Current Otorhinolaryngology Reports (2022) 10:96–107
https://doi.org/10.1007/s40136-021-00384-0

HEAD AND NECK: HUMAN PAPILLOMA VIRUS ASSOCIATED HEAD AND NECK SQUAMOUS CELL CARCINOMA (WK MYDLARZ AND C FAKHRY, SECTION EDITORS)

Immune Landscape and Role of Immunotherapy in Treatment of HPV-Associated Head and Neck Squamous Cell Carcinoma (HNSCC)

Hannah R. Turbeville[1] · Tiffany A. Toni[2] · Clint Allen[2]

Accepted: 26 November 2021 / Published online: 5 January 2022

Abstract

Purpose of Review To review and summarize recent findings on the immune system constituents relevant to the development, proliferation, and treatment of human papillomavirus (HPV)-associated head and neck squamous cell carcinoma (HNSCC), focusing on experimental therapies currently in testing or development.

Recent Findings HPV-associated HNSCC exhibits an inflamed phenotype targeted at HPV-specific antigens that may contribute to improved survival over HPV-negative HNSCC. Combinations of immunotherapy such as immune checkpoint inhibition and therapeutic vaccination take advantage of the underlying potential for T cell recognition of HPV-specific antigens to improve overall and progression-free survival.

Summary Understanding the interaction between immune cell populations and cancer cells in the tumor microenvironment of HPV-associated squamous cell carcinoma allows for the development of novel immunotherapy options for this disease. Experimental therapies currently being studied include immune checkpoint inhibition, therapeutic vaccines, and adoptive cell transfer. A combination of immunotherapies or immunotherapy with conventional chemotherapy may be the most effective option to induce clinical response and improve overall survival.

Keywords Human papillomavirus · Head and neck squamous cell carcinoma · Immune checkpoint blockade · Therapeutic vaccine · Cell therapy · Immunotherapy

Introduction

Head and neck squamous cell carcinoma (HNSCC) include a diverse collection of malignancies at multiple anatomic sites including the oral cavity, pharynx, and larynx. High-risk human papillomavirus (HPV), particularly HPV type 16, is epidemiologically and biologically linked to the development of cancer, primarily in the oropharynx. The specific reason underlying the ability of HPV to induce chronic infection and malignant transformation in the mucosa overlying tonsillar tissue to a greater extent than the mucosa from other head and neck sites is incompletely understood. Eighty percent (80%) or more of all oropharyngeal squamous cell carcinoma (OPSCC) appears to be HPV driven [1, 2]. OPSCC is now the most common HPV-associated malignancy in the USA, surpassing the incidence of HPV-associated cervical cancer [3]. HPV association confers a favorable prognosis in OPSCC. Recent studies demonstrated a three-fold reduction in cancer associated mortality [4] and a 51% risk reduction in relapse or death [5] leading to superior 5-year locoregional control and overall survival for patients with HPV-associated as compared to HPV-negative OPSCC (Table 1). Given

This article is part of the Topical Collection on *HEAD AND NECK: Human Papilloma Virus Associated Head and Neck Squamous Cell Carcinoma*

✉ Clint Allen
clint.allen@nih.gov

Hannah R. Turbeville
hrturb3@gmail.com

Tiffany A. Toni
tiffany.toni@nih.gov

[1] Department of Otolaryngology-Head and Neck Surgery, University of Michigan, Ann Arbor, MI, USA

[2] National Institute On Deafness and Other Communication Disorders, National Institutes of Health, Bethesda, MD, USA

Table 1 Comparison of HPV-positive and HPV-negative OPSCC

	HPV-associated OPSCC	HPV-negative OPSCC
Patient population	Highest in white males < 55 yoa, nonsmokers	Highest in nonwhite males > 55 yoa with tobacco/alcohol use
Treatments used	Standard of care chemoradiation, immunotherapy for relapses	Standard of care chemoradiation
Prognosis	Higher overall and progression free survival	Lower overall and progression free survival
Incidence	Increasing	Decreasing
Stage at diagnosis	T1–2 with nodal metastases	Variable
Tumor location	Base of tongue, tonsil	All sites
Histological morphology	Nonkeratinizing, basaloid, lymphoepithelial, or poorly differentiated	Moderately differentiated keratinization
TP53 Status	Wild-type TP53 targeted by HPV E6	Mutated TP53
Overall immune cell infiltration	Higher	Lower
T cell population	High total T cell/T_{reg} ratio	Low total T cell/T_{reg} ratio
Myeloid derived suppressor cells	Similar	Similar
Dendritic cells	Higher	Lower
Macrophages	More pro-inflammatory M1 macrophages	More uncommitted (M0) and anti-inflammatory M2 macrophages
Neutrophils	Lower	Higher

the differing prognosis between HPV-associated and HPV-negative oropharyngeal cancer, the American Joint Committee on Cancer (AJCC) 8th edition staging system differentiates HPV-associated OPSCC as a separate entity [6••]. HPV infection may be detected at lower frequencies in HNSCCs outside the oropharynx, but HPV-positivity does not predict effective treatment nor confer a survival advantage in these settings [7] suggesting that the low-level positivity may represent "passenger infections." The underlying reasons contributing to an enhanced response to standard anti-cancer treatment and survival observed in HPV-associated OPSCC remain under investigation but may involve differing mutation profiles between HPV-associated and HPV-negative OPSCC. HPV-associated OPSCC rarely harbors mutated TP53, and functional TP53 is critically important for cell death following exposure to chemotherapy and radiation. Another possibility is that tumor cells within HPV-associated OPSCC are more antigenic on account of expression of HPV viral antigens [8, 9]. Given the possibility of enhanced tumor cell antigenicity and immunogenicity, there is substantial clinical interest in immunotherapy as a treatment option for HPV-associated OPSCC. Understanding the different forms of immunotherapy being studied for HPV-associated OPSCC necessitates a foundational understanding of the immune cell populations involved in the detection and elimination of virally infected malignant cells. This review will summarize the function of immune cell populations in the context of the tumor microenvironment and examine recent literature on immunotherapy-based interventions for HPV-associated OPSCC.

Clinical Significance of HPV Status in HNSCC

Given the favorable prognosis following standard anti-cancer treatment observed with HPV-associated OPSCC, recent clinical studies have focused on either de-intensification of chemotherapy and radiation regimens or replacement with less toxic treatments in an attempt to avoid short- and long-term toxicity while maintaining equivalent oncologic control [10]. A randomized phase III study of subjects with HPV-associated OPSCC who did not smoke compared treatment with cisplatin or the EGFR monoclonal antibody cetuximab combined with radiation. Cetuximab plus radiation treatment did not significantly improve toxicity and was associated with worse oncologic control of disease [11]. Thus, based on current clinical data, systemic cisplatin should not be replaced with cetuximab for the treatment of HPV-associated HNSCC. Other approaches aimed at deintensification of radiation are being clinically studied, with the aims of reducing long-term tissue fibrosis and dysphagia. Initial results from the ECOG 3311 study exploring the utility of dose-reduced post-operative radiotherapy indicate that similar oncologic control and reduced toxicity was observed in patients that received 50 Gy compared to 60 Gy[12]. These encouraging results are being further explored in additional studies; DART-HPV (NCT02908477) and DELPHI (NCT03396718) are examples of ongoing clinical studies where investigators

aim to determine if lower radiation doses can be used to treat HPV-associated OPSCC. The hypothesis that HPV-associated OPSCC harbors strong T cell viral antigens and thus is highly antigenic raises the possibility that immunotherapy could be used in lieu of or as an adjuvant to de-intensified standard of care treatment.

