in 2018, which is hence a real global health challenge. The major histological types of esophageal cancer are squamous cell carcinoma and adenocarcinoma, moreover, histological subtypes and cancer incidence are closely related to geographical distribution. Although the esophageal adenocarcinoma incidence is increasing in Western countries, esophageal squamous cell carcinoma (ESCC) predominates in Asia countries, including China. Because the clinical symptoms of early esophageal cancer are obscure, more than half of the patients are in the advanced stage when they are detected. In this population of patients, palliative treatment is of great significance. However, the prognosis of patients in advanced or metastatic esophageal cancer was associated with a limited survival benefit, in fact, overall survival (OS) rate of 5 years was less than 15%. The National Comprehensive Cancer Network (NCCN) guidelines recommended cisplatin or oxaliplatin together with fluorouracil or capecitabine as first-line chemotherapy regimen for ESCC or the esophageal adenocarcinoma. The addition of epirubicin, irinotecan, or taxanes are correlated with an added benefit, but the disease control rate (DCR) for the combination chemotherapy regimens are less than 50%, and the median OS is less than 11 months, in addition, the combined
regimens are associated with severe poisonousness.\textsuperscript{6,7} Therefore, other anticancer strategies are urgently needed to improve the prognosis of these patients. In the past few decades, numerous clinical trials on targeted therapies using gefitinib, erlotinib and cetuximab showed insignificant survival benefits.\textsuperscript{8–10} Recently, it is worth noting that the successive discovery and further study of immune checkpoints, such as programmed death protein 1 (PD-1), programmed death protein 1 ligand (PD-L1), and cytotoxic T lymphocyte antigen 4 (CTLA-4), make immunotherapy served as the fourth antitumor strategies following surgery, radiotherapy and chemotherapy.\textsuperscript{11–13} Currently, immunotherapy is under extensive investigation and this review generalizes the most recent studies of immune checkpoint inhibitors for ESCC.

The mechanism of immune checkpoint

Various of co-stimulatory and inhibitory molecules form a complicated signaling pathway and involved in regulating the human immune function.\textsuperscript{14}

In these pathways, T lymphocytes play an important role in activating the immune system and against foreign pathogens as well as tumor cells. There are many immune checkpoints expressed on the surface of T lymphocytes, including programmed cell death protein 1 (PD-1) and cytotoxic T-lymphocyte antigen 4 (CTLA-4).

PD-1 belonging to the immunoglobulin superfamilly contains immunoreceptor tyrosine-based inhibitory motif (ITIM) and immunoreceptor tyrosine-based switch motif (ITSM).\textsuperscript{15} Usually, PD-1 couples with its ligands PD-L1 and PD-L2 to restrain the function of T cells in the immune system, involved in preventing autoimmune reaction and excessive inflammation, thus, protect the normal cells.\textsuperscript{16,17} Tumor-infiltrating T lymphocytes (TILs) upregulate PD-L1 expression in tumor cells by secreting interferon $\gamma$.\textsuperscript{17,18} More than 40% of ESCC and 18% of esophageal adenocarcinoma are correlated with PD-L1 overexpression, and the prognosis of patients with PD-L1 overexpression is worse than those without PD-L1 overexpression in ESCC.\textsuperscript{19,20} Tumor cells can inhibit the function of effector T cells, through the PD-1/PD-L1 pathway, and cause immune escape.\textsuperscript{17,18}

CTLA-4, also known as a T-cell receptor, belongs to the immunoglobulin superfamily. Similar to the T-cell surface co-stimulatory protein CD28, CTLA-4 competes with CD28 in binding to antigen presenting cell surface ligands CD80 (B7-1) and CD86 (B7-2), moreover, the combining capacity of CTLA-4 is stronger than CD28. CD28 is involved in the activation, proliferation, and migration of T cells, while CTLA-4 is mainly served as a suppressor, resulting in immune inhibition.\textsuperscript{21,22} For CD4+ helper T lymphocytes (Th), CTLA-4 combines with ligand B7 and decreases T cell differentiation, down-regulates the production of lymphocyte factors, thereby suppresses the cellular immune system and humoral immune system. Conversely, in CD4+ regulatory T lymphocytes (Tregs), CTLA-4 binding with B7 induces the overexpression of a variety of immunosuppressive factors, as a result, enhances the immunosuppressive effect of Tregs cells, and promotes the immune escape of tumor cells. In addition, CTLA-4 expressed on T cells also down-regulates CD80 and CD86, decreases the stimulation between T cells and antigen-presenting cells, thus blocks the activation of the immune system.\textsuperscript{22,23}

Continuous discovery of tumor-related immune mechanisms has promoted the development of immune checkpoint inhibitors, which is being the most exciting cancer treatment currently.

The application of immune checkpoint inhibitors in ESCC

PD-1/PD-L1 immune pathway inhibitors

Tumor cell ligands including PD-L1 and PD-L2 bind to the PD-1 receptor on activated T lymphocytes, inhibit the antitumor effect of T cells and lead to tumor cells immune escape. PD-1 or PD-L1 antibodies are applied to prevent the combination of PD-1 with its corresponding ligands, so as to reduce the immunosuppressive effect on effector T cells, and promote the anti-tumor effect of the body’s immune system. To date, PD-1/PD-L1 inhibitors, including nivolumab, pembrolizumab, JS001, SHR-1210, durvalumab and SHR-1316, mainly applied in ESCC (Table 1).

