BETTER UNDERSTANDING TUMOR–HOST INTERACTION IN HEAD AND NECK CANCER TO IMPROVE THE DESIGN AND DEVELOPMENT OF IMMUNOTHERAPEUTIC STRATEGIES

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Abstract: Head and neck cancers are heavily infiltrated by immune cells, the significance of which is complex. The natural immune response against head and neck tumors, including anti-human papillomavirus (HPV) T cells, and humoral responses has been clearly documented. However, during the course of tumor progression, co-option of the immune system by tumor cells for their own advantage and increased resistance of tumor cells to immune attack also occur. Inflammation and immune subversion to support angiogenesis are key factors promoting tumor growth. Only a better understanding of this tumor–host interaction will permit a rational design of new immunotherapeutic approaches combining immunostimulation with drugs endowed with the ability to counteract immunoevasion mechanisms.

Keywords: head and neck cancer; inflammation; immunosuppression; immunotherapy; tumor escape mechanism

Head and neck cancers are attractive for immunotherapeutic approaches, as epidemiologic studies have disclosed the role of immunity in controlling the growth of some of these tumors. Natural immune responses have been demonstrated against head and neck tumor-associated antigens, and some of these tumors are associated with human papillomavirus (HPV), a viral target for immunotherapy. Superficial localization of these tumors enabled direct intratumoral immunomodulation, with possible local monitoring of the tumor microenvironment during therapy. However, only a better understanding of tumor–
host interaction will permit rational design of new immunotherapeutic approaches that take into account the intrinsic resistance mechanisms of tumors and the co-option of the immune system by tumor cells for their own advantage.

**IMMUNE RECOGNITION AND CONTROL OF HEAD AND NECK CANCERS**

**Epidemiologic Studies.** Many studies have reported a relative increase in the incidence of head and neck squamous cell carcinomas (HNSCCs) in patients with acquired or iatrogenic immunodeficiency. King et al. identified premalignant lip leukoplakia in 13% of patients with renal transplants as compared with 0.6% of control age- and sex-matched individuals, and 10% of these lesions led to squamous cell carcinomas. Analysis of patients who underwent bone marrow transplantation for hematologic malignancies also demonstrated a 17.4-fold increased risk for oral cancer, which was second only to the risk of liver cancer. Some studies mention that the clinical characteristics of these induced tumors after immunosuppression are different from those of patients generally affected by this malignancy, as these tumors appeared in younger people, without male predominance, and the usual risk factors (alcohol, tobacco) associated with head and neck cancers. Compelling evidence strongly suggests that these cancers may most often be HPV-associated HNSCC. Most retrospective studies of HNSCC occurring post–hematopoietic stem cell transplantation have found indirect signs of HPV infection (koilocytosis, hyperkeratosis, and parakeratosis) and when performed, virologic analysis detected HPV in the majority of these HNSCC cases.

**Natural Immune Response against Head and Neck Cancer.** Tumor infiltrating lymphocyte (TIL) cultures from head and neck carcinomas contain T cells which, upon expansion in vitro, can lyse autologous tumor cells in a major histocompatibility (MHC)-class I restricted fashion. As with other tumors, efforts to identify novel tumor-derived antigens using serologic tools or tumor-specific cytotoxic T lymphocytes (CTLs) have resulted in the discovery of both novel HNSCC-specific tumor antigens and tumor-associated antigens shared with other tumors. Tumor antigens belonging to the group of cancer testis antigen initially identified in melanoma were shown to be expressed in many cases of HNSCC. For example, Mage A3 and Mage A4 are expressed in more than 70% of head and neck cancers, and Mage A6, Mage A1, SSX1, and Mage C2 in more than 30% of these patients. This group of antigens has been selected for clinical trials as they are specific to tumors, except for ectopic expression in germinal cells, which do not express HLA molecules and thus could not be recognized by T cells. Promising phase II clinical trials using Mage A3 led to the launch of a phase III trial in lung carcinoma using this antigen.

