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REVIEW

Next-generation treatment strategies for human papillomavirus-related head and neck squamous cell carcinoma: where do we go?

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SUMMARY

Oncogenic human papillomavirus (HPV) is currently recognised as a major risk factor for the development of head and neck squamous cell carcinomas (HNSCC). HPV is mostly detected in tumours arising from the oropharynx and more specifically from the tonsil. HPV-related tumours display clinical and molecular characteristics that are distinct from HPV-unrelated tumours, which are generally induced by alcohol and tobacco abuse. Detection of biologically active HPV in HNSCC has prognostic relevance, which warrants the separate classification of HPV-induced tumours and is a prerequisite for further optimisation of treatment protocols for this distinct group. Current guidelines for the treatment of oropharyngeal squamous cell carcinoma (OPSCC) have not incorporated specific treatment modalities for HPV-related tumours. The development of such treatment options is still in a preclinical phase or in early clinical trials. Recent data on treatment response of OPSCC have been obtained by retrospectively analysing HPV-status and indicate that patients with HPV-related tumours show a favourable prognosis, independent of the type of treatment. These patients may benefit from de-intensified treatment, which should be assessed in prospective clinical trials. The development and future use of new antiviral and immunomodulatory therapeutics may be instrumental in this approach to improve survival rates and decrease disease-and-treatment-related morbidity. In this review we will focus on present therapeutic HPV-targeting strategies and discuss future directions for de-intensified treatment of HPV-positive HNSCC. Copyright © 2011 John Wiley & Sons, Ltd.

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Abbreviations

ANP, Acyclic Nucleoside Phosphonate; AZF, Artificial Zinc Fingers; CPP, Cell-Penetrating Peptide; CIN, Cervical Intraepithelial Neoplasia; CT, Chemotherapy; EGFR, Epithelial Growth Factor Receptor; EMT, Endothelial-to-Mesenchymal Transition; E-proteins, Early proteins; FISH, Fluorescence In Situ Hybridisation; HNSCC, Head and Neck Squamous Cell Carcinoma; HPMPC, [(S)-1-(3-hydroxy-2-phosphonylmethoxypropyl)cytosine]; HPV, Human Papillomavirus; HR-HPV, High-Risk Human Papillomavirus; HSPG, Heparan Sulphate Proteoglycans; hTERT, Human Telomerase Reverse Transcriptase; ISH, In situ hybridisation; L-proteins, Late proteins; LR-HPV, Low-Risk Human Papillomavirus; OPSCC, Oropharyngeal Squamous Cell Carcinoma; OSCC, Oral Squamous Cell Carcinoma; PI, Protease Inhibitors; pRb, Retinoblastoma Tumour Suppressor protein; RNAi, RNA interference (small interfering RNA); RRP, Recurrent Respiratory Papillomatosis; TORS, Transoral Robotic Surgery; T_{regs}, CD4+/CD25+ regulatory T-cells; UCSCC, Uterine Cervical Squamous Cell Carcinoma; VLP, Virus-Like Particles.

INTRODUCTION

Head and neck cancer is a serious health care problem in many parts of the world [1]. The vast majority of head and neck cancers are squamous cell carcinomas originating from the mucosal epithelium lining the oral cavity, nasal cavity, pharynx and larynx [2]. In 2008, head and neck squamous cell carcinomas (HNSCC) were estimated to cause 480 000 new cancer cases and 273 000 cancer deaths worldwide [1]. Despite the fact that advances have been made in diagnosis and treatment, mortality rates have only marginally

decreased over the last decades and the 5-year survival rate currently ranges between 40%–60% [3]. Approximately 80%–90% of HNSCC develop in patients with a history of alcohol and tobacco abuse, including tobacco and betel quid chewing and snuff dipping [4]. These factors are also responsible for the process of 'field cancerisation' in the entire head and neck region [5], leading to multiple primary tumours in up to 40% of patients [6]. Patients without exposure to these risk factors account for 10%–20% of HNSCC. These tumours are predominantly associated with viral carcinogenesis, including infection with EBV in nasopharyngeal carcinomas [7] and, to a greater extent, infection with oncogenic human papillomavirus (HPV) in the oropharynx, in particular in the lingual and palatine tonsils. In the last decade, the incidence of HPV-related oropharyngeal squamous cell carcinoma (OPSCC) has increased relative to the total group of HNSCC [4,8]. Infection rates in OPSCC range from 20% to more than 90% in different studies, depending on geographical factors and the detection method used [9–12].

In this review, we will present the clinical and molecular features of HPV-positive HNSCC. Subsequently we will focus on the current knowledge of potential anti-HPV strategies and discuss the most promising modalities for the treatment of HPV-positive HNSCC.

METHODS

Besides relevant articles selected from the general literature concerning HPV-related carcinogenesis and references therein, specific literature on treatment options for HPV-related HNSCC was obtained by a bibliographical search in PubMed, Medline and Embase, from inception to May 2011, using the search term (HPV OR papillomavirus OR papilloma) AND (HNSCC OR 'head and neck cancer' OR oropharyngeal OR oropharynx OR oral OR pharyngeal OR pharynx OR buccal OR base of tongue OR tongue OR tonsillar OR tonsil OR floor of mouth OR mouth OR vallecula) AND (treatment OR antiviral OR therapy) AND (cancer OR carcinoma OR tumour OR tumour OR neoplasm). This search yielded 1246 results in PubMed, 137 in Medline and 309 in Embase. Based on inspection of the title and/or abstract of these publications, 63 relevant papers on treatment options and some references therein were included in this review. Moreover, ongoing clinical trials concerning new therapeutic options for HPV-related HNSCC were identified from the Cochrane Controlled Trial Register and from the US National Institute of Health Clinical Trials (www.clinicaltrials.gov), yielding five relevant results.

