Immunotherapy for head and neck cancer: advances and deficiencies

Anna-Maria De Costa[a,b] and M. Rita I. Young[a,c,d]

The concept of immunotherapy as a treatment for cancer patients has been in existence for decades. However, more recent immune therapeutic approaches have involved targeting of tumor-specific antigens. Although improvements have been made in using such immune stimulatory treatment strategies for a variety of solid cancers, the use of these strategies for patients with head and neck squamous cell carcinoma (HNSCC) is lagging behind. Immunotherapeutic approaches for HNSCC are particularly complicated by the profound immune suppression that is induced by HNSCC, which potentially decreases the effectiveness of immune stimulatory efforts. Trials involving patients with various solid cancers have shown the enhanced effectiveness of combining various immunotherapeutic approaches or combining immunotherapy with chemotherapy or radiation therapy. Treatment of HNSCC with such combination approaches has not been extensively investigated and has the added challenge of the need to overcome the HNSCC-induced immune suppression. This study focuses on clinical trials that have tested immunotherapeutic approaches for HNSCC patients and the challenges associated with such approaches. In addition, it will call attention to immunotherapeutic strategies that have been shown to be successful in the treatment of other solid cancers to identify potential strategies that may apply to the treatment of HNSCC. *Anti-Cancer Drugs* 22:674–681
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[a]Research Services, Ralph H. Johnson Veterans Affairs Medical Center, Departments of [b]Microbiology and Immunology, [c]Otolaryngology-Head and Neck Surgery and [d]Medicine, Medical University of South Carolina, Charleston, South Carolina, USA

Correspondence to M. Rita I. Young, PhD, Research Service (151), Ralph H. Johnson Veterans Affairs Medical Center, Charleston, SC 29401, USA
Tel: +843 789 6707; fax: +478 274 5761;
e-mail: rita.young@va.gov

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Introduction

Head and neck squamous cell carcinomas (HNSCC) are aggressive malignancies, with traditional treatment options involving surgery or, more recently, radiation plus chemotherapy. Despite advances in treatment options and attempts at organ preservation, the success rate of treatment has not improved significantly, and current treatments have typically resulted in debilitating effects with reduced quality of life [1]. An alternative or adjuvant treatment approach being tested for various malignancies is immunotherapy, although studies testing effectiveness of novel immunotherapeutic approaches for HNSCC are lagging.

As with other malignancies, the rationale for considering immunotherapy for HNSCC is based on the expression of antigens that are either selectively expressed on malignant versus normal tissues or expressed in increased levels. These antigens include the mucin MUC-1, epidermal growth factor receptor (EGFR), the RAGE and GAGE families of tumor antigens, NY-ESO-1, and others (Table 1) [2–5]. To increase the effectiveness of immunotherapeutic strategies, efforts are under way to improve the identification of candidate tumor antigens that cause T cell responses. One such effort involves the use of a newly developed, automated, two-dimensional chromatography system PF2D (Beckman Coulter, Inc., Fullerton, California, USA) that fractionates the proteome of human tumor tissues [4]. Advances in this area have the potential to reveal more immunogenic proteins to target, leading to stronger and more enduring responses to therapy.

Immunotherapeutic approaches hinge on the ability of the immune system to recognize these tumor antigens as foreign and develop a response, humoral and/or cellular, against the malignant tissue. Patients with HNSCC have been shown to mount antibody responses to antigens, which are expressed on the tumor tissue. Reactivity includes antibody responses to the mucin MUC-1, with increased serum levels of MUC-1 and antibodies to MUC-1 in patients with more advanced disease and nodal involvement [6]. Antibodies to p53, which is frequently mutated in HNSCC, are also detectable in the serum of HNSCC patients and are indicative of nodal disease involvement [7]. In addition to humoral responses to HNSCC, patients can mount cellular immune responses to the tumor. Increased intraepithelial $CD8^+$ tumor-infiltrating lymphocytes (TILs) in HNSCC metastases, and increased numbers of $CD20^+$ B cells in involved lymph nodes, are associated with a better prognosis [8]. In addition, both $CD4^+$ and $CD8^+$ T cells isolated from patients with HNSCC can be activated in response to tumor antigens [4]. Antigen processing and cross-presentation by dendritic cells to $CD8^+$ T cells can stimulate their reactivity to tumor antigens. The potential