Other tumor antigens recognized by antibodies or T lymphocytes are mutated proteins (Caspase 8 or P53). The P53 is mutated in more than 80% of HNSCC, and anti-P53 CD4+ and CD8+ T cells were detected against both wild type and mutated P53 neoantigens. Interestingly, none of the tumors derived from patients, where anti-P53 CTL could be generated, adequately present the peptide recognized by the T cells. This suggests immunoselection of epitope loss variant after natural induction of immune response.

Patients with tumors expressing HPV16 had an increased frequency of CD8+ T cells specific for peptides derived from the oncogenic HPV E7 protein, compared with those patients with tumors negative for HPV or normal controls. However, as already observed in cervical carcinoma and other tumors associated with HPV, the frequency of the immune response against HPV is low in HNSCC and is not comparable with other anti-viral (such as Epstein-Barr virus, cytomegalovirus) T cell responses. Chronic infection with HPV may have led to immune-tolerance and anergy of anti-HPV T cells.

The immunogenicity of human tumors based on results previously obtained in melanoma and renal cell carcinoma could thus be extended to head and neck cancer.

**TUMOR ESCAPE MECHANISMS AND IMMUNE SUBVERSION**

Until now, immunotherapy has had low impact in the clinical care of patients with head and neck cancer or other tumors. Lessons from cancer vaccine clinical trials taught us that immune responses elicited in patients with cancer were lower than those observed in healthy subjects,
suggesting a mechanism of immune system tolerance occurring in cancer patients. In addition, even when high levels of antitumor cytotoxic T cells were induced, this did not lead to the cure of tumors, which may be due to the development of resistance mechanisms of tumor cells to immune attack.

Resistance of Tumors to Immune Attack

Defect in Antigen Processing Machinery for HLA-Class I Membrane Expression. The CD8+ T cells recognize a complex composed of short peptides (8–10 amino acids) associated with HLA class I molecules, whereas CD4+ T cells interact with long peptides (12–23 amino acids) bound to HLA class II molecules. Peptides linked to HLA class I molecules are derived from intracellular proteins which are degraded by a multienzymatic complex, the proteasome composed of various constitutive (δ, MB-1, ζ) or inducible subunits (LMP2, LMP7, LMP10). The peptides generated are then transported to the endoplasmic reticulum by the associated transporter antigen processing (TAP) molecules and then loaded onto the HLA class I molecule with the help of various chaperones (tapasin, BiP, calnexin, calreticulin, ERp57, etc). All these molecules involved in the processing of the antigen are part of the antigen processing machinery (APM) and are regulated by interferon (IFN)γ. Defects in either the HLA class I genes or any component of the APM will lead to a decrease in the expression of the HLA I peptide complex at the cell membrane.

The mean frequency of total HLA class I antigen loss in primary and metastatic head and neck cancer is 15% and 40%, respectively. Structural defects in APM are rare, whereas functional abnormalities of the APM components are frequent in HNSCC cells, and could thus explain the defect in HLA class I membrane expression. Gangliosides derived from HNSCC have been shown to downregulate various MHC class I components. These abnormalities may account for the finding that many HNSCC cell lines are resistant in vitro to CTL lysis, and that this recognition is restored by incubation of tumor cells with IFNγ. The expression of most APM components correlated with the extent of CD8+ T cell infiltration in tumor lesions, and downregulation of APM components is associated with unfavorable outcomes in head and neck carcinomas.

Expression of Immune Receptors by Tumor Cells Which Activation Stimulates Anti-Apoptotic Pathways. Recent studies have shown that HNSCC cell lines express TLR-4, an innate immune receptor which binds to lipopolysaccharide (LPS). LPS binding to TLR4 enhanced tumor proliferation, activated phosphatidylinositol 3-kinase/Akt pathway, and induced nuclear NF-κB translocation. The activation of these 2 anti-apoptotic pathways may explain that TLR4 triggering protected tumor cells from lysis mediated by natural killer (NK) cells. The relevance of this observation is substantiated by the constant presence in HNSCC tumors of bacteria and fungi which could interact with TLR4.

