HPV-associated Oropharyngeal Cancer

Matt Lechner[1,2], Jacklyn Liu[1], Liam Masterson[3], Tim Fenton[4,5]

[1] UCL Cancer Institute, University College London, 72 Huntley Street, London, WC1E 6DD, United Kingdom.
[2] Department of Otolaryngology-Head and Neck Surgery, Stanford University School of Medicine, Palo Alto, CA, USA.
[3] Department of ENT, Cambridge University Hospitals NHS Trust, Cambridge, United Kingdom
[4] School of Biosciences, Stacey Building, University of Kent, Canterbury, CT2 7NJ, United Kingdom.
[5] School of Cancer Sciences, Faculty of Medicine, University of Southampton, Southampton, SO16 6YD, United Kingdom.

Correspondence to m.lechner@ucl.ac.uk or t.fenton@soton.ac.uk

Abstract (200 max.)

Human papillomavirus (HPV)-associated oropharyngeal squamous cell carcinoma (OPSCC) has one of the most rapidly increasing incidences of any cancer in high-income countries. The most recent edition of the UICC/AJCC staging system separates the HPV-associated entity from its HPV-negative counterpart to account for the improved prognosis seen in the former. Indeed, with its improved prognosis and predilection for younger individuals, recent and ongoing clinical trials emphasize the potential for treatment deintensification as a means to improve patient quality of life while maintaining high survival outcome. In addition, due to its distinct biology, targeted and immunotherapies have become an area of particular interest. Importantly, OPSCC is often detected at an advanced stage due to the lack of symptoms in early stage disease; therefore, there is also a need for the identification and validation of diagnostic biomarkers to aid in the earlier detection of disease. Here, we present a summary of the epidemiology, molecular biology and clinical management of HPV-associated OPSCC in an effort to highlight important advances in the field. Ultimately, there is a need for an improved understanding of its molecular basis and clinical course to guide efforts toward early detection, precision care and improved outcomes.

Introduction

Oropharyngeal squamous cell cancer (OPSCC) comprises cancers of the tonsils, base of tongue, soft palate and uvula (Figure 1b). Like other head and neck squamous cell carcinomas (HNSCC), OPSCC has historically been linked to alcohol and tobacco use. However, while smoking cessation in high-income countries has led to a decline in HNSCC over the past twenty years, carcinogenic human papillomavirus (HPV) infection has emerged as an important risk factor that has driven an increase in OPSCC. More specifically, HPV now accounts for 71% and 51.8% of OPSCC cases in the USA and UK respectively.[1–4] Of these, 85 - 96% are caused by HPV16 and are therefore expected to be preventable by prophylactic HPV vaccines known to be effective in preventing HPV-associated cervical neoplasia and now being administered to both boys and girls in several countries.[4,5] The most recent edition of the American Joint Committee on Cancer (AJCC) staging system defined HPV-associated and non-HPV-associated OPSCC as separate entities, with distinct molecular profiles, tumour characteristics and outcome.[6] Importantly, the former is associated with a more favourable prognosis.[7]

Epidemiology: rising incidence, particularly in men

OPSCC has one of the most rapidly rising cancer incidences in high-income countries.[8,9] Increasing rates of disease have been observed in the UK, US, across Europe, New Zealand and in parts of Asia.[9–19] In both the UK and the US, male rates of oropharyngeal cancer have overtaken those of cervical cancer (Figure 1A; adapted from Lechner *et al*.).[8] Globally, the pooled prevalence of HPV in OPSCC was recently reported to be 33%; however, prevalence varies considerably depending on the geographic region, with estimates ranging from 0% in South India to 85% in Lebanon.[20]

HPV-positive OPSCC is more prevalent in non-smokers and non-drinkers, compared to HPV-negative OPSCC, however a substantial history of smoking and drinking use remains prominent and the former is significantly associated with worse outcome.[21,22] Furthermore, sexual behavior is an established risk factor for HPV-positive OPSCC with a strong association between lifetime oral sex partners and incidence of disease.[2,23] As mentioned above, this may partially

account for the observed gender disparity as men are more likely than women to report increased numbers of sexual partners.[24] A significantly increased risk of oral HPV infection is associated with an increased number of recent oral and vaginal sex partners.[24]

While rates of both HPV-positive and -negative OPSCC have increased over the past two decades, there is evidence to suggest that the former is increasing at a faster rate. In Denmark, a three-fold increase for HPV-associated OPSCC between 2000 and 2017 was observed, compared to a two-fold increase for HPV-negative disease [13]. Comparatively, a more rapid increase in HPV-positive HNSCC, particularly tonsillar SCC, was observed in Taiwan, compared to HPV-negative HNSCC.[11] In Italy, the incidence of HPV-associated OPSCC increased from 16.7% between 2000-2006 to 46.1% between 2013-2018.[14] While lower-middle income countries of South Asia and Sub-Saharan Africa bear the vast majority of the global HPV-associated cervical cancer burden, epidemiological reports on HPV-positive OPSCC are scarce and it remains unclear whether similar rising trends are absent or thus far undetected in these regions.[25]

From the handful of reports available, it appears that the prevalence of HPV in OPSCC in Sub-Saharan Africa at least is low, with very few cases of HPV-positive OPSCC reported to date despite high rates of HPV-associated cervical cancer.[26–30] In their investigation of HPV-associated OPSCC in Mozambique, Blumberg *et al* propose that one potential contributing factor to the low prevalence of HPV-positive OPSCC in their cohort (14.5%) is the limited practice of oral sex in the region.[26] This has been reitereated by Rettig *et al*, who observed low rates of oral HPV infection among HIV-infected individuals in Northwest Cameroon and attribute this, at least in part, to relatively low rates of oral sexual behaviours.[27]

Historically, the majority of HPV-associated OPSCC occur in men, which may be due to differences in immune susceptibility and infection transmissibility through sexual activities, although this has yet to be fully elucidated.[4,31–33] However, an increase in incidence has been observed in Caucasian women in the US.[32] In their recent meta-analysis of twelve studies, Mariz *et* al observed similar prevalence of HPV-driven OPSCC in both males and females, despite the majority of the assessed OPSCC patients being male.

The prevalence of HPV-associated OPSCC was previously reported to decrease with increasing age, however, the burden of disease has begun to shift toward older men as a result of the birth cohort effect.[33,34] In one study, the median age has increased from 53 to 58 years between 1998 and 2013 while another study reported a similar increase, from 52 to 59 years between 2002 and 2017.[35,36] A rapidly increasing incidence in white men above 65 years of age has been observed and nearly 10% of cases have been reported in those above 70 years of age.[32,36] Nevertheless, increased rates of disease continue to be evident in both younger and older adults and, while the burden is shifting toward older adults, the majority of cases remain in those under 65 years of age.[31,37,38]

In the US, a higher prevalence of HPV-associated OPSCC has been observed in Caucasians when compared to racial minorities.[39] In an analysis of the National Cancer Database, a higher proportion of Caucasian OPSCC patients were HPV-positive.[40] In a recent analysis of the SEER database, there was a significant increase in rates of oropharyngeal cancer in Caucasian and Hispanic men, and men of other ethnicities, but a decrease in Black men. However, Faraji *et al* has reported a significantly more rapid increase in the prevalence of HPV-positive tumours in Black and Hispanic Americans compared to White Americans.[9,32] It may be postulated that Black men have experienced a greater decrease in HPV-negative disease compared to Caucasian and Hispanic men resulting in the observed relative increase in HPV-positive disease, however, this has yet to be confirmed. In parallel with the increased incidence in Caucasian men in the US, higher socioeconomic status is also associated with increased rates of HPV-positive disease.[40]

Importantly, the majority of epidemiological studies on HPV-associated OPSCC have been conducted in the US and are not necessarily generalizable to other parts of the world, where differences in culture and custom may influence the various lifestyle factors that play a role in HPV-associated OPSCC aetiology. As such, further studies in diverse and particularly non-Western regions are needed in order to inform region-specific guidelines particularly with regard to clinical management and targeted public health measures.

Epidemiology: prophylactic HPV vaccination and HPV-associated OPSCC

There remains a need for improved cancer prevention in parallel to ever-changing societal norms. At present, there are no screening methods for earlier detection of OPSCC so prevention can only be robustly achieved through large-scale vaccination. HPV vaccination has been offered to girls for nearly two decades and has led to decreased rates of cervical cancer. One might argue that the herd immunity established through this may preclude the need for further vaccination in boys, considering the cost associated with such a mass vaccination program. However, the universal vaccination of girls will likely not completely mitigate the risk to boys and consequent development of HPV-related cancers.[41] Indeed, such policy must take into account the population of men who have sex with men, as well as those who have sexual partners from regions where a comprehensive vaccination program, even in girls, does not exist. Furthermore, variability in vaccination uptake due to practical, societal and cultural barriers to vaccination will likely continue to hinder the ability for populations to achieve the necessary levels of immunity to prevent future malignancy.

Therefore, several countries have now extended nationwide vaccination programmes to boys, including Australia, Austria, Germany, Italy, New Zealand, the UK and the US. Australia was one of the first countries to implement a gender-neutral programme and has demonstrated significantly high vaccination uptake with 75.9% and 80.2% of boys and girls, respectively, completing a 3-dose regimen.[42] In comparison, half of US adolescents in 2018 had completed the recommended three-dose regimen and nearly one-third were unvaccinated.[43] In the UK, school-based vaccination was extended to include boys in September 2019. In the subsequent academic year, the first of a 2-dose vaccination regimen was given to 59.2% and 54.4% of girls and boys.[44] Importantly, due to school closures as a result of the ongoing Covid-19 pandemic, the programme's roll-out was interrupted. Therefore, the true uptake from this first year of a gender-neutral vaccination programme in the UK has yet to be determined.

Barriers to vaccination persist, including parental concerns over vaccine safety, socioeconomic factors and an overall lack of awareness.[8,45–47] In a survey of 725 US adults between 27 and 45 years of age, only 36% of responders were aware that HPV causes non-cervical cancers.[48] In a separate survey of roughly one thousand UK parents with children in school Years 5 to 7 (aged 9 to 12), prior to the extension of vaccination to boys in 2019, only half had heard of HPV and under

25% knew that the HPV vaccination would be offered to boys.[49] From this study, it was shown that proper education of parents led to roughly two-thirds of parents indicating they would vaccinate their child while only 10% would not. This implies that the provision of due information to parents by healthcare providers and Public Health administrators can lead to a high level of vaccine acceptance. Further education can help to assuage additional concerns for those who are undecided and demonstrate so-called 'flexible hesitancy.'[49] Importantly, improved knowledge on the part of healthcare providers is needed in order to effectively implement large-scale vaccination programmes. In a recent survey of healthcare professionals in the UK, over a third of participants indicated the need for improved training with 76% reporting that they felt adequately informed.[50] In a survey of GPs in the UK, while 74% recognized HPV as a risk factor of OPSCC, less than half were aware that being male was a risk factor for HPV-associated OPSCC.[51]

With regard to the efficacy of vaccination in preventing OPSCC, a recent report has demonstrated a substantial increased risk of developing malignancy in those who are not vaccinated compared to those who are.[52] Importantly, such conclusions may be premature as the effects of herd immunity as a result of female vaccinations is a significant confounder and the true effects of gender-neutral vaccination are still emerging. Nevertheless, this result is encouraging and reflects the efficacy of vaccination against oral HPV infection, which has been demonstrated in several reports. In their study of over 7,000 young women in Costa Rica, Herrero *et al* demonstrated a 93.3% decrease in oral HPV 16/18 infection due to vaccination.[53] A subsequent study of 2,627 US adults, the prevalence of oral HPV16/18/11 infection was significantly lower in vaccinated men compared to unvaccinated men.[54] This was similarly demonstrated in an analysis of the National Health and Nutrition Examination Survey (NHANES) data between 2009 and 2014, where vaccinated adults had a significantly lower prevalence of oral HPV 6/16/18/11 infection.[55]