Role of the Immune System in Oncogenesis and Tumor Proliferation

As the genotype of cancer cells gradually diverges from those of the surrounding normal cells through accumulation of genomic alterations via genomic instability or APOBEC mutagenesis, antigens are created that can be recognized by the immune system [13, 14]. In HPV-associated malignancies, mutations may arise due to genomic instability inherent to malignant cells, or though activity of an endogenous antiviral cellular response that introduces mutations into HPV and host DNA and RNA called apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like, or APOBEC. These immune system targets include tumor-specific neoantigens and tumor-associated antigens comprised of differentiation, cancer testes, and overexpressed normal antigens [15, 16]. Tumor antigens, if naturally processed and presented on cell surface HLA molecules by the tumor cell's antigen presentation machinery, can be detected by cytotoxic T cells (CTLs) [17]. CTLs can then kill virally infected tumor cells through T cell receptor (TCR) detection of tumor cell surface HLA-antigen complexes. A positive correlation exists between an increased number of predicted neoantigens, tumor homogeneity, and progression-free survival (PFS) in several cancer types [18]. This indicates that tumors which harbor more neoantigens expressed across all tumor cell subpopulations respond best to immunotherapy. The ability of the immune system to recognize antigens and eliminate the corresponding malignant cells through antigen presentation, priming and infiltration of T cells, and cell death (leading to additional antigen release, perpetuating this process) has been termed the cancer-immunity cycle [19].

Constitutive expression of HPV16 E6, E7, and possible expression of other early oncogenic genes, depending on the status of HPV integration into the host genome, may make it more antigenic than its HPV-negative counterpart [8]. Naturally processed and presented T cell antigens restricted to HLA-A*02 have been identified for both HPV16 E6 and E7, and it is likely that other T cell antigens restricted to other HLA alleles exist [20]. Expression of these antigens known to be naturally processed and presented suggests that strong viral antigens may be present in every HPV-associated HNSCC. Yet clearly these tumors escape anti-tumor immunity and progress in many cases. Multiple mechanisms have been described that facilitate immune evasion. One process termed "immunoediting" describes the selection of tumor cells clones best able to escape T cell detection and elimination through loss of one or more components of interferon response or antigen expression, processing, or presentation [21, 22]. In a progressing tumor, the tumor subclones remaining have escaped T cells through genetic or epigenetic mechanisms and are able to progress. Loss of function mutations or epigenetic loss of expression in JAK and STAT proteins, interferon receptors, and loss of heterozygosity of HLA molecules have all been described as mechanisms that contribute to T cell escape [23–25].

Independent of the selection of tumor cell clones more resistant to immune detection, several mechanisms of immune escape driven by the tumor microenvironment (TME) exist. Physical barriers to effective T cell immunity exist within the TME including hypoxia, low pH, high interstitial pressure, and a lack of nutrients needed for immune cell function [26, 27]. Additionally, adaptive immune resistance refers to the interferon inducible expression of programmed death-ligand 1 (PD-L1) on tumor and stromal cells, which in turn binds programmed death receptor-1 (PD-1) expressed on activated T cells. PD-pathway activation is an inhibitory signal for activated T cells. Thus, activated IFN producing T cells can self-induce anergy through PD-1 expression. Tumors take advantage of this negative feedback loop designed to help prevent uncontrolled immune activation. Expression of cytotoxic T-lymphocyte antigen-4 (CTLA-4) on tumor infiltrating immune cells can occur independently of adaptive immune resistance, but also contributes to an exhausted T cell phenotype unable to exert effective anti-tumor immunity [17, 28]. Monoclonal antibodies that block the binding and function of PD-1, PD-L1, and CTLA-4 are the basis of Food and Drug Administration–approved immune checkpoint blockade (ICB) immunotherapy [29••, 30, 31]. Yet best current laboratory and clinical evidence suggests that ICB can only unleash pre-existing antigen-specific T cells that are being functionally suppressed by the interaction of immune checkpoint proteins. There is no evidence that ICB activates *new* antigen-specific immune responses. This explains why the subset of patients most likely to respond beneficially to ICB immunotherapy harbors tumors that are already immune inflamed at baseline [32]. However, more than half of all relapsed HNSCC demonstrate evidence of immune inflammation and clinical responses rates for ICB are 25% or less in most studies. There are clearly diverse mechanisms of immune escape beyond expression of immune checkpoints that likely contribute to therapeutic resistance observed with ICB. Enhancement of anti-tumor immunity "beyond immune checkpoint blockade" is now a

major research focus, with utilization of novel therapeutic approaches designed to expand or replace HPV-specific T cell immunity.

Immune Cell Populations, Distribution, and Activity Profiles in HPV-Associated OPSCC

Components of the local tumor microenvironment (TME) include stromal cells, infiltrating immune cells, extracellular matrix (ECM) proteins, and physical/chemical parameters. The interaction between these components creates a unique environment that contributes to tumor proliferation and resistance to treatment strategies [27]. Overall, HPV-associated OPSCC harbors more immune infiltration than HPV-negative HNSCC (Table 1; Fig. 1A) [33]. Recently, Cillo et al. demonstrated that populations of CD8 + T cells and regulatory T cells (Tregs) in both HPV-associated and HPV-negative HNSCC have many shared characteristics, whereas other immune cell populations (conventional CD4 + T cells, B cells, and myeloid cells) are more divergent [34]. It is possible that the presence of viral antigens priming differential immune responses through antigen presenting cells is a core mechanism underlying these differences [34, 35].

T-lymphocytes

T cells are the major effector cell type for anti-tumor immune responses. Many clinical studies use quantification of T cell