Nivolumab is a high-affinity, humanized IgG4 monoclonal PD-1 antibody.\textsuperscript{24} ATTRACTION-01 trial is an open multicenter phase II clinical study focused on the safety and antitumor efficacy of nivolumab in esophageal cancer, there are 65 patients with advanced ESCC recruited, these patients were refractory or could not tolerate chemotherapy, the objective response rate (ORR) was 17.2%, including 3 patients achieved complete response (CR) and 8 patients were partial response (PR), and the median duration of response (DOR) was 11.17 months. The 1-, 1.5- and 2-year OS rates were 45.3%, 25.0% and 17.2%, respectively, with progressive-free survival (PFS) rates of 1-, 1.5- and 2-year of 10.3%, 8.6% and 8.6%, respectively; only 17 (26%) patients had grade 3–4 adverse events (AEs) and there were no treatment-related deaths.\textsuperscript{25,26} This clinical trial shows that
Table 1 List of ongoing clinical studies with immune checkpoint therapies for ESCC or malignant tumors including ESCC

| Checkpoint inhibitors | Tumor | Treatment | Line | Phase | Primary endpoint | NCT Number |
|-----------------------|-------|-----------|------|-------|-----------------|------------|
| PD-1 inhibitor        | ESCC  | Pembrolizumab + chemoradiation | Neoadj. | Ib    | Safety          | NCT03792347 |
| PD-1 inhibitor        | ESCC  | Pembrolizumab + chemoradiation | Neoadj. | II    | pCR rate        | NCT02844075 |
| PD-1 inhibitor        | Esophageal or gastroesophageal cancer | Pembrolizumab + epacadostat | Neoadj. | II    | Anti-tumor immune response, AE | NCT03592407 |
| PD-1 inhibitor        | Esophageal and gastric cancer | Pembrolizumab + chemoradiation | Neoadj. | II    | pCR rate        | NCT03064490 |
| PD-1 inhibitor        | ESCC  | SHR-1210+ radiation | Neoadj. | II    | pCR rate        | NCT03200691 |
| PD-1 inhibitor        | ESCC  | Pembrolizumab + chemoradiotherapy | Adj. | II    | 1-year RFS rate | NCT03322267 |
| PD-L1 inhibitor       | ESCC  | Durvalumab vs placebo | Adj. | II    | DFS            | NCT02520453 |
| PD-1 inhibitor        | ESCC  | SHR-1210+ apatinib + irinotecan/paclitaxel liposome + nedaplatin vs placebo + paclitaxel + cisplatin | 1st | II    | PFS            | NCT03603756 |
| PD-L1 inhibitor       | ESCC  | SHR-1316+ irinotecan liposome + fluorouracil vs fluorouracil + cisplatin | 1st | III   | PFS, OS        | NCT03732508 |
| PD-1 inhibitor        | Esophageal cancer | Pembrolizumab + Cisplatin +5-Fluorouracil vs Placebo + Cisplatin +5-Fluorouracil | Neoadj. | II    | PFS, OS        | NCT03189719 |
| PD-1 inhibitor/CTLA-4 | ESCC  | Nivolumab + Fluorouracil + Cisplatin or Nivolumab + Ipilimumab vs Fluorouracil + Cisplatin | 1st | III   | PFS, OS        | NCT03691090 |
| PD-1 inhibitor/CTLA-4 | ESCC  | Nivolumab ± Ipilimumab | 2nd | II    | OS            | NCT03416244 |
| PD-1 inhibitor        | ESCC  | SHR-1210+ apatinib | 2nd | II    | ORR           | NCT03736863 |
| PD-1 inhibitor        | ESCC  | SHR-1210+ nivolumab | 2nd | II    | ORR           | NCT03766178 |
| PD-1 inhibitor        | Esophageal cancer | Nivolumab vs docetaxel/paclitaxel | 2nd | III   | OS            | NCT02564263 |
| PD-1 inhibitor        | Esophageal cancer | Pembrolizumab vs docetaxel/paclitaxel/irinotecan | 2nd | III   | OS            | NCT03099382 |
| PD-1 inhibitor        | Esophageal cancer | SHR-1210 vs docetaxel/irinotecan | 2nd | III   | OS            | NCT02971956 |
| PD-1 inhibitor        | Esophageal cancer | Pembrolizumab | Salvage | II | ORR | NCT02642809 |
| PD-1 inhibitor        | Esophageal cancer | Pembrolizumab + radiation | – | I | AE | NCT03474640 |
| PD-1 inhibitor        | Advanced malignancies (including esophageal cancer) | JS001 | – | I | AE | NCT01938612 |
| PD-L1 inhibitor       | Advanced solid tumors (including esophageal cancer) | Durvalumab | – | I | AE | NCT03766178 |
| PD-1 inhibitor        | ESCC  | Nivolumab + carboplatin/paclitaxel + radiation | – | I/II | Safety | NCT03278626 |
| PD-1 inhibitor        | Esophageal cancer | Nivolumab + palliative radiotherapy/definitive chemoradiotherapy/ neoadjuvant chemoradiotherapy | – | I/II | AE | NCT03544736 |
| PD-1 inhibitor        | ESCC  | JS001 | – | Ib/II  | ORR | NCT02915432 |
| PD-L1 inhibitor/CTLA-4 | Metastatic squamous cell Cancer (including ESCC) | Durvalumab + tremelimumab + stereotactic body radiotherapy (SBRT) | – | I/II | DLT | NCT03212469 |
Nivolumab has a controllable safety and durable antitumor activity in advanced ESCC. Recently, the latest results of phase III clinical trial ATTRACTION-03 are as follows: Nivolumab exposed a remarkable extension in OS when compared with chemotherapy (docetaxel or paclitaxel) in unresectable advanced or recurrent esophageal cancer patients who were refractory to or intolerant of fluoropyrimidine and platinum-based combination therapy.\(^2\) Several studies concentrated on the treatment of esophageal cancer with nivolumab are currently underway (Table 1).