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Table 1 Main tumor antigens targeted by immunotherapy

MUC-1 [3]
EGFR [3,79–81]
RAGE family members [2,82]
GAGE family members [2]
NY-ESO-1 [3]
Carcinoembryonic antigen [3,83]

MUC-1, mucin; EGFR, epidermal growth factor receptor.

of γ/δ T cells to react to tumor has not been extensively studied. Recently, a subset of γ/δ T cells expressing the natural killer (NK) cell-associated molecule CD56 has been shown to exhibit cytolytic reactivity to HNSCC. Although both $CD56^-$ and $CD56^+$ γ/δ T cells express the cytotoxicity-regulatory molecules NKG2D and CD94, only $CD56^+$ γ/δ T cells show cytolytic activity toward HNSCC likely involving the perforin–granzyme pathway [9]. These studies indicate that HNSCC is a good candidate for the development of immunotherapeutic approaches, as HNSCC expresses tumor antigens and the immune system has the potential to react against these antigens.

Immunotherapeutic approaches

Cytokine treatment approaches

Despite the above-described potential reactivity toward tumor antigens, these immune responses have not proven to be sufficient to prevent tumor progression. Therefore, various strategies have been investigated in an attempt to enhance immune reactivity toward cancer. Earlier studies focused on the use of interleukin (IL)-2 as a cytokine immune therapy. These studies showed that the treatment of HNSCC patients with IL-2 increases cytokine levels, intratumoral levels of NK cells, and the activity of TIL [10,11]. A more recent trial showed that perilymphatic administration of IL-2 significantly lengthened disease-free and overall survival of patients with HNSCC, while avoiding toxic effects [12]. In an early study of combination therapy with interferon (IFN)-α2a and IL-2, 18% of patients achieved a partial response, though treatment was associated with substantial toxicity [13]. In conjunction with cisplatin and 5-fluorouracil, IFN-α treatment of squamous esophageal cancer resulted in a 61% response rate coupled with significant side effects [14]. Early studies testing the effectiveness of IFN-γ treatment showed immunological and varying levels of clinical responses [15]. However, further investigation into IFN-γ treatment of HNSCC has not been pursued. IL-12, when administered intratumorally into HNSCC patients, increases the B cell levels within the tumor, stimulates B cell proliferation, increases B cell expression of IFN-γ, and skews the plasma antibody profile toward a Th1 phenotype [16]. Despite IL-12-associated toxicity, this treatment strategy also showed a redistribution of NK cells, lymphocytes and monocytes from the peripheral blood to lymph nodes, and increased lymph node IFN-γ expression levels [17]. Clinical studies are ongoing involving administration of IRX-2, a cell-derived mixture of cytokines including IL-1β, IL-2, IL-6, IL-8, IFN-γ, tumor necrosis factor (TNF)-α, granulocyte colony-stimulating factor, and granulocyte macrophage colony-stimulating factor (GM-CSF), to patients with HNSCC [18]. IRX-2 treatment of peripheral blood mononuclear leukocytes-derived human dendritic cells resulted in an increased expression of co-stimulatory markers and markers for maturation and migration [19]. Although early studies with immunotherapeutic administration of cytokines to HNSCC patients have given optimistic results, none of these treatment strategies has yet been established in the clinic (Table 2).

Cytokine immunotherapeutic strategies have become more recognized as treatments for several other cancers. Despite toxicity and heterogeneity of response, IL-2 therapy is used for the treatment of renal cell carcinoma and melanoma, and it has generated favorable responses in the setting of non-Hodgkin's lymphoma and leukemia. Current research focuses on predicting which patients will respond favorably to IL-2 treatment and on altering the IL-2 molecule to produce lower toxicity [20]. IFN-α has been used successfully as an adjuvant in the treatment of melanoma and some hematologic cancers, though treatment with an antidepressant and aggressive hydration is often necessary to combat side effects [21]. GM-CSF treatment of stages III and IV melanoma after surgical resection has been shown to prolong median survival by more than 2 years [22]. In addition, an oncolytic adenovirus expressing the cytokine GM-CSF has been tested in patients with a multitude of cancers [23]. Further investigation into these promising treatment strategies is necessary to expand them to HNSCC patients.