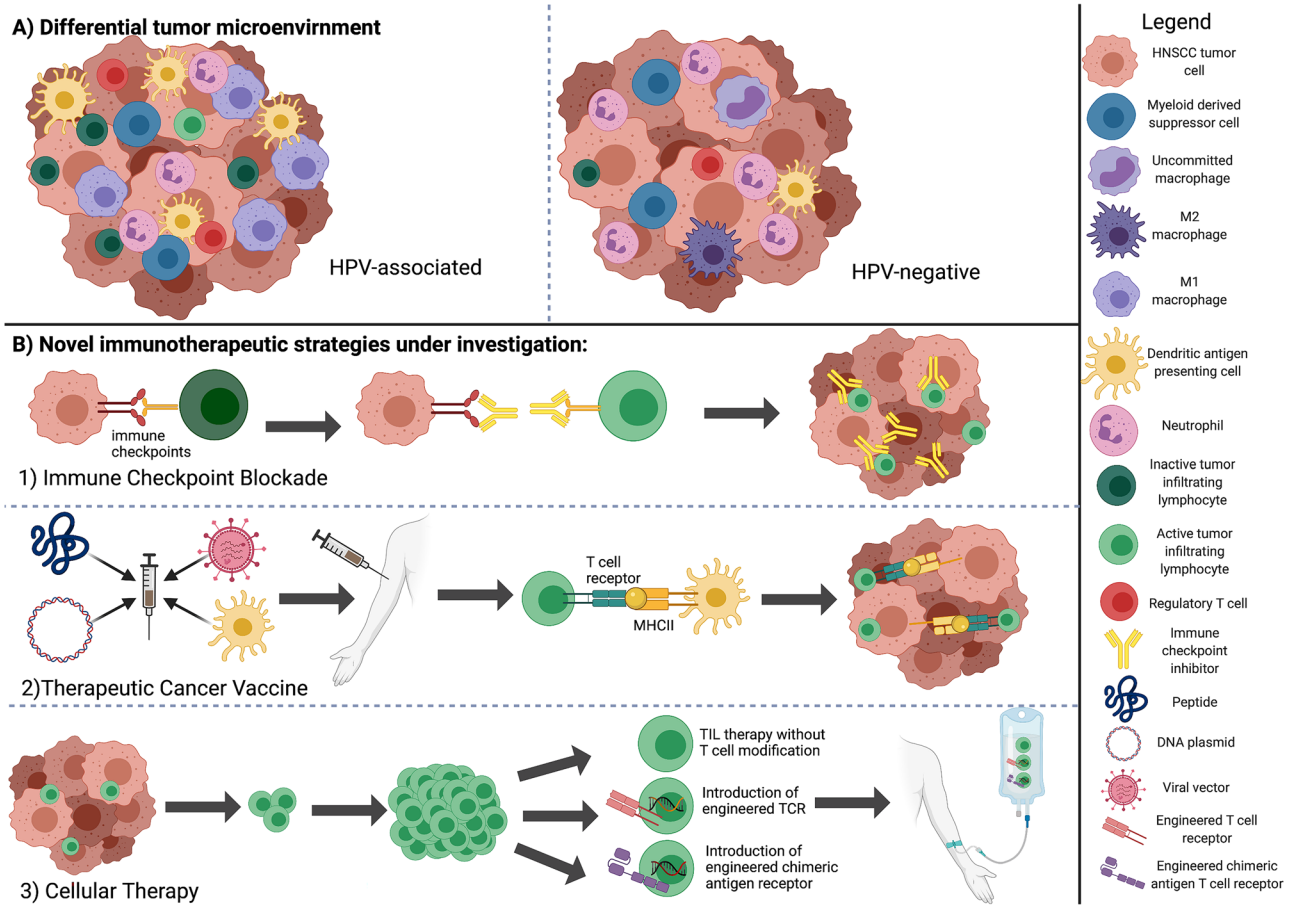

Fig. 1 Immunotherapy approaches under investigation to harness the immune inflamed phenotype of HPV-associated head and neck cancer. HPV-associated HNSCC has a relatively immune-inflamed phenotype. **A** HPV-associated HNSCC has a larger proportion of dendritic cells, T cells, and M1 macrophages present within the tumor microenvironment as compared to HPV-negative HNSCC, which harbors larger numbers of neutrophils and uncommitted or M2 macrophages. **B** Novel immunotherapy approaches for the treatment of HPV-associated HNSCC include immune checkpoint blockade, therapeutic cancer vaccines, and cellular therapy. Immune checkpoint blockade utilizes antibodies that inhibit T cell suppressive signaling. Therapeutic cancer vaccines introduce antigens to prime T cells against the tumor and can be peptide, live vector, nucleotide, or cell-based. Finally, cellular therapy introduces autologous T cells with or without an engineered T cell receptor or chimeric antigen receptor that are reinfused back into the patient after expansion. Created with BioRender.com

infiltration and effector function as an endpoint for demonstrating therapeutic efficacy. This typically includes histologic evaluation of T cell localization as well as functional assays such as production of cytokines including interferon-γ (IFN-γ) and tumor necrosis factor-α (TNF-α). Overall, more CD3 + T cells are found in HPV-associated tumors compared to HPV-negative tumors, including increases in CD4 + T cells, CD8 + T cells, and FoxP3 + Tregs [33, 35–37].

Numerous studies have demonstrated that increases in CD8 + tumor infiltrating lymphocytes (TIL) are associated with better prognosis in HPV-associated HNSCC [38, 39]. There is also evidence that CD8 + T cells are more active in HPV-associated HNSCC as measured by an increase in CD137, a costimulatory molecule expressed on activation, and effector cytokines such as IFN-γ [35, 37]. When T cells become activated, they also express negative regulatory surface receptors to modulate their activity, including PD-1 and LAG3. HPV-associated HNSCC demonstrates increased expression of these "exhaustion" markers, supporting the presence of a more active T cell phenotype and a mechanism that could be exploited to ensure prolonged T cell activation (i.e., immune checkpoint inhibitors) [33–35]. Similarly, CD39 is an immunosuppressive surface receptor that is expressed on antigen-specific T cells in colon and lung cancers and is highly expressed in HPV-associated HNSCC. Increased expression of CD39 correlated with increased overall survival in these patients reiterating the clinical importance of an active T cell phenotype [35].

The ratio of total T cells to Tregs is much higher in HPV-positive tumors compared to HPV-negative tumor [39]. Infiltration of Tregs is driven in part by HPV E7 and increases with tumor progression [36, 40]. Multiple studies have demonstrated positive or negative correlation with treatment outcomes and survival based upon Treg localization within the TME [36, 39, 41–43]. The discrepancy in prognostic value may be explained by the presence of two subpopulations of Tregs, determined by the cytokine profile of the surrounding TME [44]. Treg infiltration also correlated with CTLA-4 expression in HPV-associated HNSCC, suggesting an immunoregulatory role that could be exploited by immunotherapy [33].

Myeloid Cells

Myeloid cells are critical mediators of innate immune activation and are required for development of an adaptive immune response but can also be polarized to display immunosuppressive function. Myeloid derived suppressor cells (MDSCs) are a unique population of cells that promote tumor growth and perpetuate local immunosuppression [45]. MDSCs mediate suppression of effector immune cell function through multiple mechanisms including production of nutrient depleting arginase and suppressive cytokines such as TGF-β [46, 47]. Both HPV-associated and HPV-negative HNSCC are heavily infiltrated with MDSCs [48]. Notably, chemoradiation and HPV-targeted vaccination treatment strategies may reduce intratumoral MDSC populations [49, 50]. Additionally, STAT3 signaling within myeloid cells mediates some or most of their immunosuppressive function. Accordingly, therapeutic STAT3 inhibition may improve responses to chemoradiation through inhibition of immunosuppressive myeloid cell function [51]. This approach is under clinical study.

Consistent with an immune-inflamed phenotype, the macrophage profile of the HPV-associated HNSCC TME seems to be largely pro-inflammatory. HPV-associated OPSCC harbors increased pro-inflammatory M1 macrophages and decreased uncommitted (M0) and anti-inflammatory M2 macrophages [35, 52]. Increased M1 macrophages and an increased M1/M2 ratio correlated with better prognosis among HPV-associated HSNCC patients [52]. This is consistent with prior studies showing that immunosuppression is associated with M2 infiltration and reduction in patient survival [53]. Contrarily, another study demonstrated total infiltration of macrophages as measured by CD68 expression is increased in HPV-associated OPSCC and correlates with decreased recurrence-free and overall survival, likely due to promotion of tumor growth, production of immunosuppressive cytokines, and receptor-mediated inhibition of T cell activity via PD-1 and CTLA-4 [54, 55].

Dendritic cells are key antigen presenting cells (APCs) for T cell activation. Samples from HPV-associated OPSCC show significantly increased infiltration of dendritic cells [37]. RNAseq data in HPV-associated OPSCC show increases in transcription of genes for dendritic cell marker CD103, as well as those for several of their effector cytokines including IL-12 and IL-23, compared to HPV-negative tumors [35]. This is mechanistically consistent with data indicating increased cytotoxic T cell infiltration and activation.

Studies of granulocytic myeloid cells in HNSCC are limited despite demonstration that neutrophilic cells represent the most frequent tumor infiltrating immune cell type [56]. This is likely associated with the difficulty of studying neutrophilic cells as they do not survive cryopreservation and have relatively low numbers of RNA transcripts within each cell [57]. Infiltration of neutrophilic cells appears to be reduced in HPV-associated OPSCC compared to HPV-negative HNSCC [52, 58, 59]. Histologically, tumor infiltrating neutrophilic cell density correlates negatively with survival [52]. A recent study suggested that HPV E7 may partially inhibit neutrophil infiltration through downregulation of IL-8, a potent neutrophil attracting chemokine [58].

Current Immunotherapy Strategies in HPV-Associated OPSCC

Currently, standard-of-care treatment for newly diagnosed HPV-associated OPSCC includes surgical resection, radiation, and chemotherapy. Immunotherapy is not yet

incorporated into up-front definitive treatment. Immunotherapy strategies have been studied for HPV-associated OPSCC, along with HPV-negative HNSCC, in the setting of locoregional or distant disease relapse, and include ICB, therapeutic vaccines and cellular therapy (Fig. 1B).