The CCR7 binds to CCL19 and CCL21 and as these chemokines are highly expressed in lymph nodes, they create a gradient between peripheral tissue and the lymph node. An upregulation of CCR7 was shown in head and neck tumors and this was associated with nodal metastasis, which may be secondary to chemotaxis of tumor cells by chemokine gradient. In addition, activation of tumor cells by autocrine or paracrine-secreted CCL19 or CCL21, stimulates the pro-survival PI3K/Akt pathway. Blockade of CCR7-mediated signaling may enhance the efficacy of platinum- and epidermal growth factor receptor (EGFR)-based therapies.

Anergy and Tolerization of Immune Cells. Various studies have shown that NK cells and T cells, especially CD8+ T cells, are functionally impaired in patients with head and neck cancer. Indeed, as already observed in other tumors, T cells exhibited decreased proliferation in response to mitogens or antigens, compromised signaling through T cell receptors, and, upon stimulation with tumor antigens, decreased ability to mediate cytotoxicity against tumor targets or to produce Th1-type cytokines. This functional impairment was more pronounced in patients with advanced cancer than in early disease. In line with these results, peripheral antitumor CD8+ T cells from patients with head and neck cancer have an apoptotic phenotype as they bind to annexin, express CD95, and have a decrease of the T cell receptor (TCR)-associated zeta chain, with higher frequency than non antitumor CD8+ T cells in the same patients. This suggests that T cells directed against tumors are specifically inactivated during cancer progression. Tumor cells may have induced this defect in the function of antitumor T cells by...
various mechanisms. First, Galectin-1 has been shown to be increased in HNSCC cell lines.\textsuperscript{32} This molecule inhibits T cell function by promoting T cell apoptosis and blocking T cell activation.\textsuperscript{33} The cytoidal effects of galectin-1 on immune cells probably results from the binding of galectin-1 and its cross-linking on T cell-surface glycoproteins, including CD2, CD3, CD7, CD43, and CD45.\textsuperscript{34} The targeted inhibition of galectin-1 expression in vivo renders mice resistant to tumor challenge, a process requiring an intact CD4\textsuperscript{+} and CD8\textsuperscript{+} T cell response.\textsuperscript{33}

Second, PDL-1, a B7 family molecule which binds to PD-1, has been shown to induce apoptotic death of activated tumor-reactive T cells.\textsuperscript{35} More than 60\% of freshly isolated HNSCC express PDL-1. Administration of B7-H1 blocking monoclonal antibody with adoptive T cell therapy in a murine squamous cell carcinoma model cured 60\% of animals.\textsuperscript{36}

Third, head and neck–derived tumor cells or tumor-derived microvesicles (MV) present in the plasma of patients with head and neck cancer express Fas-L and trigger apoptotic death of activated T cells expressing Fas.\textsuperscript{37,38} In addition, tumor-derived MV were shown to downmodulate NKG2D expression on NK cells and CD8\textsuperscript{+} T cells, inhibiting their cytolytic function.\textsuperscript{39}

Fourth, various cytokines (such as interleukin[IL]-2, IL-5, IL-7, IL-15, IL-21) involved in T cell proliferation bind to cytokine receptors, which share a common gamma chain. After cytokine binding to their receptor, this gamma chain recruits and phosphorolyates Jak3, leading to the induction of STAT 5 and anti-apoptotic bcl-2 family protein which protects T cells from apoptosis. Prostaglandin E2 secreted by head and neck tumor cells downmodulates Jak3 expression, which is associated with impaired IL-2–dependent signal transduction and proliferation.\textsuperscript{40} Transforming growth factor (TGF)-\beta and IL-10 produced by head and neck tumor cells could also suppress antigen-specific CD8\textsuperscript{+} T cell function.\textsuperscript{41,42}

**Co-Optation of the Immune System by Tumor Cells for Their Growth**

*Inflammation and Head and Neck Cancer: Role of Cytokines.* Tumor development and progression is not driven exclusively by the accumulation of genetic changes in the cancer cells,\textsuperscript{43} but rather requires a permissive and supportive tumor microenvironment which may be favored by chronic inflammation,\textsuperscript{44,45} a state often preceding the development of cancer.