Antibody treatment approaches

Research into individual cytokine treatments has to a large extent fallen out of favor in recent years, possibly owing in part to the treatment-associated toxicity. Instead, treatments involving targeting of tumor antigens are now more commonly tested. Studies with malignancies other than HNSCC have led the way in using antibodies as a form of immune therapy. Unfortunately, the number of such studies targeting HNSCC has been comparatively minimal. The most prominent tumor antigen that has been targeted on HNSCC has been the EGFR. EGFR is overexpressed in both premalignant oral lesions and HNSCC [3]. Treatment of HNSCC patients with nimotuzumab, a humanized antibody against EGFR that blocks EGFR phosphorylation, has been shown to decrease tumor cell proliferation and results in objective clinical responses [24]. These antitumor effects have been observed in the absence of side

Table 2 Cytokine therapies

IL-2 [10,11]
IFN-γ [15]
IL-12 [16,17]

IFN, interferon; IL, interleukin.

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effects such as skin rashes, which can be common with other anti-EGFR antibodies such as cetuximab [25,26]. Interestingly, these studies showed an absence of a detectable lymphocyte infiltration as a result of the antibody treatment, despite the clinical responses. Studies to determine the mechanisms by which anti-EGFR antibodies might mediate their effects showed not only a blockage of EGFR phosphorylation and downstream signaling, but also the involvement of the classical complement system. EGFR antibodies can trigger complement-mediated tumor lysis [27]. This cytolytic capacity could be further enhanced by combining antibodies directed against different nonoverlapping epitopes. Researchers are also attempting to develop monoclonal antibodies to target other HNSCC-associated antigens. A phase I trial involving the treatment of medullary thyroid cancer patients with a humanized antibody targeting carcinoembryonic antigen, a TAA present in most cases of HNSCC, showed evidence of antitumor activity with minimal toxicity [28]. Ex-vivo treatment of HNSCC tumor specimens with an antibody targeting extracellular matrix metalloproteinase inducer resulted in a significant reduction in tumor ATP levels (58%) compared with reduction in ATP levels of tumors treated with cetuximab (33%) (Table 3) [29].

Studies to determine whether combinations of antibodies targeting EGFR and either chemotherapy or other forms of immunotherapy could result in increased antitumor effectiveness are also in development. For example, using cetuximab together with the EGFR tyrosine kinase inhibitors gefitinib or erlotinib in a human xenograft cancer model induced a greater level of regression and a lengthier time to tumor recurrence than when the EGFR-targeting treatments were used individually [30]. Treatment of colorectal cancer patients with two different antibodies directed against EGFR and vascular endothelial cell growth factor (VEGF), respectively (cetuximab and bevacizumab), together with the topoisomerase 1 inhibitor irinotecan resulted in improved clinical responses as compared with treatment with the antibodies alone [31]. Such studies still need to be expanded in HNSCC patients. Studies with esophageal cancer patients showed increased clinical effectiveness by combining cetuximab with cisplatin and 5-fluorouracil chemotherapy, as compared with the chemotherapy treatment alone [32]. Another combination study showed that anti-EGFR-reactive cytotoxic T cells that were induced by culture with dendritic cells pulsed with a novel immunogenic modified EGFR peptide had increased antitumor lytic activity when combined with anti-EGFR antibodies (cetuximab) [27]. Current trends clearly involve the use of such antibody-mediated treatment strategies in combination with other immune or nonimmune therapies so as to enhance clinical effectiveness, as monotherapies such as antibody-targeting of EGFR could have lower than expected effectiveness in part owing to shown mutations in the target, EGFR [33]. Unfortunately, compared with other studies with colorectal or lung cancers, such combined approaches for the treatment of HNSCC patients have been understudied.

Table 3 Antibody therapies

Antibody therapies alone
Anti-EGFR (cetuximab) [25]
Anti-EGFR (nimotuzumab) [24]
Antibody with combination therapies
Cetuximab plus radiotherapy [84]
Cetuximab, cisplatin, and radiotherapy [85]
Cetuximab, cisplatin, and 5-FU [32]

5-FU, 5-fluorouracil; EGFR, epidermal growth factor receptor.

