Head and Neck Cancer Clinics
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HPV and Head and Neck Cancers

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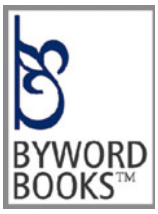

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A Note on the Series

Head and Neck Cancer (HNC) is a major challenge to public health. Its management involves a multidisciplinary team approach, which varies depending on the subtle differences in the location of the tumour, stage and biology of disease and availability of resources. In the wake of rapidly evolving diagnostic technologies and management techniques, and advances in basic sciences related to HNC, it is important for both clinicians and basic scientists to be up-to-date in their knowledge of new diagnostic and management protocols. This series aims to cover the entire range of HNC-related issues through independent volumes on specific topics. Each volume focuses on a single topic relevant to the current practice of HNC, and contains comprehensive chapters written by experts in the field. The reviews in each volume provide vast information on key clinical advances and novel approaches to enable a better understanding of relevant aspects of HNC. Individual volumes present different perspectives and have the potential to serve as stand-alone reference guides. We believe these volumes will prove useful to the practice of head and neck surgery and oncology, and medical students, residents, clinicians and general practitioners seeking to develop their knowledge of HNC will benefit from them.

Manipal, Karnataka, India — Rehan Kazi
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Foreword

Dr. Jatin P. Shah

The publishers (Byword Books) and the Series Editors Drs. Rehan Kazi and Raghav Dwivedi are to be complimented for conceiving and introducing the 'Head and Neck Cancer Clinics' series, a much-needed educational and informational resource on focused topics with contributions from world leaders in the specialty of head and neck oncology. The selection of topics and volume editors is crucial to the success of this series, and the volumes published to date, or to be published as listed, clearly reflect that this series is bound to succeed and here to stay.

The current volume on 'Human Papillomavirus and Head and Neck Cancers' is very timely and much needed to bring to fore the many misconceptions and lacunae in knowledge about this 'new entity' in the arena of clinical care of head and neck cancer patients, by all involved specialists. The editors, Drs. Carole Fakhry and Gypsyamber D'Souza, from the Johns Hopkins Medical Center, have assembled an outstanding group of authors in this volume, all of whom are world leaders in their area of interest, with their contributions culminating into an up-to-date status report. Clearly, this is the most concise treatise on this topic with state-of-the-art information available today.

The global incidence of HPV infections is rising, and so is the incidence of HPV-related cancers in the head and neck, particularly in the oropharynx (OPC), while there is a steady decline in tobacco-induced OPCs. Misconceptions about the role of HPV-positivity and promiscuity are sensitive, personal and social issues and need

societal, patient and family education. An excellent chapter on these questions addresses these very issues. While the mode of acquiring HPV infection is well established in numerous studies, the continued persistence of HPV virus for years and its eventual role in the development of a malignant tumour remains an active area of basic research. Similarly, detection assays for HPV presence on cytological specimens are desperately needed, to avoid invasive tissue sampling for accurate diagnosis.

HPV-related OPCs are inherently favourable cancers and have excellent prognosis, regardless of the treatment given. The current focus is thus on reduction of treatment-related toxicities and sequelae, while maintaining the same therapeutic outcomes. Future research will focus on vaccines, immune modulation and anti-HPV-specific molecular targeting. While administration of vaccines in young age has reduced the incidence of anogenital HPV infections, by induction of protective antibodies, its role in the prevention of OPCs remains to be elucidated. In addition, much work needs to be done in the development of therapeutic DNA vaccines which induce T-cell responses against HPV-infected cells. This may improve survival in HPV-related cancers.

The authors and editors of this volume are to be congratulated for putting together this comprehensive, up-to-date and thoughtful, yet concise, compendium on HPV-related head and neck cancers. The readers will have a clear picture on the status of current research on this relatively new disease entity.

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Foreword

Dr. Patrick Gullane

It is an honour to have been invited to write a foreword to this timely Head and Neck Series that highlights and summarizes the current knowledge of this challenging epidemic of HPV-related head and neck cancer.

The list of contributors reads like a 'who's who' of experts from a variety of backgrounds, many of whom I know as personal friends.

For this book, Drs. Fakhry and D'Souza have selected a most balanced and outstanding group of experts who thoughtfully and skilfully present a very comprehensive and concise overview of our present-day knowledge of this subject. They have included a very detailed overview of the anatomical sites, an understanding of the epidemiology of HPV-induced cancers, the molecular biology, diagnosis, clinical management, role of vaccines and a 'peep into the future' as well as a detailed section on the most frequently asked questions which have been overlooked by so many of us for years.

The material is presented in a clear and concise manner that includes both critical and contrasting points of view with a very complete list of references for the trainee and reader to explore.

This text chronicles the current depth of understanding, necessary for implementing the best form of therapy in a multidisciplinary fashion that optimizes the best outcome and attempts to highlight the need to de-intensify therapy and enhance quality of life by diminishing morbidity for this patient population. It is a very valuable contribution to head and neck oncology and should be compulsory reading for

residents and trainees across our three disciplines of surgery, radiation and medical oncology, as well as for practising oncologists.

The pages of this text are filled with innumerable facts, educational pearls, insightful treatment approaches and instructive diagrams. I am delighted to single out the very complete Chap. 7 which provides the reader with a knowledge and viewpoint that while biology, diagnosis and treatment are imperative, the most important future contribution is 'in prevention' through education and vaccines.

Armed with the knowledge contained in this text, clinicians should feel confident that they can provide the patient with a HPV+ diagnosed cancer the best care known to our specialties at this time.

My congratulations therefore to the editors and authors for their impressive contributions which have culminated in a most comprehensive reference text.

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Preface

This book serves a snapshot of the current knowledge base in this young and growing field. As human papillomavirus (HPV) causes a rising number of oropharyngeal cancers in the USA and abroad, understanding the biological, clinical, and social implications of this infection has become increasingly important for head and neck practitioners. This book reflects the multidisciplinary nature of the scientific and clinical questions involved in this disease. Experts in epidemiology, diagnosis, and treatment of HPV-related oropharyngeal cancer present in-depth reviews, which will help to improve the reader's understanding of this topic. The authors provide insight for answers of common patient and provider questions about HPV infection and related disease, and highlight remaining questions to be answered in the coming years. We hope you enjoy this comprehensive summary of the quickly evolving area affecting an increasing number of patients.

Baltimore, MD, USA

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Abbreviations

3DCRT	3-Dimensional conformal radiotherapy
4DRT	4-Dimensional radiation therapy
ACIP	Advisory Committee on Immunization Practice
AIDS	Acquired immunodeficiency syndrome
AJCC	American Joint Committee on Cancer
APC	Annual percent change
ART	Adaptive radiotherapy
CDC	Centers for Disease Control and Prevention
CDDP	cis-Diamminedichloroplatinum
CI	Confidence interval
CIN	Cervical intraepithelial neoplasia
CRT	Chemoradiotherapy
CTL	Cytotoxic T-lymphocyte
DC	Dendritic cell
DNA	Deoxyribonucleic acid
EBV	Epstein-Barr virus
ECE	Extracapsular extension
ECOG	Eastern Cooperative Oncology Group
EGFR	Epidermal growth factor receptor
eHNS	"Endoscopic" head and neck surgery
EROPC	Environmentally and smoking-related oropharyngeal cancer
FDA	Food and Drug Administration
FNA	Fine-needle aspiration
HAART	Highly active antiretroviral therapy
HC2	Hybrid capture 2
HDAC	Histone deacetylase
HIM	HPV in men
HIV	Human immunodeficiency virus
HNC	Head and neck cancer
HNSCC	Head and neck squamous cell carcinoma
HPV	Human papillomavirus
HPVOPC	Human papillomavirus-associated oropharyngeal cancer
HR	Hazard ratio
IFN	Interferon

IGRT	Image-guided radiation therapy
IL	Interleukin
IMRT	Intensity-modulated radiation therapy
IRF	Interferon regulatory factor
ISH	*In situ* hybridization
Ki-MSV	Kristen murine sarcoma virus
LAHNC	Locally advanced head and neck cancer
LC	Langerhans cells
LCR	Long control region
LRC	Locoregional control
LVI	Lymphovascular invasion
MHC	Major histocompatibility complex
NCICCTG	National Cancer Institute of Canada Clinical Trials Group
NHANES	National Health and Nutrition Examination Survey
NPC	Nasopharyngeal carcinoma
NTCP	Normal tissue complication probability
OAR	Organs at risk
OPC	Oropharyngeal cancer
OPSCC	Oropharyngeal squamous cell carcinoma
OR	Odds ratio
ORN	Osteoradionecrosis
OS	Overall survival
PAF	Population-attributable fraction
PC	Pharyngeal constrictor
PCR	Polymerase chain reaction
PD-1	Programmed cell death-1
PFS	Progression-free survival
PNI	Perineural invasion
PR	Prevalence ratio
PS	Performance status
PTV	Planning target volume
PY	Pack-years
qrtPCR	Quantitative real-time PCR
Rb	Retinoblastoma
RR	Relative risk
RT	Radiotherapy
RTOG	Radiotherapy Oncology Group
SCC	Squamous cell carcinoma
SEER	Surveillance, Epidemiology, and End Results
SIR	Standardized incidence ratio
SPT	Second primary tumor
SRS	Sinai Robotic Surgery
ST	Sequential therapy
TAP	Transporters associated with antigen processing
TGF	Transforming growth factor

TILS	Tumor infiltrating lymphocytes
TLM	Transoral laser microdissection/microsurgery
TNF	Tumor necrosis factor
TORS	Transoral robotic surgery
TPF	Docetaxel, cisplatin, 5-fluorouracil
URR	Upper regulatory region
VLP	Viral-like protein
WHO	World Health Organization

Anatomical Sites and Subsites of Head and Neck Cancer

1

Ryan Li, Nishant Agrawal, and Carole Fakhry

Head and neck squamous cell carcinoma (HNSCC) represents the sixth most common solid tumour malignancy with more than 500,000 cases worldwide and 52,000 newly diagnosed cases annually [1]. These tumours arise from the mucosa of the upper aerodigestive tract, which holds important functional roles for respiration, speech and swallowing. Both surgical and non-surgical treatment modalities achieve a cure for ~50 % of HNSCC patients at 5 years; [2] however, these same treatment regimens can also be debilitating, both functionally and cosmetically. An understanding of the anatomical origins of HNSCC tumours is paramount to making an appropriate diagnosis, for treatment and for post-treatment tumour surveillance in all cases.

HNSCCs of the upper aerodigestive tract originate within the boundaries of the following anatomical sites: the nasopharynx, oral cavity, oropharynx, hypopharynx and larynx. Carcinomas of the nasal cavity and paranasal sinuses are not described in this chapter. Whereas other histological types of malignancies also occur in these regions, as well as in salivary glands, thyroid, and skin of the head and neck, the focus of this book is on squamous cell carcinomas, which comprise a majority of head and neck cancers. Here we delineate the boundaries of the nasopharynx, oral cavity, oropharynx, hypopharynx and larynx, as well as the subsite divisions within each of these regions [3]. Appreciation of the anatomical boundaries is critical in identification of the site of origin, as misclassification has serious implications on prognosis, choice of treatment modality and survival.

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Nasopharynx

The anterior border of the nasopharynx is the posterior choanae, or posterior margin of the nasal cavity. The lateral walls of the nasopharynx include the torus tubarius opening of the Eustachian tubes and the fossae of Rosenmuller. The nasopharynx inferiorly is continuous with the oropharynx, and the division between these two anatomical spaces is a horizontal plane drawn through the soft palate. The superior surface of the soft palate comprises the floor of the nasopharynx.

The remaining sites of HNSCC are described below, with additional subsites outlined.

Oral Cavity

The anterior border of the oral cavity is defined by the intersection of the red lip (vermillion) and skin. The oral cavity site is bounded laterally by the cheek (buccal) mucosa. The posterior limit of the oral cavity is the intersection of the hard and soft palates superiorly, and the V-shaped line of circumvallate papillae of the tongue inferiorly.

The eight subsites of the oral cavity are potential sites for the origin of HNSCC tumours and include the following: (i) the upper and lower vermillion lips; (ii) the buccal mucosa, which includes both cheek and inner lip mucosal linings; (iii) the lower alveolar ridge, which includes the mucosa lining the lateral and medial surfaces of the mandibular arch ending posteriorly at the mandibular ramus; (iv) the upper alveolar ridge, which includes the mucosa lining the lateral and medial surfaces of the maxillary arch; (v) the retromolar trigone mucosa, which lines the ascending mandibular ramus posterior to the third molar; (vi) the floor of mouth mucosa overlying the mylohyoid and hyoglossal muscles and also lining the undersurface of the tongue; (vii) the hard palate mucosa; and (viii) the oral tongue, which includes all portions of the tongue anterior to the circumvallate papillae. Understanding the oral cavity subsite boundaries is critical when assigning a tumour to an oral cavity or oropharyngeal origin, as the posterior limit of many oral cavity subsites abuts an oropharyngeal subsite. However, there are occasions when the true site of origin is not clinically discernible.

Oropharynx

The anterior border of the oropharynx is the intersection of the soft palate and the hard palate superiorly, and the circumvallate papillae inferiorly. Superiorly, a horizontal plane drawn through the posterior soft palate divides the oropharynx from the nasopharynx cephalad. A horizontal plane through the hyoid bone divides the oropharynx from the hypopharynx inferiorly.

The seven primary subsites of the oropharynx include: (i) the palatine tonsils; (ii) the base of tongue, which includes the posterior third of the tongue behind the

circumvallate papillae; (iii) the oral surface of the soft palate and uvula; (iv) the posterior pharyngeal wall; (v) the lateral pharyngeal walls; (vi) the mucosa of the anterior and posterior tonsillar pillars; and (vii) the glossotonsillar sulcus. Human papillomavirus (HPV)-related HNSCC tumours of the oropharynx originate most commonly in the lymphoid tissue of the palatine and lingual (base of tongue) tonsils.

Tumours of the oropharynx may extend into adjacent sites and subsequently be misclassified.

Hypopharynx

The superior border of the hypopharynx is the same horizontal plane through the hyoid bone that delineates the inferior limit of the oropharynx. A horizontal plane through the inferior border of the cricoid cartilage delineates the lower limit of the hypopharynx.

Subsites of the hypopharynx include: (i) the pyriform sinuses; (ii) the lateral pharyngeal walls; (iii) the posterior pharyngeal wall; and (iv) the postcricoid region.

Larynx

The larynx is a complex structure that can be divided broadly into supraglottic, glottic and subglottic regions. The supraglottic larynx extends from the lingual surface of the epiglottis superiorly, to the laryngeal ventricle just above the true vocal folds inferiorly. The junction of the base of tongue and lingual surface of epiglottis is the vallecula. Tumours of the base of tongue may invade this region and beyond into the supraglottic larynx, again potentially rendering anatomical classification of tumour origins inaccurate.

The subsites of the supraglottis include: (i) the suprahyoid epiglottis (superior to a horizontal plane through the hyoid bone); (ii) the infrahyoid epiglottis; (iii) the laryngeal surfaces of the aryepiglottic folds; (iv) the arytenoids; and (v) the false vocal folds and ventricles.

The subsites of the glottic larynx include: (i) the anterior commissure; (ii) the posterior commissure; and (iii) the true vocal folds.

The subglottis is the laryngeal space just below the true vocal folds, with a horizontal plane through the inferior border of the cricoid cartilage as the lower limit.

Role of Tobacco and Alcohol in Head and Neck Cancer

Tobacco and alcohol usage have been largely recognized as the major risk factors for HNSCC of the oral cavity, oropharynx, hypopharynx and larynx [4]. Recent estimates of tobacco usage by the Centers for Disease Control (CDC) report that 45.3 million adults in the USA are currently cigarette smokers, comprising 19.3 %

of the adult population [5]. From 2005 to 2010—the most recent census period examined—there was a decline in the prevalence of smokers that equated to approximately 3 million fewer smokers; however, among active smokers a clear trend towards fewer cigarettes smoked daily has not been apparent [5]. State and federal smoking cessation initiatives are striving actively to meet a goal of <12 % smoking prevalence in adults by 2020 [6].

The incidence of HNSCC has declined significantly over the past several decades, as the prevalence of tobacco smoking has decreased. In 1965, 42.4 % of adults in the USA were smoking tobacco; as of 2010, this number has been more than halved [5, 7]. As explained in the remainder of this book, the declining incidence of HNSCC has not followed a predictably direct correlation with decreased tobacco smoking, largely because of the emergence of high-risk HPV infections in the pathogenesis of cancers in certain head and neck subsites [8]. Nevertheless, with over 45 million adults in the USA continuing to smoke, the burden of tobacco-related HNSCC continues to be a major problem.

The role of tobacco and alcohol in the pathogenesis of HNSCC has been studied extensively from an epidemiological perspective, with a challenging task of accurately describing the independent effects of these two exposures. First, the habits of tobacco and alcohol usage frequently co-vary [9]. Second, quantification of usage amounts is often obtained in a retrospective fashion, which is inherently biased. The abstraction of the medical records varies markedly in the level of detail of reported alcohol and tobacco exposure. It has become clear that the presence of both risk factors—alcohol and tobacco—is multiplicative for the risk of HNSCC rather than simply additive in nature [9–12].

A 1988 case–control study of oral cavity and oropharyngeal HNSCC patients published in *Cancer Research* illustrated the classic association between tobacco and alcohol usage and HNSCC risk [9]. The case group in this comparison (oral cavity and oropharyngeal HNSCC patients) and the control group (non-HNSCC patients of similar age, gender and race) each comprised over 1,000 patients surveyed in four cities in the USA. Cigarette, pipe and cigar use was associated with an increased odds ratio (OR) of developing cancer, after adjusting for concomitant alcohol usage, age and race. More specifically, the risk increased with longer duration of tobacco usage and increasing number of cigarettes smoked. The increased ORs were highest in smokers of cigarettes for age >40 years in men (3.6; 95 % CI 2.3–5.6), and in women who smoked more than 40 cigarettes daily (6.2; 95 % CI 3.6–11.3). Of note, users of smokeless tobacco were frequently also smokers, making it impossible to separate the risks associated with smoking and smokeless forms of tobacco. Nevertheless, the historical data associating smokeless tobacco with oral cancer risk is unequivocal, with attributable risks ranging from 6.6 to 68 % worldwide, depending upon the local prevalence of smokeless tobacco usage [13–16]. Importantly, cessation of smoking for ≥10 years decreased the risk of HNSCC to that of a lifelong never-smoker, a powerful finding in promoting public anti-tobacco policy.

The relationship between alcohol usage and risk of HNSCC of the oral cavity and oropharynx was less dramatic than that with tobacco. [9] After adjusting for tobacco

use, moderate levels of alcohol intake (1–14 drinks per week) did not impose a significantly increased risk of cancer. The risk of cancer associated with alcohol became apparent only at higher amounts. Consuming greater than two drinks daily increased the OR to 3.3 (95 % CI 2.0–5.4). When hard liquor, beer and wine intake were studied separately—large amounts of hard liquor and beer consumption appeared to increase the risk of cancer to a greater degree than wine, although fewer patients with high wine consumption were available for this analysis [9].

Perhaps the most telling evidence from this study was the multiplicative effect of concomitant tobacco and alcohol consumption on HNSCC risk, a finding consistent in subsequent studies, which emphasizes a synergistic carcinogenic effect [9–12]. Men and women consuming more than four drinks daily and smoking >40 cigarettes daily for 20 years had an ~38-fold (OR 37.7) and >100-fold increase in odds of HNSCC, respectively (OR 107.9; 95 % CI not reported). From these data, an estimated 74 % of oral cavity and oropharyngeal HNSCC cases were attributable to tobacco and alcohol consumption [9].

Studies done in more recent years have sought to elucidate the independent effects of tobacco and alcohol consumption with greater power, with site-specific analysis of cancer cases of the oral cavity, oropharynx, larynx and hypopharynx. Hashibe et al., in 2007, performed a pooled-data analysis of 15 case–control studies comprising HNSCC patients and primarily age-, sex- and race-matched controls with detailed data on smoking and alcohol consumption habits [17]. Never-users of tobacco among drinkers ($n=1{,}072$) in HNSCC patients and controls ($n=5{,}575$) were identified, with never-users of tobacco strictly defined as lifelong abstainers from any tobacco products. Likewise, tobacco users with no history of alcohol consumption were identified in HNSCC cases ($n=1{,}598$) and controls ($n=4{,}051$). Never-drinkers who reported any cigarette smoking history carried a two-fold increase in odds of HNSCC (OR 2.13; 95 % CI 1.52–2.98) when compared to never-drinkers who were also never-smokers. A dose–response increase in odds of HNSCC was observed when evaluating frequency, duration and intensity of cigarette smoking. The risk for laryngeal site HNSCC among never-drinkers was high even among lower frequency smokers (1–10 cigarettes per day; OR 5.72; 95 % CI 3.41–9.60), and it increased in a dose–response fashion with a greater frequency and duration of smoking. By contrast, in the oral cavity only the smokers of longest duration among never-drinkers had an increased risk of oral cavity HNSCC (>31 years of smoking; OR 2.28; 95 % CI 1.19–4.37). From these data the authors asserted that 24 % of the risk of HNSCC in never-drinkers was attributable to tobacco consumption [17].

As described earlier, the independent risk of HNSCC attributable to alcohol consumption has been less clear. In the same Hashibe et al. 2007 study, among never-users of tobacco with any alcohol consumption history the odds of HNSCC were only increased in individuals reporting ≥3 drinks per day (OR 1.82; 95 % CI 1.10–2.99), or >50 drink-years, a measure of cumulative drinking [17]. Interestingly, a small increased risk of HNSCC of the oral cavity was evident in never-smoking drinkers of one or more drinks daily with a dose–response increase in risk with increasing exposure (p-trend = 0.032), whereas such risk was present only for

laryngeal HNSCC when ≥5 daily drinks were consumed (OR 3; 95 % CI 1.7–5.2). It could be theorized that oral cavity and pharyngeal mucosal linings sustain more prolonged exposure to alcohol compared with the larynx for the same level of consumption, although this is unproven. In this pooled analysis the attributable risk of HNSCC due to alcohol consumption in never-users of tobacco was only 7 %, again supporting a weak role of isolated alcohol consumption in the development of HNSCC [17].

The multiplicative effect of tobacco and alcohol exposure has remained consistent in the study of HNSCC risk factors, whereas only a weak HNSCC risk is associated with alcohol consumption alone [18]. Some studies suggest that alcohol increases the solvency of tobacco compounds, potentiating their carcinogenic effects [19]. Counselling HNSCC patients on healthy living must address the frequently concurrent habits of tobacco and alcohol consumption. Furthermore, treatment outcomes data stress cessation of tobacco and alcohol consumption in patients newly diagnosed with HNSCC. For example, in HNSCC patients receiving either primary or adjuvant radiation therapy, Fortin et al. found tumour 5-year local control rates to be superior in former smokers relative to active smokers (80 % vs. 67 %, respectively; $p<0.01$) [20]. Likewise, active smokers and drinkers showed poorer local control rates compared with non-smoking and non-drinking counterparts. The term 'former smokers' included patients who smoked up until the initiation of radiation therapy. It is possible that comparison of former smokers with a longer period of cessation to active smokers would have shown an even greater disease-free survival advantage associated with tobacco and alcohol cessation.

Similarly, some studies have shown the increased risk of second primary tumours, cancer progression and death in patients who continue to smoke during the course of radiation therapy [21–24]. Gillison et al. reviewed the tobacco history of oropharyngeal HNSCC patients enrolled in two organ-preservation trials, comparing treatment outcomes after various combined chemoradiation regimens [24]. Both higher locoregional failure rates and reduced overall survival were observed in patients with >10 pack-years of smoking, compared with those with ≤10 pack-years—independent of HPV tumour status. Additionally, smoking during radiation therapy decreased both progression-free and overall survival [24].

The Role of High-Risk HPV Infection in Non-oropharyngeal Head and Neck Cancers

HPV infections hold an established role in the pathogenesis of many anogenital cancers and an emerging role in HNSCC in the past two decades [25]. Clinically, HPV is detected by *in situ* hybridization (ISH) of high-risk types or p16 immunohistochemistry used as a surrogate marker for HPV. HPV-related HNSCCs primarily originate in the oropharynx, most commonly in the palatine and lingual tonsil subsites in which an estimated 25–70 % of cases are HPV-related [25–29]. The role of HPV in HNSCC of non-oropharyngeal sites is controversial and probably limited. Studies examining the prevalence of HPV infection in various HNSCC sites

have reported a three to 10-fold higher rate in oropharyngeal primaries compared with other head and neck sites [25, 27, 30–32].

Reporting the prevalence of HPV in HNSCC of various sites requires accurate classification of the primary tumour's anatomical origin. This may prove difficult in some cases, for which the original primary site classification may no longer be verifiable. Another possible explanation for some of the evident discrepancy in the prevalence of HPV in HNSCC is that studies may use detection techniques, which have different sensitivity and specificity to the clinical tests used to detect HPV, and are vulnerable to contamination. In a single-institution retrospective study, 259 HNSCC tumour samples were tested for the presence of HPV DNA using a combination of polymerase chain reaction (PCR) for the L1 HPV genomic region followed by HPV type-specific probes and Southern blot hybridization, PCR probes for E6 and E7 HPV genomic DNA sequences, and ISH for the localization of HPV DNA [24]. These methods are discussed in more depth in subsequent chapters. The investigators found 57 % (34 of 60) of oropharyngeal primary tumours positive, and a much lower proportion of oral cavity (12 %, 10 of 84), hypopharyngeal (10 %, 2 of 21), laryngeal (19 %, 16 of 86), and nasopharyngeal (0 %, 0 of 2) tumours with detectable HPV [25]. A subsequent large multinational study retrospectively assessed head and neck tumours for the presence of HPV DNA in oral and oropharyngeal cancers [32]. DNA extracted from tumour samples was applied to an HPV-specific PCR reaction. Subsequent PCR products were analysed by enzyme immunoassay and Southern blot hybridization, using HPV type-specific probes. In this analysis 18.3 % (26/142) of oropharyngeal primaries and 3.9 % (30/766) of oral cavity primaries were found to have detectable HPV DNA in biopsy specimens. After adjusting for sex, age, tobacco and alcohol consumption, regression modelling for the risk of HPV detection in oropharyngeal tumour samples versus oral cavity samples showed an OR of 4.9 (95 % CI 2.6–9.1). Whereas these and the prior studies clearly show that HPV-related HNSCC occurs predominantly in the lymphoid tissue of the oropharynx, the small percentages of cases that occur in non-oropharyngeal sites, such as the oral cavity may actually be correctly classified. Alternatively, tumours located in anatomical subsites adjacent to the oropharynx may actually represent extension of tonsillar or base of tongue HNSCC into non-oropharyngeal sites—for example, the supraglottic larynx and oral tongue. If misclassified as laryngeal or oral cavity primaries respectively, positive HPV DNA testing will be interpreted as non-oropharyngeal HPV-related HNSCC.

Alternatively, the presence of HPV may not always suggest a causal role for the virus. In fact, the detection threshold of current HPV DNA testing protocols may on occasion be overly sensitive to the presence of small and (possibly) trivial quantities of HPV DNA that may not be oncogenic [33]. In this situation detection of HPV DNA in non-oropharyngeal samples represents a biologically irrelevant finding.

To further examine the role of HPV in oral cavity HNSCC, Lingen et al. studied a large cohort comprising 409 oral cavity HNSCC tumour samples to describe the prevalence of oncologically relevant HPV infections in this subgroup of HNSCC [34]. Whereas HPV DNA testing can confirm the presence of HPV genomic DNA within tumour samples, assaying for the expression of HPV oncogenes E6 and E7

(E6/E7) provides more definitive evidence that an HPV infection is contributing to tumorigenesis. Additionally, ISH can test for HPV DNA localized to tumour cell nuclei, a prerequisite for expression of HPV oncogenes E6/E7. All tumour samples in this study underwent immunohistochemical analysis for p16 overexpression, a surrogate marker of E7 protein activity that has high sensitivity for detecting HPV-associated tumours, but lower specificity. This was followed by ISH testing [34]. Purified tumour RNA was then assayed by quantitative real-time PCR (qrtPCR) for HPV E6/E7 expression.

Approximately 11 % (46 of 409) of tumours were p16-positive. Approximately 6 % (24 of 409) of oral cavity HNSCC samples were positive for E6/E7 expression, and 20 of 24 of these had positive ISH testing, confirming localization of oncogenic DNA to tumour cell nuclei with subsequent expression of HPV oncogenic proteins. As in HPV-related oropharyngeal HNSCC cases, these cases were more likely to occur in men with earlier tumour T stage, with poorer histopathological differentiation, and more frequently basaloid histological appearance [34]. These distinct epidemiological and clinicopathological characteristics of HPV-related HNSCC cases are examined later in this book. Of the 24 HPV-related oral cavity HNSCC cases in the Lingen et al. study, subsite classification included 16.7 % (4 of 24) tumours originating on the dental alveolar process, 12.5 % (3 of 24) on the hard palate, 25 % (6 of 24) on the oral tongue, 4.2 % (1 of 24) on the lip, 37.5 % (9 of 24) on the floor of mouth, and 4.2 % (1 of 24) on the gingiva [33]. The authors emphasize the likelihood is low that the majority of these tumours represented misclassification of oropharyngeal primaries invading oral cavity subsites, as many of these tumours were classified in oral cavity subsites remote from the base of tongue and palatine tonsils. If the results of the Lingen et al. 2012 study are extrapolated to population-based HPV rates in oral cavity HNSCC, even this small proportion (6 %) of oral cavity HNSCC patients may represent a large number of cases that are similar to HPV-related oropharyngeal HNSCC epidemiologically and clinically [34–36].

Another controversial topic relates to the role of HPV in nasopharyngeal carcinomas (NPC). Histologically, individual NPC cases are classified into one of three World Health Organization (WHO) types: I—keratinizing squamous cell carcinoma; II—non-keratinizing; and III—non-keratinizing undifferentiated [37]. The Epstein–Barr virus (EBV) is recognized as the primary environmental exposure associated with endemic NPC of the WHO II and III types, particularly in the Asian population. The question arose regarding a potential role for HPV in select NPC cases, particularly of the WHO I type that are not frequently associated with EBV infection. Type I NPC comprises ~25 % of NPC cases in North America, whereas EBV-associated type III NPC comprises approximately 95 % of NPC cases in endemic southern China [37]. In the USA, Lo et al. studied 183 NPC cases for prevalence of oncologically relevant HPV infection in 2010 [38]. Eighteen of 183 cases were WHO type I. All tumour samples underwent DNA extraction, followed by PCR for amplification and detection of E6 and E7 HPV oncogenic proteins. ISH was also performed for both HPV and EBV intracellular localization. Finally, p16 immunohistochemistry was performed on tumour tissue samples. Specimens were available for HPV testing in eight of the WHO type I NPC cases,

and 22 of the WHO types II/III cases. By p16 immunohistochemistry, 87.5 % (7 of 8) of WHO type I cases were positive, and 90.0 % (18 of 20) of WHO types II/III cases were positive, an insignificant difference. By ISH, 50 % (4 of 8) of WHO type I and 5 % (1 of 20) WHO type II/III cases were positive for nuclear localization of HPV. Conversely, 25 % (2 of 8) of the WHO type I and 60 % (12/20) of the WHO type II/III cases were EBV positive by ISH. Possibly because of the small power of this study, the difference in EBV ISH positivity was not significant ($p = 0.21$), whereas HPV ISH positivity occurred more frequently in the WHO type I group ($p = 0.015$) [38]. To date, no studies have been conducted that have assembled larger cohorts of NPC cases for HPV analysis, to provide more conclusive evidence of an association. A recent study of 45 non-keratinizing NPC cases suggested that presumed HPV-associated NPC cases might represent oropharyngeal primary tumours with extension into the adjacent nasopharyngeal site [39]. Whereas 76 % (34 of 45) of cases were EBV ISH positive, only 9 % (4 of 45) were HPV positive by ISH. A review of the staging information from three of the HPV-positive tumours confirmed that these involved both the oropharyngeal and nasopharyngeal sites. Multi-institutional collaboration should be able to assemble larger cohorts of rare NPC cases in North America for further elucidation of an HPV role.

Although HNSCC has significant morbidity and mortality, it is associated with modifiable risk factors. Given the major risks factors of tobacco, alcohol and HPV in the tumorigenesis of HNSCC, concurrent efforts to reduce tobacco and alcohol consumption with current HPV vaccination may help reduce the future burden of HNSCC.

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Epidemiology of Oral HPV Infection and HPV-Associated Head and Neck Cancer

2

Kristina R. Dahlstrom and Erich M. Sturgis

Introduction

Tobacco and alcohol exposure are the traditional risk factors for malignancies of the head and neck, and account for approximately three-quarters of all cases worldwide and half of the cases in the USA [1]. Infection with human papillomavirus (HPV), particularly type 16, has also been established as yet another important risk factor [2–5]. The overwhelming majority of HPV-associated head and neck cancers (HPV-HNSCCs) arise from the oropharynx [2, 6]. HPV-positive oropharyngeal cancer (OPC) represents a growing aetiologically distinct subset of head and neck cancers, with unique epidemiological, clinical and molecular characteristics that differ from those of HPV-negative cancers.

