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Immune modulation of head and neck squamous cell carcinoma and the tumor microenvironment by conventional therapeutics

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Abstract

Head and neck squamous cell carcinoma (HNSCC) accounts for more than 600,000 cases and 380,000 deaths annually worldwide. While human papilloma virus (HPV)-associated HNSCCs have better overall survival compared to HPV-negative HNSCC, loco-regional recurrence remains a significant cause of mortality and additional combinatorial strategies are needed to improve outcomes. The primary conventional therapies to treat HNSCC are surgery, radiation, and chemotherapies; however multiple other targeted systemic options are used and being tested including cetuximab, bevacizumab, mTOR inhibitors, and metformin. In 2016 the first checkpoint blockade immunotherapy was approved for recurrent or metastatic HNSCC refractory to platinum based chemotherapy. This immunotherapy approval confirmed the critical importance of the immune system and immuno-modulation in HNSCC pathogenesis, response to treatment, and disease control. However, while immuno-oncology agents are rapidly expanding, the role that the immune system plays in the mechanism of action and clinical efficacy of standard conventional therapies is likely underappreciated. In this article, we focus on how conventional and targeted therapies may directly modulate the immune system and the tumor microenvironment to better understand the effects and combinatorial potential of these therapies in the context and era of immunotherapy.

Introduction

Head and neck squamous cell carcinoma (HNSCC) accounts more than 600,000 cases and 380,000 deaths annually worldwide.(1) In the United States, HNSCC is the sixth most common cancer, and 63,000 patients are diagnosed and approximately 13,000 deaths occur from the disease every year.(2) In addition to the classical risk factors of tobacco and alcohol use, oropharyngeal squamous cell carcinoma (OPSCC) is currently the most common head and neck cancer in the United States due to infection with high-risk human papilloma virus (HPVs) strains including HPV 16, 18, 31, 33, and 45. Different from HPV-negative HNSCC, HPV-associated HNSCC mainly occurs in younger patients. Within the oropharynx the status of HPV infection is usually identified by the surrogate marker p16, which is upregulated by with HPV infection. However importantly, for sites outside of the oropharynx p16 status does not necessarily correlate with HPV positivity. Of note, p16, also known as p16INK4a or cyclin-dependent kinase inhibitor 2A, is a cell cycle regulator and endogenous tumor suppressor which is upregulated as a counter-regulatory mechanism to the loss of cell cycle control and inactivation of the retinoblastoma protein (pRb) by the HPV E7 protein. Fortunately, p16-positive OPSCCs are associated with longer survival and better treatment outcomes.(3) Indeed, p16-negative and p16-positive OPSCCs are considered as two distinct types of tumors in the 8th edition of TNM-classification and staging by American Joint Commission on Cancer (AJCC).

The primary curative therapeutic options for previously untreated HNSCC are surgery with or without adjuvant radiation or chemoradiation as indicated by pathology, definitive radiation alone, or definitive chemoradiation. Standard surveillance is to then obtain imaging at 12 weeks post-treatment to assess for response and then follow with routine physical exam, nasopharyngolaryngoscopy, and additional imaging as indicated. However, among all comers approximately 50% of patients will eventually develop a local or regional recurrence and despite advances in treatment, the five-year survival rate remains low(4,5). Moreover, treatment is associated with significant long-term toxicity and morbidity(4,5). Traditionally, systemic chemotherapies and cetuximab are used for relapsed refractory or metastatic disease with limited improvement in long term survival. Importantly, the anti-programmed cell death-1 (PD-1) antibodies pembrolizumab and nivolumab were FDA approved to treat platinum refractory recurrent or metastatic HNSCC in 2016 (6,7). Responses and activity of anti-PD-1 agents is seen in patients with HPV-positive tumors and HPV-negative tumors; however, objective response rates to checkpoint blockade immunotherapy (CBI) remain low on the order of 16-25% (6,7). Of note an anti-PD-1 agent as a first-line therapy was recently demonstrated to improve overall survival compared to cetuximab and chemotherapy in recurrent or metastatic HNSCC whose tumors overexpress PD-1(8). As immunotherapy is now FDA approved with demonstrated activity in metastatic HNSCC, there is a large national and international effort to understand the role of the immune system and immuno-modulation in head and neck cancer. The demonstrated activity of immunotherapy in HNSCC has prompted a re-evaluation of the mechanisms of action of conventional therapies and highlights the important role that the immune system may play in the clinical efficacy of conventional therapies. Here, we overview conventional and targeted therapies, including chemotherapies, radiotherapy, cetuximab, and others as they relate to immune modulation of HNSCC and the tumor microenvironment to better understand the immune-context of these therapies and develop strategies to improve outcomes for patients with HNSCC (Figure 1).

1. Immunomodulatory Action of Chemotherapy in HNSCC

Immune Effects of Chemotherapy

Cytotoxic chemotherapies are frequently used in HNSCC in combination with radiation therapy for locally advanced disease and alone for recurrent or metastatic disease. Chemotherapies directly inhibit cell division or proliferation in a variety of ways, including interference with DNA replication, protein function, or microtubule formation. Because of myelosuppressive effects, chemotherapy is generally thought to be immunosuppressive, causing lymphopenia and neutropenia. Recent research suggests, however, that certain cytotoxic chemotherapies may also have important immunostimulatory effects.

Preclinical models suggest that chemotherapy is more effective in an immunocompetent host, with decreased efficacy of cisplatin and paclitaxel in immunodeficient mice.(9) Mechanistically certain chemotherapies can increase antigen presentation and can reduce expression of PD-L2, leading to increased T cell activation.(10,11) Additionally chemotherapies have been shown to increase the cytotoxic effects of CTLs and induce immunogenic cell death (ICD).(12-14) Specific chemotherapies certainly have differential effects on the immune system for example: platinums can increase T-cell activation by dendritic cells through downregulation by the STAT6 pathway, while docetaxel may decrease regulatory T cell populations to enhance anti-tumor immunity.(15,16) Moreover, taxanes, platinums, and 5-FU, all used frequently in HNSCC, have been shown in animal models to decrease myeloid derived suppressor cells (MDSCs), which can enhance anti-tumor immunity.(17-19) Interestingly, alterations observed in HNSCC patients could be used as potential biomarkers to guide the use of or avoidance of certain chemotherapy or chemo-immunotherapy combinations(20) such as: anthracyclines (e.g. doxorubicin) and TOP2A protein overexpression; Taxanes (e.g. paclitaxel) and TUBB3/TLE protein overexpression; fluoropyrimidines (e.g. 5-fluorouracil) and TS protein overexpression; platinum analogues (e.g. cisplatin) and ERCC1 protein overexpression; nucleoside analogues (e.g. gemcitabine) and RRMI protein overexpression; and alkylating agents (e.g. temozolomide) and MGMT protein overexpression. Given the ability of chemotherapy to decrease tumor burden while potentially modulating immune responses, combinations of chemotherapy and immunotherapy are under investigation in HNSCC.

