Published in final edited form as:

Clin Cancer Res. 2018 January 01; 24(1): 6–13. doi:10.1158/1078-0432.CCR-17-1261.

Head and Neck Carcinoma Immunotherapy: Facts and Hopes

Theresa L. Whiteside

University of Pittsburgh Cancer Institute and University of Pittsburgh School of Medicine, Departments of Pathology, Immunology and Otolaryngology Pittsburgh, PA 15213

Abstract

Cancer of the head and neck (HNC) is a heterogeneous disease of the upper aerodigestive tract, encompassing distinct histological types, different anatomical sites and HPV+ as well as HPV^{neg} cancers. Advanced/recurrent HNCs have poor prognosis with low survival rates. Tumor-mediated inhibition of anti-tumor immune responses and a high mutational burden are common features of HNCs. Both are responsible for the successful escape of these tumors from the host immune system. HNCs evolve numerous mechanisms of evasion from immune destruction. These mechanisms are linked to genetic aberrations, so that HNCs with a high mutational load are also highly immunosuppressive. The tumor microenvironment of these cancers is populated by immune cells that are dysfunctional, inhibitory cytokines and exosomes carrying suppressive ligands. Dysfunctional immune cells in patients with recurrent/metastatic HNC can be made effective by the delivery of immunotherapies in combination with conventional treatments. With many promising immune-based strategies available, the future of immune therapies in HNC is encouraging, especially since methods for genetic profiling and mapping the immune landscape of the tumor are being integrated into a personalized approach. Efficiency of immune therapies is expected to rapidly improve with the possibility for patients' selection based on personal immunogenomic profiles. Non-invasive biomarkers of response to therapy will be emerging as a better understanding of the various molecular signals coopted by the tumors is gained. The emerging role of immunotherapy as a potentially beneficial addition to standard treatments for recurrent/metastatic HNC offers hope to the patients for whom no other therapeutic options exist.

Keywords

head and neck cancer; immune suppression; immunotherapy; immunogenetics

Introduction

Squamous cell carcinoma of the head and neck (HNSCC) accounts for about 3% to 5% of all cancers in the US, with an estimated frequency of 61,760 new cases in 2016 and 13, 190 deaths (1). More than 600,000 cases of HNSCC are diagnosed annually worldwide (2). Primary risk factors for HNSCC include tobacco, alcohol and human papilloma virus (HPV)

Corresponding Author: Theresa L. Whiteside, PhD, University of Pittsburgh Cancer Institute, Hillman Cancer Center, 5117 Centre Avenue, Suite 1.27, Pittsburgh, PA 15213, Phone: (412) 624-0096, FAX: (412) 624-0264, whitesidetl@upmc.edu.

Conflict of Interest

The author has no conflicts of interest.

infection. Despite considerable progress made in the use of chemotherapy, radiation, and targeted therapies, the treatment of advanced or recurrent head and neck cancers (HNC) remains largely ineffective. The five-year survival rate of patients with HNC has not improved for many years and remains at 50% for patients with locoregionally advanced disease (3). The development of drug resistance continues to be a major therapeutic hurdle. Recurrent/metastatic HNCs that do not respond to platinum-based chemotherapy progress very rapidly and have very poor prognosis with no other therapeutic options available. The development of new therapeutic strategies for HNC is an unmet need with the highest priority. Immune-based therapies appear to offer a new, potentially effective strategy that could alter the therapeutic landscape of HNC. By preventing tumor immune escape and stimulating anti-tumor immune responses to keep the residual tumor cells in check, immune therapies are effective in prolonging patients' survival. Also, tumors that become resistant to chemo- or radiotherapy often remain sensitive to immune-mediated mechanisms.

The hypothesis underpinning the use of immunotherapies for HNC patients with advanced or recurrent disease assumes that ineffective anti-tumor immune responses can be made effective by an initial tumor ablation followed by delivery of immunological agents that selectively block inhibitory mechanisms and stimulate anti-tumor immune responses. There are currently numerous therapeutic strategies available for testing this hypothesis, and some are being tested in clinical trials for HNC patients.

The tumor microenvironment in HNC

The HNC, like all other cancers, results from a stepwise accumulation of genomic instability, chromosomal aberrations and genetic mutations (4). Within cancer tissues, arising mutant cells strive for resources and space, avoid immune surveillance and, in collaboration with the extracellular matrix (ECM) elements, establish their own unique niche. The niche, in addition to neoplastic cells, incorporates the tumor stroma (fibroblasts, endothelial cells, pericytes, mesenchymal cells); immune cells (T, NK, B lymphocytes, macrophages, PMNs, mast cells); blood vessels and a host of immunoinhibitory soluble or membrane-bound factors (5). The cells within the tumor microenvironment (TME) are re-programmed by the tumor to aid its progression or to shield cancer cells from the host immunity. Seen in this context, the TME is a dynamic complex of cells and soluble factors contributing to tumor drug resistance, interfering with oncological therapies and promoting tumor cell growth. *In situ* studies of human tumors suggest that each tumor creates its own TME that distinguishes it from other tumors with the same histopathology. Most HNCs share the squamous cell origin; yet because they arise in various tissue locations such as the throat, larynx, nose, sinuses, the oral cavity or the oropharynx, these cancers are highly heterogeneous, and the TME of each individual tumor is unique. Further, HNCs are either HPV^{+} or HPV^{neg}, and the viral origin further introduces differences that are reflected in different susceptibility of these tumors to therapy and in patient survival (6). The HPV^{+} cancers have better outcome than HPV^{-} HNCs, and it has been speculated that this reflects better activity of the immune system conditioned by the virus. High levels of Treg and $PD1^{+}$ T cells in HPV^{+} patients were shown to correlate with favorable outcome (7, 8), suggesting that strong reactivation of immune response to the virus is being tempered by the host immune system. Interestingly, the benefit of HPV infection extends only to HPV^{+}

oropharyngeal cancers and not to other infection sites (9), an indication that local immune response to the virus and the TME shape cancer outcome. The TME is never static and always changes to deal either with host defense or with therapeutic interventions. Similar to HPV-1 infection, therapies are known to alter the content of the TME (10). Thus, a primary HNC examined prior to any therapy has different phenotypic and functional profile from that of the same tumor examined after chemo-, radio- or targeted therapy. It follows that changes induced by conventional therapies in the cellular and molecular content of the TME might be useful as biomarkers to inform selection of anti-tumor immune therapies.

The immune profile of HNC

Immune dysfunction in HNC has been extensively reviewed (5). It encompasses many different phenotypic and functional changes in immune cells that occur with a different frequency in patients with early vs late disease stages (11). HNC is one of the most immunosuppressive human tumors. While accumulations of $CD3^+CD4^+$ or $CD3^+CD8^+$ effector T cells in the tumor correlate with better prognosis in some studies (7), others report selective apoptosis of activated tumor antigen (TA)-specific $CD8^+$ T cells, rapid turnover of effector T cells, functional paralysis of T, B, NK or dendritic cells (DC), all of which predicts poor outcome (11, 12). Accumulations of regulatory T cells (Treg) or of myeloid-derived suppressor cells (MDSC) or of adenosine-producing regulatory B cells (Breg) at the tumor site or in the peripheral circulation of HNC patients also associate with poor outcome (13, 14).

