Immunotherapy for head and neck cancer patients: shifting the balance

Annelies W Turksma
Boudewijn JM Braakhuis
Elisabeth Bloemena
Chris JLM Meijer
C René Leemans
Erik Hooijberg

Immunotherapy (2013) 5(1), 49-61
Abstract

Head and neck squamous cell carcinoma is the sixth most common cancer in the western world. Over the last few decades little improvement has been made to increase the relatively low 5-year survival rate. This calls for novel and improved therapies. Here, we describe opportunities in immunotherapy for head and neck cancer patients and hurdles yet to be overcome. Viruses are involved in a subset of head and neck squamous cell carcinoma cases. The incidence of HPV-related head and neck cancer is increasing and is a distinctly different disease from other head and neck carcinomas. Virus-induced tumors express viral antigens that are good targets for immunotherapeutic treatment options. The type of immunotherapeutic treatment, either active or passive, should be selected depending on the HPV status of the tumor and the immune status of the patient.
Head & neck squamous cell carcinoma

Incidence & tumor site
The majority of malignancies arising in the head and neck region are squamous cell carcinomas. Head and neck squamous cell carcinoma (HNSCC) is the sixth most common cancer in the world [1,2]. HNSCCs arise from the mucosal linings of the oral cavity, pharynx and larynx. The incidence of HNSCC is not evenly distributed over the world; the highest incidence is in the western world. Approximately 60% of patients are male, although for the past few decades the percentage of female patients has been increasing [3]. The overall incidence in The Netherlands has been steadily increasing, especially with oropharyngeal cancer for males and females, and oral cavity cancer for females [4]. Two-thirds of patients present with advanced carcinoma.

Risk factors
Until recently the two main risk factors for the development of HNSCC were tobacco use and excessive alcohol consumption [5]. A third and increasingly important risk factor is a persistent infection with HPV [6–8]. In the USA, the proportion of HPV-positive oropharyngeal tumors is estimated to be 47%, while this is approximately 30% in The Netherlands [9].

Treatment
Early-stage, relatively small tumors are treated by surgery or radiotherapy. Advanced tumors are treated by surgery followed by postoperative chemo-/radio-therapy or definitive chemo-/radio-radiation with surgical salvage [6,10]. In the last few decades, advances have been made in surgical and radiotherapeutic techniques that have increased the quality of life of HNSCC patients [6].

Prognosis
Prognosis of HNSCC patients is largely dependent on tumor stage, HPV status and tobacco use [6]. Tumor stage is defined according to the TNM system, which is based on the extent of the tumor, spread to the lymph nodes and presence of distant metastasis. Patients with early-stage disease have a relatively good prognosis. Patients with HPV-positive tumors generally have a better prognosis than those with HPV-negative tumors [11–15]. There are many types of HPV strains of which over 15 types are classified as high-risk types. High-risk HPV is more likely to develop into cancer. HPV type 16 is present in 90–95% of HPV-positive HNSCC tumors [9].

The 5-year survival rate for advanced HNSCC patients is relatively low at approximately 40–50%. In the past few decades there has been little improvement in this survival rate making the development of novel therapy modalities of crucial importance. Immunotherapy following standard treatment might be an attractive adjuvant treatment modality.

Here, we review immunotherapeutic approaches that have been applied for the treatment of HNSCC and suggest a number of potential improvements.
**Molecular classification**

**HPV-negative HNSCC tumors**

For the molecular classification of HNSCC tumors a distinction should be made between HPV-positive and -negative tumors. HPV-negative HNSCC tumors form a heterogenic group [6] with disruptions in several signaling pathways. In many of these tumors the p53 and retinoblastoma (RB) pathways are disrupted, leading to limitless uncontrolled replication [16–18]. Changes have also been found in growth factor signaling; for example through the EGFR receptor (EGFR) and TGF-β pathways. In HNSCC the disruption in the EGFR pathway is often due to receptor overexpression or mutations [19]. TGF-β is an inhibitory regulator whose receptor can be downregulated in HNSCC tumors [20]. Mutations in SMAD-2, -3 and -4, which are downstream of the TGF-β receptor pathway, also play a role in the mutagenesis in HNSCC tumors [6]. The PI3K–Akt pathway is another pathway that is important in tumor cell growth and survival [21]. In HNSCC, mutations in PI3KCA and PTEN have been found. This can lead to the activation of protein kinase B and subsequently to apoptosis resistance [6].