Combinations of Chemotherapy and Immunotherapy

To date, most of the large trials combining chemotherapy and immunotherapy have been in non-small cell lung cancer (NSCLC). In a cohort of the CheckMate-012 trial, 56 patients with previously untreated NSCLC were treated with nivolumab in combination with one of three cytotoxic regimens (cisplatin/pemetrexed, cisplatin/gemcitabine, or carboplatin/paclitaxel). The combination was shown to be feasible, without unexpected toxicities. Two year overall survival in the patients receiving carboplatin/paclitaxel and nivolumab 5 mg/kg was promising at 62%.(21) Cohort G of the phase 2 KEYNOTE-021 study randomized 123 patients with non-squamous NSCLC to carboplatin and pemetrexed with or without pembrolizumab; improved response rates were seen with the pembrolizumab combination (55% vs 29%).(22) This led to accelerated approval of the combination by the FDA. The phase 3 KEYNOTE-189 trial confirmed these results, showing improved overall survival (HR 0.49, p < 0.001), progression free survival (HR 0.52, p < 0.001), and response rates (47.6% vs 18.9%) with carboplatin/pemetrexed/pembrolizumab compared to chemotherapy alone in patients with non-squamous NSCLC. Benefit was seen across all levels of PD-L1 expression.(23) More recently, the addition of pembrolizumab to carboplatin and paclitaxel or nab-paclitaxel in squamous cell carcinoma of the lung was shown to improve both progression free survival (HR 0.56, $p < 0.001$) and overall survival (HR 0.64, $p < 0.001$)(24); this regimen was FDA approved in October 2018. No large trials combining chemotherapy with immunotherapy have been published at this time HNSCC. Early results from the phase 3 KEYNOTE-048 trial (NCT02358031) were recently presented. In this trial, patients with recurrent/metastatic HNSCC who had not yet received systemic therapy for recurrent/metastatic disease were randomized between pembrolizumab, pembrolizumab in combination with cisplatin or carboplatin and 5-FU, and standard of care cetuximab/platinum/5-FU. Single agent pembrolizumab was found to improve overall survival compared to chemotherapy in patients with PD-L1 CPS ≥ 1; pembrolizumab combined with chemotherapy improved survival in the total population.(25) Another phase 3 trial in a similar setting is CheckMate 651 (NCT02741570) which is comparing the combination of two immunotherapy agents, nivolumab and ipilimumab, to standard therapy with cetuximab/platinum/5-FU. These trials will help define the use of chemo-immunotherapy in HNSCC.

2. Immunomodulatory Action of Radiation in HNSCC

Immunological Effects of Radiation on Tumor Microenvironment

Radiation therapy (RT) is given to approximately 50% of patients during the course of cancer treatment. It is known that radiation can induce DNA damage and ER stress via production of reactive oxygen species, leading to mitotic catastrophe and cell death. Radiation also induces cell death via intrinsic and extrinsic apoptotic pathways including upregulation of FAS expression on the cell surface.(26) Furthermore, radiation is able to induce immunogenic cell death (ICD) of cancer cells through damage-associated molecular patterns (DAMPs) – pattern recognition receptors. One such DAMP molecule is high mobility group protein B1 (HMGB1), a ligand for

TLR4, which is released by radiation and successively activates the innate immune response and changes the cytokine profile towards an immune stimulatory phenotype in the tumor microenvironment.(27) More importantly, radiation can activate antigen-specific anti-tumor immune responses. One of the most important signatures induced by radiation is upregulation of major histocompatibility complex (MHC) I surface expression(28) which occurs in part via activation of the mTOR pathway.(29) Radiation-induced IFNs also contribute to increased MHC I expression.(30) This is a crucial step for enhancing tumor-specific immune responses as many tumors downregulate or lose MHC I expression to evade the endogenous immune response. Radiation also enhances activation and migration of DCs, improving antigen cross-presentation in the lymph node or secondary lymphoid organs.(31)

Moreover, radiation can increase the density and infiltration of TILs, including CTLs involved in lysing tumor cells, by altering the expression of cell adhesion molecules and chemokines. For example, the expression of cell adhesion molecules, such as intercellular adhesion molecule 1, vascular adhesion molecule 1, and E-selection, on the cell surface of endothelium are enhanced by radiation.(32-34) These cell adhesion molecule and chemokines induced by radiation can help with immune cell extravasation and infiltration into the tumor microenvironment.(35,36)

However, radiation can also increase Treg populations in the tumor microenvironment through increased TGF-β secretion, contributing to immunosuppression.(37,38) Additionally radiation can induce the expression of immune checkpoint ligands, including PD-L1, on tumor cells which could be a dynamic response to inflammation and induced anti-tumor immunity versus an inherent immunosuppressive effect of radiation therapy. Thus, it is critical to harness the immunogenic properties while blocking the immunosuppressive effects of radiation therapy.

Taken together, radiation can augment systemic antigen-specific anti-tumor immune responses by inducing; 1) release of tumor antigens via inflammatory cell death, 2) activation and migration of DCs, 3) enhanced cross-presentation of tumor antigens via upregulation of MHC I, and 4) increased density of TILs, leading tumor-specific T cell activation and proliferation (Figure 1).