HUMAN PAPILLOMAVIRUS AND TUMOURIGENESIS

Human papillomavirus

Human papillomaviruses are non-enveloped viruses, containing circular double-stranded DNA of approximately 8 kb, that are highly epitheliotropic and known to infect both mucosal and cutaneous epithelia [13]. Papillomaviruses are species-specific and the human papillomavirus family can be classified into five genera and subdivided into 31 species and 120 types [14]. A subgroup of 15 HPV types is linked to the development of malignant lesions of mucosal and cutaneous epithelia, and is considered to comprise high-risk (HR) HPVs [15]. All HR-HPVs belong to the alpha-genus, including HPV-16 and HPV-18, which are found in ~50% and ~20% of cervical malignancies, respectively [16]. Differences in the capacity to deregulate cellular protein function by viral oncogenes E6 and E7 account for the carcinogenic properties of HR-HPV in comparison with low risk (LR) HPVs [17,18]. LR-HPV types, such as HPV-6 and HPV-11, are often found in benign mucosal lesions and are only sporadically associated with carcinomas. Human lesions in which HPV types of the alpha-genus appear to be involved are summarised in Table 1.

Human papillomavirus replication and integration

The HPV life cycle is linked to the differentiation of the infected epithelial cell. HPV infection is initiated by binding of the virion L1 protein to heparan sulphate proteoglycans (HSPG) on segments of the basement membrane, which are exposed at sites of (micro)injury. This induces conformational changes and L2 cleavage finally resulting in binding of the L1 capsid protein to a so far undetermined cell surface receptor [19]. The cell adhesion receptor α6-integrin has been implicated to be this receptor [20], but does not seem to be essential for HPV infection. However, α6-integrin might be a matrix component closely associated with HSPG [21]. The circular HPV DNA comprises 8 genes, coding for six early (E) and two late (L) proteins (Figure 1). The E-proteins regulate and facilitate virus-replication and are expressed early after infection [22]. Oncoproteins E6 and E7 have a direct effect on several essential cellular processes, such as cell cycle and apoptosis

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Table 1. Involvement of human papillomavirus types of the alpha-genus in benign and malignant human lesions. The major human papillomavirus types for the different lesions are indicated in bold

Lesion	HPV types found	References
Head and neck benign		
Focal Epithelial Hyperplasia	13, 32	[176,177]
Sinonasal papilloma	6, 11, 18	[178,179]
Laryngeal papilloma and dysplasia	**6**[1)], **11**, 16, 18	[180,181]
Oral leukoplakia and lichen planus	6, 16, 18, 31, 33	[182,183]
Head and neck malignant		
Oropharyngeal squamous cell carcinoma	6, 11, **16**, 18, 31, 33, 35	[52], this review
Oral squamous cell carcinoma	**16**, **18**	[52,60]
Laryngeal squamous cell carcinoma	6, 11, **16**, 30	[52,60]
Sinonasal carcinoma	**16**, 18	[179]
Anogenital		
Anogenital[2)] benign lesions[3)]	6, 11, **16**, 18, 31, 33, 53, 56, 58, 66, 83	[36,184]
Anogenital[§] (squamous cell) carcinoma	6, 11, **16**, 18, 31, 33, 45	[36]
Cervical intraepithelial neoplasia and uterine cervical squamous cell carcinoma	6, 11, **16, 18**, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, 70, 73, 82	[16,37]
Adenocarcinoma *in situ* and uterine cervical adenocarcinoma	**16**, **18**, 33, 35, 45, 51, 58, 59	[185,186]
Cutaneous		
Common skin warts	2, 3, 7, 10, 27, 28	[15,187]
Periungual squamous cell carcinoma	16, 26, 33, 51, 56, 73	[188,189]

[1)]The major HPV types for the different lesions are indicated in bold;[2)]including anal, vulvar, vaginal and penile lesions; [3)]including warts, lichen sclerosis, squamous cell carcinoma *in situ*, adenocarcinoma *in situ* and intraepithelial neoplasia.

regulation. E6 promotes degradation of p53 through interaction with E6-associated protein (E6AP), an E3 ubiquitin ligase, and subsequent ubiquitination and proteasomal degradation. Amongst others, this alters transcription of p53 target genes and activates human telomerase reverse transcriptase (hTERT), resulting in cell survival and ultimately in genetic instability [23,24]. The oncoprotein E7 binds to the unphosphorylated retinoblastoma tumour suppressor protein (pRb), which promotes the release of transcription factor E2F, leading to activation of the cell cycle and transition through the G1/S-phase, needed for DNA-replication [25–27]. As a consequence, $p16^{INK4A}$ is upregulated but is unable to properly inhibit the cell cycle. Expression of oncoproteins E6 and E7 is tightly regulated by E2, the main regulator of viral gene transcription [28]. Molecular studies have shown that integration of HPV often leads to a disruption in the E1/E2 open reading frame and concurrent loss of the E4 and E5 and parts of the E2 and L2 genes [29]. E2 function can moreover be abrogated by epigenetic alterations of the viral genome such as methylation of the E2 binding site in the long control region [30]. Absence of E2 function results in upregulation of the expression of oncoproteins E6 and E7, which in turn leads to uncontrolled cell cycle progression (see Figure 1).

