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Immunotherapy for head and neck cancer: advances and deficiencies

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Abstract

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. While 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 lessens 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 review 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 demonstrated as successful in the treatment of other solid cancers in order to identify potential strategies that may apply to the treatment of HNSCC.

Keywords

head and neck cancer; HNSCC; immunosuppression; immunotherapy; suppressor cells; tumor antigens

Introduction

Head and neck squamous cell carcinomas (HNSCC) are aggressive malignancies, and treatment options have traditionally involved surgery or, more recently, also 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 [2–5]. To increase the effectiveness of immunotherapeutic strategies, efforts are underway 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 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 that 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 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, as well as increased numbers of CD20⁺ B cells in involved lymph nodes, are associated with a better prognosis [8]. Also, 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. Less well studied has been the potential of γ/Δ T cells to react to tumor. Recently, a subset of γ/Δ T cells expressing the NK cell-associated molecule CD56 has been shown to exhibit cytolytic reactivity to HNSCC. While 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 treatment of HNSCC patients with IL-2 increases cytokine levels, intratumoral levels of NK cells, and the activity of tumor-infiltrating lymphocytes (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 IFN- α 2a and IL-2, 18% of patients achieved a partial response, though treatment was associated with substantial toxicity [13]. In conjunction with cisplatin and 5-FU, 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 demonstrated 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 levels of B cells 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-1 β , IL-2, IL-6, IL-8, IFN- γ , TNF- α , G-CSF, and GM-CSF, to patients with HNSCC [18]. IRX-2 treatment of PBML-derived human dendritic cells resulted in an increased expression of co-stimulatory markers as well as markers for maturation and migration [19]. While early studies with immunotherapeutic administration of cytokines to HNSCC patients have shown promise, none of these treatment strategies has as yet been established in the clinic.

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 altering the IL-2 molecule to produce lower toxicity [20]. Interferon- α has been used with success 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 stage III and IV melanoma after surgical resection has been shown to prolong median survival by greater 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 in order to expand them to HNSCC patients.

Antibody treatment approaches

Research into individual cytokine treatments have to a large extent fallen out of favor in recent years, possibly due 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 epidermal growth factor receptor (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 anti-tumor effects have been observed in the absence of side 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 non-overlapping epitopes. Researchers are also attempting to develop monoclonal antibodies to target other HNSCC-associated antigens. A Phase I trial involving treatment of medullary thyroid cancer patients with a humanized antibody targeting CEA, a TAA present on most cases of HNSCC, showed evidence of antitumor

activity with minimal toxicity [28]. *Ex-vivo* treatment of HNSCC tumor specimens with an antibody targeting EMMPRIN (extracellular matrix metalloproteinase inducer) resulted in a significant reduction in tumor ATP levels (58%) compared to reduction in ATP levels of tumors treated with cetuximab (33%) [29].

Studies are also in development to determine if combinations of antibodies targeting EGFR and either chemotherapy or other forms of immunotherapy could result in increased anti-tumor effectiveness. 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 VEGF, respectively (cetuximab and bevacizumab), together with the topoisomerase 1 inhibitor irinotecan resulted in improved clinical responses as compared to 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 to 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 anti-tumor lytic activity when combined with anti-EGFR antibodies (cetuximab) [27]. Current trends clearly involve the use of such antibody-mediated treatment strategies in combinations with other immune or non-immune therapies so as to enhance clinical effectiveness, as monotherapies such as antibody-targeting of EGFR could have lower than expected effectiveness in part due to demonstrated mutations in the target, EGFR [33]. Unfortunately, compared to other studies with colorectal or lung cancers, such combined approaches for the treatment of HNSCC patients have been understudied.

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 epithelial cell adhesion molecule (EpCAM) on tumors and with the other arm 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 of 17 patients. A different approach to stimulate immune reactivity against HNSCC was to vaccinate patients following 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 to 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.