In addition to total dose or biologically equivalent radiation dose, different fractions sizes or treatment schedules could alter immune responses. As each fraction of radiation induces a signaling cascade, the resultant effects on the immune system could certainly depend on whether hypofractionation with 1-5 fractions is delivered versus standard conventional fractionation in 30-35 fractions. With regard to tumor control, evidence suggests that alternative fractionation schedules may improve outcomes. RTOG 9003 (NCT00771641) randomly assigned stage III/IV HNSCC patients to: 1) Standard fractionation (SFX; 70 Gy/35 daily fractions/7 weeks), 2) Hyperfractionation (HFX; 81.6 Gy/68 twice-daily fractions/7 weeks), 3) Accelerated fractionation with split (AFX-S; 67.2 Gy/42 fractions/6 weeks with a 2-week rest after 38.4 Gy), 4) Continuous accelerated fractionation (AFX-C; 72 gy/42 fractions/6 weeks). At 5 years, only HFX improved local-regional control and overall survival without increasing long-term toxicity.(39) In the MARCH-meta analysis randomized trials comparing conventional RT with hyperfractionated or accelerated RT showed that altered fractionated RT is associated with improved overall survival and progression-free survival in patients with HNSCC.(40) An updated meta-analysis confirmed that hyperfractionated RT is a standard treatment for locally advanced HNSCC, along with concomitant chemoradiotherapy.(41) Given these findings it is certainly possible that optimal induction of immune responses depends not only on the radiation dose but radiation fractionation employed. Thus the role that radiation fractionation may play in differential modification of immune responses deserves further evaluation.

Combination of Radiation Therapy and Immunotherapy

Based on the diverse immunomodulatory effects of radiation, the combination of RT and immunotherapy is under intense investigation.(42,43) Phase 1/2/3 randomized trials of RT with concurrent and adjuvant anti-PD-1/PD-L1 immunotherapy with concurrent chemotherapy in patients with advanced/intermediate-risk HNSCC and numerous other clinical trials of RT combined with immunotherapy are underway (see Table 1). These clinical trials include combination therapies in the two different settings; definitive/locally advanced curative setting and metastatic/refractory setting, which will lead us to understand more effective combination strategies of radiation and immunotherapy for different stages of HNSCCs.

Regarding timing and sequencing, concurrent administration of radiotherapy and immunotherapy is commonly being tested. However, sequential therapy might be able to enhance treatment efficacy and reduce toxicities, particularly in the setting of concomitant chemotherapy. Both orders, radiotherapy prior to immunotherapy and immunotherapy prior to radiation, have potential to enhance the activity of each other. Further investigation is required to clarify the best timing and sequencing. An ongoing phase 2 randomized trial (NCT02777385) is currently evaluating the efficacy of concurrent versus sequential pembrolizumab, cisplatin and IMRT in stage III-IVb HNSCC.

The use of immunotherapy agents in the maintenance setting is not a current standard among patients treated with curative intent. This approach could keep a basal immune response against tumor higher, helping to eliminate residual tumor cells earlier and minimize the risk of recurrence. Several clinical trials are ongoing to check the efficacy of nivolumab (NCT02764593, NCT03349710), pembrolizumab (NCT02892201, NCT02841748, NCT03040999), avelumab (NCT02952586, NCT02999087), and atezolizumab (NCT03452137) in adjuvant/maintenance setting. In one of the ongoing trials RTOG3504 (NCT02764593), the feasibility of adjuvant nivolumab at 3-12 months post-RT was evaluated. An interim report showed that patients were able to tolerate continuing immunotherapy for up to a year, demonstrating that maintenance immunotherapy is feasible in this population.(44)

Development of loco-regional recurrence or a second primary tumor is unfortunately a relatively frequent event in patients with HNSCC. Treatment with a curative-intent surgical resection or re-irradiation are the primary options for these patients. Reirradiation in some cases with the addition of concurrent chemotherapy or cetuximab has been demonstrated to improve loco-regional control and may improve survival, although patients need to be selected appropriately(45). Given the relatively limited toxicity of immunotherapy, reirradiation with immunotherapy has a potential to improve the efficacy of reirradiation and clinical trials are ongoing to evaluate this in patients with recurrent HNSCC. In order to minimize toxicity from large field re-irradiation, stereotactic body radiation therapy may be quite useful in this setting. Indeed, the phase 2 randomized trial RTOG 3507 (NCT03546582) is evaluating whether the addition of pembrolizumab to stereotactic body radiation therapy (SBRT) reirradiation improves the progression-free survival for patients with recurrent or new second primary HNSCC.

Impact of HPV status on Radiation induced immuno-modulation in Head and Neck Cancer

HPV-status in HNSCC can strongly influence responses to therapy. Interestingly, HPV-positive HNSCC has been reported to be more radiosensitive *in-vivo* but not *in-vitro* when compared to HPV-negative disease(46). Thus, the status of HPV infection can be a biomarker for radiotherapy. Indeed, variations in HPV function within HPV-positive patient subsets was recently correlated with radiation sensitivity and associated with survival.(47,48) Gleber-Netto FO et al., recently analyzed and evaluated the expression pattern of 582 HPV-correlated genes from the 80 oropharyngeal squamous cell carcinomas from the cancer genome atlas (TCGA)(48). The authors identified two distinct expression profiles within HPV-positive tumors and a significant difference in 5-year OS between these two groups of HPV-positive tumors. Furthermore, alterations in HPV associated genes was found to translate to a differential sensitivity to radiation therapy when tested using *in-vitro* models(48). These findings demonstrate that HPV status can impact radiation sensitivity and that even within HPV positive tumors that subset likely exist with differential sensitivity to radiation therapy.

The underlying tumor microenvironment in HNSCC is dependent on the pathogenesis and mechanism of malignant transformation, namely alcohol, tobacco, or viral etiology. Thus HPV status can also impact the development of anti-tumor immune responses and presence or composition of tumor associated immune cells. Specifically there has been reported to be an increased immune infiltrate and inflammatory cytokines in the HPV-positive tumor microenvironment, which may contribute to the better tumor clearance after irradiation, although confirmation of these findings and mechanisms for this difference require further investigation.(49,50)

One common feature of locally advanced HNSCC is the occurrence of tumor hypoxia, which strongly attenuates the efficacy of radiotherapy and is a negative prognostic factor.(51) Radiation-induced DNA damage is decreased in the absence of oxygen due to lower production of reactive oxygen species, leading to radioresistance.(52) It has been shown that HPV-positive and HPV-negative tumors display a similar degree of hypoxia, and both HPV-positive and HPV-negative HNSCC cell lines demonstrate decreased radiosensitivity in hypoxic conditions.(53) Hypoxia modifiers, such as nimorazole, which can increase free radical formation, have been used to overcome radioresistance. It is effective for both HPV-positive and HPV-negative cell lines *in vitro*, but clinical studies showed that it was only effective on HPV-negative tumors *in vivo*.(54,55) Ultimately, differences in biochemical characteristics between HPV-positive and HPV-negative tumors suggest that distinct treatment strategies may be required for these two different types of tumors and this is reflected in the different AJCC staging systems used for these distinct disease entities.