Pembrolizumab, a strong affinity with PD-1, is a genetically engineered human IgG4-k monoclonal antibody. Pembrolizumab has shown significant antitumor activity and safety in patients with advanced malignancies. KEYNOTE-028 trial is a phase I/II clinical study; the 23 esophageal cancer patients with standard treatment failure enrolled were PD-L1 positive (PD-L1 combined positive score (CPS) ≥1, in which CPS meant that the number of PD-L1 staining cell divided by the total number of viable tumor cells and multiplied by 100), and 78% of patients were ESCC. With a median follow-up of 7 months, the ORR was 30% and the median DOR was 15 months, and the ORR was 28% in the subgroup of ESCC patients (5/18). The incidence of grade 3 treatment-related AEs was 17%. There is No grade 4 AEs or treatment-related deaths occurred.\(^2\) However, a phase II KEYNOTE-180 trial of advanced/metastatic esophageal cancer further assessed the safety and antitumor activity of pembrolizumab. Among the 63 patients with ESCC, the ORR was 14.3%, the DCR was 40%, the median PFS was 2.1 months, and the median OS was 6.8 months. Only 12.4% of patients experienced grade 3–5 treatment-related AEs, and 1 patient died of pneumonitis.\(^2\) Recently, in the global phase III KEYNOTE-181 trial, pembrolizumab compared with chemotherapy (paclitaxel, docetaxel, or irinotecan) as second-line therapy for advanced esophageal cancer were performed. In the subgroup with ESCC, median OS was 8.2 months with pembrolizumab and 7.1 months with chemotherapy (hazard ratio [HR] =0.78, \(P= 0.0095\)), with 12 months, 24 months -OS rates of 39% vs 25%, and 23% vs 12%, respectively, and PFS rates at 12 months at of 21% vs 7% respectively. In the PD-L1 CPS≥10 subgroup (n=220), median OS was 9.3 months with pembrolizumab, while median OS was 6.7 months with chemotherapy (HR=0.69, \(P= 0.0074\)). In the pembrolizumab group, the 12 months -PFS rate (21% vs 7%) and the OS rate at 12 months (43% vs 20%) were higher than the chemotherapy group. Compared with chemotherapy,

| Table 1 (Continued). |
|----------------------|
| **Tumor**           | PD-L1 inhibitor/CTLA-4 inhibitor | PD-L1 inhibitor | PD-L1 inhibitor | PD-L1 inhibitor | PD-L1 inhibitor | PD-L1 inhibitor/CTLA-4 inhibitor |
|                     | ESCC                           | ESCC            | ESCC            | ESCC            | ESCC            | ESCC                         |
| **Checkpoints inhibitors** | Durvalumab ± tremelimumab + chemotherapy | avelumab + pembrolizumab + chemotherapy | avelumab + pembrolizumab | avelumab + pembrolizumab | avelumab + pembrolizumab | avelumab + pembrolizumab + chemotherapy |
| **Phase** | Primary endpoint | NCT Number |
| Phase III | II | AE, DLT |
| Line | Treatment | NCT02735239 | NCT02647785 | NCT0232340 | NCT02018751 | NCT02639065 | NCT03737400 |
| 1 | Durvalumab ± tremelimumab + chemotherapy | NCT02639065 | NCT03477813 | NCT03477200 | NCT03737400 |

**Abbreviations:** ESCC, esophageal squamous cell carcinoma; PD-1, programmed cell death protein 1; PD-L1, programmed death ligand 1; CTLA-4, cytotoxic T-lymphocyte antigen 4; pCR, pathological complete response; AE, adverse events; RFS, recurrence-free survival; DFS, disease free survival; PFS, progression free survival; OS, overall survival; ORR, objective response rate; DLT, dose limiting toxicity.

\(^2\) Jiao et al. Dovepress.
fewer patients with pembrolizumab had experienced drug-related AEs.\textsuperscript{30} The results of this trial have brought hope to immune checkpoint inhibitors as a new second-line treatment for ESCC. In addition, there are still several clinical studies focusing on pembrolizumab as a first-line or second-line treatment for esophageal cancer (Table 1).

JS001 is a new recombinant humanized IgG4 monoclonal PD-1 antibody developed by China. In an open, multi-center phase Ib/II trial, 34 patients of metastatic ESCC were eligible for evaluated efficacy; 8 patients achieved PR, 14 patients achieved stable disease (SD). In the 10 patients of PD-L1 positive (PD-L1 CPS $\geq$1), only 2 (20\%) people were PR; and in the 24 patients of negative PD-L1 (PD-L1 CPS < 1), 6 (25\%) people achieved PR.\textsuperscript{31} The preliminary results showed that the clinical efficacy of JS001 was not related to PD-L1 expression and this study is still ongoing. Other studies include a phase I trial of JS001 for patients with advanced esophageal cancers (NCT03474640).

SHR-1210 is a humanized IgG4-kappa monoclonal PD-1 antibody with high affinity and selectivity developed by China. MoH et al reported that SHR-1210 had durable anti-tumor activity and controllable safety.\textsuperscript{32} In a phase I clinical study of advanced ESCC that is refractory or intolerant to chemotherapy, SHR-1210 was administered to 30 patients, the results revealed an ORR of 33.3\%, DCR of 56.7\%, and median PFS of 3.6 months. However, 23 (76.7\%) patients developed reactive capillary hemangioma and 3 (10\%) patients developed grade 3 treatment-related AEs (2 pneumonitis and 1 increased cardiac troponin I).\textsuperscript{33} The incidence of reactive capillary hemangioma is high, mainly because SHR-1210 is an effective agonist of human vascular endothelial growth factor 2 that can activate vascular endothelial cells, and promote the occurrence of hemangioma.\textsuperscript{34} These discoveries make the application of SHR-1210 and vascular endothelial growth factor inhibitor as a new combined anti-tumor strategy. The initial outcomes showed SHR-1210 had a manageable toxicity and promising antitumor activity; in addition, multiple studies involving SHR-1210 are ongoing (Table 1).

