Immunotherapy in head and neck cancer

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Abstract: Head and neck squamous cell carcinoma (HNSCC) comprise a diverse group of malignancies including tumors caused by tobacco and alcohol as well as an increasing number of human papillomavirus (HPV)-associated cancers. There has been recent improved understanding on how HNSCC develops by evading the immune system. The U.S. Food and Drug Administration (FDA) recently granted accelerated approval to the checkpoint inhibitor pembrolizumab for the treatment of recurrent or metastatic HNSCC. We provide a comprehensive review of immune escape mechanism in HNSCC and latest developments in immunotherapy in HNSCC with a focus on checkpoint inhibitors, therapeutic cancer vaccines, and adoptive cellular therapies.

Keywords: Checkpoint inhibitor; head and neck cancer; immunotherapy; programmed cell death-1 (PD-1); pembrolizumab

Introduction

Head and neck cancers comprise a diverse set of malignancies that originate from nasopharynx, oral cavity, oropharynx, hypopharynx, larynx, and salivary gland. It is estimated that they will account for 61,760 new cancer cases and 13,190 cancer deaths in the United States in 2016 (1). Besides tumors of salivary glands, the majority of head and neck cancers are squamous cell carcinomas and is the focus of this article.

Tobacco (including smokeless tobacco) and alcohol use are the most predominant risk factors for head and neck squamous cell carcinoma (HNSCC) and they work synergistically. Human papillomavirus (HPV) infection is now widely accepted as another cause for HNSCC, particularly in the oropharynx, with an increasing incidence in developed countries (2,3). Epstein-Barr virus (EBV), another oncogenic virus, is associated with endemic nasopharyngeal cancer in the Mediterranean and Far East (4).

For localized disease without detectable lymph node involvement, surgery or radiotherapy are effective treatment options. Radiation therapy is preferred for laryngeal cancer to preserve vocal cord function whereas surgery is recommended for small lesions in the oral cavity to avoid long-term complications associated with radiation such as xerostomia and severe dental decay. However, two-third of patients with HNSCC present with stage III or IV disease. Despite optimal local therapy, 30–50% of patients will have local or regional recurrence (5). Patients with locally or regionally advanced disease, that is, disease with a large primary tumor and/or lymph node involvement, are treated with curative intent with combined-modality therapy including surgery, radiation, and/or chemotherapy. Nearly a third of all patients with HNSCC will eventually develop distant metastases. The cornerstone of recurrent or metastatic HNSCC treatment is palliation with a platinum-based therapy (6). Cetuximab, an epidermal growth factor receptor (EGFR) inhibitor, was approved by the U.S. Food and Drug Administration (FDA) in 2011 for recurrent or metastatic HNSCC as it demonstrated survival superiority

when used with platinum plus 5-fluorouracil (5-FU) (7). Nonetheless, recurrent or metastatic HNSCC still has a poor prognosis with low response rate to current systemic treatment (methotrexate, 5-FU, cisplatin/carboplatin, docetaxel/paclitaxel, or cetuximab) and short duration of response with a median survival between 6 to 10 months (8).

Head and neck cancer treatment can cause significant side effects. Its close proximity to vital organs for speech, swallow, and breathing impacts patient's quality of life after surgical resection or radiation. Radiation therapy can be limited by acute oral mucositis and has long-term complications including loss of taste, decreased tongue mobility, xerostomia, and neck fibrosis (9,10). Complications of chemotherapy vary with specific regimen but frequently include myelosuppression, nausea and vomiting, nephrotoxicity and ototoxicity (platinum-based), and neuropathy (taxanes). Adverse effects with cetuximab include dermatological toxicity (e.g., pruritus, rash, nail changes), diarrhea, and infusion reactions (11). Severe hypomagnesemia and cardiac arrest can also occur.