Gene-expression profiling resulted in the identification of four different subgroups of HNSCC patients [22]. One of these subgroups had a relatively poor prognosis compared with the other three and was characterized by high-level expression of TGF-α, bisphenol-A, P-cadherin, laminin γ2 and collagen XVII-α. Expression of these genes has also been correlated with poor prognosis in other cancers [22].

**HPV-positive tumors have a unique molecular profile**

At the molecular level HPV-positive tumors are different from HPV-negative tumors. A very important difference is seen in the frequencies of TP53 mutations [23,24]. Mutations in TP53 are rarely seen in HPV-positive tumors, whereas these are often found in HPV-negative tumors. In HPV-positive tumors the viral oncoproteins E6 and E7 are responsible for the induction and maintenance of cell cycle disruption. E6 binds to p53, resulting in ubiquitination and proteasome degradation of p53, thereby losing a cell cycle checkpoint. On the other hand, E7 destabilizes the RB protein that normally acts as an inhibitor of cell cycle transcription factors [25,26]. Loss of function of RB contributes to cell cycle progression [27].

**Immune system: innate & adaptive**

The immune system, consisting of two distinct parts, protects us from pathogens such as bacteria and viruses, and also plays a role in immune surveillance of aberrant cells and arising tumors. The innate immune system is responsible for the first line of defense. It clears pathogens in a nonspecific way; for example, through the action of NK cells, macrophages and the complement system. The innate immune system also aids in the activation of the adaptive immune system, which targets pathogens and tumor cells in a very specific way. The adaptive immune system makes use of antigen-specific T cells and antibodies, produced by B cells. Cell surface-expressed proteins form good targets for antibodies. Ex vivo-produced antibodies specific for proteins expressed on tumor cells are in use in immunotherapeutic strategies.
Processed fragments of intracellular proteins are presented on MHC molecules to T cells by APCs such as dendritic cells (DCs). This subsequently leads to T cell proliferation and elimination of pathogens or tumors [28, 29]. MHC class I is present on all nucleated cells in the body, whereas MHC class II is present on immune cells such as DC, B cells and T cells. In humans, the MHC complexes are called HLA.

T cells can be roughly divided into CD8+ cytotoxic T cells (CTL) and CD4+ helper T cells. Both are necessary for an effective antitumor immunity. CTL kill virus-infected cells and tumor cells, in a granzyme/perforin- dependent way and produce IFN-γ [30]. T helper cells activate CTL and recruit cells from the innate immune system, such as macrophages and mast cells [31]. Naive CD4+ T cells can differentiate into several subtypes: Th1, Th2, Th17 and Tregs; Th1 CD4+ T cells produce IL-2 and IFN-γ, Th2 CD4+ T cells mainly produce IL-4 and IL-5, Th17 CD4+ T cells mainly produce IL-17, and Tregs mainly produce IL-10 and TGF-β. IL-10 can inhibit the production of IFN-γ and IL-2, which are proinflammatory cytokines produced by Th1 cells. IL-10 can also inhibit antigen presentation capabilities of APCs. TGF-β can cause immune suppression and angiogenesis, and has been found to suppress T cell proliferation [30]. These cytokines influence T cell behavior and thereby antitumor immunity. IL-2 produced by Th1 cells can stimulate T cell proliferation and activation.

**Tumor-associated antigens**

Tumor-associated antigens (TAAs) can be derived from proteins utilized to promote transformation and tumor genesis to ascertain a malignant phenotype. Multiple types of TAA can be present in tumor cells; for example, overexpressed, neo, mutated self or viral antigens. Tumors express a number of proteins important for survival or replication of cells. Most of these antigens are hardly expressed in nondondiving normal tissue, although expression can be found in normal cells with proliferative capacity such as B and T cells, and stem cells (examples of such antigens are survivin [32] and telomerase [33]). Examples of aberrantly expressed proteins are the so-called cancer testis antigens, such as melanoma-associated antigen and B melanoma antigen. Proteins resulting from mutations in the tumor suppressor gene TP53 form antigens that can be recognized by T cells [34]. Tumors can arise under the influence of viral infections. A number of viruses are known to induce tumor growth; examples are HPV, Epstein–Barr virus (EBV), Human T-lymphotropic virus-1 and HBV [35]. HPV and EBV are involved in tumor genesis of a number of head and neck cancers. The HPV-derived oncoproteins E6 and E7, and EBV-derived oncoproteins LMP and EBV nuclear antigen [36,37] are considered ideal candidates for immunotherapy since they are nonself antigens, and essential for tumor genesis.