The major structural protein L1 of the HPV capsid is sufficient for self-assembly into a capsid, but entry of the virus into the cell is co-dependent on L2, the minor structural protein [19,31].

Under normal circumstances, HPV maintains an episomal state, and infection with HPV is transient. In a recent prospective cohort study, the reported average duration of active episomal infection in the uterine cervix appears to be approximately 8 months [32]. Although uterine cervical HPV infection

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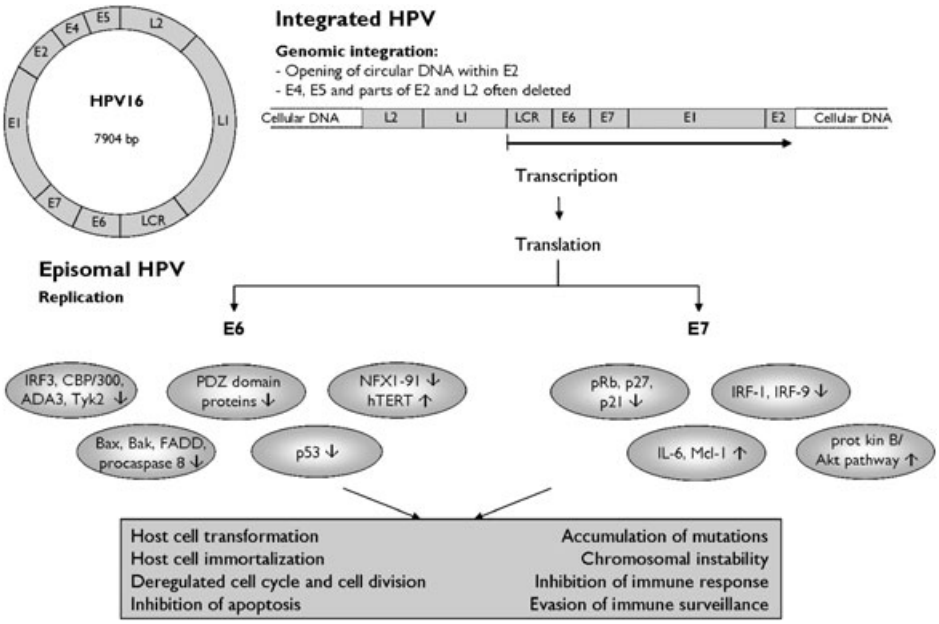

Figure 1. Structure of circular episomal and linear integrated HPV DNA. The HPV genome is usually present in many episomal copies in the nucleus of infected cells. In the transition to cancer, viral DNA often integrates in 1 or more copies into the host genomic DNA. During this process, the ring structure of the HPV-DNA molecule is most often opened within the E2 reading frame, frequently leading to deletion of E4 and E5 and part of E2 and L2. The subsequent upregulation of E6 and E7 oncoproteins results in deregulation of cell signalling pathways, which, amongst others, leads to increased cellular proliferation and inhibition of apoptosis. Based on [172–175]

prevalence decreases with increasing age [33], it is unclear whether age affects the duration of infection. Persistent infection, however, might lead to integration of the virus [34,35]. Numerous investigations have shown an etiological relationship between infection with HR-HPV infection and the development of uterine cervical squamous cell carcinomas (UCSCC) and other anogenital squamous cell carcinomas [36,37]. More than 90% of UCSCC contain and express HR-HPV sequences, which are predominantly present in an integrated form [38,39]. HPV-16 is the most common HPV type and is detected in more than 50% of UCSCC, followed by HPV-18, HPV-33 and HPV-45 [37,40] (Table 1).

The precise relationship between HR-HPV integration and head and neck carcinogenesis is less clear, partly because primary premalignant lesions of the oropharynx are seldom detected. Although controversial data have been reported [41–44], integration of HR-HPV in OPSCC is a prevailing finding.

High-risk human papillomavirus in head and neck squamous cell carcinoma

Patients with a history of HPV-related anogenital carcinomas, patients seropositive for HPV-16, and husbands of patients diagnosed with uterine cervical dysplasia or carcinoma *in situ* all show increased risk rates for developing OPSCC [45–47].

The involvement of HPV in head and neck tumourigenesis was first proposed by Syrjänen *et al*. [48], who showed histopathological features of HPV infections in 40% of patients, and HPV-positive nuclei in 20% of patients using immunohistochemistry. Since then many studies have provided evidence that infection with HR-HPV is a significant independent risk factor for HNSCC and is associated with high-risk sexual behaviour [9,10,49–51]. HR-HPV positive tumours are most frequently found in the oropharynx and are associated with HPV-16 in >90% of cases [9,49,52,53]. Because patients with OPSCC often present with metastatic disease at first diagnosis, information on the persistence of oropharyngeal HPV infections and premalignant lesions in this region is scarce [54–56]. HPV prevalences of less than 1% have been found in tumour-negative tonsillar tissue samples, screened for HPV with PCR [51,57,58].

HR-HPV detection and tumour characteristics

The reported overall incidence of HPV in OPSCC ranges from less than 20% to more than 90% in different studies. This variation depends on several factors,

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including geographical features, sample preparation and detection methods used but also the amount and manner of tobacco consumption depending on geographical location [9,12,54,59–61]. It has been shown that not all tumours tested positive for HPV DNA can be regarded as etiologically HPV-related [50,62]. A clinically relevant infection, that is, a transcriptionally active infection should be present, which can be demonstrated by detectable expression of the viral oncogenes E6 and E7 [63]. This correlates strongly with overexpression of the CDK inhibitor $p16^{INK4A}$, which is considered a reliable surrogate marker for HR-HPV infection in most cases [41,64]. A reliable algorithm for HPV detection should thus start with $p16^{INK4A}$ detection, followed by *in situ* hybridisation (ISH) and/or RT-PCR analysis of E6/E7 transcripts after HPV typing [41], as suggested by two recent reports [59,65]. A representative example of these analyses is shown in Figure 2.

