Published in final edited form as:

Oral Oncol. 2014 September ; 50(9): 780–784. doi:10.1016/j.oraloncology.2013.09.010.

Tailored immunotherapy for HPV positive head and neck squamous cell cancer

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SUMMARY

Human papilloma virus (HPV) associated oropharynx carcinoma (OPC) is increasingly common, with a distinct biology from HPV negative OPC. In spite of this better prognosis, morbidity is significant and treatment related after effects can be debilitating. Because the foreign viral proteins that drive HPV+ cancers are known, there are multiple options for tailored immune therapies. Herein we review the immunologic basis for disease and emerging immune therapies. The oncogenesis of HPV+ SCCHN goes beyond cell cycle deregulation, and relies on the immune escape through (E5, E6, and E7) downregulating antigen processing, interferon response, as well as STAT-1 signaling. Individual susceptibilities to HPV infection may vary. The treatment of HPV+ cancers has had a wide range of successes and failures. Perhaps the shining example of immunoprevention has been the L1 protein vaccines developed for cervical cancer prevention, however this vaccine has not been beneficial for people already infected. Therefore multiple strategies have been employed in the cancer therapeutic realm for people with existing disease. These agents range from peptides, to viral vectors, to adoptive cell therapy. In this review we consider the work done in both SCCHN and cervical cancer, as these therapeutic targets are the similar. The listed studies are not exhaustive, but rather illustrate experimental design and approach.

Keywords

Human papilloma virus; Head and neck squamous cell cancer; Immunotherapy; Vaccine

Introduction

HPV positive oropharyngeal cancer incidence is increasing both in the US population and worldwide over the last several decades. Although HPV positive cancers have a 60–80%

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Conflict of interest statement

None declared.

reduced risk of death when compared with HPV negative oropharyngeal cancers, the increased incidence HPV positive cases is increasingly important in terms of total cancer mortality in SCCN [1]. This near epidemic increase heralds an immediate need to develop better therapies to treat this disease.

Effective immune response to HPV infection

As with many viral infections, many people are exposed yet most clear the infection due to immune mediated clearance of infected cells. Most healthy individuals mount a Th1, Th2, and CTL reaction to viral HPV epitopes, both early (E2, E6, and E7) as well as late (L1). Clearance of HPV associated lesions has been correlated with HPV specific circulating CD4+ and CD8+ T cells [2]. Interestingly, even in patients with progressive HPV infection, those who have an HPV-specific Th1 mediated response appear to have a more mild clinical course [3]. In the majority of patients local HPV infection can eventually be cleared through a combination of specific T cell mediated immunity. HPV-E7 T cells from HPV-infected OPSCC are elevated, as compared to HPV-negative OPSCC or healthy individuals but are functionally impaired, as described below [4].

Mechanisms of immune escape

In a subset of patients this HPV infection persists that can lead to the development of cancer. Persistence is likely multi-factorial. In certain immune suppressed patients a dampened immune response likely contributes to this persistence. Oral HPV prevalence is increased in HIV positive patients even when controlling for other risk factors (adjusted OR = 2.1) and correlates with decreased CD4 count [5]. However, even in otherwise healthy patients, HPV can persist. HPV viral gene mechanisms have been shown to aid in some immune tolerance. Immune escape is one of the key processes that are required to allow HPV infection persistence, eventually leading to cellular malignant transformation. The main viral proteins associated with immune escape are E5, E6, and E7. E5 plays a role in inhibiting the innate immune response by interacting with HLA-I heavy chain, resulting in reduced cell surface HLA-I [3,6–8]. E7 expression has been shown to down regulate cell expression of HLA class I, as well as expression of transporter associated with antigen processing (TAP) [9]. The mechanism of has been proposed to occur by E7 interacting with IRF-1 and disrupting its control of these key target genes for antigen expression [10]. HPV E6 inhibits the STAT-1 pathway which is critical signaling pathway involved in many process leading to cellular immune response. Using multiple mechanisms the early viral genes preferentially alter the infected epithelial cell as a means to prevent immune detection and recognition by antiviral T cells [11].