HPV-positive tumours generally arise in the oropharynx, most commonly from the tonsils and base of tongue [7, 8]. HPV has also been associated with tumours at other head and neck sites, but to a lesser extent [7, 8]. Of an estimated 85,000 cases of OPC that occurred worldwide in 2008, around 22,000 were likely attributed to HPV infection [9]. The overall population-attributable fraction (PAF) was 25.6 %, in which the PAF refers to the proportion of cases of OPC that could theoretically have been prevented in the absence of oral HPV infections [9]. Higher fractions were observed in more developed regions and among men [9].

While the overall incidence of head and neck cancer has been decreasing over the past several decades, the incidence of OPC has been increasing both in the USA and elsewhere [10–15]. The decrease for most head and neck sites is likely due to a decrease in tobacco use [16], whereas the increase for oropharyngeal sites corresponds to an increase in HPV in recent decades [7, 17–20], thought to be a result of changing sexual practices [21].

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Epidemiology of Oral HPV Infection

Prevalence

HPV is the most common sexually transmitted infection worldwide [22, 23], although the prevalence of oral HPV infection is considerably lower than the prevalence of genital HPV infection [24–26]. National estimates have found prevalence rates of 43 % for cervical HPV infections and 23 % for infection with high-risk HPV types [27, 28]. Oral HPV prevalence is generally less than 10 %; [24, 29] although some—such as older age groups, heavy smokers and drinkers, and individuals with high number of oral sex partners—have prevalence estimates above 10 % [24].

Mucosal HPV types are categorized as either low-risk or high-risk based on their carcinogenic potential of cervical HPV infections. Some low-risk HPV types have strong associations with benign anogenital lesions or warts, whereas others have no apparent clinical manifestations. High-risk HPV types have strong epidemiological association with anogenital and oral malignant lesions and are therefore thought to cause malignant transformation of squamous epithelial cells. Studies have consistently shown that HPV16 is the type most often associated with OPC [24, 26, 30–34].

Several lines of evidence have established the oncogenic potential of HPV in HNSCC, and since the early 1990s, HPV DNA has been consistently identified in many head and neck cancers [2, 3, 5, 35–37]. A systematic review and a meta-analysis have confirmed that more than 90 % of HPV-HNSCC involve HPV16 [7, 38]. Considerable heterogeneity has been observed between geographical regions with respect to HPV prevalence in head and neck tumours, although in all regions the oropharynx is the most common HNSCC site associated with HPV [7]. Among series from the 1970s and 1980s, ~47 % (95 % CI 41–53 %) of oropharynx tumours in North America were positive for HPV vs. 28.2 % (95 % CI 24–32 %) in Europe [7]. Although prevalence estimates in Asia were generally high (overall 46 %; 95 % CI 33–60 %), these studies may suffer from selection bias due to certain cases being preferentially included, poor sample quality, and small sample sizes that may have led to an overestimation of prevalence rates [7]. North American and European cohorts composed of patients chiefly from the 1990s revealed HPV prevalence rates in oropharynx tumours of ~50 %, whereas more recent reports revealed prevalence rates in the 70–80 % range (Fig. 2.1) [3, 7, 12, 19, 38–40].

Genital HPV infections are common; however, the proportion of healthy individuals with an oral HPV infection is much smaller. Approximately 7 % of the population of the USA is infected with oral HPV, with estimates lower in developing countries [24, 29, 41]. In a systematic review of the literature, Kreimer et al. found an overall global prevalence of oral HPV infection in healthy individuals of 4.5 % (95 % CI 3.9–5.1 %) [29]. The prevalence of oral infection with carcinogenic HPV types was 3.5 % (95 % CI 3.0–4.1 %), whereas the prevalence of oral HPV16 infection was 1.3 % (95 % CI 1.0–1.7 %), with HPV16 accounting for

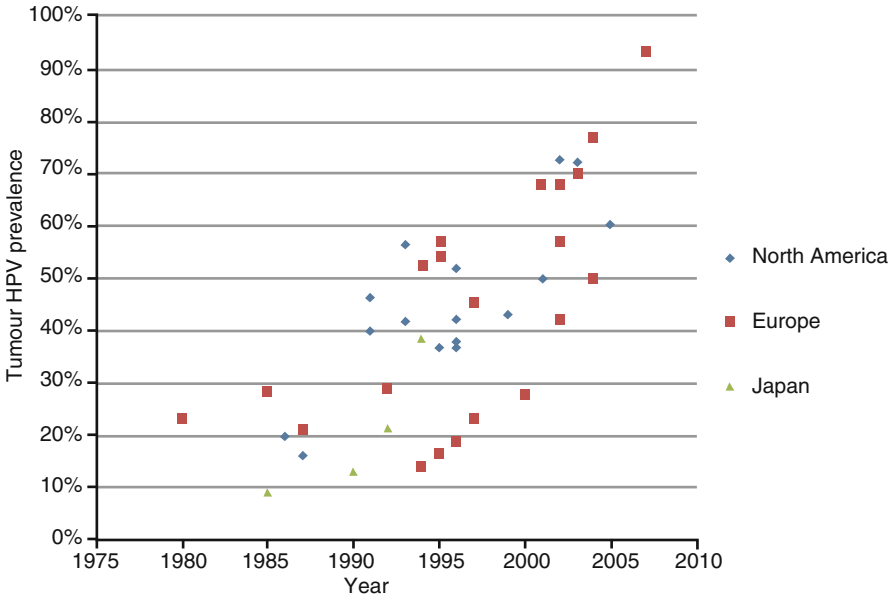


Fig. 2.1 Summary of the prevalence of HPV positivity in oropharyngeal cancer cases reported in the literature by year of case diagnosis and geographical region. Findings are graphed by the median year of case enrollment in each study and restricted to studies that enrolled cases within ≤11 years or reported HPV prevalence within calendar-year strata of ≤10 years. Studies referenced in the figure include cases diagnosed during the 1970s [20], 1980s [20, 114, 135–137], 1990s [2, 20, 37, 79, 82, 138–148] 2001–2003 [20, 89, 149–155], and 2004–2009 [12, 26, 81, 114, 147, 156] (Adapted from D'Souza and Dempsey [157])

33 % of all high-risk infections [29]. Additionally, the prevalence of oral infection with any HPV type was higher among populations from developing nations than among populations from developed nations (7.3 %; 95 % CI 5.6–9.3 % vs. 3.6 %; 95 % CI 3.0–4.2 %), as was the prevalence of oral infection with HPV16 (4.3 %; 95 % CI 2.9–6.3 % vs. 0.7 %; 95 % CI 0.5–1.1 %) [29]. In a nested study within the multinational HPV in Men (HIM) study that included 1,680 males from Brazil, Mexico and USA, the prevalence of oral infection with any carcinogenic type of HPV among all study subjects was 1.3 %, whereas the prevalence of oral infection with HPV16 was 0.6 % [41]. Among men from the USA, the prevalence of oral infection with an oncogenic HPV type was 1.6 %, and the prevalence of oral infection with HPV16 was 0.7 % [41]. A cross-sectional study from the National Health and Nutrition Examination Survey 2009–2010 estimated the prevalence of any oral HPV infection to be 6.9 % (95 % CI 5.7–8.3 %) among healthy individuals aged 14–69 years in the USA, with the prevalence almost three times as high among men as among women (unadjusted prevalence ratio [PR], 2.80; 95 % CI 2.02–3.88) [24]. HPV16, the most common type found, was present in 1 % (95 % CI 0.7–1.3 %) of individuals and was more common among men than women (PR 5.4; 95 % CI 2.1–13.8).

Risk Factors

Risk factors for oral HPV incidence and persistence are not yet well understood, but several studies have explored risk factors for prevalent oral HPV. The most important risk factors for oral HPV appear to be increasing age, sexual behaviours, male sex, current cigarette use, and host immunosuppression.

Among adults, oral HPV prevalence increases with age [24, 25, 41, 42]. In the HIM study, the lowest prevalence (3.2 %) was among those aged 18–24 years and the highest prevalence (6.1 %) was among those aged 55–74 years [41]. In a population of 1,000 students from the Ohio State University aged 18–30 years, the prevalence of oral HPV infection was 2.4 % (95 % CI 1.4–3.4 %) [42]. Moreover, a bimodal pattern has been observed in the largest population-based study to date with respect to age: individuals in the age groups of 30–34 years and 60–64 years had the highest prevalence rates (PR 7.3 %; 95 % CI 4.6–11.4 % and 11.4 %; 95 % CI 8.5–15.1 %, respectively) [24]. It is currently unclear whether higher prevalence rates in older age groups are due to incident infections or to persistent infections caused by waning immunity in these age groups.

Sexual behaviours, including oral–genital, oral–oral and oral–anal contact, have all been associated with oral HPV infection in cross-sectional studies [24, 31, 34, 42]. In particular, risk for a prevalent oral HPV infection is increased among individuals who have ever had oral sex and is associated with increasing numbers of lifetime and recent oral sex partners. Gillison et al. observed significant trends for increasing prevalence of oral HPV infection with increasing number of recent and lifetime oral, vaginal and any sex partners (p for trend <0.001 for all categories) in a population-based cross-sectional survey of NHANES data in USA [24]. Specifically, the prevalence was six times as high among individuals who had had at least 21 lifetime oral sex partners compared with those who had never had oral sex and three times as high among those with at least two oral sex partners in the past 12 months compared with those who had none (PR 6.1; 95 % CI 2.9–13.0 and 3.0; 95 % CI 1.8–5.2, respectively) [24].

These trends are consistent with other cross-sectional studies conducted in several different populations, including college-aged men in the USA, participants in the Multicenter AIDS Cohort Study, and sex workers [31, 33, 34, 42, 43]. For example, among college-aged men and young adults aged 18–30 years in the USA, the odds of having a prevalent infection increased with increasing numbers of recent oral sex partners, as well as open-mouth kissing partners [31, 42]. Likewise, among HIV-negative men who have sex with men participating in the Multicenter AIDS Cohort Study, an increasing trend was seen for oral HPV prevalence, which was in parallel with an increasing number of oral–genital and oral–anal sex partners [43]. This study found that men who reported having five or more oral–genital sex partners in the past 6 months had three times the risk of oral HPV (95 % CI 1.5–6.3; p for trend = 0.005), as did men who reported five or more oral–anal sex partners in the past 6 months (95 % CI 1.5–5.9; p for trend = 0.002) when compared with men who had no oral–genital or oral–anal sex partners in the past 6 months, respectively. Finally, among women sex workers in Peru, having oral sex three or more times per

week with a regular partner increased risk compared with having oral sex less than three times per week (p = 0.06) [43].

Tobacco use has also been found to be associated independently with oral HPV infection [24, 41]. Kreimer et al. found that the risk was almost three times as high among current users of tobacco as among non-users (OR 2.7; 95 % CI 1.5–4.7) [41]. Furthermore, a dose–response effect is apparent for intensity of tobacco use and oral HPV prevalence [24]. Smoking has been shown to have immunosuppressive effects and has been associated with higher prevalence and viral load among women with cervical HPV infection, as well as risk of progression of cervical disease [44, 45]. Smoking has also been shown to increase the persistence of oral HPV infection [26]. These associations between oral HPV infection and smoking appear to remain significant after adjustment for sexual behaviours [24]. However, effect modification may occur, and one study suggested that the association with smoking was evident among women but only marginal among men [24].

HIV-positive individuals are more likely to have a prevalent oral HPV infection than HIV-negative individuals, and HIV-positive women are more likely than HIV-negative women to have both an oral and a cervical HPV infection, suggesting that immunosuppression leads to increased susceptibility for or persistence of HPV [26, 30, 34, 46]. Interestingly, initial studies suggest that the use of highly active antiretroviral therapy (HAART) may increase clearance of oral HPV infection, which raises the concern that greater life expectancy in HIV-positive individuals successfully treated with HAART may provide a longer period during which HPV-related OPC can develop [26].

Influence of Population-Level Changes in Sexual Behaviour

Societal changes in sexual behaviour over time are a likely contributing factor to the increase in incidence of HPV-related head and neck cancer. Although longitudinal data on changes in sexual behaviour are limited, some studies suggest that certain changes in behaviour, such as decreased age at sexual debut and increased number of lifetime sex partners, have occurred. Turner et al. analysed survey data of sexual behaviours among the US population spanning the twentieth century [21]. Less than 10 % of women born before 1930 reported having sexual intercourse before age 18 years. On the other hand, ~30 % of those born during 1944–1949, and well over half of those born during 1968–1973 reported this behaviour [21]. Likewise, the number of sex partners also increased over time: less than 7 % of women and 34 % of men born before 1930 reported having five or more lifetime sex partners, whereas 23 % of women and 50 % of men born in the 1960s reported five or more lifetime sex partners [21].

More recently, in the USA, age at first sexual intercourse and number of sex partners have largely displayed slight reversal of previous trends [47–50]. In Europe and Australia, on the other hand, age at first intercourse has continued to decrease, with a concomitant increase in lifetime number of sex partners [51–54]. The most recent data from the USA suggest that whereas the prevalence of having had sex before age

13 years and of having had four or more sex partners was decreasing among adolescents since 1991, this trend has levelled off in the past several years [55]. Currently, almost half of high school students report ever having had sexual intercourse, and 15 % report having had four or more sex partners [55]. The current epidemic of HPV-positive OPC may be a reflection of sexual behaviours among individuals born in the 1960s and 1970s and indicates a latency period of several decades to develop an HPV-related malignancy. Unfortunately, temporal data on oral sexual behaviours are lacking; however, sexual behaviours tend to be highly correlated.

Transmission of Oral Human Papillomavirus

Transmissibility of HPV to the genital region is high, and transmission generally occurs early on in a sexual partnership; [56–58] however, the transmissibility of HPV to the oral cavity is unclear. The oral cavity has not been included in most HPV studies to date, and generally, they have had either small sample sizes or included couples with long-term relationships [59, 60]. Although the prevalence of oral HPV infection is less than that of genital HPV infection, studies are needed to clarify whether this is because of reduced exposure and/or greater resistance of the oral cavity and pharynx to infection with HPV. Alternatively, the difference may be because of inadequacy of current assays of HPV infection in the oral cavity and oropharyngeal region, where the ultimate sites of malignancy (tonsils and base of tongue) are not easily accessible for direct testing for HPV; much of the current prevalence data relies on more proximal sampling in the mouth (oral cavity), or rather limited/superficial testing of the oropharynx.

Women with a cervical HPV infection are more likely to have an oral HPV infection, although not necessarily of the same type [29, 42, 45, 61]. Fakhry et al. found that among women with simultaneous cervical and oral HPV infections, agreement in the type of HPV at both sites was greater than would have been expected by chance ($p < 0.001$) [30]. This might suggest that these infections are not completely independent of each other, or could reflect separate oral and genital HPV infection from the infected genitals of a partner. In another study, the majority of women with oral HPV infection were HPV-positive at the cervix (71 %), but less than 10 % of women with cervical HPV infection also had an oral HPV infection [42].

Widdice et al. found low type-specific concordance between genital and oral sites among couples; however, their study population consisted of only 25 couples [59]. Kero et al. also found low concordance between partners in the Finnish Family HPV study, but their study was limited in that it included only women in their third trimester of pregnancy and their spouses [60].

The main mode of oral HPV transmission appears to be through sexual contact, although vertical transmission from mother to child may also occur. The results from studies reporting vertical transmission rates are conflicting; some studies suggest that vertical transmission is uncommon (<5 %) [62–65], whereas others suggest that it is common (>20 %) [66–68]. Although the prevalence of oral HPV infection in newborns is low, maternal HPV positivity remains a significant risk

factor for infant HPV infection; however, type-specific concordance is generally low [62, 66, 69]. Whereas HPV DNA has been detected in the oral cavity of newborns, the mode of transmission in such cases is currently unclear. The main mode appears to be through an infected birth canal; however, HPV DNA has been detected in amniotic fluid, cord blood and placental tissue, suggesting that *in utero* transmission may be possible [67, 69–71]. It is well documented that laryngeal papillomatosis (papillomas of infants arising in the larynx and attributable to infection with HPV6 and HPV11) occurs most commonly in infants whose mothers have genital papillomas [72–74]. Furthermore, planned caesarean section for pregnant women with known genital warts reduces the risk of HPV transmission to their babies [75].

Oral Human Papillomavirus—Natural History

The natural history of oral HPV infections is not clearly understood and few prospective studies have been conducted. In a 6-month longitudinal study of 136 HIV-positive and 63 HIV-negative women, the cumulative prevalence of oral HPV infection was 33 % among the HIV-positive and 15.3 % among the HIV-negative women [26]. An infection was considered persistent if it was detected at both the baseline and the 6-month follow-up visit. Among the HIV-positive women, 55 % of infections persisted whereas among the HIV-negative women, 60 % of infections were persistent [25]. Furthermore, oral HPV infections were as likely to persist as cervical infections among both groups. Risk factors for persistent oral HPV infections included the following: current smoking (OR 8.0; 95 % CI 1.3–53), age above 45 years (OR 20; 95 % CI 4.1–83), CD4 cell count <500 cells/μl (OR 6; 95 % CI 1.1–26), and current use of HAART therapy (OR 12; 95 % CI 1.0–156) [26]. In the Finnish Family HPV Study, a prospective cohort study of 329 families followed for 6 years, 17 % of pregnant women had a prevalent oral HPV infection at baseline [76]. HPV16 was the most prevalent type detected, with 10.5 % of women being positive at baseline. Persistent infections were detected in 74 (21 %) women. The most common types to persist were HPV6 and HPV16, with 56 women having a persistent HPV16 infection with a mean duration of 18.6 months (range 2.1–81.2 months) [76]. Ever having used oral contraceptives and a second pregnancy during the 6-year follow-up period were protective factors for persistent infections; however, it is unknown whether these effects are because of immunological changes in the women or are surrogate markers for low-risk sexual behaviours, including few sexual partners [76].

Epidemiology of HPV-Related Head and Neck Cancer

Role of Human Papillomavirus

Ample evidence exists that HPV16 is associated with an increased risk of OPC; ORs from case–control studies range from 3.6 to 230 [2–5, 35, 37, 77–85]. An early landmark nested case–control study by Mork et al. found that serologically

HPV16-positive patients had more than 14 times the risk of OPC and almost three times the risk of tongue cancer compared to serologically HPV16-negative patients (OR 14.4; 95 % CI 3.6–58.1 and OR 2.8; 95 % CI 1.2–6.6) [35]. Likewise, Smith et al. found that positivity for HPV16 E6 and E7 antibodies was associated with 73 times the risk of an oropharyngeal tumour compared with seronegativity for these antibodies (OR 72.8; 95 % CI 16.0–330) [77]. In a matched case–control study that included 120 patients and 120 controls, OPC cases were 60 times as likely as cancer-free controls to be seropositive for HPV16; 59 % of OPC patients and only 9 % of the cancer-free controls were seropositive for HPV16 [78]. In a nested case–control study that included 100 patients with OPC and 200 cancer-free controls, the presence of an oral HPV16 infection was associated significantly with OPC (OR 14.6; 95 % CI 6.3–36.6) [3]. Whereas 32 % of cases were positive for an oral HPV16 infection, only 4 % of controls were positive. Furthermore, seropositivity for the HPV16 L1 capsid protein was associated with a 32-fold increased risk of OPC compared with seronegativity (OR 32.2; 95 % CI 14.6–71.3) [3]. An International Agency for Research on Cancer multicentre study that included 1,670 patients with oral cavity and OPC and 1,732 control subjects found that the presence of HPV16 E6 or E7 antibodies was associated with an increased risk of both oral cavity and OPC (OR 2.9; 95 % CI 1.7–4.8 for oral cavity, and OR 9.2; 95 % CI 4.8–17.7 for oropharynx) [4]. Most importantly, several groups have shown within population-based registries a dramatic increase in HPV-positive OPC among archived tumour specimens over the past two decades [12, 19, 40, 86].

Risk Factors for HPV-Positive Head and Neck Squamous Cell Carcinoma

Numerous case series have established that patients with HPV-positive OPCs have unique demographic and behavioural characteristics. Patients with HPV-positive OPC tend to be middle-aged white men and of higher socioeconomic status [36, 39, 87–90]. Further, patients with HPV-positive OPC are more often non-smokers and non-drinkers than patients with HPV-negative OPC; tobacco and alcohol do not appear to be risk factors for this disease [36, 87, 90]. However, sexual behaviours do appear to be associated with this disease, and patients with HPV-positive OPC as a group have a higher number of sex partners—in particular oral sex partners—than HPV-negative patients [3, 36, 87, 90, 91]. HPV-positive OPCs tend to occur in either the tonsils or base of tongue rather than other sites of the oropharynx, such as the soft palate or posterior oropharynx, and they tend to be of non-keratinizing histologies, including basaloid, lymphoepithelial, or poorly differentiated [92]. Finally, patients with HPV-positive OPC tend to present for medical care because of nodal metastases, and the initial work-up tends to reveal a small primary tumour with multiple positive nodes (typically classified as T1-2 N2b-c) [92].

Sexual behaviour is an important risk factor for HPV-positive OPC, with increasing numbers of both lifetime sex partners and oral sex partners increasing risk for HPV-positive OPC. One study showed that among individuals with OPC, those who

had HPV DNA-positive tumours were more likely to have had multiple sex partners and practise oral sex than were those who had HPV DNA-negative tumours [4]. Among 284 patients with OPC and 477 cancer-free controls, seropositivity for HPV16 capsid antibodies was associated with a greater risk of OPC (OR 6.8; 95 % CI 3.0–15.2), and decreasing age at first sexual intercourse and increasing number of sex partners were also associated with a greater risk of OPC [5]. A study from the International Head and Neck Cancer Epidemiology consortium, which included 5,642 patients with head and neck cancer and 6,069 controls, found an increased odds of OPC with six or more lifetime sex partners (OR 1.3; 95 % CI 1.0–1.5) and four or more lifetime oral sex partners (OR 2.3; 95 % CI 1.4–3.6) [91]. This same study found an increased odds of tonsil cancer with four or more oral sex partners (OR 3.4; 95 % CI 1.3–8.5), ever having had oral sex among men (OR 1.6; 95 % CI 1.1, 2.3), and early age at sexual debut (OR 2.4; 95 % CI 1.4–5.1) [91]. Odds of base of tongue cancer was increased among men who had ever had same-sex sexual contact (OR 8.9; 95 % CI 2.1–36.8), among women who had ever had oral sex (OR 4.3; 95 % CI 1.1–17.6), and among individuals with two lifetime sex partners, compared with one partner (OR 2.0; 95 % CI 1.2–3.5) [91].

The role of tobacco and alcohol in HPV-positive OPC is still unclear; studies have shown conflicting results [3–5, 78]. When D'Souza et al. limited their case–control analysis to subjects without a history of tobacco or alcohol use, having an oral HPV16 infection was even more strongly associated with OPC (OR 43.7; 95 % CI 4.2–452.7) (as was seropositivity for HPV16 L1 protein [OR 44.8; 95 % CI 5.9–338.5]), compared with those who did not have evidence of HPV16 infection. Whereas heavy users of tobacco and alcohol had an increased risk of OPC, no evidence of synergy between HPV and tobacco and alcohol use was found [3]. The results from this study indicate that OPCs develop along two separate pathways—one resulting from the carcinogenic effects of tobacco and alcohol and another induced by HPV. This premise is supported by results from another study, which found that the risk of pharyngeal cancer among never smokers and light users of alcohol was 30 times as high among those who were HPV16 seropositive as it was among those who were HPV16 seronegative (OR 32.5; 95 % CI 13.3–79.5 for never smoking and OR 29.1; 95 % CI 11.3–74.9 for <3 alcoholic drinks per week) [93]. Whereas a dose–response relationship between tobacco and alcohol use was observed among HPV16-seronegative subjects, no such relationship was seen among HPV16-seropositive patients [93]. This is in contrast to results of a study by Smith et al., which showed a synergistic effect between alcohol and tobacco use and HPV-positive OPC [94]. These workers found an increased risk of OPC with increasing tobacco/alcohol use regardless of HPV16 serostatus (p for trend <0.001 for HPV16-seropositive subjects; $p < 0.001$ for HPV16-seronegative subjects). Another study found a greater than multiplicative interaction for smoking and HPV seropositivity (joint effects OR 8.5; 95 % CI 5.1–14.4), indicating that smoking may increase the risk of HPV-positive head and neck cancer; however, this study suffered from poor/imprecise case definition, which resulted from the inclusion of patients with non-OPC [5].

It is unclear whether having had a cervical cancer increases the risk of a subsequent HPV-related OPC. In initial studies the standardized incidence ratio (SIR)

appears to be increased, although the effects of smoking are difficult to control for; [95–97] increased risk of smoking-related cancers, including OPC, in cervical cancer patients may be explained by tobacco exposure [95, 96, 98]. Interestingly, husbands whose wife had a history of cervical cancer also appear to be at increased risk for developing tonsillar cancer (SIR > 2) and tongue cancer [99]. Furthermore, among men, the risk of a subsequent oral/pharyngeal malignancy after an anogenital cancer or a subsequent anogenital cancer after an oral/pharyngeal malignancy appears to be greater than expected [100]. This risk appears greatest for never-married men.

HIV-positive patients are at increased risk for HPV-related cancers, including OPC. In HIV/AIDS-positive men, the relative risk (RR) of tonsillar cancer was 2.6 (95 % CI 1.8–3.8) compared to the expected number of cases [101]. Chaturvedi et al. found an increased risk of HPV-positive cancer, including OPC, among persons with AIDS (SIR 1.6; 95 % CI 1.2–2.1) [102]. This risk increased with increasing levels of immunosuppression; [102] this has been confirmed in other studies (SIR 4.1; 95 % CI 2.1–7.4) [103]. Engels et al. found a SIR of 1.7 (95 % CI 1.1–2.5) for oral cavity/pharynx cancer among HIV-positive individuals, and the risk increased significantly after AIDS diagnosis (RR 3.6; 95 % CI 1.6–8.2 for AIDS vs. pre-AIDS) [104]. Although 60–80 % of HIV-positive individuals smoke, tobacco per se may not account fully for these increases in risk of OPCs among patients with HIV [104].

Trends in Incidence of Head and Neck Squamous Cell Carcinoma

In 2012, approximately 13,000 individuals in the USA were diagnosed with pharyngeal cancer, of which 2,400 died, while at the same time approximately 11,000 individuals were diagnosed with tongue cancer 2,000 died [105]. These absolute numbers are provided annually in a report from the American Cancer Society on the basis of incidence data collected by cancer registries participating in the National Cancer Institute's Surveillance, Epidemiology and End Results (SEER) programme and the Centers for Disease Control's (CDC's) National Program of Cancer Registries, representing ~89 % of the population of the USA, and on US mortality data from the CDC's National Center for Health Statistics [105]. Unfortunately, significant site admixture occurs in this annual, 'cancer statistics' overview, in which all subsites of pharyngeal cancer (not only oropharynx but also the less common hypopharynx and nasopharynges) are listed as the single site 'pharynx,' and in which base of tongue sites (lingual tonsil or oropharyngeal tongue) are often listed together with oral tongue sites (mobile or oral cavity tongue) as the single site 'tongue' [105]. Regardless of these site admixtures, and even taking into account the growing and ageing US population, a clearly increasing incidence over the past decade remains of both pharyngeal and tongue cancers [16].

Following the Surgeon General's warning and mounting evidence regarding the association between cigarette smoking and cancer, per capita tobacco consumption and cigarette current smoking prevalence rates have declined relatively steadily

since the mid-1960s [16, 106]. Because of these changes, currently more Americans are former smokers than are current smokers (~90 million Americans are members of one of these two groups); in fact, many tobacco-associated malignancies will be diagnosed in former smokers [16]. In 1965, more than half of men and one-third of women in the USA were currently smoking, whereas presently less than a quarter of men and less than one-fifth of women currently smoke [106]. As might be expected for diseases with a long initiation associated with chronic tobacco exposure, the primary public health goal of reducing lung cancer incidence did not begin to occur until the late 1980s [16, 107]. Similar to the incidence of lung cancer, the incidences of laryngeal, oral cavity and hypopharyngeal cancers have declined since the late 1980s; however, during this same period, the incidence of OPC initially plateaued but subsequently rose dramatically [10, 16, 107, 108]. These complex trends in OPC incidence are consistent with the control of one aetiological exposure (smoking), but the emergence of a second unrelated aetiological exposure during the same period.

As early as 2005, a heterogeneity in the trends in age-adjusted head and neck cancer incidence rates was noted; rates for tongue and OPC were increasing or stagnant, but rates for laryngeal, hypopharyngeal and oral cavity cancer were decreasing significantly [10]. With more careful site stratification, it has become apparent that OPC age-adjusted incidence is rising dramatically (estimated at 5 % annual per cent change [APC]) in the USA, while oral-cavity cancer incidence is falling (approximately by minus 2 % APC) [108]. Similar trends have been documented in both Europe and Canada [13, 109, 110]. Furthermore, the increase in OPC incidence appears to be primarily among middle-aged (40–59 years) white men [108]. Amazingly, these trends among white men have completely eliminated the dramatic racial disparity that existed in oral cavity and pharyngeal cancer incidence in the USA [107, 111]. Whereas the incidence trends for these sites among white men are greatly influenced by HPV-positive OPC, other races have experienced declining incidence rates over the same time period on account of a decreasing prevalence of both smoking and alcohol consumption among these groups [111].

Evidence from the demographic, behavioural, pathological and clinical characteristics of patients with OPC demonstrate HPV as the cause of the increasing incidence of this disease. Whereas many behavioural researchers have suggested that oral sex prevalence has risen over the past three decades, reliable data on the trend in prevalence of oral sex behaviours are lacking [21]. However, as described above, it is clear that smoking prevalence has been dropping since the 1960s, and as expected, tobacco-associated malignancies, such as head and neck and lung cancer, have subsequently declined in incidence, with the notable exception of OPC [13, 16, 107–110]. More specifically, it appears that the increasing incidence of OPC is restricted to the oropharyngeal sites where tumours tend to be HPV-positive (base of tongue and tonsil), as opposed to the sites where tumours tend to be HPV-negative (the posterior oropharynx and soft palate) [112]. In an analysis of SEER data, Chaturvedi et al. found that the age-adjusted incidence of OPC at HPV-related sites (base of tongue, tonsils, lingual tonsil and Waldeyer ring) increased significantly between 1973 and 2004, particularly in the most recent time period studied (APC

from 2000 to 2004, 5.22; $p < 0.016$) [108]. In contrast, the age-adjusted incidence of OPC at HPV-unrelated sites, which in this study was restricted to the oral cavity, decreased or remained stable during this same time period. Data from the National Program of Cancer Registries and the SEER programme showed a 3 % annual increase in potentially HPV-associated cancers of the tonsil and base of tongue during 1998–2003, whereas the APC for potentially HPV-unassociated sites generally decreased during the same time period [113]. More recently, using OPC tumour specimens from participants from Hawaii, Los Angeles and Iowa in the SEER Residual Tissue Repositories Program, Chaturvedi et al. reported that HPV prevalence in oropharyngeal tumours increased from 16.3 % in the 1980s to 72.7 % in the 2000s (Fig. 2.2) [114]. This resulted in a 225 % population-level increase in HPV-positive OPC (from 0.8 cases per 100,000 individuals in 1988 to 2.6 per 100,000 in 2004), and a concomitant 50 % decrease in HPV-negative OPC (from 2.0 cases per 100,000 individuals in 1988 to 1.0 per 100,000 in 2004) [114].

These studies and others have shown that the increase in incidence of OPCs is particularly apparent among men, younger individuals and white people [8, 14, 108, 113, 114]. Frisch et al. examined data from the SEER programme and found that the age-adjusted incidence of tonsil squamous cell carcinoma (SCC) increased among men younger than 60 years during 1973–1995 (2.7 % for blacks and 1.9 % for whites), whereas no such increases occurred for other oral SCCs or among women [115]. Limiting the SEER data to individuals aged 20–44 years resulted in similar findings [11]. During 1973–2001, the APC was 3.9 for tonsil SCC and 1.73 for base of tongue SCC, whereas no significant increase was observed for other pharynx sites among white people aged 20–44 years [11]. Another study found that during 1973–2006, the proportion of OPC patients aged 40–59 years increased from 35 to 45 %, while the proportion of OPC patients aged 60–79 years decreased from 52 to 40 % [112]. Studies from Scandinavia have shown similar incidence trends for OPC [12, 15, 20],

In an analysis of data from the Danish Cancer Registry, the incidence of HPV-related head and neck cancers increased significantly between 1973 and 2007 and was especially pronounced for tonsillar cancer among men <60-years-old (APC 6.3; 95 % CI 5.4–7.2) [15]. During the same time period, the incidence of HPV-unrelated cancers decreased significantly among men (APC −2.0; 95 % CI −2.3 to −1.7), whereas it remained stable among women (APC 1.1; 95 % CI 0.5–1.7). Another study (from Stockholm, Sweden) found that the incidence of tonsillar cancer has been increasing significantly over the past several decades and the proportion of HPV-positive tonsillar cancers has also been rising [12]. The incidence of tonsillar cancer increased from 0.74 cases per 100,000 person-years to 1.65 cases per 100,000 person-years during 1970–2006, whereas the proportion of HPV-positive tumours increased from 23 % in the 1970s to 79 % during 2000–2007. Analysis of the Cancer Incidence in Five Continents Database for the period 1998–2002 (CI5IX) showed increasing incidence rates for pharynx cancer among both men and women in the Scandinavian countries [116] in contrast to the sex differences observed in the USA.