Durvalumab is a monoclonal immunoglobulin IgG1xantibody against PD-L1. 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 treatment-related AEs; Nineteen patients were evaluated for efficacy, with 1 patient PR and 6 patients SD.\textsuperscript{35} Currently, This study and other phase I/II clinical studies of durvalumab for esophageal cancer are still ongoing (Table 1).

SHR-1316, a humanized immunoglobulin IgG4 PD-L1 monoclonal antibody designed and produced in China, and has entered into various clinical studies in solid tumors. A multicenter, multi-country phase II clinical study of irinotecan/5-fluorouracil chemotherapy in combination with SHR-1316 in the treatment of advanced ESCC is well underway (Table 1), and the results including the primary endpoint of PFS are expecting.

**CTL4 immune pathway inhibitor**

In the Early stage of immune response, CTLA-4 plays a major role in regulating T cell proliferation, which is mainly located in lymph nodes. The CTLA-4 inhibitor combines with the CTLA-4 receptor and prevents the CTLA-4 receptor from binding to the B7 ligand on the antigen presenting cell surface, promoting T cell activation and proliferation to exert an anti-tumor effect. When T cells in peripheral tissues were activated, PD-1 was up-regulated and exerting immunosuppressive effect.\textsuperscript{36} Therefore, the immunosuppressive response mainly occurred in peripheral tissues for PD-1/PD-L1 antibody; CTLA-4 pathway inhibitors cause more severe autoimmune diseases than PD-1/PD-L1 pathway inhibitors, which limits the clinical application of CTLA-4 inhibitors. Commonly used CTLA-4 inhibitors including ipilimumab and tremelimumab are mainly applied to clinical trials of malignant melanoma, non-small cell lung cancer and other solid tumors. To date, ipilimumab is approved for the treatment of advanced malignant melanoma because it can significantly improve the OS of patients with metastatic malignant melanoma.\textsuperscript{37} However, ipilimumab did not distinctly improve OS in patients with NSCLC, and the incidence of grade 3-4 immune-related AEs was as high as 47\%.\textsuperscript{38} Moreover, a phase II trial (NCT01585987) about ipilimumab monotherapy for advanced gastric cancer and gastroesophageal junction cancer failed and was terminated.\textsuperscript{39} Currently, the efficacy of CTLA-4 inhibitors in esophageal cancer remains unclear; And there are few studies on CTLA-4 inhibitors monotherapy for esophageal cancer, mostly CTLA-4 inhibitors combined with PD-1/PD-L1 inhibitors (Table 1).

**Biomarkers predicting the efficacy of immunotherapy for ESCC PD-L1**

The significance of PD-L1 expression level in tumor cells is still controversial. Most scholars believe that PD-L1 expression in tumor cells is the most reasonable biomarker to predict...
the therapeutic efficacy of PD-1/PD-L1 inhibitors.\textsuperscript{12,19,40,41} Feihrenbacher et al indicated that the expression of PD-L1 was correlated with the therapeutic effect of PD-1/PD-L1 inhibitors.\textsuperscript{42} In the KEYNOTE-180 trial, patients with PD-L1 CPS $\geq 10$ had a higher 6-month-PFS rate (22\% vs 10\%) and 9-month-PFS rate (14\% vs 5\%) than those with PD-L1 CPS $<10$.\textsuperscript{29} And the results of phase III KEYNOTE-181 trial revealed significant survival benefit in patient with PD-L1 CPS $\geq 10$ with pembrolizumab. However, in a clinical trial of JS001, the preliminary results revealed that there was no relationship between clinical efficacy and PD-L1 expression.\textsuperscript{31} Besides, Huang et al showed that the expression of PD-L1 was not significantly associated with ORR and DCR in the clinical trial of SHR-1210 for esophageal cancer.\textsuperscript{33} Some patients with negative PD-L1 expression are effective in immune checkpoint inhibitors treatment, while some patients with positive PD-L1 expression are ineffective, in which these inconsistent results are mainly because of the heterogeneity of PD-L1 tumor expression, different samples submitted for examination and inconsistent detection standards, etc. In conclusion, it remains uncertain whether the tumor PD-L1 expression level is correlated with the efficacy of immunotherapy, and more evidence is needed to confirm the relationship between them in the future.

**Tumor mutation burden (TMB)**

TMB refers to the total amount of non-synonymous mutations in the tumor gene coding region; the higher TMB means the more neoantigens generated by tumor mutations, the more tumor-infiltrating T lymphocytes, and the stronger anti-tumor immune response.\textsuperscript{43} Between different cancer classes, TMB was highly variable and ranged from 0.001 per megabase(Mb) to more than 400 per Mb.\textsuperscript{44} And median numbers of TMB in esophageal cancer was lower than that in lung cancer and melanoma.\textsuperscript{44} Several studies have shown that both PFS and OS are prolonged with the increase of TMB with immunotherapy, and TMB has the potential to be a biomarker to evaluate the efficacy of immunotherapy.\textsuperscript{41,45–47} Rizvi and colleagues indicated that TMB had a strong correlation with clinical response in non-small lung cancer treated with PD-1 inhibitors.\textsuperscript{48} Likewise, long-term benefit was also associated with a higher TMB in melanoma patients treated with CTLA-1 inhibitors.\textsuperscript{49} Besides, Greally et al analyzed the relationship between TMB and survival in 62 patients of immunotherapeutic esophageal cancer, including 8 patients of ESCC; this clinical study found that the optimal critical value was 7.3 per Mb and indicated that patients in the high TMB group obtained significant survival benefits.\textsuperscript{50} The numbers of ESCC patients were few in this study, and prospective randomized clinical studies are needed to identify and validate the optimal cut-offs value of TMB in ESCC that effectively predict response to immune checkpoint inhibitors in the future.

