

# Advances in T-cell checkpoint immunotherapy for head and neck squamous cell carcinoma

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**Abstract:** Head and neck squamous cell carcinoma (HNSCC) has been found to be a complex group of malignancies characterized by their profound immunosuppression and high aggressiveness. In most cases of advanced HNSCC, treatment fails to obtain total cancer cure. Efforts are needed to develop new therapeutic approaches to improve HNSCC outcomes. In this light, T-cells “immune checkpoint” has attracted much attention in cancer immunotherapy. It has been broadly accepted that inhibitory T-cell immune checkpoints contribute to tumor immune escape through negative immune regulatory signals (cytotoxic T-lymphocyte-associated antigen 4 [CTLA-4], programmed cell death 1 [PD-1], B7-H3, and B7-H4, etc). Current data suggest that PD-1 and CTLA-4 receptors can inhibit T-cell receptors and T-cell proliferation. Blockade of PD-1/PD-L1 and/or CTLA-4/CD28 pathways has shown promising tumor outcomes in clinical trials for advanced solid tumors like melanoma, renal cell cancer, and non-small cell lung cancer. The present review attempts to explore what is known about PD-1/PD-L1 and CTLA-4/CD28 pathways with a focus on HNSCC. We further discuss how these pathways can be manipulated with therapeutic intent.

**Keywords:** immune checkpoint, PD-1/PD-L1, CTLA-4, HNSCC, immunotherapy

## Introduction

It has been projected that 450,000 new cases of head and neck squamous cell carcinoma (HNSCC) are diagnosed worldwide each year, which is the sixth most common cancer among human malignancies.<sup>1</sup> Of these diagnoses, ~90%–95% fall into the histological subtype of HNSCC,<sup>2</sup> including nasopharyngeal carcinoma (NPC), oropharyngeal carcinoma, laryngeal carcinoma, and hypopharyngeal carcinoma. Unlike many other epithelial malignancies, the majority of HNSCC present at a locally advanced stage with 5%–10% cervical lymph node metastases. If diagnosed at an early stage, HNSCC could be treated with traditional surgery, also radiation plus chemotherapy. For recurrent or metastatic (R/M) HNSCC conditions, cytotoxic chemotherapy remains the standard therapeutic option. Recommended first-line chemotherapy regimens include cisplatin or carboplatin plus 5-Fu, cisplatin or carboplatin plus docetaxel or paclitaxel, etc. Adding cetuximab, an epidermal growth factor receptor (EGFR) monoclonal antibody (mAb), could improve survival compared with palliative chemotherapy alone, but resulting in a median survival of only 10.1 months. A number of recent studies have focused on immunotherapy which is considered to be an alternative treatment approach for cancers in general, while several studies are underway testing the effectiveness of immunotherapeutic strategies in HNSCC which is commonly understood as an immunosuppressive disease. Despite approaches targeting growth factors and receptors such as epidermal growth factor receptor (EGFR), cell surface associated or polymorphic epithelial

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mucin (MUC-1), the receptor for advanced glycation end products (RAGE), G antigen (GAGE) families, melanoma-associated antigens A (MAGE-A) and various cytokines and tumor antigens<sup>3-5</sup> interfering with known molecular pathways activated in HNSCC have been extensively studied; only few and limited benefit has been seen to be sufficient to prevent tumor progression. Since around 2010, inhibitory checkpoint molecules have been considered as new targets for cancer immunotherapies due to the effectiveness of two checkpoint inhibitor drugs that were initially indicated for advanced melanoma – Keytruda (pembrolizumab), targeting programmed cell death 1 (PD-1) receptor,<sup>6</sup> and Yervoy (ipilimumab), targeting cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4),<sup>7</sup> which called attention to the possibility of T-cell checkpoint immunotherapy for HNSCC. In this review, we will summarize the latest developments of T-cell checkpoint immunotherapy in HNSCC, with a particular focus on PD-1 and CTLA-4.

