REGULATORY T CELLS: WHAT ROLE DO THEY PLAY IN ANTITUMOR IMMUNITY IN PATIENTS WITH HEAD AND NECK CANCER?

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Abstract: Advances in the treatment modalities for head and neck squamous cell carcinoma (HNSCC) over the last 20 years involving surgery, radiotherapy, chemotherapy, and immunotherapy are not fully reflected in increases in the 5-year survival rates, mainly due to locoregional recurrences and to a lesser extent, distant metastasis. This can, in part, be attributed to the fact that HNSCC induces severe depression of a patient’s immune system. Recent advances in understanding the complex host–tumor interactions have led to the identification of a distinct suppressor cell population known as regulatory T cells that play a crucial role in maintaining T-cell tolerance to self-antigens. Here, we present a critical review of our understanding of the involvement of regulatory T cells in controlling the T-cell immune response in tumor occurrence and progression in HNSCC with an emphasis on current and future immunotherapeutic approaches involving regulatory T cells. ©2008 Wiley Periodicals, Inc. Head Neck 30: 251–261, 2008

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The role of anti-tumor immunity in relation to head and neck squamous cell carcinoma (HNSCC) has been intensely studied in the past few years. It is now becoming clear that the complex interaction between HNSCC and immune cells plays an important part in determining tumor growth and progression.1 In the early 1980s, it was thought that immune cells were under suppression by a distinct subset of “suppressor” T lymphocytes;2 however, the existence of these cells was always doubted by some as was the concept of any distinct “suppressor” cell population. A major boost for the existence of the suppressor or regulatory concept came from Sakaguchi et al in 1995, who clearly demonstrated that self-tolerance could be induced by a subset of CD4+ T lymphocytes, which were eventually termed regulatory T cells (Treg cells).3 Over the past decade it has become clear that these cells are involved in the control of all aspects of immune regulation, with a particular focus on T-cell suppression,
from transplantation to maintenance of normal pregnancy.4

HNSCC, in common with most other tumors, results in a suppressed immune system, with an altered serum cytokine profile and immune cells that function aberrantly. For instance, CD4+ T lymphocytes, obtained from the peripheral circulation or tumor tissues of patients with HNSCC, have demonstrated functional abnormalities, which correlate with this immunosuppressive state.5 The fact that these abnormalities are detected systemically and not only in the tumor region show the importance of the tumor:immune system interaction. The main aim of this article is to review the rapidly expanding field of regulatory T cells and highlight the relevance of these cells in head and neck cancer. Specifically, it describes what Treg cells are and how they contribute to an antitumor immune response. Finally, there is a brief overview of the involvement of Treg cells in the immunotherapeutic strategies as an adjuvant modality in the treatment of patients with head and neck cancer.

IMMUNOSUPPRESSION AND HNSCC

HNSCC is a very good example of a solid tumor that induces severe depression of a patient's immune system. This is likely to result not just because of products secreted by the tumor but also as a by-product of many of the factors that are known to increase the risk of carcinogenesis such as alcoholism, smoking, presence of human papillomavirus 16 and 18, and poor nutrition.6 Katz7 highlighted the need to appreciate the general well-being of the immune system over a decade ago; however, it has only been in the past few years, particularly since Treg cells were defined and tumor vaccination studies using dendritic cell (DCs) have started, that people have become seriously interested in the complex interactions of tumor and host factors. The reason for the interest has been that many vaccination and immunotherapy trials on solid tumors have not worked8 largely because of an inappropriate milieu of cytokines and immune cells both within the tumor microenvironment and the periphery.

During the last few years evidence has emerged on defects in the immune system in patients with head and neck cancer and the association of HNSCC with immunosuppression. Young et al10 in 1996 analyzed the mechanisms of immune suppression in HNSCC by studying tumor and lymph node tissue samples from 273 patients. They showed significantly increased levels of immune inhibitory mediators transforming growth factor-β (TGF-β), prostaglandin E2, granulocyte macrophage colony stimulating factor (GM-CSF), and interleukin (IL-10) secreted by the tumor. They concluded that the mechanisms of immune suppression in HNSCC are associated with an altered function of intratumoral CD4+ T-lymphocytes and reduced cell influx of intratumoral CD8+ and CD4+ T-lymphocytes. Furthermore, Lang et al.,10 having divided the generation of an effective antitumor T-cell response into the 3 key stages of adhesion, recognition, and co-stimulation, showed that the absence of B7 protein co-stimulatory molecule or the MHC antigens by tumor cells both resulted in the failure to induce activation and proliferation of T-cell clones in vitro. Subsequently, work has shown that immune suppression operates at a number of other, non-mutually exclusive points in the recognition and stimulation pathway summarized in Table 1.

