



Crosstalk Between PD-1/PD-L1 Blockade and Its Combinatorial Therapies in Tumor Immune Microenvironment: A Focus on HNSCC

Weimin Lin[†], Miao Chen[†], Le Hong, Hang Zhao* and Qianming Chen

State Key Laboratory of Oral Diseases, West China Hospital of Stomatology, Sichuan University, Chengdu, China

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*Correspondence:

Hang Zhao
zhaohangahy@scu.edu.cn

[†]These authors have equally
contributed to this work

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Head and neck squamous cell carcinoma (HNSCC) is the sixth most common malignancy worldwide with a poor prognosis and high mortality. More than two-thirds of HNSCC patients still have no effective control of clinical progression, and the five-year survival rate is <50%. Moreover, patients with platinum-refractory HNSCC have a median survival of <6 months. The significant toxicity and low survival rates of current treatment strategies highlight the necessity for new treatment modalities. Recently, a large number of studies have demonstrated that programmed cell death protein-1 (PD-1) and its ligand, programmed cell death protein ligand-1 (PD-L1) play an essential role in tumor initiation and progression. PD-1/PD-L1 blockade has shown a desired and long-lasting therapeutic effect in the treatment of HNSCC and other malignancies. However, only a small number of patients with HNSCC can benefit from PD-1/PD-L1 blockade monotherapy, while the majority of patients do not respond. To overcome the unsatisfactory therapeutic effect of PD-1/PD-L1 blockade monotherapy, combining other treatment options for HNSCC (including chemotherapy, radiotherapy, targeted therapy, and immunotherapy) in the treatment scheme has become a commonly used strategy. Herein, the potential mechanisms underlying the crosstalk between PD-1/PD-L1 blockade and its combinatorial therapies for HNSCC were reviewed, and it is hoped that the improved understanding of the crosstalk process would provide further ideas for the design of a combinatorial regimen with a higher efficiency and response rate for the treatment of HNSCC and other malignancies.

Keywords: cancer immunotherapy, combined therapy, PD-1, PD-L1, head and neck squamous cell carcinoma

INTRODUCTION

Head and neck squamous cell carcinoma (HNSCC) includes squamous cell carcinomas that occurs in the nasopharynx, oropharynx, hypopharynx, and throat. Its incidence rate is ranked 6th among common malignancies worldwide (1). Tumor recurrence and metastasis occurs in more than 50% of patients with HNSCC within three years. Only few choices exist for the treatment of recurrent or metastatic (R/M) HNSCC, leading to its poor prognosis. In addition, a higher proportion of patients with R/M HNSCC often have tumor-related symptoms, including pain, hemorrhage, respiratory,

and nutritional disorders, which seriously affect the quality of life of patients and the choice of follow-up treatment (2). In 2016, the US Food and Drug Administration (FDA) approved the PD-1-targeted monoclonal antibodies (mAbs), pembrolizumab, and nivolumab, for the treatment of R/M HNSCC. The new edition of the National Comprehensive Cancer Network incorporated these two drugs for the treatment of HNSCC (3). Compared with traditional therapies, the emerging PD-1 blockade immunotherapy exhibited an encouraging therapeutic efficacy for patients with advanced HNSCC (4).

Due to the inherent genetic inheritance and high somatic mutation in HNSCC, alterations in the protein repertoire can be introduced, which usually result in the expression of neoantigens that can be recognized by T cells and tumor cell clearance by immune surveillance system (5, 6). In addition, in addition to the neoantigen pathway generated by cancer genome mutations, HNSCC associated with HPV infection can also be detected by T cells due to the viral antigen expression, thereby eliciting an anti-tumor immune response (7). Similarly, recent studies have revealed that HNSCC exhibits an enriched tumor immune landscape, and when HNSCC showed a clinical progression, the immune response elicited by the strong immunogenicity of HNSCC is usually suppressed. Immunosuppressive cells, such as bone marrow-derived suppressor cells (MDSCs), M2-type tumor-associated macrophages (TAMs), and regulatory T cells (Tregs) can build an immunosuppressive state in HNSCC tumor microenvironment (TME). At the same time, with the introduction of the concept of immune checkpoints, a series of inhibitory immune checkpoints including PD-1, CTLA-4, TIM-3, IDO, KIR, and TIGIT, have been proved to be involved in the construction of HNSCC immunosuppressive microenvironment (8). The blockade of immune checkpoint PD-1/PD-L1 could reduce the population and activity of MDSCs and Tregs (9), and restore the cytotoxicity of T cells and NK cells (10), ultimately relieving the tumor immune escape and inhibiting tumor growth.

A high expression of PD-L1 was commonly observed in HNSCC cells (40–70%) (11, 12), coinciding with the upregulation of PD-1 on the majority of CD8⁺ tumor-infiltrating lymphocytes (TILs) (11). Yu et al. found that PD-1 expression increased in samples from patients with HNSCC, in comparison with normal oral mucosa samples (13), which implied that the PD-1 blockade should be more effective at boosting the antitumor immune response in HNSCC. However, data from recent clinical trials revealed a modest overall response rate (ORR) to the PD-1 blockade of less than 20% (4, 14), and a lack of dramatic responses in most patients (15) when compared with the more impressive ORRs of up to 57% in other advanced/pretreated indications, such as non-small cell lung cancer and melanoma (16, 17). Thus, only a small number of patients with HNSCC can benefit from PD-1/PD-L1 blockade therapy.

A growing number of preclinical and clinical trials have confirmed that traditional cancer therapies can promote the release of tumor-associated antigens (TAAs), increase antigen presentation, as well as enhance the expression of PD-1/PD-L1 axis. In the treatment of tumors with PD-1/PD-L1 blockade, combination with other therapies can provoke the antitumor immune response, which helps to improve the efficacy of

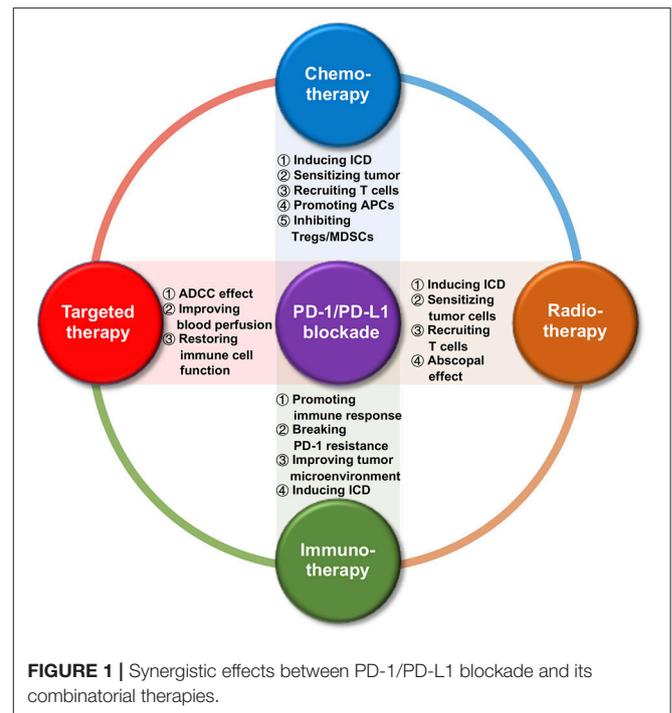


FIGURE 1 | Synergistic effects between PD-1/PD-L1 blockade and its combinatorial therapies.

