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INVITED REVIEW

Future Directions in Research, Treatment and Prevention of HPV-Related Squamous Cell Carcinoma of the Head and Neck

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Abstract The causative relationship between high-risk HPV and OSCC is well-established. HPV-associated OSCC represents a distinct disease entity compared to tobacco-associated ones. These virus-associated cancers continuously express the HPV E6 and E7 viral oncogenes even in advanced stages, and repression of viral oncogene expression can prevent the growth or survival of cancer cells. This finding raises the possibility that even late-stage HPV-associated OSCC can be cured by HPV-targeted approaches, such as medicines that interfere with the expression or function of viral oncoproteins, and therapeutic vaccines that elicit a cytolytic immune response to cells expressing these oncoproteins. The demonstration that high-risk HPVs are causally associated with a subset of OSCC has allowed the development of preventive and therapeutic strategies aimed at reducing the incidence and mortality of this disease. The better outcome of HPV-associated OSCC raises the question as to whether similar results can be achieved with less treatment. An important aim of novel approaches for favorable-prognosis, HPV-associated cancers will be minimization of devastating side effects of intensified treatment developed for poor prognostic subsets. Clinical trials are studying the potential for de-escalation of radiation therapy in HPV + OSCC in the setting of different chemoradiotherapy regimens. The role of cetuximab in HPV-associated OSCC needs to be explored in prospective clinical trials. This review summarizes the main events of HPV-induced carcinogenesis with an emphasis on the implications of these carcinogenic mechanisms on research, treatment and prevention of HPV-associated OSCC.

Keywords HPV · OSCC · Vaccines · Targeted therapies · De-intensification

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Introduction

An increase in incidence and survival of oropharyngeal squamous cell carcinoma (OSCC) in the United States has been attributed to an epidemic of high-risk human papillomavirus (HPV) infection. This increase in incidence parallels an increase in incidence of sexual habits associated with virus transmission over the past 4 decades [1, 2]. In fact, the annual number of OSCC is expected to exceed the annual number of cervical cancers by the year 2020 if these incidence trends continue [3].

HPV-induced carcinogenesis has been extensively researched in the most widely accepted HPV-related malignancy, namely cervical cancer. HPV-associated cancers continuously express the HPV E6 and E7 viral oncogenes even in advanced stages, and repression of viral oncogene expression can prevent the growth or survival of cervical cancer cells [4]. This finding raises the possibility that even late-stage HPV-associated cancers can be cured by HPV-targeted approaches, such as medicines that interfere with the expression or function of viral oncoproteins and therapeutic vaccines that elicit a cytolytic

immune response to cells expressing these oncoproteins. This review summarizes the main events of HPV-induced carcinogenesis with an emphasis on the implications of these carcinogenic mechanisms on research, treatment and prevention of HPV-associated OSCC. The demonstration that high-risk HPVs are causally associated with a subset of OSCC has allowed the development of preventive and therapeutic strategies aimed at reducing the incidence and mortality of this disease. Furthermore, novel therapeutic strategies and the role of targeted therapies in this disease are discussed.

