Immunology and Immunotherapy of Head and Neck Cancer

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Abstract
The immune system plays a key role in the development, establishment and progression of head and neck squamous cell carcinoma (HNSCC). A greater understanding of the dysregulation and evasion of the immune system in the evolution and progression of HNSCC provides the basis for improved therapies and outcomes for patients. HNSCC cells evade the host immune system through manipulation of their own immunogenicity, production of immunosuppressive mediators, and promotion of immunomodulatory cell types. Through the tumor’s influence on the microenvironment, the immune system can be exploited to promote metastasis, angiogenesis and growth. In this chapter, a brief overview of key components of the immune infiltrating cells in the tumor microenvironment is provided, reviewing immunological principles related to head and neck cancer, including the concept of cancer immunosurveillance and immune escape. Current immunotherapeutic strategies and emerging results from ongoing clinical trials are presented.

Introduction
Cancer immunotherapy is based on the premise that tumors can be recognized as foreign rather than as self and can be effectively attacked by an activated immune system. A greater understanding of the dysregulation and evasion of the immune system in the development and evolution of head and neck squamous cell cancers (HNSCC) should lead to improved therapies and outcomes for patients. There has been a recent renaissance in the idea that nascent premalignant cells are destroyed by the immune system before tumor formation can occur (termed immune surveillance). Derangements in the immune system or alterations in the transformed cells may allow immune escape which then enables the cancer to manifest. Tumors themselves produce cytokines such as transforming growth factor-beta (TGF-β), interleukin (IL)-6, and IL-10, which suppress cell-mediated antitumor immunity, while activation of STAT1 is suppressed (1, 2). Inflammatory transcription factors such as NF-κB (nuclear factor kappa-light-chain-enhancer of
activated B cells) and STAT3 (signal transducers and activators of transcription), are aberrantly activated in tumor cells and are intensively studied as possible targets for therapeutic intervention.

Tumor progression depends upon the acquisition of traits that allow cancer cells to evade immune surveillance and an effective immune response. HNSCC is an immunosuppressive disease, with lower absolute lymphocyte counts than healthy subjects (3), impaired NK cell activity (4, 5), and poor antigen-presenting function (6, 7). Impairment of tumor infiltrating T lymphocytes has also been reported in HNC and other cancers (8, 9) with a strong impact on clinical outcome (10). In addition, suppressive regulatory T cells (Treg) have been linked to HNSCC tumor progression. Treg cells secrete suppressive cytokines such as TGF-β and IL-10, express CTLA-4, and correlate with tumor progression (11). Therefore, immunomodulatory therapies that overcome immune suppressive signals in HNSCC patients have therapeutic promise. These include cancer vaccines using tumor peptide antigens, or viral, bacterial and DNA-based vectors - as well as tumor antigen-specific monoclonal antibodies (mAb). The recent clinical efficacy of FDA-approved mAb targeting immune checkpoint receptors, including anti-CTLA-4 and anti-PD-1, provide further promise for patient benefit as positive clinical data emerge.

**Cancer Immunosurveillance and Immunoediting**

The idea of immune system control of malignant cells was first proposed by Paul Ehrlich in 1908. The “cancer immunosurveillance” hypothesis was then introduced by Burnet and Thomas, who suggested that tumor cells must have recognizably different antigens than normal cells and therefore have the potential for immune clearance. Conflicting experimental results led many to abandon the idea of cancer immunosurveillance for several decades, until several key discoveries have led to a revival of the hypothesis. First was Herberman’s discovery of the NK cell in the 1970s which seemed to provide innate immune protection from tumor (12). The discovery of IFN-γ and its pro-apoptotic effect on tumor growth gave additional support to the potential for immune clearance of cancer cells (13, 14). Mice with genetically induced immunodeficiency were found to be more susceptible to both spontaneous and chemically induced tumors. In immunodeficient HIV-1 infected patients, a higher risk of HPV-associated HNC has been suggested (15, 16). In addition, pharmacologically immunosuppressed organ transplant recipients demonstrate increased risk of many tumors with no known viral etiology such as lung, head and neck (17), pancreatic, endocrine, colon cancer and melanoma (18). Cancer immunoediting suggests a dynamic evolutionary progress whereby immune surveillance of cancers provides selective pressure on tumor cells and negatively selects for cells that can evade the immune system (19). Thus, successful tumor formation occurs only after the cancer has discovered a means by which it can evade the immune system.

**Immune Escape and Immunosuppression in Head and Neck Cancer**

In order to establish effective immunotherapies, understanding the different pathways of the tumor immune evasion is necessary. First, HNSCC cells reduce their inherent immunogenicity (Table 1) and second, they actively suppress the antitumor immune response (Figure 1). A key component for the immune system’s recognition of different or altered cells is the HLA (human leucocyte antigen) complex, which presents processed tumor antigenic peptides to T lymphocytes (7). Tumor cells can reduce T cell-mediated recognition by altering HLA class I expression. Recently, mutations in specific HLA alleles, β2 microglobulin (β2m), and antigen processing machinery (APM) components, has been observed in large-scale next generation HNSCC sequencing efforts, such as The Cancer Genome Atlas, or TCGA (20), paralleling lung cancer. Chromosomal (21) and regulatory expression defects (6) in the HLA/APM-encoding genes themselves can cause selective loss of HLA and APM component expression in substantial fraction of HNSCC and are correlated with poor prognosis (22, 23).