PD-1/PD-L1 blockade. Thus, combinatorial immunotherapy represents a promising approach to boost antitumor activity in HNSCC as well as other malignancies. Therefore, it is especially important to understand the dynamic changes in the TME after standard treatment in order to design a rational combination treatment plan involving PD-1/PD-L1 blockade (18). In this review, we summarized its combination with other current treatment options for HNSCC (including chemotherapy, radiotherapy, targeted therapy, and immunotherapy) from their crosstalk in the TME (Figure 1) to the ongoing clinical trials (Table 1), in order to better understand the mechanism of interaction between PD-1/PD-L1 blockade and other cancer therapies, and to provide further ideas for the design of combinatorial regimen with a higher efficiency and response rate in HNSCC and other malignancies.

COMBINED WITH CHEMOTHERAPY

Chemotherapy, which has been known to induce systemic immunosuppression due to the bone marrow toxicity, is used as a traditional therapy to control the growth of tumors and block the proliferation of tumor cells due to its cytotoxicity (19). However, recent studies have found that many chemotherapeutic agents exerted a stimulating effect on the antitumor immune response beyond their cytotoxicity, thereby aiding the immune system in the elimination of tumor cells (20). From the tumor side, chemotherapy can promote the release of a series of TAAs by dying cells and increase the sensitivity of tumor cells to cytotoxic immune cells to promote inflammatory response. Also, from the immune side, multiple types of chemotherapeutic agents have been shown to promote lymphocyte infiltration

TABLE 1 | Ongoing clinical trials (registered in ClinicalTrials.gov) involving combinatorial PD-1/PD-L1 blockade therapy in recurrent/metastatic head and neck squamous cell carcinoma as of June 2018.

Clinical Trials.gov identifier	Mechanisms of action	Regimen (treatment arms)	Phase	Estimated enrolment	Primary completion date
NCT03085719	Anti PD-1 Radiotherapy	Pembrolizumab + Radiation	2	26	2020/10/31
NCT03317327	Anti PD-1 Radiotherapy	Nivolumab + Radiation	1,2	20	2023/11/2
NCT02289209	Anti-PD-1 Radiotherapy	Pembrolizumab + Reirradiation	2	48	2018/12/1
NCT03313804	Anti-PD-1 Radiotherapy	Nivolumab or Pembrolizumab or Atezolizumab + Radiation	2	57	2018/6/30
NCT03058289	Anti-PD-1 Chemotherapy	A: INT230-6 (Cisplatin + Vinblastine + Cell Permeation Enhancer) B: Anti-PD-1 + INT230-6	1,2	60	2019/7/1
NCT02358031	Anti-PD-1 Chemotherapy	A: Pembrolizumab B: Pembrolizumab + Cisplatin+5-fluorouracil C: Cetuximab + Cisplatin + 5-fluorouracil	3	825	2018/12/31
NCT02710396	Anti-PD-1 Chemotherapy	A: Pembrolizumab B: Pembrolizumab + Carboplatin + Nab-paclitaxel C: Pembrolizumab + Carboplatin + Pemetrexed	2	90	2019/3/31
NCT02759575	Anti-PD-1 Chemotherapy Radiotherapy	Pembrolizumab + Cisplatin + Radiation	1,2	47	2020/1/1
NCT03040999	Anti-PD-1 Chemotherapy Radiotherapy	A: Pembrolizumab + Cisplatin + Radiation B: Placebo + Cisplatin + Radiation	3	780	2021/4/16
NCT02819752	Anti-PD-1 Chemotherapy Radiotherapy	Pembrolizumab + Cisplatin + Radiation	1	36	2018/3/1
NCT03082534	Anti-PD-1 Anti-EGFR	Pembrolizumab + Cetuximab	2	83	2019/5/27
NCT02646748	Anti-PD-1 JAK inhibitor PI3K- δ	A: Pembrolizumab + Itacitinib(JAK Inhibitor) B: Pembrolizumab + INCB050465(PI3K- δ Inhibitor)	1	237	2019/6/1
NCT03532737	Anti-PD-1 Anti-EGFR Chemotherapy Radiotherapy	Pembrolizumab + Cisplatin/Cetuximab + Intensity modulated radiotherapy (IMRT)	2	50	2021/9/30
NCT02764593	Anti-PD-1 Anti-EGFR Chemotherapy Radiotherapy	A: Nivolumab + Cisplatin + IMRT B: Nivolumab + High Dose Cisplatin + IMRT C: Nivolumab + Cetuximab + IMRT D: Nivolumab + IMRT	1	120	2019/3/1
NCT03051906	Anti-PD-L1 Anti-EGFR Radiotherapy	Durvalumab + Cetuximab + IMRT	1,2	69	2022/1/1
NCT03292250	Anti-PD-L1 Anti-CTLA-4	Durvalumab + Tremelimumab	2	259	2020/1/1
NCT02834013	Anti-PD-1 Anti-CTLA-4	Nivolumab + Ipilimumab	2	707	2020/8/31
NCT03463161	Anti-PD-1 IDO Inhibitor	Pembrolizumab + Epacadostat	2	30	2020/3/1
NCT03325465	Anti-PD-1 IDO Inhibitor	A: Pembrolizumab B: Pembrolizumab + Epacadostat	2	44	2020/6/1
NCT03358472	Anti-PD-1 IDO Inhibitor	A: Pembrolizumab B: Pembrolizumab + Epacadostat C: Cetuximab + Cisplatin or Carboplatin + 5-fluorouracil	3	625	2021/1/27
NCT03343613	Anti-PD-L1 IDO Inhibitor	LY3381916 (IDO1 Inhibitor) + LY3300054 (Anti-PD-L1)	1	290	2019/9/1
NCT02903914	Anti-PD-1 Arginase Inhibitor	A: INCB001158 (Arginase Inhibitor) B: Pembrolizumab + INCB001158	1,2	346	2019/5/1

(Continued)

TABLE 1 | Continued

Clinical Trials. gov identifier	Mechanisms of action	Regimen (treatment arms)	Phase	Estimated enrolment	Primary completion date
NCT03454451	Anti-PD-1 Anti-CD73	A: CPI-006 (CD73 Inhibitor) B: CPI-006 + CPI-444 (Adenosine-A2A Receptor Inhibitor) C: Pembrolizumab + CPI-006	1	378	2022/3/1
NCT03162224	Anti-PD-L1 Vaccine	Durvalumab + MEDI0457 (a HPV DNA vaccine)	1b/2a	40	2020/3/2
NCT02432963	Anti-PD-1 Vaccine	Pembrolizumab + p53MVA Vaccine (modified vaccinia virus Ankara vaccine expressing p53)	1	19	2018/4/1
NCT03260023	Anti-PD-L1 Vaccine	Avelumab + TG4001 (a HPV vaccine)	1,2	52	2020/5/1
NCT02526017	Anti-PD-1 Anti-CSF-1R	A: Cabiralizumab B: Nivolumab + Cabiralizumab	1	295	2019/5/1
NCT02452424	Anti-PD-1 Anti-CSF1R	Pembrolizumab + PLX3397	1,2	80	2018/5/1
NCT02335918	Anti-PD-1 CD-27 Agnoist	Nivolumab + Varilumab	1,2	175	2019/4/1
NCT02475213	Anti-PD-1 Anti-B7-H3	Pembrolizumab + Enoblituzumab	2	75	2018/8/1
NCT02952989	Anti-PD-1 Fucosylation Inhibitor	Pembrolizumab + SGN-2FF (Fucosylation Inhibitor)	1	308	2019/12/1
NCT03474497	Anti-PD-1 IL-2 Radiotherapy	Pembrolizumab + IL-2 + Radiotherapy	1,2	45	2020/7/1
NCT03518606	Anti-PD-L1 Anti-CTLA-4 Chemotherapy	Durvalumab + Tremelimumab + Metronomic Vinorelbine	1,2	150	2020/12/29
NCT02551159	Anti-PD-L1 Anti-CTLA-4 Anti-EGFR Chemotherapy	A: Durvalumab B: Durvalumab + Tremelimumab C: Cetuximab + 5-fluorouracil + Cisplatin + Carboplatin	3	823	2018/12/31
NCT02643303	Anti-PD-L1 Anti-CTLA-4 TLR3 agonist	Durvalumab + Tremelimumab + Poly ICLC(a TLR3 agonist)	1,2	102	2022/8/1
NCT03019003	Anti PD-L1 Anti-CTLA-4 Chemotherapy	Durvalumab + Tremelimumab + Azacitidine	1B/2	59	2020/7/1
NCT03283605	Anti-PD-L1 Anti-CTLA-4 Radiotherapy	Durvalumab + Tremelimumab + Stereotactic Body Radiotherapy (SBRT)	1,2	45	2019/12/1
NCT03085914	Anti-PD-1 IDO-inhibitor Chemotherapy	A: Pembrolizumab + Epacadostat + mFOLFOX6 (oxaliplatin, leucovorin, 5-fluorouracil) B: Pembrolizumab + Epacadostat + Gemcitabine + Nab-paclitaxel C: Pembrolizumab + Epacadostat + Carboplatin + Paclitaxel D: Pembrolizumab + Epacadostat + Pemetrexed + Platinum E: Pembrolizumab + Epacadostat + Cyclophosphamide F: Pembrolizumab + Epacadostat + Gemcitabine + Platinum G: Pembrolizumab + Epacadostat + 5-fluorouracil + Platinum	1,2	421	2021/4/1
NCT03236935	Anti-PD-1 NO Synthase Inhibitor	Pembrolizumab + L-NMMA	1	12	2019/2/1
NCT03245489	Anti-PD-1 Anti-platelet	A: Pembrolizumab + Clopidogrel + Acetylsalicylic Acid Followed by Pembrolizumab alone B: Pembrolizumab alone Followed by Pembrolizumab + Clopidogrel + Acetylsalicylic Acid	1	20	2020/12/30
NCT02636036	Anti-PD-1 Oncolytic Virus	Nivolumab + Enadenotucirev	1	30	2019/3/1