Non-specific Activation: Immune Checkpoints

ICB, which reverses adaptive immune resistance, can lead to activation of existing T cell clones that are being "held back" by the expression of immune checkpoints. ICB leads to increased activity of many different T cell clones, some of which may target tumor antigens [60, 61]. This explains why patients that lack evidence of immune activation or PD-L1 expression in their tumors at baseline are less likely to clinically respond to ICB. Given the non-specific activation of T cell clones with ICB, immune overactivation and loss of self-tolerance to noncancerous tissue lead to a variety of immune-related adverse events [62]. Immune checkpoint blockade targeting PD-1 is currently the only FDA-approved immunotherapy for recurrent or metastatic HPV-associated OPSCC. FDA approval for PD-blockade was initially granted for second-line treatment of recurrent or metastatic HNSCC with pembrolizumab or nivolumab based upon the results of the KEYNOTE-012 and CheckMate 141 clinical studies, respectively [63, 64]. Checkmate 141 demonstrated superior survival with nivolumab treatment compared to investigators' choice systemic chemotherapy. KEYNOTE-012 demonstrated greater survival with pembrolizumab compared to expected historical controls for the second-line setting [65]. This changed with completion of the KEYNOTE-048 trial that demonstrated superior survival and reduced treatment-related toxicity with pembrolizumab, alone or in combination with systemic chemotherapy, compared to the EXTREME (cetuximab/platinum/fluorouracil) regimen for first-line therapy in patients with newly relapsed disease [29]. Positivity for p16, a surrogate marker for HPV-association, was not a significant predictor of response to pembrolizumab alone or in combination with chemotherapy. In other words, response rates of about 20–30% and survival are similar between patients with relapsed HPV-associated OPSCC and HPV-negative HNSCC [66]. These data suggest that the improved treatment responses to standard anti-cancer treatments observed with HPV-associated OPSCC in the up-front definitive setting are lost in the setting of relapsed disease treatment with ICB-based immunotherapy. The reasons for this are poorly understood but may involve selection of tumor cells clones following chemotherapy and radiation with genetic or epigenetic changes that make them highly resistant to treatment.

Another immune checkpoint is CTLA-4, which leads to T cell inhibition when engaged. CTLA-4 is primarily located intracellularly in Tregs and activated T cells. It has two ligands expressed on APCs, which it shares with the stimulatory T cell receptor, CD28 [67]. The homology between CTLA-4 and CD28 leads to an immunomodulatory balance that can fine tune the T cell response [68]. CTLA-4 blockade has not gained FDA approval for treatment of HNSCC because of associated toxicity observed in trials, possibly due to autoimmunity generated by tipping the balance towards CD28 [69]. Tremelimumab is a CTLA-4 inhibitor that showed some early benefit in combination with PD-1 blockade (durvalumab) for low PD-L1 expression tumors [70]. However, the larger, phase III EAGLE study demonstrated no additional benefit of tremelimumab beyond that observed with duvalumab alone [71••].

This is where we stand as of the writing of this manuscript: patients with newly diagnosed HPV-associated OPSCC are treated with standard anti-cancer treatments, albeit with greater chance of disease specific and overall survival compared to HPV-negative HNSCC. Patients with relapsed HPV-associated OPSCC are treated with pembrolizumab alone or in combination with chemotherapy (depending upon the combined positive PD-L1 score) with the hopes of unleashing the activity of one or more T cell clones specific for HPV. ICB certainly provides clinical benefit for a subset of patients, but there remain a significant proportion of patients that do not gain clinical benefit from these therapies and/or experience intolerable side effects. Thus, current research is focusing on the study of more specific immunotherapies designed to specifically induce, expand, or replace HPV-specific T cells. These investigational treatments are broadly categorized as therapeutic anti-cancer vaccines or cellular therapies.

Therapeutic Vaccines

If there is no pre-existing population of T cells capable of targeting HPV and recognizing cancer cells, immune checkpoint blockade is unlikely to be successful [72]. Vaccines may be a treatment approach to induce new or expand existing HPV-specific T cells. Preventive vaccines focus primarily on stimulating production of circulating antibody, thereby preventing initial oncogenic viral infection. Alternatively, therapeutic vaccines act to eliminate HPV infected cells by delivering antigen to APCs for stimulation of a specific T cell response. While no therapeutic vaccines have yet gained FDA approval, there are numerous ongoing clinical studies evaluating the ability of therapeutic vaccines to induce HPV-specific T cells and clinical responses, largely in patients with relapsed HPV-associated SCC. A portion of these studies highlighting the different vaccine types are summarized in Table 2. These vaccines can incorporate peptide, nucleic acid, whole cell, or live vectors. The most crucial step of these vaccines is antigen processing. In general, vaccines that stimulate antigen presentation that most closely

Table 2 Summary of selected therapeutic vaccine clinical studies for HPV-associated HNSCC

Category	Vaccine	Antigen	Concomitant immunostimulant or therapy	Cancer type	HPV status	Phase	Reported % of patients that developed antigen specific T cell response	Clinical disease improvement	Reference
Peptide/protein based	GL-0810	HPV16	Montanide, GM-CSF	HNSCC	+	I	80%	No complete or partial response	[87]
	GL-0817	MAGE-A3	Montanide, GM-CSF	HNSCC	+ or −	I	67%	No complete or partial response	[87]
	ISA101	synthetic HPV16 E6/7 peptides	Nivolumab	Multiple sites including HNSCC	+	II	Variable increase that did not correlate with clinical disease improvement	Overall response rate of 33% with a median duration of response of 10.3 months	[88•]
	p16 (INK4a)	P16_37-63	Montanide ISA-51 VG	Multiple sites including HNSCC	+	I/II	70%	All patients had stable or progressive disease	[89]
Nucleic acid based	MEDI0457 DNA plasmid	HPV16 E6/7	IL-12*1 patient treated with ICB	HNSCC	+	1/II	86%	1 patient with metastatic, progressive disease was treated with concomitant ICB and had a durable complete response	[90]
	GX-188E DNA plasmid	HPV16/18 E6/7	Fms-like tyrosine kinase-3 ligand	Cervical cancer	+	II	78%	15% complete response at 24 months	[91]
	AMV002 DNA plasmid	HP16 E6/7	None	HNSCC	+	I	83.3%	Not reported	[92]
Cell based	KLH-pulsed dendritic cells	HPV16/18 E7	None	Cervical cancer	+	I	80%	Not reported	[93]
Viral/vector	PRGN-2009 gorilla adenovirus vector	35 non-HLA restricted HPV 16/18 epitopes	Bintrafusp alfa	HNSCC	+	I/II*data not yet released	In preclinical murine model, 100% treated mice demonstrate antigen-specific response	Preclinical murine data showed reduction in tumor volume	[94, 95]
	TG-4001 vaccinia Ankara vector	HPV16 E6/7	IL-2	Cervical intraepithelial neoplasia	+	II	Not reported	48% at month 6	[96]
	ADXS11-001listeria monocytogenes vector	HPV16 E7	Cisplatin	Cervical cancer	+ or -	II	Not reported	Similar median progression free survival and overall response rates	[97]

approximates natural processing and presentation of tumor antigen may result in an enhanced T cell response. Although complete, unprocessed tumor-associated proteins are difficult to transport into APCs, they undergo natural processing and presentation ensuring that the same antigen is presented on both the vaccine stimulated APCs and tumor stimulated APCs. Conversely, vaccines based on minimal epitopes, such as a small peptide fragment, allow for easier production and delivery of the peptide payload but are restricted by the patient's HLA type. Nucleic acid–based vaccines must be taken up by APCs and translated into protein before presentation, producing the most natural response. However, they have lower immunogenicity than protein-based vaccines and in the case of RNA-based vaccines, are unstable and can be difficult to mass-produce [73]. Dendritic cell vaccines require preparation of personalized clones of DCs that present a specific antigen through RNA electroporation or pulsation with whole tumor cells [74].

Live vector vaccines use a live virus or bacteria to deliver nucleic acid or protein antigen to APCs for natural processing and presentation. Safety is a concern with live vector vaccines due to their innate pathogenicity. Another concern with administration of these vaccines is the existence of natural host immunity to the vector, leading to rapid elimination before the vaccine can induce antigen-specific T cell immunity. This also results in the inability to design vaccines with multiple doses, in contrast to protein or nucleic acid-based vaccines. To circumvent these issues, many live vector vaccines are engineered from non-human viruses with genomic edits to eliminate their ability to replicate [73].