Interestingly, in the TME of HNC patients, CD25+FOXP3+ Treg overexpress CTLA-4, PD-1, TIM-3 and TGF-β-associated LAP on the cell surface and up-regulate suppressor functions (15). This finding implicates Treg in downregulation of anti-tumor immunity. Molecular mechanisms these Treg or other regulatory cells utilize for mediating immune suppression in HNC are listed in Table 1. The list includes inhibitory pathways known to be overexpressed in HNC and to contribute to local or systemic immune suppression. Further, activation of these inhibitory pathways exerts strong suppressive effects on various immune cells and their development. Figure 1 illustrates how immunomodulatory ligands or cytokines which are elevated in HNC can skew differentiation of T cells, contributing to immune dysfunction. Subversive effects of ADO and prostaglandin E_2 (PGE_2) on immune cell functions in HNSCC are well documented (16, 17). Recently, the presence of JAG1, a Notch ligand, in the TME of HNC has been noted and its negative impact on immune cells is under study (18). Also, tumor-derived exosomes, the smallest of extracellular vesicles (30–150nm) in plasma of HNC patients, carry most of the above listed inhibitory ligands and inhibit immune cell functions. Exosomes, conveyors of suppressive molecules from the tumor to immune cells may potentially serve as predictors of response to therapy or of outcome and are of special interest in the context of cancer immunotherapy.

Current clinical efforts to diminish tumor-induced immune suppression in HNC

Given the extent and a variety of immune dysfunction mechanisms operating in HNC (Table 1), it is not surprising that efforts to counteract tumor-induced suppression and achieve immune reconstitution have been only moderately successful to date.

Cytokines

were the first immunotherapies tested in HNC (19). In 1994, beneficial effects of peri-tumoral delivery of interluekin-2 (IL-2) in oral HNC were reported (20). Further, in a randomized phase III trial of IL-2 given perilymphatically after surgery to patients with the oral cavity cancers, >25% improvement in OS at 5 years was reported (21). In contrast, systemic delivery of IL-2 in another trial study was ineffective (22). Perilymphatic delivery of IRX-2, a mixture of low-dose IL-2, IL-1β, IL-6, IL-8, IFN-γ, TNF-α, G-CSF, GM-CSF was tested in a Phase II study for previously untreated, resectable patients with stage II-IV HNSCC (23). Toxicities were tolerable. IRX-2, given preoperatively for 21 d in combination with cyclophosphamide and indomethacin as immune adjuvants, was effective, inducing responses in 16% of patients with evidence for increases in lymphocytic infiltrates in the responding tumors (24). In the randomized Phase II trial, the safety and efficacy of IRX-2 delivered in the neoadjuvant setting was confirmed, and the immune benefits were correlated to improved 5-year survival (25). Systemic administration of other cytokines and interferons to HNC patients demonstrated limited efficacy and had significant toxicity (26). It is worth noting that perilymphatic delivery of cytokines with intent to alter the environment of the tumor-draining lymph nodes appeared to be more successful and better tolerated than systemic delivery of cytokines. Among cytokines tested in HNC, IL-6 emerged as a key player in modulating anti-tumor immune responses, and it may be potentially useful as a prognostic biomarker in HNC (27).

Cancer vaccines

are designed to activate tumor-antigen presentation by antigen presenting cells (APC) to T cells. Vaccines using tumor antigens (TAs) that are tumor-specific and essential for tumor cell survival are preferable. Since no such non-mutated tumor-specific antigens are available for HNC, whole tumor cell vaccines or vaccines targeting tumor-associated antigens were used, all of which yielded modest results. Vaccination strategies included protein or peptide vaccines, DNA based vaccines encoding TAs or recombinant viral or bacterial vector-based vaccines containing TA-encoding DNA (28). The HPV vaccines containing bacterial vectors, e.g., vaccinia-based E6/E7 vaccines, showed that HNC patients were able to generate virus-specific CTLs, but this did not translate into robust anti-tumor responses or better outcome (29). Interestingly, a recent report on RNA-seq and WGS in HPV^+ HNCs showed that ¾ of these cancers downregulate expression of E6 and instead express E2 (30). This would suggest that E1-E2 might be better therapeutic targets than E6/E7 and could explain limited efficacy of current therapeutic vaccines for HPV^+ cancers. In contrast to therapeutic vaccines, prophylactic vaccines for HPV, aimed at eliciting virus-neutralizing Abs to prevent initial infection, are showing promising results (31). Systemic or intratumorally-delivered

vaccines containing ex vivo generated autologous DC pulsed with synthetic peptides (32) or loaded with tumor-derived proteins or tumor cells (33) were tested for toxicity and efficacy in patients with HNC . They were found to be nontoxic, feasible but rather laborious to prepare, and largely not efficacious despite multiple repeated deliveries to patients with advanced HNC (32). In aggregate, experience with therapeutic HNC vaccines suggests that the immunogens selected for vaccinations and/or adjuvants used to improve vaccine immunogenicity were not effective in the tolerogenic TME of HNC.

Monoclonal antibodies (MoAbs)

targeting the tumor, its components and products or tumor-induced regulatory cells are the most widely used immunotherapy to date in HNC. Cetuximab, a mouse–human chimeric immunoglobulin G1 (IgG1) Ab targeting epidermal growth factor receptor (EGFR) was approved by FDA for therapy of HNC in 2006. The EGFR is overexpressed in 80% - 90% of HNSCC and upon binding of EGF promotes tumor cell proliferation, angiogenesis and metastasis. However, cetuximab therapy is effective in only 10–20% of patients with HNC (34). Mechanisms underlying differential clinical responses to cetuximab are unrelated to the EGFR expression levels. Cetuximab mediates antibody-dependent cytotoxicity (ADCC) and, in addition to activating NK cells for cytotoxicity, it promotes NK-DC cross-talk, up-regulates the antigen processing machinery in DC and priming of TA-specific CD8+ T cells (35). Cetuximab also increases the frequency and suppressor functions of $CD4^+CD39^+CD25^+$ Treg but only in HNC patients who are non-responders to therapy (36). As Treg suppress functions of NK cells which mediate ADCC and of newly-induced TA-specific CTLs, expansion of Treg by cetuximab might account for the patients' unresponsiveness to therapy. Panitumumab, a fully humanized MoAb specific for EGFR was used for therapy of patients with recurrent or metastatic HNC in the phase III SPECTRUM trial comparing cisplatin and fluorouracil ± panitumumab (37). The results showed improved progression-free survival (PFS) but not overall survival (OS). This contrasted with the results of the EXTREME study, where patients were randomized between cisplatin (or carboplatin) and 5-fluorouracil with or without cetuximab. In this trial, OS was improved in patients receiving chemoimmunotherapy (38). The outcome differences observed between panitumumab and cetuximab, both of which bind to the EGFR, illustrates the complexity of the immune interactions in the TME, calling attention to the need for a better understanding of cellular/molecular mechanisms that are invoked by immunotherapies in patients with HNC.