On the other hand, cells with complete loss of HLA may evade immune response by T cell recognition but represent a strong trigger for NK cell activation, as the absence of HLA removes a key inhibitory signal for NK cells. Therefore, tumor cells must employ multiple mechanisms to realize immunoevasion while avoiding total loss of HLA expression. Endogenous antigens are processed (degraded into peptides) through the cytoplasmic immunoproteasome. Antigenic peptides are transported to the endoplasmic reticulum by the transporter associated with antigen processing (TAP1/2) heterodimer of the antigen processing machinery (APM) where they associate with HLA class I heavy chains (24).
HNSCC cells that express HLA I and tumor antigen can still evade T cell recognition through decreased expression or mutation of APM components, but still maintain moderate HLA I expression in order to avoid recognition by NK cells. In addition to oncogenic EGFR expression and mitogenic signaling, immunosuppressive effects may result, including downregulation of HLA, antigen processing machinery (APM) components and STAT1 activation, while leading to suppressive STAT3 signaling, cytokines and ligands on HNSCC cells.

Another important group of molecules that emerged in the focus of research is the group of immune checkpoint receptors (ICR). As part of the immune system’s control mechanisms against overreactive functions during inflammatory responses and to limit autoimmunity, this mechanism can be exploited in the tumor microenvironment. Several receptors have been identified that are expressed on exhausted, dysfunctional lymphocytes, including cytotoxic T lymphocyte-associated antigen-4 (CTLA-4), lymphocyte-activation gene-3 (LAG-3), T cell immunoglobulin and mucin protein-3 (TIM-3), and programmed death-1 (PD-1). The ligand for PD-1, PD-L1 (B7-H1, CD274) is upregulated in multiple tumor cell lines including HNSCC (25), and induces a loss of function of cytotoxic T cells (CTL) (26). CTLA-4 is a member of the B7 receptor family expressed by CD4+, CD8+ and regulatory T cells (Treg) (27), which competes with CD28 to stimulatory ligands CD80 and CD86. LAG-3 is another receptor that was shown to enhance Treg function (28). TIM-3 is a marker or a mediator for immunosuppression is still investigated (29), but studies have correlated TIM-3 expression levels with poor clinical outcome (30). Understanding these mechanisms has facilitated further establishment of immunotherapies, as outlined below.

Establishment of a cancer promoting tumor microenvironment

The fact that some cancers arise at sites of chronic inflammation was first noted by Virchow over a century ago. Infiltration of inflammatory mediators and a complex milieu of cytokines including TGF-β, IL-6, IL-10, GM-CSF, IL-1β, IL-23, and TNF-α as well as chemokines, which are “chemotactic cytokines” may be exploited by tumor cells. More recent developments link many of those cytokines to the formation of suppressive immune cells like myeloid derived suppressive cells (MDSC), regulatory T Cells (Treg), tumor associated macrophages (TAM) and their effectors, which are exploited and promoted by the tumor microenvironment.

Cytokines

Cytokines that suppress immune function are known to be produced by HNSCC cells (31). TGF-β suppresses NK and T cell activation and is a key cytokine in the differentiation of regulatory T cells, known as Treg (32). IL-6 signals via STAT3 to inhibit DC maturation, NK cell, T cell, neutrophil, and macrophage activation (33) and has been correlated with recurrence and survival in HNSCC (34). STAT3 is a transcription factor that is also involved in several other immunosuppressive pathways such as IL-10 signaling (35), suppression of dendritic cells (36), downregulation of IL-12 (37), and generation of regulatory T cells (38). PGE₂ is a prosurvival, proangiogenic molecule that is produced by many cancers including HNSCC (39-41). VEGF, which is primarily thought of as a promoter of angiogenesis, is overexpressed in 90% of HNSCC (42) and functions to increase the ratio of immature to mature DC in the tumor microenvironment which is thought to lead to T cell dysfunction and inactivation (43). Toll-Like Receptors (TLR) stimulate the production of proinflammatory cytokines such as TNF-α, IFN-γ with a T cell stimulating effect resulting in a type 1 helper (Th1) response.

Cellular immune components of the tumor microenvironment: myeloid-derived suppressor cells, regulatory T cells, and tumor associated macrophages

Myeloid derived suppressor cells (MDSC) are a diverse cellular population of myeloid origin with T cell suppressive functions (44). Initial studies in HNSCC found that MDSC inhibit activated T cells. Also MDSC produce nitric oxide and reactive oxygen species that catalyze the nitration of the TCR which inhibits TCR:HLA interaction, signaling and subsequent activation (45). Treatments such as antibody depletion, retinoic acid, gemcitabine and STAT3 blockade, that diminish MDSC, restore immune surveillance, increase T cell activation and improve efficacy of immunotherapy. The basal levels
of MSDC increase with age and may contribute to increased tumor frequency and growth rate increase with age (46).