Despite the recent introduction of HPV vaccination programmes for boys in several countries and a demonstrable efficacy against oral HPV infection, HPV-associated OPSCC rates are likely to rise further over the next 20-30 years before the full benefits of a vaccination programme can manifest. Indeed, Zhang et al recently forecasted that based on current vaccination rates in the USA, HPV-associated OPSCC incidence will continue to climb significantly among older individuals

between now and 2045, with a meaningful reduction confined to those below the age of 56, who are already at a lower risk of diagnosis and among whom the protective effects of vaccination will begin to manifest.[56] Consequently, significant human and broader societal costs are to be expected. In the UK, it has been estimated that roughly £2 billion will be spent on treatment for OPSCC in men, between 2019-38. Taking into account loss of workplace productivity due to illness, the cost increases to more than £18 billion.[57] Therefore, until the benefits of vaccination emerge, it is paramount that resources are put into improving public awareness of HPV-associated OPSCC and supporting public health initiatives in order to curb the substantial costs on human life and the wider society. This may also involve support for the development of novel early detection strategies, such as the use of peripheral blood for the detection of HPV16-E6 antibodies.[58]

HPV-driven carcinogenesis and the hrHPV oncogenes

Human papillomaviruses are non-enveloped viruses with circular double-stranded DNA genomes of approximately 8 kilobase pairs. Over 200 HPV types have been identified, all of which infect and complete their productive life-cycle in either cutaneous or mucosal epithelia. Of these, the World Health Organisation currently classifies 14 mucosal HPV types (HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 59, 66 and 68) as 'high-risk', due to clear experimental and epidemiological evidence implicating them in cancer causation, with HPV16 accounting for at least 85% of HPV-associated OPSCC. [59] The productive HPV16 life-cycle is intimately linked to the terminal differentiation of keratinocytes in stratified mucosal epithelia, while carcinogenesis occurs in the context of persistent infection (postulated to be favoured in the immune privileged microenvironment of the tonsillar crypts[60]) and represents an exit from productive viral replication.[61,62] The stepwise changes to viral and host gene expression and alterations to the host genome that are associated with carcinogenesis in the cervix have been studied extensively and are summarized in Figure 2A. HPV-associated carinogenesis is largely driven by two viral early genes (E6 and E7, often referred to as HPV oncogenes), whose normal function is to trigger cell cycle entry in the basal layer of the epithelium and therefore to permit viral genome replication. Increased expression of E6 and E7 is often associated with integration of hrHPV DNA into the host genome, although carcinogenesis can

occur in the absence of integration (whole genome sequencing of 103 HPV-positive OPSCCs reported evidence of viral integration in 74% of cases, with the remaining tumours harbouring episomal HPV[63]; a similar frequency of integration to that seen in HPV16+ cervical cancers).[64] Similar to cervical cancer, disruption of another viral gene, E2, which acts to repress expression of E6 and E7 during productive infection, is frequently observed in OPSCCs harbouring integrated HPV and has been linked to unfavourable prognosis in OPSCC.[65] Consistent with these findings is the observation that the physical state of the HPV genome is of clinical significance in HPV-positive OPSCC, with a recent study of 84 cases reporting shorter overall survival and evidence of decreased anti-tumour immune responses in patients displaying HPV gene expression from integrated copies (i.e. those in which chimeric viral/host mRNA sequences could be detected), compared with those displaying no evidence of integration.[66]

Much research has gone into understanding the molecular mechanisms by which E6 and E7 exert their effects to induce cell cycle entry and DNA replication in host cells; effects which in the case of the hrHPV types can, in combination with alterations to the host genome, result in malignant transformation of the host cell through enabling many of the hallmarks of cancer defined by Hanahan and Weinberg (Figure 2B).[67,68] The two best characterized oncogenic activities of hrHPV E6 and E7 are the induction of p53 and pRb degradation respectively. The removal of these critical tumour suppressor proteins results in loss of cell cycle checkpoints triggered by DNA damage and uncontrolled licencing of DNA replication, which together result in genomic instability and resistance to programmed cell death (apoptosis).[69–74]

Epigenetic reprogramming establishes oncogene addictions in HPV-transformed cells.

While inhibition of pRb function has long been recognized a key oncogenic property of epigenetic reprogramming of the host cell via the pRb-independent induction of two lysine demethylases, KDM6A and KDM6B. These chromatin-modifying enzymes exert broad effects on gene expression, including the derepression of Homeobox (HOX) genes: master regulators of development normally silenced by Polycomb group (PcG) proteins.. In addition to these effects on chromatin state and derepression of PcG targets, further examples of epigenetic reprogramming by

HPV include E6-dependent modulation of micro-RNAs and other non-coding RNAs[75–77] which act as regulators of gene expression, and the modulation of DNA methylation, which has been linked both to upreguation of DNA methyltransferases DNMT1 and DNMT3A in HPV+OPSCC[78] and to the direct interaction of HPV16 E7 with DNMT1[79–82] . It has been proposed that suppression of pRb function by E7 is necessary to prevent induction of an oncogene-induced senescence (OIS)-like response triggered by this reprogramming, rendering HPV-transformed cells dependent on the ongoing expression of the HPV oncogenes, as demonstrated by genetic loss-of-function experiments in primary cultures from cervical cancer.[83,84] This oncogene addiction has stimulated efforts to inhibit E6 and/or E7 as a therapeutic strategy, although this has proven challenging due to their lack of intrinsic enzymatic activity.[85] Encouraging progress has been made in exploiting the HPV oncoproteins as targets for therapeutic vaccines however (see '*Emergence of immunotherapies for the treatment of HPV+ OPSCC'*).

The epigenetic reprogramming of HPV-transformed cells by the E7-KDM6B axis also results in dependence on the $p16^{INK4A}$ tumour suppressor protein (hereafter 'p16', one of two cell cycle inhibitory proteins encoded by the PcG-regulated *CDKN2A* gene), due to its ability to suppress cyclin-dependent kinase (CDK4 and CDK6) activity, which in uninfected cells is required to relieve pRb-mediated inhibition of cell cycle progression.[86,87] The dependence on p16 to limit CDK4/6 activity in HPV-transformed cells is in striking contrast with many other tumour types, including ER^+ breast cancer for example, in which CDK4/6 inhibition has proven to be a highly successful therapeutic strategy.[88] This oncogenic role for the p16 tumour suppressor highlights the cellular re-wiring induced by HPV and the importance of understanding this for the rational design of targeted therapeutic strategies in HPV-positive disease. The functional requirement for p16 in HPV-transformed cells is also likely key to its utility as a clinical biomarker for diagnosis of HPV-positive OPSCC (see '*Clinical presentation and diagnosis*'), as it is much less likely to be lost or downregulated than a protein with deleterious or neutral effects on tumour cell fitness. Dependency on a second tumour suppressor protein ($p21^{CIP1}$) is also established downstream of E7-directed epigenetic reprogramming, in this case the induction of $p21^{CIP1}$ expression from the *CDKN1A* gene by KDM6A is needed to limit the rate of DNA replication driven by the Proliferating Cell Nuclear

Antigen (PCNA) and therefore to avoid lethal replication stress.[89] The rewiring of cell cycle control caused by E6 and E7 is represented in Figure 2C, which also highlights the fact that in this updated model of HPV oncogene function, the upregulation of p16 seen in HPV-positive cancers is due to induction of KDM6B by E7 not (as is often assumed) to the inhibition of pRb.[86]

Many other cellular proteins are targeted by the HPV oncoproteins, a comprehensive discussion of which is beyond the scope of this review. We have summarised some of these additional activities in Figure 2B and the reader is referred to numerous detailed reviews for further information, including.[68,90–93]

Somatic alterations and mutational processes in HPV-positive OPSCC reflect disease aetiology

Despite the ability of sustained E6 and E7 expression to initiate tumorigenesis, progression to carcinoma requires acquisition of somatic alterations in the host genome. HPV-negative HNSCCs harbour more copy number alterations than HPV-positive HNSCCs, suggesting a lower degree of genomic instability in HPV-positive disease, while single nucleotide variant (SNV) burdens appear similar between HPV-positive and HPV-negative HNSCC, at a median of approximately 2-3 mutations per megabase across the genome.[94–97] *TP53* (the gene encoding p53) is the most frequently mutated gene in HPV-negative OPSCC, occurring in at least 75% of cases but *TP53* mutations are rarely observed in HPV-positive disease, almost certainly due to the aforementioned inhibition of p53 function by E6 and thus an ability of the virus to phenocopy this genetic hit[94,97–100]. It is important to note however, that p53 loss is not entirely equivalent to *TP53* mutation, which can bestow gain-of-oncogenic function on the protein. Indeed, *TP53* mutations are seen in a subset of heavy smokers with HPV-positive OPSCC and have been associated with poor prognosis in these patients.[97] Smoking-associated *KRAS* mutations typical of those seen in lung squamous carcinoma have also been reported in HPV-positive OPSCCs from patients with >10 pack years smoking history.[94,97,101]

While somatic mutations attributable to tobacco-smoking and ageing predominate in HPV-negative OPSCC, a high proportion of mutations in HPV-positive disease (at least in the majority of HPV+ OPSCC patients who are not heavy smokers) are now thought to be caused by the off-target

DNA editing activity of one or more apolipoprotein-B mRNA editing catalytic polypeptide-like (APOBEC3) enzymes, whose physiological function is to suppress viral replication by deaminating cytosine bases in the context of single-stranded DNA or RNA.[95,96,102] Two of the seven human APOBEC3 enzymes (APOBEC3A and APOBEC3B) have been implicated in the cellular response to HPV infection, with evidence linking APOBEC-mediated editing of the viral genome to clearance of infection, at least in the cervix.[103] Sequencing of matched host exomes and viral genomes from HPV-postive OPSCC suggests that in cases where the APOBEC response is induced but fails to clear the virus however, off-target APOBEC activity against the host cell genome accounts for many of the somatic mutations seen in the tumour[104] (for detailed reviews see Smith and Fenton 2019,[105] Fenton 2021,[106] and Warren *et al* 2017[107]).

Activation of PI3K signalling in HPV-positive OPSCC: mechanisms and clinical significance

A key consequence of APOBEC activity activity against the host genome in HPV-positive OPSCC appears to be the generation of oncogenic point mutations in *PIK3CA*, which encodes the p110α catalytic subunit of the class 1A phosphoinositide 3'-kinase (PI3K).[95,96,102] Activation of the phosphoinositide 3'-kinase (PI3K) signaling pathway by somatic mutation and/or copy number alterations of *PIK3CA* is a key feature of HPV-positive OPSCC and appears to occur early in carcinogenesis.[96,108,109] Detection of activating mutations in PI3K components (*PIK3CA*, *PIK3C2B*, *PIK3R1*) and downstream mediators in the PI3K/mTOR pathway (*MTOR*, *RICTOR*) or inactivating mutations in the negative regulators, *PTEN*, *TSC1* or *TSC2* in metastatic tumours have been associated with longer OS in HPV-positive OPSCC patients,[110] while *PIK3CA* mutations were associated with increased risk of tumour recurrence in HPV-positive OPSCC patients receiving first-line chemoradiation in the setting of deintensification trials.[111] *PIK3CA* (mutation or amplification) has also been associated with dramatically prolonged disease-specific (HR = 0.23, p = 0.0032) and overall survival in HNSCC, specifically amongst patients taking regular (≥2 days/week for at least 6 months) non-steroidal anti-inflammatory drugs in a retrospective study, including those with HPV-positive disease; potentially due to increased activity of cyclooxygenase in *PIK3CA*-altered tumours.[112] While this intriguing observation requires confirmation in larger HNSCC cohorts, *PIK3CA*

mutation has also been associated with benefit from NSAIDs in colorectal cancer patients, potentially due to the induction of cyclooxygenase-2 activity by PI3K signalling.[113,114] Loss-of-function mutations in *PTEN* (which encodes the $PI(3)P_3$ phosphatase that reverses the reaction catalysed by class 1 PI3K) are significantly enriched in primary HPV-positive OPSCC, as are loss-of-function mutations in *CYLD* which encodes a ubiquitin ligase, and gain-of-function mutations in the receptor tyroinse kinase *FGFR3*, both of which can also result in activation of PI3K signalling.[96]

Other significantly mutated genes in HPV-positive OPSCC include those in pathways targeted by HPV oncogenes and those encoding regulators of gene expression