Young24 and Whiteside22 have reviewed this subject in HNSCC recently. These reviews divide the mechanisms behind evasion of immune surveillance into 2 groups. Either the tumor cells are poor stimulators of immune cells and hence are simply not recognized, and/or they actively interfere with immune function and survival of the immune cells. Multiple ways have been observed on how HNSCC tumors "hide" from surveillance with the most common being loss of human leukocyte antigen (HLA) class I molecules and hence no tumor-associated antigen (TAA) expression,21 poor expression of co-stimulatory molecules on the tumor cell surface,20 the production of immunosuppressive factors by tumor cells and the pres-

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Abbreviations: Th, T-helper; IL, interleukin; HLA, human leukocyte antigen.
ence of immune-suppressive cells such as Treg cells and, more recently, subsets of natural killer (NK) cells.\textsuperscript{25} (Table 1).

Dysfunctional DCs are another example of how tumors can hinder an immune cell response. The role of DCs in HNSCC has been recently reviewed by Dunn et al.\textsuperscript{26} Briefly, DCs are the most potent antigen presenting cells (APCs), and their interaction with T cells plays a major “pace-maker” role in the immune system. Recent findings have shown that regulatory DCs can be derived from the various factors secreted by tumor cells resulting in inhibition of T-cell proliferation and a cytokine profile that downregulates subsequent antitumor effects. Dysfunctional DCs can also induce the expansion of T cells with regulatory phenotype. Such findings have been demonstrated in HNSCC\textsuperscript{25} as well as in other tumor types.\textsuperscript{27,28} More recently, Kryczek et al.\textsuperscript{29} showed that Treg cells trigger high levels of IL-10 production by DCs which stimulates their expression of B7-H4, and the adoption of an immunosuppressive phenotype.

Hence, it is now widely accepted that regulatory T cells or Treg cells represent the earlier-named “suppressor T-cell” population and that these cells play a pivotal role in controlling the immune response. A review of current knowledge is given below.

**CURRENT UNDERSTANDING OF REGULATORY T CELLS**

The seminal work of Sakaguchi and his team described CD4\textsuperscript{+} T cells that continuously express the alpha chain of IL-2 receptor (CD25).\textsuperscript{3} These cells were shown to be important in inducing and maintaining peripheral self-tolerance, and actively preventing autoimmune diseases by the suppression of self-reactive T cells. A corollary of these functions is that they are also thought to diminish T-cell immunity to TAA and play a major role in preventing successful vaccination and immune-based therapies.\textsuperscript{23}

The use of the surface activation marker CD25 as a specific marker for Treg cells is limited because of the fact that it is also upregulated on activated effector T cells. However, it is possible to subdivide CD4\textsuperscript{+} T cells expression of CD25, as either low/intermediate/or high. The “high” group has been shown to also express the forkheadbox transcription factor P3 (FOXP3) in humans. These CD4\textsuperscript{+} CD25\textsuperscript{high} FOXP3\textsuperscript{+} T cells have a potent suppressive/regulatory capacity both in vivo and in vitro and are now commonly the cell group referred to as Treg cells.\textsuperscript{30} In mice, the entire population of CD4\textsuperscript{+} CD25\textsuperscript{+} T cells seems to confer regulatory activity, while in humans only the CD4\textsuperscript{+} CD25\textsuperscript{high} population have a similar strong regulatory function.\textsuperscript{31} Treg cells have been, and still are, under intense investigation. For instance, even some CD8\textsuperscript{+} T cells share important markers such as CD25 and FOXP3, and/or the same mode of action utilizing IL10, and have also been suggested as a distinct Treg population.\textsuperscript{32,33} Thus the search for definitive markers and an understanding of CD4\textsuperscript{+} Treg trafficking continue.