**Microsatellite instability–high status (MSI-H)**

Microsatellite instability-high status (MSI-H), also known as deficiencies mismatch repair (dMMR), is caused by mutations in the mismatch repair proteins MLH1, MSH2, PMS2, and MSH6, and induces more neoantigen emergence to increase immune cell infiltration.\textsuperscript{51} Previous study on immunotherapy for colorectal cancer found there was a positive association between MSI-H and high TMB.\textsuperscript{52} Le et al showed that the status of mismatch repair could predict clinical benefit of pembrolizumab and discovered that dMMR tumors were associated with prolonged PFS compared with mismatch repair-proficient tumors, regardless of the origin tissue of cancer.\textsuperscript{53,54} So far, the NCCN guidelines have recommended pembrolizumab as second-line or subsequent therapy for MSI-H or dMMR esophageal cancer.\textsuperscript{6} Although the incidence of MSI-H in ESCC is rare and only about 8\%, this biomarker is very important and may affect the efficacy of immune checkpoint inhibitors.\textsuperscript{55}

The analysis and verification of biomarkers that predict the efficacy of immune checkpoint inhibitors will optimize the selection of eligible patients with esophageal cancer for immunotherapy, and promote the individualization and precision of immunotherapy. To date, the prediction effect of single biomarker is limited, more attention should be paid to the combined prediction models of multiple biomarkers in evaluating the efficacy of immune in the future.

**The combination therapy**

In recent years immunotherapy combined with other anti-tumor strategies have attracted more attention, especially in malignant melanoma and non-small cell lung cancer. However, there is still a long way of immunotherapy in ESCC.

The most common combination of immune checkpoint inhibitors is PD-1/PD-L1 pathway inhibitors united with CTLA-4 pathway inhibitors. A global phase II clinical study of nivolumab alone or combined with ipilimumab for patients with advanced ESCC is ongoing.\textsuperscript{56} In addition, multiple clinical studies of PD-1/PD-L1 inhibitors combined with CTLA-4 inhibitors for the treatment of esophageal cancer are also underway (Table 1).
Radiotherapy plays a significant role in the comprehensive treatment of ESCC. Radiotherapy can induce immune-mediated abscopal effects by re-recruiting T cells to enter the microenvironment, promoting the secretion of cytokines and enhancing the expression of tumor antigens. Herrera et al proved that radiotherapy can directly induce the DNA damage in tumor cells, stimulate more tumor immuno-associated antigens releasing, and increase the infiltration of tumor T lymphocytes; however, radiotherapy can also up-regulate the PD-L1 expression of tumor cells and inhibit the anti-tumor activity of effector T cells, leading to radiotherapy resistance. Radiotherapy combined with immunotherapy can not only improve the sensitivity of radiotherapy, but also harness the immune system to improve cancer therapy. Currently, multiple phase I/II clinical study of radiotherapy combined with immune checkpoint inhibitors for esophageal cancer is underway (Table 1).

Chemotherapy is an aggressive therapy to destroy rapidly growing cells in the body. Recent studies have shown that chemotherapy may have stimulated the immune system, which has the potential to induce favorable immunogenic conditions in tumor microenvironment. Chemotherapy combined with immunotherapy can reverse the immunsuppression to some extent, improve the cross-presentation of tumor antigens, promote the proliferation of effector T cells, and enhances the anti-tumor function of the immune system. Several clinical studies to explore the best combination of the two therapies are currently underway (Table 1).

**Evaluation system of immunotherapy**

Traditionally, the Response Evaluation Criteria in Solid Tumor (RECIST) has been used to evaluate the efficacy of antitumor treatment, but it is inappropriate to utilize this system in immunotherapy. The anti-tumor response of immunotherapy is unobvious and persistent; although the tumor will continue to grow in the early stage of treatment, the patient can gain long-term survival benefits. Immunotherapy cause an innate antitumor immune response, this is usually accompanied with the increase of tumor load at the early stage of treatment, the appearance of new antigen and the subsequent continuous disease stabilisation; therefore, the pseudoprogession of tumor are always discovered at the early stage of immunotherapy, and the RECIST may underestimate the efficacy of immunotherapy, leading to early termination of treatment. For this reason, the immune-related response criteria such as irRC, irRECIST, iRECIST and imRECIST emerged. In those evaluation criteria, the presence of new lesions does not necessarily indicate disease progression in the case of reduction of primary lesions, but may activate the immune response within the tumor. Whereas, the immune-related response criteria are rarely used in clinical studies, and its accuracy and feasibility need to be further verified.

**Hyper progression**

Immune checkpoint inhibitors have shown promising anti-tumor activity, however, several studies showed accelerated disease progression in some patients treated with PD-1/PD-L1 inhibitors, which was known as hyper-progressive disease. This new phenomenon is mainly correlated with the dilation of Tregs, depletion of compensatory T cells, restructuring of pro-tumorigenic immune cell subsets, activation of aberrant inflammation or activation of oncogenic signaling. Hyper-progressive disease has been observed in NSCLS, melanoma and other malignancies; nevertheless, in the immunotherapy of ESCC, hyper-progressive disease has not been found so far.

**Liquid biopsy**

Liquid biopsy can obtain an extensive amount of information about the tumor through a simple blood sample, which is simple and non-invasive compared with surgical biopsies. Nicolazzo et al showed that PD-L1 expression in circulating tumor cells was correlated with poor clinical outcomes. Nicolazzo et al showed that PD-L1 expression in circulating tumor cells was correlated with poor clinical outcomes. In addition, Koeppel et al also demonstrated that liquid biopsy could be applied to determine TMB using circulating cell-free DNA, particularly in cases where tumor biopsy was not accessible or had been resampled. Dynamic evaluation of immunotherapy by liquid biopsy has shown certain advantages in other tumors, and it is expected to be applied and confirmed in ESCC.