Genes involved in epidermal differentiation, including *ZNF750*, *KMT2D*, *EP300*, *RIPK4* and *NOTCH1* are significantly mutated in HPV-positive OPSCC, as are various components of the p53 (although as noted above, very rarely *TP53* itself) and pRB pathways targeted by E6 and E7, including mutation or loss of *RB1* (the gene ecoding pRb) in as many as 40% of HPV-positive OPSCCs.[94,96] In a recent genomic analysis of 157 OPSCCs, 73 of which were HPV-positive and for which long-term clinical follow-up data were available, *NOTCH1* mutations were associated with significantly shorter OS specifically in the HPV-positive cases.[97] This observation, together with data showing that *Notch1* inactivation generates higher-grade tumours in a mouse model of HPV16 E6/E7-driven HNSCC suggests that even though *NOTCH1* expression is suppressed by E6, mutational inactivation may lead to a greater effect on the pathway and therefore to the development of more aggressive tumours.[115,116] The importance of overcoming host immunity to viral infection is evident also in the frequent appearance of mutations in components of the interferon response, including *DDX3X*, *TRAF3*, *IFNGR1*, *NFKBIA, TGFBR2, EP300* and *KMT2D*; again these are alterations that are selected for despite the suppression of the pathway at multiple levels by HPV oncoproteins.[117]

EP300 and *KMT2D* both encode chromatin-modifying enzymes, *NFKBIA* encodes a negative regulator of the Nuclear Factor kappa B (NFκB) transcription factors and *DDX3X* encodes a regulator of RNA metabolism and the transcription factor genes *ZNF750*, *CASZ1* and *TAF5* are also significantly mutated in HPV-positive OPSCC.[100] The somatic alteration of these transcriptional

regulators, together with the effects of E7 on *KDM6A*, *KDM6B* and *DNMT1* discussed aboe emphasizes the importance of host cell re-wiring during HPV-driven carcinogenesis; a phenomenon evident from the multiple studies that have defined gene expression signatures for HPV+ OPSCC or pan-tissue expression signatures for HPV-associated malignancies.[96,118,119]

Anti-tumour immune responses in HPV-positive OPSCC:

In non-viral malignancies, major histocompatibility complex (MHC)-loaded peptides generated by nonsynonymous somatic mutations in expressed genes are the primary means by which anti-tumour T-cell responses are induced, and the success of immunotherapy is associated with both the overall number (closely linked to tumour mutation burden) and clonality (the fraction of tumour cells in which a given neoantigen is present) of such neoantigens.[120,121] During tumour development, cells that express highly immunogenic neoantigens may be eliminated; a process known as immunoediting.[122] In HPV-associated cancer, all tumour cells are exquisitely dependent on the expression of the viral oncogenes, E6 and E7, thus these proteins serve as an indispensable source of tumour-specific antigens to which anti-tumour immune responses can be mounted. Human papillomaviruses however, have evolved many mechanisms by which to evade host immune responses, from 'passive' mechanisms, such as limiting infection to outside the basement membrane of the epithelium and restricting high gene expression and virion production to the upper layers, where few immune cells are found, to active suppression of host cell interferon responses and antigen presentation.[123] As discussed earlier, during progression of persistent infection to malignancy, E2-mediated control of viral gene gene expression in the basal layer is lost and invasive tumours also breach the basement membrane, therefore the active suppression of host immune responses to the virus is critical for HPV-positive tumour cells to avoid immune destruction. Key mechanisms include the selective retention of certain MHC class 1 components (HLA-A and HLA-B) in the Golgi apparatus through direct interaction with the Golgi-resident HPV16 E5 protein, which inhibits recognition of E5-expressing cells by CD8+ (cytotoxic) T-cells[124–126] and the inhibition of MHC class 1 gene expression by HPV16 E7.[127–129]

In spite of these, and numerous other mechanisms by which HPV oncoproteins interfere with antigen processing and presentation (reviewed in Steinbach and Riemer 2018[123]), the majority of HPV-positive OPSCCs show evidence of ongoing intratumoural HPV16 E6 and/or E7-specific T-cell mediated immune responses.[130,131] The presence of such responses appears strongly prognostic, with Welters et al reporting a 37-fold increased chance of disease-specific survival in those HPV DNA-positive OPSCC patients from whose tumours they could isolate HPV16-specific T-cells, the majority of which were CD4+ and produced cytokines (IFNγ and TNFα, IL2, IL-17) consistent with anti-tumour (Th1/Th17) T-cell polarization.[131] In further work, the same group have implicated subsets of effector memory (CD161+) T-cells with high levels of cytokine production and a recently-identified CD163+ dendritic cell subtype (DC3) as key mediators of these HPV-specific responses in HPV-positive OPSCC.[132,133] HPV-specific T-cells have also been identified in blood from patients with HPV-positive OPSCC, with circulating E7-specific CD8+ T-cells associated with longer disease-free survival.[134,135]

While these studies on HPV-specific immune responses identify clear prognostic information, such analyses require *ex vivo* culture and functional assays and so pose difficulties for translation into routine use as clinical biomarkers for predicting therapeutic response.[136] Prognostic information can also be gained from less refined analyses of the tumour immune microenvironment and circulation in HPV-positive OPSCC patients. Total (CD3+) T-cell tumour infiltration is an independent prognostic indicator of improved overall survival, local progression-free survival and distant metastasis-free survival in HPV-postive OPSCC[137,138], and in those tumours displaying a mutational signature attributable to tobacco smoking, immune infiltrates are significantly reduced, offering a potential explanation for the aforementioned association between smoking and poor prognosis.[22,139] T-cell infiltration and activation (assessed based on gene expression patterns) is also significantly higher in HPV+ OPSCC than in other HPV+ HNSCCs in the TCGA cohort, possibly explaining the greater survival benefit conferred by HPV in the oropharynx than at other HNSCC subsites.[140,141] Similarly, a comparative analysis of HPV-positive OPSCC and HPV-positive cervical cancer revealed differences in the tumour immune microenvironment related to anatomical site, with HPV-positive

OPSCCs harbouring a higher CD4+:CD8+ ratio (reflecting a higher CD4+:CD8+ ratio in tonsils versus cervical epithelium) and greater numbers of CD4+CD161+ cells.[132]

In addition to the DC3 cells mentioned abvove, other immune cell types have also been associated with prognosis in HPV-positive OPSCC. Tumour-infiltrating B-cells are commonly observed in HPV-positive OPSCC, and a recent study reported CD20+ B-cell infiltration to be a superior prognostic marker than HPV-positivity or CD8+ T-cell infiltration in OPSCC.[142,143] Tumour-associated macrophages (TAMs) are associated with poor prognosis in many tumour types including OPSCC, however macrophage infiltration has been associated with improved progression-free survival in HPV-positive OPSCC treated with definitive radiotherapy + chemotherapy.[144] It is possible that skewing of macrophage polarization towards the inflammatory M1 phenotype due to high levels of IFNγ-producing T-cells in HPV-positive OPSCC is responsible for this favourable association (reviewed in Welters et al 2020 [145]).

Upregulation of the immune checkpoint protein, Programmed Death Ligand 1 (PD-L1) has been observed at higher frequencies in HPV-positive versus HPV-negative OPSCC. In some cases this appears to be due to HPV genome integration close to the PD-L1 (*CD274*) gene.[146,147] The increasing use of PD1/PD-L1 checkpoint blockade in HNSCC patients (see below) will shed further light on the extent to which HPV-positive tumours depend on this mechanism of immune suppression. Another immune checkpoint protein, natural killer group 2 member A (NKG2A) is expressed at higher levels in HPV-positive OPSCCs in which an HPV-specific immune response can be detected and is found on tissue-resident (CD103+) CD8+ T-cells, which have been linked to favourable prognosis in HPV-positive OPSCC and other cancer types. NKG2A antibodies are at an earlier stage of clinical development than anti-PD1/PD-L1 but have shown some promising results (reviewed in Welters et al 2020[145]).

Clinical presentation and diagnosis

OPSCC most commonly presents as a neck mass or sore throat, but may also present as dysphagia, visualized mass, globus sensation, odynophagia or otalgia.[148] The majority of patients present with early-stage disease (T1 or T2) and nodal metastasis. Clinical presentation of OPSCC

449 can be easily confused with other common benign conditions, however, it is recommended that
450 asymptomatic neck masses be evaluated with ultrasound and fine needle biopsy to confirm.[149]
451 OPSCC are comprised of tumours located at the posterior pharyngeal wall, the soft palate, the
452 tonsillar complex and the base of tongue. The latter two are most common, with up to 96% found in
453 tonsillar-related areas.[20,150] Of note, there exists a subset of head and neck cancers, which present
454 with cervical lymphadenopathy only. These carcinoma of unknown primary are rising in incidence,
455 attributed to the increasing rates of HPV-related OPSCC.[151] With this, the presence of p16 and/ or
456 HPV DNA in the metastatic lesion has been shown to indicate the oropharynx as the site of origin.[151–
457 153]

458 In general, clinical examination per the UK's National Multidisciplinary Guidelines involves
459 flexible direct endoscopy of the upper aerodigestive tract and cross-sectional imaging.[154] Both
460 PET/CT and MRI are recommended, the former for primary tumour staging and to assess soft tissue
461 spread, and the latter to determine the extent of nodal disease and bony invasion as well as for the
462 detection of distant metastases to the lung and liver.[155] Conversely, in the US, F-FDG PET/CT is the
463 main modality used to assess the extent of the tumour and presence of metastases, although MRI
464 may be used to assess the extent of local invasion.

465 In order to accurately discriminate between HPV positivity and negativity, use of a robust test
466 is required. A combination of p16 immunohistochemical staining and high risk HPV *in situ*
467 hybridization (ISH) has demonstrated acceptable sensitivity (97%) and specificity (94%) and can be
468 used on formalin-fixed paraffin-embedded tissue.[156] Especially as efforts are being made to de-
469 escalate treatment in HPV-positive cases, accurate diagnosis is paramount. While the AJCC 8th
470 edition recommends using p16 IHC only as surrogate for HPV status, it has been found that p16-
471 positivity is not sufficient to detect transcriptionally active HPV in all cases. In a recent study, patients
472 who were p16-positive/HPV-negative had significantly reduced five-year survival (33%) Cancer
473 stage was reduced in 95% of p16+/HPV− patients despite having a mortality rate twice (HR 2.66
474 [95% CI: 1.37–5.15]) that of p16+/HPV+ patients under new TNM8 staging criteria..[6] As such, a
475 second ISH test has been recommended in the UK as standard practice (UK Royal College of
476 Pathologists).

There are several variants of squamous cell carcinoma, the majority of which can be categorized into keratinizing and nonkeratinizing, with or without maturation (Figure 1C). The majority of non-keratinising SCCs are associated with transcriptionally active high risk HPV.[157] This HPV exposure increases risk, regardless of tobacco and alcohol habits. On the other hand, while keratinizing SCC is the most common OPSCC subtype, only 15-25% of keratinizing SCCs are HPV-positive. These tumours resemble stratified squamous epithelium with varying degrees of architectural and cytological abnormalities, such as the formation of keratin pearls. The invasion pattern at the advancing front has been shown to be a significant and independent predictor of local recurrence and overall survival. Importantly, clinical and histological appearance, as well as management and prognosis vary between the different subtypes of OPSCC. Other less common subtypes include basoloid SCC, papillary SCC, lympoepithelial carcinoma, adenosquamous carcinoma, spindle cell carcinoma and verrucous SCC. Basoloid and papillary SCC as well as lymphoepithelial carcinoma are generally associated with transcriptionally active, high-risk HPV infection in the oropharynx.[158–163]

In general, clinical prognostication is based upon tumour size and nodal status, positive margins, and grade (well, moderate and poorly differentiated), including invasion front grade, which involves the degree of keratinization, pleomorphism, mitotic rate, invasion pattern and host response.[164] There is a significant positive relationship with proliferative index. Other independent prognostic factors for local recurrence and overall survival include invasion pattern (cohesive or non-cohesive) as well as perineural and lymphatic invasion.[165] With regard to depth of invasion compared to tumor thickness in determining the AJCC's T-category, a retrospective study conducted by Dirven and coworkers demonstrated no significant difference.[166] Lymph node involvement and extracapsular/extranodal extension may also serve as prognostic factors, although the evidence here is less clear.[167–170] While Bauer *et al* and Freitag *et al* have reported reduced survival with extracapsular extension, Tian *et al* did not observe a significant association with overall, locoregional recurrence-free nor distant metastasis-free survival.[171–173] In a cohort of patients treated with transoral surgery and neck dissection, Sinha *et al* found that metastatic node number was an independent predictor of outcome, while extracapsular spread was not.[170] Elicin *et* al suggest that

extracapsular extension may serve as a surrogate of nodal volume, which itself appears to serve a greater prognostic role.[168] Lymph node ratio has also been investigated and, while significantly associated with survival in HPV-negative OPSCC, appears to be a weaker prognosticator in HPV-positive disease.[174] The authors, here, propose that the prognosis of HPV-positive disease may depend more on the extent of the primary tumour than nodal spread. While the determination of extracapsular spread has generally relied on post-operative histopathology, the use of CT imaging has been recommended for use in the initial prognostic work-up. However, its predictive capacity is controversial, with previous studies reporting only moderate specificity and low sensitivity, as well as poor positive and negative predictive values.[175–177] Nevertheless, Carlton *et al* have found that the identification of three or more imaging criteria improves specificity and postivie predictive value, while Aiken *et al* have found that the presence of necrosis independently and significantly correlates with pathologically-proven extracapsular spread.[176,177] More recently, a study of thirty-one patients assessed with contrast-enhanced CT demonstrated good sensitivity etween 81-85% and excellent interobserver agreement.[178] Altogether, whether or not extracapsular spread remains a useful clinical prognostic factor, considering the challenges associated with the radiologic prediction of extranodal pathology, is unclear. The heterogeneity of data presented thus far and the contradicting results warrant further large-scale and multi-centre studies in order to guide clinical management.