**SUBTYPES, MODE(S) OF ACTION, AND TRAFFICKING**

The presence of different markers and levels of maturation of Treg cells strongly suggest that regulatory T cells are not a homogeneous group, but that subtypes exist with distinct regulatory activities. Recently, 2 major groups of Treg cells have been described: naturally occurring regulatory T cells (nTreg), and peripherally induced regulatory T cells (iTreg) (Table 2).

<table>
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<tr>
<th>Phenotype</th>
<th>CD4\textsuperscript{+} CD25\textsuperscript{+} FOXP3\textsuperscript{+}</th>
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<tr>
<td>Origin</td>
<td>Thymus</td>
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<tr>
<td>Mechanism of suppression</td>
<td>Cell-Contact in vitro, Cell-Contact, soluble cytokines (IL10, TGF-β), PD-1 pathway in vivo</td>
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<td>Other markers</td>
<td>GITR, CTLA-4, CD127, Nrp1, LAG-3.</td>
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<td>CD25 mAb, denileukin diftitox, cyclophosphamide, GITR mAb, CTLA-4 mAb</td>
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<tr>
<th>Phoenotype</th>
<th>CD4\textsuperscript{+} CD25\textsuperscript{+} IL10\textsuperscript{+}</th>
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<tr>
<td>Origin</td>
<td>Peripheral</td>
</tr>
<tr>
<td>Mechanism of suppression</td>
<td>IL10, TGF-β, IFN-γ</td>
</tr>
<tr>
<td>Other markers</td>
<td>CTLA-4, CD122</td>
</tr>
<tr>
<td>Depleting agents</td>
<td>IL10 mAb, TGF-β mAb, cyclophosphamide</td>
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Abbreviations: Treg, regulatory T cell; IL, interleukin; TGF, transforming growth factor; mAb, monoclonal antibody.
nTreg could have a further subtype that is peripherally induced, whereas iTreg cells are divided into Tr1 and Th3 Treg cells. When either of these groups is stimulated by antigen, or just T-cell receptor (TCR) and CD28 ligation, they can cause immune suppression, eg, suppress autoimmune disease, tumor immunity, or graft rejection.34 But these 2 groups of Treg cells differ in their origin and mode of action, as well as their activation requirements and relative efficacy.35

nTreg cells are those commonly referred to in the literature as Treg cells and classically express the FOXP3 transcription factor.36–38 Several other markers are also associated with nTreg; however, none of them is exclusively linked to the suppressive function of Treg cells, and can certainly be expressed by activated CD4+ cells, which also express CD25 (CD4+CD25+ non-Treg). Such molecules include the glucocorticoid-induced tumor-necrosis factor (GITR) receptor,39 cytolytic T lymphocyte associated antigen 4 protein (CTLA-4),40 low/negative expression of cell surfaceCD127,41,42 and neuropilin-1, a semaphorin III receptor, that has been recently described for the identification of Treg cells from both naïve and recently activated CD4+CD25+ non-Treg cells in humans.43 Another molecule that has been identified is LAG-3, an MHC class II binding CD4 homolog, which was found to be selectively upregulated in Treg cells.44 A recent study described CD4+CD25high Treg cells identified by MHC class II expression as a distinct mature and functional Treg population associated with high FOXP3 expression.45 However, using the HLA-DR marker results in a population that is much smaller than the CD25high population and hence hinders large-scale cell therapy or immunotherapy. On the other hand, Strauss et al46 managed to expand human purified CD4+CD25high T cells using Rapamycin, creating an ex vivo pure population with potent suppressive activity.

nTreg cells follow the normal T-cell development pathway via the thymus and, although expressing a typical alpha/beta TCR, it is thought they escape deletion possibly through “leaky” clonal deletion.47 It is also not fully understood how nTregs are activated, although there is a definite need for TCR engagement, the presence of CD28 and cell–cell contact with APC. Recently it has also been shown that CTLA-4 expression intensifies the TGF-β signal at the point of contact, hence bringing about suppression of responding cells.48 Moreover, IL-2 expands nTreg cells and there is a dependency on IL-2, both in vitro and in vivo, for activation and sustained CD25+ expression.49 It has been shown that these cells suppress the proliferation of other CD4+ helper and CD8+ effector T cells in vitro via a cell contact–dependent (T-T interaction), dose-dependent, and cytokine-independent mechanism.50,51 However, in vivo, the picture is more complex.52 A recently observed mechanism by which nTreg cells exert their suppressive actions is through the negative co-stimulator programmed death-1 (PD-1) mediated pathway after induction by NK cell–primed DC.53 This mechanism of suppression through APC adds to the many complex suppressive mechanisms by which nTreg can function in vivo (Figure 1).