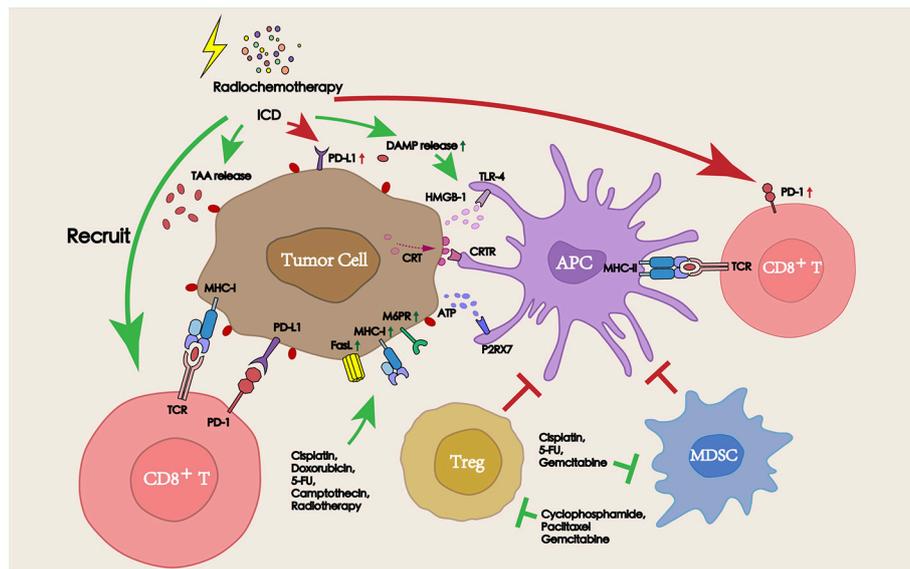


FIGURE 2 | Interactions between radiochemotherapy and PD-1/PD-L1 blockade in the TME. Radiochemotherapy can increase the release of TAAs and DAMPs, improve the expression of PD-L1 on the tumor surface. Some chemotherapeutic agents can also deplete immunosuppressive cells (Tregs and MDSCs), promote the function of APCs, and increase the sensitivity of tumors to cytotoxic immune cells, thereby improving the therapeutic efficacy of PD-1/PD-L1 blockade immunotherapy.

into the tumor site, deplete immunosuppressive cells, and directly increase the activity of antigen presenting cells (APCs). The improvement in the TME following treatment with chemotherapeutic agents suggests an attractive synergy between cytotoxic chemotherapy and PD-1/PD-L1 blockade (Figure 2).

Improvement of Tumor Immunogenicity

Some antineoplastic drugs including 5-fluorouracil (5-FU) and cisplatin could increase the expression of MHC I on the surface of tumor cells (21). Moreover, the expression of tumor antigens (carcinoembryonic antigen, CEA) could also be promoted by 5-FU (21, 22). Treatment with platinum and paclitaxel could increase the production of macrophage chemoattractant protein-1 (MCP-1) thus recruiting infiltrating macrophages into the tumor site (23).

Using chemotherapeutic agents, some types of cell death have been demonstrated to induce an immune response against antigens released from dying cells, commonly referred to as immunogenic cell death (ICD): the dying tumor cells can release a series of damage-associated molecular patterns (DAMPs), including calretectin (CRT) (24), adenosine triphosphate (ATP) (25), and high mobility group box 1 (HMGB1) (26), which plays an important role in the immune response elicited by ICD. DAMPs that are exposed to a large number of dead tumor cell surfaces or released into the TME can be recognized by APCs surface receptors such as toll-like receptor-4 (TLR-4), calretectin receptor (CRTR), and purinergic receptor P2RX7 (20), thereby promoting the maturation and proliferation of APCs, and sequentially leading to the activation of cytotoxic lymphocytes.

Sensitization of Tumor Cells

A variety of chemotherapeutic drugs can upregulate the expression of death receptors on the surface of tumor cells, thereby increasing the sensitivity of tumor cells to the attack from immune cells expressing death receptor ligands.

Cyclophosphamide chemotherapy could sensitize tumor cells to the tumor necrosis factor-related apoptosis-inducing ligand receptor (TRAILR) dependent CD8⁺ T cell-mediated immune attack, resulting in the suppression of tumor growth (27). Furthermore, treatment with 5-FU was demonstrated to upregulated FasR expression and sensitized tumor cells to low-avidity cytotoxic lymphocytes (28). In addition, paclitaxel, cisplatin, doxorubicin could upregulate the expression of mannose-6-phosphate receptor (M6PR) on the surface of tumor cells, thereby increasing the permeability of cell membrane to granzyme B, making the cytotoxicity of lymphocytes to tumor cells independent of perforin (29).

Recruitment of Effector Cells

The recruitment and infiltration of effector cells to the tumor site determines the efficiency of the antitumor immune response and the prognosis of PD-1/PD-L1 blockade immunotherapy. Preclinical investigations suggested that chemotherapy acts as a promoter and facilitator for the recruitment and infiltration of effector cells. The administration of low-dose cisplatin and paclitaxel could also trigger the recruitment of macrophages and tumor-specific CD8⁺ T-cells into the tumor site (30). Thus, the infiltration of CD3⁺ T cells into the tumor mass increased after preconditioning with cisplatin, markedly enhancing the efficacy of adoptively transferred cytokine-induced killer (CIK) cells (31). In addition to promoting the homing of transferred lymphocytes to secondary lymphoid organs and tumor mass,

treatment with cyclophosphamide treatment also favored the homeostatic proliferation/activation of transferred B and T lymphocytes (32). Moreover, sequential chemotherapy (cisplatin and gemcitabine) in mice previously treated with adenovirus-based immunotherapy increased the number and activity of both systemic and intratumoral CD8⁺ T cells (33).