The most recent edition of the AJCC staging guidelines, based on the International Collaboration on Oropharyngeal Cancer Network for Staging (ICON-S) cohort study, differentiated OPSCC based on HPV-status, as determined by p16 overexpression (Table 2).[179] With changes made to N staging in particular, many patients with HPV-positive disease were assigned to a lower stage as a result. Furthermore, this update reserves stage IV for metastatic disease only. These changes, amongst others, enabled improved survival discrimination, which is especially important in the era of treatment de-intensification.[180,181] However, while the updated system overall has been shown to be prognostically superior to the previous edition, its ability to discriminate between stage groups, particularly II and III and between III and IV, is controversial. Therefore, implementation of the staging system in clinical practice requires further adaptation, taking into consideration other

prognostic factors including the aforementioned as well as those mentioned in the following discusion.[182,183]

It is important to reiterate that there exists a subgroup of patients who are p16-positive but HPV DNA-negative, with significantly worse prognosis compared to HPV DNA-positive.[6,184] Therefore, as mentioned above, determination of HPV status should make use of both p16 expression and ISH-mediated detection of high risk HPV DNA. In addition, other tumour and patient factors may be necessary considerations to improved prognostication. A recent study, which conducted recursive partitioning of the Radiotherapy Oncology Group (RTOG)-0129, established low, intermediate and high risk groups based on HPV status, tobacco exposure and extent of lymph node disease.[22] Low risk patients are HPV-positive with low tobacco exposure or a history of smoking $\leq$10 pack-years in addition to 1 ipsilateral lymph node less than 6cm; intermediate risk are patients, who are HPV-positive with a history of smoking >10 pack-years and advanced lymph node disease or HPV-negative with low tobacco exposure and <T4; high risk patients are HPV-negative with a history of smoking >10 pack-years or T4 disease. A recent retrospective analysis of this cohort assessing 5-year survival demonstrated robustness of this stratification, with persistent differences in OS and PFS.[185] Taking into account a second, independent cohort (RTOG-0522), combined OS for low, intermediate and high risk was 88.1, 69.9 and 45.1%, respectively and PFS was 72.9, 56.1 and 42.2%, respectively. The authors, here, recommend therapeutic deintensification for the low risk group.

Crucially, a recent analysis of the National Cancer Database found anatomic subsite to be an independent prognostic factor.[150] However, the current AJCC guidelines, whilst stratifying for HPV status, do not consider subsite. This is important as tonsillar and base of tongue SCC are more frequently HPV-positive, compared to other sites. Indeed, the prevalence of HPV in these sites appears to be less with roughly 19-22% of tumours positive for HPV, compared to 56-70% for tonsillar and base of tongue OPSCC.[186,187] Furthermore, the prognostic value of HPV at other sites appears to be less robust, calling into question the appropriateness of the present AJCC staging system at these sites.[187] A more comprehensive, and potentially more accurate, prognosticator,

559 which takes into account subsite, as well as patient history with particular regard for smoking history
560 as discussed above, on top of current AJCC staging, warrants continued investigation.
561
562 *Treatment and follow-up of HPV-positive OPSCC*
563 Treatment of OPSCC typically involves surgical excision, primary radiotherapy or
564 chemoradiotherapy (see Table 3 for UK Recommendations).[154] Historically, surgical excision has
565 been achieved by open surgery, however due to associated cosmetic and functional morbidities, this
566 has largely been replaced by less invasive techniques for early stage disease, such as transoral
567 laser microsurgery (TLMS) and transoral robotic surgery (TORS). Primary radiotherapy and
568 chemoradiotherapy are also widely used, where standard of care consists of 66-70 Gy radiotherapy
569 with concurrent platinum-based chemotherapy, typically cisplatin-based.
570 Despite the favourable prognosis for HPV-positive OPSCC, 10-25% of patients will develop
571 recurrence, the majority of whom will recur within the two years and some up to five years. Thus, the
572 need for a robust and effective monitoring protocol is crucial. Typical follow-up involves regular
573 clinical examinations. The National Comprehensive Cancer Network recommends examinations
574 every one to three months in the first year, then every two to six months in the second year, every
575 four to eight months up to year five then subsequently once per year.[188] However, even with regular
576 clinical examinations, the ability to detect disease recurrence is limited.
577 HPV DNA has been shown to be a useful biomarker for the monitoring of post-treatment
578 disease. In a recent prospective study, continued detection of tumour type HPV DNA in oral rinses
579 following completion of treatment was predictive of locoregional recurrence and lower 2-year overall
580 survival. Although prediction of distant metastasis was weaker, the authors suggest that oral and
581 plasma HPV DNA detection could potentially be combined to provide an effective biomarker for
582 treatment response and risk of progression.[189] In plasma samples, circulating HPV DNA (ctHPVDNA)
583 has proven to be an extremely sensitive means of detecting recurrence.[190] In a recent study of 115
584 patients, two consecutive positive tests had a positive predictive value of 94% and negative
585 predictive value of 100%. Therefore, this approach may allow for earlier detection of recurrence and,
586 as a result, may improve the efficacy of salvage treatment thereafter.

Outcomes with primary TORS/TLMS +/- adjuvant radiotherapy or adjuvant chemotherapy in recent clinical trials

Until recently, OPSCC was generally treated with primary radiotherapy due to the significant morbidity associated with open surgery. However, with substantial advances in surgical technology, minimally invasive approaches (i.e. TORS or TLMS) have become the mainstay of OPSCC treatment.[191] A recent study by Sinha *et al.*, assessing the efficacy of TORS demonstrated high 5-year survival with DFS, DSS and OS rates of 85%, 93% and 90%, respectively. The recurrence rate was 20% and mainly due to distant metastasis; in addition, 90% of recurrences occurred within the first two years. Minimal post-treatment morbidity was observed; in the absence of indications for gastrostomy, only 4% of patients had a gastrostomy tube.[192]

Importantly, most cases of OPSCC treated with TORS or TLMS include adjuvant radiotherapy and, in a minority, additional chemotherapy.[193] As such, appropriate risk stratification is needed to safely de-escalate and thus capitalize on the reduced post-treatment morbidity offered by minimally invasive surgical techniques. As demonstrated by both Jackson *et al* and Carey *et al*, adjuvant therapy lowers the risk of local and regional recurrence, however, no significant differences in overall survival have been observed due to high salvage rates.[194,195] Indeed, while patients, who do receive upfront adjuvant therapy may relapse, salvage treatments are generally successfully, resulting in excellent survival rates. This is of especial importance due to the various toxicities associated with adjuvant radio/chemoradiotherapy. Jackson *et al* observed a greater risk of gastrostomy in patients who received adjuvant therapy. In their study on quality of life in patients who received TORS alone, Sethia *et al* demonstrated higher quality of life and superior functional outcome at 6 months as the side effects of adjuvant therapy, including xerostomia, odynophagia and oral thrush likely contribute to worse patient-reported outcomes.[196]

In cases where adjuvant radiotherapy is indicated, reducing radiation dose in patients with favourable risk factors (i.e. negative margins, early stage) can help to improve treatment-associated morbidity while maintaining efficacy. In patients with negative margins and minimal smoking history, Ma *et al* demonstrated that reducing adjuvant radiation dose from 60-66 Gy to 30-36 Gy leads to

improved swallowing and overall quality of life outcome while maintaining excellent 2-year locoregional control, progression free and overall survival rates (96.2%, 91.1% and 98.7%, respectively).[197] Alternatively, the AVOID study demonstrated that avoiding the resected primary tumor site and only targeting at-risk neck areas at reduced radiation dose in early-stage patients may be safe and can also result in high 2-year local control and survival rates (98.3%, 100%, respectively).

The safety and efficacy of de-intensified adjuvant therapy following TORS is currently evaluated further through ongoing trials, such as PATHOS and ECOG3311.[198] ECOG3311 presented updated reports both at ASCO2020 and ASCO2021, respectively, showing that primary TORS and reduced PORT without chemotherapy appears sufficient re the oncologic outcome at 35 months follow up, with favorable QOL and functional outcomes, in intermediate risk HPV-positive OPSCC.[199,200] As well, both the SIRS and MINT trials (NCT02072148, NCT03621696, respectively), will further help to confirm the accuracy of using pathological characteristics (i.e. extracapsular spread, lymphovascular invasion, perineural invasion, surgical margins and tumour stage) for the allocation of treatment, with particular regard for the omission of adjuvant therapy in low-risk patients. Reduced dose adjuvant radiation in high-risk patients will also be further investigated in both DART-HPV (NCT02908477) and DELPHI (NCT03396718).

Outcomes for primary radio/chemoradiotherapy in recent clinical trials

While positive results have been seen with minimally invasive surgical approaches, primary radiotherapy or chemoradiotherapy are still widely used. More recently, efforts to de-escalate radiation dose have demonstrated excellent outcome and improved morbidity rates. In two studies, Chera *et* al demonstrated high pathologic response to a reduced-dose IMRT regimen with concurrent low-dose cisplatin for early-stage disease.[201,202] Excellent 3-year local and regional control were also observed with a 3-year overall survival rate of 95%. Importantly, this de-intensified regimen led to favorable long-term functional outcome and quality of life.[203] For late-stage disease (stage III/IV), induction chemotherapy followed by reduced-dose chemoradiotherapy has proved to be a promising approach for improving treatment-associated morbidity while maintaining high survival rates.[204–207]

643 Indeed, prescribing radiation dose based on the extent of pathologic response to induction
644 chemotherapy takes appropriate advantage of the radiosensitive nature of certain tumours,
645 improving both survival outcome and long-term functional outcome, including swallowing, nutritional
646 status and BMI and overall quality of life.

647 With regards to the necessity of concurrent chemotherapy, results from one study show that
648 radiotherapy alone may be sufficient for HPV-positive disease. Indeed, while radiotherapy alone was
649 detrimental to p16-negative/HPV DNA-negative patients, there was no significant difference in
650 survival for p16-positive/HPV DNA-positive patients.[208] However, in addition to HPV status, the
651 extent of disease may be an additional important factor when considering the exclusion of
652 chemotherapy. In their retrospective analysis of over six hundred patients, Hall *et al* found that
653 concurrent chemotherapy reduced the risk of metastases in high risk (i.e. AJCC 7th edition T4 and/or
654 N3) HPV-positive OPSCC but not in low-risk disease.[209] Conversely, in a recent randomized phase
655 II trial of low-risk HPV-positive OPSCC, the addition of concurrent cisplatin led to improved disesase-
656 free survival, in comparison to those who received radiotherapy alone.[210] With this, a conclusion
657 cannot be drawn regarding the safety and efficacy of excluding chemotherapy from primary
658 treatment.