After their production and maturation either directly or after non-Treg activation, Treg cells are thought to be activated by the tumor itself through IL-10 and TGFβ production, as well as through immature DCs (iDCs). Thereafter, Treg cells suppress effector cells by either direct cell contact or inhibitory molecules production. DCs also play a suppressive role against effector T cells (CTL) through their immature subsets or IL-10 production, having been stimulated by the tumor or by Treg cells.
In comparison, iTreg cells can be divided into 2 further subgroups: Tr1 or CD4\(^+\) IL-10\(^+\) FOXP3\(^+\) cells, and Th3 or CD4\(^+\) TGF-β\(^+\) cells. Both subsets are generated from CD4\(^+\) CD25\(^-\) precursors under the influence of cytokines and co-stimulatory milieu during the peripheral T-lymphocyte response to antigens.\(^{54}\) Their activation is better understood requiring MHC class II-bound ligands alone\(^{35}\) unlike nTreg cells that need both TCR ligand and co-stimulation for full functional activation. iTreg exert their effect in vitro and in vivo predominantly, although not exclusively, through a cytokine-dependent mechanism, by secretion of inhibitory cytokines such as IL-10 and TGF-β\(^{55}\) that can also induce further iTreg differentiation\(^{56,57}\) and even nTreg differentiation.\(^{58}\) Furthermore, other recent data have shown that iTreg can also target negatively regulate innate immunity, by suppressing NK cell function in vivo in a cytokine-dependent manner principally through TGF-β; this is demonstrated most convincingly in a mouse tumor model.\(^{59}\)

Other suggested regulatory cell subtypes include the CD8\(^+\) regulatory T cells mentioned earlier. These are similar to CD4\(^+\) T cells and are defined according to their specific markers or mode of action (eg, CD8\(^-\) CD25\(^+\) and CD8\(^-\) IL10\(^+\) cells).\(^{32,33}\) Indeed, CD8\(^-\) CD25\(^+\) T cells are reported to share the same phenotypic and functional features of CD4\(^-\) CD25\(^+\) T cells in human\(^{32}\); however, the CD4\(^+\) subset is both more prevalent and thought to be the most influential.

The above mentioned data summarize the different subsets of Treg cells and their proposed modes of action; however, the interrelation between these subtypes remains poorly understood. Recent observations have suggested that nTreg cells are involved in the differentiation of iTreg in vitro\(^{60}\) and in vivo.\(^{61}\) This process is thought to be important early in life, reducing in adulthood when thymus functions regress, although the half life of nTreg cells is yet to be determined. In this context, the bone marrow is suggested as a “homing” compartment for Treg cells where they can proliferate and expand. This hypothesis by Wei et al\(^{62}\) was based on observations regarding CD8\(^-\) memory T cells\(^{63,64}\) and the fact that more than 25% of all CD4\(^+\) T cells are phenotypically and functionally Treg cells in both normal human and murine bone marrow.\(^{65}\)

Trafficking of Treg cells is also under current re-evaluation. Certain chemokine receptors and integrin molecules are implicated in this process: CXCL12 mediates bone marrow Treg-cell traffick-
and, paradoxically, in those patients with no evidence of disease following curative therapy when compared with normal controls. The authors suggest that the latter observations support a hypothesis that the disrupted lymphocyte "homeostasis" caused in response to the tumor, demonstrated by the increased Treg cell number, does not normalize after curative treatment. This work also showed the tendency of Treg cells in PBMCs to be more prone to undergoing apoptosis than other CD4+ T lymphocytes, a trait identified previously by others but still controversial.\(^77,78\) Finally, the paper concluded that Treg cells are possibly responsible for the downregulation of TCR-mediated signaling in the CD8+ and non-Treg CD4+ T cells, thus hindering their effector and helper functions, respectively. This conclusion was made after confirmation of the lower expression of zeta chain, which determines the ability of T cells to signal via TCR engagement, in the peripheral circulation of patients with HNSCC.