HPV-Induced Malignant Conversion: Targets for Therapeutic Intervention

Over 200 papillomaviruses types have been identified in many organisms, including humans, and this number will probably increase. These are small non-enveloped DNA viruses which give rise to a large spectrum of epithelial lesions with low malignant potential such as "warts" or "papillomas". There is, however, a subset of HPVs, the "high-risk" HPVs, which cause pre-cancerous lesions. Human papillomavirus (HPV) is nearly ubiquitously present in humans, but only a small fraction of people infected with high-risk HPVs will eventually develop cancer and often decades after the original infection. The molecular progression model of HPV-driven malignant conversion was first elucidated in cervical cancer, the most extensively studied HPV-associated malignancy. The integration of high-risk HPV DNA into the cellular genome disrupts the expression of the main viral transcription/replication factor E2 that functions as a transcriptional repressor of E6 and E7 main viral oncogenes [5]. As a consequence, E6 and E7 oncogenes are continuously expressed in cervical cancer cells and create a competent state for DNA replication. E6 binds and degrades p53 through a ubiquitin-mediated process and E7 binds and destabilizes pRB and related proteins. The functional inactivation of p53 and pRb tumor suppressor pathways induces genomic instability which increases the oncogenic potential of cells. In addition, E6 protein interferes with DNA repair enzymes while E7 oncoprotein can inhibit centrosome synthesis and cause alterations in structure and number of chromosomes. The majority of cervical carcinomas contain wild-type p53 and Rb genes. Therefore, the p53 and pRB tumor suppressor pathways are dormant but active in these cells due to the continuous expression of E6 and E7 genes [6]. It is noteworthy that, despite the acquisition of cellular mutations during malignant progression, repression of E6 and E7 expression in cervical carcinoma cell lines is sufficient to induce cell growth arrest or apoptosis [6]. Repression of E6 and E7 viral oncogene expression using antisense strategies in cervical carcinoma cell lines also results in several-fold inhibition of proliferation [7].

Several lines of epidemiologic and laboratory evidence suggest that high-risk HPVs, especially type 16, are associated with a subset of OSCC. We infected human oropharyngeal squamous cancer cell lines 147T and 090 (HPV16 DNA+) and 040T (HPV DNA-negative) cells with retroviruses that expressed a short hairpin RNA (shRNA) targeting the HPV16 E6 and E7 genes or a scrambled-sequence control shRNA [8]. In 147T and 090 HPV16+ cells, shRNA-mediated inhibition of HPV16 E6 and E7 expression reduced the E6 and E7 mRNA levels by more than 85 % compared with control cells that expressed a scrambled-sequence shRNA. E6 and E7 repression led to (1) restoration of p53 and pRB protein expression, (2) increased expression of p53-target genes (i.e. p21 and FAS), (3) decreased expression of genes whose expression is increased in the absence of functional pRb (i.e. DEK and B-MYB), and (4) induced substantial apoptosis in HPV16+ cells compared with the control shRNA-infected cells (from 13.4 % in uninfected to 84.3 % in infected 147T cells and from 3.3 % in uninfected to 71.2 % in infected 090 cells). This study was the first to show that HPV-induced malignant conversion in OSCC mimics the cervical carcinogenesis model and it also provides experimental evidence that HPV is causally associated with OSCC. In addition, similar to cervical cancer, it seems that continuous expression of E6 and E7 viral oncogenes is required to maintain the malignant phenotype of HPV + oropharyngeal carcinoma. Contrary to cervical cancer, transcription of HPV-16 E6/E7 mRNA in tonsillar carcinomas can occur in the absence of HPV DNA integration and the virus may exist predominately in episomal form [9]. It is unclear how the virus remains in cancer tissues in the episomal form with a high copy number. A study by Van Tine et al. [10] showed that HPV E2 protein may serve as an "anchor" to bind episomal HPV to cellular mitotic spindles. In tobacco-induced OSCC abrogation of p53 and retinoblastoma pathways occurs via mutation and genetic/epigenetic alterations, respectively. In HPV-associated OSCC, functional inactivation of p53 and pRb pathways by the viral oncoproteins obviates the need for mutational inactivation of p53 and pRb genes. Strati and colleagues [11] studied the individual contribution of E6 and E7 oncogenes to head and neck carcinogenesis by using transgenes that provide direct expression of the HPV16 E6 and E7 proteins to the head and neck tissues of mice. The authors found that a conditional deletion of Rb in the same tissues did not recapitulate all E7-mediated phenotypes. The authors reported that pRb-independent functions of E7 may also play an important role in head and neck carcinogenesis.