Cytokines are examples of molecules that play a major role in the development and efficiency of the immune system but have been hijacked by tumor cells for their own profit. Proinflammatory cytokines (eg, IL-6, TNF\textsubscript{\alpha}, IL-1, IL-8) are rapidly induced after infection or cellular stress and trigger the release of hepatic acute phase protein after the onset of inflammation. In many preclinical models, cytokines showed antitumor activity by a direct inhibitory effect on the growth of tumors or by activating immune cells.\textsuperscript{46} This antitumor activity was essentially observed in grafted murine tumors, where cytokines induce acute inflammation. During the natural history of cancer, tumor cells became progressively resistant to the inhibitory activity of cytokines and these molecules often switched toward a protumor molecule.\textsuperscript{47–49} This passage of antitumor activity to protumor role often coincides with the production of cytokines by tumor cells.\textsuperscript{50} Head and neck tumor cells constitutively released IL-6, IL-8, and TNF\textsubscript{\alpha},\textsuperscript{51–53} and activation of some receptors such as TLR4 on tumor cells increased the production of these cytokines.\textsuperscript{24}

Concentrations of various proinflammatory cytokines (eg, IL-6, TNF\textsubscript{\alpha}, IL-8) or soluble receptors (eg, sIL-2R\alpha) induced by these cytokines are increased in the serum of patients with head and neck cancer and correlated with poor outcome.\textsuperscript{54–58}

A direct role of cytokine in the pathogenesis of head and neck tumors is supported by the fact that smoking, a major risk factor in head and neck cancer, elicits chronic inflammation associated with high serum IL-6 levels compared with healthy donors.\textsuperscript{54} In addition, Betel quid (BQ) chewing, which is popular in Taiwan, India, and many Southeast Asian countries, has a strong association with the risk of oral leukoplakia and oral cancer and is known to induce keratinocyte inflammation by stimulating the production of TNF\textsubscript{\alpha}, IL-6, IL-8, and granulocyte-macrophage colony-stimulating factor (GM-CSF) by keratinocytes.\textsuperscript{59} In addition, functional polymorphism affecting gene expression of IL-6, IL-8, and TNF\textsubscript{\alpha} are strongly associated with an increased risk for oral cancer.\textsuperscript{60}

Various biologic activities of cytokines may explain the protumor role of these molecules. For example, they can act as growth factors for tumor cells and protect tumor cells from...
apoptosis. They may facilitate tumor cell invasion acting on extracellular matrix and proteases. They also favor angiogenesis as endothelial cells express receptors for these proinflammatory cytokines. In addition, activation of the transcription factor NF-κB by these proinflammatory cytokines leads to amplification of the production of these cytokines and of other pro-angiogenic molecules such as vascular endothelial growth factor (VEGF). Genetic or pharmacologic inhibition of NF-κB inhibited expression of IL-1α, IL-6, IL-8, GRO-1, and VEGF by HNSCC cells and tumorigenesis of xenografts in immunodeficient mice. The NF-κB also may be inducibly activated in epithelial cells by exposure to carcinogens present in tobacco products.

All these proinflammatory cytokines are functionally linked to a newly discovered Th17 CD4\(^+\) helper cell lineage characterized by their production of IL-17, IL-6, and TNFα. The IL-17 may exhibit protumour activity via its role in the induction of proinflammatory cytokines, in the stimulation of metalloproteases and in the promotion of angiogenesis. IL-15 also increases the production of proinflammatory cytokines directly or via the upregulation of IL-17. Using microarray analysis, the expression of IL-15 in biopsies of oral cancer was associated with the development of metastasis in these patients. We found intratumoral expression of IL-15 in patients with head and neck cancer and showed that the soluble alpha chain of IL-15 receptor was produced by head and neck tumor cell lines. Interestingly, soluble IL-15Rα did not act in vitro as an IL-15 antagonist but rather as an enhancer of IL-15-induced proinflammatory cytokines (IL-6, TNFα, IL-17). Increased serum soluble IL-15Rα concentrations were found in patients with head and neck cancer and were closely correlated with poor clinical outcome both in terms of locoregional control and survival, even on multivariate analysis. This protumour activity of soluble IL-15Rα based on amplification of the intratumoral inflammatory reaction is an original mechanism developed by tumor cells to subvert the immune system. As already observed for other cytokines, IL-15, up until now, has been viewed as an antitumor cytokine secondary to its role in the activation of CD8\(^+\) T cells and NK cells. During the progression of tumor, the proinflammatory protumoral activity of IL-15 seems to mask the possible beneficial effect of this cytokine for the host.