Despite significant advancements in treatment of HNSCC in recent decades such as transoral robotic surgery (TORS), more precise intensity-modulated radiation (IMRT), and cetuximab, morbidity and mortality for patients with HNSCC, especially recurrent or metastatic HNSCC, remains high. Agents with less toxicity and better efficacy are needed. The FDA recently granted accelerated approval to pembrolizumab, a checkpoint inhibitor with improved side effect profile, tolerability, and promising durable antitumor activity, for second-line recurrent or metastatic HNSCC treatment. In this article, we will review immune escape mechanism in HNSCC, FDA approval for pembrolizumab, and latest developments in immunotherapy in HNSCC with a focus on pembrolizumab and checkpoint inhibitors, therapeutic cancer vaccines, and adoptive cellular therapies.

Immune escape in HNSCC

HNSCC is known to be an immunosuppressive disease with spontaneous apoptosis and down-regulation of effector T cells (12-14), defective antigen-processing mechanism, and thus impaired recognition of cancer cells by T cells (15), lower absolute lymphocyte counts (16), and compromised natural killer cell function (17). Patients who are immunosuppressed either with HIV infection or post-solid organ transplant are at increased risk of developing HNSCC (18-20).

The lack of immune control has been recognized as an emerging hallmark of cancer (21). Allison and his colleagues found that simply occupying T-cell antigen receptor (TCR) is not sufficient to fully activate T-cells; a second signal such as binding of T-cell CD28 molecule is necessary (22). This led to the discovery of various co-stimulatory receptors and co-inhibitory receptors for the regulation of T-cell activation. Perhaps the need for two immunologic signals, co-stimulatory/co-inhibitory signals, in addition to binding of TCR, helps to prevent overacting inflammatory response to a stimuli or mistaking self as a dangerous invader while providing appropriate protection to the host. Besides CD28, multiple other co-stimulatory/activating receptors have also been identified, including CD137, CD40, OX40 (CD134), and lymphocyte activation gene-3 (LAG-3) (23,24). Co-inhibitory receptors are also known as immune checkpoints and are responsible for preventing chronic autoimmune or inflammatory state. Some cancer cells manage to present certain immune checkpoints and by doing so skip the immune surveillance. By inhibiting immune checkpoints, one can unblock the negative immune regulation and allows the immune system to fight cancer.

Two of the most studied checkpoint mechanisms are cytotoxic T-lymphocyte-associated protein-4 (CTLA-4) and programmed cell death-1 (PD-1) receptors. CTLA-4 binds to the same ligands (CD80 and CD86) as does CD28 do but with higher affinity; therefore, the presence of CTLA-4 overcomes effects of stimulatory CD28 and causing immune suppression (25). PD-1 receptors present on the surface of T lymphocytes and when activated, inhibit T-cell activation (26). PD ligand 1 (PD-L1) is the main ligand for PD-1 and can be found on antigen presenting cells as well as tumor cells. Binding of PD-L1 to PD-1 activates the latter and results in down regulation of immune function. PD-1 has another ligand called PD ligand 2 (PD-L2) and has similar effect as PD-L1. Both the CTLA-4 and PD-1:PD-L1 pathways have been shown to be commonly present in HNSCC (27,28).

Immunotherapy—checkpoint inhibitors

In August 2016, the FDA granted accelerated approval for pembrolizumab (Keytruda®, Merck & CO. Inc., Kenilworth, USA) in treatment of recurrent or metastatic HNSCC with progression on or after platinum-containing chemotherapy (29). This indication is based on tumor response rate and durability of response observed from a multicenter, nonrandomized, open-label phase 1b study,

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KEYNOTE-012 (NCT01848834). The recommended dose is 200 mg every 3 weeks administered as an intravenous infusion over 30 minutes.