In another recent study from the same laboratory, Albers et al\(^79\) also using a relatively small cohort of HNSCC patients, demonstrated that Treg cells localize to tumor tissue by confirming their enrichment in TILs when compared with PBMCs. This observation was particularly interesting because the subtype of Treg cells found in the tumor mass was almost exclusively FOXP3+, GITR+, and CTLA-4+ while the majority of cells in PBMCs were non-Treg CD4+CD25+ T cells (ie, activated CD4+ T cells). The authors suggested that the population of FOXP3+, GITR+, and CTLA-4+ Treg cells distinguishes activated CD4+ T cells from Treg cells. The authors also suggested that Treg cells may be responsible for the unresponsiveness of effector cells (CD3+CD8+) in TILs and PBMCs of tumor patients. Interestingly, Treg cells were also found to be elevated in the tumor tissue and peripheral blood of nasopharyngeal carcinoma patients, in combination with a significant decrease of CD4+ T cells.\(^80\) The concept of an inverse correlation between Treg cells and CD8+ effector T cells and their cytokine-expressing cell Tc1 and Tc2 populations was further investigated by Chikamatsu et al.\(^81\) This study similarly showed lower percentages of CD4+ T cells and higher levels of CD4+CD25\(^{high}\) T cells in the peripheral blood of 42 HNSCC patients, confirming the altered immune status.

The observations by Whiteside and her group on HNSCC were largely shared by Curiel et al,\(^66\) in the largest reported human cancer study involving a cohort of 104 patients with ovarian cancer (\(n = 104\)) in which CD4+CD25+ T cells similarly expressing FOXP3, GITR and CTLA-4, inhibited TAA-specific immunity in vitro and in vivo, and permitted tumor growth. The same study also demonstrated that Treg cells migrate into tumors expressing the CCR4 receptor in vivo and in vitro using the CCR4 ligand-chemokine CCL22. Furthermore, Treg cell numbers correlated with poor survival and were shown to be significant predictors of death even after controlling for disease stage and surgical debulking. Similarly, the work of Kono et al in gastric and esophageal cancer showed that an increase in specific CD4+CD25\(^{high}\) Treg cells correlated with tumor stage, poorer survival rates, and recurrence. This study was the first to report a link between Treg numbers and patient survival. One striking difference between the work of Kono et al and Schaefer et al\(^69\) was that Treg numbers were reduced significantly after 2 months of curative treatment; perhaps reflecting some tissue-specific differences.\(^82\)

In contrast to these results, Tartour and his group\(^83\) showed that tumor-infiltrating CD4+CD69+ T cells positively correlated with a better prognosis in patients with HNSCC. In their study of 84 patients, infiltration by CD4+FOXP3+ T cells and tumor size were shown to be the only 2 significant prognostic factors related to better locoregional control. These results are supported by the conclusion from recent studies in follicular lymphoma\(^84\) and Hodgkin’s lymphoma\(^85\) that also showed tumor infiltrating CD4+FOXP3+ Treg cells to be associated with better survival. This apparent confusion regarding the role of Treg in patient prognosis could be explained by the heterogeneity of CD4+ T cells and indeed Treg cells or the nature of the tumor type, or some combination of the 2. Further studies are needed to establish a clearer role for Treg cells as a prognostic marker.

Lymph node enrichment with Treg cells has also been demonstrated in recent studies. Linehan and his group were 1 of the first to show that tumor-draining lymph nodes (TDLNs) contain Treg cells.\(^75\) In line with these results, Viguier et al\(^70\) investigated human metastatic melanoma lymph nodes and reported their enrichment with CD4+CD25+FOXP3+ Treg cells with inhibitory functions, contributing to the local immunosuppressive status. However, contrasting results emerged from the ovarian cancer study described previously,\(^66\) where TDLNs showed similar figures of Treg cells enrichment as lymph nodes obtained from noncancer patients. Furthermore, the accu-
mulation of Treg cells in TDLN was less frequent in later stages of the disease, showing preferential recruitment of Treg cells to the tumor mass and associated ascites. The discrepancy between the above-mentioned studies is not clear; however, the nature of the specific tumor is most likely to be important, ie, what factors are released directly or indirectly and hence the specific subset of Treg cell recruited. Apart from the study by Albers et al, who reported that the levels of Treg cells in noninvolved lymph nodes from tumor patients were similar to those in PBMCs from such patients, there is no other work studying Treg cells in TDLNs of patients with HNSCC.