In addition to altering the antigen processing in the epithelial cell, HPV persistence likely depends the complex factors and pathways involved in immune clearance. For instance, when individuals are divided into groups that clear versus groups that allow persistence there are differences in T-cell reactivity to early vs. late antigens. In general in individuals where HPV persists there are reduced responses to early antigens as compared to late antigens. This in part explains the phenomenon of persistent HPV epithelial infection, where early antigens are only expressed once HPV is already invasive. In chronic premalignant lesions HPV L1 decreases and in SCC lesions HPV L1 staining is completely absent [12].

This is the histologic basis for the observation that L1 capsid preventative vaccines are ineffective in established HPV+ SCC. It also appears that the majority of healthy controls and cervical cancer patients are able to mount a systemic Th1 response against L1 capsid epitopes [13]. Therefore, successful therapeutic targeting will likely involve strategies that enhance an individual's ability to clear cells that express early viral oncogenes.

Therapies

Since HPV cancers contain foreign viral proteins and we are beginning to understand immune escape by these cells it may be possible to design therapies that can enhance clearance of this virus induced cancer. To this end our lab in 2005 examined if patients with HPV+ cancer had circulating T-cells to the early gene E7. In this series of experiments, the peripheral blood of both HPV positive and negative oropharyngeal squamous cell cancer patients were examined for T-cell subsets. The frequencies of HPV E7 specific T cells, as measured by HLA-A0201 tetramer were increased in HPV positive SCCHN patients. Since E7 downregulates expression of HLA-I, we added exogenous IFN-gamma to an HLA matched HPV positive SCCHN cell line to increase expression of antigen-processing machinery (APM). In the presence of IFN-gamma, antigen-processing machinery was induced; as a result the patients HPV E7 specific T-cells had improved cytolytic activity. E7 mediated immune suppression through APM downregulation was reversed when antigen processing was restored [4]. In this section we will discuss many current trials with therapies that could act to enhance HPV-specific immune clearance.

Immunomodulators

The toll like receptors (TLRs) are important for recognizing pathogen associated molecular patterns (PAMPs), acting as a danger signal important for initiating the innate and eventually adaptive immune responses. Imiquimod is a topical TLR 7 agonist, approved for a variety of skin cancers and VIN. Dendritic cells have the most robust response to imiquimod therapy [14]. In a study of 52 patients with grade 2 or 3 vulvar intraepithelial lesions were enrolled and randomized to receive either placebo or topical imiquimod to the vulvar lesions twice weekly for 16 weeks. Of the 26 patients treated with imiquimod, 21 had partial response of 25% decrease in lesion size. Additionally, the imiquimod group had greater histologic regression, HPV clearance, and symptom improvement. Of the entire study group, three patients progressed to invasion. In the imiquimod group nine patients had a complete and durable response [15]. In a follow-up study the same group showed that imiquimod induced HPV clearance in VIN, and that this strongly correlated with histologic normalization as well as decreased immune infiltrate [16]. Additionally, imiquimod has been used successfully in combination with HPV peptide vaccine for VIN with response rates of up to 63% [17]. TLR agonists have not undergone clinical trials in SCCHN; however, it may be possible that other less toxic TLR agents may have similar efficacy in HPV+ SCCHN.

Cytokines

In spite of clinical responses systemic cytokine therapy has proven to be overly toxic for long-term administration. The most common cytokines used in cervical cancer and OPSCC therapy are IL-2, IL-12, GM-CSF, and IFN-alpha. Due to toxic systemic effects, local

administration seems to be better tolerated [18] IL-12 may improve cetuximab efficacy in triggering NK cell ADCC. NK cell mediated cell lysis of cetuximab coated SCCHN cells was improved with IL-12 pretreatment. IL-12 co-treatment with cetuximab seems to increase ADCC via increased IFN-gamma production [19]. IRX2 is a physiologic combination of TH1 cytokines (IL-2, IL-1beta, IFNgamma, and TNF-alpha). In a phase II trial of neoadjuvant therapy, 27 SCCHN patients stage II to IVa were treated with minimal acute toxicity. Overall survival was 69% at 36 months, and high lymphocyte tumor infiltrate seemed to be correlated with improved overall survival [20]. Other local cytokine preparations may have immunological effect. In low-grade cervical LSIL patients, topical application of GM-CSF solution was well tolerated. Immune correlates demonstrated increased APC and cytotoxic T cell infiltrates. Anti HPV 16 VLP responses were increased after GM-CSF treatment [21].