Immune checkpoint blockade in HNC

Current Immunotherapy of HNC is focused on targeting T-cell inhibitory receptors that function as immune checkpoints responsible for maintaining the balance between activation and inhibition of immune responses. Tumors have learned to co-opt the immune checkpoints as a major mechanism of resistance. The inhibitory receptors, CTLA-4, PD-1 and others, act as breaks guarding against the danger of excessive, potentially dangerous, T-cell activation. In the TME, numbers of these receptors on T cells increase as does their suppressive function (15). The immune checkpoint inhibitors (ICIs), that block CTLA-4 (e.g., ipilimumab) or PD-1 (e.g., nivolimumab, pembrolizumab), release the breaks imposed by

tumor-derived signals and allow for T cells to resume their immune activities. In phase III clinical trials (CHECKMATE-141 or KEYNOTE-012) anti-PD1 Abs were shown to successfully rejuvenate anti-tumor immunity and produce durable clinical responses in a subset of HNC patients with refractory/metastatic HNC (39, 40). The overall clinical experience from several trials with different ICIs indicates that only some patients (~15%) with refractory/metastatic HNC achieved durable remissions and prolonged survival (41). Although most patients with advanced HNC do not respond to ICIs, PD-1 targeted therapies are emerging as standard of care for platinum refractory/metastatic HNC. This said, it is important to note that some of the observed clinical responses occurred in HNC patients whose tumors expressed minimal levels or no PD-L1 (39). Thus, it is unclear why only some patients respond to PD-1/PD-L1-targeted therapy. Given the lack of any therapy for HNC patients who do not respond to anti-PD-1 Abs, there is an urgent unmet need to identify the molecular determinants in the TME that are responsible for resistance to ICIs.

Targeting other immunoinhibitory mechanisms in HNC

Subversion by the tumor of immune checkpoints is but one way of orchestrating immune escape. The therapeutic efficacy of ICIs may be limited because immune dysfunction in the TME of HNC is mediated by other tumor-driven mechanisms (see Table 1). Immunohistochemistry of HNC specimens shows that these tumors produce a wealth of immunoinhibitory factors, including IL-10, TGF-β, arginase, PGE_2 and others (5). Among these, TGF-β has been shown to attenuate activity of CD8+ T cells, skew the differentiation of CD4+ helper T cells (Th1) toward Treg and Th17 cells (Figure 2), which promote tumor growth and limit development of central memory T cells (42). About 50% of HNC patients lose SMAD4 activity via inactivating mutations or loss of heterozygosity (LOH), which leads to elevated TGF-β levels and HNC formation in mice (43). Thus, in the TME of HNC, TGF-β significantly contributes to immune dysfunction and is an important therapeutic target. The ADO pathway is a well-known contributor to immune dysfunction in HNC (44) and is viewed as a significant barrier to effective immunotherapies. Pharmacologic inhibitors, siRNAs or antibodies specific for the components of this pathway or for ADO receptors show efficacy in pre-clinical studies and are entering the clinical arena. Several strategies for blocking ADO, including inhibitors of ectonucleotidases, CD39 and CD73, are currently in clinical trials. Data from a recent trial with MEDI9447, a MoAb which blocks CD73 and ADO synthesis, showed that the observed inhibition of tumor growth was associated with the reversal of ADO-mediated T-cell suppression (45). In the TME of HNC, ADO may operate in synergy with the COX-2/PGE_2 pathway, which is overexpressed in HNC and has been linked to HNC progression and poor outcome (15). PGE_2, a product of COX-2 activity, binds to four G-protein coupled receptors on responder cells and its signaling leads to cAMP-dependent suppression of immune cell functions (15). Attempts to *in vivo* block PGE_2-mediated suppression by using COX-2 inhibitors (e.g., rofecoxib, celecoxib) in HNC induced anti-tumor effects and restored anti-tumor immunity, but were abandoned due to unacceptable toxicity (46). More recently, the chronic use of non-steroidal anti-inflammatory drugs (NSAIDs) was reported to be associated with improved DFS and OS in patients with stage III colorectal cancer (CRC) whose tumors overexpressed COX-2

(47). It has been suggested that chronic intake of aspirin might have similar salutary effects in HNC.

Activation of immune cells in HNC

As immune checkpoint inhibitors release T cells from tumor-imposed suppression, survival and anti-tumor functions of these "rescued" cells need to be maintained and fostered. To this end, another strategy has emerged for enhancing positive co-stimulatory pathways, such as CD40, OX40 or CD137, and providing cytokines, e.g., IL-2, IL7, IL-12 or IL15 for immune cell activation and expansion. Agonists for CD40 (CP-870,893), OX40 (MED16469) and CD137 (BMS 663519 and PF 05082566) are being investigated in clinical trials, some designed for HNC patients. These agonists are frequently being used in combination with cetuximab or nivolumab in clinical trials, based on the evidence that agonistic anti-CD137 MoAbs potentiate T cell anti-tumor functions and stimulate NK activity (48). It is worth noting that immune cells in the TME that co-express stimulatory CD137 and inhibitory receptors such as PD-1 could be readily re-directed toward activation pathways by immunotherapies (49). Toll-like receptor (TLR) agonists, such as TLR-8, induce maturation and cross-priming of DC and up-regulate NK cell dependent lysis of tumor cells in combination with cetuximab (50).

Adoptive cell therapy in HNC

Adoptive cell-based therapies (ACT) used for treatment of cancer involve transfer of *ex vivo* expanded tumor-reactive T cells into patients. Prior to transfer, cultured T cells can be modified or engineered to improve their *in vivo* anti-tumor activity. This form of immunotherapy has been infrequently used in HNC patients, because of limitations related to expansion of TA-specific T cells and the paucity in HNC of well- defined molecular targets that could be effectively used for engineering of T cells to increase their recruitment to tumors and boost their anti-tumor cytotoxicity. In an early phase I clinical trial for HNC patients with recurrent/ metastatic HNC, 35% of patients who were treated with T cells controlled tumor growth (51). In a more recent trial, adoptive T-cell transfers after chemotherapy were performed in patients with resectable HNC, and the patients who received T-cells had improved OS (52).

Cancer stem cells in HNC

In HNC, cancer stem cells have been linked to treatment failure, resistance to therapy, recurrence and metastasis (53). These small populations of highly tumorigenic, self-renewing, therapy-resistant cells are CD44+, have high levels of active aldehyde dehydrogenase ($ALDH^{high}$) and are sensitive to immunotherapy with CSC-primed CD8+ T cells *in vitro* and *in vivo* (54). These and other results from *in vivo* studies targeting stem cells in mice, suggest that immunotherapy may be effective in eliminating this subset of chemotherapy-resistant tumorigenic cells (55).