**HNSCC & immune evasion**

The immune system is capable of recognition and eradication of aberrant cells. However, once a malignancy has emerged, the tumor microenvironment is often immunosuppressive. Evasion mechanisms employed by the tumor will emerge sooner or later, thus escaping from immune surveillance [38,39]. This surveillance is mainly based on the capability of T cells to eradicate aberrant cells, since it is unclear whether naturally occurring antibodies
play a role. Immunotherapy is designed to aid the immune system in tipping the balance towards tumor elimination.

**Tumor-infiltrating lymphocytes & Tregs**

High numbers of tumor-infiltrating lymphocytes (TIL) have been identified as a good prognostic factor for HNSCC patients [40]. In order for lymphocytes to reach the tumor beds, extravasation from the blood vessel is needed. To do so, T cells adhere to the vessel wall via endothelial adhesion molecules, such as intercellular adhesion molecules. Tumors generate new blood vessels in order to grow in a process called angiogenesis. However, newly formed blood vessels in tumors are exposed to angiogenic factors that downregulate adhesion molecules [41]. This makes it more difficult for lymphocytes to reach the tumor beds.

Not all types of lymphocytes are associated with a good prognosis. The presence of high percentages of Tregs in and around the tumor has been associated with a poor prognosis [42]. Tregs can accomplish suppressive functions by producing cytokines such as IL-10 and TGF-β, inducing indoleamine 2, 3-dioxygenase production in DCs via CTLA-4 or inducing apoptosis in T cells directly via perforin [43]. In HNSCC patients the percentage of Tregs in the peripheral blood was found to be higher than in healthy controls [44]. The percentage of Tregs is also higher in and around the tumor than in the peripheral blood of these patients and is IL-10 and TGF-β mediated [45].

**Tumor evasion of apoptosis**

Resistance to apoptosis is a way by which tumor cells may escape immune surveillance. T cells can induce apoptosis by binding to Fas present on tumor cells. Downregulation of Fas has been found in a small percentage of HNSCC cell lines, but a correlation with survival has not been found [46–48]. This may be explained by the overexpression of proteins that inhibit apoptosis, such as cFLIP [49,50] and survivin [51]. Overexpression of cFLIP correlates with clinical stage and may be an important prognostic factor, and possibly also a target in anticancer therapies [49]. Conversely, expression of Fas ligand by aberrant tumor cells may induce apoptosis of T cells.

**Tumor-expressed galectins regulate T cell survival**

Galectins are part of the lectin family, which can bind to β-galactoside with their carbohydrate recognition domain. Several galactins have been associated with HNSCC; in particular galectin-1 overexpression is associated with metastatic disease [52]. Galectin-1 is an important regulator of immune response as it plays a role in T cell homeostasis and survival [52]. By blocking T cell activation, galectin-1 can inhibit Th1-type responses against the tumor [53]. Inhibiting galectin-1 would therefore be an attractive way to increase antitumor responses.
Tumor-expressed nuclear factors block apoptosis & cytokine production