E6 and E7 exert their oncogenic potential via activation of several signaling pathways, such as the WNT/β-catenin pathway [12]. We repressed E6/E7 genes in HPV16-

positive oropharyngeal cancer cell lines and cervical cell lines SiHa (HPV16+) and HeLa (HPV18+) to measure the cytoplasmic and nuclear beta-catenin levels and beta-catenin/Tcf transcriptional activity. Silencing of HPV E6 and E7 genes induced a substantial reduction in nuclear beta-catenin levels. Luciferase assay revealed that transcriptional activation of the Tcf promoter by beta-catenin was lower after silencing. The protein levels of beta-catenin are tightly regulated by the ubiquitin/proteasome system. We showed that Seven in absentia homologue (Siah-1) proteins are involved in nuclear accumulation of beta-catenin. Thus, E6 and E7 are involved in beta-catenin nuclear accumulation and activation of Wnt signaling in HPV-induced cancers. Activation of Wnt signaling pathway by viral oncoproteins has been previously reported for Epstein Barr virus (EBV) and Kaposi's sarcoma–associated herpesvirus [13].

The improved mechanistic understanding of the molecular basis of the HPV-associated OSC promises to open a new era of dealing effectively with these diseases.

HPV-Targeted Therapy

Vaccines

Prophylactic Vaccines

HPV-associated head and neck squamous cell carcinomas can be prevented by vaccines designed to induce appropriate HPV-specific immune responses. The concept behind a prophylactic vaccine is to stimulate the immune system to elicit adequate neutralizing antibody response prior to, or upon exposure to, high-risk HPVs, to prevent establishment of persistent infection. Animal studies suggest that virus-neutralizing antibodies protect against persistent infection. It is expected that this effect would subsequently abolish the development of invasive cancers induced by high-risk HPVs. Neutralizing antibodies physically prevent the virus from attaching to host cells by binding to neutralizing epitopes on viral surfaces. HPV capsid structural proteins represent the most common neutralizing epitopes. Therefore, recombinant HPV virus-like particles (VLPs) are assembled by overexpression of major capsid HPV protein L1, which displays neutralizing epitopes. Because VLPs mimic authentic virions but are non-infectious [14], immunization of animals with VLP protects from experimental infection with the homologous animal papillomavirus. By this production method, two HPV prophylactic vaccines have been manufactured and have subsequently received approval from the U.S. Food and Drug Administration (FDA). The quadrivalent vaccine (Gardasil Merck & Co., Inc.) protects against HPV types 6, 11, 16 and 18. It was first licensed in 2006 for use in females ages 9–26 years old for the prevention of cervical, vaginal and vulvar cancers. Clinical trial data demonstrated the effectiveness of the vaccine in preventing genital warts in males and in 2009 clinical indications for the vaccine were expanded to include males in this age range (Centers for Disease Control and Prevention (CDC) 2010b). Because more recent studies have also shown the effectiveness of the vaccine in prevention of anal pre-cancers, licensure was expanded to also include anal cancer prevention [15]. Several randomized clinical trials have demonstrated that the quadrivalent vaccine elicited high levels of neutralizing antibody and significantly reduced the incidence of persistent HPV16 and HPV18 infections and associated moderate-to-high grade cervical intraepithial neoplasia CIN2/3 [16]. A very high vaccine efficacy (98 %) has been demonstrated for the prevention of anal, cervical, vaginal, and vulvar pre-cancers in HPV16/18-naïve individuals [17, 18]. Efficacy was lower (50–78 %), as expected, when analyses also included individuals previously infected with vaccine-type HPV at the time of vaccination [18].

The second HPV vaccine, Cervarix®(HPV2), is a bivalent vaccine that protects against HPV types 16 and 18. This vaccine received licensure for use in the US in 2009 for the prevention of cervical cancers (US Food and Drug Administration, 2009). This bivalent vaccine does not protect against genital warts because it does not cover low risk (6, 11) HPV types. Similar to quadrivalent vaccine, the bivalent vaccine is very effective (97 %) in the prevention of HPV16/18-associated cervical pre-cancers in HPV naïve individuals while lower efficacy is demonstrated when individuals already infected with HPV16 or 18 are included in the analyses [18, 19]. The bivalent vaccine has not been tested in clinical trials for efficacy against other HPV-associated cancers/pre-cancers such as those of vagina, vulva or anus. Structural similarities between quadrivalent and bivalent vaccine indicate that the bivalent should also be effective in preventing the other anogenital cancers induced by HPV16 and 18. However, these other diseases are not included as approved clinical indications for the bivalent vaccine.