659 Therefore, at present, the pursuit of treatment de-escalation should remain in the confines of
660 a well-designed clinical trial per a recent American Society for Radiation Oncology (ASTRO)
661 consensus paper.[211] Ongoing and future studies may further provide the necessary evidence to
662 update standard-of-care. These include, for early-stage disease, the EVADER trial, which aims to
663 determine survival outcome with reduced dose radiotherapy with or without concurrent
664 chemotherapy. The safety of hypofractionated radiation therapy with concurrent chemotherapy as
665 well as that of SABR boost and de-escalated chemoradiation will be further investigated by HYHOPE
666 (NCT04580446) and SHORT-OPC (NCT04178174), respectively. The Quarterback trials
667 (NCT01706939, NCT02945631) aim to determine the survival outcome of reduced dose
668 radiotherapy in late-stage disease (stage III or IV), in addition to acute and long-term toxicities. The
669 results from these studies and others will enable a better and more comprehensive understanding

of de-escalated primary radio/chemoradiotherapy and provide the necessary evidence to potentially influence standard-of-care.

TORS or primary radio/chemoradiotherapy

A retrospective query of the National Cancer Database did not demonstrate any significant difference in overall survival in HPV-positive OPSCC patients who received either primary TORS or primary radiotherapy.[193] However, while survival may be similar between the two methods, differences in their respective toxicity profiles and consequent morbidities are important considerations in the clinical decision-making process.

Importantly, prior to the ORATOR trial, there had been no prospective studies investigating differences in outcome between TORS/TLMS alone and primary chemoradiotherapy.[212] The ORATOR trial was not able to determine definitive differences in survival between these two treatment modalities due to its modest sample size, and the study did demonstrate similar outcomes in quality of life between the two approaches and identified a spectrum of treatment-specific toxicities.[213] However, the trial only reported one-year swallowing and oncologic outcome data. Importantly, the authors observed a risk of bleeding associated with TORS, but multi-institutional approaches to TORS with large patient numbers showed low rates of severe bleeding.[214,215] As such, both treatment options should be presented to the patient at present. A second study, ORATOR2, is currently underway to further confirm these findings and determine survival outcomes in a larger cohort.

Targeted therapies investigated in recent trials

Recent and ongoing clinical trials are investigating the efficacy of targeted therapy as neoadjuvant, concurrent or adjuvant therapy in addition to conventional surgery, radiotherapy or chemoradiotherapy. Two prospective randomized-controlled trials investigated the use of the anti-epidermal growth factor receptor (EGFR) mAb, cetuximab, as replacement for cisplatin in an effort to reduce treatment-related toxicities and morbidities. While the side-effect profile remained similar, there was poorer locoregional control and an increased incidence of distant metastases; furthermore,

there was a reduction in overall and progression-free survival.[216,217] While *EGFR* is amplified in a majority of head and neck cancers, there is likely an important difference in expression pattern for oropharyngeal cancers, specifically.[218] Genomic studies have not demonstrated clonal selection of mutated or amplified EGFR in HPV-positive tumours, in contrast to HPV-negative tumours. However, it has been shown to be upregulated through gene fusion.[94,219]

Along a different vein, one study has demonstrated the safety of an induction chemotherapy regimen consisting of de-intensified chemotherapy in combination with the antiviral, ribavirin, and the EGFR (ErbB) family inhibitor, afatinib, in patients with locally advanced HPV-associated OPSCC.[220] Biologically, the authors postulate that the anti-tumour action of afatinib occurs through inhibition of ErbB2 (HER2/neu) signaling, which is oncogenically dysregulated through the action of the E6 protein. While promising, further investigation is needed to better understand the biological mechanism of this combination as well as its efficacy as an alternative, de-intensified induction therapy approach.

Emergence of immunotherapies for the treatment of HPV+ OPSCC

Raising *de novo* or potentiating existing immune responses to viral antigens (particularly E6 and E7) in HPV-associated malignancies is a tantalizing and long-sought prospect for immunotherapy. The many and varied approaches to immunotherapy for HPV-associated cancer that have been developed over the past 20 years are covered in detail elsewhere; we will highlight some recent clinical trials in HPV-positive OPSCC here but it is important to note that thus far, only inhibition of the PD1/PD-L1 immune checkpoint has been approved for clinical use.[221–223] The anti-PD1 antibodies, nivolumab and pembrolizumab were first approved by the US Food and Drug Administration (FDA) for metastatic, platinum-refractory HNSCC (regardless of HPV status) based on the phase III trials CHECKMATE 141 and KEYNOTE-040, respectively, and pembrolizumab was recently FDA-approved as a first-line monotherapy in HNSCC patients with metastatic or unresectable disease and tumour PD-L1 expression, based on the phase III KEYNOTE-048 trial.[224–226] The above trials all incuded both HPV-positive and HPV-negative patients and several systematic reviews have recently investigated possible associations between HPV status and outcomes, with

three studies suggesting increased ORR and OS in HPV+ patients,[227–229] with one suggesting a stronger relationship in the context of PD-L1 blockade and another[230] reporting no association between HPV status and response or survival. All four studies highlight the need for further research into this important question and point to a current lack of data on the relationship between HPV status and PFS in patients receiving adjuvant anti-PD1/PD-L1 therapy. Several studies have recently reported modest response rates in HNSCC patients receiving neoadjuvant anti-PD1/PD-L1 blockade, with higher ORR to neoadjuvant nivolumab observed in patients with HPV-positive tumours in the CHECKMATE-358 trial.[231] A combination of neoadjuvant nivolumab and radiotherapy acheived a high rate of complete pathological responses among a cohort of 21 patients with locally advanced HNSCC, 16 of whom had HPV-positive disease.[232] The authors of this study noted the high rate of major pathological responses to radiotherapy alone among HPV-positive patients in this trial, indicating the need to determine the contribution of each single modality to these reponses. They also noted the unsuitability of radiologic response as an indicator of pathological response in this context, given the relatively short treatment window of six weeks. In addition to the already approved immune checkpoint inhibitors, the anti-PD-L1 mAb durvalumab is being investigated in multiple trials as a neoadjuvant therapy, with the CIAO phase 1b trial recently reporting promising activity in a cohort of 28 OPSCC patients, 24 of whose tumours were p16-positive but with no increased benefit the addition of anti-CTLA4 blockade.[233] Furthermore, atezolizumab is currently in phase III clinical trials for HNSCC as adjuvant monotherapy for locally advanced disease.[234] Anti-PD1 therapeutics are also being investigated in conjunction with the anti-CTLA4 mAb tremelimumab (NCT03618134, NCT03410615). Given the particularly strong Treg infiltration in HPV+ OPSCC and the evidence from mouse models that the anti-tumour activity of CTLA4 antibodies is due at least in part to the induction of antibody-dependent cell-mediated cytotoxicity (ADCC) against Tregs (which express high levels of CTLA4). it will be interesting to see the efficacy of CTLA4 blockade in this disease.[139,235]

Therapeutic vaccines based on E6 and/or E7 have long been investigated as treatments for cervical cancer, unfortunately thus far without significant clinical success. A number of therapeutic vaccines have entered trials for HPV-positive OPSCC however, with numerous studies now including

754 combination with a checkpoint inhibitor or other immune modulator.[223,236] Of the few trials that have
755 so far reported outcomes, a phase II trial combining nivolumab with an HPV16 E6/E7 peptide vaccine
756 (ISA 101) reported a response rate of 36% and median survival of 17.5 months among the 22
757 patients with HPV-positive OPSCC, which compares favourably with trials evaluating nivolumab
758 monotherapy.[237] MEDI0457 (a DNA vaccine encoding E6 and E7 antigens from HPV16 and HPV18,
759 administered together with DNA encoding IL-12 to act as an adjuvant in a phase I/IIa trial) induced
760 durable HPV-specific immune responses in 18 of 21 patients with locally advanced $p16^{+}$ HNSCC
761 and one patient who developed metastatic disease had a complete, rapid and durable response to
762 subseqeunt nivolumab treatment.[238] Other ongoing trials include: HARE-40, a phase I/II dose
763 escalation trial based in the UK which is determining the safety of an E7-targeting mRNA vaccine
764 delivered in combination with an agonistic CD40 antibody designed to enhance antigen presentation
765 by dendritic cells (NCT03418480); a phase I open label trial investigating MAGE-A3/HPV-16
766 targeting peptide vaccines as well as a first-in-man phase I/II trial investigating the novel E6/E7-
767 targeting vaccine, HB-201 with or without concurrent checkpoint inhibition (NCT04180215,
768 NCT03669718). The results of the above trials and others will be crucial in shaping the continued
769 and promising progress of immunotherapy for HPV-positive OPSCC.
770
771 *Future directions for targeted therapy*
772 Ultimately, it seems that with currently-available therapies, the de-escalation research
773 question in HPV-positive OPSCC is primarily one of chemoradiation dose de-escalation as opposed
774 to altered chemotherapeutic regimes. It is worth noting however, that the vast majority of molecular
775 data from HPV-positive OPSCC has thus far been derived from primary tumours, over 80% of which
776 are typically eliminated with chemoradiation. Key challenges are to identify the 15-20% of primary
777 tumours that are at high risk of recurrence and to determine effective treatments for recurrent
778 disease, in which two-year survival remains at 40%.[239] To this end, sequencing of 51 primary HPV-
779 positive OPSCCs, 16 of which recurred, together with 12 metachronous recurrent HPV-positive
780 OPSCCs (including seven cases in which matched primary tumours were also sequenced) was
781 undertaken, with the intriguing observation that recurrent tumours share genomic aberrations such

as *TP53* mutations that are almost exclusive to HPV-negative disease amongst primary HNSCC.[240] Consistent with this finding was the recent discovery of a gene expression profile associated with poor prognosis in HPV-positive OPSCC that bears similarities to HPV-negative HNSCC. Interestingly, HPV E6 and E7 expression did not vary between good and poor prognosis HPV-positive subgroups; instead the viral E1^E4 transcript, which functions during later stages of the productive HPV replication cycle but which has not previously been implicated in cancer, displayed significantly increased expression in tumours belonging to the good prognosis subgroup. The reasons for this remain unclear but might be linked to increased radiosensitivity in cells expressing E1^E4.[241] Given the findings from these studies, it will be important to determine whether cells derived from recurrent HPV-positive OPSCCs display the same dependence upon ongoing HPV oncogene expression as those derived from primary tumours since if not, this may have implications for the efficacy of HPV-targeted therapies (e.g. therapeutic vaccines) in advanced disease. Finally, in the largest genomic study of distant metastases in HPV-positive OPSCC to date, targeted cancer gene sequencing was conducted on samples from 26 metastatic tumours, revealing a potentially higher frequency of *PRKDC* mutations compared with primary tumours. *PRKDC* encodes the DNA-Dependent Protein Kinase Catalytic Subunit (DNA-PKcs), which is essential for the repair of DNA double-strand breaks by non-homologous end joining (NHEJ), thus the authors speculate that these metastatic tumours may respond to therapies such as PARP inhibitors, which exploit DNA repair defects.[110] Indeed, the PARP inhibitor Olaparib is currently being assessed as a radiosensitizer with the aim of improved logoregional control (NCT02229656). However, it will important to determine whether these tantalizing observations hold true in larger cohorts of recurrent and metastatic HPV-positive OPSCC and to develop preclinical models representative of these tumours.

Conclusion

The differentiation of HPV-associated OPSCC by the AJCC from its HPV-negative counterpart cements its distinct biology and improved prognosis. Its preference for younger individuals emphasizes the need for continued efforts to treat patients such that post-treatment quality of life is high. Novel targeted therapies, which improve on the associated morbidity and

mortality with current standard of care, will eventually include immunotherapies due to the fact that HNSCC displays particular immune sensitivity. Recent and ongoing clinical trials emphasize the potential for treatment deintensification as a means to improve patient quality of life while maintaining high survival outcome. While more trials are needed, it is apparent that such strategies can lead to excellent morbidity and mortality rates, and as such, patients who are eligible should be considered for such studies.

Importantly, there is still a need for further research into identifying and validating diagnostic, prognostic and predictive biomarkers in order to improve early detection, stratify patients for potential treatment deintensification or otherwise better allocate to current standard of care and in future, targeted therapies and immunotherapies.