## T-cell recognition and immune checkpoints

### T-cell activation

It is now established that various components in the immune system play pivotal roles in protecting humans from cancer. It has been broadly recognized that T-cell-dependent immune response specific to tumor antigens plays a crucial role in tumor immune surveillance and that the critical effector cells of adaptive antitumor immunity are the activated CD8<sup>+</sup> cytotoxic T-lymphocytes (CTLs).<sup>8</sup> T-cells have been the primary focus of cancer immunotherapy primarily due to their ability to initiate diverse immunoreactions via CD4<sup>+</sup> helper T-cells that provoke innate and adaptive immunity.<sup>9</sup> To evoke their proper activation, two sets of signals are required from antigen presenting cells (APCs) regulating T-cell survival, proliferation, and immune response in the lymph node. In a normally functioning immune system, the first signal initiates through binding of T-cell receptor (TCR) and a matching antigen packaged onto major histocompatibility complex (MHC) proteins on APCs. However, this interaction is not sufficient for complete T-cell activation and tumor cytotoxicity. A second co-stimulatory signal is needed to promote T-cell clonal expansion and cytokine secretion. The best understood co-stimulatory signal pathways are engagements of CD28 on T-cells with CD80 or CD86 (B7 superfamily genes) on APCs. In order to ensure that T-cell activation can only be stimulated by appropriate antigens and maintain their immunologic homeostasis, T-cell-mediated immunity is simultaneously controlled by co-inhibitory signals which are transduced by

immune inhibitory checkpoints expressed upon T-cells such as CTLA-4 and PD-1.<sup>10</sup> Under physiologic conditions, a balance between co-stimulatory and co-inhibitory signals is crucial to determine whether T-cells are activated or become anergic to the specific antigens displayed on the MHC molecules. These immune checkpoints are responsible for immune homeostasis and the maintenance of organ-specific tolerance in normal tissue, protecting organs from unnecessary damage while immune system could still eliminate pathogens efficiently.<sup>11</sup> Therefore, immune checkpoint molecules highly expressed in the tumor microenvironment are one of the main reasons for tumor immune escape and therapy failure. In this case, the balance between co-signals is greatly biased toward the side of inhibitory factors, resulting in T-cell functions dampened and antitumor responses significantly restricted.

## Immune checkpoints

Tumors develop numerous strategies to avoid detection and eradication by the host immune system. An enhanced understanding of the precise activators and inhibitors of the immune system has brought about therapeutic advances in cancer treatment. Currently, there are several “targetable” immune checkpoint blockade molecules. Lymphocyte activation gene 3 works to suppress the immune response via regulatory T-cells (Tregs) and direct effects on CD8<sup>+</sup> T-cells. T-cell immunoglobulin domain and mucin domain-3 triggers cell death to negatively regulate Th1/Tc1 functions upon binding its ligand galectin-9. Among all the inhibitory checkpoint molecules, two that have been shown to be clinically effective are PD-1 and CTLA-4.

### PD-1 and PD-L1/2

PD-1 (CD279) is a 288 amino acid type I transmembrane protein encoded by the *PDCD1* gene on chromosome 2 in humans. It presents not only on effector T-cells, but also on activated myeloid lineage cells such as monocytes, dendritic cells (DCs), and natural killer (NK) cells, suggesting its contribution to other important immune cell functions.<sup>12,13</sup> PD-1 comprises an extracellular IgV region, a transmembrane domain, and an intracellular tail containing an immune tyrosine-based inhibitory motif followed by an immune receptor inhibitory tyrosine-based switch motif.<sup>12</sup> PD-1 receptor has two ligands, PD-L1 and PD-L2.<sup>14</sup> PD-L1 (B7-H1/CD274) is a type I transmembrane protein of the CD28 family encoded by the CD274 gene on human chromosome 9. It is found constitutively on APCs, non-hematopoietic cells, and nonlymphoid organs.<sup>12</sup> PD-L2 (B7-DC/CD273) is expressed only upon professional APCs, which is in line with its

function of regulating T-cell priming. Compared to PD-L2, PD-L1, with a broader expression profile, is involved in delivering negative signals of T-cell activation and regulating cytokine expression and secretion. Through binding with the two ligands of PD-1 receptor, PD-1 delivers an inhibitory signal to shut down T-cell function. Many studies recently showed that the expression of PD-L1 is closely related to tumor grade in several types of malignancies and has become a new diagnostic and prognostic biomarker for tumors.<sup>10</sup> PD-L1, highly expressed on tumor cells,<sup>15–21</sup> binds with TCR PD-1, negatively regulates T-cell response, resulting in tumor antigen-specific T-cells-induced apoptosis and anergy, and makes the cancer cells evade immune surveillance and killing.