**Induction and Recruitment of Immunosuppressive Cells**

**Regulatory T cells.** Regulatory CD4\(^+\)CD25\(^+\) T cells (Treg), have emerged as the dominant T-cell population governing peripheral self-tolerance by inhibiting effector T cells. Because the CD3\(^+\)CD4\(^+\)CD25\(^+\) phenotype also identifies activated T cells, new markers such as Foxp3 have been described which, at least in mice, seems to discriminate activated and regulatory CD4\(^+\)CD25\(^+\) T cells. Experiments performed in vitro indicate that cytokines such as TGF-β and IL-2 are essential for the generation of Treg. Immature or dysfunctional antigen presenting cells, often present in tumor microenvironment, may favor the induction and/or cell expansion of this cell type. The mechanisms of suppression used by Treg are variable and, depending on the microenvironment, involve: (1) cell-to-cell contact-independent soluble factors, such as immunoinhibitory cytokines (IL-10, TGF-β) or adenosine; (2) the cell contact-dependent accumulation of cAMP in target cells or target cell death mediated by perforin/granzyme B or death receptor-ligand interactions; (3) the competition for IL-2, between Treg and responders leading to death by starvation of responder cells; (4) results obtained using 2-photon microscopy to follow the behavior of the cells in lymph nodes indicate that Tregs could also interact with DCs preventing them from forming stable contacts with conventional T cells. In animal models, removal of CD4\(^+\)CD25\(^+\) T cells improved immune mediated tumor clearance and enhanced the response to immunotherapy. Due to their role in inhibiting adaptive and innate immunity (NK cells), regulatory T cells were considered as an immunosuppressive mechanism induced by the tumor to counteract antitumor immunity, and several studies have reported poor prognostic value of the presence of Treg in other tumors. As observed in other cancers, the frequency of regulatory CD4\(^+\)CD25\(^+\) T cells was significantly elevated in the peripheral blood of patients with HNSCC, compared with healthy donors. An enrichment of CD4\(^+\)CD25\(^+\) T cells among tumor-infiltrating lymphocytes derived from head and neck cancer has also been reported. Unexpectedly, our group found that tumor infiltration by regulatory Foxp3\(^+\)CD4\(^+\) T cells was positively associated with a better locoregional control of the tumor in patients with head and neck cancer. In this study, multivariate analysis showed that...
the only significant prognostic factors related to locoregional control were T stage and Treg infiltration of the tumor. Other studies subsequently seem to confirm our results. Frequency of regulatory Foxp3\(^+\) CD4\(^+\) T cells was shown to be higher in patients with HNSCC with no evidence of disease after oncologic therapy than in patients with active disease. The presence of a high number of HNSCC-infiltrating CD4\(^+\)CD25\(^+\) lymphocytes was associated with a good prognosis in patients with head and neck cancer. In patients with colorectal carcinoma, follicular lymphoma and Hodgkin lymphoma, it is now well established that a high number of intratumoral Treg is also associated with longer disease-free survival and overall survival even in multivariate analysis. These results are in line with preclinical studies of Erdman et al\(^96\) obtained in APC\(^{Min/+}\) mice, where the tumor suppressor gene, adenomatous polyposis coli (APC), was inactivated, which led to the formation of intestinal adenomas and recapitulated early events in human colorectal cancer. Adoptive transfer of Treg into these mice resulted in the prevention of intestinal adenomas and regression of established tumors that correlated with a decrease in proinflammatory cytokines. In this case, the beneficial effect of Treg may be related to its ability to dampen inflammatory response.