Figure Legends

Figure 1: a) Directly age-standardised rates per 100,000 population of newly diagnosed cases of cervical and oropharyngeal cancer in the UK and the US. UK Office for National Statistics Cancer Data: Directly age-standardised rates per 100,000 population of newly diagnosed cases of cancer; for male oropharyngeal cancers (blue dotted line) and cervical cancers (blue solid line) from 1995 to 2016 (2016 data released on 25/5/2018). Male oropharyngeal cancers included base of tongue (International Classification of Diseases for Oncology, 3rd Edition [ICD-O-3] topography code C01), uvula (C05), tonsil (C09.0-09.9), oropharynx (C10.0-10.9), stratified for different types of squamous cell carcinoma (as for the US data). Cervical cancers (C53). US Surveillance, Epidemiology, and End Results (SEER) data: Observed age-standardised rates per 100,000 population of newly diagnosed cases of cancer; for oropharyngeal cancers among men (yellow dotted line) and cervical cancers (yellow solid line) from 1995 to 2014 from registries within the Surveillance, Epidemiology, and End Results (SEER) program. Oropharyngeal cancers included base of tongue (International Classification of Diseases for Oncology, 3rd Edition [ICD-O-3] topography code C01.9), lingual tonsil (C02.4), soft palate not otherwise specified (NOS; C05.1), uvula (C05.2), tonsil (C09.0-09.9), oropharynx (C10.0-10.9), and Waldeyer's ring (C14.2), stratified for different types of squamous cell carcinoma (histologic codes: 8052/3; 8053/3; 8070/3; 8071/3; 8072/3; 8073/3; 8074/3; 8075/3; 8076/3; 8077/3; 8078/3; 8083/3; 8084/3; 8094/3; 8051/3). Cervical cancers (C53) included all histologic subtypes. **b)** Basic anatomy of the oropharynx; HPV-positive OPSCC tropic for base of tongue (i.e. anterior 2/3rds), soft palate and tonsil. **c)** Clockwise from top-left: Non-keratinising SCC. Non-keratinising SCC with p16 stain; morphology is monomorphic, ovoid, hyperchromatic with inconspicuous cytoplasm. Additionally, exihibits increased mitosis, apoptosis and comedo-type necrosis. Keratinizing SCC: typically with filiform projections, a thickened, normal appearing stratified squamous epithelium, hyperparakeratosis and keratin plugging. Basaloid SCC: variable foci of squamous differentiation. Papillary SCC with early invasion, exhibits predominant filiform processes with minimal/absent keratinization, frequent mitosis and full thickness dysplasia with basaloid cell morphology. Spindle cell carcinoma: biphasic tumour composed of SCC and malignant spindle cell component, exhibits polypoid growth.

Figure 2. a) Major events in the development of HPV-driven malignancy based on the well-established stepwise model of cervical carcinogenesis. **b)** Schematic showing how HPV-driven oncogenic processes act to enable seven of the eight Hallmarks of Cancer originally defined by Hanahan and Weinberg and how we are attempting to disable some of these hallmarks using targeted therapeutics in recent or ongoing clinical trials in HPV-positive OPSCC (based on Hanahan and Weinberg,[67] Mesri et al,[68] Lechner and Fenton.[242]) **c**) Updated model of cell cycle perturbation by the HPV oncogenes E6 and E7 as proposed by McLaughlin-Drubin, Munger and colleagues, see main text for details. Cell cycle inhibitors ($p16^{INK4A}$ and $p21^{CIP1}$), upon which HPV-transformed cells become dependent are starred.

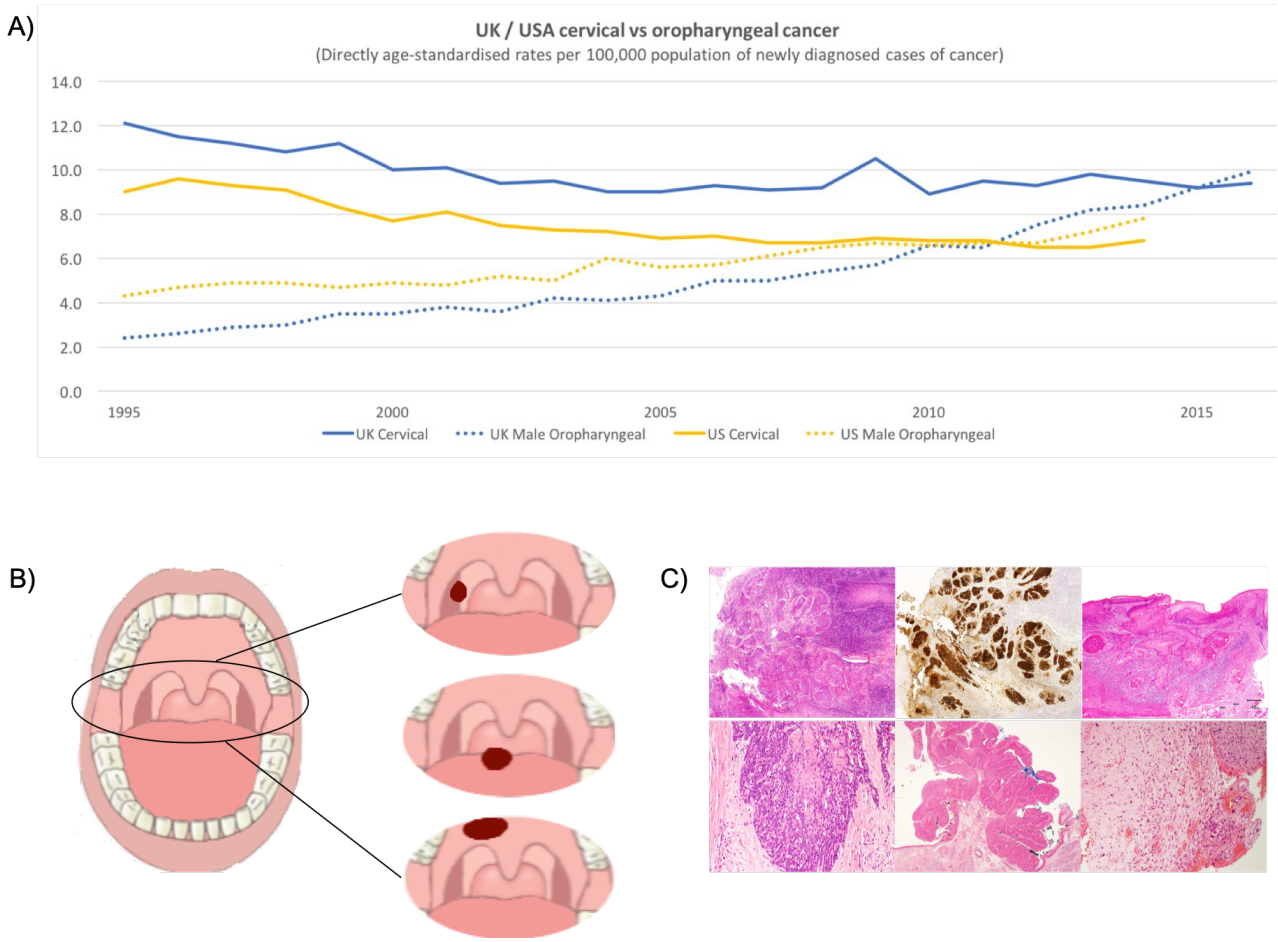
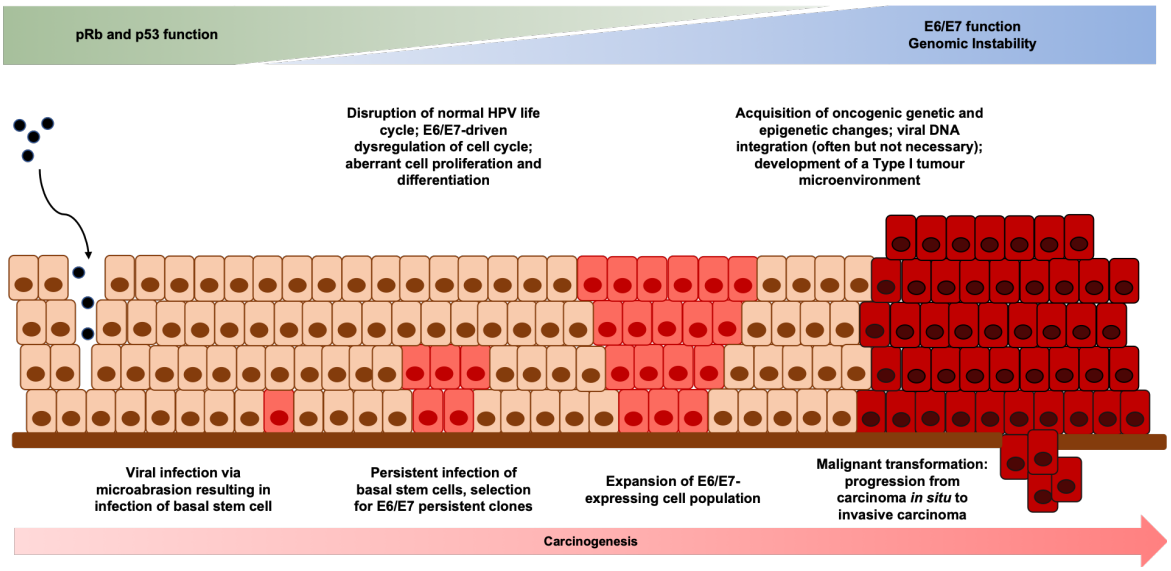
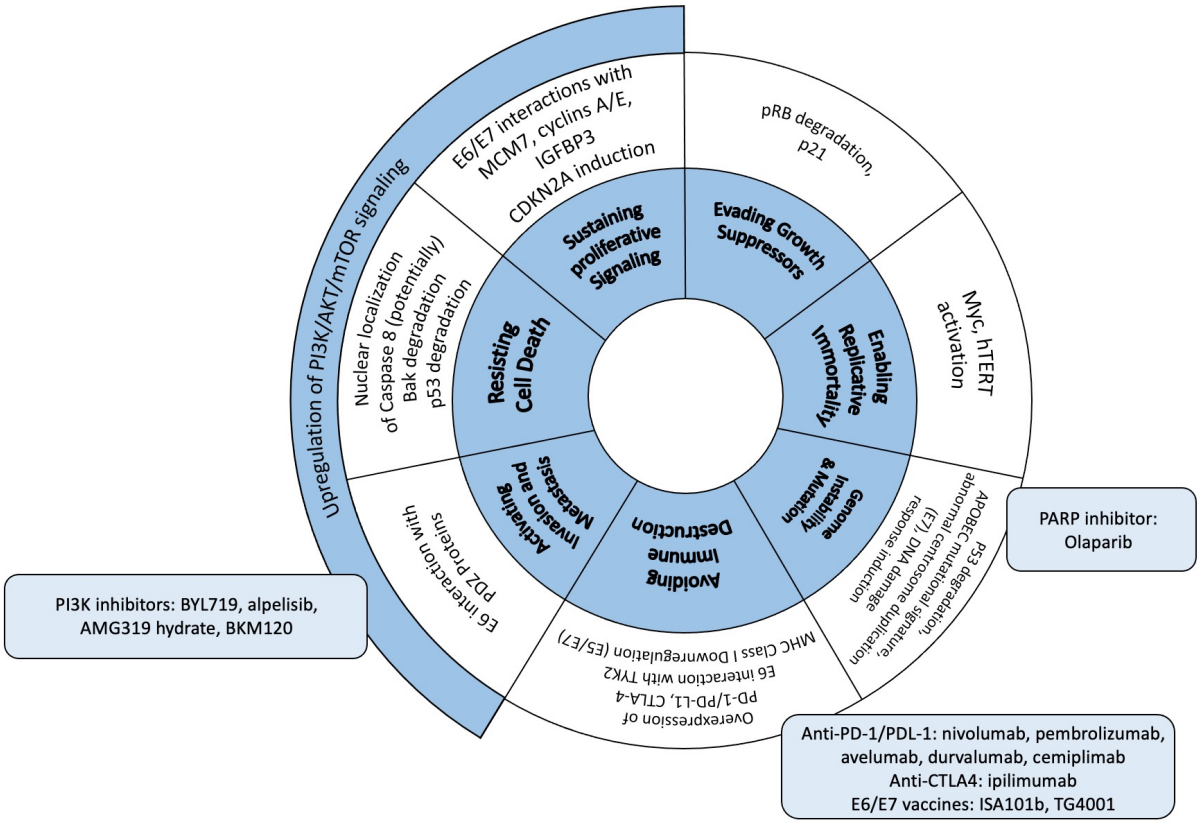

A)
UK / USA cervical vs oropharyngeal cancer
(Directly age-standardised rates per 100,000 population of newly diagnosed cases of cancer)
14.0
12.0
10.0
8.0
6.0
4.0
2.0
0.0
1995
2000
2005
2010
2015
UK Cervical
UK Male Oropharyngeal
US Cervical
US Male Oropharyngeal
B)
C)

Figure 1.