Inhibition of Immunosuppressive Cells

Chemotherapeutic agents, especially when applied at low-dose, may selectively inhibit immunosuppressive cells (including Tregs and MDSCs) with little adverse effects on immune effector cells.

Intratumoral and splenic Tregs in multiple types of tumors could be depleted after treatment with cisplatin, gemcitabine treatment (34), while paclitaxel chemotherapy selectively decreased the size of the Treg population rather than that of other subsets including cytotoxic T cells by upregulating Fas and Bcl-2/Bax mediated apoptosis (35, 36). Moreover, metronomic chemotherapy with paclitaxel could deplete Tregs and simultaneously inhibit tumor angiogenesis, instead of displaying direct cytolytic effects (37). Furthermore, the application of metronomic chemotherapy involving cyclophosphamide in patients with advanced tumors not only depleted Tregs, but also restored the function of T cells and NK cells (38).

Various chemotherapeutic agents showed the selective depletion of MDSCs at non-cytotoxic doses, including 5-FU, paclitaxel, gemcitabine, doxorubicin, and platinum (22). Apart from the depletion of MDSCs, treatment with ultralow-dose paclitaxel treatment also promoted the maturation of MDSCs into DCs, which exhibit a higher expression of MHC II and CD86/CD40 co-stimulatory molecules (39). A sequential treatment with gemcitabine and cisplatin could also prevent the increase in the number of systemic immunosuppressive cells induced by adenoviral-based immunotherapy. After immunotherapy, the number of MDSCs, Tregs and B cells in spleen was significantly upregulated, while the addition of chemotherapy reduced the number of those immunosuppressive cells (33).

Promotion of APCs Functions

Several chemotherapeutic agents may promote antigen presentation by directly stimulating the maturation and activation of DCs. Treatment with low-dose cyclophosphamide could increase the potency of DCs with respect to antigen presentation and cytokine secretion (40). Cyclophosphamide at a non-myeloablative doses could also promote the compensatory myelopoiesis and alter DCs homeostasis, thus leading to a higher secretion of IL-12 and a lower secretion of IL-10, and eventually priming the T cell activation and inhibiting Tregs (41). When transfected with low dose 5-FU-treated tumor RNA, DC activation markers increased, leading to a significant increase in the IFN- γ producing T cells in tumor-bearing mice (42). Moreover, the expression of the co-stimulatory factors, such as CD80, CD86, CD40, and MHC-II, on DCs could also be stimulated by various chemotherapeutic agents including vinblastine, paclitaxel, doxorubicin, and methotrexate at nontoxic concentrations, which directly enhanced DCs

maturation and their activation of T cells, eventually initiating a more effective antitumor immune response (43, 44).

Combining Chemotherapy With PD-1/PD-L1 Blockade

Although various chemotherapeutic agents have been shown to promote antitumor immune responses, tumor cells tend to acquire resistance after chemotherapy, which may be caused by tumor immune escape. Some chemotherapeutic drugs can lead to a high expression of PD-1/PD-L1 through the MAPK/ERK kinase pathways in patients with HNSCC (45), thereby promoting the tumor immune escape. The high expression of PD-1/PD-L1 as a result of chemotherapy provides the possibility of combining chemotherapy with PD-1/PD-L1 blockade. A large number of preclinical and clinical studies have confirmed that combination therapy is superior to monotherapy in controlling tumor growth and improving survival time.

In a syngeneic murine model of HNSCC, the concurrent use of cisplatin and PD-1/PD-L1 blockade delayed tumor growth and enhanced survival without significantly reducing the number or function of TILs or increasing the cisplatin-induced toxicity (46). Moreover, taxane and 5-FU treatment upregulated the expression of PD-L1, thus inhibiting the function of antigen-specific T cells and promoting PD-L1-mediated T cell apoptosis in breast cancer cell (47). A significant increase of PD-L1 expression was observed after docetaxel, platinum, and fluorouracil (TPF) treatment. The mean density of tumor-infiltrating CD8⁺ T cells also significantly increased after TPF treatment (45). Also, PD-L1 expression was significantly upregulated in patients with HNSCC after carboplatin chemotherapy, suggesting that these patients may benefit from the sequential PD-1/PD-L1 immunotherapy (48). In addition to increasing PD-1/PD-L1 expression and improving the TME, some chemotherapeutic agents may also induce the production of immunosuppressive cells. Despite the immunostimulatory effect of cyclophosphamide, it could also induce an increase of MDSCs (CPM-MDSCs) (49), leading to an immunosuppressive effect. Doxorubicin combined with cyclophosphamide chemotherapy could increase the number of MDSCs in breast cancer patients (50). Combining with PD-1/PD-L1 blockade could eliminate the CPM-MDSCs-mediated immunosuppression, enhance antigen-specific immune responses, increase the intratumoral ratios of CD8⁺/Tregs and CD4⁺ Foxp3⁺/Tregs, and prolong the CPM inhibition of Tregs, thus leading to a long-lasting antitumor effect (51, 52).

In summary, the above evidence suggests a strong synergistic effect between chemotherapy and PD-1/PD-L1 blockade immunotherapy. Regrettably, no studies have been conducted to compare the effects of different combinations of chemotherapy drugs with PD-1/PD-L1 blockade so far. However, low-dose, metronomic chemotherapy is recommended in this review because tumor-associated antigen presentation is promoted while the patient's immune system function can be better protected. Similarly, some chemotherapeutic drugs, such as gemcitabine, paclitaxel, have been shown to promote immune responses at tumor sites in a variety of ways in the treatment

of other solid tumors, thereby synergistically interacting with PD-1/PD-L1 blockade. As common chemotherapeutic drugs for HNSCC, they are also supposed to exhibit a similar synergistic effect with PD-1/PD-L1 blockade in HNSCC, but further clinical analysis still needs to be carried out. Therefore, combining PD-1/PD-L1 with chemotherapy can be an effective strategy to improve clinical outcomes.

COMBINED WITH RADIOTHERAPY

Radiotherapy, alone or in combination with chemotherapy plays an important role in the treatment of early and advanced HNSCC by locally controlling of tumor progression. While destroying tumor cells, radiotherapy can also damage immune cells in the affected area. Similar to chemotherapy, radiotherapy has been generally considered to inhibit the immune system in the past. Recent studies have found that radiotherapy could also induce antitumor immune responses similar to chemotherapy. While destroying tumor cells and tumor stromal cells, radiotherapy could promote the release of a large number of TAAs (53). At the same time, radiotherapy could also induce ICD, thereby promoting the activation and maturation of DCs, and upregulating MHC I expression on tumor cells (54, 55). Thus, T-cell priming in draining lymphoid tissues dramatically increased after radiotherapy, leading to the reduction of the primary tumor or distant metastases in a CD8⁺ T cell-dependent fashion (56). In addition, the increase in TILs after radiotherapy also demonstrated the activation of the immune system following radiotherapy. Therefore, considering the immunostimulatory properties of radiotherapy, a combined application of radiotherapy and immunotherapy is feasible (57).