To explain these apparent contradictory results about the prognostic value of Treg in patients with cancer, some data suggest that they may depend on the stage of the tumors, the ratio between effector and regulatory T cells, and the precise phenotype of regulatory T cells. For example, the ability of Treg cells to produce IL-10 and control inflammation is lost in the course of progressive disease in a mouse model of hereditary colon cancer, and instead these cells switch to the production of IL-17. This emphasizes the plasticity of regulatory T cells and their possible conversion to a CD4\(^+\)Foxp3\(^+\)Th17 phenotype with proinflammatory activity.

Other regulatory T cells called Tr1 not expressing Foxp3 and with suppressive activity on effector cells mediated by IL-10 also have been described in patients with head and neck cancer. Tumor-derived prostaglandin E\(_2\) (PGE\(_2\)) controls the induction and expansion of Tr1 cells which are responsible for the suppression of antitumor response.\(^{100}\)

**Myeloid-derived dendritic cells.** Myeloid derived suppressive cells (MDSC) represent a heterogeneous population of myeloid cells comprising granulocytes, immature macrophages, dendritic cells (DCs), and other myeloid cells at earlier stages of differentiation. They can be identified in mice by the expression of CD11b and Gr-1 markers\(^{101}\) and in humans by a CD34\(^+\)CD33\(^+\)CD13\(^+\)CD11b\(^+\)CD14\(^-\)HLA-DR\(^+\) phenotype. They are recruited by tumor-derived soluble factors, such as TGF-\(\beta\), IL-10, VEGF, IL-6, GM-CSF, GCSF, and PGE\(_2\). These cells themselves produce and secrete many proinflammatory mediators, thereby maintaining the autocrine feedback loop and sustaining their numbers in tissues. The MDSC-dependent generation of reactive oxygen species (ROS) and peroxynitrite causes nitration of tyrosines in the T cell receptor and CD8 molecules, preventing the ability of T cells to respond to specific peptides.\(^{103}\)

The MDSCs, present in tumors, constitutively express iNOS and arginase 1, which will generate peroxynitrites under conditions of limited L-arginine availability. The MDSC may also produce immunosuppressive cytokines (eg, IL-10, TGF-\(\beta\)) and upregulate indoleamine-2,3-dioxigenase (IDO) involved in the catabolism of tryptophan, an amino acid essential for T cell differentiation.\(^{101,104}\)

In head and neck cancer, Pak et al\(^{105}\) first reported accumulations of CD34\(^+\) cell-derived myeloid cells with immunosuppressive activity in the peripheral blood of HNSCC patients. These immune inhibitory CD34\(^+\) cells were also present within the cancer mass of patients with HNSCC and in oral squamous cell carcinomabearing mice.\(^{106}\) In head and neck cancer, GM-CSF, G-CSF, and VEGF seems to play a key role in MDSC recruitment and expansion, as tumor cells derived from this cancer secrete GM-CSF, G-CSF, and VEGF. Blockade of GM-CSF inhibited tumor cell invasion in nude mice resulted in a decrease of intratumor myeloid cell recruitment.\(^{107}\)

**Immune Subversion to Support Angiogenesis.** Angiogenesis is a fundamental step in tumor growth and progression, and solid tumors have to induce angiogenesis to obtain the required nutrients and oxygen. Otherwise, tumors do not grow beyond 2 to 3 mm in diameter. In head and neck cancer, angiogenesis is significantly triggered by VEGF, IL-8, and fibroblast growth factor (FGF).\(^{64,108}\) Production of VEGF and IL-8 by tumors may thus lead to the increase of...
angiogenesis, but it also seems that many immunologic cells and mediators in the tumor microenvironment contribute to tumor angiogenesis, which constitutes another example of immune subversion. As already mentioned, activation of NF-κB by proinflammatory cytokines will stimulate the expression of genes (eg, VEGF, IL-8) involved in angiogenesis. Activation of STAT3, a signal transducer and activator of transcription, by IL-6 will lead to the production of VEGF, IL-17 and IL-23, major regulators of inflammation, also promote angiogenesis in a variety of models.