Pembrolizumab is an IgG4 monoclonal antibody against PD-1 receptor therefore blocks tumor cell's immune-suppressing ligands, PD-L1 and PD-L2, from interacting with PD-1 on T cell and helps to restore immune response. It has been approved previously by the FDA for patients with unresectable or metastatic melanoma and in patients with metastatic non-small cell lung cancer (NSCLC) who have positive PD-L1 expression. KEYNOTE-012 study explored the use of pembrolizumab in patients with other advanced solid tumors including triple-negative breast cancer, HNSCC, urothelial tract cancer, and gastric cancer (NCT01848834). A total of 192 patients aged 18 years or older with confirmed diagnosis of recurrent (not amenable to locally curative options) or metastatic HNSCC received pembrolizumab in this study (30). Exclusion criteria include active autoimmune disease that required immunosuppression, evidence of interstitial lung disease, ECOG ≥2, CNS metastases, HIV, hepatitis B or C, and additional progressing malignancies (31). The initial (I) cohort enrolled PD-L1-positive patients (n=60) whereas expansion (E) cohort enrolled patients regardless of PD-L1 status (n=132). Pembrolizumab 10 mg/kg every 2 weeks (I) or 200 mg every 3 weeks was given for up to 24 months until disease progression, or unacceptable toxicity. Accelerated approval by the FDA was granted as the clinical trial showed an objective response rate (ORR) of 16% (95% CI, 11–22%) in the 174 patients (I =53, E =121) who had progression after platinum-containing chemotherapy (32). Twenty-three (82%) of responding patients have responses of 6 months or longer, with several lasting for more than 2 years and a complete response rate of 5%. Similar ORR was demonstrated in both the initial cohort and expansion cohort when calculated separately regardless of PD-L1 status (31,33). The median response duration has not been reached. It was also noticed that ORR was 22% (95% CI, 12–34%) in HPV-positive patients and 16% (95% CI, 10–23%) in HPV-negative patients when counting all responding patients regardless of previous treatment status (30). However, caution should apply when reaching final conclusions on how HPV status affects response rate to PD-1 inhibitor given the small number of patients.

Pembrolizumab has a relatively tolerable side effect profile. Treatment-related adverse events occurred in 122 (64%) of the patients (30). Adverse events experienced by patients with HNSCC were similar to those occurring in patients with melanoma or NSCLC, with the addition of facial edema (10% all grade, 2% grades 3–4). Most common adverse reactions (reported in ≥20% of patients) were fatigue, decreased appetite, and dyspnea; 12% patients had grade 1–2 pruritus (31). Of the 192 patients receiving pembrolizumab for recurrent or metastatic HNSCC, 23 (12%) had a grade 3–4 adverse event including increased alanine aminotransferase, increased aspartate aminotransferase, hyponatremia, atrial fibrillation, congestive heart failure, diarrhea, lymphopenia, musculoskeletal pain, and neck abscess. Clinically significant immune-mediated adverse reactions such as endocrinopathies (hypophysitis, thyroid disorders, and type 1 diabetes mellitus), colitis, hepatitis, nephritis, and pneumonitis can also be associated with pembrolizumab (34). Immune-related adverse events usually are reversible if recognized promptly, however delayed recognition can lead to severe toxicity (35). Depending on the grade of toxicity, treatments involve withholding or permanently discontinuing the medication and/or administration of corticosteroids for immunosuppression.

Pembrolizumab in head and neck cancer is currently under further investigation in multiple advanced trials. Preliminary results from the first 50 out of 172 patients enrolled in a phase 2 trial (KEYNOTE-055, NCT02255097) evaluating pembrolizumab for HNSCC with progression after platinum and cetuximab found an ORR of 18.0% (95% CI, 8.6–31.4%) confirming findings from KEYNOTE-012 (36). One (2%) patient died because of a treatment-related adverse event in this trial whereas none in KEYNOTE-012. As a part of the accelerated approval condition, Merck is conducting a multicenter, randomized phase 3 trial to assess if pembrolizumab has superiority over standard therapy for recurrent or metastatic HNSCC with disease progression on or after platinum-containing chemotherapy (KEYNOTE-040, NCT02252042). The study uses pembrolizumab as the experiment group with investigator's choice (IC) of standard treatment (methotrexate, docetaxel, or cetuximab) as the control arm and will be looking at overall survival (OS) for primary endpoint. KEYNOTE-048 is another phase 3 clinical trial currently recruiting patients with recurrent or metastatic HNSCC with no prior systemic therapy (NCT02358031). This study will be evaluating pembrolizumab as first line treatment for recurrent or metastatic HNSCC with enrolled patients randomized to either of the three groups—pembrolizumab monotherapy, pembrolizumab + platinum + 5-FU, or