In patients with HNSCC, fractionated conformal radiation increased the number of circulating CD8⁺ T cells, and significantly decreased serum CXCL10 and CXCL16 levels. Moreover, radiation-induced tumor lysis led to a decrease in tumor CXCL10 secretion, while the levels of CXCL16 secreted by the APCs into the circulation increased, thereby attracting T cells to the TME and increasing the number of TILs (58). However, radiotherapy also increased the number of MDSCs, Tregs, and T cells expressing checkpoint receptors (particularly PD-1) (58, 59). Interferon- γ (IFN- γ) produced by CD8⁺ T cells after ionizing radiation was responsible for mediating the upregulation of PD-L1, resulting in a PD-1/PD-L1-mediated CD8⁺ T cell inactivation and depletion (59, 60). Furthermore, the increased secretion of transforming growth factor- β (TGF- β) after radiotherapy contributed to the infiltration of Tregs into the TME and then suppressed the immune response (61, 62). Stereotactic radiotherapy in combination with PD-1 blockade could reduce the number of Tregs and increase the proportion of CD8⁺ T cells, thereby enhancing the cytotoxicity toward tumor cells (63). Combinatorial therapy could also enhance the specific antitumor immune response by cross-presenting TAAs (63), inhibit the local accumulation of MDSCs, and reconstruct the TME (64). In a murine orthotopic model of HNSCC, fractionated conformal radiotherapy could sensitize anti-PD-L1-resistant tumors, and a combination of radiotherapy with PD-L1

blockade resulted in a significant enhancement in tumor control and improvement in survival when compared with monotherapy (65).

PD-1/PD-L1 blockade in combination with radiotherapy could also suppress the metastatic tumors via abscopal effect. A combination of ablative radiotherapy with PD-1 blockade led to a 66% reduction in the size of non-irradiated tumors in mice with melanoma (66). In mice with breast cancer undergoing ionizing irradiation together with PD-L1 blockade, not only the tumor at the radiotherapy site but also the metastatic tumors outside the irradiated site were significantly reduced, and a long-lasting immune memory was elicited (67). These synergistic effects could be attributed to the fact that radiotherapy promoted the release of a large number of TAAs, thereby activating the immune system and inhibiting tumors outside of radiotherapy site.

COMBINED WITH TARGETED THERAPY

Several commonly used IgG1 antibodies (represented by cetuximab) have been shown to modulate the TME via antibody-dependent cellular cytotoxicity (ADCC) and promote the antitumor immune response (68, 69). Distinct from the tumor targeted IgG1 antibodies, anti-angiogenic drugs could regulate the blood perfusion into the tumor site, adjusting the oxygen concentration and the pH, and eventually affecting the TME. Combining targeted drugs with immune checkpoint inhibitors resulted in a satisfactory antitumor efficacy. Herein, the mechanisms of tumor/vascular targeting drugs in combination with PD-1/PD-L1 blockade for the treatment of HNSCC are described below (Figure 3).

Tumor Targeting mAbs

EGFR was highly expressed in various types of tumor tissues, and the overexpression of EGFR could be detected in more than 90% of HNSCC cases (70). Cetuximab, a humanized IgG1 mAb targeting EGFR, was approved by the FDA in 2006 for the treatment of head and neck malignancies. The crosstalk of ADCC effect and host immune response suggested the feasibility of combining cetuximab with immune checkpoint inhibition therapy (69).

NK cells and DCs represent the main immune cells that mediate the ADCC effect. The antibody IgG Fc receptor (Fc γ RIIIa) is mainly expressed on the NK cells, therefore, when cetuximab specifically binds to the antigen on the target cell through its Fab segment, the naked Fc segment binds to Fc receptors on NK cells. The antibody could act as a bridge between target cells and NK cells, activating NK cells and promoting the release of cytotoxic mediators (perforin and granzymes), eventually leading to the lysis of target cells (71). Except for NK cells, the IgG Fc receptor (Fc γ RIIa) has been found to be expressed on DCs and neutrophils, which may also mediate several important downstream immunological responses (72). TAAs released by the lysed tumor cells can be cross-presented by DCs to cytotoxic T cells, thereby triggering the activity of cytotoxic T cells (73). In addition, activated NK cells can secrete cytokines to promote DCs maturation. In turn, the stimulation of NK cells can be greatly enhanced through the stimulation

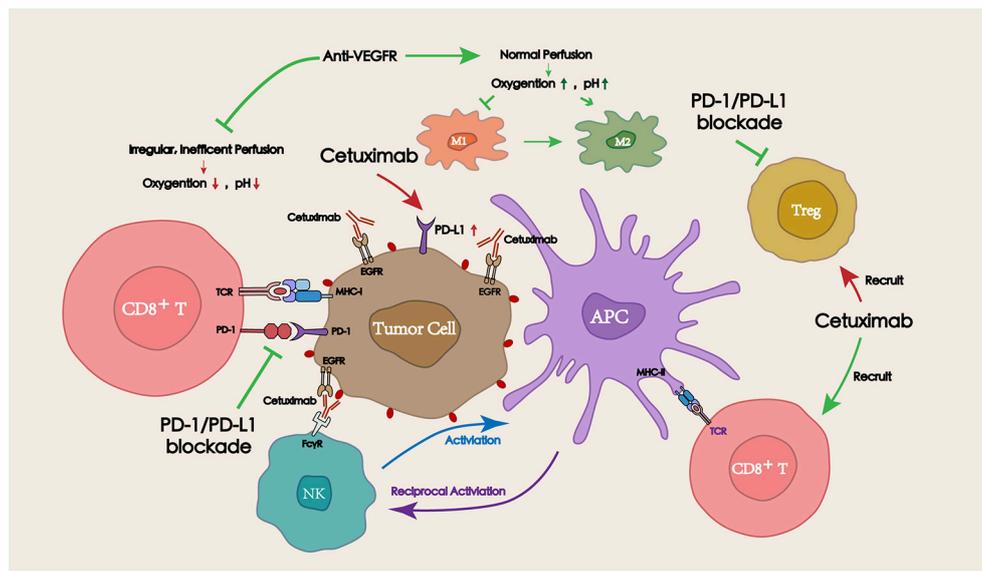


FIGURE 3 | Interactions between targeted therapies (including tumor targeting IgG mAbs and vascular targeting drugs) and PD-1/PD-L1 blockade in the TME. Tumor targeting IgG mAbs (represented by cetuximab) can recruit and activate NK cells via ADCC effect, thereby lysing tumor cells. Activated NK cells can also promote antitumor immune responses by secreting cytokines to facilitate the crosstalk with dendritic cells (DCs) and other immune cells (macrophages, other NK cells). Appropriate doses of vascular targeting drugs can restore blood perfusion at the tumor site, and then improve the hypoxic and acidic TME, which is beneficial for the function of antitumor immune cells.

by cytokines and surface molecules from mature DCs (72). Moreover, the crosstalk between NK cells and other immune cells is important for promoting the infiltration of cytotoxic T cells into the tumor site, which perform their lytic activity on tumor cells and further stimulate long-term immune responses (74).

Similar to chemotherapy and radiotherapy, PD-1 expression on CD8⁺ TILs significantly increased in patients treated with cetuximab; this was inversely correlated with the clinical outcome of cetuximab therapy (75). The recruitment of immunosuppressive cells after cetuximab treatment might also explain the resistance to EGFR-targeted therapy. Among patients who exhibit poor responses to cetuximab treatment, a significant increase in the number of Tregs in peripheral blood and TILs was observed (76). Moreover, there was a negative correlation between the activation of NK cells and the expansion of the Treg population, which in turn inhibited the ADCC effect (77). In a patient with poorly differentiated HNSCC, a clinical remission of invasion was observed after a combined application of cetuximab and nivolumab for 6 months (78). These evidences suggested that the anti-EGFR-resistant patients might benefit from PD-1 blockade immunotherapy. Notably, PD-L1 expression was significantly lower in EGFR-mutant tumors than that in EGFR-wild-type tumors; therefore, patients with EGFR mutations might not benefit from the combination therapy. Therefore, a combined PD-1 therapy is not recommended as a regimen to enhance the treatment efficacy in these patients (79, 80).