Future of immunotherapy for HNC

After several years of initial experience with immunotherapy of HNC, it is possible to predict that within next few years it will become fully integrated into the spectrum of conventional HNC therapies. Immune therapies emerge as non-toxic, highly specific, targeted treatments that can be used as monotherapies or in combination with conventional therapies or drugs that block tumor escape. Immunotherapies given to HNC patients can ameliorate or restore compromised TA-specific immune responses and decrease/eliminate tumor-induced suppression of immune effector cells (Table 2). They can induce TA-specific memory responses which might be able to prevent tumor recurrence and provide long-term survival benefits. They are effective in silencing cancer stem cells. The limited beneficial effects of ICIs in HNC emphasize the fact that pervasive immune suppression is the major barrier to effective therapies in HNC. This has intensified the search for new actionable immune checkpoints within the TME of HNC and the development of new therapies that will be needed for more effective restoration of immune competence. HNC is a heterogeneous disease, encompassing HPV+ and HPV^{neg} cancers among other clinicopathological subtypes, all requiring distinct therapeutic approaches. Immunotherapy offering a wide variety of therapeutic strategies will be especially useful in meeting this need.

There are many challenges to making immunotherapy a standard of care in HNC. Foremost is the selection of immune therapy likely to benefit the patient. Extensive genetic, molecular and immunological evaluations of the TME in HNC (5, 56) suggest that immune therapies, while rational in view of the existing prevalent immune suppression in this cancer, will have to be carefully selected to fit with the suppression profile of each tumor and with the previous or concurrent therapy being administered to each patient (Table 3). No biomarkers are currently available to inform either the selection of therapy or outcome. It appears, however, that immunotherapy of HNC will be greatly facilitated by technical advances in the field of immunogenetics. Recent findings from deep sequencing of the HNC genome indicate that a large diversity of genetic alterations exist in these cancers (57). Most of these alterations seem to fall within a few major biological pathways, as if those pathways that best promote tumor growth are aberrantly activated via genetic alterations. There is evidence, for example, that molecular signaling of *PIK3CA*, the most commonly mutated oncogene in HNC (58), is involved in regulating activity of the COX-PGE_2 pathway, the most immunoinhibitory molecular axis in the TME of HNC (17). In the near future, the ability to identify immune defects resulting from genetic aberrations in HNC will enable us to map the immunogenetic profiles of each HNC. This in turn will allow for profiling of specific immune dysfunctions in the TME and for selection of immune therapies that are likely to correct the existing defects. The ability to identify patients who can benefit from immune therapy will improve outcome. Further, using whole genome sequencing to identify point mutations or other genetic aberrations that can be recognized by T cells provides motivation for producing new vaccines that target neoantigens with high efficiency and are strongly immunogenic. Additional advantages will come from the discovery of novel biomarkers of outcome or response to therapy. Already technologies are available for establishing immune-cell specific signatures of tumors using gene microarrays, flow

cytometry, RNAseq and Ab-based protein arrays. The availability of these data will be critical in search for and validation of biomarkers of prognosis, response to therapy or outcome. The development of simple blood tests for assessments of the mutational burden or for emergence of microsatellite instability (MSI) after chemotherapy is in progress (59) and will greatly facilitate linking of genetic alterations to changes in the specific molecular pathways signaling immune dysfunction. Overall, high mutational activity in HNCs bodes well for the application of immunogenetic approaches to personalize future immune therapies based upon silencing of the major immunosuppressive molecular pathways driven via the identified genetic alterations.

Acknowledgments

This study was supported in part by NIH grants R01 CA168628 and R21 CA205644 to TLW.

References

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2015. CA Cancer J Clin. 2015; 65:5–29. [PubMed: 25559415]
2. Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer. 2015; 136:E359–86. [PubMed: 25220842]
3. Cooper JS, Pajak TF, Forastiere AA, Jacobs J, Campbell BH, Saxman SB, et al. Postoperative concurrent radiotherapy and chemotherapy for high-risk squamous-cell carcinoma of the head and neck. N Engl J Med. 2004; 350:1937–44. [PubMed: 15128893]
4. Vogelstein B, Kinzler KW. Cancer genes and the pathways they control. Nat Med. 2004; 10:789–99. [PubMed: 15286780]
5. Ferris RL. Immunology and Immunotherapy of Head and Neck Cancer. J Clin Oncol. 2015; 33:3293–304. [PubMed: 26351330]
6. Weinberger PM, Yu Z, Haffty BG, Kowalski D, Harigopal M, Brandsma J, et al. Molecular classification identifies a subset of human papillomavirus--associated oropharyngeal cancers with favorable prognosis. J Clin Oncol. 2006; 24:736–47. [PubMed: 16401683]
7. Badoual C, Hans S, Rodriguez J, Peyrard S, Klein C, Agueznay Nel H, et al. Prognostic value of tumor-infiltrating CD4+ T-cell subpopulations in head and neck cancers. Clin Cancer Res. 2006; 12:465–72. [PubMed: 16428488]
8. Lukesova E, Boucek J, Rotnaglova E, Salakova M, Koslabova E, Grega M, et al. High level of Tregs is a positive prognostic marker in patients with HPV-positive oral and oropharyngeal squamous cell carcinomas. Biomed Res Int. 2014; 2014:303929. [PubMed: 24864233]
9. Chakravarthy A, Henderson S, Thirdborough SM, Ottensmeier CH, Su X, Lechner M, et al. Human Papillomavirus Drives Tumor Development Throughout the Head and Neck: Improved Prognosis Is Associated With an Immune Response Largely Restricted to the Oropharynx. J Clin Oncol. 2016; 34:4132–41. [PubMed: 27863190]
10. Lesterhuis WJ, Punt CJ, Hato SV, Eleveld-Trancikova D, Jansen BJ, Nierkens S, et al. Platinum-based drugs disrupt STAT6-mediated suppression of immune responses against cancer in humans and mice. J Clin Invest. 2011; 121:3100–8. [PubMed: 21765211]
11. Reichert TE, Strauss L, Wagner EM, Gooding W, Whiteside TL. Signaling abnormalities, apoptosis, and reduced proliferation of circulating and tumor-infiltrating lymphocytes in patients with oral carcinoma. Clin Cancer Res. 2002; 8:3137–45. [PubMed: 12374681]
12. Hoffmann TK, Dworacki G, Tsukihiro T, Meidenbauer N, Gooding W, Johnson JT, et al. Spontaneous apoptosis of circulating T lymphocytes in patients with head and neck cancer and its clinical importance. Clin Cancer Res. 2002; 8:2553–62. [PubMed: 12171883]