PD-1/PD-L1 signaling pathway is involved in the process of immune regulation through several distinct mechanisms. The ligation of PD-L1/PD-L2 to PD-1 inhibits the PI3K/AKT pathway and downregulates expression of the antiapoptotic gene Bcl-xl to promote T-cell apoptosis.<sup>22</sup> The binding of PD-1 and PD-L1 restricts naive T-cell migration and accumulation in APCs and downregulates TCR, which prevents effective antigen presentation.<sup>23</sup> PD-1–PD-L1/2 ligation upregulates expression of gene PTEN causing blockade of Akt/mTOR/S6 pathway, and converts Th1<sup>+</sup>CD4<sup>+</sup> T-cells to become Foxp3<sup>+</sup> Tregs that restrain cell-mediated immunity, which is in line with exhaustion of tumor infiltrated lymphocytes (TILs) in the tumor microenvironment.<sup>24</sup>

## CTLA-4

CTLA-4 or CD152 was first discovered to belong to the immunoglobulin super family when researchers were screening the cDNA library.<sup>25</sup> A later study showed that CTLA-4 knockout mice suffered from massive lymphoproliferation and severe autoimmune disease resulting in tissue destruction and death within 3–4 weeks of age, which demonstrated that the CTLA-4 receptor is an important negative co-stimulatory signal for T-cell activation and proliferation.<sup>26,27</sup> Currently, it is well established that CTLA-4 is a CD28 homologue with >30% of similar sequence expressed exclusively on the surface of T-cells upon activation, but with a much higher binding affinity for CD80 (B7.1) and CD86 (B7.2) than CD 28 (about 10–40 fold).<sup>28</sup>

The engagement of CTLA-4 and CD80/86 competes with that of CD28 causing direct inhibition of antigen presenting followed by T-cell anergy.<sup>29–31</sup> Besides “stealing” B7 from CD28, other mechanisms of CTLA-4 as an inhibitory signal for immune response have also been proposed. Some studies suggest that engagement of CTLA-4 with B7 itself may

transduce inhibitory signals that antagonize the stimulatory signals from CD28 and TCR.<sup>32–34</sup> CTLA-4 may increase T-cell mobility resulting in decreased effective antigen presentation.<sup>35</sup> In vitro and in vivo studies have shown that deficiency of CTLA-4 in Tregs leads to systemic lymphoproliferation, fatal T-cell-mediated autoimmune disease, increased IgE production, and furthermore, potent tumor immunity. Constitutive expression of CTLA-4 by CD4<sup>+</sup> Tregs is critical for their immune-suppressive function.<sup>36,37</sup>

## Correlation of immune inhibitors expression in HNSCC with tumor prognosis

Of late, many studies have shown that the expression of immune checkpoint is demonstrably correlated with tumor grade or prognosis in several types of malignancies and has become a new biological index for tumor detection and prognosis.<sup>38</sup> The expression of CTLA-4 and PD-1 in Tregs of HNSCC tumor tissue is higher than that in peripheral blood, which confirms that Tregs in tumor tissues have more immune suppression features.<sup>39</sup> Montler et al investigated the expression of CTLA-4 and PD-1 on effector T-cells from peripheral blood and tumor tissue in 29 HNSCC patients after surgery, and the data suggested that the two proteins were increased significantly in all T-cell subsets isolated from tumor compared with peripheral blood lymphocytes (PBLs) from the same patient.<sup>40</sup> And, the level of PD-1 is even higher on effector CD4<sup>+</sup> and CD8<sup>+</sup> T-lymphocytes of human papilloma virus (HPV)-positive HNSCC patients. Malm et al showed that the PD-1 expression in healthy peripheral blood donors is generally under 15%, while the rate surged to over 50% of both the CD4<sup>+</sup> and CD8<sup>+</sup> T-cells among the PBLs, draining lymph nodes and TILs in the tumor microenvironment in HNSCC patients.<sup>41</sup> The ligand of PD-1, PD-L1, has been shown in different solid tumors including HNSCC and studies have found that PD-L1 was present in 66%–87% of HNSCC primary tumors.<sup>42</sup> Chikamatsu et al demonstrated that the expression of PD-L1 on myeloid-derived suppressor cells in the peripheral blood of HNSCC patients was higher than that in the healthy control group.<sup>43</sup>