Human papilloma virus, which is associated with HNSCC, is also a target of immune reactivity. The recent FDA approval of an HPV prophylactic vaccine for young females 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 pre-clinical murine squamous cancer model aiming to

define the immune requirements for treatment of established HNSCC, reactivity that can result in tumor clearance required both CD4⁺ and CD8⁺ responses toward HPV⁺ tumors [38]. Also currently under consideration is the possibility of vaccination of men so as to prevent cancer. A recent survey showed that to be effective, such vaccination efforts would need to introduce more education about the vaccine, and demonstration of safety and efficacy in trials involving males [39].

While 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 $\alpha 1$ 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 FDA approved administration of a vaccine composed of monocyte-derived dendritic cells pulsed with a recombinant fusion protein consisting of prostatic acid phosphatase (PAP), a prostate cancer tumor antigen, and GM-CSF for the treatment of castration-refractory metastatic prostate cancer, as its administration resulted in a lengthening in 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]. Also, vaccination of HNSCC patients with DNA encoding the heat shock protein hsp65 resulted in some 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 as well as within regional lymph nodes could uncover the responses that are key to clinical effectiveness since clinical responsiveness is likely to be due to a multitude of immune responses, of which only some are measured in individual studies.

HNSCC-induced defects in immune function

HNSCC-derived soluble immune inhibitory mediators

While 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 to results of the pre-clinical studies that formed the foundation for the clinical trials. Some of the marginal responsiveness to these immune stimulatory therapies is likely to be due to 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 4 risk factors was the extent of immune suppression of the HNSCC patients [44]. The mechanisms of immune suppression are multifocal. HNSCC produces several immuneinhibitory mediators including prostaglandin E₂ (PGE₂), transforming growth factor- β (TGF- β), vascular endothelial cell growth factor (VEGF), IL-6, and IL-8 [45–47]. These mediators have long been known to be inhibitory toward T-cell functions [48–50]. The release of TGF- β and PGE₂ 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 by

HNSCC of VEGF, PGE₂ and TGF- β . These mediators induce a shift in dendritic cell cytokine production to contribute to a more tolerogenic phenotype. They also alter expression of the chemokine receptors that are important in allowing dendritic cell migration to tumor-draining lymph nodes. In these studies, the dendritic cell dysfunction induced by HNSCC was overcome by blocking PGE₂ 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 (EGCG) have been shown to inhibit the expression of HNSCC-derived indoleamine 2,3-dioxygenase (IDO), 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 EGCG, 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.

HNSCC 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 within HNSCC patients is greater than 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, 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 [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 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 in animal tumor models have shown that these cells can also differentiate into endothelial cells [71]. In order 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 treatment of HNSCC patients with the active hormone 1,25-dihydroxyvitamin D₃ [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 (MDSC). MDSC, which are found in a multitude of tumor types including HNSCC, mediate their immune suppressive activity via reactive oxygen species [75]. Their suppressive activity can be blocked by blocking NADPH 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 PGE₂ 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. 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.

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. While very few in number, there have been some efforts to block immune inhibition while stimulating immune reactivity. For example, one study with previously untreated HNSCC patients tested peritumoral and perilymphatic administration of a commercial preparation of natural cytokines (multikine) containing IL-2, IL-1, GM-CSF, interferon- α , TNF- α , TNF- β , IL-3, IL-4, IL-6, IL-8, IL-10, macrophage inflammatory protein and G-CSF [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.

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 due to the minimal 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 treatment of HNSCC have lagged significantly compared to trials using immunotherapeutic strategies for treatment of other solid cancers such as breast, lung and colorectal cancers. Studies with other malignancies have demonstrated the advantages of combining immunotherapeutic strategies with either chemo or radiation therapies, or using combinations of immunotherapeutic approaches. However, a great deficiency persists in use of these combined therapies for 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. While 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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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]

Table 2

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]

Table 3

Cytokine therapies

IL-2 [10,11]
IFN- γ [15]
IL-12 [16,17]

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 <i>in vitro</i> -expanded T-cells [35]

Table 5

Overcoming immune suppression

1,25-dihydroxyvitamin D ₃ [72,73] cyclophosphamide, indomethacin plus cytokines [77,78]