Multidisciplinary comprehensive treatment is important in theAnti-tumor therapy. At the time of initial diagnosis, most patients with ESCC are in advanced or metastatic stage and their disease progressed rapidly. Present existence treatment regimens have limited benefits for patients. For patients with advanced/metastatic ESCC who have failed in standard treatment, immunotherapy shows persistent anti-tumor activity and manageable safety profile, which creates a promising prospect for the application of immunotherapy in ESCC. At present, there are seldom biomarkers to accurately predict the effect of.
immunotherapy; to some extent, PD-L1, TMB, MSI-H and other biomarkers are related to the efficacy of immunotherapy, and more prospective trials are needed for further study. The evaluation of immunotherapy efficacy by multiple biomarkers may become a direction for future research. The RECIST system is limited in the evaluation of the immunotherapy efficacy, and the optimal evaluation criteria for immunotherapy are being explored. In order to evaluate the most appropriate time window for the combination of immunotherapy and traditional anti-tumor strategies to maximize the anti-tumor benefit of combination therapy and minimize the adverse reactions, a number of studies in ESCC patients are currently under investigation (Table 1). Anyway, immunotherapy is an exciting treatment in the emerged antitumor strategies.

Acknowledgments
This work was supported by the National Natural Science Foundation of China (81372436), and Science and Technology Open Cooperation Project of Henan Province (182106000062).

Disclosure
The authors report no conflicts of interest in this work.

References

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018;68(6):394–424. doi:10.3322/caac.214922
2. Zhang HZ, Jin GF, Shen HB. Epidemiologic differences in esophageal cancer between Asian and Western populations. Chin J Cancer. 2012;31(6):281–286. doi:10.5732/jcj.011.10390
3. Domper Arnal MJ, Ferrandez Arenas A, Lanas Arbeloa A. Esophageal cancer: risk factors, screening and endoscopic treatment in Western and Eastern countries. World J Gastroenterol. 2015;21(26):7933–7943. doi:10.3748/wjg.v21.i26.7933
4. Tew WP, Kelsen DP, Ison DH. Targeted therapies for esophageal cancer. Oncologist. 2005;10(8):590–601. doi:10.1634/theoncologist.10-8-590
5. D’Journo XB, Thomas PA. Current management of esophageal cancer. J Thorac Dis. 2014;6(6 Suppl 2):S253–S264.
6. National comprehensive cancer network: NCCN clinical practice guidelines in oncology. Esophageal and esophagogastroduodenal junction cancers v.1. 2019. Available from: https://www.nccn.org/professionals/physician_gls/f_guidelines.asp. Accessed July 16, 2019.
7. Wiedmaw MW, Mosnjar N. New and emerging combination therapies for esophageal cancer. Cancer Manag Res. 2013;5:133–146. doi:10.2147/CMAJ.S32199
8. Suntharalingam M, Winter K, Ison D, et al. Effect of the addition of cetuximab to paclitaxel, cisplatin, and radiation therapy for patients with esophageal cancer: the NRG oncology RTOG 0436 phase 3 randomized clinical trial. JAMA Oncol. 2017;3(11):1520–1528. doi:10.1001/jamaoncol.2017.1598
9. Zhao C, Lin L, Liu J, et al. A phase II study of concurrent chemoradiotherapy and erlotinib for inoperable esophageal squamous cell carcinoma. Oncotarget. 2016;7(35):57310–57316. doi:10.18632/oncotarget.9809
10. Dutton SJ, Ferry DR, Blazey JM, et al. Gefitinib for oesophageal cancer progressing after chemotheraphy (COG): a phase 3, multicentre, double-blind, placebo-controlled randomised trial. Lancet Oncol. 2018;19(5):894–904. doi:10.1016/S1470-2045(14)70024-5
11. Robert C, Thomas L, Bondarenko I, et al. Ipilimumab plus dacarbazine for previously untreated metastatic melanoma. N Engl J Med. 2011;364(26):2517–2526. doi:10.1056/NEJMoa1104621
12. Topalian SL, Hodi FS, Brahmer JR, et al. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. N Engl J Med. 2012;366(26):2443–2454. doi:10.1056/NEJMoa1200690
13. Brahmer JR, Tykodi SS, Chow LQ, et al. Safety and activity of anti-PD-L1 antibody in patients with advanced cancer. N Engl J Med. 2012;366(26):2455–2465. doi:10.1056/NEJMoa1200694
14. Raufi AG, Klempner SJ. Immunotherapy for advanced gastric and esophageal cancer: preclinical rationale and ongoing clinical investigations. J Gastrointest Oncol. 2015;6(5):561–569. doi:10.3978/j.issn.2078-6891.2015.037
15. Parry RV, Chenmitz JM, Frauwirth KA, et al. CTLA-4 and PD-1 receptors inhibit T-cell activation by distinct mechanisms. Mol Cell Biol. 2005;25(21):9543–9553. doi:10.1128/MCB.25.21.9543-9553.2005
16. Keir ME, Butte MJ, Freeman GJ, Sharpe AH. PD-1 and its ligands in tolerance and immunity. Annu Rev Immunol. 2008;26:677–704. doi:10.1146/annurev.immunol.26.021607.090331
17. McDermott DF, Atkins MB. PD-1 as a potential target in cancer therapy. Cancer Med. 2013;2(5):662–673. doi:10.1002/cam4.106
18. Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. Nat Rev Cancer. 2012;12(4):252–264. doi:10.1038/nrc3239
19. Ohigashi Y, Sho M, Yamada Y, et al. Clinical significance of programmed death-1 and programmed death-1 ligand-2 expression in human esophageal cancer. Clin Cancer Res. 2005;11(8):2947–2953. doi:10.1158/1078-0432.CCR-04-1469
20. Derks S, Nason KS, Liao X, et al. Epithelial PD-L2 expression marks Barrett’s esophagus and esophageal adenocarcinoma. Cancer Immunol Res. 2015;3(10):1123–1129. doi:10.1158/2326-6066.CIR-15-0046
21. Chen DS, Melilman I. Elements of cancer immunity and the cancer-immune set point. Nature. 2017;541(7637):321–330. doi:10.1038/nature21349
22. Qureshi OS, Zheng Y, Nakamura K, et al. Trans-endocytosis of CD80 and CD86: a molecular basis for the cell-exclusive function of CTLA-4. Science. 2011;332(6029):600–603. doi:10.1126/science.1202947
23. Peggs KS, Quezada SA, Chambers CA, Korman AJ, Allison JP. Blockade of CTLA-4 on both effector and regulatory T cell compartments contributes to the antitumor activity of anti-CTLA-4 antibodies. J Exp Med. 2009;206(8):1717–1725. doi:10.1084/jem.20082492
24. Hamanishi J, Mandai M, Ikeda T, et al. Safety and antitumor activity of anti-PD-1 antibody, nivolumab, in patients with platinum-resistant ovarian cancer. J Clin Oncol. 2015;33(34):4015–4022. doi:10.1200/ JCO.2015.62.3397
25. Kudo T, Hamamoto Y, Kato K, et al. Nivolumab treatment for esophageal squamous-cell carcinoma: an open-label, multicentre, phase 2 trial. Lancet Oncol. 2017;18(5):631–639. doi:10.1016/S1470-2045(17)30181-X
26. Kitagawa Y, Doki Y, Kato K, et al. Two year survival and safety update for esophageal squamous cell carcinoma treated with nivolumab (ATTRACTION-1:ONO-4538-07). Ann Oncol. 2017;28(suppl_5). Available from: https://oncologypro.esmo.org/Meeting-Resources/ESMO-2017-Congress/Two-year-survival-and-safety-update-for-esophageal-squamous-cell-carcinoma-treated-with-nivolumab-ATTRACTION-1-ONO-4538-07. Accessed July 16, 2019.
27. Opdivo® (Nivolumab) demonstrates a significant extension in overall survival versus chemotherapy in patients with unresectable advanced or recurrent esophageal cancer in Phase III clinical study; 2019. Available from: https://www.ono.co.jp/en/news/pdf/sm_cn190109.pdf. Accessed July 16, 2019.