A)


pRb and p53 function
E6/E7 function
Genomic Instability
Disruption of normal HPV life cycle; E6/E7-driven dysregulation of cell cycle; aberrant cell proliferation and differentiation
Acquisition of oncogenic genetic and epigenetic changes; viral DNA integration (often but not necessary); development of a Type I tumour microenvironment
Viral infection via microabrasion resulting in infection of basal stem cell
Persistent infection of basal stem cells, selection for E6/E7 persistent clones
Expansion of E6/E7-expressing cell population
Malignant transformation: progression from carcinoma in situ to invasive carcinoma
Carcinogenesis

B)


Upregulation of PI3K/AKT/mTOR signaling
E6/E7 interactions with MCM7, cyclins A/E, IGFBP3
CDKN2A induction
pRB degradation, p21
Myc, hTERT activation
Nuclear localization of Caspase 8 (potentially)
Bak degradation
p53 degradation
E6 interaction with PDZ proteins
Overexpression of PD-1/PD-L1, CTLA-4
E6 interaction with TYK2
MHC Class I Downregulation (E5/E7)
p53 degradation, aberrant centrosome duplication (E7), DNA damage response induction
APOBEC mutational signature
Sustaining proliferative Signaling
Evading Growth Suppressors
Enabling Replicative Immortality
Genome Instability & Mutation
Avoiding Immune Destruction
Activating Invasion and Metastasis
Resisting Cell Death
PI3K inhibitors: BYL719, alpelisib, AMG319 hydrate, BKM120
PARP inhibitor: Olaparib
Anti-PD-1/PDL-1: nivolumab, pembrolizumab, avelumab, durvalumab, cemiplimab
Anti-CTLA4: ipilimumab
E6/E7 vaccines: ISA101b, TG4001

C)

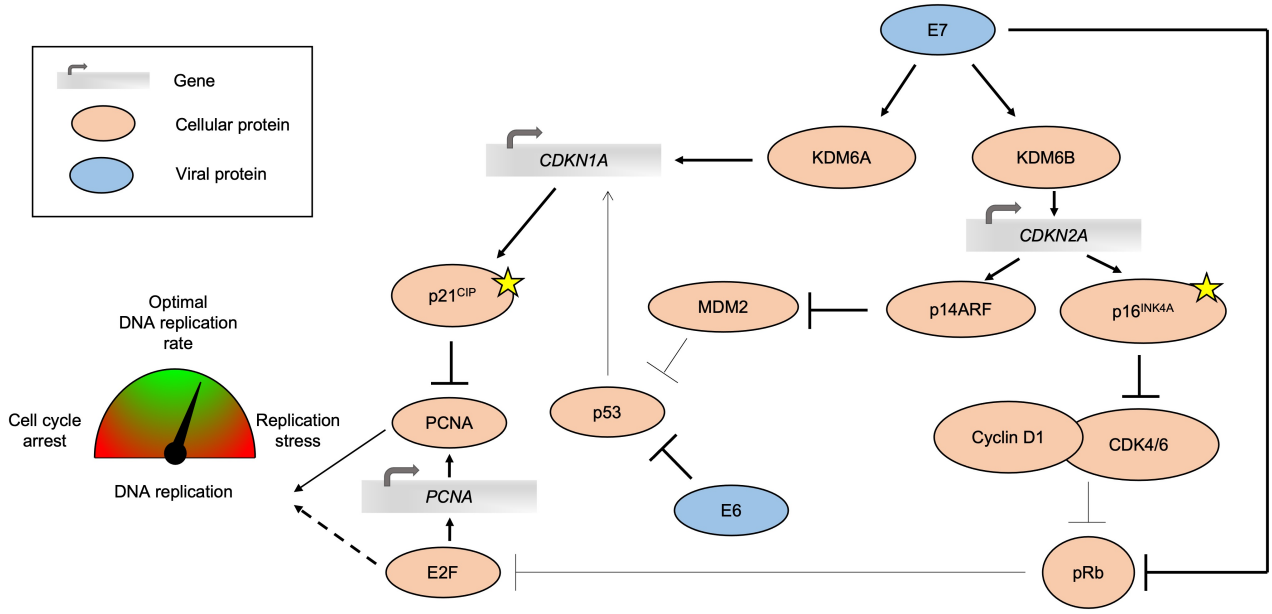

Gene
Cellular protein
Viral protein
E7
CDKN1A
KDM6A
KDM6B
CDKN2A
p21CIP
MDM2
p14ARF
p16INK4A
Optimal
DNA replication
rate
Cell cycle
arrest
Replication
stress
DNA replication
PCNA
p53
Cyclin D1
CDK4/6
PCNA
E6
E2F
pRb

Figure 2.

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

		HPV-positive OPSCC	***HPV-negative OPSCC***
Patient Characteristics	Age	59	60 ($p < 0.001$)[243]
		incidence of HPV-positive OPSCC increasing in older men	
	Sex	86.9% male	76.8% male ($p < 0.001$)[243]
	Ethnicity	90% Caucasian	75.9% Caucasian ($p < 0.001$)[243]
	Smoking	Similar (rising incidence of HPV-positive OPSCC in smokers, as well as non-smokers)[243]	
	Alcohol	HPV-negativity associated with greater alcohol use[7]	
	Sexual history	High number of sexual partners a risk factor for HPV-positive OPSCC[7]	
Incidence	Per 100,000	4.62	1.82[243]
Tumour Characteristics	Site	Greater preference for oropharynx (94.2% HNSCC); specifically base of tongue and tonsil[2]	Less preference for oropharynx (72.8% HNSCC)[243]
	Stage (AJCC 7th)	Early stage (T1-2); frequently with nodal metastasis at presentation[157]	All stages (T1-4)[243]
	Histopathology	Immature, basal-like/basaloid, non-keratinizing [157]	Frequently keratinizing SCC
Prognosis	Cancer-specific mortality	aHR = 0.40 ($p < 0.001$)[243]	
Biological Characteristics	Genetic Mutations	More frequent aberration of DNA damage response pathways, *FGF* and *JAK/STAT* signaling as well as immune-related genes (*HLA-A/B*); *PIK3CA* mutations more commonly observed[94]	Aberration of *TP53* and cell cycle pathways (eg. Loss of *CDKN2A*); oxidative stress regulation more frequently mutated[94]
	Other Aberrations	p53/Rb1 degradation by E6/7[242]	

Table 2a. AJCC 8th edition TNM Staging for HPV-positive oropharyngeal squamous cell carcinoma

T Category	**T Criteria**
T0	No primary identified
T1	Tumour 2cm or smaller in greatest dimension
T2	Tumour larger than 2 cm but not larger than 4 cm in greatest dimension
T3	Tumour larger than 4 cm in greatest dimension or extension to lingual surface of epiglottis
T4	Moderately advanced local disease. Tumour invades the larynx, extrinsic muscle of tongue, medial pterygoid, hard palate or mandible or beyond. **mucosal extension to lingual surface of epiglottis from primary tumours of the base of the tongue and vallecula does not constitute invasion of the larynx*
T Suffix	**Definition**
(m)	Select if synchronous primary tumours are found in single organ
cN Category	**cN Criteria**
NX	Regional lymph nodes cannot be assessed
N0	No regional lymph node metastasis
N1	One or more ipsilateral lymph nodes, none larger than 6cm
N2	Contralateral or bilateral lymph nodes, none larger than 6cm
N3	Lymph node(s) larger than 6cm
N Suffix	**Definition**
(sn)	select if regional lymph node metastasis identified by SLN biopsy only
(f)	Select if regional lymph node metastasis identified by FNA or core needle biopsy only
pN Category	**pN Criteria**
NX	Regional lymph nodes cannot be assessed
pN0	No regional lymph node metastasis
pN1	Metastasis in 4 or fewer lymph nodes
pN2	Metastasis in more than 4 lymph nodes
N Suffix	**Definition**
(sn)	Select if regional lymph node metastasis identified by SLN biopsy only
(f)	Select if regional lymph node metastasis identified by FNA or core needle biopsy only
M Category	**M Criteria**
cM0	no distant metastasis
cM1	Distant metastasis
pM1	Distant metastasis, microscopically confirmed

Table 2b. AJCC 8th edition prognostic groups for HPV-positive oropharyngeal squamous cell carcinoma

cT	**cN**	**cM**	**Stage**
T0-2	N0 or N1	M0	I
T0-2	N2	M0	II
T3	N0-2	M0	II
T0-4	N3	M0	III
T4	N0-3	M0	III
Any T	Any N	M1	IV
pT	**pN**	**pM**	
T0-2	N0 or N1	M0	I
T0-2	N2	M0	II
T3 or T4	N0 or N1	M0	II

T3 or T4	N2	M0	III
Any T	Any N	M1	IV

Table 2c. AJCC 8th edition lymphovascular invasion coding for HPV-positive oropharyngeal squamous cell carcinoma

Component of LVI Coding	**Description**
0	LVI not present (absent)/ not identified
1	LVI present/ identified, NOS
2	Lymphatic and small vessel invasion only (L)
3	Venous (large vessel) invasion only (V)
4	Both lymphatic and small vessel and venous (large vessel) invasion
9	Presence of LVI unknown/indeterminate

Table 3. UK Treatment Recommendations for HPV-positive OPSCC (not yet updated for AJCC 8th edition staging guidelines).[154]

		Early Stage (T1 or T2, N0)*	Late Stage (T3 or T4, N0; T1-4, N1-3)
Open Surgery	Paramedian mandibulotomy (PM)	Not typically recommended; TORS/TLM resection or definitive RT instead	• Usually PM,TCP for tongue base resections, G/LR not frequently used; mandibulectomy for tumours with gross bony involvement • Lip-splitting mandibulotomy usually required for adequate visualization • Reconstruction by radial artery free or anterolateral thigh free flaps • Used in cases of surgical salvage • Adjuvant CRT or PORT usually required • Modified or selective neck dissection recommended
	Mandibulectomy		
	Trans-cervical pharyngotomy (TCP)		
	Glossotomy (G) /lingual release (LR)		
Transoral Surgery	Transoral robotic surgery (TORS)	• T1/T2, potentially T3*; ipsilateral selective neck dissection recommended, N0 treated electively • Adjuvant RT/CRT to reduce risk of recurrence depending on tumour features	Limited to early stage disease
	Transoral laser microdissection (TLM)		
Radiotherapy	Radical	• 70 Gy/ 35 fractions (hypo-fractionated: 65-66 Gy/30 fractions) • prophylactic RT to ipsilateral cervical lymph nodes for lateralised tumours, both sides for non-lateralised tumours	Only if patient is unfit for CRT (e.g. >70 years of age, poor performance status)
	Intensity modulated radiotherapy	In clinical trials for de-escalation in definitive and adjuvant settings	
Chemoradiotherapy		70 Gy (2 Gy fractions) with concurrent cisplatin standard of care	

Adjuvant therapy	Chemoradiotherapy	• For positive or close resection margins or extra-nodal extension of lymph nodes; or other high-risk features (lymphovascular or perineural invasion) • Post-operative RT can be with or without concurrent chemotherapy	Improved outcome for patients with extra-capsular invasion and/or microscopically involved surgical resection margins around primary tumour; not recommended for those >70 years of age or with poor performance status

Table 4a. Ongoing and Recently Completed Clinical Trials for the Management of HPV-associated Oropharyngeal Squamous Cell Carcinoma