## Oropharynx squamous cell carcinoma (OPSCC) and PD-1/PD-L1

Besides the usual risk factors of tobacco and alcohol addiction, an increasing proportion of OPSCC is related to the most common high-risk HPV genotype, HPV-16, accounting for 5%–20% of the total HNSCC cases worldwide.<sup>44</sup> Given the recognized significance of HPV as an emerging etiological

risk factor for OPSCC, several investigators recently managed to correlate the expression of PD-L1 with HPV status. In the study of Lyford-Pike et al, 59% of the 20 analyzed HPV-HNSCC samples displayed PD-L1. PD-L1 expression was present in 70% HPV mRNA-positive cases versus 29% in those that were HPV-negative.<sup>45</sup> In a study of 181 OPSCC patients, Ukpo et al found that 46.4% of the samples were positive of PD-L1 expression with rates of 49.2% and 34.1% in the HPV (+) and HPV (-) groups, respectively. The majority of CD8<sup>+</sup> TILs in HPV (+) OPSCC samples produce the cytokine interferon- $\gamma$ , which upregulates PD-L1 expression on the surface of tumor cells. Twenty eight cases of recurrence and distant metastases showed PD-L1 expression of 43% and 70%.<sup>46</sup> Furthermore, with the novel tissue microarray technique, an even higher rate of PD-L1 expression could be identified.

### Oral cavity squamous cell carcinoma (OSCC) and PD-1/PD-L1

Both patients with OSCC and actinic cheilitis (AC), an oral premalignant lesion, presented much higher percentage of PD-1 expression in peripheral blood and lesions than normal individuals. And there is an increased proportion of PD-1 in tumor cells from OSCC patients than lesion cells from patients with AC. In all,  $21.1\% \pm 14.6\%$  of the lymphocytes were CD8<sup>+</sup>PD-1<sup>+</sup> T-cells in OSCC, but AC samples showed only  $0.84\% \pm 0.6\%$  CD8<sup>+</sup>PD-1<sup>+</sup> T-cells among their lymphocytes. The number of CD4<sup>+</sup>PD-1<sup>+</sup> cells was also higher in OSCC than in the AC group.<sup>47</sup> A study conducted by Zhang et al investigated PBLs of 82 OSCC patients and 25 healthy individuals using flow cytometry analysis. Their data showed a significant difference in the percentage of PD-1 and PD-L1 expression on CD4<sup>+</sup> and CD8<sup>+</sup> T-cells in PBLs of OSCC patients (CD4<sup>+</sup>PD-1<sup>+</sup>  $11.4\% \pm 1.3\%$ , CD8<sup>+</sup>PD-1<sup>+</sup>  $10.6\% \pm 1.8\%$ ; CD4<sup>+</sup>PD-L1<sup>+</sup>  $9.5\% \pm 1.6\%$ , CD8<sup>+</sup>PD-L1<sup>+</sup>  $20.8\% \pm 2.8\%$ ) and the control subjects (CD4<sup>+</sup>PD-1<sup>+</sup>  $5.5\% \pm 0.9\%$ , CD8<sup>+</sup>PD-1<sup>+</sup>  $6.7\% \pm 1.2\%$ ; CD4<sup>+</sup>PD-L1<sup>+</sup>  $5.0\% \pm 1.1\%$ , CD8<sup>+</sup>PD-L1<sup>+</sup>  $9.2\% \pm 1.6\%$ ). Moreover, the soluble PD-1 level in plasma was also higher in OSCC group than in normal controls.<sup>48</sup>

### NPC and PD-1/PD-L1

Analysis of 59 nasopharyngeal tumor specimens presented high levels of PD-1 (67.8%, 40/59) and PD-L1 mRNA (66.6%, 20/30), whereas there was no PD-L1 detected in healthy nasopharyngeal tissues. These results suggested that expression of PD-L1 was not significantly correlated with age and gender, but had strong correlations with clinical tumor stage, lymphatic metastasis, and local recurrence.<sup>49</sup>

The expression rate of PD-1 and PD-L1 has been investigated in CD8<sup>+</sup> and CD4<sup>+</sup> T-cells in 28 NPC patient samples. Twenty-eight point four percent and 3.9% of CD8<sup>+</sup> T-cells were found to be PD-1-positive in nasopharyngeal tumor tissues and controls, respectively. As for PD-L1, all the 28 NPC tissues were positive for PD-L1, while 79% (23/29) of the nasopharyngeal control subjects were found to be PD-L1<sup>+</sup>. Eighteen out of 28 cases of NPC expressed B7-H1 on EpCAM<sup>+</sup> epithelial cells.<sup>50</sup>