A subset of suppressor, regulatory T cells (termed Treg) was relatively recently identified, and exist to prevent autoimmunity. This subpopulation of CD4+ T cells also express CD25 (47, 48), CTLA-4 and CD39. Tregs promote cancer progression by causing anergy, apoptosis and cell cycle arrest of activated T cells via production of IL-10, TGF-β, and direct cell-to-cell contact (49). They also inhibit the action of dendritic cells, NK cells, and B cells (50). In HNSCC patients, Tregs are increased in peripheral blood and more potent among T cells infiltrating the tumor, resulting in an immunosuppressed state (27, 51, 52). Also, Treg numbers are inversely proportional to DC and CD8+ T cell numbers in HNSCC (53, 54). Additionally, Treg frequency is elevated in HNSCC patients after treatment, indicating that oncologic treatment increases Treg numbers (27).

Tumor associated macrophages (TAM) in the tumor microenvironment may be strongly anti-tumor, and possess a so-called M1 phenotype, which is characterized by the production of IFNγ and other type 1 cytokines. Alternatively activated macrophages (M2) force a T\textsubscript{H2} response, with production of interleukins such as IL4 and IL13 that permit tumor growth. TAM infiltrating tumors correlate with worse clinical outcome and are closely associated with the M2 phenotype. These TAMs have been demonstrated to produce EGF, IL-6, IL-10 and have been associated with angiogenesis, local tumor progression and metastasis (55). Through these immune/inflammatory cells and mediators, HNSCC induces an immunosuppressed state via multiple potent mechanisms which is a barrier to effective cancer immunotherapy (56).

**Immune evasion of HPV associated HNSCC**

HPV infection and immune evasion in HPV associated cancers is clinically relevant model for immunotherapy. A critical component in avoiding adaptive and innate immune response is HPV's interference with interferon and other signaling pathways. Interferons link the innate immunity response to the adaptive immunity response by activation of immature dendritic cells (DC), CD8+ T cells and virus specific antibody production (57, 58). Interferon alpha (IFN-α) and beta (IFN-β) have immunostimulatory properties, are produced by virally infected cells and execute their antiviral effects through inhibition of mRNA, natural killer (NK) cell stimulation and inhibition of viral protein expression (57). IFN-γ activates leukocyte migration, antigen presentation and inflammation, and is primarily produced by effector lymphocytes. Therefore, antiviral immune response critically depends on inflammatory signaling, as evidenced by the frequent inactivating mutations in the TRAF3 gene found in The Cancer Genome Atlas (TCGA) (20). Danger signals such as TLR present on inflammatory cells are able to detect so called pathogen-associated molecular patterns (PAMP) (59) to stimulate these IFN’s. Furthermore, HPV interacts with antigen presentation in order to reduce adaptive immune response, and suppresses STAT1 signaling inhibition by IFN pathways causing downregulation of HLA class I APM (59, 60). Genetic host polymorphisms (61) and even mutations, such as the recently identified 10-12% frequency of genomic alterations in HLA/TAP/β2-m antigen processing/presentation pathways (20) data may present an ultimate barrier to successful immunotherapy in these patients.

During normal immune responses, the presence of checkpoint receptors such as PD-1 or CTLA-4 limits an overly robust immune response, in order to protect from autoimmune reactivity (62, 63). In HNSCC patients, elevated PD-1 expression has been observed on CD8+ HPV+ tumor infiltrating lymphocytes (64) but unexpectedly patients with high numbers of PD1 expressing T cell infiltration showed a better 5-year overall survival (93.9%) compared to those patients with low PD-1 expressing T cell infiltration (63.6%) (64). This potentially conflicting observation may reflect a quantitatively greater overall anti-tumor immune response, since pro-inflammatory conditions can stimulate PD-L1 expression. Interestingly, PD-L1 expression of tumor tissue was not correlated to clinical outcome (64). As a result, the quality and quantity of tumor-infiltrating lymphocytes (TIL) determines the anti-tumor response. This is confirmed by recent studies correlating the number of TIL in patients with HPV positive OSCC with disease prognosis (65, 66). Badoual and colleagues also observed a higher number of tumor infiltration with regulatory T cells (Treg) (64) in HPV-positive OSCC. So far, the reasons for the better prognosis of
HPV positive patients despite all of the mentioned HPV and non-HPV associated immune evasion mechanisms remain unclear.