The HPV-associated OPSCC are now considered to comprise a separate entity with typical clinical and molecular features. Table 2 summarises the major differences between HPV-positive and HPV-negative OPSCC.

The HPV-positive OPSCC are characterised by overexpression of oncoproteins E6 and E7 leading to degradation of p53 and pRb, thereby inducing cell cycle and apoptosis deregulation. As a result, CDK inhibitors including $p16^{INK4A}$, $p14^{ARF}$, $p18^{INK4C}$ and $p21^{Cip1/WAF1}$ are upregulated, which subsequently leads to downregulation of cyclin D1 and inhibition of complex formation with CDK4 [22,62,66,67]. In HPV-negative tumours, cell cycle deregulation is established by p53 and pRb gene mutations, or alternatively by inactivation of $p16^{INK4A}$ and $p14^{ARF}$ gene expression through mutation, promoter hypermethylation or homozygous deletion [22], or activation of cyclin D1 expression via 11q13 amplification [68]. High expression of EGFR by transcriptional upregulation is generally present in this OPSCC subgroup [22,56,62,66–68]. Upregulation of EGFR expression is usually not seen in HPV-positive OPSCC [56,67,69–71].

In addition, global genome and protein scanning approaches have been and are being used to unravel DNA, mRNA, microRNA, and protein signatures specific for HPV-positive and HPV-negative OPSCC. So far, these studies revealed that HPV-positive tumours exhibit a relatively stable genome with 11q and 16q loss [72–74], and upregulate transcriptional activity of cell cycle regulators (as mentioned previously), transcription factors (e.g. TFDP2, ZNF238, TAF7L and RPA2) and DNA repair proteins (e.g. RFC4 and RFC5). Also, HPV-positive tumours show decreased expression of genes involved in immune responses (e.g. IFIT1, IFITM1-3, IFI6-16, IFI44L, OAS2 and IFN-κ) [68,75–79]. In addition, these tumours differentially express microRNAs, and for example upregulate miR-363 (belonging to the oncogenic miR-106a-363 cluster) and downregulate miR-218. A recent proteome analysis comparing HPV-positive and HPV-negative oral squamous cell carcinomas (OSCC) reported upregulation of thioredoxin and epidermal-fatty acid binding protein [80]. Thioredoxin is an important redox-mediator that stimulates cell growth and inhibits apoptosis under adverse conditions, apparently including HPV infections, as also seen in cervical carcinomas. Epidermal fatty-acid binding protein, although mainly involved in fatty acid uptake, transport and metabolism, also functions in cellular signalling,

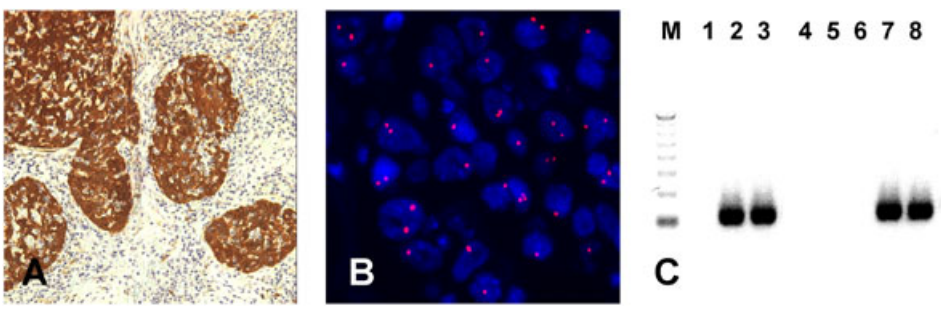

Figure 2. Representative examples of strong nuclear and cytoplasmic $p16^{INK4A}$ immunostaining (A) and punctate nuclear HPV-16 FISH signals indicating viral integration (B) shown on paraffin embedded, formalin fixed tissue sections of oropharyngeal squamous cell carcinoma. An example of E6-specific HPV-16 RT-PCR products on a 1% agarose gel, on RNA extracted from cell lines and fresh-frozen oropharyngeal squamous cell carcinoma tumour tissues, are shown in (C)

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Table 2. Clinical and molecular differences between OPSCC with or without HR-HPV *

	HPV-positive	HPV-negative
Clinical characteristics		
Preferred location	oropharynx	all sites
Degree of differentiation	poorly differentiated	moderately to well differentiated
Baseloid appearance	more often	less often
T-stage at diagnosis	T1-2	T3-4
Disease stage (TNM)	more advanced	less advanced
Average age	slightly younger than 60 years	slightly older than 60 years
Tobacco (ab)use	low	high
Alcohol (ab)use	low	high
5-year disease free survival	70-90%	30-60%
Second primary tumours within 5 years	0-10%	10-15%
Local recurrences within 5 years	10-20%	25-55%
Molecular characteristics		
E6/E7 expression	+	-
p53 downregulation	+	-
pRb downregulation	+	-
$p16^{INK4A}$ overexpression	+	- **
$p14^{ARF}$ overexpression	+	- **
$p18^{INK4C}$ overexpression	+	-
$p21^{Cip1/WAF1}$ overexpression	+	-
Cyclin D1 overexpression	-	+ ***
EGFR overexpression	-	+ ***

*Summarised from [10,22,41,56,59,62,65–68,92,94,158,174,190–192].
**Inactivated by gene mutation, hypermethylation or homozygous deletion.
***Mainly induced by gene amplification or transcriptional upregulation.

affecting differentiation, growth regulation and gene expression [80]. Although expression of 3q-specific genes has been reported as being specific for HPV-positive OPSCC, this finding remains to be confirmed, because extra copies of 3q-genes have been found in both HPV-positive and HPV-negative tumours [68,77].