### Laryngeal carcinoma and PD-1/PD-L1

To study the expression of PD-L1 in human laryngeal carcinoma tissues and peritumoral tissues, Cao et al assessed the expression of PD-L1 in 31 cases of laryngeal carcinoma and 24 cases of paraneoplastic tissues, and demonstrated that PD-L1 was significantly higher in poorly and moderately differentiated larynx squamous carcinoma than that in well-differentiated tumor tissues. Immunohistochemical analysis showed that PD-L1 was mainly expressed in blood vessels and rarely in cancer cells in poorly and moderately differentiated groups, but displayed weakly positive in blood vessels and partially positive in cancer cells of the well-differentiated group. PD-L1 expression was intensely correlated with lymphatic metastasis. The expression of PD-L1 in the surrounding tissues was significantly lower than that in cancer tissues, but the rates were in keeping with that of tumor tissues.<sup>51</sup> Automated quantitative protein analysis was utilized to examine PD-L1 protein expression on TILs in 260 laryngeal squamous cell cancer patients. Scientists speculated that superior disease-free survival and overall survival (OS) were both correlated with PD-L1 levels. And, mRNA level of PD-L1 was up-modulated in tumors compared to paraneoplastic tissues.<sup>52</sup>

### Esophageal squamous cell carcinoma (ESCC) and PD-1/PD-L1

The expression of PD-1 was detected by immunohistochemical Streptavidin-peroxidase (SP) analysis in 118 cases of ESCC (54.2%), 39 cases of adjacent atypical hyperplasia (35.9%), and 39 cases of normal esophageal tissues (2.6%).<sup>53</sup> Results of another research found that 26 of the 54 (48.15%) esophageal carcinoma tissues were positive for B7-H1 protein expression, while normal esophageal tissues were totally negative for both B7-H1 mRNA and protein expression. Scientists further explored the relationship between B7-H1 expression and prognostic clinical parameters. They found no association between B7-H1 expression and age, gender, tumor size, location, or differentiation. In contrast,



the expression of B7-H1 was significantly correlated to invasion depth, lymphatic metastasis, distant metastasis, and pT classification of the tumor.<sup>54</sup>

## HNSCC and CTLA-4 (role of CTLA-4 in HNSCC)

Jie et al isolated TILs and lymphocytes from peripheral blood from a group of HNSCC patients and discovered that CTLA-4 was displayed more frequently on intratumoral Tregs than circulating Tregs.<sup>55</sup> A study conducted among laryngeal squamous cell carcinoma patients demonstrated that intracellular CTLA-4 presented with a higher expression in CD8<sup>+</sup> lymphocytes among patients than in control subjects ( $8.2\% \pm 8.7\%$  vs  $2.3\% \pm 3.5\%$ ). These rates in CD4<sup>+</sup> and CD19<sup>+</sup> lymphocytes were also significantly higher in patients. In terms of surface CTLA-4, the difference was only detected in the case of CD8<sup>+</sup> lymphocytes ( $0.7\% \pm 0.6\%$  vs  $0.3\% \pm 0.3\%$ ).<sup>56</sup> NPC patients with lower tumor CTLA-4 expression were found to have higher 3-year OS rates (91.4%), failure-free survival rates (82.8%), and distant failure-free survival rates (85.8%) than those who fell into the high CTLA-4 expression group (81.2%, 68.0%, and 72.3%, respectively).<sup>57</sup> Expression of CTLA-4 was present in 87% (137/158) of the ESCC cases. With respect to tumor imaging metrics core, 65 patients (42.2%) fell into the negative category, while 89 (57.8%) patients were found positive for CTLA-4 expression. Scientists further speculated that the OS of CTLA-4 ESCC patients (65 months) was longer than that of the CTLA-4<sup>+</sup> group (36 months).<sup>58</sup>