Treg cells obviously do not act in isolation and, not surprisingly, groups have studied associations with other key components of the immune response. The correlation between DCs, the most potent APC, and Treg cells was studied recently in HNSCC patients. In a cohort of 45 patients, Saka- kura et al, using flow cytometry, first confirmed the increase in the percentage of circulating Treg cells in these patients. Second, they demonstrated the altered nature of DCs in the same group of patients, ie, both a lower percentage of myeloid DCs and significantly lower expression of HLA-DR, a good maturation marker, on the surface of the cells that were present. This immaturity of DCs was also shown to be related to tumor progression as HLA-DR expression was further reduced on plasmacytoid DCs in patients with advanced disease compared with DCs taken from patients with early-stage disease. Finally, a significant inverse correlation was found between DCs and Treg cells, indicating their imbalance and suggesting that immature DCs promote the appearance of Treg cells.

Conclusions regarding our current understanding of the involvement of Treg cells in patients with HNSCC are summarized in Table 3. However, questions regarding most aspects of Treg cell biology remain unanswered in most types of tumors, including HNSCC. The merit of involving Treg cells in immunotherapeutic modalities against cancers is certainly valid, but until a better understanding of the roles that Treg cells play in antitumor immunity in patients with cancer, such modalities of treatment will remain far from perfect. The current status of this approach is given below.

**FUTURE IMPLICATIONS AND IMMUNOTHERAPY**

Soon after Sakaguchi et al described Treg cells, his team published a study in which they depleted CD4+CD25+ T cells in a mouse tumor model, and this resulted, as anticipated, in strong induction of tumor immunity. This approach added a novel dimension to tumor immunotherapy (ie, targeting Treg cells). Methods used to achieve that, apart from depleting Treg cells, include blocking their trafficking, blocking their differentiation and signaling, and finally targeting the suppressive molecules that Treg cells use to confer their regulatory/suppressive function or a combination of 2 or more of these strategies.

Different approaches have been used to deplete Treg cells in mice and humans. In vivo treatment with CD25-specific antibody in mouse melanoma, leukemia, and colorectal tumor models and treatment with denileukin diftitox, a recombinant IL-2 diphtheria toxin conjugate