Although therapeutic vaccines can induce a robust, HPV-specific T cell response, this has not always translated into clinical responses. Data from one clinical study in patients with advanced cervical cancer demonstrated that clinical response may be limited by local immunosuppression within the tumor microenvironment [75]. Since the PD-1/L1 pathway has been previously established as one of the mechanisms by which these tumors evade T cell immunity, it is reasonable to consider combining the priming and activation of T cells following therapeutic vaccination with the reversal of adaptive immune resistance achieved with ICB. Preclinical and clinical studies have also demonstrated that ICB and therapeutic vaccines act in synergy with conventional chemotherapy. Animal studies demonstrate increased APC expression of T cell stimulatory ligands after cisplatin therapy with improvements in responses generated by the addition of either CTLA-4 blockade or the therapeutic vaccine ISA101 [76]. In a clinical study of patients with advanced cervical cancer, those with a stronger immune response stimulated by ISA101 in combination with carboplatin/paclitaxel lived significantly longer (16.8 months) compared to those with a weaker immune response (11.2 months). This study characterized a reduction in the population of myeloid-derived suppressor cells, which is a potential explanation for the ability of chemotherapy to reduce immunosuppression [77]. Overall, therapeutic vaccines are being studied in several combination therapy regimens, as an adjunct to surgical resection, chemoradiation, and immune checkpoint inhibition that are currently in various phases of completion.

Cellular Therapies

In some scenarios, it may not be possible to activate existing HPV-specific T cells with ICB or therapeutic vaccination and it may be necessary to *replace* the patient's immune system with one better able to exert anti-tumor immunity using cell therapy technology. Cell therapy is the provision of a pre-stimulated, autologous, reactive clone of T cells that recognize one or multiple tumor-specific antigens and can be broken down into "TIL therapy" or "TCR-engineered cell therapy." In the first method, TILs can be cultured from a tumor fragment, expanded ex vivo, and reinfused into the same patient following treatment with a conditioning chemotherapy consisting of fludarabine and cyclophosphamide [78]. Alternatively, if a T cell receptor (TCR) with known antigen-specificity and known HLA restriction is available, autologous T cells can be engineered to express such a TCR and reinfused back into the same patient following a similar chemotherapy conditioning regimen [79]. A third option exists, which includes engineering autologous T cell to express a chimeric antigen receptor (CAR) instead of a TCR. CARs recognize cell surface epitopes similar to an antibody and are useful treatment approaches for malignancies that are highly clonal and consistently express targetable cell surface molecules, such as hematopoietic cancers [80]. CAR-engineered cell therapy is less useful for epithelial malignancies where the tumor specific antigens are intracellular, and antigens are presented on the surface of tumor cells via HLA for TCR detection. Furthermore, many of the cell surface antigens expressed on carcinomas are widely expressed on other tissues, raising concerns about treatment-related toxicity and tumor specificity of CAR-engineered cell therapy [81].

TIL therapy approaches have now been expanded into large clinical studies for multiple tumor types [82]. A phase II trial using TIL therapy in cervical and non-cervical HPV-associated SCC yielded an overall clinical response rate of 24% that correlated with the frequency of detected HPV-reactive T cells and included one patient with HPV-associated OPSCC that remained disease free up to 51 months post-treatment [83]. However, specificity of TILs was modest, at 30%, lending support for the movement to therapy involving highly specific TCRs.

Early clinical study of HPV16 E6 or E7-specific TCR-engineered cell therapy has yielded promising results [84]. A recent study that treated patients with metastatic vaginal,

head and neck, anal, and vulvar HPV-associated cancers with an HPV 16 E7-specific, HLA-A*02:01-restricted TCR demonstrated objective responses in 50% of patients and persistence of engineered T cells in the recipient over time. Some metastatic tumors completely regressed and did not reappear for the duration of study follow-up [85••]. Interestingly, several tumors that did not respond to the TCR-engineered cell therapy displayed resistance through one or more genetic defects in components of antigen processing and presentation or IFN response. Although genetic mechanisms of resistance to T cell detection and killing remain a concern, these initial promising results indicate a promising future for cellular therapies in the treatment of HPV-associated HNSCC.

Conclusions

Preventative HPV vaccination will hopefully reduce the overall incidence of HPV-associated carcinoma, but significant impact of preventative vaccination in the short term is in question due to disparity between the current vaccination strategy and associated risk factors for HPV infection [86]. Until the incidence of HPV-associated disease is reduced by wider early vaccination rates in both genders, development of oropharyngeal malignancy secondary to chronic infection with high-risk HPV will remain a dominant clinical entity. The presence of strong viral T cell antigens in these cancers makes T cell-based immunotherapy an attractive alternative therapeutic strategy to toxic standard anti-cancer therapies. Non-specific immunotherapy in the form of PD-based ICB is currently FDA-approved for relapsed HPV-associated disease, but novel, highly HPV-specific therapeutic vaccines and cell therapies are poised to provide safe and effective treatment options in the future. Clinical study is now underway for many of these exciting new immunotherapies in the setting of relapsed disease both as singular regimens or in combination with other immune targeted strategies. As the safety and efficacy of new HPV-specific immunotherapies are established in the relapsed disease setting, continued clinical study in the upfront treatment setting in combination with standard anti-cancer treatments, such as surgery or radiation, may yield new treatment options with equivalent oncologic control of disease but with less risk of long-term treatment-related toxicity.

Funding This work was supported by the Intramural Research Program of the National Institutes of Health (NIH), National Institute on Deafness and Other Communication Disorders (project number ZIA-DC00008). This research was also made possible through the NIH Medical Research Scholars Program, a public–private partnership supported jointly by the NIH and contributions to the Foundation for the NIH from the Doris Duke Charitable Foundation (DDCF Grant #2014194), the American Association for Dental Research, the Colgate-Palmolive Company, Genentech, Elsevier, and other private donors.

Compliance with Ethical Standards

Conflict of Interest The authors declare no competing interests.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

References

Papers of particular interest, published recently, have been highlighted as:
• Of importance
•• Of major importance

1. Ramqvist T, Dalianis T. Oropharyngeal cancer epidemic and human papillomavirus. Emerg Infect Dis. 2010;16:1671–7.
2. Pytynia KB, Dahlstrom KR, Sturgis EM. Epidemiology of HPV-associated oropharyngeal cancer. Oral Oncol. 2014;50:380–6.
3. (2020) HPV and Cancer. In: www.cdc.gov. https://www.cdc.gov/cancer/hpv/statistics/cases.htm.
4. Lassen P, Lacas B, Pignon J-P, Trotti A, Zackrisson B, Zhang Q, Overgaard J, Blanchard P. Prognostic impact of HPV-associated p16-expression and smoking status on outcomes following radiotherapy for oropharyngeal cancer: the MARCH-HPV project. Radiotherapy and oncology: journal of the European Society for Therapeutic Radiology and Oncology. 2018;126:107–15.
5. Ang KK, Harris J, Wheeler R, et al. Human papillomavirus and survival of patients with oropharyngeal cancer. N Engl J Med. 2010;363:24–35.
6.•• Geltzeiler M, Bertolet M, Albergotti W, et al (2018) Staging HPV-related oropharyngeal cancer: validation of AJCC-8 in a surgical cohort. Oral Oncol. 84:82–87 . **The American Joint Committee on Cancer (AJCC) 8th edition staging system differentiates HPV-associated OPSCC from HPV-negative OPSCC, highlighting pivotal differences in the pathogenesis, evolution, and response to treatment of these two entities. HPV status is a critical modulator for immune infiltration and will likely play a role in determining response to immunotherapy.**
7. Chakravarthy A, Henderson S, Thirdborough SM, Ottensmeier CH, Su X, Lechner M, Feber A, Thomas GJ, Fenton TR. Human papillomavirus drives tumor development throughout the head and neck: improved prognosis is associated with an immune response largely restricted to the oropharynx. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2016;34:4132–41.
8. Fakhry C, Westra WH, Li S, Cmelak A, Ridge JA, Pinto H, Forastiere A, Gillison ML. Improved survival of patients with human papillomavirus-positive head and neck squamous cell carcinoma in a prospective clinical trial. J Natl Cancer Inst. 2008;100:261–9.
9. Dai M, Clifford GM, le Calvez F, Castellsagué X, Snijders PJF, Pawlita M, Herrero R, Hainaut P, Franceschi S. Human papillomavirus type 16 and TP53 mutation in oral cancer: matched analysis of the IARC multicenter study. Can Res. 2004;64:468–71.