cetuximab + platinum + 5-FU. Pembrolizumab also demonstrated promising antitumor activity in treatment-refractory advanced (unresectable and/or metastatic) salivary gland carcinoma cohort in a phase 1b clinical trial (KEYNOTE-028, NCT02054806) and is under active investigation in the phase 2 KEYNOTE-158 trail (NCT02628067) (37).

Nivolumab (Opdivo®, Bristol-Myers Squibb, New York City, USA) is another IgG4 monoclonal antibody against PD-1 receptor that has shown promise in HNSCC. More specifically, nivolumab was found to improve OS and have better tolerability when compared with methotrexate, docetaxel, or cetuximab in patients with platinum-refractory recurrent or metastatic HNSCC in a randomized, open-label, phase 3 trial CheckMate-141 (NCT02105636) (38). A total of 361 patients aged 18 years or older with recurrent or metastatic HNSCC who had disease progression within 6 months of platinum-based chemotherapy and ECOG 0–1 were randomized 2:1 to nivolumab (3 mg/kg intravenously every 2 weeks) or single-agent of IC (methotrexate, docetaxel, or cetuximab). A 30% reduction in risk of death was seen in nivolumab-treated group with a median OS of 7.5 months (95% CI, 5.5–9.1) compared to 5.1 months (95% CI, 4.0–6.0) for IC. Nivolumab was effective for HNSCC regardless of PD-L1 status, however there was a positive correlation between degree of PD-L1 expression and medication response as ORR for nivolumab-treated patients with PD-L1 ≥1%, 5%, and ≥10% was 18.2%, 25.9%, and 32.6%, respectively, and 3.3%, 2.3%, and 2.9% for IC. Treatment-related adverse events occurred in 58.9% of patients on nivolumab *vs.* 77.5% of patients on IC; of which 13.1% in nivolumab *vs.* 35.1% on IC were grade 3–4. Given these data, the FDA has granted priority review to nivolumab for recurrent or metastatic HNSCC and will be announcing their decision regarding approval no later than November 11, 2016 (39).

In addition to PD-1 inhibitors, monoclonal antibodies that target PD-L1 are under investigation. Currently there is a phase 2 study evaluating durvalumab, an Fc optimized monoclonal antibody against PD-L1, as monotherapy for recurrent or metastatic HNSCC (NCT01693562) and more studies looking into the effect of combining durvalumab with other therapies as discussed in later sections. Both avelumab and atezolizumab are IgG1 anti-PD-L1 monoclonal antibodies; both have ongoing phase 1 trials evaluate use in patients with advanced solid tumors including HNSCC (NCT01772004 and NCT01375842, respectively). A phase 2 trial of avelumab in patients with

recurrent or metastatic nasopharyngeal carcinoma is soon to open (NCT02875613).

Ipilimumab (Yervoy®, Bristol-Myers Squibb) is an IgG1 monoclonal antibody against CTLA-4 that has received the FDA approval for metastatic melanoma, and is currently being evaluated in combination with cetuximab and IMRT for locally advanced HNSCC (NCT01860430, NCT01935921). Ipilimumab blocks the interaction of CTLA-4 with its ligands, CD80/CD86, and removal of this negative regulation subsequently augments T-cell activation and proliferation. Compared to PD-1 inhibitors, ipilimumab is associated with more severe immune-related toxicity (40). Another anti-CTLA-4 antibody that has been evaluated in several clinical trials in HNSCC is tremelimumab (NCT02319044, NCT02369874, NCT02551159).