Live viral vector

Immunotherapy can be delivered through a viral vehicle, often, either as an oncolytic virus or through a viral vector expressing HPV-16 specific oncoproteins. Oncolytic viral therapy for SCCHN was administered via intratumoral HSV/GMCSF expressing construct injection in a phase I/II trial. Oncolytic HSV/GMCSF was combined with chemotherapy and radiation, as well as post-treatment neck dissection in 17 advanced SCCHN patients. Results demonstrated an 82.4% disease specific survival at a median follow up of 29 months, with no dose limiting toxicity [22]. A modified vaccinia virus TG4001 was designed to express HPV-16 oncoproteins E6 and E7 as well as human interleukin-2. A recent study of cervical cancer patients with HPV 16 related CIN 2/3 were given 3 weekly subcutaneous injections of TG4001. Ten patients were identified as clinical responders. HPV 16 clearance was associated with cytologic regression in 7 out of 10 clinical responders. Of patients without final tissue biopsy, those that did not undergo conization, 7 of 8 had cleared HPV infection, and there was no suspicion of CIN 2/3 [23].

A recent phase II trial has been submitted to the EORTC combining TG4001 and chemoradiotherapy in patients with HPV positive oropharyngeal squamous cell cancer [24].

Non-viral HPV peptide and protein vaccines

The introduction of preventative HPV vaccination programs is a positive step in HPV associated cancer prevention. Oral HPV infection prevalence is 6.9% according to recent population data with a notably higher rate in men. Infection risks correlates with an increase in number of sexual partners [25]. The possibility exists that HPV vaccination such as Gardasil and Cervarix, shown to be effective in the prevention of cervical cancer, would have a similar effect to prevent infection and subsequent HPV positive SCCHN. Much is still not known of the natural history of oral HPV infection. The effect of preventative L1 capsid vaccines on oral HPV prevalence is being evaluated as a secondary endpoint in vaccine trials in both men and women [1].

Protein vaccines are also under investigation for therapeutic approaches in people with existing disease. Highly expressed SCCHN specific antigens such as MAGE-A3 or HPV-16 derived peptides are convenient targets for immunotherapy. To prevent proteolysis and

allow HLA-I processing, Trojan type constructs have been used to facilitate antigen delivery. Five patients with advanced head and neck cancer were enrolled in a phase I study of Trojan type vaccine of HLA-I and HLA-II restricted Melanoma Antigen E (MAGE-A3) and HPV-16 derived peptides. There was a peripheral blood response to the Trojan constructs in 4 out of 5 patients and toxicity was acceptable. In spite of the immunological response, there were no patients that had a RECIST defined clinical response [26].

In a similar manner, patients with persistent cervical HPV infection were vaccinated with HPV 16 E6 and E7 long peptides along with incomplete freund's adjuvant in a 2009 study of 20 women with HPV 16 associated vulvar intraepithelial neoplasia. The entire cohort had vaccine induced T cell responses, and at one year 15 out of 19 patients had durable clinical responses (9 were complete response) [27]. In a phase II randomized placebo controlled trial of HPV16 associated E6/E7 long peptides in women with high-grade cervical intraepithelial lesions, flu-like symptoms and injection site reactions caused the trial to terminate prematurely. There was increased HPV16 specific immunity in those patients that received the vaccine as determined by IFN-gamma responses seen by ELISPOT, and cytokine profiles demonstrating significant Th1 and Th2 response [28].

In a recent mouse transgenic humanized HLA-A2 immune system model animals were challenged with cervical cancer tumors. After vaccination with HPV 16 E7 C-terminus linked to bursal disease virus like particle (VLP) there was complete tumor eradication after one dose [29].