**HPV-specific cancer immunoprevention strategies:** The most successful HNSCC-targeted immunotherapy will likely be HPV-targeted immunoprevention vaccines. The aim of the preventive vaccines is to inhibit viral infection and thus hinder cancer formation. The immunization targets the L1 capsid proteins and is realized by using virus-like particles (VLPs). Those particles provoke a humoral antibody response, and interestingly, generate a significantly stronger humoral response than natural infection (67). Several large randomized, double-blinded controlled phase III trials demonstrated high efficacy (Gardasil® 96.8-100%, Cervarix® 90.9-100%) in prevention of benign and malignant HPV associated cervical lesions (68). The effects of the vaccination on oropharyngeal lesion is net yet finally evaluated, but is expected to have promising results, considering the achieved antiviral results so far and the rising prevalence of HPV positive oropharynx carcinoma (60, 69). The GSK vaccine delivered in a randomized, placebo-controlled Costa Rican cohort demonstrated significantly reduced (nearly eliminated) oral HPV infection in the vaccine group potential benefit for reducing future OPSCC cases (70), suggesting a potential benefit for reducing future OPSCC cases. Because these prevention vaccines induce 2-3 log-fold higher L1 capsid specific Abs than natural infection, they prevent viral entry and initial infection. However, because established HPV infection leads to viral DNA integration and expression of intracellular E6 and E7 oncogenes, and loss of L1 expression, these prevention vaccines are ineffective for prior infections and are not therapeutic tools for established HPV-associated cancers.

**Immunotherapies in established HPV-related HNSCC**

Several vaccination therapies under development in HNSCC have yielded modest results to date. Peptide vaccines consist of synthesized peptides that have been designed to correspond to an epitope on a tumor antigen that binds well to the cleft of an HLA molecule. They are similar to DNA vaccines in that they are safe and inexpensive with low immunogenicity but have the added drawback of being restricted to the HLA subclass (allele) for which they were designed based on specific binding. Clinical trials are underway with a MAGE-A3/HPV-16 peptide (NCT00257738) and a LMP-2 peptide for EBV-related nasopharyngeal carcinoma (NCT00078494).

Bacterial/viral vaccines can deliver tumor antigen as well as functioning as an immune adjuvant because the immune system responses to a perceived infection. Several such vaccines are currently under development: HPV-16 E7 Listeria vaccine (71), vaccinia-based E6/E7 vaccine (72), and a vaccinia-based E2 expressing vaccine (73). Other vectors include bacterial based HPV vaccines targeting E7, which showed preclinical effects and are in clinical investigation (71). The viral vector TG4001 (encoding HPV E6/E7 and IL-2) was also used in a phase II trial of malignant lesions in combination with chemotherapy (74).

Another major avenue of immunotherapy for head and neck cancer is adoptive T cell transfer. In this approach, T cells are removed from a patient, genetically modified or treated with agents to enhance their activity, and then re-introduced into the patient with the goal of improving the immune system’s anti-cancer response. Several trials of adoptive T cell transfer techniques are currently under way for patients with head and neck cancer, including a phase II trial of TILs for HPV-associated cancers, including HNSCC at the National Cancer Institute (NCT01585428).

Dendritic cells (DC) are the most potent activators of antigen-specific T cells but DC vaccines are produced as a cellular product ex vivo, isolated from each patient and loaded with tumor antigen ex vivo. This loading can be in the form of peptides, proteins, DNA transfection, tumor cell lysates, apoptotic tumors, necrotic tumors or cell fusion. After maturation and activation with various cytokine cocktails, these DC are then introduced to the patients, usually into the tumor or into lymph nodes. Several DC-based vaccines are currently being developed for HNSCC: intratumoral injection of DC (NCT00492947), multivalent p53 DC vaccine (75) and lysyl oxidase like-4 transfected DC (76).

**Monoclonal Antibody-Based Immunotherapy of HNSCC**
Today the most widely used form of cancer immunotherapy is mAb therapy (77). Tumor antigen (TA)-targeted mAbs, cytokine-targeted mAbs, tumor necrosis factor receptor (TNFR)-family costimulatory targeted mAbs and immune checkpoint-targeted mAbs. Currently available mAbs that are being investigated in HNSCC are listed in Table 2. The most extensively studied (and FDA approved for HNSCC) of these is cetuximab, a mouse–human chimeric IgG1 anti-epidermal growth factor receptor mAb (78). EGFR is an attractive target in HNSCC because it is overexpressed in 80–90% of HNSCC and leads to tumor cell proliferation, invasion, angiogenesis, tumor survival, and consequently, poor survival and prognosis (79).