Additionally, HPV-positive cancers are typically detected at higher grade, and national trends reveal a rising proportion of higher-grade tumours for cancers of the

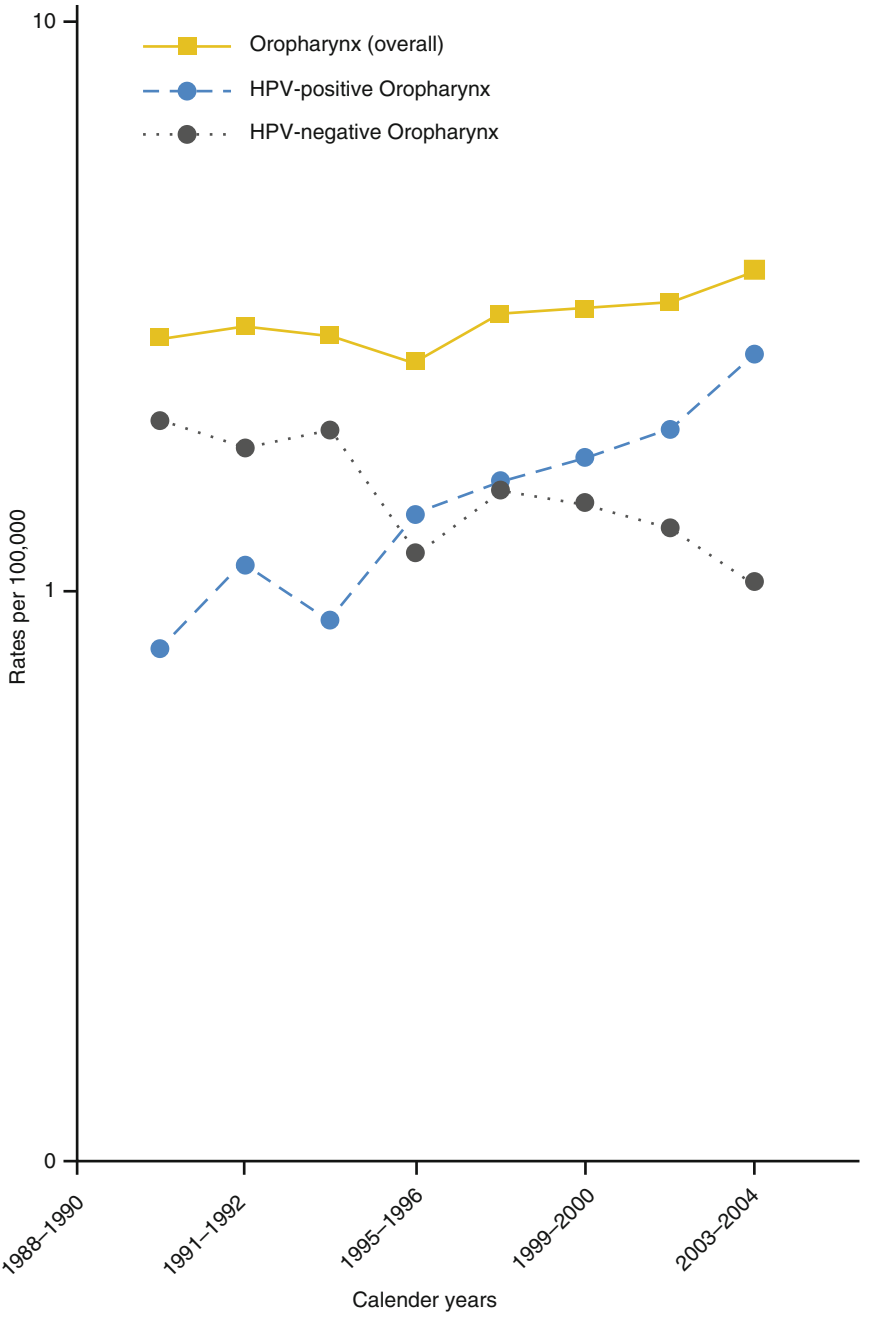


Fig. 2.2 Incidence rates for overall oropharyngeal cancer (OPC), human papillomavirus (HPV)-positive OPC, and HPV-negative OPC during 1988–2004 in Hawaii, Iowa and Los Angeles (Adapted from Chaturvedi et al. [114])

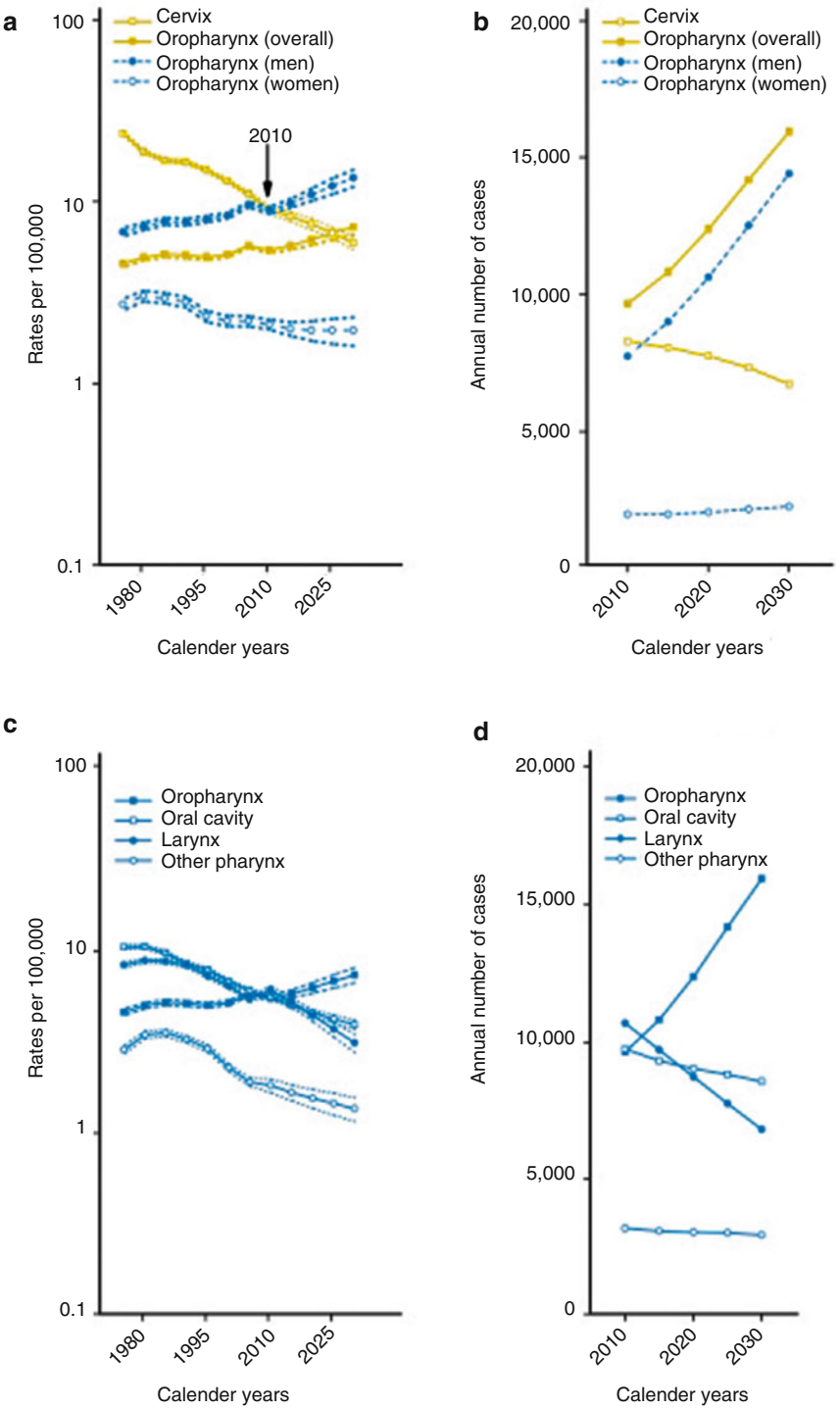
tongue and tonsil but not for cancers of other head and neck sites [112]. While the incidence of well-differentiated tumours decreased for all subsites of head and neck tumours during 1973–2006, the incidence of moderately and poorly differentiated tumours doubled for the tonsil and tongue [112]. This has resulted in a shift in the ratio of poorly differentiated to well-differentiated tongue tumours from 0.72:1 in 1975 to 1.84:1 in 2006, and in tonsil tumours from 1.4:1 in 1975 to 10.6:1 in 2006 [112].

Taken together, these findings and epidemiological trends support the increasing importance of HPV-positive OPC in the USA, in direct opposition to declining incidence of tobacco- and alcohol-associated head and neck cancer. If current trends continue, it is estimated that by 2030 almost half of all head and neck cancers will be HPV-positive OPC and that by 2020 the number of cases of HPV-positive OPC will surpass the number of cases of cervical cancer, although this reflects not only the incidence of HPV-HNSCC but also the large proportion of cervical cancers prevented by Pap screening (Fig. 2.3) [114].

Trends in Survival of Head and Neck Squamous Cell Carcinoma

In the past, patients with OPC had the poorest survival of all patients with head and neck cancer; however, SEER data show that 5-year survival for OPC patients has consistently improved since 1975 and these patients now have better survival than those with cancers at other head and neck sites (Fig. 2.4) [108]. Despite often being diagnosed at later stages of disease, patients with HPV-positive tumours tend to

Fig. 2.3 (**a**) Observed and projected incidence rates and bootstrap 95 % CIs (ages 30–84 years) for oropharyngeal cancers (OPCs) overall, OPCs among men, OPCs among women, and cervical cancers. (**b**) Projected annual number of patients (ages 30–84 years) with OPCs overall, OPCs among men, OPCs among women, and cervical cancers through the year 2030 (Adapted from Chaturvedi et al. [114]). (**c**) Observed and projected incidence rates for oropharyngeal, oral cavity, larynx and other pharynx cancers. (**d**) Projected annual number of patients with oropharyngeal, oral cavity, larynx and other pharynx cancers through the year 2030. Observed incidence rates from 1973 to 2007 from nine registries within the Surveillance, Epidemiology, and End Results programme were used in age–period–cohort models to project expected incidence through the year 2030. Projected incidence rates were applied to the 2008 US population projections to calculate the annual number of patients. OPCs included base of tongue (International Classification of Diseases for Oncology, 3rd edition [ICD-O-3] topography code C019), lingual tonsil (C024), soft palate not otherwise specified ([NOS] C051), uvula (C052), tonsil (C090-099), oropharynx (C100-109), and Waldeyer ring (C142). Oral cavity cancers included lip (C000-009), oral tongue (C020-23, C028, and C029), gum (C030-039), floor of mouth (C040-049), hard palate (C051, C058, and C059), and other and unspecified parts of the mouth (C060-C069). Laryngeal cancers included glottis (C320), supraglottis (C321), subglottis (C322), laryngeal cartilage (C323), overlapping lesion of larynx (C328) and larynx NOS (C329). Other pharynx cancers included nasopharynx (C110–C119), pyriform sinus (C129), postcricoid region (C130), hypopharynx (C130-139), and pharynx NOS (C140 and C148). OPCs included both HPV-related and HPV-unrelated (soft palate NOS and uvula) anatomical subsites because projections were conducted for all head and neck cancer sites. Oropharyngeal, oral cavity, laryngeal and other pharynx cancers were restricted to squamous cell histologies (ICD-O-3 codes 8050–8076, 8078, 8083, 8084 and 8094). Cervical cancers (C530-539) included all histological subtypes (Adapted from Chaturvedi et al. [114])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Oropharynx (overall)
Oropharynx (men)
Oropharynx (women)
2010
Rates per 100,000
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Cervix
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Annual number of cases
20,000
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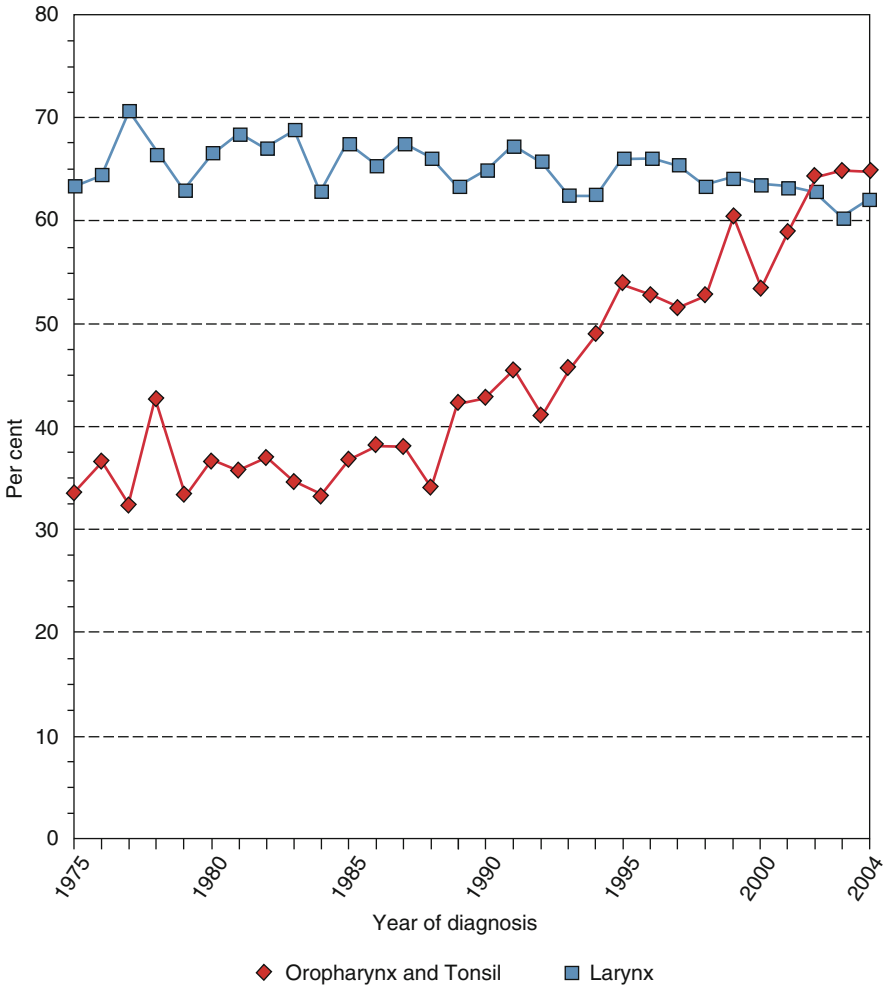

Fig. 2.4 Five-year relative survival rates by year of diagnosis for oropharynx/tonsil cancer and larynx cancer among men and women of all races and any age in the Surveillance, Epidemiology, and End Results (SEER) programme, 1975–2004 [107]. Cancer sites include invasive cases only unless otherwise noted. Survival source: SEER 9 areas (San Franscico, Connecticut, Detroit, Hawaii, lowa, New Mexico, Seattle, Utah and Atlanta). The 5-year survival estimates are calculated using monthly intervals

have better outcomes than patients with HPV-negative tumours, and HPV tumour status has been identified as an important and independent predictor of improved overall and disease-specific survival in patients with OPC. This has been shown in case series [2, 79, 82] and confirmed in a meta-analysis [117] as well as population-based studies [19, 108, 112]. Moreover, several clinical trials have shown a survival benefit for HPV-positive patients [89, 118–121].

In a case series of 253 patients with head and neck cancer, Gillison et al. found a 59 % reduction in risk of death from cancer among patients with HPV-positive tumours, compared to those with HPV-negative tumours after multivariable adjustment (hazard ratio [HR] 0.41; 95 % CI 0.2–0.9) [2]. When the analysis was limited to oropharyngeal tumours, disease-specific survival was even better among those with HPV-positive tumours, compared to those with HPV-negative tumours (HR 0.26; 95 % CI 0.07–0.98). Similarly, Ritchie et al. found that patients with HPV-positive oral cavity and oropharyngeal tumours had better overall 5-year survival than patients with HPV-negative tumours (RR = 0.4; 95 % CI 0.2–1.0) [82].

Patients with OPC have longer life expectancies, given that they are presenting at younger ages and are responding better to treatment because of the changing aetiology of the disease [122, 123]. Thus, it becomes essential to identify factors involved in HPV16 carcinogenesis that may be exploited to improve quality of life for OPC patients through alterations in treatment strategies. In a small study of 42 patients with OPC who were treated with induction chemotherapy and chemoradiation, a high HPV titre was associated with better response to induction chemotherapy as well as better overal [123] and disease-specific survival [124]. Both smoking and HPV status are strong predictors of survival in OPC patients, and initial results suggest synergy in these risk factors with worse survival among smoking than non-smoking HPV-positive OPC [119]. Hafkamp et al. found that HPV-positive tumours tended to be smaller but have more regional metastasis at presentation than HPV-negative tumours [125]. Moreover, among patients with HPV-positive tonsillar SCC, smoking was strongly associated with worse survival ($p = 0.036$); however, among smokers with tonsillar SCC, HPV status did not predict survival ($p = 0.20$) [125]. Results from this study and others suggest that tobacco use is a strong predictor of survival in addition to tumour HPV DNA status [119, 125–127].

A meta-analysis found a 28 % lower risk of death (95 % CI 0.5–1.0) and a 49 % lower risk of recurrence (95 % CI 0.4–0.7) for patients with HPV-positive compared with HPV-negative SCC of the oropharynx [117]. Improved survival was limited to HPV-positive patients with tumours of the oropharynx, suggesting a site-specific survival advantage among those with HPV-positive tumours. The higher prevalence of tobacco use among patients with non-oropharyngeal tumours could explain why the survival advantage was limited to patients with oropharyngeal tumours.

Patients with OPC have shown a trend for improved survival over time, which is possibly because of the increased proportion of HPV-positive tumours. Mehta et al.'s analysis of SEER data revealed that although the 5-year overall survival rate among individuals with well-differentiated oropharyngeal tumours improved from 63.3 to 73.1 % during 1975–2004, the improvement was even more dramatic among individuals with poorly differentiated tumours [112]. For poorly differentiated tumours, the relative 5-year survival rate increased from 35.1 % in 1975 to 55.9 % in 2004, and this improvement was even more pronounced among men. Furthermore, analysis of survival statistics by site revealed that the 5-year survival rate increased significantly for patients with poorly differentiated tumours of the tongue (31.3–58.1 %; $p < 0.001$) and tonsils (35.4–72.5 %; $p < 0.001$) but not for patients with tumours at other sites, which likely reflects the increasing proportion of HPV-positive tonsil and

base of tongue tumours. These trends were seen for lower-stage tumours as well, with the 5-year survival rate increasing from 50.5 to 74.8 % for tongue tumours ($p < 0.001$) and from 39.6 to 57.2 % for tonsil tumours ($p < 0.01$) during 1975–2004 [112].

Another analysis of SEER data found that 2-year survival for radiation-treated patients with head and neck cancers at HPV-related sites (OPCs) improved significantly more over time than did 2-year survival for patients with head and neck cancer at HPV-unrelated sites [108]. Specifically, the absolute increases in survival from 1973–1982 to 1993–2004 for localized, regional and distant stages were 9.9, 23.1 and 18.6 % for HPV-related sites, respectively, and 5.6, 3.1 and 9.9 %, respectively, for HPV-unrelated sites. Survival was significantly longer among patients with cancers at HPV-related sites than among those with cancers at HPV-unrelated sites treated with radiation during 1993–2004; this difference is thought to be due in part to the greater radiosensitivity of HPV-positive tumours [108]. A population-based study in Sweden also found improved survival for tonsil and base of tongue cancer patients, particularly since 1980 [128].

Studies have consistently shown that patients with HPV-positive tumours have markedly better survival compared to those with HPV-negative tumours. A prospective phase II clinical trial of patients with SCC of the oropharynx and larynx found that patients with HPV-positive tumours had better response to induction chemotherapy and chemoradiation, lower risk of progression, and better overall survival than patients with HPV-negative tumours [118]. Specifically, the 2-year overall survival rate was 95 % (95 % CI 87–100 %) for patients with HPV-positive tumours and 62 % (95 % CI 49–74 %) for patients with HPV-negative tumours, with a difference of 33 % (95 % CI 18.6–47.4 %). The 2-year progression-free survival rate was 86 % (95 % CI 74–99 %) for HPV-positive patients and 53 % (95 % CI 36–67 %) for HPV-negative patients, with a difference of 33 % (95 % CI 12.7–53.3 %). Furthermore, tumour HPV status was associated independently with survival outcomes, and patients with HPV-positive tumours had a 64 % lower risk of death (HR 0.36; 95 % CI 0.15–0.85) and a 73 % lower risk of progression (HR 0.27; 95 % CI 0.10–0.75) than patients with HPV-negative tumours in multivariable analysis [118].

Likewise, a recent retrospective analysis of patients with SCC of the head and neck in a large phase III trial (Radiation Therapy Oncology Group 0129) found that among patients with SCC of the oropharynx, those with HPV-positive tumours had a 58 % lower risk of death than patients with HPV-negative tumours after multivariable adjustment (HR 0.42; 95 % CI 0.27–0.66) [119]. This same study also found that HPV-positive patients had a better progression-free survival than HPV-negative patients (HR 0.49; 95 % CI 0.33–0.74) [119].

Although studies firmly support a survival advantage for HPV-positive OPC, discrepancies exist in the estimated ratios, with HRs ranging from 0.2 to 0.6 for overall survival in multi-institutional clinical trials [122]. This may be due in part to methods for determining tumour HPV status as well as the smoking status of these patients. Nevertheless, these studies show that HPV is a strong independent predictor of both overall and progression-free survival among patients with oropharyngeal cancer.

Implications for Prevention and Use of the HPV Vaccine

Two Food and Drug Administration-approved prophylactic vaccines are currently available to protect against infection with the HPV types responsible for 70 % of cervical cancers and 90 % of HPV-positive non-cervical cancers (HPV16 and HPV18). The bivalent vaccine manufactured by GlaxoSmithKline (Cervarix) protects against HPV16 and HPV18, whereas the quadrivalent vaccine manufactured by Merck (Gardasil) protects against these HPV types and an additional two types—HPV6 and HPV11. Whereas clinical trials of these vaccines initially focused only on women, because of the high global burden of cervical cancer and other HPV-positive cervical lesions, the vaccines have more recently been shown to prevent anogenital warts in men, anal pre-cancer in men and women, and are expected to prevent HPV-positive oropharyngeal cancer as well.

Both of the HPV vaccines are highly immunogenic. In Australia, where 65 % of eligible women have been vaccinated, the incidence of genital warts has reduced significantly [129]. Between 2004 and 2009, a 59 % reduction was seen in the incidence of genital warts among women aged 12–26 years in Australia (the ages that were eligible for free vaccination) [129]. Interestingly, a significant 39 % reduction was also found in the incidence of genital warts among heterosexual men aged 12–26 years in 2007. In areas where vaccine coverage rates are high, such as Australia, herd immunity would confer protection to heterosexual boys; however, this benefit is not observed when vaccination rates are low in women and does not extend to homosexual men [130]. Although the Merck vaccine has been recommended for use in girls and young women in the USA since 2006 [131], only about half of girls in the targeted age range have received one dose, and only 32 % have received all three doses of the vaccine [132]. In 2011, the vaccine was recommended for routine use in boys [133]. Given that the main burden of OPC in developed countries is among men and is expected to increase, it would seem imperative to vaccinate both boys and girls.

Screening and early detection of OPC has the potential to significantly reduce morbidity and mortality for patients with OPC; however, no test currently exists to screen for oropharyngeal abnormalities. Recently, Fakhry et al. evaluated an oral Pap test equivalent, but results were disappointing, in that tonsillar HPV infection was not associated with cytological abnormalities consistent with HPV infection; the main obstacle was difficulty in adequately testing the tonsillar epithelium [134]. Unfortunately, HPV-positive premalignant and early cancerous lesions occur deep within tonsillar crypts and base of tongue (lingual tonsils) and as such appear to be inaccessible for visible diagnosis or existing oral cytological techniques.

Summary Points

- Oral HPV infects ~7 % of the population of the USA. Prevalence of HPV infection increases with age and is twice as common in men as in women. Factors associated with oral HPV infection include oral–genital sexual contact, number of lifetime oral sex partners, and possibly smoking.

- HPV, in particular HPV16, is an important risk factor for OPC. ORs for the association between HPV and head and neck cancer from case–control studies have ranged from 3.6 to 230.
- The risk factor profiles for HPV-positive and HPV-negative OPC show differences. HPV-positive cancer tends to be associated with sexual behaviours, whereas HPV-negative cancer is associated with tobacco and alcohol use.
- The prevalence of HPV positivity among patients with OPCs in the USA has increased from 16 % in the 1980s to >70 % since 2000. This is because of both decreasing tobacco use and an increase in the incidence of HPV-OPC.
- The incidence of HPV-positive OPC is increasing, especially among middle-aged white men, and this increase is thought to be caused by an increase in oral HPV infection. This is in contrast to a decrease in the incidence of HPV-negative OPC and cancers at other head and neck sites.
- Changes in sexual behaviour, namely a decrease in age at sexual debut and an increase in number of oral sex partners, may explain the increase in incidence in HPV-positive OPC.
- Although HPV-positive OPCs are often diagnosed at later stages, patients with HPV-positive oropharyngeal tumours have improved survival compared with patients with HPV-negative tumours. HPV status and smoking are both strong predictors of survival in patients with OPC with HPV-positive tumours associated with better, and smoking associated with worse, survival.

It is hoped that the current HPV vaccines will substantially reduce the risk of HPV-associated oropharyngeal cancer in vaccinated populations.

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Frequent Behavioural Questions with an HPV-Positive Malignancy of the Head and Neck

3

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An initial diagnosis of a head and neck malignancy is sometimes followed by a sense of relief when it becomes known that the malignancy is human papillomavirus (HPV)-positive. This is because HPV-positive malignancy is associated with improved prognosis. However, patients and their partners often wrestle with complex social and behavioural questions. Unfortunately, the answers to many of these questions have not been examined to date. At best, the most appropriate response is to say, 'We do not know; however, initial evidence suggests that …', with the caveat that our knowledge of oral HPV infection can only be extrapolated from related research on anogenital HPV infection. Because these cancers have implications for past, present and future relationships between patients and their partners, it is essential that any advice given to patients must be considered carefully.

This chapter addresses common patient concerns relating to behaviours associated with HPV-positive head and neck cancer and oral HPV in the format of questions and answers, reviewing what is currently known and what remains unknown about the acquisition, transmission of oral HPV infection and progression to cancer.

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How Did I Get an Oral HPV Infection?

- HPV is a sexually transmitted infection that can infect the epithelium of the oropharynx and anogenital tract [1].
- The natural history of genital HPV is well understood and serves as a model for what we may expect the natural history of oral HPV to be. Genital HPV infections are common among sexually active young adults, and include cervical, vaginal and penile infection. More than 80 % of sexually active adults are infected at some point during their lifetime, although many of them are unaware that they were ever infected [2, 3]. Most people clear these genital infections on their own within a year or two [2, 4, 5]. Among those who do not clear their infections, persistent infection can lead to premalignant and malignant genital lesions [6–8].
- Oral HPV infection is not as common as genital HPV infection; the latter is more common among men than women [9–13]. Initial studies suggest that most people clear their oral HPV infections on their own within a year or two but, in some, oral HPV infection persists [14, 15].
- Oral HPV is more common in individuals who have ever performed oral sex, and who have increasing numbers of lifetime oral sexual partners [13, 16, 17]. However, oral HPV infection has also been (more rarely) reported in people who deny having ever performed oral sex [14, 16, 18].
- The presence of oral HPV infection is not a marker of promiscuity. Some people with oral HPV infection report never having performed oral sex, or have had only a few lifetime oral sex partners [13, 16, 17, 19]. Current estimates indicate that ~10 % of men and 4 % of women aged 14–69 years in the general population of the USA have a prevalent oral HPV infection [13], and a much higher proportion will probably have been exposed at some time in their life. It is believed, although it has not been shown, that whereas most sexually active individuals are exposed to HPV infection orally, most people clear these infections on their own with a year or two [15, 20].

Is Oral HPV Infection and HPV-Positive Head and Neck Cancer Caused by Oral Sex?

Oral–Genital Contact

- Several studies have shown that people who have a larger number of partners with whom they have oral sex have increased odds of the following:
  - Prevalent oral HPV infection [16, 17]
  - Incident oral HPV infection [21]
  - HPV-positive head and neck squamous cell carcinoma (HNSCC) [22, 23].
- This suggests that HPV is probably transmitted by oral sex. However, it is important to understand that many types of sexual behaviour are collinear (i.e. people

with a larger number of partners for one type of sexual behaviour tend to have a larger number of partners for other types of sexual behaviour). Therefore, it is difficult to determine which specific behaviours are responsible for the presence of HPV in the mouth and/or pharynx.

Oral–Anal Contact

- Anal HPV is common and it is possible that oral–anal sexual contact (rimming) may be associated with transmission of HPV to the oropharynx [24]. One study on gay men showed that having a large number of rimming partners was associated with higher oral HPV prevalence; however, further research is needed to determine whether rimming can, indeed, transmit HPV infection from the anus to the mouth and throat [25].

Oral–Oral Contact

- It is not known whether open-mouth kissing (i.e. deep kissing or French kissing) can transmit oral HPV infection. Three studies reported an association between open-mouth kissing and oral HPV prevalence, even among those who reported never having performed oral sex [13, 16, 26]. However, these studies were limited by small sample sizes. Oral HPV has been detected in children who have not had sexual exposure, but prevalence among younger adolescents (12–15 years of age, 1.5 %) and older adolescents (16–20 years of age, 3.3 %) is relatively low despite the common practice of deep-kissing among these age groups [18, 27].

When Did I Get an Oral HPV Infection?

- We do not know the usual time from oral HPV infection to development of HPV-positive HNSCC because the presence of oral HPV infection is clinically asymptomatic. However, extrapolating from what is known about the natural history of anogenital HPV, it is believed that individuals presenting with HPV-positive HNSCC today probably acquired the infection many years (probably >10 years) earlier [28, 29].
- Oral HPV infection is detected not only in individuals in their twenties (the peak time of sexual activity for most adults), but also in older people, among whom it has an even higher prevalence [13]. In a recent US population-based study, oral HPV prevalence in both men and women was most common among 30–34-year-olds (7.3 %) and 60–64-year-olds (11.4 %), although these estimates probably do not indicate when these infections were first acquired. Whether the second peak in prevalence of infection (among 60-year-olds) is related to recent sexual activity or immune-related factors (i.e. re-activation in the context of being immunocompromised) is unknown.

Does a Diagnosis of HPV-Positive HNSCC Indicate Past or Present Promiscuity?

- It is important to remind patients that being diagnosed with HPV-positive HNSCC does not imply that either partner was/is unfaithful, or that either partner has a 'risky' sexual past.
- Although it is not as common as genital HPV infection, many people will probably be exposed to oral HPV infection during their lifetime [13, 30]. We suspect that despite being exposed to oral HPV infection, few have persistent infection and even fewer develop malignancy [2, 14].
- It is our opinion that oral HPV infection must persist for many years for cancer to occur, although at present data are scarce on the natural history of oral HPV infection. A recent study detected antibodies to HPV oncogenes in some HPV-positive HNSCC cases >10 years before cancer was diagnosed, suggesting that the oral HPV infections were acquired and the disease process began many years before diagnosis [29].

Are There Non-sexual Routes of Transmission of Oral HPV Infection?

Vertical Transmission

- Several studies have detected a low prevalence of HPV DNA in the mouths of infants born to women with cervical HPV infection (0–0.4 %) [31, 32]. These data support the possibility that oral HPV could be transmitted to infants during childbirth from mothers with an active genital HPV infection.
- Oral HPV infections in infants and young children, however, appear to clear quickly [33]. In addition, oral HPV infection is rare among children aged 7–12 years, suggesting that an infection in early life from vertical transmission is likely to clear and is not a probable source of persistent infection [34].

Casual Transmission

- Oral HPV infection does not appear to be transmitted casually. Sharing utensils and drinks, hugging, kissing on the check, and sharing a bathroom are not associated with the transmission of oral HPV infection.

Will I Transmit This Infection to Others?

Family and Friends

- Oral HPV does not appear to be transmitted casually.
- Currently, there is no evidence to suggest that oral HPV is transmitted non-sexually (except potential vertical transmission from mothers to newborns, which is fairly uncommon and an unlikely source of persistent infection).

Spouse or Long-Term Sexual Partners

- HPV is a sexually transmitted infection—couples who have been intimate have probably already been exposed to each others' sexual infections [35]. Therefore, we do not recommend changing current sexual behaviours in established monogamous relationships.
- Spouses of patients with HPV-positive HNSCC have probably been exposed to HPV themselves (from current and/or former partners), though they might not have an active oral HPV infection [36]. Thus, there is no need to change sexual behaviour with a current spouse or long-term partner.
- Women with HPV-positive HNSCC and female partners of men with HPV-positive HNSCC should undergo routine gynaecological screening for cervical HPV-related abnormalities (as currently indicated for all women >30 years of age) [37].