3. Immunomodulatory Action of Cetuximab in HNSCC

The anti-tumor effects of cetuximab have primarily been attributed to the blockade of EGFR signaling resulting in single agent activity, activity in combination with chemotherapy, as well as enhancement of radiation-induced cytotoxicity.(56) However, recent studies have demonstrated that cetuximab also has robust immunomodulatory activities. The cetuximab antigen-binding site region (Fab) region binds EGFR on tumor cells while the constant region (Fc) binds to the CD16 receptor (i.e. FcγRIII) on myeloid cells and natural killer cells (NKCs). Antibodies themselves are designed to stimulate innate and adaptive immune systems, resulting in fixation and activation of the complement system, Fc receptor engagement, and antibody-dependent cell-mediated toxicity (ADCC)(57). Recruited myeloid cells can directly exert lytic effects on tumor cells, as well as modify the maturation, activation, and function of dendritic cells, B-cells and T-cells in the tumor microenvironment via cytokines including interleukin (IL)-10, transforming growth factor (TGF)-β, tumor necrosis factor (TNF)-α, IL-6 and interferon (IFN)-γ. In oropharynx SCC, crosstalk between dendritic cell (DC)-NKC is also modulated by stimulator of interferon genes (STING), an endoplasmic-reticulum associated adaptor protein. EGFR blockade with cetuximab and STING activation increased the maturation markers CD86, CD83, and HLA-DR and PD-1 ligand (PD-L1) on DC, when given alone and in combination(58).

Tumor antigens liberated by dying tumor cells are presented by macrophages and DCs to naïve cytotoxic T lymphocytes (CTLs) that can acquire EGFR-specificity(59), or specificity to other tumor associated antigens resulting in an anti-tumor adaptive immune response and epitope spreading. Release of perforin and granzyme B by CTLs induces membranolysis, activation of caspases, and subsequent apoptosis of tumor cells.(57) In a cetuximab neoadjuvant therapy trial, patients exhibited upregulated CD107a and CD137 on tumor-infiltrating NKCs and upregulated perforin and granzyme B on peripheral blood NKCs.(60) Furthermore, NKC surface expression of CD137 correlated with clinical response to neoadjuvant cetuximab.(60)

Cetuximab binding to EGFR-expressing cancer cells also results in complement-dependent cytotoxicity via C3b deposition, formation of C5b-C9 complex, and resultant osmotic lysis of the target cell(61,62). In support of these mechanisms, patients with HNSCC who exhibit higher baseline ADCC activity and EGFR expression are more likely to have a complete response with cetuximab and radiotherapy.(63)

However, the recently published RTOG 1016 (NCT01302834) provides us with considerable data regarding cetuximab combined with RT which may have important implications for combining radiation with other monoclonal antibodies. 849 patients with HPV-positive oropharyngeal cancer were randomly assigned to receive either cisplatin with RT or cetuximab with RT. Unexpectedly, overall survival on the cetuximab arm was significantly inferior to the cisplatin arm. Overall rates of serious adverse events (grade 3-5) were similar for patients in both groups although toxic side effects were different.(64) Importantly we must re-evaluate the direct mechanism of 'radiosensitization' between these drugs. Cisplatin impairs DNA repair and enhances DNA damage after irradiation by directly binding to DNA resulting in classical radiosensitization. On the other hand, cetuximab functions indirectly as a 'radiosensitizer', altering growth and cell signaling pathways to cause cell cycle dysregulation, apoptosis, or activate immune responses as described above. However, cetuximab does not directly increase DNA damage from radiation therapy and similarly checkpoint blockade immunotherapy does not directly enhance DNA damage from radiation therapy. Thus these monoclonal antibodies do not function as classical radiosensitizers and instead may enhance loco-regional control through alternative mechanisms in combination with radiation therapy. RTOG 1016 as well as similar trial reported at ESMO (Abstract LBA9_PR) highlight and confirm that the standard therapy for advanced HPV-positive oropharyngeal cancer remains concurrent cisplatin with RT. The results of these studies and associated differential mechanisms of radiosensitization raise important questions which need to be carefully addressed when using immunotherapy with concurrent radiotherapy in the definitive setting.

The Immunosuppressive Tumor Microenvironment and Resistance to Cetuximab

Tumor-infiltrating lymphocytes (TILs) are observed to have upregulated expression of immune checkpoint receptors including PD-1, cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), T-cell immunoglobulin and mucin domain 3 (TIM-3) and lymphocyte-activation gene 3 (LAG-3) which can paradoxically indicate activation as well as exhaustion, or anergy depending on the magnitude and chronicity of expression. Nonetheless, an EGFR-mediated immunosuppressive tumor microenvironment has been described where co-inhibitory signals are upregulated at the interface between tumor and T cells or antigen-presenting cells (APCs) and T cells.(57) In patients treated with cetuximab, CD8+ TILs expressed increased levels of PD-1 and TIM-3 over the course of cetuximab therapy.(65) PD-1 ligation by PD-L1 on tumor cells results in T cell receptor signaling inhibition, and TIM-3 stimulation results in T cell exhaustion.(65) Cetuximab-treated patients also exhibit an increase in circulating and intra-tumoral CD4+CD25+Foxp3^{high} regulatory T cells (Treg) expressing CTLA-4. CTLA-4, when expressed by T cells, binds B7 expressed on antigen-presenting cells and induces a coinhibitory “signal 2” which destines the T cell to an anergic fate.(66) Increased circulating and intratumoral CTLA-4+ Treg correlate with worse oncologic outcome in HNSCC patients treated with cetuximab.(66) Of note, overexpression of PD-L1 is observed in a majority of patients with *recurrent* HNSCC. Seiwart et al. screened 104 patients with recurrent or metastatic HNSCC and identified PD-L1 positivity in 78%.(7) Ferris et al. found PD-L1 expression in 57% of patients with recurrent HNSCC.(67) Taken together, these data indicate that HNSCC recurrence involves hijacking of immunosuppressive pathways in order to evade immune-mediated cell death.(68)