28. Doi T, Piha-Paul SA, Jalal SI, et al. Safety and antitumor activity of the anti-programmed death-1 antibody pembrolizumab in patients with advanced esophageal carcinoma. J Clin Oncol. 2018;36(1):61-67. doi:10.1200/JCO.2017.74.9846

29. Shah MA, Kojima T, Hochhauser D, et al. Efficacy and safety of pembrolizumab for heavily pretreated patients with advanced, metastatic adenocarcinoma or squamous cell carcinoma of the esophagus: the Phase 2 KEYNOTE-180 study. JAMA Oncol. 2018. 5(4):546-550. doi:10.1001/jamaoncol.2018.5441

30. Takashi K, Kei M, Eric F, et al. Pembrolizumab versus chemotherapy as second-line therapy for advanced esophageal cancer: Phase III KEYNOTE-181 study. Journal of Clinical Oncology. 2019;37:no. 4_suppl. 2-2. doi:10.1200/JCO.2019.37.4_suppl.2.

31. Xu R-H, Wang F, Shi J, et al. Recombinant humanized anti-PD-1 monoclonal antibody (JS001) as salvage treatment for advanced esophageal squamous cell carcinoma: preliminary results of an open-label, multi-cohort, phase Ib/II clinical study. J Clin Oncol. 2018;36(4_suppl)116. doi:10.1200/JCO.2018.36.4_suppl.116

32. Mo H, Huang J, Xu J, et al. Safety, anti-tumor activity, and pharmacokinetics of fixed-dose SHR-1210, an anti-PD-1 antibody in advanced solid tumours: a dose-escalation, phase 1 study. Br J Cancer. 2018;119(5):538-545. doi:10.1038/s41416-018-0100-3

33. Huang J, Xu B, Mo H, et al. Safety, activity, and biomarkers of SHR-1210, an anti-PD-1 antibody, for patients with advanced esophageal carcinoma. Cancer Res. 2018;78(24):1296-1304. doi:10.1158/0008-5472.CCR-17-2439

34. Finlay WJ, Coleman JE, Edwards JS, Johnson KS. Anti-PD1 ‘SHR-1210’ abnormally targets pro-angiogenic receptors and this polypsistency can be ablated by paratope refinement. Mabs. 2019;11(1):26-44. doi:10.1080/19420862.2018.1550321

35. Ishuchi H, Nogami N, Kozuki T, et al. Phase I study to evaluate the safety and tolerability of MED4736, an anti-programmed cell death ligand-1 (PD-L1) antibody, in Japanese patients with advanced solid tumors. J Clin Oncol. 2018;36(15_suppl)3039. doi:10.1200/JCO.2018.33.15_suppl.3039

36. Ribas A. Tumor immunotherapy directed at PD-1. N Engl J Med. 2012;366(26):2517-2519. doi:10.1056/NEJMMe1205943

37. Hodi FS, O’Day SJ, McDermott DF, et al. Improved survival with ipilimumab in patients with metastatic melanoma. N Engl J Med. 2010;363(8):711–723. doi:10.1056/NEJMoa1003466

38. Reck M, Bondarenko I, Luft A, et al. Ipilimumab in combination with paclitaxel and carboplatin as first-line therapy in extensive-disease small-cell lung cancer: results from a randomized, double-blind, multicenter phase 2 trial. Ann Oncol. 2013;24(1):75–83. doi:10.1093/annonc/mds213

39. Bang Y-J, Cho JY, Kim YH, et al. Efficacy of sequential ipilimumab monotherapy versus best supportive care for unresectable locally advanced/metastatic gastric or gastroesophageal junction cancer. Clin Cancer Res. 2017;23(19):5671–5678. doi:10.1158/1078-0432.CCR-17-0025