Consequently, treatment with cetuximab could drive ADCC and other immune effects, thus leading to immunosuppression by initiating immune checkpoints (PD-1/PD-L1 axis). When

combined with PD-1 blockade, cetuximab treatment resulted in the recruitment of immune cells to the TME, while PD-1/PD-L1 blockers could attenuate the Tregs or MDSCs-mediated inhibition of effector T cells and NK cells. However, in patients with EGFR mutations, the expression of PD-L1 is downregulated, and a combined PD-L1 blockade therapy might not achieve satisfactory therapeutic effects. Therefore, the combinatorial therapy is considered to be an ideal therapeutic strategy for the treatment of EGFR-wild-type HNSCC.

Vascular Targeted Drugs

The formation of tumor vessels is crucial for the process of tumor growth and metastasis, which is regulated by various cytokines, such as vascular endothelial growth factor (VEGF), angiostatin, and endostatin. Moreover, it is associated with the regulation of complex factors such as the hypoxic microenvironment, tumor suppressor genes, and oncogenes. VEGFR is highly expressed in HNSCC and associated with tumor progression and metastasis. As an independent prognostic factor, VEGFR overexpression is negatively correlated with the survival rates. Therefore, VEGFR has attracted increasing attention as an important target in HNSCC (81).

Although vascular targeting drugs treatment can prevent the development of tumors, the hypoxic environment, and acidosis environment caused by the treatment may not only affect the tumor cells, but also seriously compromise normal immune clearance function of the immune effector cells. This leads to the preferential recruitment of immunosuppressive cells that promote tumor growth, such as Tregs, MDSCs, and M2-type

TAMs (81). Clinical evidence suggested that vascular targeting therapy increased PD-L1 expression and led to tumor resistance to anti-angiogenic therapy (82). The dose of vascular antagonists is closely related to its influence on the TME. The targeting of the tumor vasculature at lower vascular-normalizing doses but not high anti-angiogenic doses by an anti-VEGFR2 antibody resulted in the polarization of TAMs from an immune inhibitory M2-like phenotype toward an immune stimulatory M1-like phenotype. Moreover, it facilitated the infiltration of CD4⁺ and CD8⁺ T cells into the tumor site, thus synergizing with cancer vaccine immunotherapy (83). Normalized tumor vessels facilitated the infiltration of effector T cells, while reducing MDSC accumulation. In addition, improvements in blood perfusion polarized TAMs to an immune stimulatory M1-like phenotype, consequently re-engineering the TME and improving the efficacy of cancer immunotherapy (84). Standard (high doses) of sorafenib treatment modestly delayed the growth of tumors, but also induced hypoxia, thus increasing the tumor infiltration of Tregs, MDSCs, and M2-type TAMs, as well as the expression PD-L1. When combined with PD-L1 blockade, the activation and tumor infiltration of cytotoxic CD8⁺ T cells were enhanced (85). Furthermore, a clinical study in patients with metastatic renal cell carcinoma revealed that atezolizumab (an anti-PD-L1 antibody) combined with bevacizumab increased the number of intratumoral CD8⁺ T cells and the migration of antigen-specific T cells (86).

In summary, vascular targeting therapy can improve the TME and might enhance the therapeutic efficacy of immunotherapy, providing a new strategy for the design of PD-1/PD-L1 blockade combination therapies.

COMBINED WITH IMMUNOTHERAPY

Pembrolizumab and nivolumab as the only immunotherapeutic drugs approved by the FDA for the treatment of platinum-refractory recurrent/metastatic HNSCC, have shown promising prospects for the treatment of advanced HNSCC. However, the immune escape mechanisms of tumors are quite complex, which always involves in multiple factors in the TME. Therefore, for different patients, personalized combined immunotherapy regimens according to pathological reports are supposed to be designed to achieve higher response rates and therapeutic effects. At present, a series of therapeutic schedules involving the combination of the PD-1/PD-L1 blockade with other co-inhibitory/co-stimulatory checkpoints or other immunotherapies are being explored (Figure 4).

Co-inhibitory Checkpoints

The activation of co-inhibitory immune checkpoints can produce a series of inhibitory signals, resulting in the inhibition of the corresponding immune response. To improve the antitumor efficacy, mutual combinations of immune checkpoint inhibitory antibodies are commonly adopted. Among these, the most widely studied is the synergistic interaction between cytotoxic T lymphocyte-associated antigen-4 (CTLA-4) blockade and PD-1 blockade.

CTLA-4

CTLA-4 (CD152) is similar to PD-1 as an immune checkpoint molecule, which is expressed on the surface of activated CD4⁺ and CD8⁺ T cells and exerts a negative regulatory role in the initial stage of T cell activation. Since CTLA-4 ligands (CD80 and CD86) are only expressed on APCs, but not on the tumor cell surface, the CTLA-4-mediated inhibition of T cell activation occurs in secondary immune organs (lymph nodes). Unlike CTLA-4, PD-1 mainly acts on the immune microenvironment, thus a combined blockade of CTLA-4 and PD-1 may produce a synergistic effect (87).

The analysis of peripheral blood and tumor tissue specimens in patients receiving anti-PD-1, anti-CTLA-4, or the combinatorial therapy demonstrated that CTLA-4 blockade induced a proliferative signature in a subset of memory T cells. Conversely, PD-1 blockade resulted in changes in the expression of genes involved in T cells or NK cells functions (88). In a phase 1 study in patients with advanced melanoma, the ORR was 61% in the group receiving both ipilimumab and nivolumab vs. 11% in the group receiving ipilimumab and placebo, demonstrating that a combination of the two could significantly improve the ORR (89). In the treatment of refractory HNSCC with ipilimumab and nivolumab, complete remission was achieved after 4 months of therapy (90). A series of clinical trials combining CTLA-4 and PD-1/PD-L1 blockades for the treatment of HNSCC confirmed the superiority of the combinatorial therapy vs. monotherapy (Table 1).

TIM-3

Similar to CTLA-4 and PD-1, T cell immunoglobulin-3 (TIM-3) is also one of the most studied inhibitory checkpoints. TIM-3 blockade could effectively induce antitumor immune responses by enhancing T cell effects and depleting MDSCs in a murine HNSCC model (91).

In an anti-PD-1 resistant murine tumor model, the high expression of TIM-3 on T cells was detected in TILs. Moreover, TIM-3 positive expression was also significantly correlated with anti-PD-1 treatment time. The expression of TIM-3 was low before and during the treatment-sensitive period, while it significantly increased after drug resistance (92). Treatment with anti-TIM-3 antibodies after PD-1 blockade overcame anti-PD-1 resistance and significantly increased median survival time in tumor-bearing mice. In the further clinical study, TIM-3 was also found to be highly expressed on the surface of TILs in patients with resistance to PD-1 blockade therapy, suggesting that a combined anti-TIM-3 therapy had the potential to overcome resistance to anti-PD-1 therapy (92, 93). In patients with advanced HNSCC, PD-1 blockade might promote the expression of TIM-3, which triggered the resistance to anti-PD-1 therapy in the TME. To further explore the feasibility of TIM-3 blockade for overcoming PD-1 resistance, PD-1 blockade combined with TIM-3 blockade was applied in murine HNSCC models. This combinatorial therapy significantly improved the antitumor effect when compared with anti-PD-1 monotherapy (18).