Cellular immune stimulatory approaches

In addition to administering antibodies that target tumor antigens, combination treatments involving stimulation of cellular immune reactivity have been used to stimulate immune reactivity in cancer patients. In a small study, patients with recurrent HNSCC received adoptive therapy with autologous peripheral blood mononuclear leukocytes that had been opsonized during culture with catumaxomab, an antibody that binds with one arm to epithelial cell adhesion molecule on tumors and with the other arm to $CD3^+$ T cells [34]. Such an approach showed significant toxicity at high cell dose numbers but good tolerability and some clinical responses when lower numbers of $CD3^+$ cells were administered. In a separate trial, patients with unresectable HNSCC were vaccinated with irradiated autologous tumor plus GM-CSF and then received adoptive transfer of their in-vitro-expanded lymph node cells consisting of both $CD4^+$ and $CD8^+$ cells [35]. This combined active and adoptive immunization scheme resulted in limited toxicity and some degree of clinical response in 5 out of 17 patients. A different approach to stimulate immune reactivity against HNSCC was to vaccinate patients after surgical treatment with autologous tumor cells that were antigenically modified by infection with Newcastle disease virus [36]. This trial comparing preconditioning treatment with IL-2 alone with vaccination with virus-modified autologous tumor plus IL-2 showed that vaccination increases levels of tumor-reactive T-cells, increases anti-tumor delayed-type hypersensitive responses, and prolongs long-term survival that was associated with the increased immune reactivity (Table 4).

Table 4 Cellular immunotherapies

Adoptive T-cell immunotherapy
Anti-EpCAM (catumaxomab)-coated PBML [34]
Active vaccination therapy
IL-2 followed by virus-modified HNSCC [36]
Hsp65-DNA [43]
Combinations of active and passive immunotherapy
Irradiated autologous tumor + GM-CSF followed by adoptive transfer of in-vitro expanded T cells [35]

EpCAM, epithelial cell adhesion molecule; GM-CSF, granulocyte macrophage colony-stimulating factor; HNSCC, head and neck squamous cell carcinomas; IL, interleukin; PBML, peripheral blood mononuclear leukocytes.

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Human papilloma virus (HPV), which is associated with HNSCC, is also a target of immune reactivity. The recent US Food and Drug Administration approval of an HPV prophylactic vaccine for young women aims at preventing HPV-associated diseases such as genital warts and cervical cancer [37]. Whether this vaccination effort results in a concurrent reduction in the incidence of HNSCC will be determined with time. In a preclinical murine squamous cancer model aiming to define the immune requirements for the treatment of established HNSCC, reactivity that can result in tumor clearance required both $CD4^+$ and $CD8^+$ responses toward HPV^+ tumors [38]. In addition, the possibility of vaccination for men so as to prevent cancer is currently under consideration. A recent survey showed that to be effective, such vaccination efforts would need to introduce more awareness about the vaccine, and show safety and efficacy in trials involving males [39].

Although there have been advancements in cellular immune therapies for the treatment of HNSCC, these strategies have been more extensively investigated for the treatment of other solid cancers. Thymosin α1 has been tested in melanoma patients as an approach to stimulate T-cell reactivity to tumor [40]. Monocyte-derived dendritic cells pulse with tumor lysates have been tested in patients with malignancies such as renal cell carcinoma as a means to stimulate T-cell activity [41]. Recently, the Food and Drug Administration approved the administration of a vaccine composed of monocyte-derived dendritic cells pulsed with a recombinant fusion protein consisting of prostatic acid phosphatase, a prostate cancer tumor antigen, and GM-CSF for the treatment of castration-refractory metastatic prostate cancer, as its administration resulted in an increase in the median survival by 4.1 months [42]. This groundbreaking advancement inspires renewed confidence in the field of cancer immunotherapy and should serve to encourage amplified research into expanded applications of this treatment strategy, such as in the setting of HNSCC.

Perplexities and discrepancies

Although not easy to explain, some clinical trials have shown discrepancies between immunological and clinical responses to immunological treatment approaches. For example, treatment of HNSCC patients with the anti-EGFR antibody, nimotuzumab, resulted in clinical responses in most patients without having a detectable stimulation of lymphocyte infiltration [24]. In addition, vaccination of HNSCC patients with the DNA encoding the heat shock protein, Hsp65, resulted in stabilization of disease despite the absence of an antibody response or either a T-cell proliferative or IFN-γ response to Hsp65 [43]. It is possible that a broader evaluation of immune parameters within the tumor mass and within regional lymph nodes could uncover the responses that are key to clinical effectiveness as clinical responsiveness is likely to be because of a multitude of immune responses, of which only some are measured in individual studies.