Future Partners

- Many patients with HPV-positive HNSCC do not have detectable viral infection after treatment and therefore probably cannot transmit the infection after therapy [38]. On the other hand, some patients continue to have HPV detectable in exfoliated oral cells after therapy [38].
- It is theoretically possible that HPV can be transmitted to future partners. The level of risk is unknown.
- Advice on appropriate protection should be given to new partners, as for the prevention of any sexually transmitted infection.
- Inconsistent or lack of condom use and barrier protection during oral–genital and genital–genital contact has been associated with increased odds of oral HPV infection and HPV-positive HNSCC [13, 22, 39]. Given this association and the knowledge that consistent condom use has been shown to reduce the risk of anogenital HPV infection, the use of barrier protection during new sexual encounters may decrease the risk of transmitting infection during oral sex [40].

Is My Spouse/Partner at Increased Risk of Contracting This Cancer? What Should He/She Do?

- Some studies have suggested that spouses of cervical cancer patients have a ~2–3-fold increased risk of developing HPV-positive HNSCC [41–43]. Examples in the literature are rare of both members of a couple being diagnosed with HPV-positive HNSCC [44].
- Partners of patients with HPV-positive HNSCC may have slightly higher rates of other HPV-positive cancers than the general population, such as anal, penile/cervical, and/or oropharyngeal cancers, but these are currently rare and chances of developing these cancers remain low overall [42].

- Screening guidelines for HPV-positive malignancies of the head and neck currently are unavailable. Therefore, with the exception of cervical cancer screening guidelines, there are no additional recommendations for sexual partners or spouses of patients with HPV-positive HNSCC.

Will I Always Have This Oral HPV Infection?

- Before treatment, many patients with HPV-positive HNSCC have HPV DNA detectable in their oral exfoliate cells; this DNA is probably sloughed off from the tumour and does not necessarily represent a virus particle capable of infecting someone else [22, 45].
- After treatment, many patients no longer have HPV DNA detectable in their oral exfoliate cells, but it is difficult to say whether the infection is 'cleared' (gone) or whether the HPV is dormant as a latent infection somewhere in the body, controlled by the immune system and not actively expressed [38].

Will HPV Vaccines Help?

- Currently, two HPV vaccines licensed to prevent HPV infections are available in the USA. The quadrivalent HPV vaccine (Gardasil, Merck) protects against HPV types 6, 11, 16 and 18, which cause most HPV-positive cancers as well as genital warts. The bivalent HPV vaccine (Cervarix, GlaxoSmithKline) protects against the cancer-causing HPV types 16 and 18.
- These vaccines prevent individuals from becoming infected with the above types of HPV if/when they are exposed to the virus [46, 47]. Unfortunately, they do not clear an infection acquired prior to vaccination.
- These vaccines have not been tested against oral HPV infection. However, because individuals with an HPV-positive HNSCC are already infected with HPV, and neither of the HPV vaccines helps to clear a current HPV infection, these vaccines are not part of treatment regimens for current cancers.
- Existing clinical trials of the HPV vaccines have focused on their efficacy in preventing HPV-positive anogenital diseases such as cervical cancer [48, 49], but their utility against oral HPV infection is unknown. However, given that such a large proportion of HNSCCs are caused by HPV16 (which the vaccines target), researchers are hopeful that the vaccine might be beneficial in preventing HPV-positive HNSCC as well [50, 51].
- Whereas the efficacy of Gardasil or Cervarix has not been evaluated in clinical trials of HNSCCs in humans, several studies using animal models do suggest that HPV vaccination could provide protection against oral HPV infection with vaccine-type HPV strains [52, 53].

Summary Points

- A common route of oral HPV transmission is through oral–genital contact; however, having an oral HPV infection is not a marker of promiscuity and it does not mean that a current partner was unfaithful.
- Oral HPV is not casually transmitted; you cannot become infected sharing utensils and drinks or kissing on the cheek.
- For partners of patients with HPV-positive HNSCC, it is likely that couples have already shared whatever infections they have a long time ago (i.e. during the first year of their relationship). Patients need not change sexual behaviour with their current spouse or a long-term partner.
- Many (but not all) patients with HPV-positive HNSCC no longer have HPV DNA detectable in their mouths after cancer treatment.
- Existing HPV vaccines (Gardasil and Cervarix) will not help to clear current HPV infection but can protect against new infections. Both vaccines are recommended for boys and girls between the ages of 9 and 26 years. Their efficacy in patients with HPV-positive HNSCC is unknown at present.

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The Molecular Biology of HPV-Related Head and Neck Cancer

4

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Human Papillomavirus Infection

Human papillomaviruses (HPVs) are epitheliotropic, non-enveloped, circular deoxyribonucleic acid (DNA) viruses of ~7,900 base pairs. There are over 100 distinct subtypes of HPVs that infect squamous epithelia and cause both benign and cancerous lesions [1, 2]. Benign lesions caused by HPV include both mucosal and epithelial warts.

In the 1800s, condyloma acuminata, or genital warts, were regarded as infectious manifestations of venereal disease [2]. Shope and Hurst in 1933 were the first to elucidate the infectious transmission of HPV in the form of papillomas in cottontail rabbits [3]. Further studies in rabbits by Rous and co-workers in 1935 and 1936 described the malignant progression of the papilloma to an epithelial carcinoma [4–6]. These investigators inoculated domestic rabbits with papillomatous tissue from Shope's cottontail rabbits and malignant progression was noted in 7 of the 10 experimental rabbits [4–6]. Four of the rabbits later developed regional lymph node metastases and one developed lung metastases. Over the next several decades, HPVs were linked to human cancers, specifically cervical and anal cancer. More recently, HPV was identified as a causative agent in oropharyngeal cancer [7–9].

Mucosal HPV subtypes are categorized into 'high-risk' and 'low-risk' types on the basis of their potential to induce malignancy in cervical cancer. Most HPV types are considered low-risk, including types 6 and 11 which cause anogenital warts and laryngeal papillomatosis. Warts do not progress to malignancy even if left untreated [2]. High-risk HPV types are classified on the basis of strong epidemiological associations in the literature with cervical cancer [10]. Eleven HPV types are classified consistently as high-risk (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58) [11], whereas the oncogenic potential of seven other HPV types has been suggested in some studies, but not

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established [11]. Among the high-risk types, HPV16 is the most common and closely associated with oropharyngeal squamous cell carcinoma (OPSCC) [12], accounting for up to 95 % of all current HPV-positive OPSCC in North America [13].

Life Cycle of HPV

The HPV life cycle is linked closely to the inherent differentiation process of the host epithelium. High-risk HPV infects and replicates within the basal cells of stratified squamous epithelium. In normal stratified epithelium, the basal cell layer is adjacent to the basement membrane and contains the only actively dividing cells within the epithelium, including stem cells. When basal cells divide, two daughter cells are created. One becomes a new basal cell and the other migrates away from the basal cell layer and differentiates [1, 14]. The differentiating cells eventually exit the cell cycle, resulting in death and desquamation [15]. The model of carcinogenesis in the cervix suggests that for HPV infection to occur, microabrasions or epithelial wounds must be present, thus exposing the basal cell layer and basement membrane to the virus [16].

The process of viral uptake into the host cell, encapsidation, and nuclear import of the viral genome is not entirely understood. However, evidence has shown that once HPV binds to the basement membrane, the virion undergoes a conformational change, allowing it to enter the cell. The HPV capsid protein L2 is cleaved, exposing the N-terminal L2 epitope, and thus allowing for the transfer of the capsid to the epithelial cell surface [17]. Most HPV types enter the cell via a clathrin-dependent endocytic mechanism; however, data are inconclusive [18]. After the HPV virion enters the cell, the circular, episomal HPV genome is replicated in the host nucleus to ~100 copies per cell [15, 17]. During mitosis, the episomal virus genomes are partitioned into two daughter cells. One daughter cell becomes a basal cell while the other enters the differentiation process. The HPV DNA then assembles into virions that contain the L1 and L2 capsid proteins in the uppermost layer of the differentiating epithelium, and mature viruses are shed with desquamated cells [2].

It takes many years for an HPV infection to lead to malignancy. A key step in this malignant transformation is thought to be integration of the HPV DNA into the host DNA; [19] a process that is poorly understood. Investigators have shown that both integrated and episomal HPV DNA are found in OPSCC, making it difficult to determine the role of HPV integration in carcinogenesis [20]. However, variable numbers of full-length or partially deleted HPV genomes, ranging from one to >500 head-to-tail or head-to-head sequences, are found integrated into the host genomes in HPV-positive cancers [20]. It is also known that HPV-infected cells that express E6 and E7 from integrated HPV sequences have a survival advantage over those cells with episomal HPV DNA sequences [21, 22]. The E6 and E7 proteins, which will be discussed in more detail later, are largely responsible for the immortalization of the host cell and malignant transformation.

The HPV Genome

Whereas HPV-related OPSCC is a relatively new discovery, the HPV genome has been studied vigorously in models on cervical cancer. The HPV genome is organized into the following three regions: An E or early gene region, an L or late gene region, and the upstream LCR or long control region, also known as the upper regulatory region (URR; Fig. 4.1). The 1 kb LCR is a non-coding region which contains *cis* elements that regulate viral replication and gene expression. The 4 kb E region encodes oncoproteins and other non-structural proteins. The 3 kb L region encodes two capsid proteins, L1 and L2. The E and L regions are numbered according to size—the smaller the number, the larger the open reading frame [13].

The E6 and E7 genes encode oncoproteins that promote virus replication and immortalization of the human cell. The E6 oncoprotein binds to and inactivates the tumour suppressor protein p53, which is responsible for cellular apoptosis and delaying a cell's entry into the S-phase of the cell cycle while DNA is being repaired [23]. Similarly, the E7 oncoprotein binds to the tumour suppressor pRb, releasing the transcription factor E2F which activates DNA synthesis by promoting the transcription of genes involved in this process [24, 25]. As a compensatory mechanism, cellular p16 is strongly induced by these events and produces surrogate measures of transcriptionally active oncogenes, as in HPV-negative OPSCC the tumour suppressor p16 protein is usually inactivated [26]. Therefore, once E6 has inactivated the cell cycle control by p53, the action of E7 allows the cell to replicate even in the presence of damaged DNA [27].

E2, another early gene, is a DNA-binding transcription factor that interacts with ACCN6GGT motifs in the LCR, or URR. E2 thereby modulates viral gene expression by acting as a transcriptional repressor in host keratinocytes [28, 29]. In

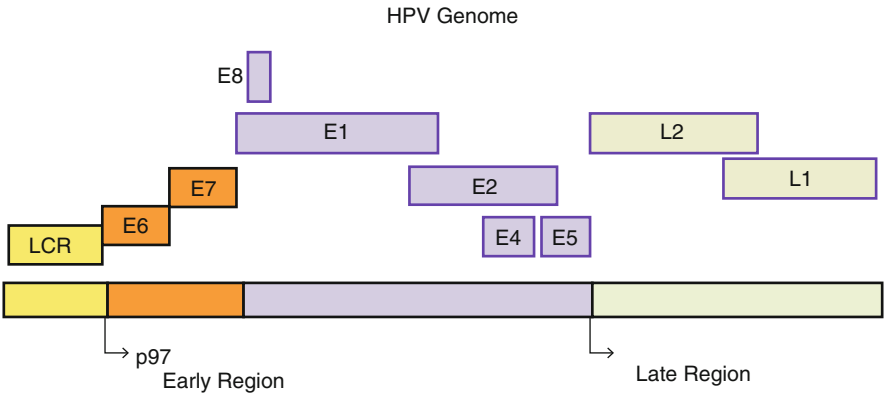

Fig. 4.1 The HPV16 double-stranded DNA genome. The long control region (LCR) depicts the 1 kb structure, which is the non-coding upper regulatory region (URR) that regulates viral replication and gene expression. The early (*E*) gene region is a 4 kb structure that encodes for the oncoproteins E6 and E7, as well as the proteins E8, E1, E2, E4 and E5. The late (*L*) gene region is a 3 kb structure that encodes the capsid proteins L1 and L3. p97 is a major early promoter of transcription [2, 13]

high-risk HPVs, E2 can function as a transcriptional activator as well [30]. In addition to its function as a modulator of gene expression, the E2 protein also tethers the viral genome to the host chromosomes during mitosis, thereby ensuring that HPV DNA is partitioned equally into the daughter cells [31].

The E2 protein also associates with the viral DNA helicase E1, allowing for extrachromosomal viral DNA replication [32]. This interaction allows HPV to retain a stable and low copy number, which permits the virus to evade the host immune system and persist in undifferentiated basal cells [1]. The E2 protein regulates the early viral promoter that controls the expression of E6, E7 and E1 proteins [33, 34]. These functions of E2 are crucial for the life cycle and replication of the virus in the host cell. Upon integration of the HPV DNA into the genome, the E2 gene is disrupted frequently, resulting in the loss of E2 expression and increased expression of the E6 and E7 proteins [34]. Therefore, loss of the E2 repressor function is a crucial step in the malignant transformation of cells.

Once the epithelial cells containing the HPV DNA differentiate, the amount of proteins encoded by the E1^E4-spliced mRNA and the E5 gene is increased. This leads to a rise in the viral copy number from ~100 to several thousand per cell [35]. The E1^E4-spliced mRNA consists of the first five codons of E1 fused to the open reading frame of E4 [36]. Studies have shown that E1^E4 protein induces the breakdown of the cytoplasmic cytokeratin network in the host keratinocyte, which is thought to assist in the release of the virion from the infected human cell [37]. E1^E4 is therefore expressed in the later stages of HPV infection and is necessary for viral replication.

In most papillomaviruses, the E5 protein is responsible for regulation of cell growth and the viral life cycle. In bovine papillomavirus type 1, the E5 open reading frame encodes a major transforming viral protein [38]. E5 transforms cells by activating the receptor tyrosine kinases, such as platelet-derived growth factor, thereby playing a crucial role in the viral life cycle. Disruption of E5 expression in non-HPV models affects the life cycle of the virus [39]. However, in the case of HPV, integration of the viral genome into the host cell leads to loss of E5 expression [40]. The HPV E5 protein is generally not detected in HPV-associated cervical cancers, suggesting that in humans the E5 protein is not necessary for malignant transformation [27, 38].

During cellular differentiation, the late viral promoter is activated, which leads to the synthesis of late proteins L1 and L2. L1 and L2 are capsid proteins that are expressed late in cellular differentiation and are highly immunogenic. Because of their immunogenicity, these proteins are expressed when the cell is highly differentiated and therefore they are not as closely monitored by the host immune system [4, 41]. Furthermore, L2 has properties that allow it to suppress the maturation of Langerhans cells (LCs), which are crucial for the functioning of the immune system [42]. Once the virions are shed with desquamated cells, the capsid proteins are responsible for viral persistence, both by avoiding inflammation and by adhering to host cells. After integration, L1 and L2 are not expressed, thus no ‘infectious’ virions are believed to derive from HPV-infected OPSCC [1].

HPV Oncoproteins

Our knowledge about the malignant transformation of oropharyngeal cells infected by HPV is extrapolated from cervical cancer models. Drawing from these models, there are two HPV proteins that have a well understood oncogenic process. As previously mentioned, loss of E2 repressor function may lead to de-regulated expression of E6 and E7, thereby promoting malignant progression [34]. Investigators have shown that re-expression of E2 in cervical cancer cell lines causes tumour growth suppression [39]. Not only does this support the role of E2 in maintaining a non-carcinogenic HPV infection, it demonstrates the importance of E6 and E7 expression in the maintenance of the transformed cancerous phenotype.

The high-risk HPV E7 protein is ~100 amino acids long and contains an LXCXE motif, which mediates binding of E7 to the retinoblastoma (Rb) family of pocket proteins, and induces the ubiquitin-mediated proteasomal degradation of pRb, p107 and p130 (Fig. 4.2) [43, 44]. This helps push the cell into S-phase by releasing the E2F transcription factor from the pRb–E2F complexes [24]. The Rb family of pocket proteins plays a crucial role in controlling the cell cycle by monitoring the checkpoint between the G1 and S-phase of the cell cycle. The pRb binds to E2F, a transcription factor, making E2F unavailable for cell cycle promotion [24]. However, when E7 binds to pRb, the E2F transcription factor is released from its attachment with pRb and is able to promote cell cycle progression. Additionally, high-risk E7 proteins

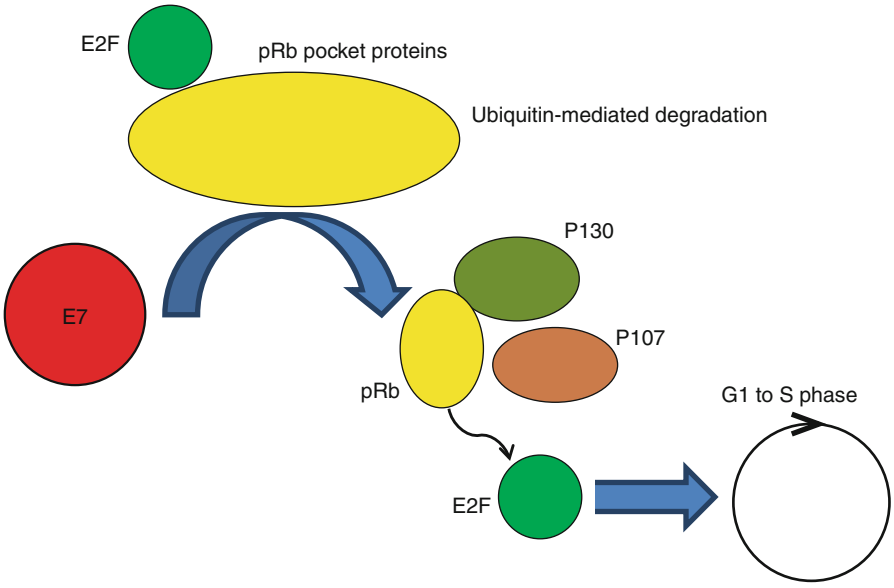

Fig. 4.2 Schematic diagram of the interaction between E7 and pRb. E7 binds pRb and the pRb family of pocket proteins, causing ubiquitin-mediated proteasomal degradation of pRb, p107 and p130. By inactivating the pRb–E2F complex, the E2F is released and overexpressed, resulting in upregulation of cell cycle progression from the G1 to S phase, thereby leading to cellular proliferation

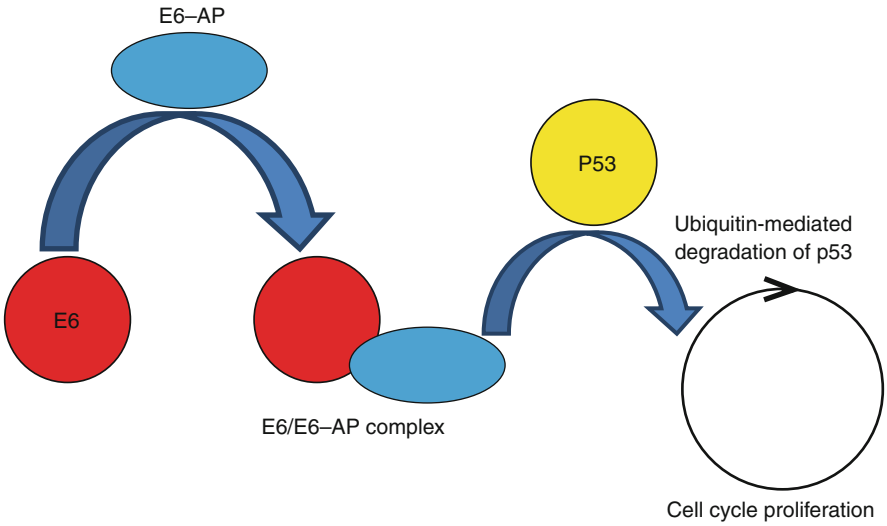

Fig. 4.3 Schematic diagram of the interaction between E6 and p53. E6 initially binds with E6AP, a necessary protein for p53 interaction. This newly formed complex acts as an ubiquitin–protein ligase that targets p53 for proteosome-mediated degradation. The inactivation of p53 leads to unregulated cell proliferation

bind to other cellular targets, including cyclin-dependent kinase inhibitors—p21 and p27—to further disrupt cell cycle control [45, 46]. Other cellular proteins that interact with E7 include cyclins A and E, p600, and histone deacetylases (HDACs), further contributing to cell cycle deregulation [1]. The low-risk E7 proteins, however, bind to pRb and its family members but are unable to promote their degradation.

The effect of E7 on pRb leads to the stabilization of p53, underscoring the importance of the E6 protein, which degrades p53 [47]. E6 is a small protein of ~150 amino acids and acts as a scaffold for other cellular factors that are important in oncogenesis. E6 degrades wild-type and mutant p53, thereby inhibiting apoptosis. E6 associates with the E6–AP, and this complex acts as an ubiquitin–protein ligase that targets p53 for proteasome-mediated degradation (Fig. 4.3) [47]. The C-terminal domain of high-risk HPV E6 also interacts directly with proteins containing PDZ-binding domains, including hDlg and hScrib [48]. High-risk E6 also interferes with the function of transcription factors involved in cellular processes, such as growth, differentiation and cell cycle progression by binding to regions of the co-activators ADA3, p300 and CBP [49].

High-risk HPV E6 proteins further contribute to immortalization of the host cell by promoting telomerase activity [50, 51]. In normal human cells, each round of DNA replication leads to degradation of the chromosomal telomeric terminus. Telomere shortening in normal cells restricts the immortal proliferation of the cell. In cells that require a limitless number of cell divisions, such as stem cells, telomere erosion is prevented. Telomerase, a ribonucleoprotein, prevents telomere erosion and thereby allows for cellular immortalization. In human cells, it has been shown that a catalytic telomerase subunit, hTERT, facilitates this immortalization [52].

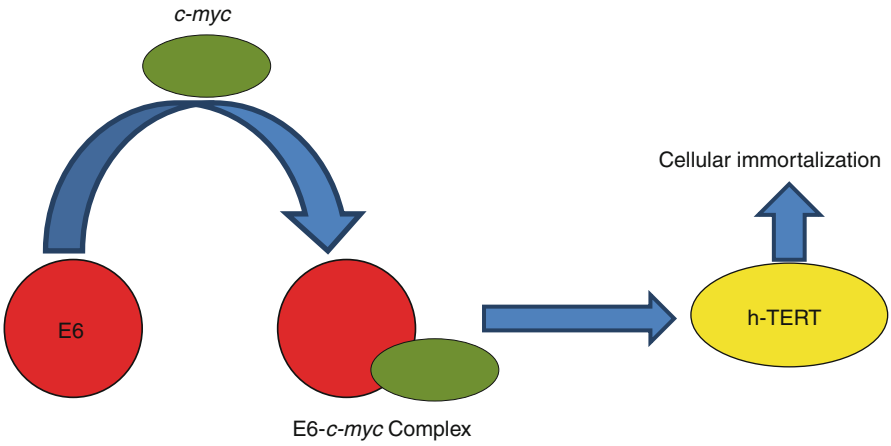

Fig. 4.4 Diagram depicting the role of high-risk E6 in activating telomerase expression. E6 binds to *c-myc*, forming a complex that activates the expression of hTERT. hTERT is a catalytic telomerase subunit that prevents telomere erosion and therefore leads to cellular proliferation and immortalization

hTERT expression is not only important in human stem cells, but is a crucial component in human tumour-like cells *in vitro* [52]. High-risk E6 proteins activate hTERT, thereby preventing telomere shortening and contributing to cellular immortalization (Fig. 4.4). Specifically, E6 binds to *c-myc*, an hTERT promoter fragment, forming an *E6–c-myc* complex, which then activates the expression of hTERT [53].

Expression of high-risk HPV E6 and E7 in host cell keratinocytes promotes their immortalization [2, 30]. Dürst and colleagues developed an *in vitro* model for human epithelial cell carcinogenesis to elucidate the role of HPV in cervical cancer [54]. These investigators showed that human epidermal keratinocytes become immortalized upon transfection of HPV16 DNA; however, they do not necessarily undergo malignant conversion. They added Kristen murine sarcoma virus (Ki-MSV), a virus containing an activated K-*ras* oncogene, to HPV-infected cells, which induced malignant transformation. This transformation was further enhanced by the addition of glucocorticoid. These findings from human epithelial cells in culture showed that malignant conversion requires other host factors, such as activated ras oncogenes in addition to HPV infection [54]. The high-risk E6 and E7 proteins also promote chromosomal instability by inducing centrosome abnormalities and foreign DNA integration and other mutagenic events in the cell [55].

Immune System Response to HPV Infection and Carcinogenesis

HPV has developed multiple mechanisms to evade recognition and elimination by the immune system, contributing to its survival and persistence, often through intimate and interpersonal contact. This process may contribute to HPV latency prior to

the induction of HPV-related malignancy. HPV has evolved to have a low copy number and limited protein synthesis while the host cell is in the undifferentiated state, reduced tumour antigen presentation, and subsequent adaptive immunity [56]. In contrast, once the cells undergo terminal differentiation and are no longer under strict immune surveillance, the number of HPV virions produced by the cell increases greatly. The host immune system has evolved to more closely monitor cellular damage and mutations in the undifferentiated cells and stem cells, compared with those cells that are differentiated with a finite life cycle [42].

For HPV to persist, it must repress both innate and adaptive immune responses [1]. HPV evades the innate immune response in several ways. One method is by suppressing the transcription of genes targeted by interferon, including *Stat*-1, and both the E6 and E7 proteins are involved in this process. HPV is a non-lytic virus, meaning that it does not induce cell lysis, viraemia, or other triggers of the inflammatory response that might facilitate antiviral immunity [48]. HPV suppresses the release of inflammatory cytokines, including interleukin (IL)-1, IL-6, tumour necrosis factor (TNF)-alpha and transforming growth factor (TGF)-beta [57]. Concurrently, it increases the expression of anti-inflammatory cytokines such as IL-10 [57].

As with all viruses, HPVs must be cleared by cell-mediated immune responses. However, HPVs have several mechanisms to disrupt the function and productivity of the cell-mediated response. First, LCs are the major dendritic cell (DC) subset found in squamous mucosal epithelium and are responsible for triggering the influx of CD8+ and CD4+ lymphocytes [58]. The infiltration of LCs and DCs is inhibited by the inflammatory cytokines promoted by HPV [59]. Furthermore, the L2 capsid protein suppresses maturation, migration and cytokine secretion by LCs [58].

HPVs also evade the cell-mediated immune response by downregulating expression of major histocompatibility complex (MHC) I [56]. Several HPV proteins have been implicated in this downregulation, including E5, E6 and E7 [60]. E7 may inhibit the transporters associated with antigen processing (TAP), which play a crucial role in MHC presentation of antigenic peptides to antiviral T-cells at the cell surface [61]. It has also been shown in bovine papillomaviruses that E5 prevents the expression of MHC I to the cell surface and causes retention of MHC I within the cell [60]. HPVs also inhibit the activity of natural killer cells, contributing to the viral avoidance of the immune system (Fig. 4.5) [56].

HPV Persistence and Host Factors

It is important to distinguish the persistence and maintenance of HPV infection from HPV carcinogenesis. For HPV to reproduce and reinfect other cells, its DNA must be present in an episomal state in a differentiating cell and continue to produce the L1 and L2 capsid proteins [42]. Studies with cervical as well as oropharyngeal cancers have shown that in these cancers, the HPV DNA is almost always integrated into

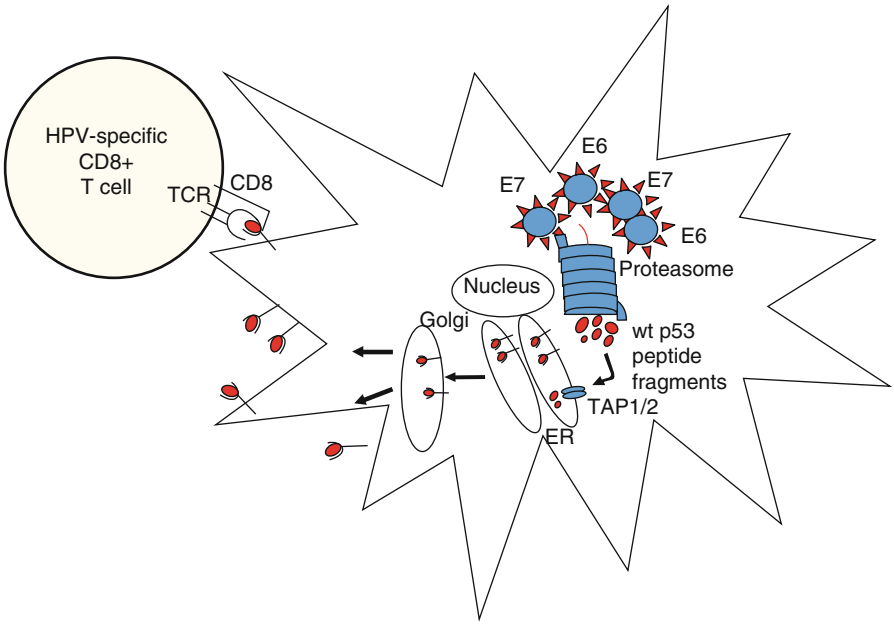

Fig. 4.5 HPV-infected cell lysis by cytotoxic T lymphocytes

the host genome, and therefore unable to replicate and produce virions [20]. Thus, once a cell infected by HPV becomes malignant, the virus can no longer reproduce.

The extended temporal lag between the onset of infection and carcinogenesis shows the ability of HPVs to survive as a virus in a latent or dormant state for several years. During this time period, however, a number of genetic abnormalities accumulate, leading to viral instability, all of which contribute to malignant transformation [2]. This understanding is crucial to the notion that HPV infection is necessary, but not sufficient, for malignant transformation. Indeed, transfection of full-length HPV16 DNA into normal squamous cells must be accompanied by additional factors, some of them genetic, to permit an invasive malignant phenotype, in addition to immortalization. Such host factors, as discussed previously, include *ras* oncogenes or P13K alterations [2, 54].

The various factors that contribute to malignant transformation in patients with HPV infection are not entirely understood. In the cervical cancer model, HPV carcinogenesis is affected by host and environmental factors, such as co-infection with *Chlamydia trachomatis*, smoking and possibly oestrogen. Studies on patients with HPV-positive OPSCC have shown that smoking increases the risk of productive HPV infection, disease recurrence and poorer prognosis [13, 62, 63]. However, it is unknown whether smoking increases the risk of developing cancer once a person is already infected with HPV.

Immunosuppression is known to increase susceptibility and persistence of viral infections. Co-infection with human immunodeficiency virus (HIV) has been also

shown to promote HPV oncogenesis directly. Specifically, in studies on women with cervical cancer, the HIV-encoded tat protein enhances the expression of the HPV E6 and E7 proteins [64, 65]. This observation is consistent with the increased prevalence of HPV-associated OPSCC in HIV-positive individuals [66].

Factors that contribute to HPV persistence and malignant transformation, other than immunosuppression and genomic instability within the host cell, remain unknown. HPV-related cervical cancer provides a guide to understanding the oncogenic mechanism of HPV in the oropharynx; however, important differences exist. For one, HPV-positive cervical cancer is characterized by a progression of premalignant lesions, ranging in intensity from cervical intra-epithelial neoplasia (CIN) I–III [67]. Moderate dysplasia (CIN II) and severe cervical dysplasia or carcinoma *in situ* (CIN III) have the potential to progress to malignancy if left untreated [68]. This model of premalignant progression has not been shown clearly in the oropharynx. Although cervical cancer models provide a framework for our understanding of HPV, much remains unknown regarding the oncogenic mechanism of HPV in the oropharynx.

Summary

The biology of HPV is centred on its ability to invade epithelial cells and persist in a non-immunogenic state, replicating its viral genome using host cell machinery and rapidly turning over and shedding with desquamated cells. In most cases, the host's innate and adaptive immune surveillance systems identify the virus and resolve the HPV infection. However, in some cases, HPV infection persists over many years, allowing for the accumulation of cellular damage and genomic instability. This genomic instability, induced in part by high-risk HPV E6 and E7 oncoproteins, leads to the malignant transformation of infected cells. Each viral protein contributes to this malignant transformation of cells, which is a terminal or abortive event in the life cycle of high-risk HPV. The importance of persistence of HPV and the multiple factors that contribute to its malignant progression remain crucial areas of investigation in HPV-related oropharyngeal cancer.

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The Diagnosis of HPV-Related HNSCC: Recognition of Its Microscopic Appearance and the Use of Ancillary Detection Assays

5

William H. Westra and Justin A. Bishop

Introduction

Squamous cell carcinoma (SCC) of the head and neck (HNSCC) has long been regarded as a homogeneous disease entity. Important distinctions between anatomical sites and natural histories have, in large part, been ignored, given the uniformity of histopathology and response to treatment. Recent work suggests considerable differences between some HNSCCs that go beyond variations related to tumour site and stage. In particular, a subset of HNSCC is now known to be associated with HPV [1, 2]. These HPV-related HNSCCs (HPV-HNSCCs) occur more frequently in younger, male patients. They occur most frequently in the oropharynx, and are associated with improved clinical outcomes [3, 4]. In effect, recognition of this HPV association has amounted to the identification of a new and distinct disease entity.