Several studies have explored the association between oncogenesis and CTLA-4 single nucleotide polymorphisms (SNPs), namely +49A/G (rs231775), -1661A/G (rs4553808), -318C/T (rs5742909), -1722T/C (rs733618), and CT60G/A (rs3087243) SNPs, etc.<sup>59,60</sup> Kämmerer et al found that the genotype CTLA-4 (-1661A/G) was detected more frequently in patients with OSCC than in healthy controls, and that several combinations of SNPs were found only in patient tissues.<sup>61</sup> However, a study conducted by Wong et al demonstrated that there was no significant difference in CTLA-4 SNPs between OSCC and control groups. They found that the genotype CTLA-4 A/A was correlated with a younger onset age and lower survival rate.<sup>62</sup> A hospital-based case-control study and meta-analysis results suggest that in the Chinese Han population, the CTLA-4 +49 G>A polymorphism was not associated with the risk of developing esophageal cancer.<sup>63</sup> Another case-control study among the Chinese population showed that there was significant association in the frequency of CTLA-4 +49 A/G and NPC risk.<sup>64</sup> Therefore, the

correlation between CTLA-4 SNPs and their roles in cancerogenesis in HNSCC remains to be further studied.

## Checkpoint inhibitors as novel strategies for HNSCC and clinical outcome

Immunotherapies against existing tumors include diverse approaches, ranging from stimulating effector mechanisms to eliminating inhibitory mechanisms.<sup>38</sup> In conventional cancer immunotherapy, the aim is focused on “putting the weights on the stimulatory side”. However, unfortunately, it is difficult to provide sufficient stimulatory co-signals to exceed heavily inhibitory factors in the tumor microenvironment. Even though such methods could be performed on patients in clinical practice, their adverse effects associated with over-activation of immune cells in non-tumoral organs would be extremely severe.<sup>65</sup> As mentioned above, immune checkpoint molecules, which belong to inhibitory co-signals, exist to counteract stimulatory co-signals. Currently, several mAbs targeting those molecules have been developed to “decrease the weights from the inhibitory side” and have shown impressive promise in Phase I–III clinical trials for advanced solid tumors like melanoma, renal cell cancer, and non-small cell lung cancer.<sup>66–68</sup> Unlike conventional antibodies used for the treatment of tumors, antibodies that block immune checkpoints do not bind directly to the tumor cells, but target lymphocyte receptors or their ligands to modulate their antitumor activity. Among these immune checkpoint blockades, ipilimumab (anti-CTLA-4 antibody), nivolumab, and pembrolizumab (anti-PD-1 antibody) have been designed and used in some solid tumors to block co-inhibitory signaling, recovering the CTL from anergy in the antitumor immune response.<sup>69,70</sup> It has been reported that, compared to traditional modalities, those Abs display superior clinical efficacies with long-term disease remission and increased objective response rates in selected patients. The anti-CTLA-4 mAb ipilimumab was first approved as a new agent by the US Food and Drug Administration (FDA) for the treatment of high-risk and metastatic postoperative melanoma. In addition to anti-CTLA-4, mAbs targeting PD-1 and its ligand PD-L1 have also been investigated as important therapeutic tools in several early phase clinical trials. Based on intriguing preclinical data from many groups, several ongoing clinical trials (Table 1) are investigating the role of immune checkpoint blockades in R/M HNSCC including single agent and combinations with tumor vaccines, chemotherapy, or radiation.<sup>71</sup> Each of these categories will be discussed briefly below.