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<td>Tumor</td>
<td>83</td>
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Abbreviations: HNSCC, head and neck squamous cell carcinoma; PBMC, peripheral blood mononuclear cells; TIL, tumor infiltrating lymphocytes; DC, dendritic cells; TCR, T cell receptor; TGF-β, tumor growth factor-β; FOXP3, forkheadbox transcription factor P3.
DAB\textsuperscript{389}IL-2 that selectively eliminate Treg cells in human PBMC,\textsuperscript{91} managed to deplete Treg cells effectively and showed enhanced tumor immunity and even tumor regression in both mice and humans.\textsuperscript{72,92} Chemotherapeutic drugs such as cyclophosphamide and cyclosporine have also been shown to reduce Treg cells’ numbers and function in vivo in mouse tumor models\textsuperscript{74,93} but such approaches are yet to be established in humans. Collectively, the above methods have been used alone or, more often, in conjunction with other conventional immunotherapeutic modalities. For example, elimination of Treg cells through a single dose of denileukin diftitox (DAB\textsuperscript{389}IL-2) followed by vaccination with RNA-transfected DCs significantly improved the stimulation of tumor-specific T cell responses in renal cell carcinoma patients (n = 7) when compared with vaccination alone (n = 4). No apparent clinical toxicity or evidence of autoimmunity was observed; however, Treg depletion was transient (<2 months) and the use of denileukin diftitox (DAB\textsuperscript{389}IL-2) was restricted to a prevaccination setting as it has also abrogated DC-mediated activation of T cells in an in vitro assay.\textsuperscript{91} Furthermore, another study combined Treg cell depletion by anti-CD25 mAb with IL-21 secreting cellular vaccine (a TS/A mammary adenocarcinoma cell genetically modified to secrete IL-21, a member of the IL-2 cytokine family that promotes proliferation, cytotoxic activity, and IFN-\(\gamma\) production by murine and human CD8\textsuperscript{+} effector T cells).\textsuperscript{94} The results showed an induction of a strong antitumor effect using CD8\textsuperscript{+} cells, NK cells, and IFN-\(\gamma\), and induced long-term protective immunity in a mouse bearing TS/A parental cell micrometastases model\textsuperscript{95} that otherwise could not be established using a single modality alone. Another study by Kudo-Saito et al.\textsuperscript{96} used multimodal therapy to eliminate well-established lymphoma, melanoma, and colon adenocarcinoma murine tumor cell lines. A viral vaccine given with a concomitant use of anti-CD25 mAb to deplete Treg cells yielded a better antigen-specific immune response and improved T-cell immune response specific for a self antigen as well as those specific for a non-self antigen. However, external beam radiation was needed to eliminate the tumor as Treg depletion and the vaccine were not sufficient to stimulate the immune system to eradicate the established tumors. The study also showed the reduction of GITR\textsuperscript{+}/CTLA-4\textsuperscript{-} Treg cells and an increase in the percentage of activated DCs, again supporting an important link between these cells types as suggested by Sakakura et al.\textsuperscript{5} Other potential strategies in targeting Treg cells can be achieved by blocking the trafficking of these cells through CCL22-specific antibody or by targeting the suppressive molecules associated with Treg cells such as FOXP3, GITR, CTLA-4, TGF-\(\beta\), and IL-10. Treatments with GITR and CTLA-4-specific antibodies have been used in mouse models. GITR-specific antibody induced tumor regression in mice bearing MethA-induced sarcoma and colon carcinoma (CT26),\textsuperscript{97} while treatment with CTLA-4-specific antibody not only caused tumor regression but also increased survival in mice bearing MethA-induced sarcoma.\textsuperscript{98} In humans, blocking CTLA-4 in vivo resulted in objective tumor regression or stabilization of the serum tumor marker CA125 in some patients who were vaccinated with HLA-A2 restricted peptide or irradiated autologous GM-CSF-secreting tumor cells in human metastatic melanoma and metastatic ovarian carcinoma trials, respectively.\textsuperscript{99,100} However, this treatment resulted in a severe, but manageable, autoimmune diseases in these patients. This established side effect of depleting Treg cells or associated molecules has also been observed in animal studies.\textsuperscript{101,102} Indeed, a variety of autoimmune diseases such as thyroiditis, insulinitis, gastritis, and autoimmune diabetes have all been associated with Treg cells function and depletion.\textsuperscript{103} Another setback in depleting Treg cells is that they soon recover in number unless followed by other immune or nonimmune therapies. In addition, the anti-CD25 mAb also depletes activated tumor-reactive CD25\textsuperscript{+} effector T cells that may be counter-productive. Moreover, Treg cell elimination did not produce tumor regression in all mouse and human trials.\textsuperscript{97,100} One explanation for these observations is that other suppressive cells could be functioning in that particular tumor condition, such as the CD8\textsuperscript{+} suppressor cells, NK-T cells, and/or \(\gamma\)\(\delta\)T cells.\textsuperscript{104} Another explanation points to the dose, methods, and combinations of the different modalities used to target Treg cells, emphasizing the need to establish well-controlled studies to optimize treatment.\textsuperscript{23} Almost all the data regarding the use of Treg cells in immune-based therapies are conducted in non-HNSCC tumor models, which is surprising because of the prevalence of other in vivo studies. It is highly likely that the lessons learned from these studies could be applied in tumors of the head and neck; however, specific trials are also needed in HNSCC models.
Stimulation of immune reactivity is a valid and tempting option; however, to achieve this goal one first needs to overcome immune suppression induced by HNSCC. Multimodal therapy approach incorporating the manipulation of Treg cells, as discussed above, is proving to be beneficial. In HNSCC, multiple treatment combinations, although still not involving Treg cells manipulation, are gaining support in clinical practice.

In summary, as our understanding of the failure of immune surveillance to control tumor progression and tumor escape mechanisms is improving, specific immunotherapy protocols are gaining wider approval. Targeting Treg cells is indeed 1 of the promising recent approaches to improve the efficiency of anticancer treatment modalities. Its use in conjunction with other traditional tumor therapy and conventional immunotherapy reflect that the complexity of the host–tumor interactions and the fact that immunodeficiency in HNSCC is multifactorial can only be overcome by the combination of different treatment modalities.

REFERENCES