10. Rühle A, Grosu A-L, Nicolay NH. De-escalation strategies of (chemo)radiation for head-and-neck squamous cell cancers-HPV and beyond. Cancers. 2021. https://doi.org/10.3390/cancers13092204.
11. Mehanna H, Robinson M, Hartley A, et al. Radiotherapy plus cisplatin or cetuximab in low-risk human papillomavirus-positive oropharyngeal cancer (De-ESCALaTE HPV): an open-label randomised controlled phase 3 trial. Lancet (London, England). 2019;393:51–60.
12. Ferris RL, Flamand Y, Weinstein GS, et al. Phase II randomized trial of transoral surgery and low-dose intensity modulated radiation therapy in resectable p16+ locally advanced oropharynx cancer: an ECOG-ACRIN Cancer Research Group Trial 2021;(E3311). JCO JCO.21.01752.
13. Linnemann C, van Buuren MM, Bies L, et al. High-throughput epitope discovery reveals frequent recognition of neo-antigens by CD4+ T cells in human melanoma. Nat Med. 2015;21:81–5.
14. Petljak M, Alexandrov LB, Brammeld JS, et al. Characterizing mutational signatures in human cancer cell lines reveals episodic APOBEC mutagenesis. Cell. 2019;176:1282-1294.e20.
15. Matsushita H, Vesely MD, Koboldt DC, et al. Cancer exome analysis reveals a T-cell-dependent mechanism of cancer immunoediting. Nature. 2012;482:400–4.
16. Simpson AJG, Caballero OL, Jungbluth A, Chen Y-T, Old LJ. Cancer/testis antigens, gametogenesis and cancer. Nat Rev Cancer. 2005;5:615–25.
17. Rooney MS, Shukla SA, Wu CJ, Getz G, Hacohen N. Molecular and genetic properties of tumors associated with local immune cytolytic activity. Cell. 2015;160:48–61.
18. McGranahan N, Furness AJS, Rosenthal R, et al. Clonal neoantigens elicit T cell immunoreactivity and sensitivity to immune checkpoint blockade. Science (New York, NY). 2016;351:1463–9.
19. Chen DS, Mellman I. Oncology meets immunology: the cancer-immunity cycle. Immunity. 2013;39:1–10.
20. Evans C, Bauer S, Grubert T, Brucker C, Baur S, Heeg K, Wagner H, Lipford GB. HLA-A2-restricted peripheral blood cytolytic T lymphocyte response to HPV type 16 proteins E6 and E7 from patients with neoplastic cervical lesions. Cancer immunology, immunotherapy : CII. 1996;42:151–60.
21. Dunn GP, Bruce AT, Ikeda H, Old LJ, Schreiber RD. Cancer immunoediting: from immunosurveillance to tumor escape. Nat Immunol. 2002;3:991–8.
22. Urosevic M, Dummer R. Human leukocyte antigen-G and cancer immunoediting. Can Res. 2008;68:627–30.
23. Dunn GP, Bruce AT, Sheehan KCF, et al. A critical function for type I interferons in cancer immunoediting. Nat Immunol. 2005;6:722–9.
24. Sharma P, Hu-Lieskovan S, Wargo JA, Ribas A. Primary, adaptive, and acquired resistance to cancer immunotherapy. Cell. 2017;168:707–23.
25. Tran E, Robbins PF, Lu Y-C, et al. T-cell transfer therapy targeting mutant KRAS in cancer. N Engl J Med. 2016;375:2255–62.
26. Lim AR, Rathmell WK, Rathmell JC. The tumor microenvironment as a metabolic barrier to effector T cells and immunotherapy. eLife. 2020. https://doi.org/10.7554/eLife.55185
27. Bhat AA, Yousuf P, Wani NA, et al. Tumor microenvironment: an evil nexus promoting aggressive head and neck squamous cell carcinoma and avenue for targeted therapy. Signal Transduct Target Ther. 2021;6:12.
28. Ahmadzadeh M, Johnson LA, Heemskerk B, Wunderlich JR, Dudley ME, White DE, Rosenberg SA. Tumor antigen-specific CD8 T cells infiltrating the tumor express high levels of PD-1 and are functionally impaired. Blood. 2009;114:1537–44.
29.•• Burtness B, Harrington KJ, Greil R, et al. Pembrolizumab alone or with chemotherapy versus cetuximab with chemotherapy for recurrent or metastatic squamous cell carcinoma of the head and neck (KEYNOTE-048): a randomised, open-label, phase 3 study. Lancet (London, England). 2019;394:1915–28. **The KEYNOTE-048 trial was instrumental to the decision to recommend Pembrolizumab as first-line treatment for recurrent HNSCC, which is the first and only FDA-approved immunotherapy treatment for HNSCC to date.**
30. Ferris RL, Blumenschein GJ, Fayette J, et al. Nivolumab vs investigator's choice in recurrent or metastatic squamous cell carcinoma of the head and neck: 2-year long-term survival update of CheckMate 141 with analyses by tumor PD-L1 expression. Oral Oncol. 2018;81:45–51.
31. Hodi FS, O'Day SJ, McDermott DF, et al. Improved survival with ipilimumab in patients with metastatic melanoma. N Engl J Med. 2010;363:711–23.
32. Chen DS, Mellman I. Elements of cancer immunity and the cancer-immune set point. Nature. 2017;541:321–30.
33. Mandal R, Şenbabaoğlu Y, Desrichard A, et al. The head and neck cancer immune landscape and its immunotherapeutic implications. JCI insight 2016;1:e89829.
34. Cillo AR, Kürten CHL, Tabib T, et al. Immune landscape of viral- and carcinogen-driven head and neck cancer. Immunity. 2020;52:183-199.e9.
35. Gameiro SF, Ghasemi F, Barrett JW, Koropatnick J, Nichols AC, Mymryk JS, Maleki Vareki S. Treatment-naïve HPV+ head and neck cancers display a T-cell-inflamed phenotype distinct from their HPV-counterparts that has implications for immunotherapy. Oncoimmunology. 2018;7:e1498439.
36. Kindt N, Descamps G, Seminerio I, Bellier J, Lechien JR, Mat Q, Pottier C, Delvenne P, Journé F, Saussez S. High stromal Foxp3-positive T cell number combined to tumor stage improved prognosis in head and neck squamous cell carcinoma. Oral Oncol. 2017;67:183–91.
37. Partlová S, Bouček J, Kloudová K, et al. Distinct patterns of intratumoral immune cell infiltrates in patients with HPV-associated compared to non-virally induced head and neck squamous cell carcinoma. Oncoimmunology. 2015;4:e965570.
38. Balermpas P, Rödel F, Rödel C, et al. CD8+ tumour-infiltrating lymphocytes in relation to HPV status and clinical outcome in patients with head and neck cancer after postoperative chemoradiotherapy: a multicentre study of the German cancer consortium radiation oncology group (DKTK-ROG). Int J Cancer. 2016;138:171–81.
39. Punt S, Dronkers EAC, Welters MJP, et al. A beneficial tumor microenvironment in oropharyngeal squamous cell carcinoma is characterized by a high T cell and low IL-17(+) cell frequency. Cancer immunology, immunotherapy : CII. 2016;65:393–403.
40. Russell S, Angell T, Lechner M, Liebertz D, Correa A, Sinha U, Kokot N, Epstein A. Immune cell infiltration patterns and survival in head and neck squamous cell carcinoma. Head Neck Oncol. 2013;5:24.
41. Liang Y, Liu H, Su Y, Zhang T, Chu M, Liang L, Liao G. Foxp3 expressed by tongue squamous cell carcinoma cells correlates with clinicopathologic features and overall survival in tongue squamous cell carcinoma patients. Oral Oncol. 2011;47:566–70.
42. Al-Qahtani D, Anil S, Rajendran R. Tumour infiltrating CD25+ FoxP3+ regulatory T cells (Tregs) relate to tumour grade and stromal inflammation in oral squamous cell carcinoma. Journal of oral pathology & medicine: official publication of the International Association of Oral Pathologists and the American Academy of Oral Pathology. 2011;40:636–42.
43. Seminerio I, Descamps G, Dupont S, de Marrez L, Laigle J-A, Lechien JR, Kindt N, Journe F, Saussez S. Infiltration of FoxP3+ regulatory T cells is a strong and independent prognostic factor in head and neck squamous cell carcinoma. Cancers. 2019. https://doi.org/10.3390/cancers11020227.