Anti-EGFR mAb mediate antigen-specific immune responses to targeted tumors through two major mechanisms (Figure 4): direct killing via lytic immune cell (NK cell or monocytes) and complement fixation, or opsonization of tumor for phagocytosis and subsequent antigen processing. The latter would induce TA-specific cytotoxic T lymphocytes (CTL) to recognize and lyse tumor cells. One of the most direct methods by which antibodies can cause tumor lysis is via antibody-dependent cellular cytotoxicity (ADCC) mediated by NK cells and probably monocytes and neutrophils. The extent of ADCC is heavily influenced by genetic polymorphisms in FcγRIIIa, also known as CD16 (80), however confirmatory clinical data in HNSCC patients are lacking. In addition to direct activation of NK cell lysis of tumor cells, TA-specific mAbs can elicit CD8+ T cell responses to tumor-derived antigens through interaction with FcγRs on antigen-presenting cells (APC). This antigen-specific T cell activation was noted in 78% of patients treated with trastuzumab for breast cancer and this activation seemed to correlate positively with clinical response (81). Specific T cell activation has been demonstrated in HNSCC patients treated with cetuximab (82, 83), alone or in combination with cisplatin chemotherapy. In addition to extensive clinical and correlative immune response data using cetuximab, MEHD7945A, an anti-HER3/EGFR human mAb targeting human epidermal growth factor receptor 3 (HER3) and EGFR is currently being tested in a phase I/II clinical trials for HNSCC (NCT01577173, NCT01911598).

Imune Checkpoints and Inhibitors

T cell activation occurs through a combination of T cell receptor engagement and co-stimulatory molecules. The duration and extent of immune responses, for example to infections, is regulated by “immune checkpoints” or inhibitory pathways which prevent excessive inflammatory responses as well as development of autoimmunity. Immune checkpoints have also been shown to play an important role in the tumor microenvironment and can be manipulated as a mechanism of tumor immune evasion (84). The immune checkpoint pathways are mediated by ligand and receptor interactions, and examples include cytotoxic T-lymphocyte antigen-4 (CTLA-4) and its ligands CD80 and CD84 and programmed death-1 (PD-1) and its ligands PD-L1 and PD-L2. Blocking anti-CTLA-4 Ab therapy results in rejection of murine cancers (85). A mAb against CTLA-4, ipilimumab, was the first drug in this class to demonstrate clinical benefit and was approved by the FDA for patients with metastatic melanoma (86). Tremelimumab is also available for CLTA-4 targeting. More recently, anti-PD-1 or PD-L1 Abs have demonstrated clinical efficacy, alone (87-89) or in combination with ipilimumab (90).

PD-L1 pathway targeting in HNSCC

Programmed death receptor-1 (PD-1, CD279), a 55 kD type I transmembrane protein, is a member of the CD28 family of T-cell co-stimulatory receptors that also includes CD28, CTLA 4, ICOS, and BTLA. PD-1 contains an intracellular membrane proximal immunoreceptor tyrosine inhibitory motif (ITIM) and a membrane distal immunoreceptor tyrosine-based switch motif (ITSM). Two ligands specific for PD-1 have been identified: PD-L1 (B7-H1/CD274) and PD-L2 (B7-DC/CD273). PD-L1 and PD-L2 have been shown to down-regulate T-cell activation upon binding to PD-1 in both murine and human systems. PD-1 delivers a negative signal, suppressing type 1 based antitumor immunity (91) by the recruitment of SHP-2 to the phosphorylated tyrosine residue in the ITSM in its cytoplasmic region, skewing the immune response away from a beneficial “type 1” response. PD-1 is primarily expressed on activated T cells, B cells, and myeloid cells. PD-1 blockade has the potential to activate anti-self T cell responses, but these responses are variable and dependent upon various host genetic factors.
Tumor immune evasion can occur by high tumor expression of PD-L1 and/or tumor immune infiltration by PD-1+ T lymphocytes. Preliminary analyses indicate that PD-L1 is expressed in 50-60% of HNSCC, and that tumor infiltration by PD-1+ Treg may be more common for HPV-negative HNSCC. Strome and colleagues reported membrane and or intracytoplasmic PD-L1 expression in 66% (16 of 24) of HNSCC. Badoual and colleagues reported tumor infiltration by PD-1+ CD8+ and PD-1+CD4+ lymphocytes was more common among HPV-positive than HPV-negative HNSCC. In 33 (55%) of 64 HNSCC, high levels of PD-L1 expression were observed, but there was no association between PD-L1 expression and tumor HPV status (64). Jie and colleagues observed higher expression of immune-checkpoint receptors (CTLA-4 and PD-1) in intratumoral T_{reg} cells than on matched peripheral blood samples from 27 patients with HNSCC. These data strongly support a role for PD-1 inhibition in the therapy of HNSCC.

**Checkpoint receptor targeted mAbs in combination with cetuximab**

Cetuximab therapy alters expression of checkpoint receptors on circulating and intra-tumoral TIL. Specifically, the frequency of Treg suppressor cells that express CTLA-4 and PD-1 are enriched in the tumor microenvironment (2013). Furthermore, cetuximab therapy increased the frequency of CD4+CD25^hiCD39^hiFOXP3^+ Treg (p=0.01), indicating that this treatment expands Treg in patients with HNSCC. CTLA-4+/CD39^hi cells were significantly increased among the majority of CD4^hiFOXP3^+ Treg from patients prior to and after cetuximab treatment, indicating that CTLA-4 targeting may provide enhanced benefit in cetuximab treated patients (92). Recent data in NSCLC indicates that the EGFR pathway may contribute to regulation of PD-L1 expression (93), a finding corroborated in HNSCC (Concha-Benavente and Ferris, unpublished data).