13. Chikamatsu K, Sakakura K, Whiteside TL, Furuya N. Relationships between regulatory T cells and CD8+ effector populations in patients with squamous cell carcinoma of the head and neck. Head Neck. 2007; 29:120–7. [PubMed: 17103408]
14. Figueiro F, Muller L, Funk S, Jackson EK, Battastini AM, Whiteside TL. Phenotypic and functional characteristics of CD39high human regulatory B cells (Breg). Oncoimmunology. 2016; 5:e1082703. [PubMed: 27057473]
15. Jie HB, Gildener-Leapman N, Li J, Srivastava RM, Gibson SP, Whiteside TL, et al. Intratumoral regulatory T cells upregulate immunosuppressive molecules in head and neck cancer patients. Br J Cancer. 2013; 109:2629–35. [PubMed: 24169351]
16. Whiteside TL, Jackson EK. Adenosine and prostaglandin e2 production by human inducible regulatory T cells in health and disease. Front Immunol. 2013; 4:212. [PubMed: 23898333]
17. Camacho M, Leon X, Fernandez-Figueras MT, Quer M, Vila L. Prostaglandin E(2) pathway in head and neck squamous cell carcinoma. Head Neck. 2008; 30:1175–81. [PubMed: 18642283]
18. Bell D, Hanna EY, Miele L, Roberts D, Weber RS, El-Naggar AK. Expression and significance of notch signaling pathway in salivary adenoid cystic carcinoma. Ann Diagn Pathol. 2014; 18:10–3. [PubMed: 24238845]
19. Whiteside TL, Letessier E, Hirabayashi H, Vitolo D, Bryant J, Barnes L, et al. Evidence for local and systemic activation of immune cells by peritumoral injections of interleukin 2 in patients with advanced squamous cell carcinoma of the head and neck. Cancer Res. 1993; 53:5654–62. [PubMed: 8242620]
20. Cortesina G, De Stefani A, Majore L, Forni G, Galeazzi E. Loco-regional treatment with low and high doses of interleukin-2 of head and neck squamous cell carcinoma recurrences. Acta Otorhinolaryngol Ital. 1994; 14:3–9.
21. De Stefani A, Forni G, Ragona R, Cavallo G, Bussi M, Usai A, et al. Improved survival with perilymphatic interleukin 2 in patients with resectable squamous cell carcinoma of the oral cavity and oropharynx. Cancer. 2002; 95:90–7. [PubMed: 12115321]
22. Chi KH, Myers JN, Chow KC, Chan WK, Tsang YW, Chao Y, et al. Phase II trial of systemic recombinant interleukin-2 in the treatment of refractory nasopharyngeal carcinoma. Oncology. 2001; 60:110–5. [PubMed: 11244324]
23. Egan JE, Quadrini KJ, Santiago-Schwarz F, Hadden JW, Brandwein HJ, Signorelli KL. IRX-2, a novel in vivo immunotherapeutic, induces maturation and activation of human dendritic cells in vitro. J Immunother. 2007; 30:624–33. [PubMed: 17667526]
24. Berinstein NL, Wolf GT, Naylor PH, Baltzer L, Egan JE, Brandwein HJ, et al. Increased lymphocyte infiltration in patients with head and neck cancer treated with the IRX-2 immunotherapy regimen. Cancer Immunol Immunother. 2012; 61:771–82. [PubMed: 22057678]
25. Wolf GT, Fee WE Jr, Dolan RW, Moyer JS, Kaplan MJ, Spring PM, et al. Novel neoadjuvant immunotherapy regimen safety and survival in head and neck squamous cell cancer. Head Neck. 2011; 33:1666–74. [PubMed: 21284052]
26. Urba SG, Forastiere AA, Wolf GT, Amrein PC. Intensive recombinant interleukin-2 and alpha-interferon therapy in patients with advanced head and neck squamous carcinoma. Cancer. 1993; 71:2326–31. [PubMed: 8453554]
27. Duffy SA, Taylor JM, Terrell JE, Islam M, Li Y, Fowler KE, et al. Interleukin-6 predicts recurrence and survival among head and neck cancer patients. Cancer. 2008; 113:750–7. [PubMed: 18536030]
28. Schlom J, Hodge JW, Palena C, Tsang KY, Jochems C, Greiner JW, et al. Therapeutic cancer vaccines. Adv Cancer Res. 2014; 121:67–124. [PubMed: 24889529]
29. Skeate JG, Woodham AW, Einstein MH, Da Silva DM, Kast WM. Current therapeutic vaccination and immunotherapy strategies for HPV-related diseases. Hum Vaccin Immunother. 2016; 12:1418–29. [PubMed: 26835746]
30. Nulton TJ, Olex AL, Dozmorov M, Morgan IM, Windle B. Analysis of The Cancer Genome Atlas sequencing data reveals novel properties of the human papillomavirus 16 genome in head and neck squamous cell carcinoma. Oncotarget. 2017; 8:17684–99. [PubMed: 28187443]

31. Herrero R, Quint W, Hildesheim A, Gonzalez P, Struijk L, Katki HA, et al. Reduced prevalence of oral human papillomavirus (HPV) 4 years after bivalent HPV vaccination in a randomized clinical trial in Costa Rica. PLoS One. 2013; 8:e68329. [PubMed: 23873171]
32. Schuler PJ, Harasymczuk M, Visus C, Deleo A, Trivedi S, Lei Y, et al. Phase I dendritic cell p53 peptide vaccine for head and neck cancer. Clin Cancer Res. 2014; 20:2433–44. [PubMed: 24583792]
33. Whiteside TL, Ferris RL, Szczepanski M, Tublin M, Kiss J, Johnson R, et al. Dendritic cell-based autologous tumor vaccines for head and neck squamous cell carcinoma. Head Neck. 2016; 38(Suppl 1):E494–501. [PubMed: 25735641]
34. Bauman JE, Ferris RL. Integrating novel therapeutic monoclonal antibodies into the management of head and neck cancer. Cancer. 2014; 120:624–32. [PubMed: 24222079]
35. Srivastava RM, Lee SC, Andrade Filho PA, Lord CA, Jie HB, Davidson HC, et al. Cetuximab-activated natural killer and dendritic cells collaborate to trigger tumor antigen-specific T-cell immunity in head and neck cancer patients. Clin Cancer Res. 2013; 19:1858–72. [PubMed: 23444227]
36. Jie HB, Schuler PJ, Lee SC, Srivastava RM, Argiris A, Ferrone S, et al. CTLA-4(+) Regulatory T Cells Increased in Cetuximab-Treated Head and Neck Cancer Patients Suppress NK Cell Cytotoxicity and Correlate with Poor Prognosis. Cancer Res. 2015; 75:2200–10. [PubMed: 25832655]
37. Vermorken JB, Stohlmacher-Williams J, Davidenko I, Licitra L, Winquist E, Villanueva C, et al. Cisplatin and fluorouracil with or without panitumumab in patients with recurrent or metastatic squamous-cell carcinoma of the head and neck (SPECTRUM): an open-label phase 3 randomised trial. Lancet Oncol. 2013; 14:697–710. [PubMed: 23746666]
38. Vermorken JB, Mesia R, Rivera F, Remenar E, Kawecki A, Rottey S, et al. Platinum-based chemotherapy plus cetuximab in head and neck cancer. N Engl J Med. 2008; 359:1116–27. [PubMed: 18784101]
39. Ferris RL, Blumenschein G Jr, Fayette J, Guigay J, Colevas AD, Licitra L, et al. Nivolumab for Recurrent Squamous-Cell Carcinoma of the Head and Neck. N Engl J Med. 2016; 375:1856–67. [PubMed: 27718784]
40. Chow LQ, Haddad R, Gupta S, Mahipal A, Mehra R, Tahara M, et al. Antitumor Activity of Pembrolizumab in Biomarker-Unselected Patients With Recurrent and/or Metastatic Head and Neck Squamous Cell Carcinoma: Results From the Phase Ib KEYNOTE-012 Expansion Cohort. J Clin Oncol. 2016
41. Xie X, O'Neill W, Pan Q. Immunotherapy for head and neck cancer: the future of treatment? Expert Opin Biol Ther. 2017:1–8.
42. White RA, Malkoski SP, Wang XJ. TGFbeta signaling in head and neck squamous cell carcinoma. Oncogene. 2010; 29:5437–46. [PubMed: 20676130]
43. Bedi A, Chang X, Noonan K, Pham V, Bedi R, Fertig EJ, et al. Inhibition of TGF-beta enhances the in vivo antitumor efficacy of EGF receptor-targeted therapy. Mol Cancer Ther. 2012; 11:2429–39. [PubMed: 22927667]
44. Whiteside TL. Targeting adenosine in cancer immunotherapy: a review of recent progress. Expert Rev Anticancer Ther. 2017
45. Hay CM, Sult E, Huang Q, Mulgrew K, Fuhrmann SR, McGlinchey KA, et al. Targeting CD73 in the tumor microenvironment with MEDI9447. Oncoimmunology. 2016; 5:e1208875. [PubMed: 27622077]
46. Lang S, Tiwari S, Andratschke M, Loehr I, Lauffer L, Bergmann C, et al. Immune restoration in head and neck cancer patients after in vivo COX-2 inhibition. Cancer Immunol Immunother. 2007; 56:1645–52. [PubMed: 17387473]
47. Liao X, Lochhead P, Nishihara R, Morikawa T, Kuchiba A, Yamauchi M, et al. Aspirin use, tumor PIK3CA mutation, and colorectal-cancer survival. N Engl J Med. 2012; 367:1596–606. [PubMed: 23094721]
48. Bauman JE, Grandis JR. Targeting secondary immune responses to cetuximab: CD137 and the outside story. J Clin Invest. 2014; 124:2371–5. [PubMed: 24837438]