Besides inflammation, immunosuppressive mediators may also participate in angiogenesis. An inducible enzyme responsive to cytokines and growth factors, cyclooxygenase (COX)-2, enhances the synthesis of PGE$_2$, an immunosuppressive prostaglandin. PGE$_2$ plays an important role in the growth of endothelial cells and has been demonstrated to induce angiogenesis.

The MDSCs can also promote tumor growth by supporting tumor angiogenesis, as they produce high levels of the matrix metalloprotease 9, which regulates the bioavailability of vascular endothelial growth factor. The selective deletion of the matrix metalloprotease 9 gene in these cells eliminates their ability to promote tumor growth and inhibits tumor formation.

**Interplay and Global Integration between These Various Resistance and Escape Mechanisms.** Overt inflammation and immunosuppression are often associated in the tumor microenvironment. For example, the proinflammatory cytokine IL-6 in conjunction with TGF-β will permit the differentiation of Th17 cells, which, in turn, will amplify the local recruitment of inflammatory cells and the production of other inflammatory mediators. However, IL-6 also will activate STAT3, a transcription factor overexpressed in 58.9% of head and neck cancers. Activation of STAT3 is responsible for various immunosuppressive activities, such as the blockade of dendritic cell maturation and the release of IL-10, which will inhibit T cells and macrophage activation and downregulate HLA expression. Other factors upregulated in head and neck cancer (eg, IL-10, VEGF) could also increase the expression of STAT3.

In addition, other inflammatory cytokines (eg, TNFα, IL-1) will induce cyclooxygenase-2 (COX-2), which converts arachidonic acids to PGE$_2$, a prostaglandin responsible for various immunosuppressive activities. Indeed, PGE$_2$ has been reported to enhance IL-10 production, downregulate dendritic cell function, and inhibit IL-12 production in dendritic cells. PGE$_2$ facilitated both expansion of Foxp3$^+$CD4$^+$ CD25$^+$ naturally occurring regulatory T cells (nTreg) and induction of interleukin 10$^+$ (IL-10$^+$)CD4$^+$ type 1 regulatory T cells (TTr1) in a COX-2-positive microenvironment. In addition, in mice, COX-2 induces arginase 1 expression in myeloid suppressor cells which participates in the immunosuppressive role of MDSCs. The clinical relevance of this observation is supported by the fact that an overexpression of COX-2 is a common feature of HNSCC and COX-2 production is considered as a negative prognostic factor in HNSCC.

**IMMUNOTHERAPY HAS TO BE COMBINED WITH DRUGS ENDOWED WITH THE ABILITY TO COUNTERACT IMMUNOEVASION MECHANISMS**

**Immunotherapy and Cancer Vaccines Alone Did Not Yield Significant Clinical Results in Head and Neck Cancer.** IL-2 administered as maintenance immunotherapy to patients' responder to chemotherapy showed some immunomodulation (an increase in lymphocytes and NK cells and a decrease of VEGF), but the clinical value of these changes was not assessed. In another trial, IL-2 added to neoadjuvant chemotherapy in patients with advanced head and neck cancer did not show any clinical benefit. Immunostimulation with intratumor administration of mycobacterial heat-shock protein 65 (HSP 65) encoded plasmid, virus modified or irradiated autologous tumor cells led to increased delayed type hypersensitivity (DTH) but only anecdotal clinical responses. These negative results may be explained by the profound immunosuppression present in these patients, and we could hypothesize that the combination of these immunotherapeutic approaches with drugs or molecules with the ability to concomitantly inhibit immunosuppression may improve these clinical results.