These emerging data support the incorporation of checkpoint inhibitory mAb into conventional HNSCC therapy, either to deplete Treg or to disrupt the PD-1:PD-L1 suppressive signal transmitted to CD8^+ effector T lymphocytes. These suppressed NK cells and T cells express the negative regulatory PD-1 receptor, at higher levels and generating greater inhibitory signals in tumor infiltrating lymphocytes, providing strong rationale for combining cetuximab with anti-PD-1 mAb therapy in a curative setting in which traditional cytotoxic chemotherapy may impart deleterious effect(s) on the generation and proliferation of beneficial antitumor lymphocyte responses.

**Costimulatory agonistic strategies**

In addition to blocking negative regulatory receptors on lymphocytes, another strategy has emerged, to enhance and trigger positive, co-stimulatory signals using agonistic Abs and small molecules. So far, the investigation of TNFR targeting mAb in clinical trials for HNSCC is in phase I. Because of the important costimulatory pathways for immune cell activation, substances like CP-87,893 (Pfizer), an IgG2 CD40 agonist, OX40 mAb (AZ/Immune), an IgG2 OX40 agonist or urelumab (Bristol-Myers Squibb), an IgG4 CD137 agonist, have been investigated with cetuximab or with nivolumab in clinical trials (94), which are currently enrolling HNSCC patients. TLR agonists induce the maturation and cross-priming of dendritic cells (DC) and have been shown to induce NK cell dependent lysis of tumor cells in combination with mAb such as anti-EGFR cetuximab (95).

**Integration of Immunotherapy into Clinical Practice**

The integration of this new modality into standard clinical practice must adapt to different stages and disease status of HNSCC patients, depending on the clinical needs for each population of individuals. Indeed, monoclonal Abs targeting PD-1 and CTLA-4 (and others) are being investigated in several clinical trials, now that FDA approval exists for these agents in melanoma and lung cancer. Indeed, in November 2014, the NCI funded a Clinical Trials Planning Meeting (CTPM) to facilitate rational design of combinations of immunotherapies for phase II and III randomized trials in HNSCC.

**Previously untreated, locally advanced HNSCC**

For HPV+ previously untreated, locally advanced (PULA) HNSCC, the clinical need is more targeted, less toxic therapy, and to determine the sequencing and optimal chemo-radiation regimens that do not inhibit immunotherapeutic efficacy. Specifically trials need harness this novel systemic therapy to
make an impact on the burden of uncommon, though lethal distant metastatic disease for “high-risk” advanced disease (T4, N2c/N3, >10 pk-yr smokers) HPV+ patients. Trials planned or in development include eliminating systemic cytotoxic chemotherapy by combining IMRT with cetuximab and anti-CTLA-4 mAb (ipilimumab, NCT01935921), in which the overlap of mAb exposure begins at week 5 of cetuximab/RT. In the first 6 patients accrued, 2 DLT’s were experienced (dermatologic toxicity, leading to dose reduction from 3mg/kg to 1 mg/kg of ipilimumab, RL Ferris and J.E. Bauman, unpublished results). Additionally, “intermediate risk” HPV+ and “high-risk” HPV- patients will be treated with concurrent, weekly cisplatin CRT with anti-PD-1 mAb, a natural “add-on” strategy that is in development by the RTOG for prospective evaluation in the near future.

For HPV-negative PULA HNSCC patients, disease free survival (DFS) has not improved beyond the historical 50% rate for decades – despite concomitant treatment intensification. Thus, the clinical impact of immunotherapy would be to improve DFS, given that intensification using conventional modalities has been unacceptably toxic. Intensifying therapy to enhance survival using anti-PD-1 mAb plus CRT will be tested for HPV+ disease Neoadjuvant approaches will also take advantage of tumor accessibility for serial biomarker testing. Trials sequencing anti-PD-1 before, during or after RT are being developed to evaluate complex effect(s) on immunity stimulated during these combinatorial trials.

Thus, for HPV and HPV- locally advanced HNSCC, checkpoint inhibitors (anti-PD-1 or anti-CTLA-4) are being investigated for adjuvant, postoperative PULA HPV-negative disease combined with cisplatin/IMRT, or for upfront treatment of “high risk” advanced disease stage HPV+/- PULA HNSCC, in combination with concomitant cisplatin- or cetuximab-IMRT (Table 3).