49. Srivastava RM, Trivedi S, Concha-Benavente F, Gibson SP, Reeder C, Ferrone S, et al. CD137 Stimulation Enhances Cetuximab-Induced Natural Killer: Dendritic Cell Priming of Antitumor T-Cell Immunity in Patients with Head and Neck Cancer. Clin Cancer Res. 2017; 23:707–16. [PubMed: 27496866]
50. Stephenson RM, Lim CM, Matthews M, Dietsch G, Hershberg R, Ferris RL. TLR8 stimulation enhances cetuximab-mediated natural killer cell lysis of head and neck cancer cells and dendritic cell cross-priming of EGFR-specific CD8+ T cells. Cancer Immunol Immunother. 2013; 62:1347–57. [PubMed: 23685782]
51. To WC, Wood BG, Krauss JC, Strome M, Esclamado RM, Lavertu P, et al. Systemic adoptive T-cell immunotherapy in recurrent and metastatic carcinoma of the head and neck: a phase 1 study. Arch Otolaryngol Head Neck Surg. 2000; 126:1225–31. [PubMed: 11031409]
52. Jiang P, Zhang Y, S JA, Wang H. Adoptive cell transfer after chemotherapy enhances survival in patients with resectable HNSCC. Int Immunopharmacol. 2015; 28:208–14. [PubMed: 26066298]
53. Davis SJ, Divi V, Owen JH, Bradford CR, Carey TE, Papagerakis S, et al. Metastatic potential of cancer stem cells in head and neck squamous cell carcinoma. Arch Otolaryngol Head Neck Surg. 2010; 136:1260–6. [PubMed: 21173377]
54. Visus C, Ito D, Amoscato A, Maciejewska-Franczak M, Abdelsalem A, Dhir R, et al. Identification of human aldehyde dehydrogenase 1 family member A1 as a novel CD8+ T-cell-defined tumor antigen in squamous cell carcinoma of the head and neck. Cancer Res. 2007; 67:10538–45. [PubMed: 17974998]
55. Visus C, Wang Y, Lozano-Leon A, Ferris RL, Silver S, Szczepanski MJ, et al. Targeting ALDH(bright) human carcinoma-initiating cells with ALDH1A1-specific CD8(+) T cells. Clin Cancer Res. 2011; 17:6174–84. [PubMed: 21856769]
56. Stransky N, Egloff AM, Tward AD, Kostic AD, Cibulskis K, Sivachenko A, et al. The mutational landscape of head and neck squamous cell carcinoma. Science. 2011; 333:1157–60. [PubMed: 21798893]
57. The Cancer Genome Atlas N. Comprehensive genomic characterization of head and neck squamous cell carcinomas. Nature. 2015; 517:576–82. [PubMed: 25631445]
58. Lui VW, Hedberg ML, Li H, Vangara BS, Pendleton K, Zeng Y, et al. Frequent mutation of the PI3K pathway in head and neck cancer defines predictive biomarkers. Cancer Discov. 2013; 3:761–9. [PubMed: 23619167]
59. Parsons HA, Beaver JA, Park BH. Circulating Plasma Tumor DNA. Adv Exp Med Biol. 2016; 882:259–76. [PubMed: 26987539]
60. Cheng F, Wang HW, Cuenca A, Huang M, Ghansah T, Brayer J, et al. A critical role for Stat3 signaling in immune tolerance. Immunity. 2003; 19:425–36. [PubMed: 14499117]
61. Geiger JL, Grandis JR, Bauman JE. The STAT3 pathway as a therapeutic target in head and neck cancer: Barriers and innovations. Oral Oncol. 2016; 56:84–92. [PubMed: 26733183]
62. Kortylewski M, Xin H, Kujawski M, Lee H, Liu Y, Harris T, et al. Regulation of the IL-23 and IL-12 balance by Stat3 signaling in the tumor microenvironment. Cancer Cell. 2009; 15:114–23. [PubMed: 19185846]
63. Albesiano E, Davis M, See AP, Han JE, Lim M, Pardoll DM, et al. Immunologic consequences of signal transducers and activators of transcription 3 activation in human squamous cell carcinoma. Cancer Res. 2010; 70:6467–76. [PubMed: 20682796]
64. Wang T, Niu G, Kortylewski M, Burdelya L, Shain K, Zhang S, et al. Regulation of the innate and adaptive immune responses by Stat-3 signaling in tumor cells. Nat Med. 2004; 10:48–54. [PubMed: 14702634]
65. Yu H, Pardoll D, Jove R. STATs in cancer inflammation and immunity: a leading role for STAT3. Nat Rev Cancer. 2009; 9:798–809. [PubMed: 19851315]
66. Li J, Jie HB, Lei Y, Gildener-Leapman N, Trivedi S, Green T, et al. PD-1/SHP-2 inhibits Tc1/Th1 phenotypic responses and the activation of T cells in the tumor microenvironment. Cancer Res. 2015; 75:508–18. [PubMed: 25480946]
67. Francisco LM, Salinas VH, Brown KE, Vanguri VK, Freeman GJ, Kuchroo VK, et al. PD-L1 regulates the development, maintenance, and function of induced regulatory T cells. J Exp Med. 2009; 206:3015–29. [PubMed: 20008522]

68. Amarnath S, Mangus CW, Wang JC, Wei F, He A, Kapoor V, et al. The PDL1-PD1 axis converts human TH1 cells into regulatory T cells. Sci Transl Med. 2011; 3:111ra20.