Immunotherapy—therapeutic vaccine

An increasing number of HNSCC are virally mediated with exogenous proteins and therefore place them at a unique advantage of potential benefit of therapeutic vaccination as a treatment or prevention option. Commercially available HPV vaccines (Cervarix®, GlaxoSmithKline, Philadelphia, USA and Gardasil®, Merck & CO. Inc.) are highly effective in preventing ano-genital cancers but not effective in treating existing HPV infections or HPV-associated malignant lesions. Vaccine efficiency in reducing HPV-associated HNSCC incidence is currently being evaluated; it will take longer to collect the data as HPV oropharyngeal cancers can take years to develop after initial infection (41). HPV infected cells do not express detectable level of capsid antigens, as a result HPV prophylactic vaccine does not work for preexisting HPV lesions as the vaccine consists of viral-like particles and works by inducing strong antibodies against capsid antigen L1 in recipients. HPV infected cells, however, do express E6 and E7 proteins which can be used as targets in the development of HPV therapeutic vaccines. Both viral E6 and E7 proteins are expressed early and throughout the course of infection and participate in oncogenesis (42,43).

There are products advancing to clinical trials for HPV therapeutic vaccine. VGX-3100 is a synthetic plasmid targeting HPV-16 and HPV-18 E6 and E7 protein. A phase 2 study showed histopathological regression in women with cervical intraepithelial neoplasia 2/3 treated with VGX-3100 delivered by electroporation (44). Patients treated with VGX-3100 who had lesion regression were also found to have increased CD8-positive infiltrates compared

to no change in intensity of CD8-positive infiltrates in placebo group patients who had histopathological regression. Even though the response rate in these pre-malignancies was low (50% in experimental arm *vs*. 30% in placebo group), it was the first trial to successfully demonstrate that therapeutic vaccines' ability in inducing immunity in patients with existing HPV-associated lesions. A phase 1/2a trial looking into safety, tolerability, and immunogenicity of VGX-3100 plus adjuvant interleukin-12 in patients with HPV-associated HNSCC is undergoing (NCT02163057).

Another method of viral protein delivery that is under investigation is called ADXS11, a live attenuated *Listeria monocytogenes* based immunotherapy that secretes antigen-adjuvant fusion protein consisting of HPV-16 E7. One phase 1 trial of ADXS11 in HPV-positive oropharyngeal cancer was terminated early due to dose limiting toxicity (NCT01598792) and a phase 2 trial studying modified ADXS11 as neoadjuvant to ablative TORS for patients with newly diagnosed HPV-16-positive oropharyngeal cancer is currently recruiting (NCT02002182).

In addition to viral protein E6/E7, cellular protein p16 has also been an interest of therapeutic vaccine development. Limited success with E6 and E7 vaccines could be partially due to immunoevasion and immunoediting against the viral antigen in patients with persistent HPV infections (45). Cyclin-dependent kinase inhibitor p16 is consistently overexpressed in HPV-associated cancers. The theoretical risk of systemic tissue-destructive autoimmune reaction with p16 vaccine is low given expression level of p16 in normal tissue is very low and may be contributing to aging-related pathologies (46). In the phase 1/2a clinical trial no unexpected serious adverse reaction was found (NCT01462838). Of the 26 patients with advanced HPV-associated malignancies enrolled in the study, seven have HNSCC and all noticed to have immune response after vaccination. The best clinical response observed in this trial was stable disease; tumor regression was not seen, potentially due to the advanced stage of diseases in recruited patients. Another small phase 1 trial is evaluating a p16_37-63 peptide vaccination with cisplatin-based chemotherapy to improve the prognosis and quality of life for patients with HPV-positive cancers (NCT02526316).