Head and neck squamous cell carcinoma-induced defects in immune function

Head and neck squamous cell carcinoma-derived soluble immune inhibitory mediators

Although immune therapies targeting tumor-associated antigens such as EGFR, HPV, or Hsp65 have been used in HNSCC patients, responsiveness has typically been somewhat disappointing compared with results of the preclinical studies that formed the foundation for the clinical trials. Some of the marginal responsiveness to these immune stimulatory therapies is likely to be because of the profound immune suppression that is characteristic for HNSCC patients. In fact, a prospective study analyzing risk factors for HNSCC patient survival showed that among the top four risk factors was the extent of immune suppression of the HNSCC patients [44]. The mechanisms of immune suppression are multifocal. HNSCC produces several immune inhibitory mediators including prostaglandin E_2 (PGE_2), transforming growth factor-β (TGF-β), VEGF, IL-6, and IL-8 [45–47]. These mediators have been known long to be inhibitory toward T-cell functions [48–50]. The release of TGF-β and PGE_2 from HNSCC has been shown to correlate with reduced intratumoral levels of T cells, in particular $CD8^+$ T cells [51]. More recently, dendritic cells of HNSCC patients have been shown to be defective in maturation and functionally impaired [52,53]. This impairment is attributed in part to the production of VEGF, PGE_2, and TGF-β by HNSCC. These mediators induce a shift in dendritic cell cytokine production to contribute to a more tolerogenic phenotype. They also alter the expression of the chemokine receptors that allow dendritic cells to migrate to tumor-draining lymph nodes. In these studies, the dendritic cell dysfunction induced by HNSCC was overcome by blocking PGE_2 and TGF-β. HNSCC production of other immune suppressive cytokines such as IL-6 and IL-8 has been shown to be reduced with curcumin [47]. Both curcumin and (–)-epigallocatechin-3-gallate have been shown to inhibit the expression of HNSCC-derived indoleamine 2,3-dioxygenase, an immunosuppressive enzyme that suppresses T-cell and dendritic-cell responses [54,55]. Thus, there are pharmacological means, such as with the use of cyclooxygenase inhibitors, TGF-β antagonists, curcumin and (–)-epigallocatechin-3-gallate, by which it is possible to diminish the levels or activity of immune suppressive mediators produced by HNSCC so as to enhance the effectiveness of immune stimulatory treatment, such as through dendritic cell-based tumor vaccines.

Head and neck squamous cell carcinoma induction of immune suppressive cells

In addition to producing soluble mediators that inhibit immune reactivity of HNSCC patients, HNSCC also induces immune inhibitory cells. The levels of Treg cells

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within HNSCC patients are greater than the levels in healthy controls [56,57]. A lower ratio of $CD8^+$ T cells to Tregs is associated with decreased survival [58]. In addition to secreting the immunosuppressive cytokines TGF-β and IL-10, Treg cells hydrolyze ATP to a greater extent and, in turn, cause increased levels of adenosine-mediated suppression of effector T cells. Treg homeostasis and immunosuppressive capacity depends on the inhibitory protein cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) [59–61]. Therefore, the blockade of CTLA-4 may at least partially ameliorate the immune suppression associated with HNSCC. As such, one approach that needs to be further expanded to treatment of HNSCC patients involves the use of ipilimumab and tremelimumab, both of which target CTLA-4 to potentiate antitumor T-cell responses. Ipilimumab and tremelimumab have been used successfully in treatment of malignancies such as metastatic melanoma, prostate, and bladder cancers (Table 5) [62–65].