HPV-HNSCC is also distinct at the microscopic level [5, 6]. Its morphological features stand apart from those of tobacco-related cancers, such that the recognition of HPV-HNCC begins at the microscope. This review gives details of the microscopic appearance of the more prototypic HPV-HNSCC, and draws attention to morphological variants that deviate from the prototypic HPV-HNSCCs in ways that can cause diagnostic confusion. An awareness of this appearance can (i) alert the pathologist to the presence of HPV, (ii) inform the interpretation of various histological parameters as they relate to tumour grade and the presence of invasion, and (iii) enlighten communication with the treating physician, and (iv) guide the interpretation of ancillary HPV tests.

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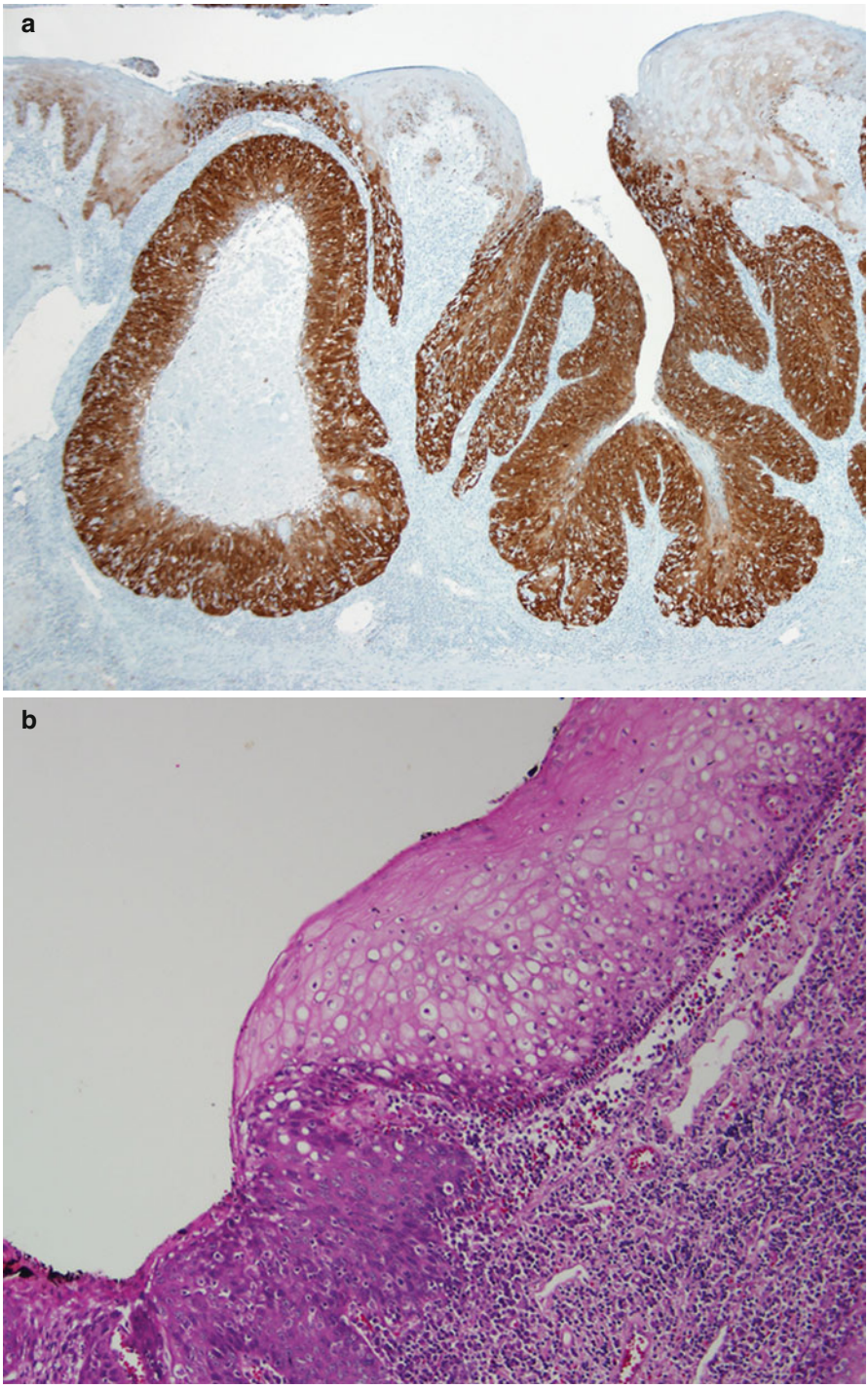
The role of ancillary testing to confirm HPV status is by no means restricted to mere prognostication. As more is understood of the unique natural history of HPV-HNSCC, applications for HPV testing will undoubtedly continue to increase. Detection of HPV is emerging as a valid biomarker for discerning the presence and progress of disease, encompassing all aspects of patient care, from early cancer detection [7], to more accurate tumour staging [8, 9], to selection of patients most likely to benefit from specific treatments [10], to post-treatment tumour surveillance [11, 12]. As for clinical research, study design and data analysis must incorporate HPV status into the next generation of clinical trials. Knowledge of HPV status is compulsory for meaningful comparison of treatment responses for patients enrolled in these trials.

No standard approach for HPV testing of clinical samples is available currently. Instead, methods of HPV testing across laboratories vary considerably, reflecting the biases and tendencies of individual investigators, and the cost-to-benefit ratio of each technique [13]. Detection strategies vary not just in design, but in their detection targets. These targets have included HPV DNA, HPV RNA, viral oncoproteins, cellular proteins, and HPV-specific serum antibodies. In the ongoing effort to establish a consensus approach for HPV testing, the challenge for the oncological community is to implement standardized HPV testing using a method that is highly accurate, technically feasible, cost-effective, and readily transferrable to the diagnostic pathology laboratory.

The Microscopic Appearance of HPV-HNSCC

HPV infection is strongly correlated with oropharyngeal location, particularly the palatine and lingual tonsils [1, 14]. This preferential targeting probably reflects complex biological interactions between HPV and the highly specialized lymphoepithelium lining the tonsillar crypts, known as the reticulated epithelium [15]. Involvement of the tonsillar surface, when it occurs, is generally a secondary phenomenon reflecting colonization of the surface epithelium, as the carcinomas spill over from the tonsillar crypts. The transition between HPV-HNSCCs and the adjacent surface epithelium tends to be abrupt without transitional zones of epithelial precursor lesions (Fig. 5.1). Indeed, the histological progression through the sequential stages of dysplasia culminating in carcinoma in situ and invasive growth that characterize non-HPV-HNSCCs is not generally evident for HPV-HNSCCs. The inability to characterize histologically the early stages of HPV-induced tumorigenesis continues to deter efforts to assess cancer risk and diagnose early cancers.

Fig. 5.1 HPV-related tumorigenesis tends to target the epithelium lining the tonsillar crypt. A p16 immunohistochemical stain is used to highlight tumour distribution in the tonsil (**a**, p16 immunohistochemistry). The transition of the tumour with the surface epithelium is abrupt without transitional zones of epithelial dysplasia (**b**, haematoxylin and eosin stain)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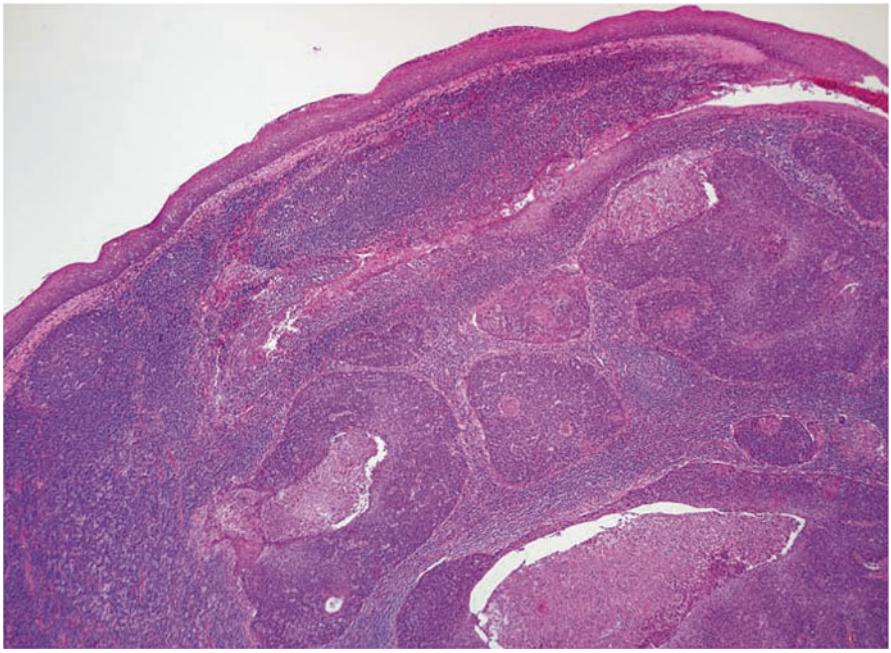

Fig. 5.2 HPV-related squamous cell carcinoma of the tonsil. The tumour grows as expanding lobules beneath the surface epithelium. Central necrosis within the tumour lobules is a frequent finding

As these carcinomas infiltrate, they tend to invade as sheets, lobules or ribbons of cells. Central necrosis within expanding tumour lobules, sometimes giving rise to cystic degeneration, is a frequent finding (Fig. 5.2). Invasive growth often does not elicit a strong desmoplastic stromal reaction. Instead, the tumour nests are often surrounded by a zone of lymphoid cells. The degree to which these lymphoid cells permeate the tumour lobules as tumour infiltrating lymphocytes (TILS) is highly variable. When the TILS are numerous, they can disrupt the tumour lobules into cords and individual cells.

The tumour cells display a high nuclear to cytoplasmic ratio, syncytial cytoplasm without intercellular bridges, and lack significant cytoplasmic keratinization. These cellular features can impart a distinct basaloid appearance (Fig. 5.3). In cytological preparations they are seen as cohesive sheets and clusters of cells with hyperchromatic, pleomorphic, and overlapping nuclei (Fig. 5.4) [16]. Their high nuclear to cytoplasmic ratio is in contrast to conventional keratinizing HNSCC, in which the keratinized cells exhibit abundant orangeophilic cytoplasm.

In lymph node metastases, the presence of cystic degeneration is a common finding in HPV-HNSCCs, and its presence should warrant strong consideration of a metastasis from the lingual and/or palatine tonsils [17]. These squamous-lined cysts of the lateral neck are sometimes clinically and histologically mistaken for

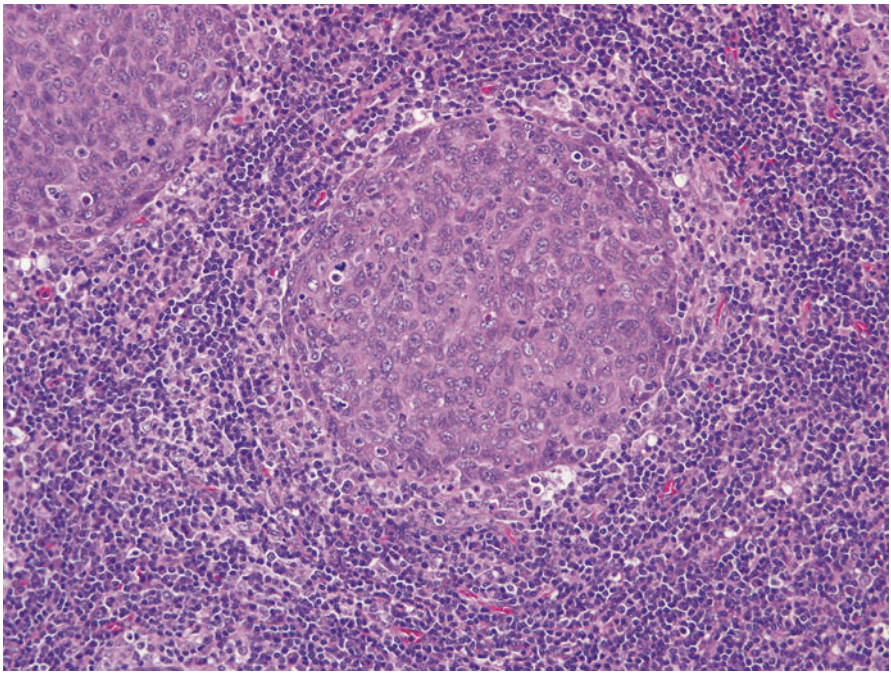

Fig. 5.3 Nests of invasive HPV-related tonsillar carcinoma in a lymphoid stroma. The absence of keratinization, cell borders and abundant cytoplasm (i.e. high nuclear to cytoplasmic ratio) often imparts a 'basaloid' appearance

branchial cleft cysts [18, 19]. Fine-needle aspiration (FNA) of the cyst contents gives rise to specimens that are highly degenerated. A definite cytopathological diagnosis may not be possible if the aspirate does not incorporate the cyst lining.

Histological Grading of HPV-HNSCC

Tumour grade is a semi-quantitative measurement of differentiation expressed as the degree to which a tumour resembles the normal tissue from which it arises. As a rule of thumb, the more poorly differentiated a tumour, the more aggressive its behaviour. HPV-HNSCCs are widely perceived as poorly or undifferentiated carcinomas. This perception is largely based on the immature appearance of a tumour cell that widely diverges from the stratified squamous epithelium that lines the surface of the tonsil. Using the surface epithelium as a point of reference for phenotypic divergence, however, may not be appropriate for those HPV-HNSCCs arising from the tonsillar crypts. HPV-HNSCCs often retain the appearance of the reticulated epithelium from which they arise, and thus might best be regarded as highly differentiated tumours on the basis of this more suitable comparison (Fig. 5.5) [5]. Recognition of HPV-HNSCC as well differentiated, not poorly or undifferentiated,

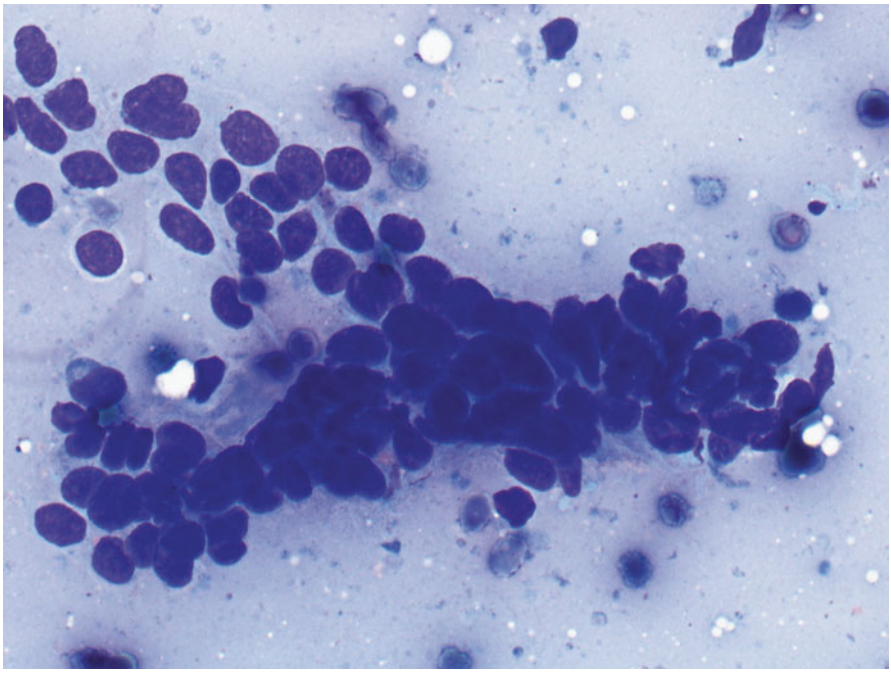

Fig. 5.4 On cytological preparations, HPV-related squamous cell carcinoma is seen as cohesive clusters of cells with hyperchromatic and overlapping nuclei. The nuclear to cytoplasmic ratio is high, and evidence of cytoplasmic keratinization often is absent

is appropriate as it more accurately reflects histogenic derivation and more fittingly associates tumour grade with expected clinical behaviour.

Few studies have tried to identify histopathological parameters that might help identify the subgroup of HPV-HNSCC associated with unfavourable clinical outcomes. Lewis et al. made a preliminary observation that the presence of anaplasia and tumour cell multinucleation are predictive of poorer clinical outcomes in patients with HPV-positive OPCs, but this initial observation has yet to be confirmed by others [20].

Carcinoma *In Situ* Versus Invasive Carcinoma

For squamous neoplasia of the head and neck, invasive tumour growth is microscopically recognized as rupture of the basement membrane, irregular extensions of tumour cells into the stroma underlying the surface epithelium, and subsequent incitement of a desmoplastic stromal reaction (e.g. fibrosis, oedema and chronic inflammation). For HPV-related OPCs, the difficulty in distinguishing carcinoma in situ from invasive carcinoma is heightened by the following factors: (i) origin from the tonsillar crypts beneath the surface epithelium; (ii) the oft absent tell-tale sign of

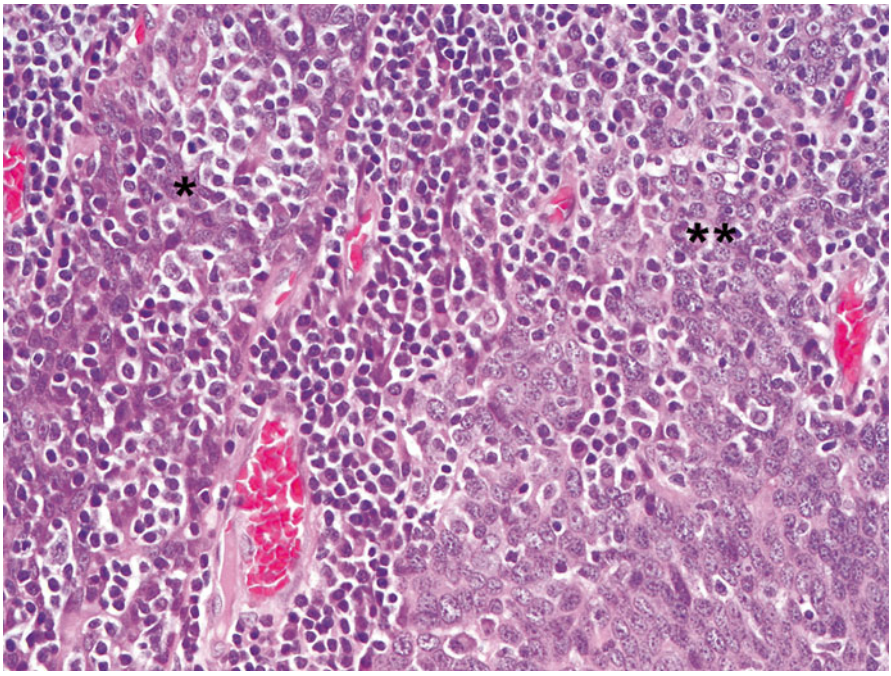

Fig. 5.5 This HPV-related squamous cell carcinoma (*right, double asterisk*) retains a phenotypic resemblance to the non-neoplastic reticulated epithelium lining the tonsillar crypt (*left, single asterisk*)

invasion, namely stromal desmoplasia; (iii) the blurred junction of the reticulated epithelium and the underlying lymphoid stroma; and (iv) the porous nature of this epithelial/lymphoid junction where the basal cell layer is incomplete and its supporting basement membrane is disrupted and non-contiguous; [21] and (v) the well recognized propensity of small, sometimes occult, tonsillar carcinomas to metastasize to regional nodes in the absence of clear-cut stromal invasion. In effect, the time-honoured microscopic approach to the recognition of tumour invasion may not be valid for those HPV-HNSCCs arising from the tonsillar crypts. Until the histological progression of HPV-related neoplasia of the tonsils is better characterized and the critical transition marking infiltrative growth is more clearly demarcated, an aggressive approach that regards all HPV-related neoplasia of the tonsils as potentially malignant, even in the absence of those histological features that have been traditionally used to diagnose invasion, may be warranted.

Morphological Variants of HPV-HNSCC

Subsets of HPV-HNSCC deviate from the morphological prototype, and these are set apart as morphological variants of HPV-related HNSCC. To date, these variants

include papillary SCC [22], adenosquamous carcinoma [23], basaloid SCC [24], lymphoepithelial-like carcinoma [25], and small-cell carcinoma [26, 27]. With the notable exception of HPV-related small cell carcinoma (*see below*), morphological variance does not seem to influence clinical behaviour. A few of these variants warrant further comment because of their propensity to be confused with other subtypes of HNSCC.

HPC-HNSCC with Basaloid Features

The lobular arrangement of compact tumour cells, with a high nuclear to cytoplasmic ratio in the absence of keratinization, confers a distinctly 'basaloid' appearance to HPV-HNSCCs. When the basaloid morphology is highly developed, HPV-related SCC may be histologically indistinguishable from the basaloid squamous variant of SCC—a variant of HNSCC that is set apart as a distinct subtype on the basis of its striking basaloid morphology and its aggressive clinical behaviour (Fig. 5.6). At the microscopic level, the distinction between HPV-HNSCC and the basaloid variant of HNSCC can be difficult, as both tumours are characterized by the lobular growth of basaloid cells. Morphological similarities aside, HPV-HNSCCs do not share the same aggressive clinical behaviour that characterizes the basaloid variant of SCC [24, 28, 29]. In their evaluation of basaloid

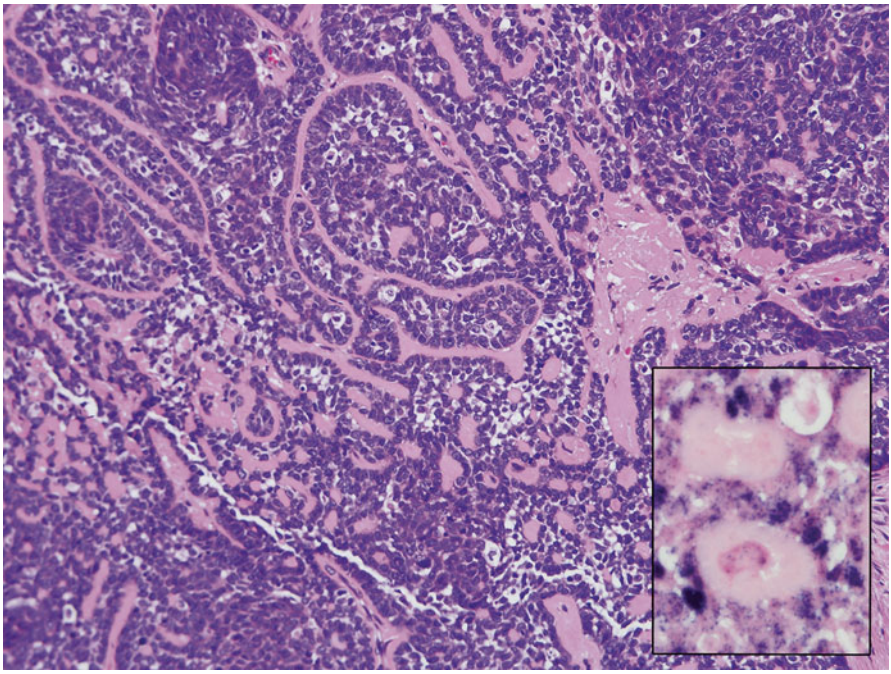

Fig. 5.6 HPV-related squamous cell carcinoma with basaloid features, including nests of basaloid cells with intercellular deposition of eosinophilic stromal matrix. The tumour cells harbour high-risk HPV, as shown with an HPV DNA in situ hybridization probe (inset)

HNSCCs, Begum and Westra found that the presence of HPV was significantly associated with improved overall survival, even though patients with HPV-HNSCCs were more likely to present with lymph nodes metastases [24]. In effect, the detection of HPV essentially downgrades what would otherwise be regarded as a high-grade HNSCC.

HPC-HNSCC with Lymphoepithelial Features

Some HPV-related SCCs demonstrate lymphoepithelial features, including tumour cells with syncytial cytoplasm, vesicular nuclei and large central nucleoli dispersed in an inflammatory background as cell clusters or single cells (Fig. 5.7) [25, 30]. When these lymphoepithelial features are highly developed, an HPV-HNSCC may be mistaken for an Epstein–Barr virus (EBV)-induced undifferentiated carcinoma of the nasopharynx. On the basis of this morphological overlap, one cannot assume an EBV-driven process by phenotype alone. Failure to recognize that the lymphoepithelial phenotype is not restricted to nasopharyngeal carcinomas may be particularly problematic when these features are encountered in cervical lymph node metastases. Assumptions about nasopharyngeal origin based solely on the morphological findings run the risk of inappropriately diverting treatment away from the oropharynx and toward the nasopharynx. Accordingly, testing for both HPV and

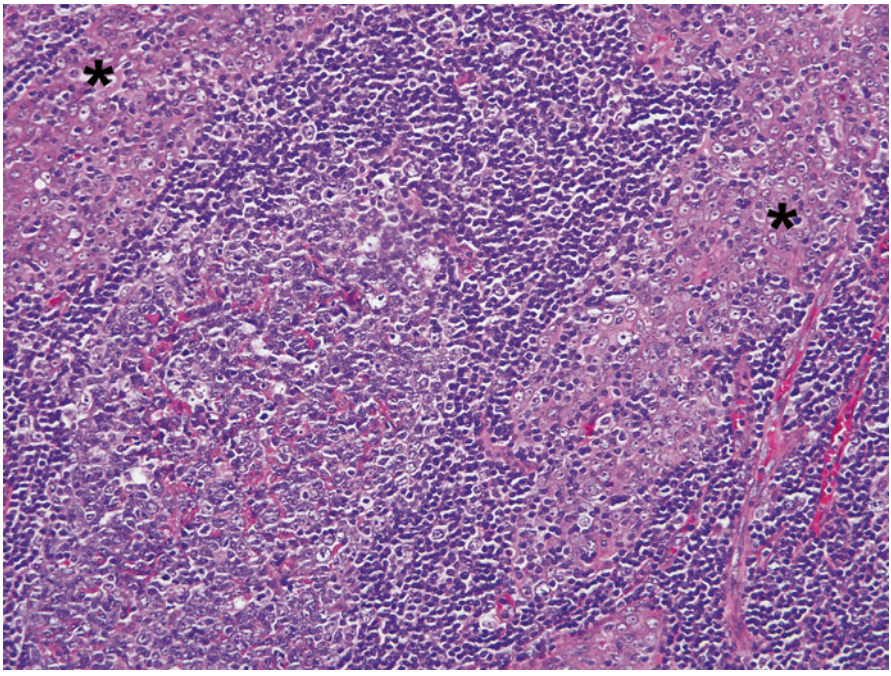

Fig. 5.7 HPV-related squamous cell carcinoma with lymphoepithelial features. Nests of tumour cells (*asterisks*) exhibit syncytial cytoplasm, vesicular nuclei and prominent nucleoli. The surrounding stroma exhibits a dense infiltrate of plasma cells and lymphocytes with a germinal centre formation

EBV is advisable when lymphoepithelial carcinomas are encountered as lymph node metastases in patients with occult primary tumours.

HPV-HNSCC with Small Cell Features

Recent reports have underscored the presence of an HPV-HNSCC variant characterized by well developed small cell features [26, 27]. This small cell variant consists of small anaplastic cells with hyperchromatic nuclei, scant cytoplasm, and immunohistochemical evidence of neuroendocrine differentiation (Fig. 5.8). Most of these cases arise in close association with a more conventional HPV-HNSCC, and the presence of HPV persists as these tumours undergo small cell transformation. Importantly, HPV does not appear to convey a favourable prognosis when its presence is detected in this small cell variant. HPV-related small cell carcinomas of the oropharynx appear to share the same aggressive clinical features of their counterpart in the uterine cervix and lung, where the small cell phenotype is associated with early distant spread and poor overall survival [26, 27]. Consequently, the small cell phenotype should be regarded as a poorly/undifferentiated form of HPV-related OPC in which tumour morphology supercedes HPV positivity as a prognostic indicator.

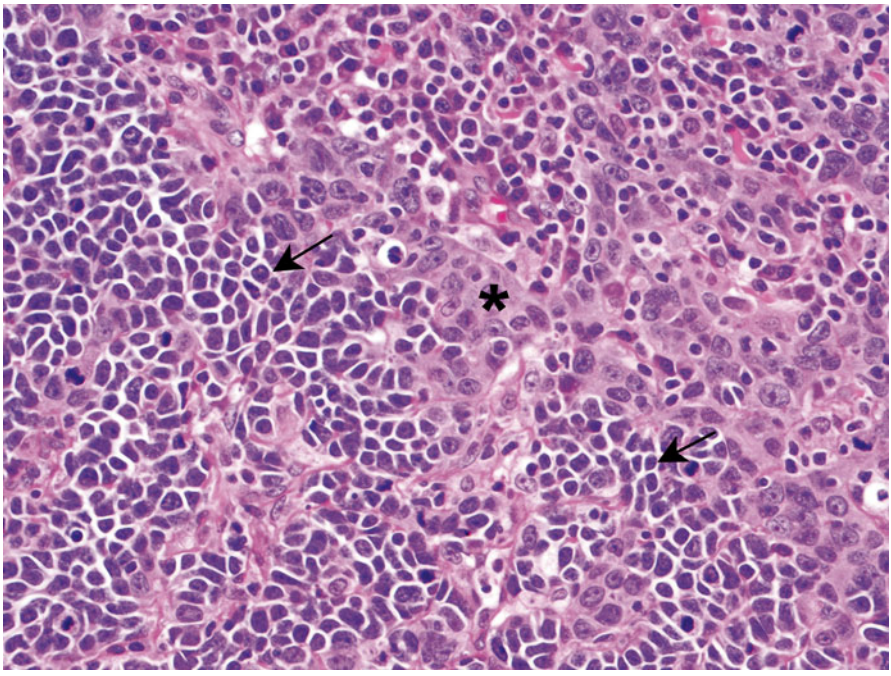

Fig. 5.8 Small cell variant of HPV-related carcinoma. This field shows cords of squamous cell carcinoma (*asterisk*) transitioning with sheets of small cell carcinoma (*arrows*). Both components were HPV-positive

HPV-Carcinomas of the Sinonasal Tract

Outside of the oropharynx, the only anatomical sub-site of the head and neck where HPV is detected in a significant percentage of carcinomas is the sinonasal tract [31, 32]. HPV has recently been detected in 21 % of sinonasal carcinomas implicating it as a relevant causative factor for carcinomas arising in the nasal cavity and paranasal sinuses [31]. Like their counterparts in the oropharynx, HPV positivity in sinonasal carcinomas tends to track with non-keratinizing SCCs; but HPV positivity is not restricted to this archetypal phenotype when dealing with sinonasal carcinomas. HPV analysis of a large and comprehensive group of sinonasal carcinomas has turned up a peculiar form of HPV-related carcinoma exhibiting features of a salivary gland carcinoma (Fig. 5.9) [31]. These tumours are characterized by solid and cribriform growth patterns, a dual population of ductal cells and myoepithelial cells, and an absence of significant squamous differentiation. Our group has tentatively designated these tumours as 'HPV-related carcinomas of the sinonasal tract with adenoid cystic-like features', drawing attention to the common tendency to confuse these tumours with adenoid cystic carcinoma on the basis of a few morphological similarities. Most HPV-related carcinomas of the head and neck are non-keratinizing SCCs that harbor HPV type 16, but initial research suggests these peculiar sinonasal tract carcinomas are usually infected with HPV type 33.