CURRENT TREATMENT OF OPSCC AND EFFECT OF HR-HPV STATUS ON TREATMENT RESPONSE

Current treatment modalities

Current international clinical guidelines for HNSCC treatment mention HPV as a risk factor for OPSCC. The American National Comprehensive Cancer Network has suggested to include HPV detection in the diagnostic work-up of these tumours [81]. However, the treatment guidelines do not offer therapeutic modalities specific for HPV-related tumours. Current therapeutic options include surgery, radiotherapy, chemotherapy (CT), immunomodulatory therapies or combinations of the foregoing. Surgery as primary treatment avoids toxicity caused by radiotherapy and CT but causes loss of function, particularly in patients with larger tumours. The development of laser surgery and transoral robotic surgery (TORS) for OPSCC reduces functional morbidity as a consequence of three-dimensional visualisation and the ability to manipulate and perform reconstruction of the oropharynx without the need for an open surgical approach [82,83]. Regional infiltration of critical structures and unacceptable loss of function after surgery can classify a tumour as functionally or technically unresectable. In those cases, radiotherapy and/or CT is the treatment of choice [84] when aiming at restoring function, however with the disadvantage of therapy-related local and systemic side effects [85]. The final choice of treatment is based upon clinical variables such as tumour

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type, localisation and stage [86], age of the patient, general medical and psychomedical condition [81] and individual preferences of the patient.

Effect of HR-HPV status on outcome

In retrospective studies, HR-HPV- and/or $p16^{INK4a}$ positive tumours have been found to respond better to multimodal therapies as compared to HPV-negative tumours, thereby favouring patient survival [41,42,53,64,87,88]. More recent retrospective studies have shown that this favourable outcome is independent of treatment modalities [89–95]. However, the heterogeneity of the HNSCC patient populations and consequent variability with regard to the HPV and/or $p16^{INK4A}$ status, as well as applied treatment protocols, has most probably negatively influenced the association between HR-HPV status and outcome in these studies. It can be anticipated that the actual difference in clinical outcome between HPV-positive and HPV-negative cases will become even more pronounced when comparing a homogeneous population of OPSCC and application of reliable detection methods for clinically relevant HPV-infections. Prospective clinical trials are required to further validate HR-HPV presence as predictive factor for therapy outcome and to determine whether treatment de-intensification might improve quality of life while preserving the favourable clinical outcome in HPV-positive OPSCC patients [96,97].

An explanation for the favourable response may lie in the fact that, although the pRb-pathway and p53-pathway are compromised in HPV-positive tumours, they retain some function, such that under the pressure of radiotherapy and/or CT, p53-mediated apoptotic pathways may still function. The presence of wild-type p53 in combination with low levels of Bcl-2/Bcl-xL and EGFR, which are features of HPV-positive tumours in non-smokers, may enhance this treatment advantage [9,53,63,67,69,71]. Moreover, limited tobacco and/or alcohol use reduces field cancerisation and the chance of developing a second primary tumour or distant metastasis in HPV-positive tumours [41,53,93], which underscores the need to investigate the effect of tobacco and alcohol exposure on the biological behaviour of HPV-positive OPSCC, as recently proposed [41,94].

Besides the overall better survival of patients with HPV-positive tumours, their treatment may be further improved by the implementation of strategies that either 1) promote the immune response to eradicate the virus, 2) inhibit viral DNA replication, 3) specifically target viral oncoproteins or 4) have an effect on deregulated signal transduction pathways specific for HPV-positive tumour cells. In the following section, we review these strategies, their mode of action and possible benefits for patients with HPV-induced HNSCC.

PROPHYLACTIC AND THERAPEUTIC ALTERNATIVES FOR HPV-POSITIVE OPSCC

Immunomodulating therapies

Vaccination

Two prophylactic vaccines, containing recombinant virus-like particles (VLP) composed of the L1 proteins of the respective HPV types [31] have been marketed recently, which are Cervarix® (GlaxoSmithKline, Brentford, Middlesex, TW8 9GS, United Kingdom) and Gardasil® (Merck, NJ, USA). Both vaccines have been FDA-approved for use in girls and young women [98,99] and Gardasil® has also been approved for use in men [100].

Cervarix® is a bivalent vaccine that protects against infection with HPV-16 and HPV-18, whereas the quadrivalent vaccine Gardasil® provides protection against HPV-6, HPV-11, HPV-16 and HPV-18. Reports indicate that Cervarix® also offers cross-protection against HPV-31, HPV-45 and HPV-52 [101,102], and Gardasil® possibly against HPV-31 [103]. More robust cross-protection may be induced by adding L2 minor capsid proteins to the vaccine [104]. Both vaccines induce high antibody titers and seem to be well-tolerated and safe and provide >90% protection in HPV-16 and HPV-18 in naïve females when given in three doses within six months [102,105]. Currently, only young HPV-naïve females are vaccinated because vaccination of women actively expressing HPV-16 or HPV-18 at study entry did not result in decreased development of cervical intraepithelial neoplasia (CIN) lesions [102,105]. However, vaccination with Gardasil® also provided >90% protection in women with evidence of past infection (seropositive and HPV DNA negative) with one or more of the HPV-types against which the vaccine is directed [106,107]. Long-term benefits of vaccination are not yet known, but it is hypothesised that vaccination could also strongly reduce the number of HPV-related OPSCC. This would indicate that HPV-naïve boys and young men should be vaccinated as well, because HPV-related OPSCC is diagnosed in males more often than in females [61,69]. However, seeing that patients usually present with HPV-related head and neck tumours from the fifth decennium of life onwards, the efficiency of vaccination in these patients will only become evident within a few decades.