STAT3 and NF-κB proteins play a role in transmission of external signals to the nucleus of the cell. Many signals from cytokines and growth factors are transferred via members of the STAT-family proteins. STAT3 expression is increased in HNSCC patients and signaling via STAT3 is required for tumor growth [54]. There are indications that STAT3 overexpression is important in the early phases of tumor formation [55]. STAT3 protein levels are not only elevated in tumor cells but also in the non-neoplastic mucosa of HNSCC patients compared with healthy donors [55]. Furthermore, it has been shown that protein levels of STAT3 are high in over 70% of early stage, but only in approximately half of late-stage tumors [56]. STAT3 activation in HNSCC patients is regulated by TGF-α binding to the EGFR and IL-6 binding to the IL-6 receptor, as well as by binding of nicotine, found in tobacco smoke, to nicotinic receptors [57]. After activation, STAT3 promotes the transcription of the antiapoptotic genes Bcl-X, [58] and survivin [59], while inhibiting IL-6, IL-8 and TNF-α transcription [60]. By inhibiting proinflammatory cytokines and promoting antiapoptotic proteins, STAT3 contributes to immune escape of the tumor cells. STAT3 can also be activated by the transcription factor NF-κB [57]. NF-κB activity in HNSCC is associated with decreased tumor cell apoptosis and increased survival and proliferation [61]. NF-κB can be activated by several stimuli, including nicotine in tobacco smoke and Toll-like receptor-activation. NF-κB positively regulates the expression of several proinflammatory cytokines, including IL-6 and IL-8, which contribute to inflammation [62]. Increased IL-6 secretion also facilitates NF-κB-induced STAT3 activation [63].

Tumor-expressed COX-2 & PGE2 leads to immune suppression

The COX enzymes catalyze the conversion of arachidonic acid to prostaglandins. COX-2 is often overexpressed in HNSCC and is related to imbalance and suppression of the immune system [64]. COX-2 plays an important role in the synthesis of PGE2 from membrane phospholipids [65], which is overexpressed in cancer cells. For head and neck cancer it has been shown that overexpression of PGE2 correlates with a poor prognosis [66]. PGE2 overexpression in tumor cells can protect the cells from apoptosis and stimulate formation of metastases and angiogenesis [67]. Furthermore, it has immune-modulating effects; PGE2 is known to enhance Th2-type and inhibit Th1-type responses. Th1-type responses are needed for an efficient antitumor response [67]. Furthermore, PGE2 secreted by tumor cells can induce apoptosis in CD4+ T cells [68]. Increased NF-κB activity results in upregulation of COX-2, which has immunosuppressive effects [69].

Dysfunctional T cells

Tumor-infiltrating T cells in HNSCC patients are less functional than T cells from healthy donors [70]. Production of cytokines such as IL-2 and IFN-γ by T cells from HNSCC patients is often compromised [71]. When the T cell receptor recognizes a peptide presented in the MHC complex, a signal will be transmitted via the ζ-chain into the cell, leading to the production of cytokines such as IFN-γ. In HNSCC patients it has been shown that the ζ-chain in T cells is downregulated, which correlates with poor prognosis [72,73].
CHAPTER 5

HLA downregulation

A method of immune evasion employed by tumor cells is via downregulation of MHC molecules, thus preventing recognition by T cells. In HNSCC, HLA class I downregulation has been found in approximately 50% of tumor samples investigated and was identified as an important prognostic factor [74–76].

Shifting the balance

Tumors have many ways of subverting the immune system. Various different immunotherapeutic strategies have been designed to shift the balance back towards tumor eradication. Below, several immunotherapeutic strategies will be discussed that have been investigated in HNSCC patients, such as skewing the immune system by using cytokines or monoclonal antibody approaches. Several cell-based therapies also show potential as adjuvant treatment for HNSCC patients. In passive immunotherapy the patient receives therapeutic antibodies or adoptive cell transfer (ACT), thereby circumventing the need to activate the patient’s immune system. Active immunotherapy, such as the administration of cytokines or DC-based vaccination, is aimed at activating the patient’s own immune system to fight the tumor.

Passive immunotherapy

Antibody approaches

At present, antibody treatment is the most widely used form of immunotherapy in cancer patients. For HNSCC patients, the present focus is on antibodies targeting the EGFR, since in 90% of HNSCCs the EGFR is overexpressed [77,78]. Triggering of the EGFR by EGF activates pathways leading to proliferation, survival and metastasis of HNSCC [79]. EGFR monoclonal antibodies competitively bind to the receptor, downregulating it, thereby inhibiting tumor cell growth and increasing apoptosis [78]. Another mechanism by which these antibodies exert their action is by inducing antibody-dependent cell-mediated cytotoxicity via the recruitment of NK cells and macrophages.