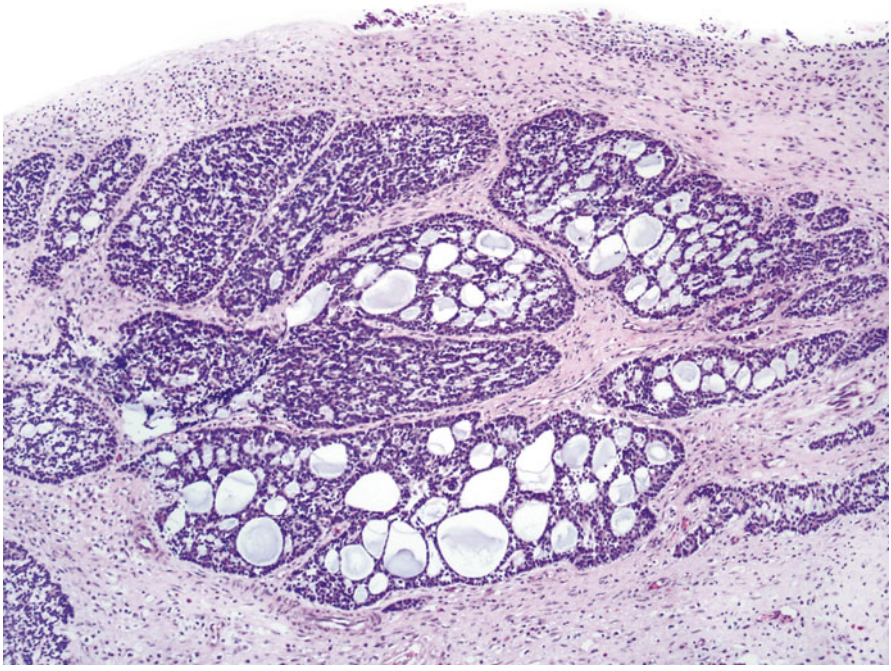

Fig. 5.9 HPV-related carcinoma of the sinonasal tract with adenoid-cystic features. The cribriforming architecture is highly reminiscent of adenoid cystic carcinoma

HPV Detection Strategies and Methods

When to Test for the Presence of HPV

HPV Testing of Non-oropharyngeal Carcinomas [OPCs]

Based on the localization of HPV-related HNSCC to the oropharynx, directives for routine HPV testing are generally restricted to those carcinomas arising from this specific anatomical site. At this site, HPV detection is regarded as a powerful biomarker, indicating a more favourable clinical outcome such that routine HPV assessment is becoming part of the standard pathological evaluation of all OPCs. Indeed, the College of American Pathologists, the American Joint Committee on Cancer, and the National Comprehensive Cancer Network have all recommended routine HPV testing as part of the standard pathological evaluation of oropharyngeal SCCs for the purpose of diagnosis and molecular tumour staging. The finding of HPV in <20 % of sinonasal carcinomas may support increased testing at this site as part of an investigational process, but expanding the scope of routine diagnostic HPV testing to non-oropharyngeal sites is not warranted until studies establish a clear relationship between HPV infection and a distinct clinical behaviour, including treatment responses. At sites that are not preferentially targeted by HPV, such as the oral cavity, larynx and hypopharynx, the likelihood that a positive HPV test truly reflects the presence of transcriptionally active and clinically relevant HPV infection may be unacceptably low (i.e. poor positive predictive value) [33, 34].

HPV Testing of Lymph Node and Lung Metastases

In malignant transformation of the tonsillar epithelium, HPV does not act through a 'hit and run' mechanism where its role is transient and limited to the initiation of tumorigenesis. Instead, the presence of HPV persists, and it is just as readily detected in metastatic implants as in the corresponding primary cancers [14]. Consequently, a lymph node metastasis is quite suitable as a substrate for HPV testing, obviating the need for additional tissue acquisition in those patients with small or even occult primary cancers. For those patients who present with neck metastases in the absence of an obvious primary tumour, HPV testing of a lymph node metastasis is an effective strategy for localizing the site of origin. In these patients, the detection of HPV in a lymph node metastasis is a reliable predictor of oropharyngeal origin [8, 9, 35]. Similarly, for the SCC in the lung of a patient with a prior HPV-related OPC, HPV detection provides a direct link between the two tumours and provides compelling evidence that the tumour in the lung represents a metastasis rather than a new lung primary [36, 37].

Methods of HPV Detection in Clinical Samples

A standard approach for HPV testing of clinical samples is lacking at present. In fact, methods of HPV testing across laboratories vary considerably, reflecting the biases and tendencies of individual investigators, and the cost-to-benefit ratio of

each technique [13, 38]. Detection strategies vary not just in design, but in their detection targets. These targets have included HPV DNA, HPV RNA, viral oncoproteins, cellular proteins, and HPV-specific serum antibodies. In the ongoing effort to establish a consensus approach for HPV testing, the challenge for the oncological community is to implement standardized HPV testing using a method that is highly accurate, technically feasible, cost-effective, and readily transferrable to the diagnostic pathology laboratory (Table 5.1).

The various tumour testing strategies that are currently available are guided by an understanding of HPV-induced malignant transformation of oropharyngeal epithelium, particularly its interaction with key components of the retinoblastoma (Rb) tumour suppressor gene pathway. The p16 tumour suppressor gene is a member of the INK4 class of cell-cycle inhibitors and represents a key component of the Rb pathway. The binding of the p16 tumour suppressor gene product with the cyclin-dependent kinases 4 and 6 blocks its interaction with the D-type cyclins, maintains the Rb gene in a hypophosphorylated state that binds E2F transcription factor and, in turn, prevents cell cycle progression [39]. In non-HPV-related HNSCC, the p16 tumour suppressor gene is usually inactivated by various genetic and non-genetic modifications, such that expression of its protein product is lost or dramatically diminished [40]. In contrast, integration of high-risk HPV into the host genome is followed by transcription of viral mRNA transcripts, translation of these transcripts into viral oncoproteins, disruption of the cellular machinery with altered expression of critical cellular proteins, including overexpression of the p16 tumour suppressor gene product. Each individual step along this pathway of HPV-driven tumorigenesis provides a unique opportunity for HPV detection. HPV detection strategies may look to detect the following: (i) HPV DNA, (ii) post-integration transcription of viral E6 and/or E7 mRNA transcripts, (iii) the viral oncoproteins E6 and E7, or (iv) altered expression of cellular proteins, such as overexpression of the p16 protein (Figs 5.10 and 5.11).

The ability to both recognize the presence of HPV and to discern its potential as a driving force of tumorigenesis is the key to developing any HPV detection strategy. For example, a given assay might be highly sensitive in its ability to detect trace amounts of HPV, but it may have no clinical value if it cannot discern an incidental virus (e.g. viral contaminant) from an active oncological agent. Evidence for transcriptional activation of the viral oncoproteins E6 and E7 is generally regarded as the gold standard method of clinically relevant HPV. In the absence of reliable immunohistochemical probes for E6 and E7 oncoproteins, detection of E6/E7 mRNA is the current standard by which the sensitivities and specificities of other detection assays are measured. Until recently, detection of E6/E7 mRNA has required RNA extraction from fresh or frozen tissues followed by polymerase chain reaction (PCR) amplification of viral RNA. Although the transfer of this technique to formalin-fixed and paraffin-embedded tissues has greatly expanded its application to clinical samples, it remains a technically challenging technique that is mainly restricted to the research laboratory. The ongoing challenge of HPV detection efforts has been to reproduce the accuracy and reliability of the PCR E6/E7 mRNA assay, using techniques that are easier and transferrable to the diagnostic laboratory.

Table 5.1 Comparison of HPV detection methods

Method	Advantages	Disadvantages
HPV DNA		
Consensus and type-specific PCR	High sensitivity	Inability to discern clinically relevant from irrelevant infections
Real-time PCR	High sensitivity and specificity Estimation of viral load	Requires complex tissue processing (e.g. microdissection, DNA extraction)
In situ hybridization	Optimized for fixed tissues Visualization of viral distribution Highly specific	Reduced sensitivity at low viral load
Hybrid capture 2	Can be applied to cytological sample No need for specimen processing	Further studies are needed to determine overall sensitivity and specificity
HPV RNA		
Reverse transcriptase PCR	Highly sensitive Highly specific	Limited to fresh frozen tissue
In situ hybridization	Highly sensitive Highly specific Can now be applied to fixed tissues	Technology not yet widely available
HPV proteins		
E6/E7 immunohistochemistry	Visualization of oncoprotein expression	Poor performance
Cellular proteins		
P16 immunohistochemistry	Optimized for fixed tissues Highly sensitive Strong correlation with HPV integration	Low specificity
Serum antibodies		
Anti-HPV protein antibodies	Minimally invasive No tissue requirement	Marker of life-time cumulative exposure to HPV
		Low sensitivity and specificity as cancer marker

Modified from Ref. [47] with permission

PCR-Based Methods Versus DNA *In Situ* Hybridization

Most laboratories that perform routine testing of clinical samples use one of two methods of HPV detection: PCR-based amplification and DNA in situ hybridization (ISH). PCR-based amplification of HPV DNA is a target amplification technique that is capable of amplifying trace DNA sequences in a biological sample which

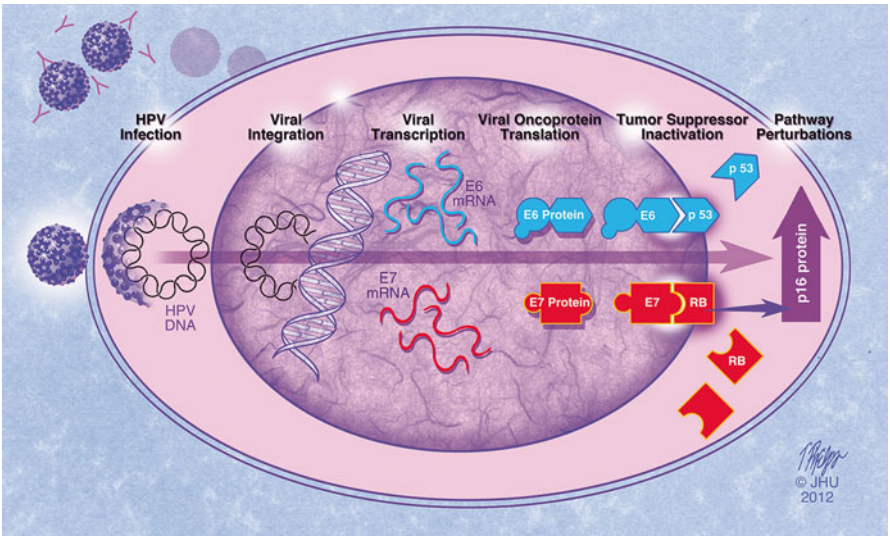

Fig. 5.10 The biology of HPV infection and malignant transformation provides several sequential points for HPV recognition. Concurrent evidence for HPV DNA, transcriptional activation and disruption of key growth regulatory pathways (e.g. retinoblastoma pathway) confirms the presence of clinically and biologically relevant HPV

contains heterogeneous cell types. The primers can be specific for individual HPV types or a target consensus sequence shared by multiple HPV types. When used as a non-qualitative technique, it provides no information regarding the abundance of a particular DNA species. DNA ISH is a signal amplification technique that utilizes labelled DNA probes complementary to targeted viral DNA sequences. The DNA probes may hybridize either to HPV type-specific DNA sequences or to a consensus sequence shared by multiple HPV types, or may be mixed in a single reaction to cover an extended range of HPV types. The performance of these two techniques is comparable, although a direct comparison suggests that DNA ISH may be a preferable tool for both practical and biological considerations for the following reasons: (i) the development of non-fluorescent chromogens allows visualization of hybridization using conventional light microscopy (Fig. 5.12); (ii) the introduction of various signal amplification steps has improved the sensitivity of ISH; (iii) adaptation of ISH to formalin-fixed and paraffin-embedded tissues has made this technique compatible with standard tissue processing procedures and consequently transferrable to most surgical pathology laboratories; and (iv) the visualization of viral distribution in tumour cells more reliably differentiates biologically relevant HPV infection from passenger virus or viral contaminant.

p16 Immunohistochemical Staining As a Surrogate Marker of HPV

Immunostaining for p16 protein has recently been regarded as a practical alternative or complementary procedure for HPV testing of OPCs, based on a high correlation between the HPV detection and p16 overexpression in recent studies

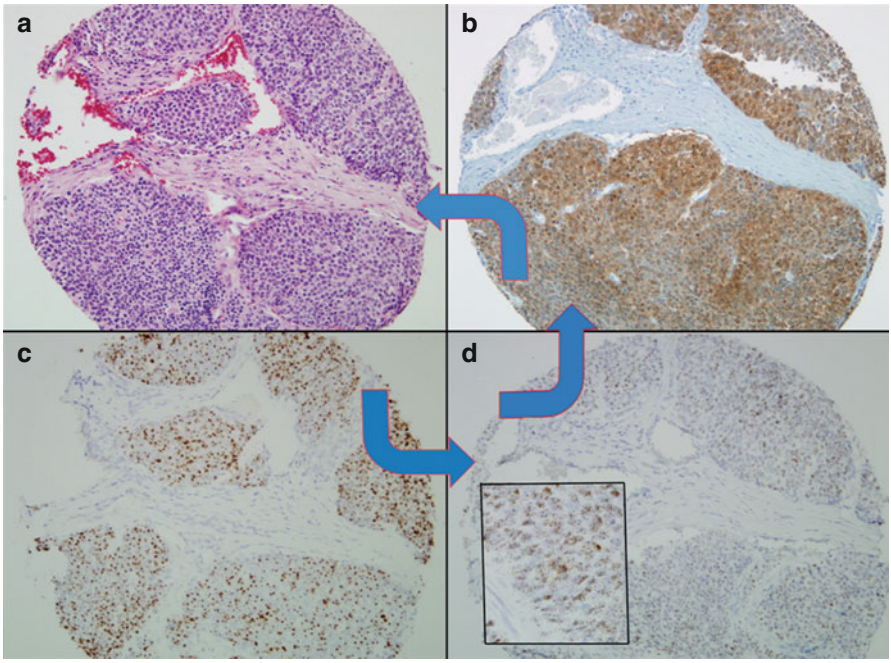

Fig. 5.11 The development of various assays permits visualization of the sequential biological steps of HPV-related tumorigenesis. The prototypic morphology of HPV-related oropharyngeal squamous cell carcinoma (**a**, haematoxylin and eosin stain) represents the culmination of viral infection, HPV DNA integration (**b**, HPV DNA in situ hybridization), viral mRNA transcription (**c**, mRNA E6/E7 in situ hybridization), and translation of viral oncoproteins driving the overexpression of the cellular p16 protein (**d**, p16 immunohistochemistry)

(Fig. 5.12) [41–43]. Indeed, the simplicity, low cost and high sensitivity of p16 immunohistochemistry have prompted consideration of replacing more intensive ISH and PCR-based methods as a stand-alone HPV test [42]. The mechanistic link between HPV DNA integration and p16 expression, however, is neither direct nor exclusive. The Rb gene may be inactivated by mechanisms other than E7 oncoprotein expression, yet still resulting in high levels of p16 expression [44].

To be truly useful as a surrogate marker of HPV infection, the interpretation of p16 immunohistochemistry must be informed by various histological, anatomical and clinical considerations [45]. First, p16 IHC may substitute for HPV testing when strong staining is present in the nucleus and cytoplasm of the tumour cells throughout all or most (>70 %) of the tumour. Focal or weak staining should be supported by other forms of HPV testing. Second, whereas the sensitivity and specificity of p16 staining as a marker of HPV infection is sufficiently high to serve as a reliable test for SCCs of oropharyngeal origin, these values are either unknown or unacceptably low for HNSCCs arising in non-oropharyngeal sites. Third, interpretation of p16 staining must be informed by the morphological features of the tumour, as outlined above. p16 IHC staining may substitute for HPV detection in those

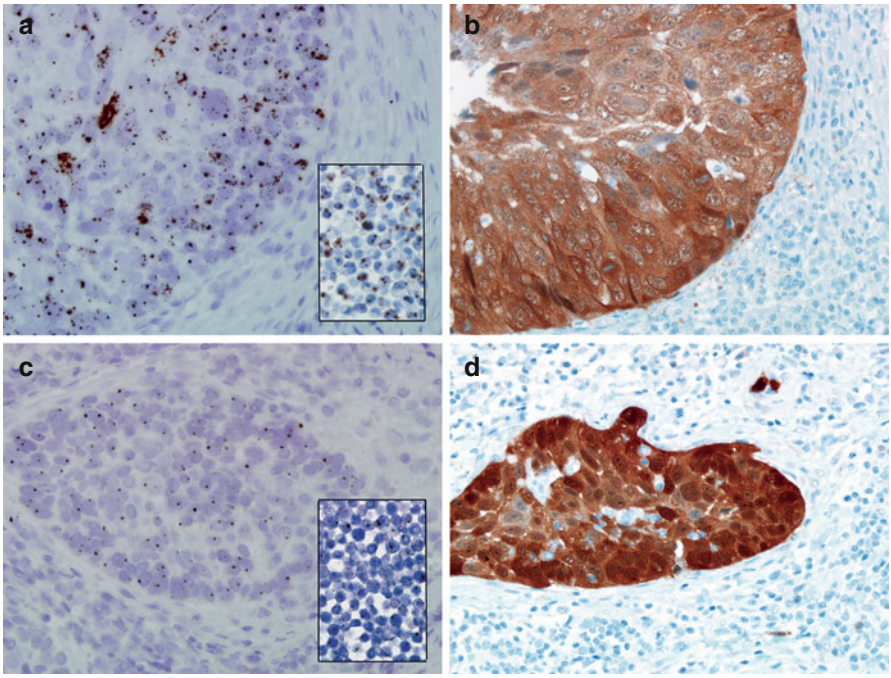

Fig. 5.12 Like DNA in situ hybridization (**a**, **c**), p16 immunohistochemistry (**b**, **d**) permits the direct microscopic visualization of HPV in the tissues. p16 immunostaining tends to be strong and diffuse whether the virus is present at high copy numbers (**a**, **b**) or low copy numbers (**c**, **d**) (inset **a**, CASKI cell line with high HPV copy number; inset **c**, SIHA cell line with low HPV copy number)

OPCs that show the typical morphology of HPV-related HNSCC. Additional HPV testing should be performed in p16-negative OPCs that exhibit classic HPV-related histomorphology, and in p16-positive OPCs that do not exhibit classic HPV morphology. Fourth, p16 IHC is currently used primarily as a prognostic indicator for patients with OPC, and any expanded clinical role for HPV detection may necessitate more stringent detection methods.

Single Versus Multimodality HPV Analysis

The power of p16 immunohistochemical staining lies in its high sensitivity for detecting all high-risk types of HPV, but it suffers from suboptimal specificity. Conversely, DNA ISH offers a high degree of specificity, but at the expense of suboptimal sensitivity. When making a head-to-head comparison between the available techniques by using HR-HPV E6/E7 mRNA expression as the gold standard for HPV status, Jordan et al. [43] reported that both p16 immunohistochemistry (sensitivity 96.8 %; specificity 83.8 %) and HPV16 ISH (sensitivity 88 %; specificity

94.7 %) showed excellent performance in HPV detection. The appropriate assay to use either singly or in combination will depend upon the clinical implications of a false-positive or false-negative test. The call for p16 immunohistochemistry as a standalone test may be appropriate in its limited role as a prognostic indicator, but may be less suitable in a more expanded role as in guiding clinical trial eligibility and clinical management. Where the stakes are truly high, such as selection of patients for 'de-escalation' or therapeutic vaccine trials based on HPV-status, the high specificity of combined p16 immunohistochemistry and HPV ISH is necessary to avoid possible under-treatment of patients with HPV-unrelated cancers.

Future Directions in HPV Testing

RNA *In Situ* Hybridization [ISH]

The ultimate goal of any developing technology for HPV detection in clinical samples is to approach the gold standard for sensitivity and specificity while maximizing efficiency, simplicity, reproducibility and transferability to the routine diagnostic laboratory. Although the most direct and compelling evidence of HPV-related tumorigenesis is the documentation of transcriptionally active HPV in tumour cells, the detection of E6/E7 transcripts is technically challenging. The recent development of RNA ISH probes complementary to E6/E7 mRNA now permits direct visualization of viral transcripts in routinely processed tissues (Fig. 5.11C) [33, 46, 47]. Testing for HPV E6/E7 transcripts by RNA ISH is an ideal platform for HPV detection in clinical samples. First, it confirms the presence of integrated and transcriptionally active virus by permitting the visualization of viral transcripts directly in tissue sections. Second, it is technically feasible and easily transferable into the diagnostic pathology laboratory. Indeed, the imminent availability of the HPV RNA ISH method to a widely available automated staining platform promises to enhance standardization across diagnostic laboratories, decrease turnaround time for large case volumes, and improve reproducibility among clinical trials. Third, the transcription of viral mRNA provides a natural target amplification step that may dramatically improve viral detection in clinical samples and clarify the status of those perplexing tumours that are p16-positive by immunohistochemistry but HPV-negative by DNA ISH [33]. Fourth, it is prognostically useful that the presence of E6/E7 mRNA transcripts is tightly coupled to the expression of other powerful prognostic markers (e.g. p16 expression), and strongly correlates with patient outcomes [46]. Use of HPV RNA ISH has so far been restricted by limited access to the technology, but broader availability may pave the way for routine implementation in the diagnostic laboratory.

Liquid Phase Assays for HPV Detection in Cytological Samples

The widespread implementation of HPV testing in the clinical arena awaits the development and refinement of methods for testing cytological specimens (e.g. FNAs of neck metastases and brushings of primary tumours). Use of aspirated cells as a substrate for HPV assessment could facilitate the diagnosis of an HNSCC, direct the search for its site of origin, predict clinical outcome, and select patients most likely to benefit from

immunology-based therapy—all while abrogating the need for tissue acquisition via a surgical procedure. The few studies that have addressed HPV testing of cytological samples have primarily tried to adapt tissue-targeted approaches (e.g. p16 immunohistochemistry and HPV ISH) to archived cytological specimens [8, 35, 48]. In most instances, HPV testing of cytological specimens is restricted to a small subset of cases in which ample cellular material is available for the construction of cell blocks. Broad-based application awaits the development of strategies that can be applied to aspirated cells without the need for high cellularity and specimen processing.

One very promising approach that does not require the processing of cytological specimens as tissue blocks involves the use of liquid phase assays that are already in widespread use for routine assessment of cervical cancer risk. Direct transfer of cytological samples into the liquid media minimizes specimen preparation and eliminates the need for specimen processing as cell blocks. The Hybrid Capture 2 (HC2) HPV DNA test is an in vitro nucleic acid hybridization assay with signal-amplification using microplate chemiluminescence for the detection of 18 oncogenic types of HPV DNA in cervical specimens. In a limited study of 24 patients, the HC2 assay was found to be highly reliable in discerning the HPV status of HNSCCs [49]. In this study population, 100 % correlation was seen between HC2 analysis of the cytological specimens (brushings and FNAs) and DNA ISH analysis of the paired surgical resection specimens (primary tumour resections and lymph node metastases): HPV was consistently detected by the HC2 assay in the 14 patients with HPV-positive HNSCCs, but it was not detected using the HC2 method in any of the cytological preparations from HPV-negative controls (non-neoplastic tonsils and lymph nodes) or the HPV-negative HNSCCs [49]. Thus, all cytological preparations were correctly classified using the HC2 assay.

Similar to the HC2 method, the Cervista® HPV HR test is a liquid phase assay that is clinically validated for HPV detection in cervical cytological specimens. Its analytical sensitivity is comparable to that of HC2 assay, but the addition of a housekeeping gene as an internal control to ensure sufficient cellularity diminishes the likelihood of false-negative results. In one feasibility study, this method was found to be effective in detecting the presence of HPV types 16 and 18 in FNAs from patients with metastatic HNSCC, again showing that HPV detection and genotyping can be achieved without the need for tissue acquisition or complex specimen processing [50].

Summary Points

- HPV-HNSCCs consistently arise from the lingual and/or palatine tonsils, are unassociated with dysplastic changes of the surface epithelium, are often non-keratinized or partially keratinized, and frequently exhibit a basaloid appearance.
- The morphology of HPV-related OPC is retained when these tumours metastasize to regional and distant sites. When encountered in cervical lymph node metastases, particularly when accompanied by cystic change, these features point to the oropharynx as the site of tumour origin.

- Determination of HPV status is important as it impacts all aspects of patient care, including prognosis, tumour staging (i.e. identifying site of tumour origin) and selection of patients most likely to benefit from certain therapeutic options.
- In the ongoing effort to establish a consensus approach for HPV testing, the challenge for the oncological community is to implement standardized HPV testing using a method that is highly accurate, technically feasible, cost-effective, and readily transferrable to the diagnostic pathology laboratory.
- Each currently used test is associated with its own unique strengths and weaknesses.
- Use of p16 immunohistochemical staining may be acceptable as method of HPV detection, provided it is used and interpreted in a defined context that takes into account certain anatomical factors, histological findings, and staining characteristics. When clinical management issues are at stake (e.g. de-intensification of therapy), other detection assays should be included.
- Development of detection assays optimized for cytological samples may open the door to more widespread implementation of HPV testing, and may obviate the need for tissue acquisition.

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Clinical Management of HPV-Related Oropharyngeal Cancer

6

Marshall R. Posner

Introduction

The demographics and prognosis of locally advanced head and neck squamous cell cancer (HNSCC) have changed dramatically over the past two decades. Epidemiological evidence has revealed a significant increase in the incidence of oropharyngeal cancer (OPC) in North America and Europe [1–3]. Molecular studies of oropharyngeal tumours have revealed that this increase is due to a rise in the incidence of tumours containing human papillomavirus (HPV), most specifically HPV16. Evidence shows that HPV16 is the molecular cause that mechanistically drives the development and viability of the cancer cells [4]. HPV-associated OPC (HPVOPC) presently accounts for ~70 % of OPC seen in the USA, and an increasing fraction of these malignancies is seen in Europe [1, 2, 5].

Our current understanding is that two clinically significant carcinogenic and biological pathways exist that cause OPC. One is environmentally and smoking-related OPC (EROPC), which is caused by smoking, alcohol and environmental carcinogens. Carcinogenesis in this entity is independent of HPV; tumours are HPV-negative and result from p53 mutations and loss of cell-cycle regulation, usually via p16 deletion, methylation or mutation. The other pathway is HPVOPC, in which carcinogenesis is driven by HPV. Carefully conducted studies have shown that high-risk HPVs are identified rarely outside the oropharynx [6, 7]. A small number of true larynx cancers occur which are identifiable as HPV16-positive; however, the biology of the rare HPV-positive tumours in the larynx may be different from HPVOPC. Some tumours classified as oral cancers, supraglottic cancers or nasopharynx cancers occur in watershed areas within and adjacent to the oropharynx and are probably misclassified because of local spread of an OPC. These are biologically-related OPCs and

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not oral cancer per se, having been misclassified. Thus, the vast majority of HPV-related HNCs are HPVOPC. In addition to having distinct biologies, HPVOPC and EROPC have responded differently to therapy [8, 9].

The Current State of the Art—Therapeutics

Existing data from clinical trials and retrospective analysis of patient materials suggests the two forms of OPC—HPVOPC and EROPC—are biologically distinct and have distinct prognoses. The relative paucity of genetic changes in HPV-positive HNC is in sharp contrast to what is observed in HPV-negative HNC, and is mechanistically related to the direct effects of viral proteins in inactivating regulators of key cellular processes [8–12]. In contrast to EROPCs, HPVOPCs usually do not contain p53 mutations [9, 11]. Similarly, p16, an inhibitor of mitosis in the Rb pathway of cell growth, is often upregulated in HPVOPC, whereas it is lost in EROPC, as a consequence of viral alterations in Rb function [9, 13, 14]. Up-regulation of p16 can be seen in about 20 % of non-HPV-related cancers, including other sites in the head and neck; however, p16 appears to be up-regulated in >95 % of HPVOPCs, making it a good screening tool and a potentially important diagnostic tool [7, 14, 15]. The biological differences in the carcinogenesis of these tumours are expressed in their distinctly different responses to therapeutic interventions.

Retrospective studies in unselected patients indicate that patients with HPVOPC have a significantly better prognosis than patients with EROPC, regardless of therapeutic intervention [16–18]. These differences are profound and only fractionally related to the improved demographics of HPVOPC patients who tend to be younger and have less exposure to smoking and alcohol [19–21]. In one retrospective study from Denmark in which radiotherapy (RT) was the sole therapy, p16 was used as a surrogate for HPV [22]. In this randomized study of a radiation sensitizer, the control arm of RT only was analysed for p16 expression. Five-year survival was 62 % among p16-positive patients compared with 26 % in p16-negative patients. Locoregional control (LRC) was 58 % versus 28 %, respectively. The data support the notion that p16-positive, and, hence by implication, HPV-positive, tumours are more responsive to RT and more likely to be cured. One caveat of the study relates to the determination of p16 positivity. A significant fraction of p16-positive tumours were not of oropharyngeal origin and HPV status was not obtained, hence the relationship of p16-positive and HPV positivity, especially HPV16 positivity, in this study is less strong than might be expected [22]. These results illustrate limitations with many studies; first because of using p16 as a surrogate marker for HPV carcinogenesis and second because of the need for uniform diagnostic criteria to help identify HPV in HPVOPC for therapeutic management.

In another trial reported by Licitra et al., a cohort of surgically treated patients were evaluated retrospectively to determine tumour HPV status [23]. Surgery alone was effective therapy for a small group of patients who received a surgical resection only and no RT. The HPVOPC surgery-only patients were spared the long-term consequences of radiation. However, selection for surgery only was not

well explained [23]. New function sparing surgical technology has increased the rate of patients with OPCs receiving surgery as a first therapy. Prior to the development of transoral laser microdissection (TLM) and transoral robotic surgery (TORS), OPC patients were treated with non-surgical therapy to spare surgical morbidity [24]. These technologies substantially reduce surgical morbidity in OPC. Several retrospective studies have been published; however, it is difficult to tease out the role of postoperative treatments in this population as most of these studies are not protocol-driven trials with clear inclusion and exclusion criteria. What is evident in these studies is that those patients with p16-positive tumours have done very well in survival and functional outcomes and HPV16 positivity and/or p16 has been predictive of this quantitatively larger overall survival (OS) than might have been expected [25].

Eastern Cooperative Oncology Group (ECOG) 2399 is a phase II study of operable patients treated with an aggressive sequential therapy (ST) regimen of induction chemotherapy followed by chemoradiotherapy (CRT) for organ preservation [26]. This study prospectively evaluated HPV status and was the first prospective study of HPV and therapeutic outcome. The investigators reported a significant difference in survival for patients with HPVOPC compared with EROPC. Sixty-two patients with OPC were treated, all the 38 HPVOPC patients were HPV16-positive as opposed to other high-risk HPV. Even among this small group of patients, OS was significantly better for the HPVOPC group. An analysis of failure in this population revealed several important features. First, LRC was much better in the HPVOPC patients. Additionally, co-morbidities and noncancer deaths were much reduced. The impact of therapy on LRC was most striking: there was a 95 % versus 67 % LRC rate in HPVOPC versus EROPC, respectively [26, 27]. Thus, LRC seems to be significantly improved in HPVOPC patients treated with induction chemotherapy and CRT compared with EROPC patients. In a trial to identify patients with a good prognosis, the University of Michigan used a single cycle of induction chemotherapy to select operable OPC patients for RT or surgery [28, 29]. They analysed 42 informative cases for HPV and HPV copy number and found that responses to induction chemotherapy correlated with HPV status, as did disease-specific survival. The relationship to copy number of HPV in the tumours was less clear although there was a suggestion that increasing copy number was associated with a better prognosis [28].

Recently, results of retrospective analyses of survival and HPV status were reported from two large phase III trials comparing CRT regimens in locally advanced HNSCC [15, 30]. In both trials there were insufficient patient numbers to report a treatment effect; however, the impact of HPV on survival, regardless of therapeutic assignment, was highly significant. The Radiation Therapy Oncology Group (RTOG) study 0129 has the most extensive data and retrospectively analysed outcomes in 323 out of 433 OPC cases [15]. In RTOG 0129, patients were randomized between CRT with accelerated fractionation with cisplatin versus regular fractionation and cisplatin. The OS and progression-free survival (PFS) at 3 years were 82 % and 74 % in HPVOPC compared with 57 % and 43 % for EROPC, respectively. A careful analysis of failure and death revealed an LRC rate of 86 % versus

65 % for HPVOPC versus EROPC, and a second primary tumour rate of 6 % versus 15 %, respectively. Non-cancer deaths also occurred in 9 % and 19 %, respectively. All these data support a better outcome for HPVOPC, much of which is found in improved LRC, some fraction of which is explained by less co-morbidity.

Tumour HPV16 status, survival and demographics in subjects with OPC treated in TAX324, a large international randomized phase III clinical trial, were also evaluated retrospectively. TAX 324 compared survival between ST with TPF or PF followed by CRT with weekly carboplatin in patients with locally advanced HNSCC [30]. The data show a significant difference in survival outcome and patterns of failure between patients with HPVOPC and EROPC and significant differences in demographic characteristics in the populations. Of the 501 patients entered on TAX 324, 264 (53 %) were identified as having OPC. Of these 264 subjects, 119 had tissue prospectively collected and 111, or 42 % of all OPC cases, were analysable for HPV16 status and constituted the study population.

The demographic data and test results for group comparisons are shown in Table 6.1. Fifty-six (50 %) patients were identified as HPV-positive (HPVOPC) and 55 (50 %) as HPV negative (EROPC). Both HPVOPC and EROPC cases were divided evenly with regard to treatment assignment and sex. HPVOPC cases were significantly younger compared to EROPC cases (56 vs. 58 years, $p = 0.02$), performance status (PS) was also significantly different between the two populations, despite selection for good PS in patients for enrolment in this trial. Thus, 77 % of HPVOPC patients were PS 0 compared with 49 % of the EROPC patients ($p = 0.003$).