**Table I** Ongoing clinical trials evaluating immune checkpoint inhibitors in HNSCC

Trial identifier	Drug	Phase	Immune checkpoint target	Disease/tumor type	Primary outcome measures
NCT01848834	Pembrolizumab (MK-3475)	IB	PD-I	R/M HNSCC	OR ORR OS PFS
NCT02105636	Nivolumab	III	PD-I	R/M HNSCC	ORR OS PFS
NCT02358031	Pembrolizumab (MK-3475)	III	PD-I	R/M HNSCC	
NCT02296684	Pembrolizumab (MK-3475)	II	PD-I	Locoregionally advanced surgically resectable HNSCC	Locoregional recurrence rates; distant failure rate
NCT02252042	Pembrolizumab	III	PD-I	R/M HNSCC	PFS, OS
NCT02255097	Pembrolizumab	II	PD-I	R/M HNSCC	ORR
NCT02289209	Pembrolizumab + RT	II	PD-I	Locoregional inoperable recurrence or 2nd primary HNSCC	PFS
NCT0233558	Nivolumab	II	PD-I	R/M nasopharyngeal cancer	Objective tumor response per RECIST criteria
NCT01693562	Durvalumab (MEDI-4736)	I/II	PD-L1	Solid tumors (including HNSCC) refractory to standard therapy	OR
NCT02499328	Durvalumab (MEDI-4736) + either AZD9150 or AZD5069	I/II	PD-L1	R/M HNSCC	Safety/efficacy ORR
NCT02291055	Durvalumab (MEDI-4736) + ADXS11-001	I/II	PD-L1	Previously treated locally advanced or metastatic cervical or HPV <sup>+</sup> HNSCC	Safety/efficacy
NCT01860430	Ipilimumab-concurrent cetuximab and IMRT	IB	CTLA-4	AJCC stage III/IVB HNSCC, excluding T1N1	Ipilimumab dose
NCT01935921	Ipilimumab-concurrent cetuximab and IMRT	I	CTLA-4	R/M HNSCC	PFS Ipilimumab dose
<b>Combinatorial immunotherapy</b>					
NCT02551159	Durvalumab (MEDI-4736) + tremelimumab	III	PD-L1 and CTLA-4	R/M HNSCC	PFS, OS
NCT02369874	Durvalumab (MEDI-4736) + tremelimumab	III	PD-L1 and CTLA-4	R/M HNSCC	OS, PFS
NCT02319044	Durvalumab (MEDI-4736) +/- tremelimumab	II	PD-L1 and CTLA-4	R/M HNSCC	ORR
NCT02262741	Durvalumab (MEDI-4736) + tremelimumab	I	PD-L1 and CTLA-4	R/M HNSCC	Safety

**Abbreviations:** PFS, progression-free survival; ORR, overall response rate; OS, overall survival; RT, radiation therapy; IMRT, intensity-modulated radiotherapy; HNSCC, head and neck squamous cell carcinoma; PD-I, programmed cell death 1; CTLA-4, cytotoxic T-lymphocyte-associated antigen 4; R/M, recurrent or metastatic; HPV, human papilloma virus; AJCC, American Joint Committee on Cancer; OR, odds ratio; RECIST, response evaluation criteria in solid tumors.

## Anti-CTLA-4

The anti-CTLA-4 antibody ipilimumab has shown the greatest clinical curative effect in patients with advanced melanoma in two randomized Phase III trials.<sup>72</sup> Some observations have recently evaluated the role of CTLA-4 as a potential therapeutic target in HNSCC. In a cohort of patients

with HNSCC (n=22), treatment with cetuximab increased the expression of CTLA-4 in the majority of intratumoral Tregs, and it was also demonstrated that ipilimumab can decrease suppression of NK cells from Tregs.<sup>73</sup> Ipilimumab is currently being assessed in clinical trials in combination with cetuximab and intensity-modulated radiotherapy in patients with

advanced HNSCC (NCT01860430 and NCT01935921).<sup>74</sup> Tremelimumab (CP-675, 206) is a fully humanized mAb specific for CTLA-4, which has been successfully used to treat patients with metastatic melanoma and some other cancers. Currently, clinical testing of tremelimumab for HNSCC in combination with other therapies including cetuximab and intensity-modulated radiation therapy (IMRT) is underway (NCT01860430).