Several clinical trials using anti-EGFR have been carried out in HNSCC patients [80–83]. Cetuximab and panitumumab are clinically approved anti-EGFR antibodies used to treat HNSCC patients; their working mechanisms are similar. Cetuximab is a human–mouse chimeric monoclonal antibody, whereas panitumumab does not contain murine components. Binding of these monoclonal antibodies results in a downregulation of EGFR expression [84]. In a randomized study with 424 patients, cetuximab in combination with radiotherapy was shown to increase overall survival of patients with locoregionally advanced HNSCC. Patients receiving radiotherapy alone had an overall survival of 29 months, whereas with patients receiving radiotherapy in combination with cetuximab it was 49 months [85].

Instead of directly targeting tumor cells, the vascular system of the tumor can also be targeted, resulting in antitumor effects. VEGF binds to the receptors present on vascular endothelial cells, thus inducing angiogenesis. Angiogenesis is of vital importance to the tumor and is, therefore, an interesting target. Bevacizumab is a humanized VEGF antibody.
Immunotherapy for head and neck cancer patients: shifting the balance

By blocking the binding of VEGF to the VEGF receptor, the antibody inhibits angiogenesis and decreases microvascular permeability. The latter has been theorized to increase the effectiveness of chemotherapeutics [86]. Bevacizumab is being tested in HNSCC patients in combination with chemotherapy [87,88].

**Adoptive cell transfer**

ACT is a form of passive immunotherapy, whereby large numbers of T cells are transferred into the patient. ACT has been studied extensively in preclinical mouse models and human clinical trials in, for example, metastatic melanoma [89]. The leukocytes used for ACT can either be derived from TIL or from the peripheral blood. Lymphokine-activated killer cells can be generated from the peripheral blood by stimulation with IL-2 and lectins. Lymphokine-activated killer cells can kill tumor cells in an unspecific manner, not targeted to a specific antigen presented on the surface of tumor cells [90]. To the present author’s knowledge, this approach has not been successfully pursued in HNSCC cancer patients. To generate sufficient numbers of effector T cells for ACT, extensive ex vivo expansion of T cells is required.

In order to attract sufficient numbers of effector cells to the tumor site, a monoclonal antibody can be used. The monoclonal antibody catumaxomab is directed against EpCAM, which is overexpressed on most HNSCC tumors. Catumaxomab binds to the Fc-γ receptors CD16 (present on NK cells and T cells) and CD64 (present on macrophages). In this way, effector cells are attracted to the tumor cells. The anti-epithelial cell adhesion molecule-coated peripheral blood mononuclear cell (PBMC) upregulated CD25, CD69 and CD83, suggestive of increased antitumor activity [91]. In a small pilot study using catumaxomab that included four patients, one patient showed stable disease for 6 months, one patient went into complete remission and two had progressive disease [92].

Besides total PBMC fractions, specific cell types can be used to enhance the antitumor effect of the immune system. Total numbers of NKT cells, a subtype of T cells, have been shown to be related to clinical outcome of HNSCC patients [93]. Low numbers of NKT cells have been correlated with a poor clinical outcome [93,94]. Based on these observations, clinical outcome may be improved by transfer of ex vivo-expanded NKT cells. In a small trial reported by Yamasaki et al., ten HNSCC patients were injected with in vitro-expanded autologous NKT cells [95]. Five out of the ten patients showed tumor regression, a partial response. The other five patients had stable disease. In a recent trial, 17 HNSCC patients were enrolled in a study comparing APC administration via the nasal mucosa with administration via the oral mucosa [96]. An increase in activated NKT cells was detected in patients receiving α-GalCer-loaded APCs administrated via the nasal mucosa. However, patients receiving α-GalCer-loaded APCs via the oral mucosa did not show increased NKT cell levels [97], underlining the importance of the administration route. Inducing NKT cell activity in HNSCC patients seems to be an effective immunotherapy for HNSCC patients.