Clinical Trials of Combined Immunomodulation and Cetuximab Therapy

In light of the immunomodulatory capabilities of cetuximab, there are multiple studies are actively investigating the safety and efficacy of cetuximab immunotherapy combinations (see Table 2). Targeting of immune checkpoint pathways (anti-CTLA-4, anti-PD-1, anti-PD-L1) as well as leveraging toll like receptor (TLR) 8 and 9, NKG2A/CD159 on NKCs, and IL-12 are all under investigation. Table 2 shows active, completed, and pending clinical trials of combined therapy of cetuximab plus a dedicated immunomodulating agent. Published results, if available, are included as well.(68-70)

A phase 1 study of motolimod, a toll-like receptor 8 agonist, by Dietsch et al. (NCT01334177) found that NK cells become more responsive to stimulation by NKG2D or FcγRIII following motolimod treatment. Ferris et al. (NCT01935921) reported on motolimod or placebo in combination with EXTREME (platinum, fluorouracil, cetuximab). In 195 patients, median PFS and OS was not significantly improved with motolimod combination (HR 0.99 [1 sided CI 0.00-1.22]; P=0.47 for PFS and HR 0.95 [1 sided CI 0.00-1.22; P=0.40). However, the authors noted significantly better PFS (7.8 vs 5.9 months; HR, 0.58; 1-sided 90% CI, 0.00-0.90; P = .046) and OS (15.2 vs 12.6 months; HR, 0.41; 1-sided 90% CI, 0.00-0.77; P = .03) in HPV-positive participants, and that patients with injection site reactions had longer PFS and OS (median PFS, 7.1 vs 5.9 months; HR, 0.69; 1-sided 90% CI, 0.00-0.93; P = .06; and median OS, 18.7 vs 12.6; HR, 0.56; 1-sided 90% CI, 0.00-0.81; P = .02), suggesting an immunological basis for these results.

A multi-institutional phase 2 study of pembrolizumab combined with cetuximab for treatment of recurrent/metastatic HNSCC is underway (NCT03082534). Eight-three patients are to be enrolled into one of four treatment arms: 1) PD-1/PD-L1 inhibitor-naïve and cetuximab-naïve patients treated with pembrolizumab + cetuximab; 2) PD-1/PD-L1 inhibitor-refractory and cetuximab-naïve patients treated with pembrolizumab + cetuximab; 3) PD-1/PD-L1 inhibitor-refractory and cetuximab-refractory patients treated with pembrolizumab + cetuximab; 4) Cutaneous HNSCC treated with pembrolizumab + cetuximab. Pembrolizumab (200 mg) is to be given every 3 weeks. Cetuximab (400 mg/m^2) is to be given weekly. The main outcome measure will be overall response rate in six months from time of study enrollment.

Multiple other additional studies are active including: a multi-institutional phase 1 study of untreated, loco-regionally advanced HNSCC patients (NCT02764593) that will examine the safety of adding nivolumab to cisplatin, cetuximab, or radiation alone; a phase 2 randomized study which will examine biweekly avelumab alone vs. alternating biweekly avelumab plus biweekly cetuximab combination therapy (NCT03494322); and a study of nivolumab plus cetuximab combination therapy which will occur in 2 phases and seeks to enroll 52 patients with recurrent and/or metastatic HNSCC (NCT03370276).

Currently, over twenty clinical trials are underway or planned that will investigate cetuximab plus immunotherapies. Cetuximab already has established activity in HNSCC in combination with chemotherapy and radiation therapy. Given that it is a monoclonal antibody with intrinsic ability to recruit innate and adaptive immunity, cetuximab represents one of the best currently available targeted drugs to combine with immunotherapies and conventional therapies to modulate the tumor microenvironment in HNSCC.

4. Immunomodulation in HNSCC by mTOR and Metformin

Recent deep sequencing approaches, including a landmark study from The Cancer Genome Atlas (TCGA) Network (71), have recently revolutionized our understating of the HNSCC mutational landscape. We learned that HNSCC lesions harbor hundreds of genomic alterations, but surprisingly, the majority of them fall within a limited number molecular pathways whose dysregulation contribute to HNSCC initiation and progression (71,72). These include mutations resulting in persistent mitogenic signaling resulting in aberrant activation of the PI3K, MAPK and JAK/STAT pathways (73). Among them, the PI3K-mTOR pathway is mutated in the highest percentage of the cases, with multiple alterations converging in the activation of PI3K/AKT/mTOR pathway in most HNSCC lesions (72). This, and extensive experimental studies in mouse models provided a rationale for multiple efforts aimed at blocking mTOR for HNSCC treatment in the clinic (reviewed in (74)). mTOR is the target of immunosuppressive therapies, such as rapamycin (sirolimus), which has been used to prevent rejections in renal transplant patients for decades, most often together with cyclosporine and corticosteroids (75). Surprisingly, however, multiple trials using single-agent rapamycin and its analogs, referred to as rapalogs, have shown no evidence of increased immunosuppression in cancer patients (76-78). Paradoxically, mTOR inhibition with rapamycin has been recently shown to increase the immune responses in the clinic, and to potentiate the activity of Immuno-Oncology (IO) agents in cancer models (79-87). Thus, it is possible that mTOR blockade may increase rather than negate the anti-tumor activity of IO agents.

Multiple mechanisms can contribute to a potential beneficial effect of combining mTOR blockers with immune checkpoint inhibitors. mTOR inhibition in HNSCC can promote apoptotic tumor cell killing (88), which can expose multiple antigens thereby increasing cancer immunity.

mTOR inhibition can also affect T cell differentiation programs, increasing the development of long-lived tumor specific memory T cells (89). Experimental studies in HNSCC suggest that simultaneous mTOR and PD-L1 inhibition reduces the tumor burden by increasing IFN-γ production in tumor-infiltrating CD8 T cells (87). On the other hand, the expression of immune suppressive cytokines secreted by Tregs and MDSCs, such as IL-10 and TGF-β, can be decreased by mTOR blockade (90-93), which can help to overcome cancer immune evasion. Thus, although counterintuitive, the use of mTOR inhibitors to suppress a key HNSCC driver pathway could be optimized to concomitantly enhance the anti-tumor immune response when combined with IO agents as a novel precision immune therapeutic strategy for HNSCC patients.