The currently available vaccines might be particularly effective in preventing HPV-associated OSCC considering that the vast majority of HPV-associated OSCC are caused by HPV16. However, the impact of these vaccines on the incidence of persistent oral HPV infection has not been studied. Data from animal models immunized against HPV16 have demonstrated a reduction in the development of HPV-oral lesions [20]. It is unclear, however, whether persistent oral HPV infection can induce premalignant changes in oropharynx, as it does in cervical carcinoma. The natural history of oral HPV infection is not well

understood and routine screening for HPV-associated OSCC is not recommended. In addition, contrary to cervical cancer, the typical progression from pre-cancerous to cancerous state is less well established in OSCC. Most vaccine clinical trials use cervical pre-cancer as an endpoint due to long lag time between HPV infection and cervical cancer. This endpoint is not easily applicable to OSCC.

Although these prophylactic vaccines deliver promise, several issues should be resolved. Firstly, the duration of protection is uncertain. Clinical trials of the quadrivalent vaccine, which have followed women out to 5 years, demonstrate that protection remains high despite the decline in neutralizing antibody titers [21]. In addition, data from the monovalent (HPV16) precursor of the quadrivalent vaccine demonstrate high protection out to 8.4 years [21, 22]. Clinical trials of the bivalent vaccine also show high clinical efficacy out to 6.4 years [21]. OSCC typically develops in individuals in their 50s and the impact of the vaccine on the incidence of the disease cannot be assessed from current ongoing clinical trials especially since duration of infection to malignant change in OSCC is not known. Longitudinal studies comparing the incidence of OSCC before and after the introduction of the vaccine may answer this question.

A second issue is that VLPs are expensive and will not be affordable in the developing world. Thirdly, the vaccines will not protect against all high-risk HPV types.

Therapeutic HPV Vaccines

Several lines of evidence underscore the importance of an intact immune system in controlling HPV infection and its associated lesions. First, most healthy individuals infected with HPV are capable of clearing the infection without any clinical manifestation. Only a minority of individuals are not capable of clearing the virus and subsequently develop HPV-associated lesions. Second, immune cell infiltrates are often found in HPV-associated regressing lesions while these cell types are absent in persistent disease. Lastly, immunocompromised individuals, such as HIV-infected patients, have documented higher rates of HPV infection and associated lesions. Since the immune system plays an important role in controlling HPV infection, therapeutic vaccine strategies have been developed. Therapeutic vaccines are aimed at treating HPV-infected cells and this can be achieved by developing a cellular T cell immune response that can recognize and eliminate these HPV-infected cells.

HPV16 E6 and E7 proteins represent ideal targets for immunotherapy. HPV16 E6 and E7 are foreign viral proteins and are more immunogenic as compared to a self-protein overexpressed in cancer cells. In addition, they are uniquely expressed by all virus-infected cells. Thus, DNA vaccines, viral vector vaccines, bacterial vector vaccines, peptide vaccines and cell-based vaccines appear attractive targets for investigation. DNA vaccines are promising candidates for therapeutic HPV vaccination in HPV-associated OSSC. A Phase I open-label, dose-escalation trial of a DNA vaccine, pNGVL4a-CRT E7, aiming to elicit immunologic responses against HPV16 E7 will be initiated at Johns Hopkins in patients with HPV-associated OSCC (NCT01493154). This promising vaccine uses a targeting strategy that conjugates the E7 antigen of HPV16 to the immunostimulatory molecule calreticulin (CRT). HPV16 E7 DNA was cloned into the pNGVL-4a plasmid backbone with amino acid substitutions at positions 24 (cysteine to glycine) and 26 (glutamic acid to glycine) of E7 which ablates the Retinoblastoma protein binding site, thereby preventing malignant transformation of transfected cells. CRT-E7 fusion protein is expressed by this plasmid under the control of the CMV promoter. The vaccine is administered via electroporation and low-dose cyclophosphamide is given 1 day before vaccination.