44. Saito T, Nishikawa H, Wada H, et al. Two FOXP3(+)CD4(+) T cell subpopulations distinctly control the prognosis of colorectal cancers. Nat Med. 2016;22:679–84.
45. Veglia F, Perego M, Gabrilovich D. Myeloid-derived suppressor cells coming of age. Nat Immunol. 2018;19:108–19.
46. Rodriguez PC, Hernandez CP, Quiceno D, Dubinett SM, Zabaleta J, Ochoa JB, Gilbert J, Ochoa AC. Arginase I in myeloid suppressor cells is induced by COX-2 in lung carcinoma. J Exp Med. 2005;202:931–9.
47. Dumitriu IE, Dunbar DR, Howie SE, Sethi T, Gregory CD. Human dendritic cells produce TGF-beta 1 under the influence of lung carcinoma cells and prime the differentiation of CD4+CD25+Foxp3+ regulatory T cells. J Immunol (Baltimore, Md : 1950). 2009;182:2795–2807.
48. Ma X, Sheng S, Wu J, et al. LncRNAs as an intermediate in HPV16 promoting myeloid-derived suppressor cell recruitment of head and neck squamous cell carcinoma. Oncotarget. 2017;8:42061–75.
49. Parikh F, Duluc D, Imai N, et al. Chemoradiotherapy-induced upregulation of PD-1 antagonizes immunity to HPV-related oropharyngeal cancer. Can Res. 2014;74:7205–16.
50. Li S, Zhang Q, Bai H, Huang W, Shu C, Ye C, Sun W, Ma Y. Self-assembled nanofibers elicit potent HPV16 E7-specific cellular immunity and abolish established TC-1 graft tumor. Int J Nanomed. 2019;14:8209–19.
51. Moreira D, Sampath S, Won H, et al. Myeloid cell-targeted STAT3 inhibition sensitizes head and neck cancers to radiotherapy and T cell-mediated immunity. J Clin Investig. 2021. https://doi.org/10.1172/JCI137001.
52. Chen X, Yan B, Lou H, Shen Z, Tong F, Zhai A, Wei L, Zhang F. Immunological network analysis in HPV associated head and neck squamous cancer and implications for disease prognosis. Mol Immunol. 2018;96:28–36.
53. Costa NL, Valadares MC, Souza PPC, Mendonça EF, Oliveira JC, Silva TA, Batista AC. Tumor-associated macrophages and the profile of inflammatory cytokines in oral squamous cell carcinoma. Oral Oncol. 2013;49:216–23.
54. Seminerio I, Kindt N, Descamps G, Bellier J, Lechien JR, Mat Q, Pottier C, Journé F, Saussez S. High infiltration of CD68+ macrophages is associated with poor prognoses of head and neck squamous cell carcinoma patients and is influenced by human papillomavirus. Oncotarget. 2018;9:11046–59.
55. Galdiero MR, Bonavita E, Barajon I, Garlanda C, Mantovani A, Jaillon S. Tumor associated macrophages and neutrophils in cancer. Immunobiology. 2013;218:1402–10.
56. Gregory AD, Houghton AM. Tumor-associated neutrophils: new targets for cancer therapy. Can Res. 2011;71:2411–6.
57. Tamassia N, Cassatella MA, Bazzoni F. Fast and accurate quantitative analysis of cytokine gene expression in human neutrophils by reverse transcription real-time PCR. Methods in molecular biology (Clifton, NJ). 2020;2087:243–60.
58. Li C, Zhao L, Wang Q, et al. Neutrophils infiltration and its correlation with human papillomavirus status in the oral squamous cell carcinoma. Cancer management and research. 2019;11:5171–85.
59. Xu L, Jin Y, Qin X. Comprehensive analysis of significant genes and immune cell infiltration in HPV-related head and neck squamous cell carcinoma. Int Immunopharmacol. 2020;87:106844.
60. Barber DL, Wherry EJ, Masopust D, Zhu B, Allison JP, Sharpe AH, Freeman GJ, Ahmed R. Restoring function in exhausted CD8 T cells during chronic viral infection. Nature. 2006;439:682–7.
61. Hashimoto M, Kamphorst AO, Im SJ, Kissick HT, Pillai RN, Ramalingam SS, Araki K, Ahmed R. CD8 T cell exhaustion in chronic infection and cancer: opportunities for interventions. Annu Rev Med. 2018;69:301–18.
62. Ramos-Casals M, Brahmer JR, Callahan MK, Flores-Chávez A, Keegan N, Khamashta MA, Lambotte O, Mariette X, Prat A, Suárez-Almazor ME. Immune-related adverse events of checkpoint inhibitors. Nat Rev Dis Primers. 2020;6:38.
63. Seiwert TY, Burtness B, Mehra R, et al. Safety and clinical activity of pembrolizumab for treatment of recurrent or metastatic squamous cell carcinoma of the head and neck (KEYNOTE-012): an open-label, multicentre, phase 1b trial. Lancet Oncol. 2016;17:956–65.
64. Gillison ML, Blumenschein GJ, Fayette J, et al. CheckMate 141: 1-year update and subgroup analysis of nivolumab as first-line therapy in patients with recurrent/metastatic head and neck cancer. Oncologist. 2018;23:1079–82.
65. Szturz P, Vermorken JB. Management of recurrent and metastatic oral cavity cancer: Raising the bar a step higher. Oral Oncol. 2020;101:104492.
66. Powell SF, Gold KA, Gitau MM, et al. Safety and efficacy of pembrolizumab with chemoradiotherapy in locally advanced head and neck squamous cell carcinoma: a phase IB study. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2020;38:2427–37.
67. Egen JG, Kuhns MS, Allison JP. CTLA-4: new insights into its biological function and use in tumor immunotherapy. Nat Immunol. 2002;3:611–8.
68. Rowshanravan B, Halliday N, Sansom DM. CTLA-4: a moving target in immunotherapy. Blood. 2018;131:58–67.
69. Wolchok JD, Saenger Y. The mechanism of anti-CTLA-4 activity and the negative regulation of T-cell activation. Oncologist. 2008;13(Suppl 4):2–9.
70. Siu LL, Even C, Mesía R, et al. Safety and efficacy of durvalumab with or without tremelimumab in patients with PD-L1-low/negative recurrent or metastatic HNSCC: the phase 2 CONDOR randomized clinical trial. JAMA Oncol. 2019;5:195–203.
71.•• Ferris RL, Haddad R, Even C, et al. Durvalumab with or without tremelimumab in patients with recurrent or metastatic head and neck squamous cell carcinoma: EAGLE, a randomized, open-label phase III study. Annals of oncology : official journal of the European Society for Medical Oncology. 2020;31:942–50. **The EAGLE study demonstrated no increased benefit with tremelimumab, a CTLA-4 inhibitor, beyond the effect of durvalumab, a PD-1 inhibitor. Given the increased immune-related toxicity with CTLA-4 inhibitors and lack of additional clinical improvement, CTLA-4 inhibition has had limited clinical applications in the treatment of HNSCC. These findings have prompted investigation of more specific T cell induction via therapeutic vaccine and cellular immunotherapy.**
72. Bonaventura P, Shekarian T, Alcazer V, Valladeau-Guilemond J, Valsesia-Wittmann S, Amigorena S, Caux C, Depil S. Cold tumors: a therapeutic challenge for immunotherapy. Front Immunol. 2019;10:168.
73. Yang A, Jeang J, Cheng K, Cheng T, Yang B, Wu T-C, Hung C-F. Current state in the development of candidate therapeutic HPV vaccines. Expert Rev Vaccines. 2016;15:989–1007.
74. Benencia F, Courrèges MC, Coukos G. Whole tumor antigen vaccination using dendritic cells: comparison of RNA electroporation and pulsing with UV-irradiated tumor cells. J Transl Med. 2008;6:21.
75. Kenter GG, Welters MJP, Valentijn ARPM, et al. Phase I immunotherapeutic trial with long peptides spanning the E6 and E7 sequences of high-risk human papillomavirus 16 in end-stage cervical cancer patients shows low toxicity and robust immunogenicity. Clinical cancer research : an official journal of the American Association for Cancer Research. 2008;14:169–77.