Due to the critical role of the PI3K-mTOR pathway in HNSCC initiation and progression, our team explored the possibility of targeting this signaling circuit for HNSCC prevention in patients with oral premalignant lesions (OPL). These efforts led to the discovery that metformin, the most widely used anti-diabetic agent, can potently block mTOR in OPL and halt their progression to HNSCC in experimental systems (94,95). Remarkably, two recent large retrospective population case-control cohort studies involving together more than 300,000 diabetic patients demonstrated a decreased HNSCC risk in patients on metformin (96,97). Based on these preclinical and epidemiological evidence, metformin is now under investigation for HNSCC prevention (NCT02581137). Of interest, recent findings also support that metformin can regulate proinflammatory cancer-promoting pathways in the tumor microenvironment. In pancreatic ductal adenocarcinoma (PDAC), metformin was shown to reduce the levels of tumor extracellular matrix (ECM) in overweight diabetic PDAC patients, which was recapitulated the exposure of pancreatic stellate cells (PSCs) to metformin *in vitro* (98). Furthermore, metformin exerts an anti-inflammatory activity by reducing the expression of inflammatory cytokines, including IL-1β, and by diminishing the polarization of macrophages to pro-tumorigenic M2 tumor associated macrophages (TAMs) *in vivo* and *in vitro* (98). Thus, by restricting the negative immune modulating role of M2-macrophages metformin may disrupt the establishment of an immune evasive pre-malignant microenvironment, thereby halting cancer progression.

In addition to this anti-inflammatory role, it was recently shown that metformin increases the number of CD8+ TILs, and that metformin can protect anti-tumoral CD8+ cytotoxic T cells from functional exhaustion in the tumor microenvironment (99). Remarkably, these resulted in increased cancer vaccine effectiveness by improving CD8+ TIL multifunctionality in response to metformin treatment (99).

Overall, the emerging data support that metformin may limit cancer progression at least in part by increasing the antitumor immune response by 1) preventing the M2 polarization of TAMs, 2) the secretion of pro-inflammatory and immune suppressive cytokines, 3) increasing cytotoxic CD8+ T cell function, and 4) preventing T cell exhaustion in the tumor microenvironment. This raises the exciting possibility of repurposing metformin, which is safely used by millions of type 2 diabetes patients, to boost the activity of immune checkpoint inhibitors (100).

5. Immunomodulatory Effects of Other Targeted Therapies

Bevacizumab, a monoclonal antibody against vascular endothelial growth factor, is FDA approved as a single agent or in combination with chemotherapy in multiple malignancies. There is evidence that VEGF inhibition can increase T-cell migration into tumors(101) and potentially improve efficacy of checkpoint inhibitors. There is also evidence of efficacy of bevacizumab in combination with atezeolizumab in renal cell carcinoma and hepatocellular carcinoma and in combination with chemotherapy and atezolizumab in non-squamous non-small cell lung cancer.(102-104) Concerns regarding the risk of hemorrhage with VEGF inhibition may limit the use of bevacizumab combinations in HNSCC, though there is an ongoing phase II trial enrolling patients with HPV or EBV associated HNSCC (NCT03074513).

There is also emerging evidence that cell cycle inhibition may be synergistic with checkpoint inhibitors. CDK4/6 inhibitors abemaciclib and palbociclib have been shown to increase antigen presentation in breast cancer cell lines, and these agents also appear to reduce regulatory T cells.(105) Based on this data, several trials are ongoing to study the combination of these agents with checkpoint inhibitors, including a phase I study combining PD-L1 inhibitor avelumab with palbociclib and cetuximab in HNSCC (NCT03498378).

In summary, the importance of the immune system in HNSCC responses to treatment and patient outcomes is now at the forefront. The approval and activity of checkpoint blockade immunotherapy in HNSCC was a pivotal event which opened entirely new opportunities and avenues for basic, translational, and clinical research. However, objective response rates to checkpoint blockade remain quite low and there is a tremendous amount of work and further investigation needed to better understand the role of the immune system in HNSCC. Here we highlighted some of the ways by which conventional therapies including chemotherapy, radiation, and cetuximab can modulate the immune system and tumor microenvironment in HNSCC. The incorporation of this knowledge and additional data from basic research, translational science, and ongoing clinical trials will hopefully elucidate mechanisms of action and the combinatorial strategies needed to improve outcomes for HNSCC patients in the era of immunotherapy.

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Table 1: Clinical trials of combined radiation therapy and anti-PD-1/PD-L1 immunotherapy

Study	Phase	Eligible Patients	Arms	Enrollment	Main Outcome(s)	Coordinating Institution	Sponsor	Status
NCT03383094	2	Locoregionally advanced HNSCC	RT + Pembrolizumab RT + Cisplatin	122 (estimated)	PFS	UC San Diego Moores Cancer Center	Merck Sharp & Dohme Corp	Recruiting
NCT03317327	1,2	Recurrent or new second primary HNSCC with prior RT	Radiation + Nivolumab	20 (estimated)	Adverse Events	Oslo University Hospital	Bristol-Myers Squibb	Recruiting
NCT03546582	2	Recurrent or new second primary HNSCC	SBRT + Pembrolizumab SBRT	102 (estimated)	PFS	RTOG Foundation	Merck Sharp & Dohme Corp	Not yet recruiting
NCT02296684	2	Locoregionally advanced HNSCC	Neoadjuvant Pembrolizumab + Adjuvant Pembrolizumab + SOC Neoadjuvant Pembrolizumab + SOC	66 (estimated)	Logoregional recurrence, distant failure rate, rate of major pathologic treatment effect	Washington University School of Medicine	Merck Sharp & Dohme Corp	Recruiting
NCT03051906	1,2	Locoregionally advanced HNSCC	RT + cetuximab + durvalumab	69 (estimated)	PFS	Azienda Ospedaliero-Universitaria Careggi	Azienda Ospedaliero-Universitaria Careggi	Not yet recruiting
NCT02999087	3	Logoregionally advanced HNSCC	RT + Cisplatin RT + Cetuximab + Avelumab RT + Cetuximab	688 (estimated)	PFS	Groupe Oncologie Radiotherapie Tete et Cou	Merck KGaA, Pfizer	Recruiting
NCT02764593	1	Locoregionally advanced HNSCC	RT + Nivolumab + Cisplatin RT + Nivolumab + Cetuximab RT + Nivolumab	40 (actual)	DLT	RTOG Foundation	Bristol-Myers Squibb	Active, not recruiting
NCT03247712	1,2	Surgically resectable HNSCC	Neoadjuvant Nivolumab + RT + Surgery + Adjuvant Nivolumab	18 (estimated)	Number of patients with unplanned delay to surgery	Providence Health & Services	Providence Cancer Center	Recruiting
NCT03673735	3	Locoregionally advanced HPV-negative HNSCC	RT + Durvalumab + Cisplatin RT + Cisplatin + Placebo	650 (estimated)	DFS	European Organisation for Research and Treatment of Cancer	None	Not yet recruiting
NCT03529422	1	Locoregionally advanced HNSCC	RT + Durvalumab + Tremelimumab	24 (estimated)	DLT, acute toxicities	UNC Lineberger Comprehensive Cancer Center	AstraZeneca	Recruiting
NCT03426657	2	Logoregionally advanced HNSCC	RT + Durvalumab + Tremelimumab	120 (estimated)	Feasibility, DLT, CD8+ T-cell Tumor Infiltration	University of Erlangen-Nürnberg Medical School	None	Not yet recruiting
NCT03509012	1	Advanced HNSCC, NSCLC, SCLC	RT + Durvalumab + Cisplatin	300 (estimated)	DLT, adverse events	Multiple	AstraZeneca	Recruiting