Overall, protein and peptide vaccination strategies have proven to be safe both in concept and clinical trials. Protein vaccines continue to be a powerful and versatile tool, in that they can be crafted to present a full range of viral epitopes available.

DNA vaccines

DNA vaccines for HPV related cancers are attractive for multiple reasons, yet are significantly limited by problems such as inadequate antigenicity. The benefits of DNA vaccines include stability, easy production, and ability to produce high expression of antigen in vaccinated cells [30]. The lack of effectiveness likely involves poor antigen presentation, strategies to remedy this include: (1) increasing the quantity of dendritic cells expressing the antigen of interest (2) improvement in antigen processing (3) optimization of DC and T cell crosstalk [30]. In spite of the inherent difficulties, there are trials underway in cervical cancer. In one phase I trial, a microencapsulated DNA vaccine (ZYC-101) consisting of multiple HLA-A2 restricted E7 epitopes was tested in CIN 2/3. The treatment was well tolerated and interestingly 33% of the patients had complete histologic responses and a majority of women had T-cell HPV specific response [31]. Multiple DNA vaccine trials have been conducted in cervical cancer, as well as combined therapeutic trials. An ongoing phase I clinical trial of the pNGVL4a-CRT/E7 (Detox) DNA vaccine in combination with Cyclophosphamide in HPV-16 associated OPSCC (NCT01493154) [32].

Tumor cellular vaccine

Tumor cellular vaccines are appealing in that the precise tumor antigens need not be identified, allowing a certain flexibility of approach. In theory this approach should be

combined with genetic modification such as cytokine expression, and optionally administered with conventional therapies. One Czech study used both TC-1 (non-metastasizing) and MK16 (metastasizing) HPV16 associated murine tumor models. The tumor cells were genetically modified ex-vivo to constitutively produce IL-12; this was used as a tumor cellular vaccine in combination with gemcitabine. This combination therapy group had the greatest cytoreductive response in both tumor types, when compared to either therapy alone [33]. This general approach is applicable to many tumor types including HPV16 associated tumors. As with other cellular vaccines, there are difficulties in mass production and purity, one of the reasons this approach has not been widely explored [18].

Dendritic cell vaccine

Dendritic cells process antigen and present it to T-Cell for immune response. Vaccines with dendritic cells exposed to HPV antigens in culture and then re-implanted are being developed to optimize T-cell specific anti-tumor immunity. The goal is to promote effective antigen presentation through mature DCs preloaded with antigen, which can be paired with other immunologics such as viral vectors, tumor lysates, and protein or peptide constructs [18]. A recent preclinical study used a murine model of cervical cancer (TC-1 HPV-16 E6/E7 expression transplantable tumors), showed that repeated vaccination with HPV-E7 linked to dendritic cells using a fusion protein containing the extra domain A (EDA) from fibronectin (natural ligand for TLR4) resulted in ability to cure 5–7 mm tumors. Additionally, immunization resulted in antitumor CD8+ T cell responses [34]. As with other cellular vaccines, great obstacles remain in terms of manufacture and administration on a commercial scale.

Bacterial vectors

Of the therapeutic bacterial vectors investigated, *Listeria Monocytogenes* has garnered the most interest. *Listeria*'s main benefits lie in its ability to infect APCs in the cytosolic compartment, allowing for MHC class I/II presentation [18]. In 2008, Sewell, worked with an HPV 16 transfected mouse model, in which animals were vaccinated with *Listeria*-based anti E7 vaccine or vehicle *Listeria* vaccine. The mice that were vaccinated with *Listeria* with E7 vector had significantly smaller tumors, thus the immunological tolerance had been partially counteracted [35]. Currently, there is a phase I study still recruiting in England of Recombinant *Listeria Monocytogenes* (Lm)-Based Vaccine Encoding Human Papilloma Virus Genotype 16 Target Antigens (ADX511-001) In Patients With HPV-16 +ve Oropharyngeal Carcinoma (NCT01598792) and a phase II trial with the same therapy is soon to be completed for CIN2/3 [36,37].