Strategies to increase the potency of vaccines include (1) using alternative administration routes, (2) eliminating the suppressive tumor microenviroment, and (3) combining the vaccine with chemotherapy. For example, administering the DNA vaccine via electroporation, instead of intramuscular needle injection, may increase virus-specific immune responses. This was shown in a clinical trial of an HIV DNA vaccine [24]. In patients with HPV-related OSCC, a high frequency of T regulatory cells that inhibit cellular immune response (Sara Pai, ASCO 2011) are often found in tumor biopsies. Low dose of the immunomodulator cyclophosphamide may decrease the frequency of inhibitory T regulatory cells [25]. Treating HPV+ tumor-bearing mice with low-dose cyclophosphamide reduced the frequency of inhibitory T cells (Sara Pai, ASCO 2011). The combination of cyclophosphamide and the therapeutic HPV vaccine developed at Johns Hopkins increased the frequency of HPV-specific immune responses against the tumors which resulted in better long-term survival in these tumor-bearing mice. Cisplatin was also combined with the HPV DNA vaccine and resulted in smaller tumor diameter and longer survival in tumor-bearing mice.

Vaccination with peptides derived from HPV antigenic proteins involves the uptake of peptide antigen by dendritic cells and presentation of the peptide antigen in association with MHC molecules. In general, peptide vaccines have poor immunogenicity and use of adjuvants can circumvent this problem. Most studies on peptide-based vaccines have aimed at enhancing vaccine potency by using adjuvants such as Granulocyte Macrophage Colony Stimulating Factor (GM-CSF) and Montanide ISA-51.

A therapeutic HPV vaccine consisting of overlapping peptide sequences that spanned both the E6 and E7 protein

was tested in a Phase I trial in patients with vulval intraepithelial neoplasia (VIN) grade III [23]. All patients mounted vaccine-induced immune responses and there was a 47 % complete response rate 40 weeks after the last vaccination dose. These responses correlated with induction of HPV-specific immunity.

HPV+ oropharyngeal cancer is associated with increased expression of p16INK4a. A phase I/IIa study of immunization with a p16INK4a peptide (amino acids: 37-63) combined with immunoenhancer Montanide ISA-51 VG in patients with advanced p16 + HPV-associated cancers (NCT01462838) is ongoing. The aim of this study is to show that vaccination with a p16INK4a peptide is safe and can induce a p16INK4a—specific T cell immune response in patients with advanced HPV+, p16INK4a+ head and neck cancer.

The usefulness of peptide immunization using epitopes derived from the processing of proteins that are preferentially expressed on tumor cells is limited because only a fraction of the patient population will express the appropriate MHC allele that restricts the corresponding T cell response. Furthermore, the extracellular proteolysis of short peptides limits the clinical efficacy of traditional peptide vaccines. In order to overcome these problems, a new generation of peptide–based vaccines named "Trojan peptide vaccines" have been developed. Trojan peptide-based vaccines are based on large peptides that contain a sequence derived from HIV-TAT and furin-cleavable linkers to join multiple HLA-I and HLA-II peptide epitopes. Trojan peptide sequence allows the entire peptide to translocate through the cell membrane to the endoplasmic reticulum and Golgi apparatus where the endopeptidase furin resides. The peptidase generates multiple HLA-I and HLA-II peptide epitopes from the Trojan peptide carrier. A phase I study of immunization with a therapeutic peptide vaccine using two novel Trojan peptide complexes composed of MAGE-A3 and HPV16 epitopes is ongoing (NCT00257738). Melanoma antigen E (MAGE-A3) was selected based on its overexpression in head and neck squamous cell carcinoma (HNSCC) and the availability of well characterized HLA-I and HLA-II epitopes. HPV16 was elected based on its high prevalence in HNSCC. In this study, Trojan peptides are solubilized in Montanide ISA 51 and GM-CSF before injection to promote dentritic cell migration to the site of vaccination (Table 1).