Another immune suppressive cell that our laboratory has shown to be in increased levels in the peripheral blood of HNSCC patients is the immature $CD34^+$ progenitor cell. These cells are mobilized from the bone marrow by tumor-derived GM-CSF and are chemoattracted into the tumor by VEGF. They mediate their immune inhibitory activity by the production of TGF-β [66–69]. Depending on the cytokine milieu, the tumor-mobilized $CD34^+$ cells can differentiate into granulocytes, monocytes, or dendritic cells [66,69,70]. Studies carried out in animal tumor models have shown that these cells can also differentiate into endothelial cells [71]. To alleviate the level of immune suppression in HNSCC patients, our studies used the strategy of taking advantage of the immature status of these $CD34^+$ cells and driving the differentiation of these inhibitory cells into stimulatory dendritic cells. This was accomplished by the treatment of HNSCC patients with the active hormone 1,25-dihydroxyvitamin D_3 [72,73]. Such a strategy not only resulted in a decrease in levels of intratumoral $CD34^+$ suppressor cells and an increase in numbers of mature dendritic cells and T cells expressing markers of activation within the HNSCC, but it also significantly prolonged the time between surgical treatment and cancer recurrence. Others have shown that HNSCC $CD34^+$ progenitor cells are induced to produce increased levels of the immune inhibitory mediator, IL-6 [74]. These $CD34^+$ cells are likely to be an earlier precursor within a spectrum of immune inhibitory cells in various stages of differentiation that include myeloid-derived suppressor cells. Myeloid-derived suppressor cells that are found in a multitude of tumor types including HNSCC, mediate their immune suppressive activity through reactive oxygen species [75]. Their suppressive activity can be blocked by blocking nicotinamide adenine dinucleotide phosphate oxidase. A newly recognized tumor-induced immune suppressive population that has been described for HNSCC patients is the immune inhibitory endothelial cell [76]. The suppressive activity of endothelial cells is induced by tumor-derived VEGF. These HNSCC-induced immune inhibitory endothelial cells mediate their immune suppressive activity through production of PGE_2 which, in turn, blocks T-cell proliferation, production of IFN-γ, perforin and granzyme B, suggesting inhibition of both T-cell helper and cytolytic functions. The blockade of VEGF through the use of bevacizumab could potentially prevent the induction of both $CD34^+$ progenitor cell and suppressive endothelial cell populations. Further investigation into the mechanisms by which HNSCC induces immune suppressive cells and strategies to block or reverse the consequent immune suppression may greatly assist the efficacy of attempts to stimulate immune rejection of HNSCC.

Table 5 Overcoming immune suppression

1,25-dihydroxyvitamin D_3 [72,73]
Cyclophosphamide, indomethacin plus cytokines [77,78]

Combining treatment to alleviate tumor-induced immune suppression with immune stimulatory approaches

Rather than attempting to use immune stimulatory strategies in an immune inhibitory environment, there has been a gradual realization that effectiveness of immunotherapy could be stimulated by alleviating the inhibitory environment that is established by the tumor. Although very few in number, there have been efforts to block immune inhibition while stimulating immune reactivity. For example, earlier, one study with untreated HNSCC patients tested peritumoral and perilymphatic administration of a commercial preparation of natural cytokines (multikine) containing IL-2, IL-1, GM–CSF, IFN-α, TNF-α, TNF-β, IL-3, IL-4, IL-6, IL-8, IL-10, macrophage inflammatory protein, and granulocyte colony-stimulating factor [77]. In addition, the treatment regimen included zinc to enhance immune reactivity plus strategies to diminish immune suppressor cells by using cyclophosphamide to block T-suppressor activity and indomethacin to block prostaglandin production [77]. These studies, which were modeled after earlier studies conducted by Hadden *et al.* [78], showed minimal toxicity, activation of T cells, and various degrees of clinical responses. With the plethora of immunotherapeutic strategies in development to either reverse immune inhibition or stimulate immunity, further research into combinations of these strategies may lead to successful therapeutic strategies that effectively develop antitumor immune responses, leading to significant clinical benefit (Table 5).

Concluding remarks: the future for immunotherapeutic approaches for treatment of HNSCC patients

There is the realization that novel treatment approaches for HNSCC patients are essential because of the minimal

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levels of improvement in patient survival over the last few decades. Immunotherapy could be a strong candidate for one such approach. However, clinical studies testing immunotherapeutic approaches for the treatment of HNSCC have lagged significantly compared with trials using immunotherapeutic strategies for the treatment of other solid cancers such as breast, lung, and colorectal cancers. Studies with other malignancies have shown the advantages of combining immunotherapeutic strategies with either chemo or radiation therapy, or using combinations of immunotherapeutic approaches. However, a great deficiency persists in the use of these combined therapies for the treatment of HNSCC patients. Approaches to actively stimulate immune reactivity also need to consider incorporating approaches that target the multitude of immune inhibitory mechanisms that are induced by HNSCC. Although the testing of these treatment approaches for HNSCC patients is lagging, future studies should take advantage of what has been learned from the results of immunotherapeutic treatment approaches that have been used in patients with other solid malignancies and then to apply this knowledge to optimize the treatment of HNSCC patients.

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