Immunotherapy—adoptive T-cell therapy

Before development of immune checkpoint inhibitors and therapeutic cancer vaccines, interests have been drawn to passive immunization, also known as adoptive T-cell transfer or therapy. Adoptive T-cell therapy involves harvesting and *ex vivo* expansion of patient's own tumor antigen specific T-cells, followed by reinfusion into the patient in hopes of enhancing a patient's antitumor immune response (47). It has potential success in HNSCC as HPV- and EBV-associated diseases have foreign (viral) antigens that are excellent targets. It has been demonstrated that EBV-specific cytotoxic T lymphocytes (CTL) therapy has antitumor activity in advanced nasopharyngeal cancers. However, the use of lymphodepleting chemotherapy prior to CTL infusion did not enhance clinical benefit (48). Clinical benefit of EBV-specific CTL in recurrent or metastatic nasopharyngeal cancer was further demonstrated in a phase 2 study where it was used as first-line therapy in combination with chemotherapy (49). Even though specific data regarding HPV-positive HNSCC is not available at this time, adoptive T-cell therapy has showed some promising results in other HPV-associated malignancy (50). In this small phase 2 study, three out of nine patients with platinum-refractory metastatic cervical cancer experienced objective tumor responses with two complete responses over 1 year. Similar phase 2 with metastatic HPV-associated carcinoma including oropharyngeal cancer has been done and is in final data collection stage (NCT01585428).

Rationale of combining immunotherapies

The new paradigm of checkpoint blockade is promising and combination of different immunotherapies opens up more potential therapeutic interventions. Different groups of checkpoint inhibitors have distinct immunologic effects; therefore by combining them, one can influence different phases of T-cell induction and maturation to achieve a stronger response. For example, blockade of PD-1 leads to gene changes regarding cytolysis and natural killer cell function whereas CTLA-4 blockade induces a proliferative upregulation of a transitional memory T cell subset (51). Higher response rate indeed were observed in patients with advanced melanoma treated with combined nivolumab and ipilimumab than either approach alone (52). Current clinical trials assessing the efficacy of anti-PD-1 and anti-CTLA-4 antibodies combination in recurrent or metastatic HNSCC are listed below (*Table 1*).

Conclusions

Significant advancements have been made in immunotherapy

Table 1 Ongoing clinical trials evaluating combined checkpoint inhibitors therapy for recurrent or metastatic HNSCC as of October 1, 2016

Study ID	Estimated number of patients	Phase	Interventions
NCT02319044	240 (ongoing study however not recruiting)	2	Durvalumab monotherapy
			Tremelimumab monotherapy
			Durvalumab + tremelimumab
NCT02369874	720	3	Durvalumab monotherapy
			Durvalumab + tremelimumab
			Standard of care*
NCT02551159	628	3	Durvalumab monotherapy
			Durvalumab + tremelimumab
			Standard of care*
NCT02741570	490	3	Nivolumab + ipilimumab
			Standard of care*
NCT02823574	315	2	Nivolumab + ipilimumab
			Nivolumab + placebo

*, standard of care: cetuximab, cisplatin/carboplatin, 5-fluorouracil. HNSCC, head and neck squamous cell carcinoma.

treatments for advanced HNSCC treatment. Better understanding of immune evasion of tumors offers more treatment options particularly with the checkpoint inhibitors. The anti-PD-1 antibody pembrolizumab has recently been granted accelerated approval for recurrent or metastatic HNSCC by the FDA as it demonstrated durable response rate. Further studies are ongoing to assess potential survival benefit. Another anti-PD-1 antibody, nivolumab, is currently undergoing FDA priority review and the decision regarding its approval is pending. Other checkpoint inhibitors (anti-PD-L1, anti-CTLA-4 antibodies) as well as therapeutic vaccine and adoptive T-cell therapy all show promising results. Clinical trial participation for recurrent or metastatic HNSCC patients should be encouraged.

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