Targeting tumor cells specifically may be a more effective method of ACT and can be accomplished by adoptive transfer of T cells genetically engineered to express antitumor T cell receptors [98]. Such strategies may also be applicable for patients with difficult-to-resect common epithelial tumors [99]. This strategy may be especially suited to HPV-positive tumors, since it can be targeted against the viral proteins E6 and E7, which are necessary for tumor cells to maintain their malignant phenotype. Recently, a number of HPV-specific T cell receptors have been isolated and tested in vitro [99–103].
Active immunotherapy

Active immunotherapy aims to stimulate the patient’s own immune system to mount an antitumor response. Such therapies are designed to stimulate cytotoxic T cells to kill tumor cells. Indirect targeting of DCs by injection of tumor lysates, peptides, DNA or viral particles has been explored in patients with cancers other than HNSCC [104]. Administration of cytokines or vaccination with DCs have been explored in HNSCC patients [28] and will be discussed here.

DC-based vaccines

DC-based immunotherapy is a form of active immunotherapy whereby DCs loaded with TAAs are used to elicit a T cell response in vivo. The number of DCs found in peripheral blood is low. However, abundantly present monocytes can be used to generate autologous DCs in vitro, thus providing a large supply of professional APCs [105,106]. Subsequently, these monocyte-derived DCs can be loaded with TAAs and transferred to the patients to elicit a T cell response against the tumor. Alternatively, an allogenic DC line might be used for vaccination purposes [107–110].

One way of loading DCs is to pulse them with a peptide of a TAA, such as p53 [34]. Disadvantages of using a single peptide for vaccination are that it is HLA-restricted and only focused on either CD4+ or CD8+ T cells. This can be circumvented by using the patient’s own tumor cells as the TAA source.

Apoptotic tumor cells have been incubated with autologous DCs in vitro [111]. Autologous DCs can also be loaded with RNA isolated from tumor cells [112] or mRNA encoding tumor antigens in combination with cytokines [113,114]. In vitro, these techniques resulted in TAA-specific T cells capable of producing IFN-γ [34,112,115]. None of these approaches have been explored as active immunotherapy in HNSCC patients.

Cytokine approaches

Cytokines play an important role in maintaining the right balance of the immune system. In a tumor environment, the cytokine balance may be disturbed. IL-10 can work as an immune suppressor, whereas cytokines such as IL-2 and IL-12 promote T cell growth. The cytokine investigated the most for immunotherapy is IL-2; several trials have been performed for HNSCC [116–122]. Given in high doses, IL-2 can be toxic [119]. Furthermore, it is a concern that high levels of IL-2 induce Treg expansion, resulting in the suppression of surrounding lymphocytes. Lower doses of IL-2 have been used and tested in a Phase III trial [120]. In this trial, patients were either given chemotherapy alone or in combination with low-dose IL-2. However, in this trial, no differences were found between the patient group receiving chemotherapy alone compared with the group receiving chemotherapy in combination with IL-2 [120].

Instead of a single recombinant cytokine such as IL-2, a mix of cytokines named IRX-2 has been tested on HNSCC patients [123]. IRX-2 is a natural cytokine mix produced by stimulating PBMCs from healthy donors and harvesting the cytokines produced, mainly consisting of IL-1, IL-2 and IFN-γ. It was injected intra lymphatically, which was well tolerated by HNSCC patients and increased their survival [124–126]. Berinstein et al. demonstrated increased numbers of TIL in patients following IRX-2 treatment. High
lymphocyte immune infiltrate correlated with decreased tumor size and improved 5-year survival [127]. Currently, the administration of recombinant IFN-α, IL-12 and GM-CSF are also being tested for use as adjuvant treatment for HNSCC patients [128,129]. Vaccination strategies employing DCs have not been explored in HNSCC patients.

**Immunotherapy in HNSCC tumors compared with other cancer types**

Compared with some other types of cancer, immunotherapy in HNSCC patients has not been extensively investigated, even though with the current standard treatments, the 5-year survival rate remains moderate at best. The majority of immunotherapeutic strategies have been applied in melanoma patients, in part owing to the immunogenic nature and accessibility of primary tumors [130]. The status of the immune system of HNSCC patients is notoriously impaired. In the peripheral blood of HNSCC patients, tumor antigen-specific T cells have been measured and were shown to rapidly undergo apoptosis [131]. Furthermore, high percentages of Tregs have been measured in HNSCC patients [132]. This may be considered an extra hurdle to overcome in immune therapeutic strategies for these patients. An appealing potential candidate for adjuvant treatment is the monoclonal antibody ipilimumab.