T cell therapy

Adoptive T cell therapy has not gained broad acceptance due to the laborious preparation and expertise required. However, a group out of Baylor recently demonstrated using a technique well adapted for mass production, that HPV E6/E7 specific CTL could be expanded in vitro. The expansion of these HPV specific T cells was promoted through contact with dendritic cells bearing HPV peptide library, as well as, IL-6, IL-7, IL-12, and IL-15. These T cells were further expanded using B cell blasts loaded with peptide library [38].

Monoclonal antibody therapy

Monoclonal antibody therapy continues to be the dominant immunologic treatment in a variety of cancer types. In head and neck cancer this is especially true, and ongoing trials and preclinical work share this focus. For SCCHN, monoclonal antibody therapy arrived with the approval of cetuximab. In vitro data suggests that cetuximab activated NK cells promote DC maturation and CD8+ T cell priming, leading to a Th1 antitumor response [39]. An unplanned subgroup analysis of the 2006 Bonner trial suggests that radiation plus cetuximab combination is beneficial over radiation alone, especially in young non-smoker males with oropharyngeal cancer [40]. Though HPV data are not available for this group of interest, based on demographic data, it is very likely that many of these patients were HPV positive.

PD-L1, the ligand for PD-1, is commonly expressed on SCCHN as well as other tumor types. The PD-1 and PD-L1 interaction is thought to mediate immune suppression and tumor progression. The reservoir for chronic oropharyngeal HPV infection is the tonsillar crypts. In a recent study, immunohistochemistry characterization of tonsillar tissue demonstrated membranous expression of PD-L1 in normal tonsil. In HPV+ SCCHN tumor specimens with heavy immune cell infiltrate there was PD-L1 expression on both the tumor cells as well as the CD68+ TAM at the lymphocyte front. PD-1 was highly expressed on the CD8+ TIL. This pairing of PD-1 (a T cell inhibitory receptor) and its ligand PD-L1 may allow for a microclimate of immune privilege. This region within the tumor may prevent adaptive antitumor response, thus blocking this interaction is a logical immunotherapy target [41]. Badoual et al. compared both HPV positive and negative SCCHN tumors. Histologically, HPV positive tumors were more heavily infiltrated with FoxP3+ as well as PD-1 positive T cells. In line with numerous other studies, HPV+ SCCHN had a better prognosis in their study patients. Furthermore, a rich PD-1 expressing TIL is more likely to respond to blockade of the PD-1/PD-L1 axis. These PD-1 positive T cells were theorized to be activated T cells, due to in vitro expression of activation markers after PD-1/PD-L1 blockade. Supporting this claim, a mouse tumor vaccination model also demonstrates increases PD-1+ T cells. It may be that these activated PD-1 expressing T-cells had previously mounted an anti-tumor immune response, and the anti-cancer potency of these T-cells may be unmasked by PD-1 inhibition. This may point to a new therapeutic target, perhaps combined with other immunotherapies specific for HPV+ SCCHN [42].

CTLA-4 is the first checkpoint inhibitor that was clinically targeted; the monoclonal antibody specific to CTLA-4 is ipilimumab. Normally, CTLA-4 inhibits T cell activation by outcompeting CD28 for binding of CD80 and CD86. Signaling via CD28 is an important means of amplifying the TCR signal [43]. At the University of Pittsburgh we have recently opened a phase I clinical trial (UPCI 12-084), combining concurrent ipilimumab plus cetuximab with radiation.

Conclusion

HPV positive oropharyngeal SCC continues to be an emerging epidemic despite preventative vaccines. The prognosis for this subset of head and neck cancers is by

comparison excellent with conventional therapy; however, treatment morbidity is significant. Immunotherapy is an attractive therapeutic option in a disease where de-escalation of treatment is under evaluation. HPV+ SCCHN specifically lends itself to this targeted immunotherapeutic approach, as the pathogenic targets are well known (E6/E7), and with new information about the role of PD-1/PD-L1 in the tumor stroma.

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