NCT03539198		Recurrent logoregional or metastatic HNSCC	Proton SBRT + Nivolumab	91 (estimated)	ORR	Mayo Clinic		Recruiting
NCT03085719	2	Metastatic HNSCC	High-dose RT + Pembrolizumab High-dose RT + low-dose RT + Pembrolizumab	26 (estimated)	ORR	Dana Farber Cancer Institute	Merck Sharp & Dohme Corp	Recruiting
NCT03283605	1,2	Metastatic HNSCC	SBRT + Durvalumab + Tremelimumab	45 (estimated)	PFS, Acute Toxicities	Centre Hospitalier de l'Université de Montréal	AstraZeneca	Recruiting
NCT03313804	2	Previously treated advanced or metastatic HNSCC or NSCLC	Immune checkpoint inhibitor + RT	57 (estimated)	PFS	University of Kentucky Markey Cancer Center	None	Recruiting

Abbreviations: DFS (disease-free survival), DLT (dose-limiting toxicity), HNSCC (head and neck squamous cell carcinoma), NSCLC (non-small cell lung cancer), ORR (objective response rate), PFS (progression-free survival), RT (radiotherapy), SOC (standard of care)

Table 2: Clinical trials of combined therapy using cetuximab and immunotherapy

Study	Phase	Eligible Patients	Arms	Mechanism of Immunomodulator	Enrollment	Main Outcome(s)	Coordinating Institution	Sponsor	Status
NCT01040832	2	R/M HNSCC failing 1st line cytotoxic therapy	Cetuximab + EMD 1201081 Cetuximab alone	TLR-9 agonist	107 (actual)	PFS	Multiple	EMD Serono	Completed. Ruzsa et al.
NCT01334177	1	R/M HNSCC failing platinum or incurable with surgery or RT	Cetuximab + VTX-2337	TLR-8 agonist	13 (actual)	DLT, characterization of immunologic response	Fred Hutchinson Cancer Research Center/University of Washington Cancer Consortium	University of Washington	Completed. Dietsch et al.
NCT01360827	1	R/M HNSCC not curable locally and not yet treated with systemic therapy or RT	EMD 1201081 + 5-FU + Cisplatin + Cetuximab	TLR-9 agonist	13 (actual)	MTD, ORR	Clinical Research Unit and Pharmacology Lab EA 3035 Institut Claudius Regaud, Toulouse, France	Merck	Terminated due to safety concerns in combination with platinum-based therapy
NCT01468896	1, 2	Unresectable R/M HNSCC	Cetuximab + recombinant IL-12	IL-12	23 (actual)	DLT, ORR	MedStar Georgetown University Hospital	National Cancer Institute	Active. 2/23 DLT events.
NCT01836029	2	R/M HNSCC not yet treated with systemic therapy	Cisplatin or carboplatin + 5-FU + cetuximab + VTX-2337 Cisplatin or carboplatin + 5-FU + cetuximab + placebo	TLR-8 agonist	175 (estimated)	PFS	Multiple	VentiRx Pharmaceuticals	Active
NCT01935921	1	Locoregionally advanced HNSCC	Cetuximab + RT + ipilimumab	anti-CTLA4	19 (actual)	DLT, ORR	University of Pittsburgh Cancer Institute	National Cancer Institute	Completed. Ferris et al.
NCT02110082	1	Advanced/metastatic CRC and incurable HNSCC	Cetuximab + urelumab	anti-CD 137	66 (actual)	Toxicities Objective response rate	Multiple	Bristol-Myers Squibb	Completed. Results pending.
NCT02124850	1	Resectable primary HNSCC	Surgery + cetuximab + motolimod Surgery + cetuximab + motolimod + nivolumab	TLR-8 agonist (motolimod) anti-PD-1 Mab (nivolumab)	24 (estimated)	Change in immune markers anti-tumor response	University of Pittsburgh Medical Center	VentiRx Pharmaceuticals	Recruiting
NCT02633800	2	R/M HNSCC not previously treated with systemic therapy	Cetuximab + platinum + patritumab Cetuximab + platinum + placebo	anti-HER3 Mab	87 (actual)	PFS	Multiple	Daiichi Sankyo, Inc.	Completed. Results submitted.
NCT02643550	1, 2	Platinum-resistant R/M HNSCC	Cetuximab + monalizumab	anti-NKG2A Mab	100 (estimated)	DLT, ORR	University of Pennsylvania	Innate Pharma	Recruiting
NCT02764593	1	Locoregionally advanced HNSCC	Nivolumab + cisplatin Nivolumab + high dose cisplatin Nivolumab + cetuximab Nivolumab + IMRT	anti-PD-1 Mab	40 (actual)	DLT	Multiple	Radiation Therapy Oncology Group, Bristol-Myers Squibb	Active
NCT02938273	1	New diagnosis locally advanced HNSCC	RT + cetuximab + avelumab	anti-PD-L1 Mab	10 (estimated)	Grade 3-5 toxicity Overall response rate	The Netherlands Cancer Institute	Merck	Recruiting