The novel antibody, ipilimumab, has been tested in patients with melanoma or prostate cancer [133,134]. This monoclonal antibody binds to CTLA-4, which becomes expressed after T cell activation, functioning as a natural break to prevent autoimmunity. This antibody is not directed against the tumor cells but is aimed at enhancing the immune response against the tumor [135]. CTLA-4 can downregulate T cell activation and proliferation. By blocking this, antitumor immunity can be promoted. This strategy might be worthwhile to investigate in the treatment of HNSCC patients since it could remove the naturally occurring inhibition following activation of (tumor-specific) T cells. Recently, the results of a study in 93 melanoma patients who received treatment with autologous T cells has been published [136]. Tumor-infiltrating T cells were isolated from the surgically removed melanoma and expanded to large numbers ex vivo. Melanoma patients were treated by adoptive transfer of these autologous T cells in combination with the cytokine IL-2. Approximately one-fifth of the treated patients achieved a complete tumor regression. The success of this treatment was correlated with the number of so-called ‘young’ CD8+ T cells. This form of passive immunotherapy may also be suited for the treatment of immune-compromised HNSCC patients.

**Virus-induced HNSCC tumors & immunotherapy**

High immune infiltrate in HNSCC tumors is correlated with a relatively good prognosis [40,137–139]. In a study by Wansom et al., increased levels of TIL were correlated with a good prognosis [139]. However, TIL levels were found to not be increased in HPV-positive tumors compared with HPV-negative tumors. Others, along with the present authors, have found that patients with HPV-positive tumors have increased circulating CD8+ T cells compared with patients with HPV-negative tumors [140,141]. Furthermore, T cells specific for HPV16 have been found around the tumor and in the peripheral blood of patients with HPV16-positive head and neck tumors [142]. Increased immunogenicity of HPV-pos-
itive tumors may, in part, be explained by HPV-specific responses. Moreover, patients with HPV-positive tumors often smoke less and use alcohol less excessively compared with patients with HPV-negative tumors, which could also play an important role in their increased immunogenicity.

Since persistent infection of high-risk HPV may ultimately lead to the formation of malignant tumors, use of HPV vaccinations might be considered to prevent the development of tumors. Prophylactic vaccines have been developed and marketed in recent years for the prevention of genital warts and cancer of the cervix, penis and anus [143,144]. At present, young girls are vaccinated to prevent persistent HPV infections. In view of the increase in HPV-positive tumors in the head and neck region, it might also be worth the effort to vaccinate young boys against high-risk HPV types.

Another type of tumor in the head and neck region is nasopharyngeal carcinoma (NPC).

---

**Figure 1. An overview of possible immunotherapeutic strategies.** A distinction is made between HPV-positive and -negative tumors. Both groups can subsequently be divided into patients with a relatively good immune status and patients with a relatively poor immune status, as depicted in the top row of the figure. Underneath the four possible patient categories the advised treatment is depicted.  
ACT: Adoptive cell transfer; DC: Dendritic cell; LAK: Lymphokine-activated killer; TAA: Tumor-associated antigen; vac.: Vaccine.
Similar to HPV-positive tumors, a virus is associated with tumor genesis, in this case EBV. In the western world, the incidence of NPC is low; however, in south China and southeast Asia, the incidence is much higher. Nearly all undifferentiated NPC are associated with EBV infection [145]. EBV antigens are attractive candidates as targets for immunotherapy since they are not expressed in healthy tissue. Viral antigens recognized by T cells include EBNA1, LMP-1 and LMP-2. In a Phase I study, autologous monocyte-derived DCs were pulsed with multiple LMP-2 peptides and injected into a lymph node. In nine of the 16 patients, LMP-2-specific T cell responses were measured, resulting in two partial responses [146]. Clinical responses remained minimal, which could be due to low expression of immunogenic virus proteins in the infected cells when the EBV is in its latent state. An appealing way to enhance immunotherapy in NPC patients is to reactivate the EBV virus, thereby enhancing immune recognition. Promising results were obtained in a pilot study where three patients with end-stage NPC received virus-activating drugs, which resulted in an increase of viral DNA levels in the circulation [147]. The increase of viral DNA levels suggest the EBV is no longer latent and can therefore be more easily targeted.