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Patients with HPV-related disease may benefit from the development of therapeutic vaccines. These vaccines are designed to induce cell-mediated immunity against the overexpressed foreign viral oncoproteins, particularly E6 and E7. There are four classes of therapeutic vaccines: 1) live-vector based; 2) peptide/protein based; 3) nucleic acid based; and 4) whole cell vaccines (for a comprehensive review see [108]). In anogenital and uterine cervical lesions therapeutic vaccination has been shown to generate specific immunological and clinical responses, including complete regression of the lesion in 22% of patients with CIN III lesions as reported in a study using a fusion protein-based vaccine [109–111]. A preclinical study using a DNA-based vaccine demonstrated that such an approach for therapeutic vaccination was efficacious in a mouse model of HPV-related HNSCC [112]. Clinical trials to evaluate the effectiveness of therapeutic vaccination in HPV-related HNSCC are ongoing [113,114].

Interferon

Interferons are cytokines that are produced by many cell types in response to infection with bacteria, viruses and parasites [115]. Two classes of IFNs can be distinguished: Class I consists of IFN-α and IFN-β, and Class II consists of IFNγ. Class I IFNs are secreted from infected cells and bind to the ubiquitously expressed heterodimeric interferon receptor. Binding of IFNα/β to the interferon receptor induces the transcription of several host cell proteins that inhibit viral replication in the infected epithelial cell, and leads to activation and the production of IFNγ in dendritic cells. IFNγ can also be produced by activated Th1 cells. Both classes of IFNs possess antiviral and antiproliferative properties. IFNγ can also activate macrophages and natural killer lymphocytes, and induce translocation of the major histocompatibility complex Classes I and II to the cell membrane [115]. The interferon response, however, is suppressed upon HPV infection because several HPV proteins (E1, E6 and E7) interfere with the IFN signal transduction cascade by binding to, for example, Tyk2 kinase, IRF-1 and IRF-3, p48 and p56 leading to downregulation of the levels of IFN-inducible genes, such as TNSFS10, IFIT1 and IFI54 [78,116,117].

Despite this, a successful immune response to HPV is generally seen in healthy individuals, as for example reported in the studies of van der Burg and co-workers [118,119], showing high frequencies of circulating CD4+ T-helper cells reacting with HPV16 E2 and E6, indicating a cell-mediated Th1 immune response. In persisting lesions, application of IFN therapy may restore antiviral defence mechanisms, thereby supporting effective treatment of HPV-infected lesions. IFN therapy proved to be beneficial in HPV infections such as condylomata acuminata [119,120], whereas the use of IFN therapy in HPV-associated anogenital intraepithelial neoplasia has been assessed in several studies with contradicting results. Improved outcome for IFN-treated patients was shown in some studies [121,122], whereas others reported no change in response rates between treated patients and controls [123,124]. This might be attributed to the fact that local application seems to achieve better responses than systemic application [120]. In addition, it seems that IFN therapy can eradicate episomal HPV infection but leads to growth advantage for cells containing integrated HPV [125,126]. IFN-induced upregulation of p56, which blocks HPV replication by binding to the E1 protein and inhibits its helicase activity, may explain this effect on episomal infection [127,128]. Loss of (parts of) E1 and E2 by viral integration, resulting in upregulation of E6 and E7 as stated previously could explain the lack of effect of IFN treatment in these cells and their selective growth advantage. On the contrary, IFN was also shown to increase viral early gene transcription in a cell model [129]. In recurrent respiratory papillomatosis (RRP) a long-term response to IFN-α therapy was seen in patients with HPV-6-related papillomas, but patients with HPV-11-related papillomas were much less responsive to IFN therapy [130]. In conclusion, the beneficial effects of IFN therapy seem to be limited to episomal infections, which limits the applicability of this therapy in HPV-positive carcinomas.

Antiviral therapy

Cidofovir

Cidofovir [(S)-1-(3-hydroxy-2-phosphonylmethoxypropyl)cytosine] (HPMPC) is a nucleoside analogue of deoxycitidine monophosphate with a remarkably broad spectrum of antiviral activities directed against DNA viruses, including HPV and polyoma [131]. After intracellular double phosphorylation, the structure resembles dCTP and can act as a competitive substrate. After removal of the diphosphate group cidofovir can be incorporated into viral DNA during replication, resulting in selective antiviral activity for those viruses encoding their own DNA polymerase. Viral DNA polymerases, for instance cytomegalovirus, display greater affinity for cidofovir than human cellular DNA polymerases. Although HPVs do not produce viral DNA polymerases, cells infected with HPV show enhanced

susceptibility to cidofovir-induced apoptosis as compared to non-infected cells for a yet unknown reason [132,133].