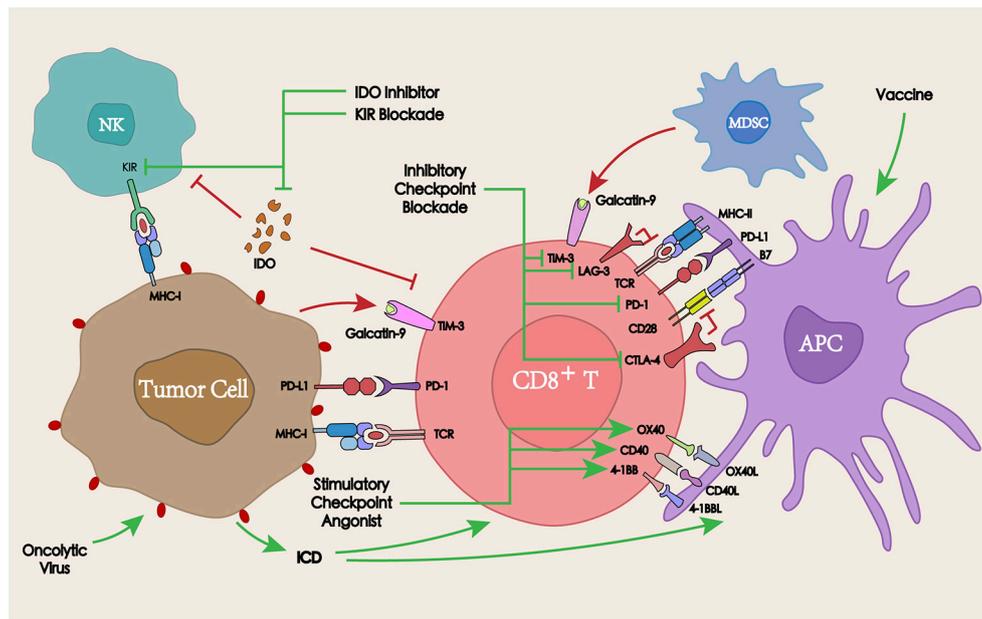


FIGURE 4 | Interaction between immunotherapy and PD-1/PD-L1 blockade in the TME. PD-1/PD-L1 blockade in combination with other co-inhibitory checkpoints, co-stimulatory checkpoints, or other immunotherapies such as tumor vaccines or oncolytic viruses may overcome the tumor resistance to PD-1/PD-L1, thereby enhancing the antitumor immune response.

Other Co-inhibitory Checkpoints

The blockade of other inhibitory immune checkpoints such as lymphocyte activation gene-3 (LAG-3), T cell immunoglobulin and ITIM domain protein (TIGIT), and their combination with PD-1/PD-L1 blockade for the treatment of malignancies are also being explored (94, 95). However, only a few studies have focused on HNSCC, and more basic experiments and clinical studies still need to be carried out.

Overall, in the treatment of HNSCC, PD-1/PD-L1 blockade may result in a further upregulation of the inhibitory checkpoints on the surface of TILs, thus supporting a circuit of compensatory signaling and potentially permitting immune escape from PD-1/PD-L1 blockade in the TME. Therefore, combining inhibitory checkpoint blockade with PD-1/PD-L1 blockade may provide an effective strategy to overcome acquired resistance to PD-1/PD-L1 blockade, as well as to increase the ORR and therapeutic efficacy.

Co-stimulatory Checkpoints

Unlike co-inhibitory checkpoints, co-stimulatory checkpoints can induce immunostimulatory effects by activating members of the tumor necrosis factor receptor superfamily on T cells (96). The most studied stimulatory checkpoint receptors and their ligands to date are mainly OX40, 4-1BB, and CD27, whose agonist in combination with PD-1/PD-L1 blockade are also being developed for the treatment of HNSCC (97, 98).

OX40

OX40 and its ligand OX40L are important co-stimulatory molecules in the immune response, which can improve the

immunosuppressive effect in the TME and enhance the cytotoxicity of the effector lymphocytes (99). In a PD-1 treatment-resistant murine model of ovarian cancer, PD-1 blockade combined with an OX40 agonistic antibody treatment led to a promising ORR of 60% (100).

CD27

Similar to OX40L, CD70 (ligand of CD27) is found on activated T cells, leading to CD27-CD70 interactions that may involve direct cellular communication between T cells and APCs (101). CD27 agonists synergized with PD-L1 blockade by enhancing CD8⁺ T cell proliferation and effector cytokine generation, while simultaneously downregulating the expression of multiple quiescence-related genes (102). In human CD27 transgenic mice, varlilumab (an CD27 agonist mAb), similarly synergized with PD-L1 blockade in protecting against lymphoma (103). An ongoing phase I/II clinical trial (NCT02335918) is designed to determine the clinical benefit, safety, and tolerability of combining varlilumab and nivolumab in advanced refractory solid tumors including HNSCC.

4-1BB

4-1BB (CD137) is expressed on a variety of immune cells including T cells, B cells, NK cells, and DCs. 4-1BB signaling can enhance the proliferation and activation of T ligands bitory PD-1 and TIM-3 checkpoints (104). For advanced giant tumors that could not be controlled by anti-PD-1 monotherapy, its combination with CD137 agonist therapy resulted in a complete

rejection (105). Moreover, a phase Ib study (NCT02179918) has already demonstrated the safety, tolerability, and clinical activity of utomilumab (a 4-1BB agonist mAb) in combination with pembrolizumab in the treatment of advanced solid tumors including HNSCC (106).

Other Immunotherapies

In addition to immune checkpoints, other therapies targeting the immune system, such as the indoleamine 2,3-dioxygenase 1 (IDO1) inhibitor, tumor vaccines, oncolytic viruses, and NK cell Ig-like receptor (KIR) in combination with PD-1/PD-L1 blockade are also under development.

IDO1 is a rate-limiting enzyme in the catabolism of tryptophan, which can induce the apoptosis or dysfunction of T cells and NK cells, thereby weakening the antitumor immune response. The inhibition of IDO1 activity could restore the function of T cells and NK cells and overcome the tumor immune tolerance (107). In patients with oral squamous cell carcinoma, PD-L1 and IDO1 were overexpressed. Moreover, the expression of PD-L1 and IDO1 was higher in patients without alcohol and tobacco history, suggesting that patients with HNSCC that do not smoke or drink may benefit more from PD-L1 and IDO1 inhibition (108). In a phase II clinical trial, a limited benefit from the PD-1 blockade was observed in selected patients with advanced soft-tissue sarcoma and gastrointestinal stromal tumors. This could be explained by an immunosuppressive TME resulting from macrophage infiltration and the activation of IDO1 pathway (109). A combined PD-L1 and IDO1 blockade could increase the percentage of CD8⁺ T cells in TILs and enhance the antitumor effects and survival time (110). A series of clinical trials combining IDO inhibitors with PD-1/PD-L1 blockade in the treatment of head and neck cancers are currently underway (NCT03463161, NCT03325465, NCT03358472, NCT03343613).

Cancer vaccines could provide antigens to activate the immune system, initiate antitumor immune responses, and overcome tumor-induced immunosuppression (111). Although tumor vaccine-induced immune responses have been observed in most studies, in clinical studies tumor vaccines were insufficient as a monotherapy. Therefore, combining tumor vaccines with other therapies such as PD-1/PD-L1 blockade might lead to better clinical outcomes (112), and several ongoing clinical trials are investigating the efficacy of tumor vaccines in combination with PD-1/PD-L1 blockade for the treatment of HNSCC (NCT03162224, NCT02432963, NCT03260023).

Oncolytic virus therapy represents a novel tumor immunotherapy that utilizes the virus to specifically replicate in tumor cells resulting in the lysis of tumor cells, which can lead to ICD and stimulate specific antitumor immune responses (113). Talimogene laherparepvec (TVEC) is a genetically modified type I herpes simplex virus engineered to selectively replicate within tumors and to produce the granulocyte-macrophage colony stimulation factor (GM-CSF), thus enhancing systemic antitumor immune responses (114). The results of a phase I clinical trial showed that combining an oncolytic virus with pembrolizumab could improve the efficacy of anti-PD-1 therapy by improving the TME, without increasing the toxicity in

patients with advanced melanoma (115). An ongoing clinical trial has been established to further evaluate the safety and efficacy of the combination of TVEC with pembrolizumab for the treatment of R/M HNSCC (NCT02626000).