69. Bornstein S, White R, Malkoski S, Oka M, Han G, Cleaver T, et al. Smad4 loss in mice causes spontaneous head and neck cancer with increased genomic instability and inflammation. J Clin Invest. 2009; 119:3408–19. [PubMed: 19841536]

70. Mandapathil M, Hilldorfer B, Szczepanski MJ, Czystowska M, Szajnik M, Ren J, et al. Generation and accumulation of immunosuppressive adenosine by human CD4+CD25highFOXP3+ regulatory T cells. J Biol Chem. 2010; 285:7176–86. [PubMed: 19858205]

71. Izumchenko E, Sun K, Jones S, Brait M, Agrawal N, Koch W, et al. Notch1 mutations are drivers of oral tumorigenesis. Cancer Prev Res (Phila). 2015; 8:277–86. [PubMed: 25406187]

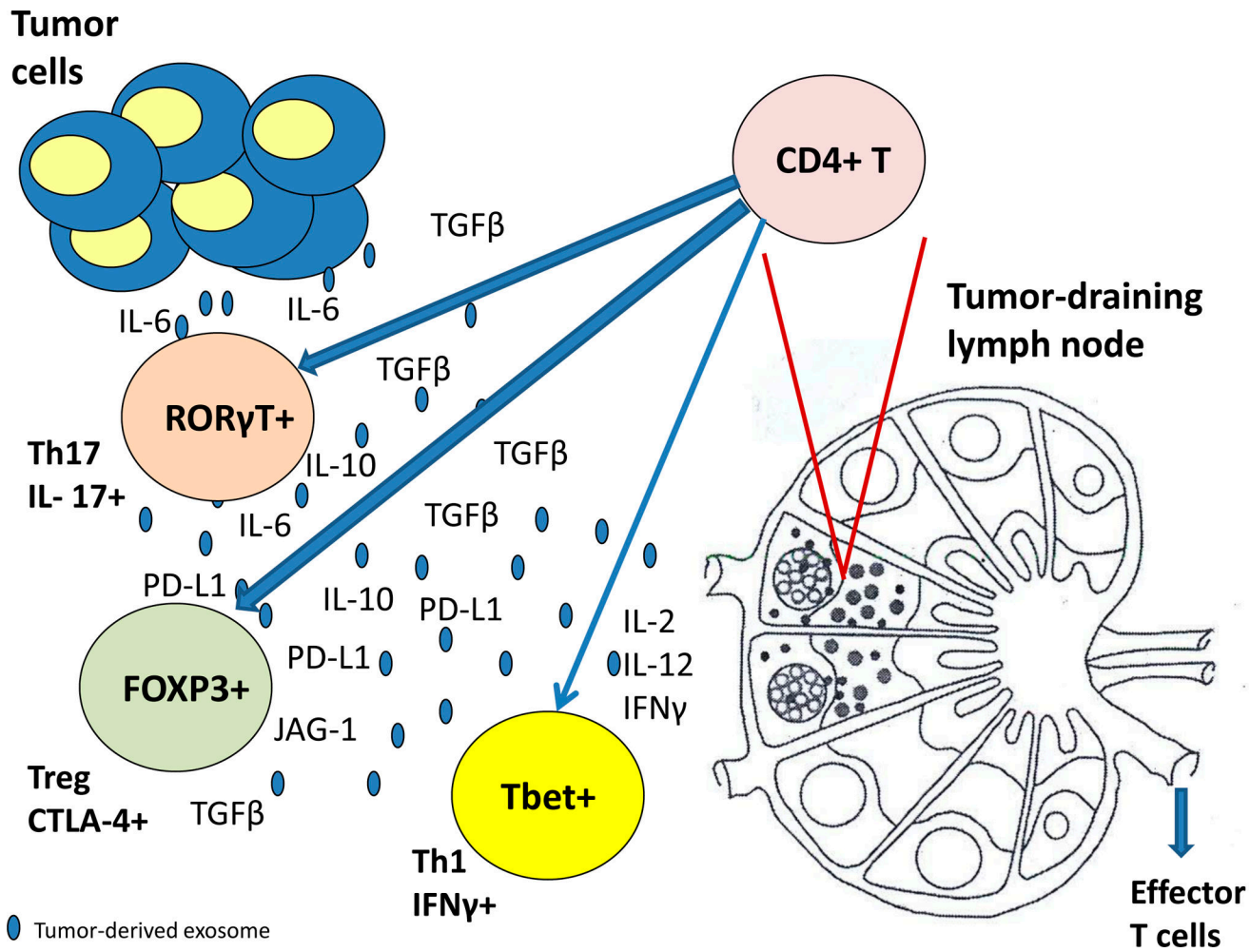

Figure 1.

The TME of HNCs is rich in lymph nodes (LNs) which drain the tumor but also serve as key immune organs for T cell differentiation, maturation and interaction with DCs presenting tumor antigens. The milieu of the tumor-draining LN is saturated by tumor-derived inhibitory factors (TGF-β, IL-10, IL-6, PD-L1, JAG-1) and is deficient in IL-2 and IFNγ. In this cytokine milieu, T cells are polarized to differentiate into Treg or Th17 lineages and away from the IFNγ-producing Th1 effector cells phenotype. As a result, tumor-infiltrating lymphocytes (TIL) are enriched in Treg and pro-inflammatory Th17 cells but lacking in Th1 anti-tumor effector cells. The skewed differentiation of T cells in the TME results in dysregulation of anti-tumor immunity. Tbet, RORγt and FOXP3 are transcription factors that determine T cell lineages. Tumor-derived exosomes carrying immunosuppressive cargoes contribute to the inhibitory environment.