Results for OS, PFS and site of failure for the 111 patients analysed for HPV16 status, independent of the treatment arm, are also shown in Table 6.1 [30]. HPVOPC

Table 6.1 Clinical stage, demographics and 5-year outcomes of HPV-positive and HPV-negative oropharyngeal cancer patients treated on TAX 324 trial

	HPV+(%) n = 56	HPV−(%) *n* = 55	p value
Median age in years (range)	54 (39–71)	58 (41–78)	0.02
T stage			0.001
T1–T2	50	20	
T3–T4	50	80	
N stage			0.03
N0–N1	23	33	
N2–N3	77	67	
Performance status (WHO)			0.003
0	77	49	
1	23	51	
Overall survival (alive)	79	31	<0.0001
Progression-free survival	73	29	<0.0001
Local regional failure	13	42	0.0006
Distant metastases	5	11	NS
Died without recurrence	9	22	0.07

and EROPC surviving patients were followed for a median of 83 months and 82 months, respectively. At the time of analysis, 79 % of HPVOPC patients were still alive, and their PFS rate was 73 %, compared with the 31 % OS and 29 % PFS for the EROPC patients (both $p < 0.0001$). The median OS time for the EROPC patients is 21 months (95 % CI 13–49 months), whereas median survival has not been reached in the HPVOPC group after almost 7 years median follow-up. The reduction in mortality was 80 % in HPVOPC compared with EROPC (HR = 0.2; 95 % CI 0.10–0.38; $p < 0.0001$). Analysis of the site of failure, as shown in Table 6.1, revealed a significant reduction in LRF (13 % vs. 42 %, $p = 0.0006$) and slightly reduced distant failure in the HPVOPC patients compared with the EROPC patients. Total disease failures showed a significant difference (16 % vs. 49 %; $p = 0.0002$) and a borderline improvement in deaths without recurrence ($p = 0.07$). These data indicate that LRC is the major parameter contributing to improved survival and that PS and co-morbidities among EROPC patients account for another fraction of mortality.

The data presented here from multiple trials clearly shows that survival is significantly better for HPVOPC than with EROPC, and that improved survival is primarily a function of HPV status and improved LRC. A fraction of this improvement is also related to a reduced co-morbidity. In Table 6.2, comparable data on OS and PFS for TAX 324, RTOG 0129 is shown for a qualitative comparison and demonstrates that survival and PFS in RTOG 0129 and TAX 324 are similar at the 3-year analysis time point and significantly better for HPVOPC [15, 30, 31]. The RTOG re-analysed their data recently and suggested that HPVOPC can be divided into good prognosis and intermediate prognosis on the basis of smoking, stage and nodal involvement [32]. The RTOG study retrospectively showed that smoking is a significant prognostic factor and smoking history is an important component of decision-making for therapy and consideration for studies. In the original analysis of RTOG 0129, a history of smoking above and below 20 pack-years (PY) correlated to meaningfully different population outcomes [15]. More recently, a review of their data suggested that current smoking was a highly negative prognostic factor, regardless of HPV status, and that a 5 PY history had a significant impact on prognosis, although the original paper suggested 20 PY was a realistic cut-off [32]. Others have reported similar results [33]. Unreported long-term follow-up from the RTOG study beyond 3 years limits the reliability of the survival data. Further, the absolute differences in survival between a 20, 10 or 5 PY history are in the order of

Table 6.2 Comparison of 3-year overall survival (OS) and progression-free survival (PFS) data for HPV-positive and HPV-negative oropharyngeal cancer (OPC) cases in two recent US Trials: RTOG 0129 and TAX 324

Study	OPC patients tested	% of all OPC tumours	Therapy	HPV+(%)	HPV-positive vs. HPV-negative	
					3-year OS (%)	3-year PFS (%)
RTOG 0129	323	75	Chemoradiotherapy	65	82 vs. 57	74 vs. 43
TAX 324	111	42	Sequential therapy	50	87 vs. 41	81 vs. 33

2–5 % at the time of analysis. So, while the survival analysis based on smoking exposure results in statistically significant comparisons of high risks between exposures, the numeric impact on survival may be trivially small and unhelpful in making treatment selections in which toxicity of therapy is a major consideration.

Other prognostic features are hard to identify in the literature. The University of Michigan recently proposed that matted nodes in the neck were a poor prognostic feature indicative of a high risk of distant metastases and local regional failure, regardless of HPV status [34]. Earlier literature suggested that positive margins and extracapsular nodal extension in tumours were poor prognostic features as were bilateral and contralateral lymph node involvement and nodal involvement lower in the neck [35]. One troublesome difficulty in staging HPV-positive patients is a high rate of cystic nodal disease, which although large and hence technically of higher stage, is not believed to be as poor a prognostic finding as solid nodes or multiple solid nodes [34].

Current Therapeutic Recommendations for HPVOPC

Patients with HPVOPC have more options for curative therapy than patients with EROPC because of improved prognosis and new technologies. Early-and intermediate-stage patients can be treated with surgery if the primary tumours are lateralized. When primary tumours involve the lateral base of tongue, exploration of the contralateral neck can provide evidence that allows a reduction of the radiation fields and sparing of the opposite neck. Standard therapy calls for adjuvant RT in stage 3 and stage 4 patients and adjuvant CRT in those with poor prognostic features [36]. The value of surgery in this setting is a reduction in radiation and chemotherapy if no poor prognostic features (e.g. multiple nodes, matted nodes extracapsular extension [ECE], lower level nodes) are identified. Preoperative testing should eliminate a clinically advanced population from surgery. Surgical therapy for midline tongue lesions remains morbid because of a bigger impact on function, early lymphatic spread and difficult margin control compared with lateralized lesions. For these tumours, adjuvant radiation must be more extensive, bilateral and morbid despite surgery; therefore, these patients should be advised non-surgical therapy. The value of surgery in HPVOPC is to provide part of the curative therapy and reduce the amount and field of radiation leading to less morbidity and late consequences for the patients [24].

For patients with advanced primary tumours or midline tongue involvement, the impact of surgery and radiation on function is less clear. A primary CRT approach with cisplatin-based CRT appears to be best. Evidence suggests that erbitux may be equivalent to cisplatin-based CRT, but this has not been established to date and a comparison trial is under way (RTOG 1016). Weekly cisplatin treatments would be more tolerable, less toxic and more likely to be completed and are a reasonable alternative to bolus cisplatin. Similarly, for patients with poor pathological findings, such as ECE, positive margin, or multiple positive lymph nodes, or lower level nodes, a postoperative CRT course is indicated on the basis of current evidence [35, 37].

Lymphovascular invasion (LVI) and perineural invasion (PNI), historically markers of poor prognosis, are lesser indications for CRT in HPVOPC and in non-smokers [35–37]. In smokers, CRT might be more likely to be helpful with these more minor indications.

In patients with poor prognostic findings, or with clinical and radiographic indications for extensive RT fields, CRT and/or high-dose RT, surgical resection may not be advantageous. Despite these patients having a technically resectable primary tumour, they should proceed with non-surgical combined modality therapy. Both ST and CRT are indicated and treatment should result in a high rate of cure in otherwise healthy patients. Evidence of advanced nodal diseases, matted nodes, low neck nodes or T4 tumours might sway decision-making towards a sequential approach because of a higher rate of distant metastases; however, evidence is lacking to support one approach over the other. For these patients, treatment decisions are based on the experience of the treating physicians and a multidisciplinary team approach.

Anecdotal evidence suggests that patients presenting with primary disease and metastases or with an early recurrence after curative CRT may be cured by a combined modality approach. Aggressive systemic chemotherapy and localized therapy to bulk disease areas or boney metastases with CRT may be effective in primary presentations. Systemic chemotherapy after removal of oligometastases or induction followed by CRT may lead to curative outcomes in first recurrences. Otherwise there is no specific therapy today for HPV-positive recurrent and/or metastatic patients. Before embarking on a curative course it is important to confirm HPV status. p16 is not adequate when assessing potential metastatic lesions as deriving from the original HPV-positive primary tumour. For example, many squamous cancers of the lung and oesophagus are p16-positive and HPV-negative [38].

Current Therapeutic Trials

In general, patients with HPVOPC are young and will live for prolonged periods. They are at high risk for long-term toxicity and mortality from therapy [39]. While the long-term consequences of chemotherapy and modern surgery for HNC are relatively constrained, high-dose RT and CRT substantially impact on local tissues and organ function and result in a significant rate of late mortality and morbidity in patients [40–44]. Studies are now being designed to reduce the impact of RT and CRT for patients. Identifying appropriate end-points and study arms which will allow an early assessment of outcomes will be problematic, particularly for equivalence studies wherein survival differences are small, and in which prolonged time periods and large patient numbers are necessary to accurately assess outcomes. For ST as given with TAX 324, 3-year PFS might be an appropriate end-point. The same may not be possible for CRT. The best example of changing outcomes in CRT trials would be R91-11, in which a premature negative conclusion regarding the efficacy of induction therapy was published with the early analysis. Late failures, toxicity and morbidity, a hallmark of upfront cisplatin-based CRT trials, led to equivalence

between induction therapy and CRT for laryngectomy-free survival at 5 years, and more importantly a non-significant relative 10 % improvement in OS in the PF induction arm compared with the CRT arm, which included an every 3-week bolus cisplatin treatment for three cycles during RT [15, 30, 41, 45].

Radiation dose reduction trials are either being planned or have been completed by the ECOG. ECOG 1308 is a phase II trial treating patients with p16-positive resectable OPC with an aggressive regimen of induction chemotherapy using weekly paclitaxel, cisplatin every 3 weeks, and cetuximab weekly for three cycles, followed by cetuximab + RT to a total radiation dose of 5400 cGy for responders. Non-responders receive standard RT with cetuximab. This trial completed accrual and is currently being analysed. Unfortunately, without a control arm evidence will be lacking to support this regimen as being equivalent to standard therapy and it should not be used in the community. ECOG is opening a randomized phase II trial in operable patients with early- and intermediate-stage disease to assess a reduced dose of RT versus a reduced dose of CRT for LRC. A similar trial of surgery with TLM is opening at Washington University and with TORS at Mount Sinai Medical Center. These studies are aimed at reducing RT-associated early and late morbidity through reduced doses.

The Mount Sinai School of Medicine surgical/radiation dose reduction trial explores a very different hypothesis. The Sinai Robotic Surgery (SIRS) trial is a surgical study in which operable patients are assessed pathologically after a TORS resection, and those with good prognostic features are followed without RT or CRT. CRT or systemic therapy is reserved for salvage therapy for those who relapse. Because of the excellent responses of HPVOPC and local control, it is hypothesized that survival will be equivalent to upfront RT and that at least 50 % patients will avoid radiation. Those with varying poor prognostic features will receive either reduced dose RT for modest features, such as LVI or PNI, or reduced RT with chemotherapy for ECE or positive margin. This is a radical departure from standard practice, which will be carefully monitored during the study and over the first 5 years of follow up. The Mount Sinai Medical Center is also leading the Quarterback Trial. This is a randomized trial in which patients presenting with localized HPV-positive disease who are inoperable, have poor prognostic features, or would not be spared CRT with an operation are treated with a course of dose-reduced TPF induction chemotherapy, followed by randomization 2:1 to CRT with 5600 cGy plus carboplatin, or 7000 cGy and carboplatin—the control arm. This is the only randomized dose reduction trial for this population. The end-points of this randomized trial are equivalence of the reduced dose RT for PFS at 3 years and reduced morbidity from the lower RT dose.

The RTOG has initiated a randomized trial to compare cisplatin to erbitux-based CRT, with full-dose RT in both arms. Although this is called a dose-reduction trial, it does not address the substantial morbidity of full-dose radiation given in both arms. Patients are likely to show little improvement in their long-term toxicity in either arm, although this will answer the question of equivalence between cisplatin and erbitux as CRT for this disease.

Future therapeutic trials will include therapeutic vaccines and immune modulators to alter or boost the immune response to HPV. It is also likely that there will be HPV-specific therapeutics developed to attack viral-specific processes, such as p53 binding,

which are necessary for cancer cell survival. Finally, molecular antiviral approaches with anti-sense DNA or silencing RNA therapies may be envisaged. Much needs to be learned before we understand how best to apply any of these approaches.

Whereas it is tempting to reduce therapy for HPVOPC, clinicians are urged not to unilaterally lower radiation doses for HPV-positive patients outside of a clinical trial. Harmful differences in outcomes with dose reduction will not be discernible in a single practice or academic centre. It is only with protocol-driven prospective clinical trials with adequate numbers that sufficient evidence will be available to make confident, evidence-based recommendations for therapy. In addition, all of the trials described are gathering tissue and biomarker data, which will inform the next generation of studies. We would urge clinicians to participate in clinical trials so that these important questions can be answered as quickly and accurately as possible for our patients.

Summary Points

- Patients with HPVOPCs are younger and healthier than those with traditionally EROPCs.
- HPVOPCs are more responsive to almost any therapy than EROPCs and have much better local and regional control. The majority of patients with HPVOPC will survive their cancer and live longer, with the consequences of curative therapy.
- Clinical investigation today is focused on improving treatment-related morbidity using new technologies and reducing long-term RT-associated toxicities. Future therapies, which are in development, will be directed at vaccines, immune modulation and anti-HPV-specific molecular targeting.

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The Role of Vaccines for HPV-Related Head and Neck Cancers

7

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The Immune System and HPV Infection

Several lines of evidence highlight the importance of a functioning immune system in controlling human papillomavirus (HPV) infection and its associated neoplasms. Harnessing the immune system to both prevent and treat HPV infections has therefore been an area of significant research effort, innovation and progress over the past decades. Although much of the research performed thus far has been on genital HPV infections, the interaction between oral HPV infection and the immune system are beginning to be explored.

First and foremost is the observation that the majority of immune-competent individuals infected with anogenital HPV infection are able to clear the infection without any clinical manifestation. In the anogenital region, only 10 % of infected individuals develop HPV-related lesions [1]. Histological examination of spontaneously regressing cervical HPV-related lesions demonstrates the infiltration of CD4+ and CD8+ T-cells, whereas these immune cells are lacking in the lesions of patients with persistent disease [2]. Initial studies on the natural history of oral HPV infection suggest that similar to anogenital HPV, immunocompetent individuals clear infection with a median time to clearance of 6.3 months [3, 4].

With most HPV infections cleared by the immune system, HPV persistence may be a sign of integration of viral DNA into the host genome, as this is a strong predictor of risk of progression from viral infection to anogenital disease [5].

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HPV late genes (L1 and L2) and some early genes (E1 and E2) are commonly deleted with viral integration; with the disruption of E2 expression, expression of the E6 and E7 oncoproteins is unregulated [6, 7]. Concurrently, E5 upregulates the expression of epidermal growth factor receptor (EGFR) within the cell [8]. This leads to the overexpression of proto-oncogenes and repression of p21 (cyclin-dependent kinase inhibitor 1A) expression, a regulatory protein that controls cell apoptosis and differentiation [8]. The interruption of cellular mechanisms that regulate apoptosis and the cell cycle results in dysregulated cell cycle proliferation, delayed cellular differentiation, increased frequency of spontaneous and mutagen-induced mutations, and increased chromosomal instability [9]. Thus, the overexpression of the viral oncoproteins, E6 and E7, drives and maintains the neoplastic process.

Immunocompromised individuals, such as organ transplant recipients on immunosuppressive medications [10] and patients infected with human immunodeficiency virus (HIV) [11] have been documented to have significantly increased rates of cervical HPV infections and of HPV-related diseases [12, 13]. Once these individuals cease their immunosuppressive medications or recover their immune cell counts, they are able to clear the virus and any associated lesions [14]. Furthermore, preclinical studies have reported that animals immunized with vaccines that elicit HPV-specific CD8+ T-cells show regression of established HPV-related cancers [15, 16].

Clinically, systemic immune responses against anogenital HPV infection are often detected as humoral responses generated against the configured L1 pentamer, but this response is weak, inconsistent and may not even protect against future re-infection [17]. For high-risk types, the seroconversion rate is 30–50 % after a documented infection [18], and in patients with HPV-associated cancer 30–50 % have detectable antibody levels against the L1 protein from the causative viral type [19]. If present, the antibody titres can persist for many years even after the anogenital infection is cleared; therefore, seropositivity is a useful marker for past infection rather than current infection. Humoral immune responses have also been detected to the ‘early’ viral proteins. Patients with cervical cancer can have detectable antibody levels to E7 [20] and HPV-related head and neck cancer (HNC) patients have detectable antibody levels to E6 or E7 [21]. Serum analysis for HPV antibodies may therefore have promise as a screening method for HPV-associated oropharyngeal cancer (OPC) [22, 23].

Instead of the humoral response, it is the cell-mediated immune responses, or HPV-specific CD4+ and CD8+ T-cells, which have been shown to be most critical in clearing established cervical lesions. The virus-specific CD4+ and CD8+ T-cells coordinate to clear chronic viral infections [24], and patients with evidence of previously cleared HPV 16 infections have strong detectable T-cell responses to viral proteins [25]. Deficits in T-cell responses have been documented in patients with cervical cancer and in patients with cervical intraepithelial neoplasia (CIN) [26, 27]. The relative contributions to this lack of T-cell response from inherent host genetic factors [28] and/or viral mechanisms to escape immune recognition are not yet known.

Viral Mechanisms to Evade the Immune System

HPV has evolved multiple mechanisms to evade host immunological responses [29, 30], thereby succeeding in causing HPV-related lesions. The first adaptive mechanism for escaping immune surveillance is the coordination of viral replication to cellular differentiation. In the uterine cervix, within the organization of stratified squamous epithelium, the degree of immune surveillance decreases considerably in the superficial, keratinized layers. HPV takes advantage of this organization by tightly regulating its own replication with differentiation of the keratinocyte. The virus evades cytotoxic T-lymphocyte (CTL) responses by expressing a minimal level of viral gene products in the keratinocytes of the basal cell layer and upregulates expression of viral gene products with differentiation and upward migration of keratinocytes, away from areas of active immune surveillance. In addition, HPV does not cause lysis of keratinocytes—rather, virions are released through the mechanical breakage of surface epithelium and thereby minimize any associated inflammatory response. In this way, HPV replication is a local phenomenon with minimal systemic immune activation.

In the tonsil, HPV infects the reticulated epithelium lining the deep tonsillar crypts. Recent data suggest that the deep crypts of tonsils may be immune-privileged sites that can inhibit the effector function of HPV-specific T-cells and thereby facilitate immune evasion at the time of initial HPV infection [31]. This provides a biological explanation of how a virus can infect a lymphoid organ, such as the tonsil and base of tongue, yet still evade immune recognition and clearance.

Viral proteins also have local inhibitory effects on inflammatory cytokines to dampen both innate and adaptive immune responses. The HPV E5 and E7 proteins downregulate expression of the major histocompatibility complex (MHC) class I molecules, which inhibits viral antigen processing and presentation to the immune system [32, 33]. E6 and E7 have also been shown to reduce expression of Toll-like Receptor 9 [34] and cytokines in cervical cancer, such as IL-8 [35], and IL-18 [36], which are all potent pro-inflammatory molecules. A blunted response to interferon (IFN)-gamma has also been observed in HPV infections [37, 38]. One mechanism for the blunted response in cervical disease is a reduction in the expression of interferon regulatory factor (IRF-1), which is a transcription factor that mediates interferon responses [39]. Because interferon signalling is a critical component in the activation of many aspects of both the innate and adaptive immune responses, as well as a potent antiproliferative agent, HPV thus disables a major mechanism of immune surveillance to oncogenic transformation.

Prevention of HPV Infection Through Vaccination

Vaccines for the Prevention of Cancer

The discovery that the L1 viral proteins self-assemble into viral-like proteins (VLPs) in the absence of viral DNA was the critical first step in developing preventive

vaccines [40]. Recombinant techniques could then be used to produce hollow VLPs, which could induce protective L1 antibody levels that can prevent new HPV infection without the risk of being exposed to an infectious virus [41].

Large-scale trials to test the efficacy of this vaccination strategy were carried out in the early 2000s, and have led to the approval of two vaccines for the prevention of HPV-related diseases and cancers. A quadrivalent (HPV types 6/11/16/18) VLP vaccine was approved in June 2006 for administration to females aged 9–26 years. Randomized, double-blinded, placebo-controlled trials evaluated the ability of the quadrivalent vaccine to prevent HPV-related anogenital diseases (genital warts, vulvar, vaginal or cervical neoplasia—FUTURE I), and high-grade cervical intraepithelial neoplasia (FUTURE II), respectively, in females [42, 43]. The trials showed 100 % protection against the development of anogenital lesions related to the four viral types. As a result, in 2007 the Centers for Disease Control (CDC) Advisory Committee on Immunization Practices (ACIP) recommended vaccination for all girls aged 11–12 years, before the age of sexual debut and the risk of exposure to HPV.

A second prophylactic bivalent (HPV types 16/18) VLP vaccine was approved in October 2009 for use in females aged 10–25 years. Although this vaccine does not include HPV types 6 and 11, a head-to-head clinical trial showed that the bivalent vaccine induced higher antibody titres against the high-risk viral types, compared with the quadrivalent vaccine [44]. Both of these vaccines are given as three doses administered over a 6-month period. The length of protection against HPV infection achieved through these vaccines is being studied currently in order to determine whether booster vaccinations would be required to maintain immune protection against HPV.

In October 2009, the quadrivalent vaccine was approved for males aged 9–26 years. A randomized, placebo-controlled, double-blinded trial reported on the safety of the quadrivalent vaccine and on its efficacy in preventing the development of HPV-related external anogenital lesions in boys and men [45]. The study enrolled 4065 healthy boys and men between 16–26 years of age across 18 countries. Subjects in a per-protocol population were documented to be negative for exposure to the HPV types at the time of enrolment, and these subjects received all three vaccinations. The intention-to-treat population included subjects whose baseline HPV exposure history was unknown and these subjects received either the vaccine or placebo. The study reported that the quadrivalent vaccine efficacy was 90.4 % against the development of genital lesions related to the HPV-6, 11, 16, or 18 viral types in HPV-naïve patients; the efficacy dropped to 65.5 % in patients with an unknown HPV exposure history. Based on these study results, in October 2011, the CDC ACIP recommended that boys aged 11–12 years receive the vaccine. These studies demonstrate that vaccinating both boys and girls is beneficial. However, the benefits of the HPV vaccine will not be appreciated for several decades, and it is unclear how the vaccine will impact oral mucosal immunity or oral HPV infection and, thus, the development of HPV-related HNCs.

Implementation of Vaccination and the Elimination of Population Risk

If high HPV vaccination rates are achieved (>85 %), herd immunity might be achievable. Indeed countries with high female vaccination coverage have seen a dramatic reduction in genital warts among boys as well as girls, because of herd immunity [46]. However, even with strong governmental recommendations for HPV vaccination, many societal and logistical barriers exist to achieving high vaccine coverage rates in the USA. A nationwide survey of 13–17-year-old girls by the CDC in 2010 found that less than half (48.7 %) had received at least one dose of the three-part HPV vaccination series, and of the teenagers who commenced the vaccination series, 30 % did not complete the series [47], Several reasons are believed to account for the low vaccination rates. These include: (i) certain parents' misconception that giving the vaccine promotes sexual activity among adolescents; [48] (ii) an inability to reach out to this target population, as it is one not served previously by immunization programmes in the past and/or not receiving any routine primary preventive care; [49] (iii) the associated high costs for the vaccine and its administration ($130 for each shot); (iv) lack of insurance coverage for the vaccination series; [50] and (v) the time commitment to receive three doses of vaccine over a 6-month period.

As increasing numbers of boys and girls receive the vaccine, the overall prevalence of HPV and its associated diseases in the population should decrease, indirectly benefiting those who may not have been vaccinated. Using disease modelling of the 14 high-risk HPV types, it is estimated that a 47 % reduction in cervical cancer would result if 50 % of the female population is vaccinated against HPV 16/18, with 25 % of the prevented cases occurring in females who never received the vaccine [51]. With >20 million Americans currently infected with HPV in the USA, and an estimated 6 million new infections to occur each year [52], vaccination programmes when implemented have great potential to reduce the burden of HPV disease and to prevent its related cancers.

However, the impact of current prophylactic vaccines on HPV-associated OPC is unknown. As vaccination programmes decrease the overall prevalence of HPV infection in the population, the trends of OPC increasing [53] may show signs of slowing or even reversing. However, none of the prophylactic vaccine studies performed thus far have evaluated oral HPV infection or oral immunity to HPV, so its impact on the incidence of HPV-associated OPC remains an open question.

Immunotherapy for the Treatment of Established HPV-Associated Disease

The commercially available preventive HPV vaccines (Gardasil and Cervarix) elicit antibody responses to the HPV viral capsid protein, L1, and can prevent viral infection of epithelial cells [41]. However, this strategy is not effective for treating

existing infections or established HPV-related diseases. Treatment of established disease requires activation of the cellular immune system, both CD4+ and CD8+ T-cells, which can recognize and eliminate virus-infected cells. This purposeful priming of the cellular immune system against an established infection has gained the moniker 'therapeutic vaccination' to distinguish it from protective vaccination strategies.

Therapeutic Vaccination Strategy

Therapeutic vaccination strategies depend on the identification of tumour-specific antigens that will determine the specificity of the targeted immune response. The HPV E6 and E7 proteins represent model tumour-specific antigens for several reasons. These oncoproteins are foreign viral proteins and, thus, are more immunogenic compared with a self-protein, which may be mutated in the cancer cell, such as p53, or upregulated aberrantly, such as Mage-A3. The proteins are encoded by the viral genome and are expressed uniquely by all virus-infected cells, and, thus every virus-related cancer cell. As the E6 and E7 oncoproteins are required for the induction and maintenance of the malignant phenotype, these proteins are expressed constitutively within the cancer cells and it is unlikely the cancer cell would downregulate expression of these proteins in order to evade an immunological response.

Administration of Therapeutic Vaccines

One of the challenges with therapeutic vaccination is generating a robust and relevant T-cell response specific to the antigen of interest. Multiple approaches to creating and enhancing this specific T-cell response have been developed. The target antigen can be encoded in a 'DNA vaccine' plasmid where it is linked to chaperone proteins. Once the DNA vaccine is administered, the plasmid is taken up by host cells, transcribed, and the target antigen is directed to cellular pathways to enhance presentation to the immune system [54]. Alternatively, a peptide encoding the antigen of interest can be administered directly. The peptide is taken up by antigen presenting cells, which in turn induce a T-cell response specific to the administered peptide.

The physical delivery method of the DNA plasmid or peptide also has a significant impact on the T-cell response generation. Simple intramuscular injection is not significantly immunogenic, so either type of vaccine can be delivered via intramuscular electroporation or intradermal gene gun, which enhances significantly the levels of antigen expression within the cell and/or increases the transfection rate of antigen-presenting cells within the skin milieu [15]. A further strategy for increasing the effective T-cell response is a combination of vaccination with chemotherapeutic agents. Induction of tumour cell apoptosis and inflammation broadens tumour antigen presentation to the immune system and increases the effectiveness of the generated T-cells [55]. All of these strategies to enhance the efficacy of therapeutic vaccines are currently under investigation in the treatment of HPV-associated HNCs.

Therapeutic Vaccines for HPV

Novel therapies utilizing these strategies for HPV-related diseases are being explored actively in the clinical realm. One promising therapeutic HPV vaccine tested thus far was a series of synthetic long peptides, which spanned both the HPV16 E6 and E7 proteins. A phase II clinical trial was completed which included 20 women with HPV16-associated grade III vulvar intraepithelial neoplasia [56]. The patients were vaccinated with these overlapping peptides every 3 weeks for a total for four vaccinations. Biopsies were performed at 3 and 12 months after the last vaccination. All patients mounted vaccine-induced immune responses and clinical responses correlated with the induction of HPV16-specific CD8+ T-cells. The majority of the immune responses were specific to the HPV16 E6 protein. A complete response was observed in 25 % (5/20) of patients 3 months after the last vaccination, and this increased to a 47 % (9/19) complete response rate 12 months after the last vaccination. This study showed for the first time that with therapeutic vaccination, clinical responses could be achieved for established HPV disease. This peptide-based approach continues to undergo evaluation in high-grade cervical disease in further clinical trials [57].

In addition to peptide-based vaccines, several groups are evaluating DNA plasmid vaccines, a strategy that allows for continued, high levels of target gene expression in transfected cells and, thus, sustained immunological responses. A preliminary safety study for a DNA vaccine against HPV16 E7 was tested in CIN [58] and successfully showed the ability to generate a specific T-cell response and cause regression of disease. The next generation of this vaccine is now being evaluated in HPV-related HNCs in an ongoing clinical trial [59]. These novel treatments are in the early phases of development, but hold great promise for the treatment of HPV-associated diseases with the same targeted precision already achieved by preventive vaccines.

Challenges and Future Directions of Immunotherapy for HPV-Associated Cancers

Although targeted immunotherapeutic strategies for the treatment of HPV-associated disease are conceptually attractive, significant challenges are expected to arise as experience is gained in early clinical testing. Even if a robust systemic cytotoxic T-cell response can be generated, the same local immune environment around the HPV-associated lesion that has allowed it to escape immune clearance may also decrease the efficacy of this induced T-cell response. Understanding the local immunological environment is therefore a critical component in devising successful clinical trials that include therapeutic vaccination strategies.

Regulatory T-Cells and Local Immunosuppression

The role of regulatory T-cells in contributing to a local immunosuppressive microenvironment and in suppressing cytotoxic T-cell function in cancers increasingly is

being appreciated in HPV-associated cervical cancer [60]. The function of these regulatory T-cells is to modulate activated T-cell function to prevent autoimmunity. However, in the setting of cancer immunotherapy, the regulatory T-cells can dampen the desired vaccine-induced T-cell responses. Both HPV-related tumours and other cancers have populations of regulatory T-cells and the presence of this T-cell population can predict lack of clinical response to therapeutic vaccination against HPV16 [61]. Therefore, groups are evaluating the combination of chemotherapeutic agents, such as cyclophosphamide, and cancer vaccines to eliminate the regulatory T-cell population to enhance vaccine-specific immune responses [55, 62].

Immune Check-Points in HPV-Associated Cancer

In addition, antigen-induced activation and proliferation of T-cells are regulated by the temporal expression and binding of both co-stimulatory and co-inhibitory receptors. The orchestrated signalling through these receptors in adaptive cellular immunity modulates the initiation, escalation and subsequent resolution of host immune responses. These immune checkpoints can represent major obstacles to overcoming tumour-specific T-cell tolerance and generating clinically meaningful tumour control. Two such co-inhibitory T-cell receptors that are the focus of intense current interest are CTLA-4 and programmed cell death-1 (PD-1). PD-1 is up-regulated significantly in HPV-associated OPC and may represent a novel target for modified immunological therapy [31].

These immune check-points have clinical significance because monoclonal antibody therapy has been developed against the receptors. Ipilimumab, a monoclonal antibody (mAb) that blocks CTLA-4, was evaluated in patients with advanced metastatic melanoma in a randomized phase III clinical trial and showed a survival benefit; however, it was associated with significant immune-related toxicities [63]. In a phase I clinical trial, blocking mAb against PD-1 (MDX-1106) was evaluated in patients with advanced metastatic melanoma, colorectal cancer, castrate-resistant prostate cancer, non-small-cell lung cancer, and renal cell carcinoma. Blocking the PD-1 immune check-point was better tolerated than CTLA-4 blockade and clinical activity was observed in all of the evaluated histologies, except for prostate cancer [64]. Furthermore, tumour cell surface, or ‘membranous’, expression of the major PD-1 ligand and PD-L1 correlated with the likelihood of response to therapy; the same expression that is seen in HPV-associated OPC [31]. Understanding and manipulating the immunosuppressive tumour microenvironment is therefore a potentially promising strategy for enhancing the efficacy of therapeutic vaccination in the future.

Conclusions

HPV is a common virus that causes a wide range of human diseases, including a growing subset of OPCs. Significant progress has been made within the past decade in designing and implementing preventive vaccination programmes against

HPV. However, a variety of societal and economic challenges impede implementation of these vaccination programmes, and the burden of HPV and its related cancers will continue until these challenges are overcome. Therapeutic vaccines, if developed, will be a potentially important tool to treat those individuals already infected and/or diagnosed with established HPV-related disease. However, a different set of challenges is uncovered with therapeutic vaccines on the basis of the biology of these tumours. As the field of cancer immunology matures, the obstacles to achieving successful immunotherapy are being revealed and strategies are being applied to address these barriers.

Summary Points

- Established cervical HPV infections are cleared naturally through T-cell-mediated immune responses (CD4+ and CD8+ T-cells) rather than humoral antibody responses.
- HPV manipulates the immunological microenvironment through multiple mechanisms to evade clearance by the immune system.
- Commercially available vaccines are highly effective in preventing primary anogenital HPV infection through the induction of protective antibodies.
- Experimental therapeutic DNA vaccines induce T-cell responses against HPV-infected cells, and are being developed in the hope of improving survival among patients with established HPV-related diseases.

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The Care of the HPV-Negative Head and Neck Cancer Patient: Presentation, Prognosis, Treatment

8

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Epidemiology of Human Papillomavirus (HPV)-Negative Oropharyngeal Cancer (OPC)

Chaturvedi et al. [1] recently described the population-based estimated incidence of HPV-positive and HPV-negative OPC in the USA using the Surveillance, Epidemiology, and End Results (SEER) database [1]. From 1988 to 2004, HPV-positive OPC increased by 225 % (95 % CI 208 – 242 %; from 0.8 per 100,000 to 2.6 per 100,000), whereas the incidence of HPV-negative cancers declined by 50 % (95 % CI 47 – 53 %; from 2.0 per 100,000 to 1.0 per 100,000). The American Cancer Society estimated that 52,140 new cases of laryngeal, oral and pharyngeal cancers would occur in 2011 [2]. A limitation of these estimates is the difficulty in ascertaining the proportion of 'oral cavity and pharynx cancers' that are OPCs *per se*.