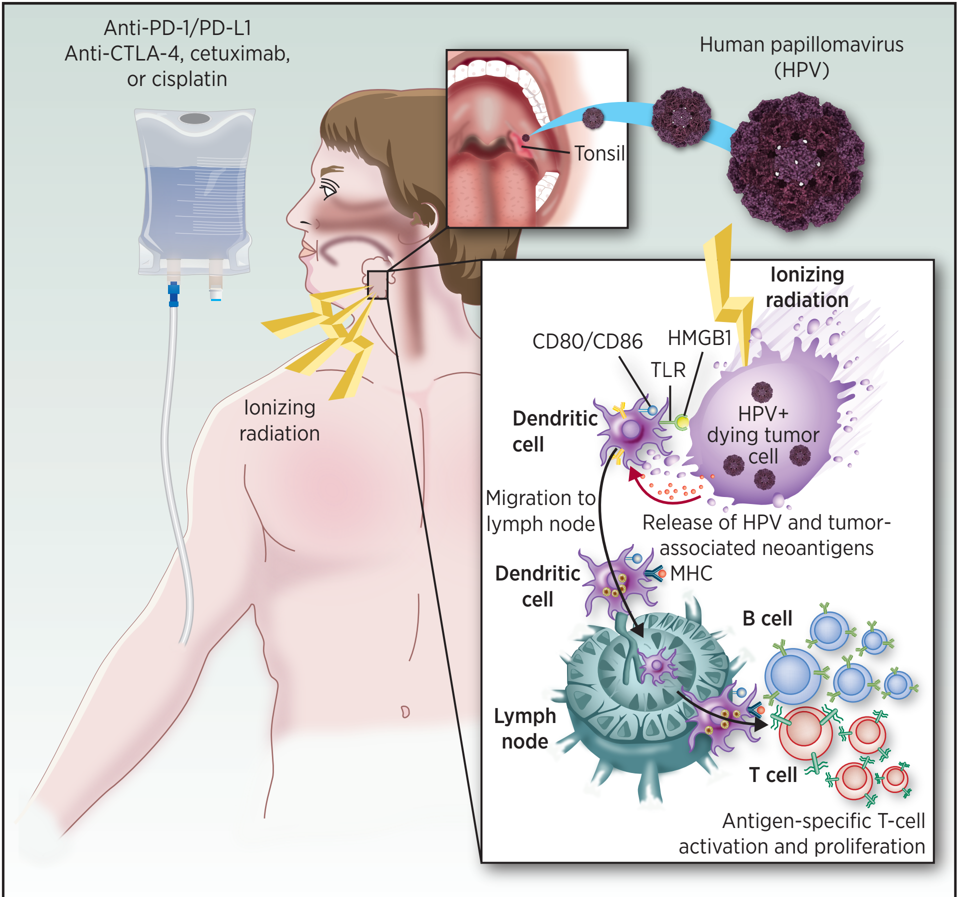
NCT02999087	3	Untreated locoregionally advanced HNSCC	RT + cisplatin RT + cetuximab + avelumab RT + cetuximab	anti-PD-L1 Mab	688 (estimated)	PFS	Centre Hospitalier Bretagne Sud, Lorient, France	Groupe Oncologie Radiotherapie Tete et Cou, Merck, Pfizer	Recruiting
NCT03051906	1, 2	Locoregionally advanced HNSCC	RT + cetuximab + durvalumab	anti-PD-L1 Mab	69 (estimated)	PFS	Azienda Ospedaliero-Universitaria Careggi	Azienda Ospedaliero-Universitaria Careggi	Not yet recruiting. Bonomo et al.
NCT03082534	2	Incurable platinum-refratory or ineligibile HNSCC	Cetuximab + pembrolizumab	anti-PD-1 Mab	83 (estimated)	ORR	UC San Diego Moores Cancer Center	Merck Sharp & Dohme Corp.	Recruiting
NCT03349710	3	R/M HNSCC not curable locally and not yet treated with systemic therapy or RT	Cetuximab + nivolumab + RT Cetuximab + placebo + RT Nivolumab + cisplatin + RT Placebo + cisplatin + RT	anti-PD-1 Mab	1,046 (estimated)	PFS	Multiple	Bristol-Myers Squibb	Recruiting
NCT03370276	1, 2	incurable R/M HNSCC	Cetuximab + nivolumab	anti-PD-1 Mab	52 (estimated)	MTD, 1-year OS	H. Lee Moffitt Cancer Center and Research Institute	H. Lee Moffitt Cancer Center and Research Institute, Bristol-Myers Squibb, Eli Lilly and Company	Active
NCT03494322	2	Incurable R/M HNSCC	Cetuximab + avelumab Avelumab alone	anti-PD-L1 Mab	130 (estimated)	DLT, ORR	University College, London	Merck	Recruiting
NCT03498378	1	Incurable HNSCC	Cetuximab + avelumab + palbociclib	anti-PD-L1 Mab(avelumab) CDK4 and CDK6 inhibitor (palbociclib)	24 (estimated)	MTD, ORR	UC San Diego Moores Cancer Center	Pfizer	Recruiting
NCT01860430	1	Locoregionally advanced HNSCC	Cetuximab + IMRT + ipilimumab	anti-CTLA4	18 (estimated)	Dosing, ORR	University of Pittsburgh Cancer Institute	National Cancer Institute, Robert Ferris	Active

Abbreviations: CRC (colorectal cancer), DLT (dose-limiting toxicities), HNSCC (head and neck squamous cell carcinoma), MTD (maximum tolerated dose), ORR (objective response rate), PFS (progression free survival), R/M (recurrent or metastatic), RT (radiotherapy)

Figure 1. Radiation-induced immune responses in head and neck cancer

Radiation induces 1) release of tumor antigens and damage-associated molecular pattern (e.g. HMGB1) via cell death, 2) activation and migration of dendritic cells to lymph node, 3) enhanced cross-presentation of tumor antigens via upregulation of MHC I, and 4) antigen-specific T cell activation and proliferation. Radiation therapy can be combined with immunotherapy (checkpoint blockade) or chemotherapy. TLR: toll-like receptor, HMGB1: high mobility group protein B1, MHC: major histocompatibility complex, PD-1: programmed cell death-1

Figure 1:


Anti-PD-1/PD-L1
Anti-CTLA-4, cetuximab,
or cisplatin
Human papillomavirus
(HPV)
Tonsil
Ionizing
radiation
Ionizing
radiation
CD80/CD86
HMGB1
TLR
Dendritic
cell
HPV+
dying tumor
cell
Migration to
lymph node
Release of HPV and tumor-
associated neoantigens
Dendritic
cell
MHC
B cell
Lymph
node
T cell
Antigen-specific T-cell
activation and proliferation

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Immune modulation of head and neck squamous cell carcinoma and the tumor microenvironment by conventional therapeutics

Sayuri Miyauchi, Sangwoo S Kim, John Pang, et al.

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