In 1998, it was shown by Andrei *et al*. that acyclic nucleoside phosphonate (ANP) analogues, such as cidofovir, show a selective antiproliferative effect in HPV-bearing tumour cell lines CK-1, SiHa, CaSki and HeLa [134]. This effect is partly induced by its non-selective toxicity to rapidly dividing cells [134,135]. Apoptosis might also be induced by accumulation of the tumour suppressor proteins p53 and $p21^{Cip1/WAF1}$ [132,133], although an increase in p53 expression was not found in the HNSCC cell line UPCI:SCC090 [136]. However, by combining cidofovir with radiotherapy, the radiosensitivity of UPCI:SCC090 and other HPV-containing cell lines could be enhanced *in vitro* [132,136], as well as *in vivo* in nude mouse xenografts [132]. CT combined with cidofovir also yielded a synergystic effect in an HNSCC cell line model [137]. One study expressed concern about using cidofovir for the treatment of RRP [138], as it demonstrated high malignancy transformation rates in rats and cell lines. In humans, this effect has not been reported, and cidofovir is already applied as an effective adjuvant therapy for HPV-induced RRP in humans [139].

For the treatment of various HPV-related lesions the route of administration may be an important factor. Cidofovir can be applied systemically or topically or injected intralesionally. Although concern was raised about possible nephrotoxicity in systemic use, this side-effect can be greatly diminished by administration of probenecid and prehydration with saline solution [140].

In a clinical setting, it was shown that local therapy with cidofovir gel resulted in complete or partial regression of uterine CIN II and III lesions [141,142] as well as vulvar and other intraepithelial neoplasms [143,144]. On the other hand, intralesional treatment with cidofovir of one patient with an invasive carcinoma in the respiratory tract and a history of RRP only lead to minor clinical effects, limited to the superficial portion of the tumour [145].

Clinical trials using cidofovir as an adjuvant therapy in cervical cancer have started [146], but trials for its application in HNSCC have to be initiated.

Interfering RNAs

The RNA interference (RNAi) can be used to inactivate gene expression and so far encouraging results have been reported for the treatment of HPV-related carcinomas *in vitro* as well as *in vivo*. Chen and coworkers, for example, reported a 50% reduction of E7 mRNA expression in HPV-6b/11 E7-expressing mouse tumour models [147]. RNAi against HPV-16 E6 and/or E7 has been shown to degrade these mRNAs leading to decreased expression of the gene products in both cervical as well as HNSCC cell line models. This resulted in restoration of pRb function and upregulation of p53 and $p21^{Cip1/WAF1}$, leading to substantial apoptotic cell death [148–150]. RNAi against HPV-18 E6 and E7 has also seemed to possess antitumour activity by retarding the growth of HeLa-cell induced tumours in NOD-SCID mice [151] and to enhance the chemotherapeutic effect of cisplatin in HeLa cells *in vitro* [152].

Molecular therapy based on cellular targets

Because inactivated tumour suppressor gene products such as p53 and $p16^{INK4A}$ are difficult to restore by molecular therapy, many studies have focussed on the identification of oncogenes and deregulated cell signalling pathways in HNSCC. Key pathways involved in HNSCC include EGFR, PI3K-PTEN-AKT, TGFβ and NF-κB signalling for which inhibitors are available, for example, the anti-EGFR antibody cetuximab [153], or being tested in several clinical trials (for reviews, see [72,73]).

In cervical cancer EGFR overexpression has been shown to negatively affect overall survival in patients treated with radiotherapy [154]. Anti-EGFR therapy using cetuximab lead to a therapeutic response in 12.5% of patients with uterine cervical SCC [155]. Also in HNSCC, including HPV-positive OPSCC, overexpression of EGFR correlates with poor prognosis [67,70,71], although only a small subgroup of HPV-positive OPSCC exhibit EGFR protein accumulation [67,71]. Large prospective trials with anti-EGFR therapy in HPV-positive HNSCC have been initiated [97], although its efficacy is most probably limited to the small subgroup of EGFR-expressing tumours.

Alternatively, the PI3K-PTEN-AKT pathway might be an efficient target because HPV-positive OPSCC show extra copies of chromosome 3q in up to two-thirds of cases [68], including the 3q26 locus, harbouring the PI3K gene.

Tumour angiogenesis and metastases are correlated to upregulation of the TGF-β and NFκB pathways [156,157]. HPV positive OPSCC have been shown to metastasise in an earlier stage compared to HPV negative OPSCC [158], indicating earlier

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endothelial-to-mesenchymal transition (EMT), which is characterised by the expression of vimentin, dowregulation of E-cadherin and upregulation of β-catenin [159]. This suggests that EMT might be related to upregulation of these pathways and that particularly HPV-positive OPSCC might be a potentially interesting group for NFκB-inhibitors, for which a clinical trial has recently started [160].

Increased degradation of cell cycle regulatory proteins p53 and pRb by the oncoproteins E6 and E7, can be inhibited by targeting the proteasomal pathway. Ritonavir, a protease inhibitor (PI) that is used in HIV-infected patients, inhibits the chemotryptic activity of the human cellular 20S proteasome while increasing the tryptic activity [161], resulting in reduced protein degradation. It was shown to enhance antitumour activity when combined with radiotherapy both *in vitro* and *in vivo* in a Hep-2 head and neck carcinoma model [161], later however shown to be contaminated with HeLa cells. The PI Lopinavir was shown to restore p53 expression and to induce apoptosis in SiHa cells [162]. Athough several clinical trials have evaluated the effectiveness of PIs in the treatment of HIV, clinical trials in the treatment of HPV-related disease have not been initiated.