## Anti-PD-1

Regarding anti-PD-1 antibodies, the humanized pembrolizumab and fully human nivolumab are IgG4 mAbs that block the binding of PD-1 receptor to PD-L1 and PD-L2. Pembrolizumab (also named MK-3475), with a high affinity for PD-1, is the first mAb targeting PD-1 approved by the FDA, which has been the most extensive immunotherapeutic strategy in HNSCC. An ongoing multicenter Phase I trial (KEYNOTE-012, NCT01848834) is the largest to date completed immune checkpoint therapy trial in HNSCC. Preliminary results show that 49.5% of patients demonstrated partial response or stable disease following treatment with pembrolizumab given a fixed dose of 200 mg every 3 weeks. Eighty six percent of these patients demonstrated durable responses.<sup>75</sup> In another ongoing clinical trial, of the 104 patients with head and neck cancer who were enrolled, the overall response rate (ORR) was 18.2%; response rates were similar regardless of HPV status.<sup>71</sup> Pembrolizumab has shown minimal adverse effects when administered in head and neck cancer patients.<sup>76</sup> Further updates on this trial reported that pembrolizumab was twice as effective as cetuximab in HNSCC, with disease control of 50% and tumor shrinkage in 57% of patients.<sup>77</sup> In a combined update of two cohorts of patients with R/M HNSCC that was heavily pre-managed, pembrolizumab resulted in a response rate of 24% and an additional 25% of patients with stable disease, which were both slightly improved compared with earlier reports.<sup>78</sup> Another trial KEYNOTE-048 evaluating pembrolizumab either as monotherapy or in combination with chemotherapy versus standard chemotherapy is underway in patients with R/M HNSCC (NCT02358031). Pembrolizumab is also currently being assessed in conjunction with re-irradiation and as part of primary treatment in multiple clinical settings (NCT02289209, NCT02296684). Some Phase II/III trials are underway to evaluate the clinical efficacy (ORR, response duration, and side effects) of pembrolizumab in R/M HNSCC (NCT02255097, NCT02252042). Nivolumab, a fully human immunoglobulin G4 PD-1 immune checkpoint inhibitor antibody, was active and generally well tolerated in patients with advanced solid tumors. In patients with HNSCC refractory to

platinum therapy, a randomized Phase III trial of nivolumab (NCT02105636) resulted in improved OS compared with treatment with the weekly methotrexate, docetaxel, or cetuximab (NCT02105636). Furthermore, a Phase II nivolumab monotherapy study for R/M NPC is currently in progress (NCT02339558).

## Anti-PD-L1

At present, representative antibodies targeting PD-L1 include BMS-936559 (also known as MDX1105), MPDL3280A, and durvalumab (MEDI-4736), which have been applied as monotherapy or adjuncts to conventional therapies in clinical trials for a variety of tumors.<sup>79</sup> Two ongoing Phase I trials, with anti-PD-L1 mAb (IgG1 isotype) MEDI-4736 (NCT01693562) and anti-PD-1 mAb (IgG4 isotype) MK-3475 (NCT01848834), have recruited cohorts of R/M HNSCC patients. The disease control rate at 6 months was 15% (18% for PD-L1-positive vs 11% for PD-L1-negative), and the ORR in patients with HNSCC was 11%. Efficacy of durvalumab, an engineered IgG1 antibody to PD-L1, has also been tested among 62 patients with R/M HNSCC. Results showed an ORR of 12%, and the duration of response ranges from 4 to 43 weeks. A second trial (NCT02291055) has combined PD-L1 inhibitor durvalumab (MEDI-4736) with ADXS11-001, live attenuated *Listeria monocytogenes* engineered to express, and subsequently elicit, an immune response against an HPV-16-E7 fusion protein.<sup>80</sup> In another randomized Phase I/II trial to assess the combination of MEDI-4738 with either AZD9150 or AZD5069 in patients with metastatic squamous cell carcinoma of head and neck (NCT02499328), the clinical safety/efficacy and ORR are being evaluated.

## Combinatorial immunotherapy clinical trials in HNSCC

Some studies are currently active or under development to investigate the efficacy of immune checkpoint inhibitors in combination with one another. Some trials evaluating the utility of MEDI-4736 monotherapy alone or in conjunction with tremelimumab (anti-CTLA-4) compared to the standard treatment for first-line R/M HNSCC are ongoing (NCT02551159, NCT02369874, NCT02319044). This is a novel perspective in the application of immune therapy, and the results as well as analysis of the outcomes are much looked forward to.<sup>81,82</sup>

## Conclusion

HNSCC has been recognized as an immunosuppressive disease. There have been multiple recent developments using immunotherapy for the management of HNSCC patients. Blockades of immune checkpoints have proved to play a

vital role in enhancing immune surveillance and tumor cell clearance, which provides oncologists with a significant set of antitumor therapies with promising potentials. Studies of immune checkpoint inhibitors should not only lead to a novel strategy for advanced HNSCC, but also for many other solid tumors. However, more investigations are required and further studies need to be better designed to explore the possibility and efficacy of combinations of different agents and still keep an eye on their side effects, thus to bring about more desired tumor outcomes.

## Acknowledgments

This work is supported by the National Natural Science Youth Foundation of China (81502348); the National Natural Science Foundation of China (81572653); and Science and Technology Department of Jilin Province Youth Foundation (20150520042JH).

## Disclosure

The authors report no conflicts of interest in this work.

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