SPECIFIC LANDSCAPE FOR HNSCC

In addition to the above-mentioned several commonly applied solid tumor treatment strategies, due to the specific etiology of HNSCC, some treatments for HNSCC have also been developed, and these therapies are blocked with PD-1/PD-L1. The efficacy of the combined application is also being evaluated.

Tobacco, alcohol consumption, and human papillomavirus infection (HPV) are considered to be the major risk factors for HNSCC. Toxic-induced and HPV-induced HNSCC are described as two different clinical entities with different oncologic pathways and prognosis in the 2017 WHO classification (116). Furthermore, there is also a high rate of somatic mutation in HNSCC (117). Therefore, the immunogenicity of HNSCC differs depending on tumor intrinsic antigen, neoantigen derived from the mutations, or the expression of the antigen induced by the HPV infection. Tumor immune microenvironment also differs in terms of the mutations load and viral infection (118). Cancer genomic analysis of different patients is important for the design of PD-1/PD-L1 blockade combinatorial therapy.

HPV Infection in Combinatorial Therapy in PD-1/PD-L1 Blockade

As HPV-induced HNSCC is considered as a distinct clinical entity in terms of clinical presentation, response to treatment and prognosis, the specificities in its microenvironment need to be more precisely characterized in order to stratify patients. Due to the specificity of HPV-induced HNSCC in clinical manifestations and response to treatment, there are significant differences in tumor immune microenvironment among HNSCC patients based on HPV infection status, thus the ORRs to PD-1/PD-L1 blockade are also different.

HPV-positive HNSCC samples showed a significantly higher number of invasive CTLs, dendritic cells and pro-inflammatory chemokines, which was considered to be positively correlated with a favorable prognosis. In addition, TIL in HPV-positive tumors has significantly higher PD-1 expression (119, 120). In patients treated with pembrolizumab for advanced HNSCC, the response rate for HPV-positive populations was 32%, compared with 14% for HPV-negative populations. Of patients that responded, the 6-months progression-free survival rate for HPV-positive population was 37%, compared with 20% for HPV-negative populations, suggesting that the efficacy of PD-1/PD-L1 blockade is related to HPV infection status (14). In the treatment of E6/E7 expressing ovarian cancer burden mice, when HPV-E6/E7 vaccine was combined with PD-L1 blockade, most tumor growth was effectively controlled (121). However, the ORR was only 33% when treating HNSCC patients with HPV vaccine and PD-1 blockade (122). To the inconsistency with the results of preclinical studies, the investigators speculated that although the HPV-16-specific T cells increased after vaccine injection, these

vaccine-induced T cell populations may be necessary, but not sufficient to enhance the ORR in combination of nivolumab, and additional immunosuppressive pathways might still exist. Further clinical trials to verify the superiority of the HPV vaccine in combination with PD-1 blockade are still needed.

Cancer Genomics for Designing Combinatorial Strategies

High-load mutations in HNSCC can lead to the expression of neoantigens and changes in TME, which may serve as new therapeutic targets. And the detection of common tumor mutation genes can also provide a reliable basis for the choice of treatment plan for patients with HNSCC. Studies on cancer genomics showed the loss of cell cycle regulation was an important driver for HNSCCs.

The most reported cell cycle regulation genes and their molecular pathways include *TP53*, *pRB*, *CCND1*, and *CKN2A* (123, 124). HPV infection also plays an important role in HNSCC oncogenesis. The E6 and E7 oncogenes in HPV can lead to mutation and inactivation of *TP53* and *pRB*, respectively (125). In fact, mutations in *TP53* are detected in most HPV-negative HNSCC cases (126). Likewise, *CDKN2A* is inactivated by mutation or methylation in most HPV-negative HNSCC cases. Also, 32% of HPV-negative HNSCCs had *CCND1* amplification whereas only 6% of that was observed in HPV-positive samples (123). Similarly, increased cell growth and proliferation pathway activation also plays an important role in HNSCC tumorigenesis. One of the key regulators of proliferation identified in HNSCC is *EGFR*. Other common mutant genes in cell growth pathway, including *PIK3CA*, *PTEN*, *FGFR*, and other growth factor receptors or their ligands, are also important for tumorigenesis (123).

PD-L1 membranous expression on tumor cells can result either from an adaptive immune phenomenon or from intrinsic oncogenic events in HNSCC. Several oncogenic mechanisms have been associated with PD-L1 expression, such as tumor-suppressive *PTEN* gene mutation or deletion, pro-oncogenic *PI3K* path activation, *AKT/mTOR*, *NF- κ B*, and *MAPK* (mitogen-activated protein kinase, *MEK/ERK*) pathways deregulation. Either adaptive immune responses or mutations from endogenous carcinogenesis-related genes in HNSCC can lead to the upregulation of PD-L1 expression on tumor cells. Several carcinogenesis mechanisms are associated with PD-L1 expression, including tumor suppressor *PTEN* gene mutations or deletions, oncogene *PI3K* pathway activation, *AKT/mTOR*, *NF- κ B* and *MAPK* pathway dysregulation (127).

Based on the researches on the HNSCC cancer genomics, several targeted drugs have been developed and extended to clinical applications such as *EGFR* which has been described above. Gene therapy drugs such as liposome or virus based *TP53* gene delivers have also been developed for treatment of HNSCC (128, 129). It has been proved that transduction of *p53* gene to HNSCC cells induced loss of cell viability while increased the immunogenicity and expression of PD-L1 on tumor cells, which synergized with PD-1 blockade (128).

FUTURE PERSPECTIVES

PD-1 blockade could prevent the negative regulatory signals generated by T cells to relieve immunosuppression and enhance the antitumor efficacy of T cells. In addition, it may also abnormally enhance the autoimmune response, thereby leading to an imbalance of immune tolerance. Common autoimmune inflammatory reactions induced by PD-1 blockade involving normal tissues includes the skin symptoms, hypothyroidism, and hyperthyroidism (130). When combined with other therapies, although the therapeutic efficacy is enhanced, more serious side effects may also be triggered, for example an increase in the incidence of fatal myocarditis (131). Targeted delivery can be achieved by loading the anti-PD-1/PD-L1 antibodies within engineered carriers, thereby reducing the side effects of the PD-1/PD-L1 blockade combinatorial therapy and improving antitumor efficacy. PD-1/PD-L1 inhibitors and other drugs could be loaded into the nanoparticles to enrich drugs at the tumor site (132), or encapsulated into the hydrogels for local treatment by peritumoral injections (133). Satisfactory therapeutic outcomes effects with reduced off-target and adverse effects have been achieved, demonstrating that smart delivery of PD-1/PD-L1 inhibitors in combination with other drugs via engineered carriers could represent a promising strategy for reducing side effects as well as enhancing antitumor efficacy.

In conclusion, PD-1/PD-L1 blockade has shown good and long-lasting therapeutic effects in the treatment of HNSCC and other malignancies, but only a small subset of patients can benefit from the monotherapy. To enhance the response rate and the therapeutic effect of PD-1/PD-L1 blockade, numerous preclinical experiments, and clinical trials exploring the combinatorial therapies involving PD-1/PD-L1 blockade are underway. In this review, we summarized the current status of the combined application of chemotherapy, radiotherapy, targeted therapy, and immunotherapy in combination with PD-1/PD-L1 blockade in the field of HNSCC. Moreover, the potential mechanisms underlying the crosstalk process between PD-1/PD-L1 blockade and combinatorial cancer therapies in the TME was described. Furthermore, it is hoped that the improved understanding of the crosstalk would provide further ideas for the design of PD-1/PD-L1 combinatorial therapies for HNSCC and other malignancies.

AUTHOR CONTRIBUTIONS

WL wrote the manuscript. MC collected the literature and generated the figures and table. LH edited and checked the manuscript format. HZ and QC reviewed the manuscript.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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