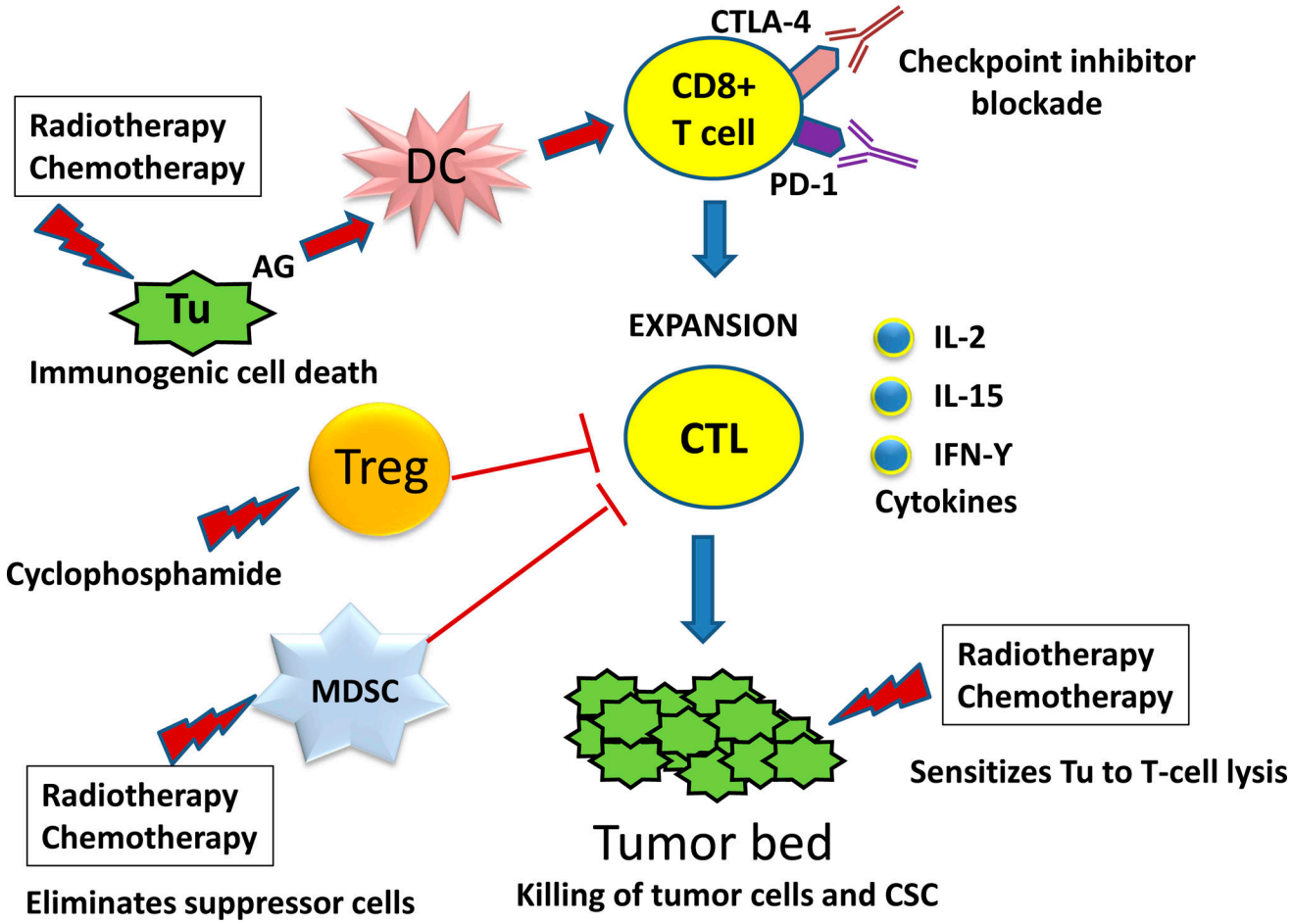

Figure 2.

The rationale for combination of immunotherapy with conventional anti-tumor therapy. The conventional therapy for HNC can (1) induce immunogenic death of tumor cells and release tumor antigens that can be successfully processed and presented to T cells by DCs; (2) eliminate or decrease the numbers of Treg or MDSC which interfere with anti-tumor activity of T cells; and (3) sensitize tumor cells to lysis by immune effector cells. The subsequent blockade of immune checkpoints (CTLA-4, PD-1) allows tumor-reactive T cells to expand and exercise anti-tumor activities. This requires further modification of the TME and the delivery of cytokines that are necessary for expansion of tumor-reactive T cells and for the maintenance of their anti-tumor functions. Ultimately, these CTLs will be responsible for elimination of residual tumor cells. Conventional therapies not only eliminate tumor cells, providing a pool of antigens for presentation to T cells, but they also set the stage for immune cells rejuvenated by immune therapies, such as ICIs, to eliminate residual tumor cells.

Table 1

Molecular pathways overexpressed in the microenvironment of HNCs mediate immune suppression

Dysregulated Signaling Pathways	Effects of signaling on immune or tumor cells	References
EGF/EGFR	↑ tumor resistance to immune attack	5
	↑ production of inhibitory factors/cytokines	
	↑ suppressor functions in Treg	
IL-6, EGF, VEGF/STAT3	↑ tumor resistance to immune attack	59 – 63
	↑ production of inhibitory factors/cytokines	
	↓ DC maturation	
	↓ cytolytic activity of CTL, NK cells	
COX-2/PGE_2	↑ tumor resistance to immune attack	
	↓ immune cell functions via cAMP-mediated signaling	16, 17
PI3K/COX-2/PGE_2	↓ survival of immune cells	
PD-1/PD-L1	↑ autocrine tumor signals, survival	15, 64 – 67
	↓ anti-tumor functions of T, B, NK cells, monocytes	
	↑ Treg expansion and suppressor functions	
TGF-β/TGF-βRI+RII	↑ tumor growth	42, 43, 68
	↓ functions of $CD8^+$Teff polarization of $CD4^+$Tcell differentiation toward Treg and TH17 cells	
Adenosine/$A_{2A}R$	↓ T cell functions via cAMP-mediated signaling	44, 69
CD39/CD73 ectoenzymes	↑ Adenosine production	44,70
	↑ $A_{2A}R$ signaling	
	↓ functions of immune cells	
Fas/FasL	↑ apoptosis of activated $CD8^+$Teff cells	12
JAG-1/NOTCH-1	↑ tumor resistance to immune attack	18, 71
	↓ functions of immune cells	
	↑ Treg proliferation	

Table 2

Key facts about HNCs and immune therapies

- HNCs evolve numerous ways of escape from the host immune system.
- Tumor microenvironment in HNC is strongly immunosuppressive.
- HNCs have high mutational activity.
- Immune profiles of HNC are heterogeneous.
- Immune therapies aim at rejuvenation of anti-tumor immunity
- Responses to checkpoint inhibitors or other immune therapies have been limited.
- Combinations of immune with conventional therapies are in clinical trials.
- Future immune therapies for HNC will be guided by immunogenetics and will be personalized.

Table 3

Future immunotherapy for HNC

1	Immunotherapy will be delivered in combination with conventional therapies (surgery, chemotherapy, radiotherapy) to take advantage of the effects of conventional therapies on: **a.** decrease in the tumor burden **b.** elimination of suppressor cells **c.** increased mutation rates **d.** increased tumor vulnerability to immune cells
2	Different immunotherapeutic strategies for HNC subtypes with different viral (e.g., HPV^{+} vs. HPV^{neg}) or clinicopathological presentations (e.g., “inflamed” vs. “non-inflamed” tumors) will be rationally selected.
3	Personalized immunotherapy for HNC based on immunogenetics and advanced profiling technologies will become a standard of care: **a.** full spectrum of genetic aberrations will be defined **b.** immune signatures of individual tumors will be established **c.** combined analysis of genetic/immunological profiles will become routine **d.** biomarkers for predicting prognosis will be identified
4	selection of optimal immune therapies will be facilitated from many available, including: ■ MoAbs ■ ICIs ■ Neoantigen-targeting vaccines ■ Cytokines ■ Stimulatory receptor agonists ■ ACT ■ Small molecule inhibitors ■ Oncolytic virotherapy ■ CSC inhibitors
5	immune/molecular/genetic monitoring of clinical responses using non-invasive “liquid biopsies” (plasma DNA, exosomes) will become feasible
6	biomarkers of outcome will be defined