De-escalation Studies: Determining Therapy Based on HPV Status

Patients with HPV-associated OSCC have better prognosis compared to their age- and stage-matched counterparts. HPV positivity confers a 60–80 % reduction in risk of death from cancer compared to similarly treated HPV negative tumors [1, 2, 26–29]. The absolute survival difference between HPV positive and negative tumors is consistently higher than 30 % across prospective studies. In addition, because HPV + OSCC is more responsive to chemotherapy and radiation relative to HPV-negative cases, organ preservation strategies may be more successful in these patients. However, current clinical guidelines do not take into account HPV status in treatment decisions. According to the National Comprehensive Cancer Network (NCCN) guidelines "HPV testing is recommended for all oropharynx tumors". According to US National Cancer Institute (NCI) and CTEP, "HPV status must be included as stratification factor for trials including oropharynx cancer patients". The better outcome of HPV-associated OSCC raises the question as to whether similar results can be achieved with less treatment. An important aim of novel approaches for favorable prognosis HPV-associated cancers will be minimization of devastating side effects of intensified treatment developed for poor prognostic subsets.

Because the radiation component of concurrent therapy is the most toxicity-producing, the following de-escalation protocols favor reduction in radiotherapy intensity. The Eastern Cooperative Oncology Group (ECOG) Phase II study (E1308) evaluates whether the increased response to platinum-based induction chemotherapy can be used to select patients who can safely receive a lower dose of intensity-modulated radiation therapy. E1308 uses 3 cycles of induction chemotherapy with cisplatin, paclitaxel and cetuximab to identify those who attain a complete response for receiving low-dose, intensity-modulated radiation therapy (IMRT) (54 Gy/27fractions). Cisplatin is substituted for cetuximab in a concurrent component of treatment. Patients who do not achieve a complete response receive standard dose (60.3 Gy) IMRT with concurrent cetuximab. In this regard, the Radiation Therapy Oncology Group study (RTOG 1016) is a Phase III non-inferiority study that will evaluate whether the substitution of cisplatin with cetuximab in concurrent chemoradiotherapy regimens employing accelerated IMRT (70 Gy/6 weeks) achieves similar survival with less short- and long-term toxicity. Patients will be stratified by smoking history. The "De-ESCALaTE HPV" is a multicenter randomized Phase III study led by Hisham Mehanna (United Kingdom) comparing cetuximab and concurrent radiotherapy to standard concurrent cisplatin chemoradiotherapy in patients with HPV-associated OSCC. The primary endpoint of the study is the incidence of acute and late toxic events. Two other studies (NCT1088802/J0988 and NCT01221753) are exploring additional de-escalation protocols. NCT1088802/J0988 is a Phase I/II study of radiation de-intensification with concomitant chemotherapy in favorable subset of HPV-associated OSCC.

Table 1 Selected clinical trials in HPV-associated OSCC

Identifier	Patient population	Intervention	Sponsor	Phase
NCT01493154	Adjuvant HPV16 + HNSCC	DNA vaccine pNGVL4a-CRT E7	Sidney Kimmel Comprehensive Cancer Center	I
NCT01462838	Advanced HPV-induced cancers	p16INK4a peptide vaccine	Oryx GmbH & Co. KG	I/II
NCT00257738	Progressive, recurrent or metastatic HPV16 + HNSCC	MAGE-A3/HPV 16 vaccine	University of Maryland	I
NCT01084083 E1308	Stage III/IV resectable HPV16 + OSCC	Induction chemotherapy followed by cetuximab (Erbitux) with low dose versus standard dose IMRT in patients with HPV-Associated resectable squamous cell carcinoma of the oropharynx	ECOG	II
NCT01302834 RTOG1016	III/IV HPV16 + OSCC	Radiation therapy with cisplatin or cetuximab in treating patients with oropharyngeal cancer	RTOG	III
De-ESCALaTE HPV	III/IV favorable subset of HPV16 + OSCC	Radiation therapy with cisplatin or cetuximab in patients oropharyngeal cancer	University of Warwick	III
NCT 01221753	Locally advanced HPV16 + OSCC	(TPF) Induction chemotherapy followed by concurrent chemoradiotherapy using a modified radiation dose	Dana Farber Cancer Institute	II
NCT1088802	Favorable subset HPV+	Radiation de-intensification with concomitant chemotherapy OSCC	Chapel Hill	I/II