**Recurrent/Metastatic HNSCC**

In R/M HNSCC, the usual disease setting in which novel therapeutics are initially tested, a proliferation of immunotherapeutic Abs and combinations has occurred (Table 2). In a phase I clinical trial investigating the anti-PD-1 monoclonal antibody pembrolizumab (MK-3475, Merck) targeting advanced/recurrent HNSCC, responses are observed regardless of HPV status, including those without detectable PD-L1 expression (96). Of 60 patients, 23 were HPV+ and 37 were HPV-; 9 had no prior systemic treatment, 10 had 1, 16 had 2, 13 had 3, and 7 had ≥4 prior regimen of treatment (5 unknown). Of the patients treated with anti-PD-1 mAb, 78.3% experienced ≥1 AE, and 46.7% reported a drug-related (DR) AE. The most common AEs reported were pruritis (6, 10%), fatigue (4, 7%), rash (4, 7%), and diarrhea (3, 5%). Response rates (PR/CR) were approximately 20%, and were similar in both HPV+ and HPV- HNSCC patients. Recurrent and metastatic (R/M) HNSCC has seen modest survival improvements by adding cetuximab to doublet chemotherapy of platinum/5-FU (EXTREME regimen, ref. (97). Whether anti-PD-1 therapy can further enhance these outcomes is a logical line of investigation (Table 3).

Adding immunotherapeutics to standard cetuximab-containing regimens is a natural line of investigation. VTX-2337 is a toll-like receptor 8 (TLR8) agonist currently being tested in a randomized phase II clinical trial in first-line R/M HNSCC, combined with platinum/5-FU/cetuximab (NCT01836029, n=175 patients). For cisplatin-refractory R/M HNSCC, two blocking anti-PD1 Abs, nivolumab (NCT02105636 (n=340 patients) or pembrolizumab (NCT02358031, n=750 patients), are being investigated in randomized phase III trials for platinum-refractory HNSCC as single agent. Anti PD-L1 (MEDI4736) has generated additional promising data (~14% response rate scored using RECIST criteria, with 24% RR in PD-L1+ patients) in a phase I trial (Fury, ESMO 2014), warranting the design of a randomized phase III trial of MEDI4736 alone or in combination with anti-CTLA-4 (tremelimumab), as compared to standard of care agents. Stratification by PD-L1 expression status is planned, and follows up on phase II trials current accruing. Another new phase III trial in the first line R/M setting (NCT02358031) will compare anti-PD-1 (pembrolizumab) alone or in combination with platinum/5-FU, vs cetuximab/platinum/5-FU (EXTREME regimen).

A different group of receptors with a modulating effect on immune cells are other checkpoint receptors such as Lag-3 or the killer-cell immunoglobulin-like receptors (KIRs). They interact with MHC I molecules and regulate immune response. Most of the receptors have a suppressing effect on the cytotoxicity, particularly “turning off” NK cells when HLA is present on tumor cells. Anti-KIR Abs thus

might remove the major inhibitory signal on NK cells. Ongoing trials are investigating an anti-KIR mAb in combination with the anti-CTLA-4 mAb ipilimumab (NCT01750580) or anti PD-1 mAb nivolumab (NCT01714739). Anti-PD-1 mAb are also being tested in various novel combinations in the phase I setting, such as nivolumab plus agonistic anti-CD137 mAb (urelumab, NCT02253992), nivolumab plus anti-LAG-3 (NCT01968109), as well as cetuximab plus urelumab. A more complete listing of trials open or in late stages of development is provided in Table 3.

**Checkpoint Inhibitors and Radiotherapy**

In addition to direct cytotoxic effects, radiotherapy may induce an immune effect important to tumor cell death (98). Preclinical data support synergy between checkpoint inhibitors and radiotherapy. Mouse models of poorly immunogenic tumors have demonstrated that concomitant administration of anti-CTLA-4 antibodies and radiotherapy results in antitumor T cell responses both in the radiation field as well as outside of it (an abscopal effect) (98, 99). PD-1 blockade after completion of radiotherapy also has been shown to induce rejection of persistent tumors in mouse models (Liang 2013). Combination PD-1 blockade and anti-CD-137 stimulation increased response to radiotherapy in a mouse model of triple negative breast cancer (100), and PD-L1 blockade concomitant with radiotherapy improved survival in comparison to either therapy alone in mouse models of glioma (101). In human subjects, case-reports support the existence of a clinically significant abscopal effect for patients with melanoma who have received ipilimumab prior to radiotherapy (102, 103). These data support a hypothesis that checkpoint inhibitors administered prior to or concomitant with radiotherapy can induce clinically significant antitumor immune responses induced by “vaccination” to tumor-specific antigens exposed during radiation-induced cell death (104). Such a phenomenon may be particularly relevant to viral-induced tumors, such as HPV-positive HNSCC and to highly genetically unstable tumors such as HPV-negative HNSCC.