**Conclusion & future perspective**

There are many types of immunotherapy, all working in different ways to shift the balance towards antitumor immunity. However, not all types are suitable for HNSCC patients. In this review we have discussed immunotherapeutic approaches that have been explored in HNSCC patients.

In Figure 1 we show an overview of potential personalized immunotherapeutic approaches, taking the HPV status of the tumor and the overall immune status of the patient into account. First, a distinction between patients with HPV-positive and -negative tumors should be made. Patients with a relatively good immune status may benefit from active immunotherapy, for example, by administration of DCs loaded with tumor antigens. Immunotherapeutic treatment of patients with HPV-positive tumors may be a sensible start, since the immune system of such patients is often less compromised. Importantly, HPV-positive tumor cells can be very specifically targeted, without collateral damage to healthy tissues.

For patients with an impaired immune system, regardless of HPV status, active immunotherapy may not be the first choice of treatment. Passive immunotherapy, in the form of adoptive transfer of *ex vivo* expanded TIL or T cell receptor transgenic T cells, might prove beneficial for such patients. These T cells can be cultured *in vitro* to increase their numbers and killing capacity prior to adoptive transfer. Boosting the immune response by taking away the naturally occurring inhibition of activated T cells with antibodies directed against CTLA-4 might proof beneficial to all HNSCC patients, especially those receiving active immune therapy.
Financial & competing interests disclosure

This work was supported by a grant from the Dutch Cancer Society (grant number VU 2007–3814). The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.
Executive summary

- The majority of malignancies arising in the head and neck region are squamous cell carcinomas. The 5-year survival rate for advanced head and neck squamous cell carcinoma (HNSCC) patients is approximately 40–50%.
- Tobacco use and excessive alcohol consumption are the main risk factors.
- Persistent infection with certain HPV types is an increasing risk factor.
- Patients with HPV-positive tumors generally have a better prognosis than patients with HPV-negative tumors.
- The immune system plays a role in immune-surveillance of aberrant cells and arising tumors.
- Proper activation of T cells is achieved by dendritic cells and cytokines.
- Overexpressed, neo, mutated self and viral antigens can serve as targets for tumor-specific T cells.
- HPV and Epstein–Barr viruses play a role in a number of HNSCCs.
- High numbers of tumor-infiltrating lymphocytes are a good prognostic factor for HNSCC patients.
- HNSCC tumors escape immune surveillance.
- Immunotherapeutic strategies should shift the balance to tumor destruction.
- Adoptive immune cell transfer is a form of passive immunotherapy.
- Adoptive transfer of (T cell receptor-transduced) tumor-specific T cells has not been tested in HNSCC patients.
- Immunotherapy in HNSCC patients has not been extensively investigated.
- Adjuvant treatment options might be best focused on passive immunotherapy such as the administration of T cells or antibodies.
- Virus-induced cancers should be considered as a separate class of tumors.
- Viral antigens derived from oncogenic viruses such as HPV or Epstein–Barr virus may serve as targets for immunotherapeutic approaches for the treatment of HNSCC.
References

Papers of special note have been highlighted as:
♦ of interest
♦♦ of considerable interest

Immunotherapy for head and neck cancer patients: shifting the balance


Summarizes the current understanding and progress in dendritic cell-based immunotherapy.


Immunotherapy for head and neck cancer patients: shifting the balance


* Reviews the influence of the tumor on the characteristics of the patient’s lymphocytes and various immunotherapeutic approaches.


CHAPTER 5


Immunotherapy for head and neck cancer patients: shifting the balance


100 Scholten KB, Ruizendaal JJ, Graf M et al. Promiscuous behavior of HPV16E6 specific T cell receptor b chains hampers functional expression in TCR transgenic T cells, which can be restored in part by genetic modification. Cell. Oncol. 32(1–2), 43–56 (2010).


◆ Reviews various methods of loading dendritic cells and targeting for immunotherapy.


Immunotherapy for head and neck cancer patients: shifting the balance


• Reports promising results in the field of adoptive T-cell immunotherapy.