**Rationale for Combining Immunotherapy with Molecules Modifying the Tumor Microenvironment and Preliminary Results.** In preclinical models, cancer vaccines targeting HPV synergize with drugs eliminating regulatory T cells. Other molecules inhibiting the interaction between
PD1 and PD-L1 or reducing the intratumoral infiltration of MDSCs have been shown to improve adoptive T cell immunotherapy.\textsuperscript{36,129}

Because inflammation is deleterious in head and neck carcinoma and also amplifies immunosuppression, inhibition of inflammation and related immunosuppression may represent a prerequisite for immunotherapy. Inhibitors of COX-2, a key enzyme at the interface between inflammation and immunosuppression, was assessed in this regard. In mice, COX-2 inhibition reduced Treg cell frequency and activity, attenuated Foxp3 expression in tumor-infiltrating lymphocytes, and decreased tumor burden.\textsuperscript{130} COX-2 inhibition also augments the efficacy of a therapeutic HPV vaccine.\textsuperscript{131} Treatment of patients with HNSCC with COX-2 inhibitors to block production of PGE\textsubscript{2} restored immune functions and increased effector T cell infiltration into the cancer mass.\textsuperscript{132,133} Unfortunately, specific COX-2 inhibitor was taken off the market due to the increased cardiovascular risk as compared to conventional nonsteroidal anti-inflammatory drugs (NSAIDs) inhibiting both COX-1 and COX-2. Interestingly, these latter compounds may substitute to specific COX-2 inhibitor for clinical use. Indeed, NSAIDs such as sulindace, which inhibits both COX-1 and COX-2, can delay the onset and severity of oral cancer in transgenic animals.\textsuperscript{134} In addition, patients with head and neck cancer treated peri-tumorally with indomethacin, a conventional NSAID, combined with low-dose cyclophosphamide to decrease regulatory T-cell activity and IL-2 or a mix of natural cytokine, led to objective clinical responses with modification of tumor microenvironment.\textsuperscript{135,136}

Up to 90\% of head and neck cancers express angiogenic factors such as VEGF produced by tumor cells or by inflammatory infiltrating tumor microenvironment.\textsuperscript{137} Reducing inflammation thus may have some impact on angiogenesis. Conversely, various immune modifications induced by anti-angiogenic therapy, such as the decrease of immunosuppressive cells (regulatory T cells, MDSC, PD1-expressing T cells), immunosuppressive cytokines (IL-10, TGF-β), transcription factor (STAT3), and an increase of interferon gamma T cells and recruitment of antitumor effector T cells within tumors, have recently been reported.\textsuperscript{136–143} All these changes will lead to a more favorable immune competent state and may contribute to the clinical benefit of this therapy. In preclinical models, anti-angiogenic molecules enhance endogenous antitumor response and synergize with immunotherapy protocols.\textsuperscript{142,144,145} Monotherapy with anti-angiogenic drugs did not show significant clinical activity in head and neck cancer and various combined therapies are under evaluation.\textsuperscript{137} The ability of anti-angiogenic to potentiate immune response warrants the development of clinical protocols combining anti-angiogenic and immunotherapy.

Various therapeutic vaccines against HPV and vaccines targeting P53 are being developed and proposed in head and neck cancer.\textsuperscript{146–149} This may represent good opportunities to address the interest of combining them with anti-inflammatory or anti-angiogenic molecules.

In addition, cetuximab, an anti-EGF-R antibody, is effective in 10\% to 20\% of patients with squamous cell carcinoma of the head and neck.\textsuperscript{150} Preliminary data suggest that the particular phenotype of immune cells in the tumor microenvironment may also influence the activity of this antibody.\textsuperscript{151}

Overall, head and neck cancer may in the future represent a cancer model to develop innovative immunotherapy approaches, as potential targets (such as HPV, tumor antigen) have been well characterized, and resistance mechanisms responsible for failure of current immune-mediated therapy (Figure 1). The availability of molecules (inhibitors of regulatory T cells and MDSC, NSAID drugs, or anti-angiogenic molecules) counteracting some immunosuppressive mechanisms will favor their combination with immunotherapy in head and neck cancer to

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**FIGURE 1.** Immunosuppressive and escape mechanisms associated with the development of head and neck cancer.
improve and potentiate immunostimulation strategies.

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