OSCC oropharyngeal squamous cell cancer, *HNSCC* head and neck squamous cell carcinoma, *IMRT* intensity-modulated radiation therapy, *ECOG* eastern cooperative oncology group, *RTOG* radiation therapy oncology group, *TPF* docetaxel, cisplatin, 5-Fluorouracil

NCT01221753 investigates the use of induction docetaxel/cisplatin/5-fluorouracil (TPF) chemotherapy followed by concomitant chemoradiotherapy with modified radiotherapy regimen in locally advanced OSCC.

Targeted Therapies

The Epidermal Growth Factor Receptor (EGFR) is an attractive molecular target for therapy in HNSCC. Cetuximab, the chimeric monoclonal antibody targeting EGFR, is approved in HNSCC in locally advanced setting combined with radiotherapy and in recurrent/metastatic disease. Bonner et al. [30, 31] conducted a Phase III study comparing high dose radiation with or without cetuximab in patients with locally advanced HNSCC. The study showed that the addition of cetuximab significantly prolonged overall survival. In this trial, 424 patients were randomized: 60 % had oropharyngeal, 25 % laryngeal, and 15 % hypopharyngeal primary tumors. Median survival times, from Kaplan–Meier estimates, were 54 months versus 28 months ($p = 0.02$), favouring the cetuximab arm. For oropharyngeal tumors that were not assessed for HPV status, the effect of cetuximab in this study was more pronounced in (1) young (<65) patients, (2) patients with good performance status (Karnofski score >90), (3) oropharyngeal primary site, (4) small primary tumors (T1–T3) and (4) positive cervical lymph nodes (N1–N3) [32]. Therefore, although unstated, the clinical phenotype that appeared to benefit the most was, in fact, consistent with HPV + disease. Despite inadequate power, this subset analysis provided some hints that HPV-associated OSCC might respond better to cetuximab.

Since this publication, however, emerging data suggest that cetuximab-RT may not benefit patients with HPV+ disease. A retrospective comparison of HNSCC patients treated with cisplatin-RT versus cetuximab-RT at Memorial Sloan Kettering showed that oropharynx patients in fact did better with cisplatin [33]. When the authors isolated HPV+ patients they found treatment with cisplatin, not cetuximab, predicted for better locoregional control and overall survival. In a RTOG0522 Phase III randomized study of concurrent accelerated radiotherapy with cisplatin with/without cetuximab for stage III/IV HNSCC, the study unfortunately did not meet its primary endpoint. Nevertheless, subset analysis of treatment effect in the p16 group showed lack of benefit from cetuximab.

The SPECRUM study led by Jan Vermorken was a randomized Phase III study of chemotherapy with cisplatin-5-Fluorouracil with and without panitumumab (human monoclonal anti-EGFR antibody) in patients with recurrent/metastatic HNSCC. A retrospective analysis confirmed benefit only in HPV-negative patients. Thus, more recent data challenge the role of cetuximab in HPV + OSCC.

Conclusions

HPV-associated OSCC represents a distinct entity from tobacco- and alcohol-related OSCC with different tumor behavior. The impact of prophylactic HPV vaccines on the incidence of HPV-associated OSCC is uncertain at this point. Clinical trials show that therapeutic HPV vaccines are able to induce HPV-specific tumor responses which correlate with tumor regression. Therapeutic HPV vaccines are currently undergoing investigations in clinical trials in OSCC. The question of de-intensification of treatment in this good prognosis subset in order to reduce toxicity appears to be of major clinical relevance and is currently being explored in clinical trials. The value of cetuximab in HPV-associated OSCC is unclear and retrospective analyses yielded conflicting results. Prospective studies will provide level I evidence regarding the value of cetuximab in HPV-positive disease. Based on retrospective analysis the value of cetuximab in HPV-associated OSC remains unclear.

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