76. Beyranvand Nejad E, van der Sluis TC, van Duikeren S, Yagita H, Janssen GM, van Veelen PA, Melief CJM, van der Burg SH, Arens R. Tumor eradication by cisplatin is sustained by CD80/86-mediated costimulation of CD8+ T Cells. Can Res. 2016;76:6017–29.
77. Melief CJM, Welters MJP, Vergote I, et al. Strong vaccine responses during chemotherapy are associated with prolonged cancer survival. Science translational medicine. 2020. https://doi.org/10.1126/scitranslmed.aaz8235.
78. Chandran SS, Somerville RPT, Yang JC, et al. Treatment of metastatic uveal melanoma with adoptive transfer of tumour-infiltrating lymphocytes: a single-centre, two-stage, single-arm, phase 2 study. Lancet Oncol. 2017;18:792–802.
79. Ramachandran I, Lowther DE, Dryer-Minnerly R, et al. Systemic and local immunity following adoptive transfer of NY-ESO-1 SPEAR T cells in synovial sarcoma. J Immunother Cancer. 2019;7:276.
80. Cappell KM, Sherry RM, Yang JC, Goff SL, Vanasse DA, McIntyre L, Rosenberg SA, Kochenderfer JN. Long-term follow-up of anti-CD19 chimeric antigen receptor T-cell therapy. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2020;38:3805–15.
81. Newick K, O'Brien S, Moon E, Albelda SM. CAR T Cell Therapy for Solid Tumors. Annu Rev Med. 2017;68:139–52.
82. National Institutes of Health Clinical Trials Using Tumor Infiltrating Lymphocyte Therapy. https://www.cancer.gov/about-cancer/treatment/clinical-trials/intervention/tumor-infiltrating-lymphocyte-therapy. Accessed 11 Aug 2021.
83. Stevanović S, Helman SR, Wunderlich JR, et al. A phase II study of tumor-infiltrating lymphocyte therapy for human papillomavirus-associated epithelial cancers. Clinical cancer research: an official journal of the American Association for Cancer Research. 2019;25:1486–93.
84. Draper LM, Kwong MLM, Gros A, Stevanović S, Tran E, Kerkar S, Raffeld M, Rosenberg SA, Hinrichs CS. Targeting of HPV-16+ epithelial cancer cells by TCR gene engineered T cells directed against E6. Clinical cancer research: an official journal of the American Association for Cancer Research. 2015;21:4431–9.
85.•• Nagarsheth NB, Norberg SM, Sinkoe AL, et al. TCR-engineered T cells targeting E7 for patients with metastatic HPV-associated epithelial cancers. Nat Med. 2021;27:419–25. **This study demonstrated successful and sustained treatment using engineered T cell therapy in a significant portion of patients with metastatic vaginal, head and neck, anal, and vulvar HPV-associated cancers. This study used the engineered HPV 16 E7-specific, HLA-A*02:01-restricted TCR in particular, but paves the way for further development of engineered T cell therapy for HPV-associated cancer.**
86. Gillison ML, Chaturvedi AK, Anderson WF, Fakhry C. Epidemiology of human papillomavirus-positive head and neck squamous cell carcinoma. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2015;33:3235–42.
87. Zandberg DP, Rollins S, Goloubeva O, et al. A phase I dose escalation trial of MAGE-A3- and HPV16-specific peptide immunomodulatory vaccines in patients with recurrent/metastatic (RM) squamous cell carcinoma of the head and neck (SCCHN). Cancer immunology, immunotherapy : CII. 2015;64:367–79.
88.• Massarelli E, William W, Johnson F, et al (2019) Combining Immune Checkpoint Blockade and Tumor-Specific Vaccine for Patients With Incurable Human Papillomavirus 16-Related Cancer: A Phase 2 Clinical Trial. JAMA Oncol 5:67–73. Combination treatment with ICB and tumor-specific vaccine yielded an overall response rate of 33% in patients with heavily pre-treated advanced HPV-associated OPSCC, demonstrating the utility of combination treatment with multiple targeted immunotherapy approaches.
89. Reuschenbach M, Pauligk C, Karbach J, et al. A phase 1/2a study to test the safety and immunogenicity of a p16INK4a peptide vaccine in patients with advanced human papillomavirus-associated cancers. Cancer. 2016;122:1425–33.
90. Aggarwal C, Cohen RB, Morrow MP, et al. Immunotherapy targeting HPV16/18 generates potent immune responses in HPV-associated head and neck cancer. Clinical cancer research: an official journal of the American Association for Cancer Research. 2019;25:110–24.
91. Youn JW, Hur S-Y, Woo JW, et al. Pembrolizumab plus GX-188E therapeutic DNA vaccine in patients with HPV-16-positive or HPV-18-positive advanced cervical cancer: interim results of a single-arm, phase 2 trial. Lancet Oncol. 2020;21:1653–60.
92. Chandra J, Woo WP, Finlayson N, et al. A phase 1, single centre, open label, escalating dose study to assess the safety, tolerability and immunogenicity of a therapeutic human papillomavirus (HPV) DNA vaccine (AMV002) for HPV-associated head and neck cancer (HNC). Cancer Immunol Immunother. 2021;70:743–53.
93. Santin AD, Bellone S, Palmieri M, Zanolini A, Ravaggi A, Siegel ER, Roman JJ, Pecorelli S, Cannon MJ. Human papillomavirus type 16 and 18 E7-pulsed dendritic cell vaccination of stage IB or IIA cervical cancer patients: a phase I escalating-dose trial. J Virol. 2008;82:1968–79.
94. Pellom ST, Smalley Rumfield C, Morillon YM II, Roller N, Poppe LK, Brough DE, Sabzevari H, Schlom J, Jochems C. Characterization of recombinant gorilla adenovirus HPV therapeutic vaccine PRGN-2009. JCI Insight. 2021. https://doi.org/10.1172/jci.insight.141912.
95. Floudas CS, Strauss J, Allen C, et al. First-in-human phase I/II trial of PRGN-2009 vaccine as monotherapy or with bintrafusp alfa in patients with recurrent/metastatic (R/M) human papillomavirus (HPV)-associated cancers (HPVC) and as neoadjuvant/induction therapy in locoregionally advanced (LA) HPV oropharyngeal (OP) and sinonasal (SN) squamous cell cancer (SCC). JCO 2021;39:TPS6092–TPS6092.
96. Brun J-L, Dalstein V, Leveque J, et al. Regression of high-grade cervical intraepithelial neoplasia with TG4001 targeted immunotherapy. Am J Obstet Gynecol. 2011;204:169.e1-169.e8.
97. Basu P, Mehta A, Jain M, Gupta S, Nagarkar RV, John S, Petit R. A randomized phase 2 study of ADXS11-001 Listeria monocytogenes-Listeriolysin O immunotherapy with or without cisplatin in treatment of advanced cervical cancer. Int J Gynecol Cancer. 2018;28:764–72.