40. Guo W, Wang P, Li N, et al. Prognostic value of PD-L1 in esophageal squamous cell carcinoma: a meta-analysis. Oncotarget. 2018;9(17):13920–13933. doi:10.18632/oncotarget.23810

41. Ott PA, Bang YJ, Piha-Paul SA, et al. T-cell-inflamed gene-expression profile, programmed death ligand 1 expression, and tumor mutational burden predict efficacy in patients treated with pembrolizumab across 20 cancers: KEYNOTE-028. J Clin Oncol. 2019;37(4):318–327. doi:10.1200/JCO.2018.78.2276
61. Sakai H, Kokura S, Ishikawa T, et al. Effects of anticancer agents on cell viability, proliferative activity and cytokine production of peripheral blood mononuclear cells. J Clin Biochem Nutr. 2013;52(1):64–71. doi:10.3164/jcbn.12-60

62. Javeed A, Ashraf M, Riaz A, Ghafoor A, Afzal S, Mukhtar MM. Paclitaxel and immune system. Eur J Pharm Sci. 2009;38(4):283–290. doi:10.1016/j.ejps.2009.08.009

63. Lin T, Song C, Chuo DY, Zhang H, Zhao J. Clinical effects of autologous dendritic cells combined with cytokine-induced killer cells followed by chemotherapy in treating patients with advanced colorectal cancer: a prospective study. Tumour Biol. 2016;37(4):4367–4372. doi:10.1007/s13277-015-3957-2

64. Yang L, Ren B, Li H, et al. Enhanced antitumor effects of DC-activated CIKs to chemotherapy treatment in a single cohort of advanced non-small-cell lung cancer patients. Cancer Immunol Immunother. 2013;62(1):65–73. doi:10.1007/s00262-012-1311-8

65. Hodi FS, Hwu WJ, Kefferd R, et al. Evaluation of immune-related response criteria and RECIST v1.1 in patients with advanced melanoma treated with pembrolizumab. J Clin Oncol. 2016;34(13):1510–1517. doi:10.1200/JCO.2015.64.0391

66. Nishino M, Jagannathan JP, Krajewski KM, et al. Personalized tumor response assessment in the era of molecular medicine: cancer-specific and therapy-specific response criteria to complement pitfalls of RECIST. AJR Am J Roentgenol. 2012;198(4):737–745. doi:10.2214/AJR.11.7483

67. Hodi FS, Ballinger M, Lyons B, et al. Immune-Modified Response Evaluation Criteria In Solid Tumors (imRECIST): refining guidelines to assess the clinical benefit of cancer immunotherapy. J Clin Oncol. 2018;36(9):850–858. doi:10.1200/JCO.2017.75.1644

68. Wolchok JD, Hoos A, O’Day S, et al. Guidelines for the evaluation of immune therapy activity in solid tumors: immune-related response criteria. Clin Cancer Res. 2009;15(23):7412–7420. doi:10.1186/1078-0432-CCR-09-1624

69. Bohnsack O, Hoos A, Ludajic K. Adaptation of the immune related response criteria: irRECIST. Ann Oncol. 2014;25(suppl_4):i369. Oxford Academic Annals of Oncology Web site. Available from: https://academic.oup.com/annonc/article/25/suppl_4/i369/2241779. Accessed July 16, 2019.

70. Seymour L, Bogaerts J, Perrone A, et al. iRECIST: guidelines for response criteria for use in trials testing immunotherapeutics. Lancet Oncol. 2017;18(3):e143–e152. doi:10.1016/S1470-2045(17)30072-4

71. Saada-Bouzid E, Defauchex C, Karabakjian A, et al. Hyperprogression during anti-PD-1/PD-L1 therapy in patients with recurrent and/or metastatic head and neck squamous cell carcinoma. Ann Oncol. 2017;28(7):1605–1611. doi:10.1093/annonc/mdx178

72. Ferrara R, Mezquita L, Texier M, et al. Hyperprogressive disease in patients with advanced non-small cell lung cancer treated with PD-1/PD-L1 inhibitors or with single-agent chemotherapy. JAMA Oncol. 2018;4(11):1543–1552. doi:10.1001/jamaoncol.2018.3676

73. Champiat S, Ferrara R, Massard C, et al. Hyperprogressive disease: recognizing a novel pattern to improve patient management. Nat Rev Clin Oncol. 2018;15(12):748–762. doi:10.1038/s41571-018-0111-2

74. Zuazo-Ibarra M, Arasanz H, Fernández-Hinojal G, et al. Highly differentiated CD4 T cells unequivocally identify primary resistance and risk of hyperprogression to PD-L1/PD-1 immune checkpoint blockade in lung cancer. BioRxiv. 2018. Available from: https://www.biorxiv.org/content/10.1101/320176v2. Accessed July 16, 2019.

75. Nicolazzo C, Raimondi C, Mancini M, et al. Monitoring PD-L1 positive circulating tumor cells in non-small cell lung cancer patients treated with the PD-1 inhibitor nivolumab. Sci Rep. 2016;6:31726. doi:10.1038/srep31726

76. Koeppe F, Blanchard S, Jovelet C, et al. Whole exome sequencing for determination of tumor mutation load in liquid biopsy from advanced cancer patients. PLoS One. 2017;12(11):e0188174. doi:10.1371/journal.pone.0188174

77. Cai LL, Wang J. Liquid biopsy for lung cancer immunotherapy. OncoLett. 2019;17(6):4751–4760. doi:10.3892/ol.2019.10166

78. Schumacher TN, Schepfer W. A liquid biopsy for cancer immunotherapy. Nat Med. 2016;22(4):340–341. doi:10.1038/nm.4074