In a recent Radiation Therapy Oncology Group (RTOG) trial (RTOG 0129), the prevalence of HPV-negative tumours was ~40 % of the total oropharyngeal study population [3]. This is a robust estimate of the prevalence of HPV-negative oropharyngeal tumours in the USA, given the multi-institutional nature of the cooperative

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group and the geographical diversity of this study population. While this number may be decreasing on account of declining tobacco use, HPV-negative OPC still represents a significant number of annual cases in the USA. Recently, Ang et al. highlighted the significant impact HPV status has on the epidemiology of OPC [3]. RTOG 0129 provided strong evidence that HPV status is an independent prognostic factor for overall survival (OS) and progression-free survival (PFS) among patients with squamous-cell OPC, highlighting the poorer survival of HPV-negative cases. An examination of therapeutic considerations of this patient population is warranted, given their poor prognosis relative to HPV-positive OPC patients.

Patients with HPV-negative tumours had significantly reduced OS and PFS, even after adjusting for demographics, T stage, N stage and smoking. While de-intensified treatment is being considered for HPV-positive patients, a pressing concern is the dismal outcome for 'high-risk' HPV-negative OPC patients. Even when treated upfront with primary radiation therapy and cisplatin, HPV-negative patients have substantially diminished outcomes, both in terms of locoregional control (LRC) and OS. In RTOG 0129 [3], patients with HPV-negative tumours had a 25.1 % reduction in OS at 3 years (57.1 % vs. 82.4 %) when compared with patients with HPV-positive tumours [3]. Locoregional relapse at 3 years was 21 % higher in patients with HPV-negative tumours: 35.1 % (95 % CI 26.4 – 43.8) versus 13.6 % (95 % CI 8.9 – 18.3 %) for HPV-positive tumours ($p < 0.001$). These poor outcomes for HPV-negative patients are remarkable, given the gradual trend toward increasing intensification of treatment with altered fractionation schema [4], concurrent chemoradiation [5], multidrug induction chemotherapy [6, 7], and targeted molecular therapies [8].

It seems that for these HPV-negative patients, simply altering the method of radiation delivery and the dosing and/or types of concurrent chemotherapy may not be sufficient to improve oncological outcomes. A new approach seems warranted.

The Role of Surgery for HPV-Negative OPC

One approach to intensify treatment for HPV-negative OPC patients would be to implement surgery as the primary therapy for these patients. In the past, surgical resection of OPC was typically permissible through disfiguring incisions and, often, mandibulotomy with significant postoperative functional deficits [9–11]. However, recently 'endoscopic' head and neck surgery (eHNS) has emerged [12], which consists of minimally invasive transoral approaches to the oropharynx with laser carbon dioxide (CO_2) microsurgery (transoral laser microsurgery [TLM]) [13] and transoral robotic surgery (TORS) [14]. This chapter reviews these new surgical approaches and explores how these evolving techniques might facilitate 'surgical-intensification' for HPV-negative OPC.

Compared with traditional 'open' head and neck surgery, eHNS of the oropharynx is performed without external incisions and obviates the need for mandibulotomy or transmandibular access. This approach can be considered 'inside-out' surgery, in that incisions start from the mucosal (inner) surface and extend outward, sparing external

skin incisions. Using a laser and microscope for TLM or the da Vinci® Surgical System (Intuitive Surgical Inc., Sunnyvale, CA, USA) for TORS, a complete resection of the index oropharyngeal tumour is performed with an oncological margin. While both TLM and TORS rely on a minimally invasive approach through the mouth, there are some significant differences in these eHNS techniques.

Another therapeutic option for this patient population is TLM, an endoscopic surgical technique performed under direct laryngoscopy, with suspension/fixation and the use of an operating microscope, microsurgical instruments and a CO_2 laser. It is an adaptive surgical technique, relying on the surgeon's understanding of the 3-dimensional anatomy of the tumour's extent and surrounding anatomy. First described in 1972, TLM has a robust literature, although few multicentre experiences have been published and no prospective coordinated clinical trial has been performed [15].

On the other hand, robotic head and neck surgery was approved for use by the US Food and Drug Administration in 2009. TORS for head and neck cancer (HNC) is performed using three arms, which are placed within the patient's mouth but controlled by a surgeon sitting at a remote console, in a 'master-slave' configuration. A suitable oral retractor is positioned in the mouth and the endoscope or camera is introduced into the pharynx followed by the two other arms carrying interchangeable 5 mm wide working instruments (e.g., grasping forceps and electrocautery). The surgeon is provided with an endoscopically derived 3-dimensional visual display that is co-located with control handles that direct movements of the robot's instruments inside the patient's body. Standard surgical instruments, including tissue forceps, an electrocautery spatula, or CO_2 and thulium laser [16, 17] are then used to perform an *en-bloc* resection of the oropharyngeal tumour. While the first paper on robotic surgery for OPC was published in 2005, there is little prospective literature examining the role of TORS within the multidisciplinary paradigm.

Both techniques provide a highly magnified view of the tumour, which allows confident resection of various tumour invaginations that are not often visualized with standard surgical techniques. Whereas numerous retrospective single-institution reports and a few important multicentre trials have generated significant enthusiasm for implementing eHNS into the multidisciplinary approach, prospective clinical evidence to support its use is limited. Recently, Adelstein and Ridge hosted a National Cancer Institute (NCI)-sponsored, R13-funded Clinical Trials Planning Meeting to discuss the role of transoral endoscopic surgery for the treatment of OPC [18].

Compared with open surgery, eHNS is minimally invasive. Numerous publications show a reduction in immediate postoperative toxicity, shorter postoperative hospitalization time, and faster functional recovery compared with open surgery [14, 19, 20]. Advocates for eHNS (TLM and TORS) in oncological surgery cite excellent functional results and argue that eHNS 'de-intensifies' the long-term toxicity that is sometimes associated with a primary radiation-based approach for OPC [14, 21].

Skeptics of TORS and TLM are wary of this approach, citing concerns about the relatively 'close' margins and the high rate of postoperative radiation therapy required after eHNS [22].

However, because of the sigmoidal shape of the normal tissue complication probability curve, treatment with a postoperative dose (60 Gy) rather than a definitive dose (70 Gy), combined with an intensity-modulated radiation therapy (IMRT) technique optimized to spare adjacent organs at risk, may significantly reduce the risk of damaging critical normal tissue for the group of patients who require adjuvant therapy.

Despite a surge in interest in eHNS by surgeons, there is no 'level-A' evidence-based clinical data to support its use. Granted, surgery followed by radiation therapy has been the established paradigm for OPC, dating back to the early-and mid-twentieth century [9, 10]. However, eHNS is a considerable technological advance in surgical technique, akin to the difference between conventional 2-and 3-dimensional conformal radiotherapy versus IMRT. This surgical approach, including the impact of margins and the role of postoperative radiation therapy following TORS and TLM, must be carefully studied, ideally in the setting of a prospective multicentre clinical trial. Accordingly, RTOG 1221, a prospective clinical trial evaluating the role of eHNS in OS for HPV-negative OPC, is currently open to accrual.

Radiation Therapy for HPV-Negative Patients

The differences in tumour response and outcome in relation to HPV status have mandated stratifications in clinical trials and changes in treatment paradigms for HNCs. As new dose de-escalation treatment plans are investigated for HPV-positive head and neck squamous cell carcinoma (HNSCC), strategies for HPV-negative patients continue to pursue treatment methods that maximize tumour control and disease-free survival in this higher-risk group. This logically has evolved into utilization of aggressive multimodality drug combinations given at maximally tolerated doses (dose-intensification strategies) and testing of novel systemic agents. This paradigm continues to evaluate altered-fractionation radiation regimens as well as the addition of targeted therapies, radiosensitizing agents, and radioprotectants. Strategies using maximally tolerated therapy combined with approaches that minimize radiotherapy side-effects and prevent overlapping toxicities may be even more important in the treatment of HPV-negative oropharyngeal patients.

A multidisciplinary approach (including surgeons, radiation oncologists, medical oncologists, clinical nurse specialists, speech and language therapists, and dieticians) should be used in the management of patients with HNSCC. Factors that influence the treatment of choice for HPV-negative patients are the primary site, AJCC stage, tumour differentiation, patient age and medical co-morbidities [23]. The treatment of choice for HPV-negative patients should, theoretically, aim to achieve the following outcomes:

- Maximize locoregional tumour control
- Sterilize subclinical micrometastatic disease
- Reduce risk of second malignancies

- Maximize preserving organ function
- Minimize acute and chronic toxicities
- Improve quality of life.

Altered Fractionation

Local recurrence remains the most common scenario for treatment failure in patients with HPV-negative HNC. In an attempt to decrease this risk, two main radiation approaches have been studied. First is hyperfractionation, which encompasses delivering two fractions a day with a reduced dose per fraction. This approach facilitates the delivery of a 10–15 % higher total radiation dose to the tumour without increasing the risk of late normal tissue toxicity. Second is accelerated fractionation, delivering >5 fractions of treatment per week by treating on weekends or delivering >2 fractions on some weekdays. Accelerated fractionation shortens the delivery period of radiation, potentially reducing accelerated repopulation of tumour clonogens that occurs 3–4 weeks after initiating treatment. Some clinical trials have combined these approaches to formulate both hyperfractionated and accelerated treatment regimens [24, 25]. According to the RTOG criteria, the costs in terms of acute toxicity have been higher—with grade 3 or higher acute toxicities increasing from 33 to 55 % compared with conventional fractionation [26–28]. These altered fractionation approaches have also shown clinical benefits in terms of improved LRC, which leads to a small but significant survival benefit at 5 years of 3.4 % (2 % for accelerated, 8 % for hyperfractionation) over the standard fractionation approach [29]. The benefit of altering fractionation is greater in local tumour control than in regional (nodal) control, and has a larger benefit in younger patients, particularly those of <50 years of age. Altered fractionation approaches appear not to have an effect on the development of distant metastases.

Intensity-Modulated Radiation Therapy (IMRT)

IMRT uses X-rays of differential fluence to protect normal tissue surrounding the target tumour. Brahme described the concept 25 years ago, and IMRT has been progressively introduced into the clinics over the past 10–15 years [30]. IMRT was first used to spare salivary gland tissue in HNC patients in phase I/II studies performed at the University of Michigan. Radiation dose to the contralateral parotid gland was 32 % compared with 93 % for the standard plans. This resulted in patients recovering 63 % of their stimulated saliva by 1 year [31, 32]. The role of IMRT in parotid gland sparing is now well established, with subsequent studies from other institutions reporting similar threshold doses; a mean dose threshold for reduction in salivary output to <25 % of the baseline was found for both stimulated (26 Gy) and unstimulated (24 Gy) saliva flow rates [33, 34]. A randomized study with 51 nasopharyngeal cancer patients receiving either IMRT or conventional RT showed

Table 8.1 Osteoradionecrosis (ORN) rates with intensity-modulated radiation therapy in head and neck cancer

Study	No. of patients	Primary tumour site	ORN (%)
Nguyen et al. [49]	83	All	1
Mendenhall et al. [50]	130	Oropharynx	3
Eisbruch et al. [51]	69	Oropharynx	6
Montejo et al. [48]	43	All	2.3
Ben-David et al. [44]	176	All	0
Studer et al. [45]	73	Oral cavity, oropharynx	1
Gomez et al. [52]	35	Oral cavity	5

that 83 % of patients in the IMRT group had recovered parotid salivary flow versus 9.5 % in the conventional group at 1 year. The global quality of life was significantly better in the IMRT group compared with the conventional group [35]. Local control and disease-specific survival were equivalent in patients given conventional treatment [36–38].

Sensorineural hearing loss has been shown to result from high doses to the auditory apparatus in the treatment of parotid tumours and those of the temporal region [39, 40]. This loss is permanent, and can result in significant cognitive impairment, depression, and reduction in functional status, particularly if it develops at a young age. Planning studies indicate that the dose to the cochlea can be reduced with IMRT [41]. Prospective studies are ongoing to determine if IMRT can prevent the development of these toxicities.

Osteoradionecrosis remains one of the most feared complications of head and neck radiotherapy because of its effect on the quality of life of the patient. The risk of osteoradionecrosis at the mandible is related to total radiation dose and to the volume of bone irradiated, the presence or absence of mandibular surgery prior to radiation, and dental extraction after radiation [42–45]. Early-onset radionecrosis is usually caused by doses of >70 Gy to the mandible, whereas delayed radionecrosis is frequently associated with bony trauma [46]. In studies offering a direct comparison between 3D and IMRT techniques, a significant amount of normal bone can be spared from high radiation dose with IMRT [47]. Preliminary studies of IMRT for HNC reported a possibly lower prevalence of osteoradionecrosis compared with historical controls treated with conventional radiotherapy techniques [48]. A recent review of 22 published reports and 5,712 patients shows that the risk of developing osteoradionecrosis among the irradiated HNC patients has declined significantly in recent years (Table 8.1) [53].

The role of IMRT for sparing the dose to the pharyngeal constrictors (PCs) and associated dysphagia is less well established. Significant late dysphagia rates (12–50 %) have been reported in a number of studies using conventional RT techniques during both chemoradiation and altered radiation fractionation [54, 55]. It is recognized that feeding tube dependency at 1 year significantly affects the quality of life [56]. Studies have reported that late dysphagia after treatment for HNC is dependent on the dose to the PCs, particularly to the superior constrictor [57, 58].

Table 8.2 Studies correlating radiation dose/organ at risk with dysphagia

Study	No. of patients	Tool/end-point	Organ at risk
Eisbruch et al. [59]	26	Videofluoroscopy	Constrictors, larynx
Feng et al. [60]	36	Videofluoroscopy, QOL	Constrictors, larynx
Jensen et al. [61]	25	H&N 35[a]	Supraglottis
Levendag et al. [57]	56	H&N 35[a]	Constrictors
Caglar et al. [62]	96	Videofluoroscopy	Constrictors
Dirix et al. [63]	53	H&N 35[a]	Constrictors, supraglottis
Caudell et al. [64]	83	Videofluoroscopy	Constrictors, larynx

[a]H&N 35 [65] is a questionnaire developed by the European Organization for Research and Treatment of Cancer (EORTC) to assess the quality of life of head and neck cancer patients. *QOL* quality of life

Other authors have shown that the dose to adjacent structures can also contribute to late dysphagia (Table 8.2). IMRT has the potential to prevent radiation-induced dysphagia by limiting the dose to the PCs and adjacent organs. Feng and colleagues [60] reported on a prospective study of the constrictor-sparing approach using IMRT in patients with OPC [55]. The authors minimized the dose to the PCs by not treating the medial retropharyngeal nodes. However, IMRT has the following drawbacks: (i) cost of treatment delivery, (ii) it uses two to three times more radiation monitor units, which results in increased total body dose caused by increased radiation leakage and scatter, (iii) more treatment fields are needed to optimize organ-sparing, which results in larger volume of normal tissue exposed to a lower radiation dose (Fig. 8.1a, b), (iv) a twofold increased risk of radiation-induced malignancies compared with 3D concomitant radiotherapy, and (v) proper delivery of IMRT for head and neck tumours requires a thorough knowledge of the complex anatomy of the region and the intricate physiology of swallowing, speech and auditory functions, among others [66]. To keep complications to a minimum a clear understanding of the pathophysiology of local and regional tumour spread is also required, as well as knowledge of the tolerance of normal tissues to irradiation, whether or not combined with chemotherapy or targeted agents. In addition, IMRT studies have not consistently shown benefits in tumour control or survival. Therefore, IMRT is not recommended where it fails to offer significant advantages in either normal tissue-sparing or dose escalation.

Image-Guided Radiation Therapy (IGRT)

The advent of advanced delivery methods over the past decade, such as conformal radiation therapy and intensity modulation, has emphasized the need to improve our ability to localize the target for treatment. Radiotherapy evolution, therefore, has been linked to improvements in medical imaging. Major technological innovations in anatomical imaging have resulted in substantial improvements in radiotherapy

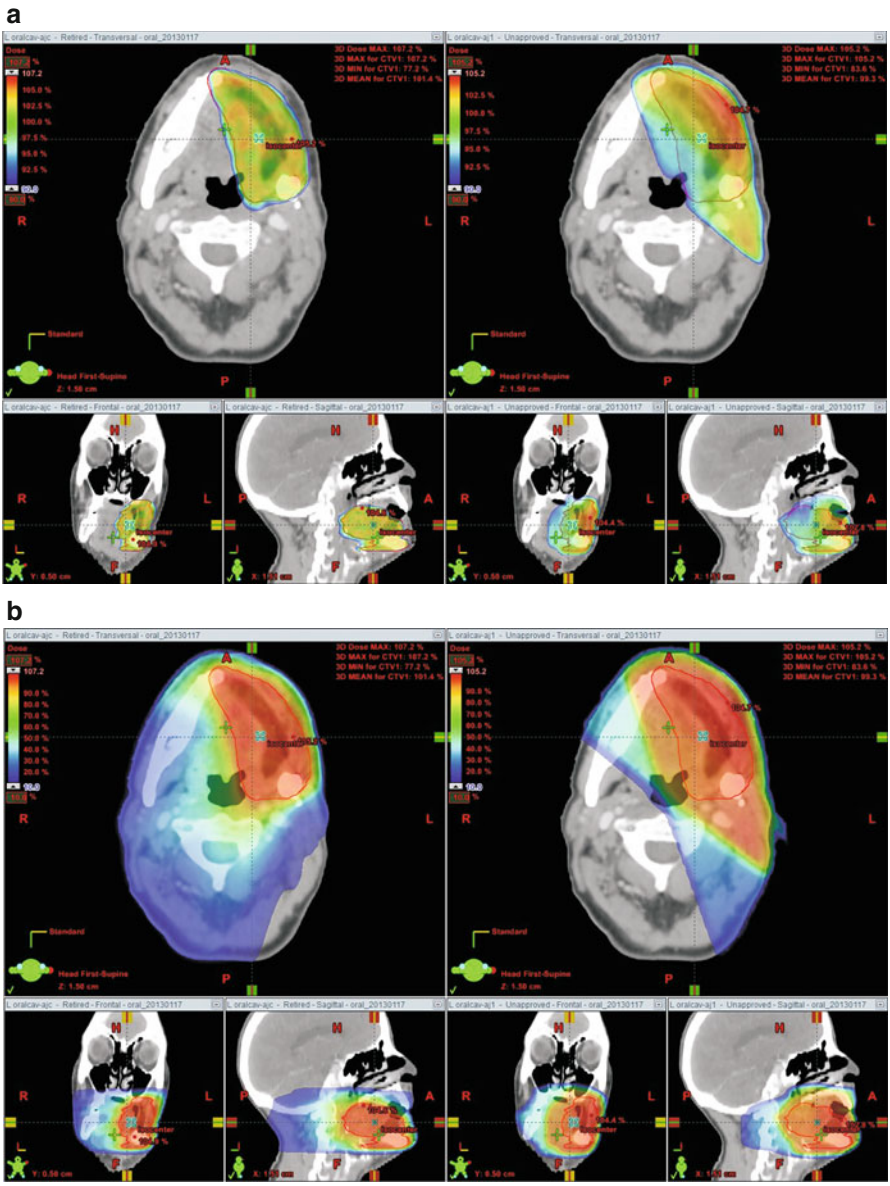

Fig. 8.1 (**a**) High-dose isodose colour wash (90–100 % of prescribed dose) for a left oropharyngeal/oral cavity tumour involving the mandible after resection (pathological stage T4N0M0) undergoing postoperative radiation using IMRT (*left-side* axial, coronal and sagittal views). Note the impressive conformality of dose in relation to the planning target volume compared to the dose delivered by 3D conformal radiation (*right-side* axial, coronal and sagittal views). High doses to the right oral cavity and posterolaterally are spared better with IMRT. (**b**) Low-dose isodose colour wash (10 % [in *blue*] to 100 % [in *red*] prescribed dose) for the same oropharyngeal/oral cavity tumour. IMRT on the *left-sided panels* (axial, coronal and sagittal views) results in significantly more tissue treated to low dose radiation than does 3D conformal radiation on *right-sided panels*. Note that more brain in the posterior fossa is exposed to low dose radiation with IMRT, which has been hypothesized by Nutting and colleagues to be the aetiology behind increased fatigue in patients receiving IMRT [38]

planning, delivery and verification. Verification is a vital link in the radiation treatment delivery cycle, especially when using IMRT, in which sharp dose gradients increase the likelihood of a geographical miss. Verification traditionally is performed both before treatment starts and regularly during treatment, and ensures that underdosing to the tumour and overdosing to the organs at risk is avoided by minimizing the systematic and random positioning errors. In addition to the conventional 2-dimensional verification using portal imaging, modern devices also enable 3D volumetric verification using kilovoltage cone beam CT (or 3D ultrasound), and *in vivo* dosimetry. Increasing the precision and accuracy of radiation delivery through IGRT is likely to result in an improved therapeutic index and reduce toxicity with potential for dose escalation and improved tumour control. Two concerns about the use of IGRT are the increased dose to the patient from additional imaging, and the resources required for its implementation and general use.

An additional technique that has developed as a natural progression of imaging used for IGRT is 4-dimensional (4D) imaging. 4D medical imagine (4DMI) includes time-resolved volumetric CT, MRI, PET, PET/CT, SPECT, and US imaging. It enables the radiation oncologist to perform real-time motion changes in soft tissue within the patient while in the treatment position and allows for more precise target localization. 4D radiation therapy (4DRT) aims to track and compensate for target motion during radiation treatment, minimizing normal tissue injury, especially to critical structures adjacent to the target, and/or maximizing radiation dose to the target. This is most commonly utilized in lung cancer patients, particularly with stereotactic body radiotherapy. Since the anatomy of the head and neck carries less motion, 4DRT is utilized sparingly.

Adaptive Radiotherapy (ART)

IMRT plans are typically based on a pretreatment CT scan that provides a snapshot of the patient's anatomy. Nevertheless, patient variations may occur during the course of treatment because of setup error and anatomical modifications. Therefore, the accuracy of IMRT delivery for HNC may be compromised during the treatment course, potentially affecting the therapeutic index [67]. In the setting of HNC, radiotherapy patients suffer significant anatomical changes because of tumour shrinkage and/or weight loss. This phenomenon creates dose changes over target volumes and organs at risk of injury, such as the spinal cord and brain. Beltran utilized cone-beam CT during treatment at the 15th and 25th fractions to determine the actual dose delivery compared with the intended dose from the initial IMRT plan generated from the pretreatment CT to sensitive structures in the treatment region [68]. Contralateral and ipsilateral parotid gland mean doses increased by 6.1 % (range −5.4, 23.5 %) and 4.7 % (range −9.1, 22.3 %), respectively, by the 25th fraction. Additionally, the maximum absorbed dose to the spinal cord by the 15th fraction had increased by 1.8 Gy. Others have shown similar results. Hansen and colleagues found that the maximum dose (Dmax) to the spinal cord increased in all 13 HNC patients studied (range 0.2−15.4 Gy; $p=0.003$) and the brainstem Dmax increased in 85 % of patients (range 0.6−8.1 Gy; $p=0.007$) [69]. Similarly, anatomical changes during treatment of HNC patients also affect the lips, oral cavity and middle neck, and alter dose distribution

and induce a loss of dose coverage to tumour volumes. Doses to clinical target volume (D95%) to gross tumour volume were reduced in 92 % of patients ($p=0.02$). These dosimetric data support the concept of identifying patients with bulk nodal disease likely to respond (shrink) during treatment, patients with tumours involving larger air cavities/sinuses, or patients with significant weight loss. These could benefit from ART. Utilizing serial cone beam CT in these patients during treatment would identify patients at risk of developing acute or long-term toxicities, or allow re-planning during treatment to ameliorate unwanted dose changes. Serial re-imaging may also improve tumour control. ART has brought an additional dimension to the management of patients with HNC and has the potential to counteract the effects of positioning errors and anatomical changes. For swift implementation of tailored and adaptive therapy, tools and procedures, such as accurate image acquisition and reconstruction, automatic segmentation of target volumes and organs at risk, non-rigid image and dose registration, and dose summation methods need to be developed and properly validated in the future.

Combined Chemotherapy and Radiation Strategies for Non-Surgical HPV-Negative Patients

Concurrent Chemotherapy

Several chemotherapeutic agents have been historically identified that act as radiosensitizers to potentiate the effect of radiation on HNC cells. Among these, the most widely used is cisplatin (cis-diaminedichloroplatinum [CDDP]). It is commonly utilized in concurrent treatment regimens for locoregionally advanced disease, both for definitive and adjuvant (post-surgical) treatment. The addition of concurrent chemoradiation therapy results in a survival benefit, estimated to be 8 % at 5 years, with a greater benefit for platinum-versus non-platinum-based regimens [70]. This has made platinum-based concurrent chemoradiation the standard of care in the treatment of HNC. However, the addition of cisplatin is also associated with a significant increase in grade 2 or higher acute toxicity, to the order of >80 % in large randomized multi-institutional trials [71]. Furthermore, the benefit of the addition of cisplatin is often confined to patients <70 years of age. Systemic agents, especially platinum compounds, also have chronic toxicity involving multiple organ systems that add to the overall cost of care. Other regimens, both single-agent and in combination, are also well recognized and widely utilized.

Induction Chemotherapy

Induction chemotherapy was first utilized in HNCs (e.g., HNSCC) in the 1970s, historically with the combination of cisplatin and 5-fluorouracil (5-FU) [72]. High overall response rates were observed in previously untreated tumours and a correlation between response to induction chemotherapy and favourable response (97.6 %)

to subsequent radiotherapy was noted [73]. Conversely, poor response to induction chemotherapy correlated with poor response (5.5 %) to radiation [73]. Since that time numerous clinical trials have shown a benefit for larynx preservation, but mixed results with respect to improvements in OS [74, 75]. The most recent meta-analysis reviewing the impact of induction chemotherapy (MACH-HN) reported in 2009 consisted of 31 induction chemotherapy trials that included 5,311 patients with a median follow-up of 6.1 years [76]. The HR of death was 0.96 (0.90 – 1.02; p = 0.18), which suggests a survival benefit associated with induction chemotherapy, and an absolute benefit of 2.4 % at 5 years. The type of chemotherapy was not associated with a significant (p = 0.23) variation of the effect: 0.90 (0.82 – 0.99) for 5-FU-platin, 1.01 (0.91–1.12) for other poly-chemotherapy, and 0.99 (0.84–1.18) for mono-chemotherapy (no trial with platinum-based agent). The HRs of death were not significantly different (p = 0.68) between trials using radiotherapy alone, surgery plus postoperative radiotherapy, or other locoregional treatment. Clear evidence was lacking of a differential effect of induction chemotherapy on survival according to age, sex, performance, stage or tumour site. In terms of the direct comparison of induction chemotherapy to concurrent chemotherapy given with radiotherapy, the meta-analysis included 6 trials (861 patients) and favoured concomitant chemoradiation (OS HR = 0.90; p = 0.15) with an absolute benefit of 3.5 % at 5 years; LRC also appeared better [76].

The question then arises about the value of induction therapy in a setting in which concurrent chemotherapy and radiation is utilized. Does it add to tumour control and survival or only increase toxicity? Recent years have witnessed a renewed interest in the use of induction chemotherapy in HNSCC, particularly for HPV-negative patients in whom improvements in LRC and distant control have been lacking. Can induction chemotherapy response allow subsequent concurrent treatment to be individualized on the basis of response and, therefore, risk? The role of induction chemotherapy was tested against concurrent chemoradiotherapy (CRT) in two key randomized controlled trials: DeCIDE and PARADIGM [77, 78]. The DeCIDE trial included only patients considered at high risk of distant metastases [77]. It assessed whether the addition of two cycles of induction TPF (docetaxel, cisplatin and 5-FU) chemotherapy to CRT (docetaxel, 5-FU, oral hydroxyurea) using 1.5 Gy b.i.d. fractions reduces the distant failure rate and improves survival in patients with advanced nodal disease (N2/N3 M0). Toxicity was high (3.5 % death rate with induction chemotherapy and 13 % did not proceed to CRT after TPF) and outcome was higher for the control group than anticipated, probably because of inclusion of HPV-positive patients. The PARADIGM phase III trial consisted of TPF induction chemotherapy in patients with previously untreated stage III–IVb HNSCC [78]. In the control arm of this study, accelerated concomitant boost radiotherapy (72 Gy in 6 weeks) was given with concurrent cisplatin 100 mg/m^2 on days 1 and 22. The experimental treatment arm consisted of three cycles of induction TPF delivered followed by response assessment. In complete pathological responders to TPF, conventional fractionation to 70 Gy with weekly carboplatin was given. All other patients received accelerated fractionation concomitant boost radiotherapy (72 Gy in 6 weeks) with concurrent once-weekly docetaxel 20 mg/m^2 for four

cycles. Unfortunately, both trials were terminated early as a result of slow accrual and did not show a survival advantage with the addition of induction TPF to CRT. Other phase III trials are ongoing in the USA and Europe, which are assessing induction chemotherapy's role in locally advanced patients.

Newer Treatment Strategies: Epidermal Growth Factor Receptor (EGFR) Inhibition

HPV-negative tumours have high-frequency epidermal growth factor receptor (EGFR) gene amplifications [79]; therefore, addition of anti-EGFR targeted agents to such tumours could be beneficial. Several mechanisms of resistance to anti-EGFR treatment have been described. The evaluation of targeted agents that would potentially overcome this resistance would be of relevance in this setting [80]. Several phase III trials comparing platinum-based chemoradiation with anti-EGFR therapy-based bioradiation are ongoing to evaluate the strategy of chemotherapy-sparing during the concurrent phase of treatment. The HN.6 study (NCT00820248), a phase III trial led by the NCI of Canada Clinical Trials Group, has recently completed recruitment to compare standard fractionation radiotherapy combined with high-dose concurrent cisplatin with accelerated fractionation radiotherapy combined with the humanized anti-EGFR monoclonal antibody—panitumumab. Results are forthcoming and could provide valuable information on the relative efficacy of an anti-EGFR agent to standard chemotherapy.

The addition of the monoclonal antibody cetuximab to radiotherapy has been shown to improve the response rate, PFS and OS when compared with radiation alone in a phase III trial in locally advanced HNSCC [8]. However, results of direct OS comparison between cisplatin-based CRT and anti-EGFR therapy-based bio-radiotherapy from prospectively conducted clinical trials are pending. Early results from such a comparison in a clinical trial are available from the TREMPLIN study [81]. In this phase II larynx-preservation study, patients with laryngeal and hypopharyngeal tumours who had achieved at least a partial response after TPF induction therapy were randomized to CRT with cisplatin or bio-radiotherapy with cetuximab. Whereas patients on the cetuximab arm had greater skin toxicity within the radiotherapy field, they encountered a lower incidence of severe toxicities and achieved better treatment compliance. The trend to a higher locoregional failure after 18 months in the cetuximab-treated patients was non-statistically significant, but, as salvage surgery was feasible in many cases, the eventual LRC was similar in both arms. In addition, metastatic recurrence showed no difference between treatment arms.

Problem of Second Primaries

Second primary tumours (SPTs) are the major threat to long-term survival after successful therapy of non-HPV-associated HNC, particularly of the lung, oesophagus and other head and neck subsites [82]. The occurrence of SPTs after successful

HNSCC treatment illustrate the concept of field cancerization throughout the upper aerodigestive tract, where environmental carcinogens, such as tobacco and alcohol, induce a field of mucosa afflicted with premalignant disease and may elevate epithelial cancer risk [83, 84].

The past decade has shown that among patients with HNSCC, the risk and distribution of SPTs differ significantly according to the subsite of the index cancer. Before the 1990s, cancers of the hypopharynx and oropharynx carried the highest excess risk of SPT. Since then, during the dramatic rise of HPV-associated cancers, SPT risk associated with oropharyngeal SCC has declined to the lowest risk level of any head and neck subsite [85]. In fact, since 1991, as the incidence of HPV-associated cancers has risen, the SPT risk has decreased significantly among patients with oropharyngeal SCC (annual percentage change in excess absolute risk of SPT −4.6 %; $p=0.03$) [85]. Therefore, while future clinical studies may be able to harness this emerging knowledge and put less emphasis on long-term surveillance strategies of HPV-associated SCC, continued active supervision of patients with classic smoking-related HPV-negative cancers will continue to be of distinct importance.

Summary

Considerable progress has been made in recent years in refining the role of both surgery and radiation. Surgical intensification with transoral endoscopic head and neck surgery may play a role in providing comparable outcomes to open surgery, and rendering HPV-negative tumours to microscopic residual disease. Improvement in radiation planning, imaging and delivery techniques, along with the discovery of new systemic treatments, coupled with the understanding of the pathogenesis of radiation toxicity may provide better primary and adjuvant therapy. In the future we will be able to better evaluate the true clinical impact of these technical and scientific breakthroughs in terms of quality of life, preservation of function and survival. It is anticipated that continued improvements in anatomical and tumour imaging, computer technology, and their integration, as well as a greater understanding of the biology of HNCs will result in a more targeted and biologically directed approach to treatment for the individual patient. Newer systemic agents may find more specific targets on HNC cells and important interactions with radiation, both for tumour sensitization and for protection of normal tissue.

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