Finally, replication of the HPV virus can be targeted. In episomal HPV infection, replication is initiated by binding of E2 to its origin of replication [54]. In human transcription factors, the most commonly found DNA binding motifs are zinc fingers. Recently, artificial zinc fingers (AZF) have been developed as a potent new inhibitor of HPV [163]. When linked to a cell-penetrating peptide (CPP) these AZF were shown to inhibit HPV-18 for 97% [164]. However, because the CPP-AZF is designed to prevent E2 from binding to its origin of replication, they are only effective in episomal HV infection.

DISCUSSION

The past decade has provided evidence for a biological association between oncogenic HPV and OPSCC. HPV-induced OPSCC show molecular and clinical features that are clearly different from tobacco-and-alcohol-induced tumours and these differences seem to underlie prognostic differences between both tumour subgroups. Independent of treatment modality, patients with HPV-positive tumours demonstrate up to 30% better survival rates. In the past decennia, intensification of treatment was the most important strategy to improve survival of patients with HNSCC. However intensification of treatment, combined with increased side effects, is finally reaching the maximum tolerance of the patient and this limits the intensity of treatment. Until now, no differentiation of therapeutic strategies has been made between the HPV-positive and HPV-negative subgroups in international guidelines on OPSCC treatment [81]. Because of the clinical and molecular differences between both groups, the question arises whether HPV-positive tumours need equally intensive treatment protocols as their HPV-negative counterparts. Moreover, additional antiviral therapeutic strategies can possibly improve survival without increasing therapy-related morbidity in HPV-positive tumours. In current and future studies. we should, therefore, aim at improving the quality of life in patients by de-intensification regimens in selected cases. Next-generation treatment strategies for HPV-associated cancers should focus on decreasing adjuvant radiotherapy and chemotherapy, whether or not combined with therapeutic options specifically targeting HPV. Assessing which therapy is most effective will finally lead to a more personalised approach for individual patients.

Immunomodulating therapies, such as IFN therapy, may have beneficial effects, but this seems to be limited to episomal infections. This conveys the need to reliably establish the integration status of HPV infection. That this criterion has not yet been met becomes apparent when observing the reported integration frequencies, which range from 0% to 100%, depending on the population studied and methods used [41,42].

Tumour-specific host responses could also be enhanced by depletion of CD4+/CD25+ regulatory T-cells (T_{regs}). Increased expression of T_{regs} was shown in patients with CIN and cervical cancer [165,166]. It is hypothesised that the enlarged population of T_{regs} suppresses HPV-specific immunity and inhibits tumour-specific T-cell responses. Upregulated T_{regs} have already been depleted using an anti-CD25 antibody, such as PC61 [167].

Other immunomodulating therapies such as imiquimod, a topical immune response modifier that has successfully been used in the treatment of anogenital lesions with episomal HPV infections [168], are thought to be unsuitable for application in HPV-related HNSCC and RRP. Application to cutaneous epithelia is known to induce local inflammatory responses and pain, which will be enhanced in mucosal epithelia. Moreover, the substance cannot be controlled to reach all tumour parts when

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topically applied, and, like IFN-therapy, will at best lead to eradication of only episomal HPV infections, whereas a large proportion of HPV-positive HNSCC show viral integration [169].

Antiviral therapies such as cidofovir and RNAi have already shown promising results and are expected to have progressive impact on the treatment of HPV-associated lesions. Cidofovir has been tested in cervical cancers and RRP, where it has been applied topically or intralesionally in most studies. It has been shown that combining IFN therapy with cidofovir could enhance the antiviral and antiproliferative effects of either substance alone, and it is postulated that adding IFN therapy could further improve the auspicious effects of cidofovir combined with CT and/or radiotherapy [170]. Furthermore, it is recommended to assess the effects of cidofovir as adjuvant therapy in the treatment of HPV-associated HNSCC in a larger, prospective clinical trial.

The RNAi treatment, although tested in mouse models, has not yet been evaluated for use in human HPV-associated HNSCC. Such studies can, however, be expected in the near future, judging from patents referring to the use of oligonucleotides in the treatment of HPV infections (see for example [171]).

Therapeutic approaches based on the molecular profile of the tumours are emerging in an adjuvant setting. However, one of the major drawbacks of such an approach is that the applicability should be assessed for each individual patient. For example, cetuximab can only be applied in a small subgroup of patients with HNSCC because HPV-positive tumours tend to show a low EGFR expression. In the current practice, Cetuximab is already used for larger non-resectable head and neck tumours, irrespective of HPV status. With regard to PIs and AZFs, no clinical studies have yet tested the applicability of these therapeutic options in the treatment of HPV-related carcinomas.

Because therapeutic vaccination is expected to have minimal side effects it can be combined with other therapeutic approaches, such as radiotherapy and/or CT, to obtain synergistic effects. However, therapeutic vaccines are still in a developmental stage.

Although a significant reduction in the burden of HPV-related diseases can be anticipated if prophylactic vaccination will live up to its promises, only HPV-naïve females are currently vaccinated. We firmly believe that young HPV-naïve boys should also be vaccinated in order to achieve optimal protection, although it needs to be validated whether vaccination is cost-effective.

In conclusion, we can state that although it has become evident that HPV-positive HNSCC have a better prognosis that their HPV-negative counterparts, the choice of therapy for these two subgroups of HNSCC will strongly depend on the outcome of ongoing clinical trials, including de-intensification protocols and implementation of treatment options based on new insights into the molecular biology of HPV-infection.

CONFLICT OF INTEREST

The authors have no competing interest.

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