**Conclusion**

Cancer immunology is a rapidly evolving field and only recently have we begun to understand the complex interaction between cancer and the host immune system. Tumor cells demonstrate several methods to exploit the immune system to help promote angiogenesis, derive pro-survival and proliferative signals, and induce metastasis and tumor progression. At the same time, cancers are able to cloak themselves from the immune system by self-modification and by immunosuppression of the host. Recent results from clinical trials show evidence for effective anticancer immunotherapies. Because of the manifold tumor evasion strategies and hence different response rates for treatments, combinational therapies are crucial to develop for cancer treatment. These insights and better understanding of the workings of the immune system have allowed the recent explosion of several promising immunotherapeutic agents that are currently in clinical use as well as under others in development.
References


Table 1: Mechanisms of immune escape in HNSCC

<table>
<thead>
<tr>
<th>Mechanism</th>
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<tbody>
<tr>
<td>Development of T cell tolerance to persistent HPV infection, or overexpressed/mutated antigens</td>
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<tr>
<td>Production of low genome copy numbers in the basal layer of the epithelium</td>
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<tr>
<td>Increased PD-L1 expression in HPV+ tumors and increased PD-1 expression in cytotoxic T lymphocytes</td>
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<tr>
<td>Downregulation of interferon regulatory factors and activated STAT1</td>
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<tr>
<td>Inhibition of inflammatory cytokines and transcription factors</td>
</tr>
<tr>
<td>Downregulation or mutation of HLA class I and antigen processing machinery (APM) components</td>
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**TABLE 2: Drug (Company) Target IgG class HNSCC development stage**

<table>
<thead>
<tr>
<th>Tumor Antigen Targeted Monoclonal Antibodies</th>
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<tbody>
<tr>
<td>Cetuximab (Bristol-Myers Squibb, Eli Lilly) EGFR antagonist IgG1 Phase III/IV</td>
</tr>
<tr>
<td>Panitumumab (Amgen) EGFR antagonist IgG2 Phase II/III</td>
</tr>
<tr>
<td>AV-203 (Aveo) HER3 antagonist IgG1 Phase I (monotherapy; cetuximab combination)</td>
</tr>
<tr>
<td>Cixutumumab (Eli Lilly) IGFR antagonist IgG1 Phase 0-II (neoadjuvant monotherapy; cetuximab combination)</td>
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<tr>
<th>Cytokine Targeted Monoclonal Antibodies</th>
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<tbody>
<tr>
<td>Bevacizumab (Genentech) VEGF neutralizing Ab IgG1 Phase III (platinum chemotherapy +/-)</td>
</tr>
<tr>
<td>Ficlatuzumab (Aveo) HGF neutralizing Ab IgG1 Phase I (cetuximab combination; cisplatin-radiation combination)</td>
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<tr>
<th>TNF Receptor Targeted Monoclonal Antibodies</th>
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<tbody>
<tr>
<td>MEDI0562 (Astra-Zeneca/Medimmune) OX40 agonist IgG2 Phase Ib</td>
</tr>
<tr>
<td>Urelumab (Bristol-Myers Squibb) CD137 agonist IgG4 Phase I</td>
</tr>
<tr>
<td>PF-05082566 (Pfizer) CD137 agonist IgG2 Phase I</td>
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<tr>
<th>Immune Checkpoint Targeted Monoclonal Antibodies</th>
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<tbody>
<tr>
<td>Ipilimumab (Bristol-Myers Squibb) CTLA4 IgG1 Phase I (cetuximab-radiation combination)</td>
</tr>
<tr>
<td>Tremelimumab (AZ/Medimmune) CTLA4 IgG2 Phase I</td>
</tr>
<tr>
<td>MEDI4736 (AZ/Medimmune) PD-L1 IgG1 Phase II</td>
</tr>
<tr>
<td>Pembrolizumab (MK-3475, Merck) PD-1 IgG4 Phase I</td>
</tr>
<tr>
<td>Nivolumab (Bristol-Myers Squibb) PD-1 IgG4 Phase III</td>
</tr>
</tbody>
</table>
Figure 1. Tumor cell immune evasion and exploitation by cellular and soluble mediators in the tumor microenvironment. Tumor cells secrete several small molecules and cytokines that depress NK, DC, and T cell function and induce immunosuppressive MDSC and regulatory T cells. MHC downregulation and defects in the antigen presentation machinery impairs T cell recognition. Fas ligand is expressed which kills T cells. Chemokine receptors aid in metastasis of the cancer cell to lymph nodes.
Figure 2. Immune escape from each step required for the development of strong antitumor immunity. Signal 1 represents TCR:HLA-peptide antigen interactions. Signal 2 represents co-stimulatory (or co-inhibitory) signals. Signal 3 indicates cytokine secretion, which may be proinflammatory, type 1 (Th1) antitumor mediators, or tumor-permissive type 2 (Th2) cytokines. Signal 4 represents cell extrinsic attracting signals to augments/amplify or suppress antitumor immunity.
Figure 3: The process of antigen presentation for recognition of tumor cells by the immune system requires fully functional antigen-processing machinery (APM), recently found to be mutated in a subset of HNSCC by the TCGA. Protein antigen is degraded into short peptide fragments by the transporter associated with antigen processing (TAP), an ATP-dependent peptide pump, into the endoplasmic reticulum, where the antigenic peptides bind to MHC molecules and progress to the cell surface. Surface MHC-peptide complexes are then recognized by antigen-specific T lymphocytes.
Figure 4. Immune cell interactions mediated by therapeutic, tumor antigen specific mAb and FcγR-bearing NK cells and DC, leading to induction of innate and adaptive antitumor immunity.