	Study Cohort	Treatment	Outcome Measures	Toxicity Profile	Reference
Efficacy of Induction Therapy					
OPTIMA	N = 62; cohort divided into low risk (≤T3, ≤N2b, ≤10 pack-year smoking history) or high risk (T4 or ≥N2c or >10 pack-year smoking history)	3 cycles carboplatin (AUC 6) + nab-paclitaxel (100mg/m^2) followed by low dose CRT (45 Gy + paclitaxel, 5-FU and hydroxyurea) or standard CRT (75 Gy)	2-year PFS = 95% (low risk group), 94% (high risk group)		Seiwert, 2019
E1308	N=80; majority stage T1-3N0-N2b, ≤10 pack-year smoking history	3 cycles cisplatin, paclitaxel and cetuximab followed by concurrent cetuximab with RT (54 Gy for complete responders or 69.3 Gy)	2-year PFS = 80%, 2-year OS = 100% for primary site complete responders to induction therapy	Fewer patients with low dose RT had difficulty swallowing solids (40 v. 89%, *P* = 0.11) or impaired nutrition (10% v. 44%, *P* = 0.025)	Marur, 2017
	N=44, stages III-IV (AJCC 7th ed.)	2 cycles paclitaxel (175 mg/m^2) and carboplatin (AUC 6) followed by IMRT (54 Gy for complete/partial responders or 60 Gy) + paclitaxel (30 mg/m^2)	2-year PFS = 92%	Grade 3 adverse events = 39%, gastrostomy tube rate = 2%	Chen et al, 2017; NCT02048020, NCT01716195
De-escalation of Chemoradiotherapy/Radiotherapy					
HYHOPE	N=24; T1-3 N0-2, ≤10 pack-year smoking history, not actively smoking, ECOG 0-2	Hypofractionated radiation therapy over 3 weeks with concurrent weekly cisplatin:	Maximally tolerated dose and fractionation (primary outcome); acute and late toxicities, locoregional		NCT04580446

		44.4 Gy in 12 fractions or 46.5 Gy in 15 fractions or 52 Gy in 20 fractions	control, PFS, QOL, feeding tube dependence		
SHORT-OPC	N=106; stage I-II	SABR boost and de-escalated chemoradiation (40 Gy in 20 fractions, concurrent cisplatin) vs. standard chemoradiation (70 Gy in 33 fractions with concurrent cisplatin)	Locoregional control (primary outcome); subacute/acute/late toxicities, OS, PFS, symptom burden, dysphagia		NCT04178174
MC1273	N=80, ≤10 pack-year smoking history, negative margins; cohort B included patients with extranodal extension	Cohort A: 30 Gy + docetaxel (15 mg/m^2) Cohort B: extranodal extension to 36 Gy	2-year locoregional tumour control = 96.2%, PFS = 91.1%, OS = 98.7%	Grade 3 or worse toxicity, pre-RT = 2.5%, 1- and 2-year post-RT = 0%	Ma et al, 2019
	N=43; T0-3N0-2cM0, minimal smoking history	60 Gy IMRT + concurrent cisplatin (30 mg/m^2)	3-year locoregional control = 100%, distant metastasis-free survival = 100%, OS = 95%	Improved preservation of QoL; 39% required feeding tube (none permanent), no ≥ grade 3 later adverse events	Chera, 2018; NCT01530997
	N=76; Hypoxia negative; T1-2, N1-2b	30 Gy IMRT with concurrent cisplatin (100mg/m^2) or carboplatin (AUC 5) and 5-FU (2400 mg/m^2)	Effectiveness of study treatment comparable to standard CRT		NCT03323463
EVADER	N=100; T1-3, N0-1, M0 (AJCC 8th)	70/56 Gy RT with cisplatin (100 mg/m^2) or 70/56 Gy RT only	Event-free survival (primary outcome); OS, local/regional/locoregional control, distant metastasis-free survival		NCT03822897

Quarterback	N=24; stage 3 or 4 without distant metastases (AJCC 7th)	56 Gy RT with concurrent carboplatin or 70 Gy with concurrent carboplatin	PFS (primary outcome), locoregional control, OS, acute toxicities, predictive biomarkers		NCT01706939
Quarterback 2b	N=65; stage 3 or 4 without distant metastases (AJCC 7th)	56/50.4 Gy IMRT	3 and 5-year PFS (primary outcomes); locoregional control, OS, acute/long term toxicities		NCT02945631
	N=75; low risk HPV-positive OPSCC (T1-2, N0-1)	MRI-guided (individualized up to 70 Gy in 33 fractions) vs. standard-of-care IMRT (individualized up to 70 Gy in 33 fractions)	Locoregional control, composite dysphagia outcome (primary outcomes); PFS, OS, DMFS		NCT03224000
	N=60; T1, 2 or 3, any N; ECOG 0-1, no distant metastases	Radiation dose de-escalation from 70 Gy to 63 Gy and 58.1 Gy to 50.75 Gy in 35 fractions; weekly carboplatin	Grade 3+ late toxicity, QOL, adverse events		NCT01088802
^{18}F FMISO PET Imaging for Treatment Allocation					
	N=33, stage III-IVb; assessment of hypoxia by ^{18}F FMISO PET imaging	No hypoxia/resolution: 10 Gy-dose reduction of IMRT to metastatic lymph nodes, standard dose to primary tumour Persistent hypoxia: standard dose to tumour bed and lymph nodes	30% received dose reduction, 2-year locoregional control = 100%, distant metastasis-free = 97%, OS = 100%	Acute grade 3 mucositis (11/33), grade 3 dysphagia (0/33), late grade 2 xerostomia (2/33)	Lee, 2016; NCT00606294
MSKCC Pilot Study	N=19; T1/2/x, N1/2a/2b, M0 (AJCC 7th); assessment of hypoxia by ^{18}F FMISO PET imaging	No hypoxia/resolution: 30 Gy IMRT with concurrent high-dose cisplatin or carboplatin/5-FU	15/19 de-escalated to 30 Gy IMRT based on pre-treatment ^{18}F FMISO PET; to date disease free = 18/19		Riaz, 2017

		Persistent hypoxia: 70 Gy IMRT with concurrent high-dose cisplatin or carboplatin/5-FU followed by neck dissection			
TORS vs. Radiotherapy					
ORATOR	N=68, ≤18 years old, ECOG 0-2, T1-2, N0-2; stratification by p16 status	70 Gy IMRT (with high dose cisplatin or modified cisplatin, cetuximab or carboplatin, if N1-2) or TORS + neck dissection with 1 cm margins (+/- adjuvant CRT)	MDADI score (swallowing related QOL at 1 year): 86.9 (radiotherapy group), 80.1 (TORS group)	More cases of neutropenia, hearing loss and tinnitus in radiotherapy group, trismus in TORS group; most common AEs were dysphagia, hearing loss and mucositis in radiotherapy group, dysphagia in TORS group	Nichols, 2019
ORATOR2	N=140; T1-2, N0-2 (AJCC 8th ed.)	De-intensified IMRT (60 Gy +/- chemotherapy) vs. TOS and neck dissection (+/- adjuvant 50 Gy IMRT)	2-year OS (primary outcome); PFS, QOL, toxicity profile		NCT03210103
De-escalation of Adjuvant Therapy					
PATHOS	N~1,100 Group A: tumours with no adverse histological features Group B: T3 (or T1-2 with additional risk factors), pN2a or pN2b, PNI or VI, histologically normal tissue margin of 1-5mm	Arm 1 (Group A): No intervention Arm B1 (Group B): post-operative RT (60 Gy) Arm B2 (Group B): post-operative RT (50 Gy) Arm C1 (Group C): post-operative RT (60	Swallowing function (MDADI), overall survival (primary outcomes); swallowing panel, QoL, DFS, locoregional control, distant metastases, acute and late toxicity		Hargreaves, 2019; NCT02215265

	Group C: any T, any N with high risk pathological features (positive (<1mm) margins, negative marginal biopsies and/or cervical lymph node extracapsular spread	Gy) with concurrent cisplatin Arm C2 (Group C): post-operative RT (60 Gy) without chemotherapy		
SIRS	Intermediate stage, stratification based on pathological prognosis (based on ECS, LVI, PNI)	Follow up without post-operative radiotherapy for patients with good prognosis, reduced dose adjuvant radiotherapy or CRT based on pathology for patients with poor prognosis	3/5-year DFS, locoregional control (primary outcomes); OS, toxicities, QOL	NCT02072148
	N=118; T0-3, N0-2b (AJCC 7^{th}), <5 positive lymph nodes, TORS primary site resection and ipsilateral neck dissection	Adjuvant radiotherapy dose reduction according to characteristics of primary site and involved lymph nodes, 50 Gy IMRT for high risk neck; 45 Gy IMRT to low risk neck with reduction of treated volume	2-year locoregional control (primary outcome), treatment-related toxicity, 2-year PFS, metastasis-free survival, OS, QoL, difference in toxicities between IMRT and IMPT	NCT03729518
MINT	N=40; Stage I-III (AJCC 8^{th}); standard of care transoral surgery of primary tumour and management of cervical lymph nodes	Arm 1 (ECE or positive margin but not pT4 or cN3): 42 Gy IMRT/IMPT and concurrent cisplatin ($100mg/m^2$)	Percent weight loss (day 1 compared to last day of radiation therapy) (primary outcome); PEG tube placements in each arm, serum creatinine changes, narcotics	NCT03621696

		Arm 2: 42 Gy IMRT/IMPT Arm 3 (cT4/pT4 or cN3): 60 Gy IMRT/IMPT, cisplatin (100 mg/m^2)	administration, QoL, disease recurrence rate (24 post-treatment)		
E3311	N=511, stage III/IVA/IVB (AJCC 7th)	TOS or TOS then low-dose IMRT or TOS then standard-dose IMRT or TOS then standard-dose IMRT with concurrent cisplatin or carboplatin	PFS, accrual rate, risk distribution, incidence of grade 3-4 bleeding events during surgery and positive margins (primary outcomes); AEs, OS, swallowing, voice, QoL		NCT01898494
AVOID	N=60, pT1-pT2 N1-3; surgical resection by TORS with favourable features at primary site	Adjuvant RT omitting tumour bed	Local control	SAE in 30%: dysphagia (3.33%), esophageal pain (1.67%), other GI disorder (1.67%), mucositis oral (5.00%), dermatitis radiation (13.33%), aspiration (3.33%), hypoxia (1.67%)	Swisher-McClure, 2020 NCT02159703
DART-HPV	N=227; gross total surgical resection and unilateral neck dissection; ECOG 0 or 1; one of: lymph node > 3 cm, 2 or more positive lymph nodes, perineural invasion, lymphovascular space invasion, T3 or T4 primary disease, lymph node	Docetaxel (15 mg/m^2) plus 30 Gy/1.5 Gy fractions twice daily or 36 Gy/18Gy fractions twice daily vs. Standard of Care	Adverse Events Rate, Locoregional control, QOL, DFS, distant failure		NCT02908477

	extracapsular extension				
DELPHI	N=384; intermediate and high risk	54/ 59.4 Gy and concurrent chemotherapy (high risk) vs. 48.8/ 55 Gy vs. standard CRT	Rate of locoregional recurrences, OS, acute/late toxicities , QOL		NCT03396718
	N=111; low to high risk	Intermediate risk: reduced-dose adjuvant radiation therapy; High risk: adjuvant radiation therapy without chemotherapy	DFS, OS, toxicities, QOL, symptom burden, dysphagia, shoulder dysfunction		NCT03875716
Targeted Therapies					
	N=43, previously untreated stage III-IV (excluded N3 or T4) disease without distant metastasis	Weekly cetuximab (250 mg/m^2) with concurrent radiotherapy (70 Gy in 35 fractions over 7 weeks to gross tumour, 50-60 Gy to subclinical target volumes)	Rate of recurrence, Adverse Events		NCT01663259
	N=70; stage III-IV, detection of KRAS-variant	Radiation and concurrent cisplatin vs. cetuximab followed by radiation and concurrent cisplatin	OS, primary tumour control, locoregional recurrence rate, acute and late toxicities		NCT04106362
	N=987, 849 randomised; T1-2, N2a-N3 or T3-4, any N, no distant metastases	IMRT (70 Gy over 35 fractions) with concurrent cisplatin (100 mg/m^2) vs. IMRT (as above) with concurrent cetuximab (400 mg/m^2 before IMRT then 250 mg/m^2 for 7 weeks)	OS (primary outcome); PFs, time to locoregional failure/distant metastasis/secondary primary cancer; adverse events	Cetuximab vs. Cisplatin: acute moderate to severe toxicity (77.4% vs. 81.5%); late moderate to severe toxicity (16.5% vs. 20.4%)	Gillison, 2019

Table 4b. Ongoing immunotherapy clinical trials for HPV-positive OPSCC

	Study Cohort	Treatment	Outcome Measures	Reference
IMvoke010	N=400; complete/partial response or stable disease to definitive local therapy	Atezolimumab or placebo	Event-free survival, OS, adverse events	NCT03452137
CITHARE	N=66; T1, N1-2 or T2-3, N0-2 (AJCC 8th)	70 Gy RT with either cisplatin or durvalumab	Rate of patients alive without progression at 12 months (primary outcome); 2-year PFS, OS, safety (NCI-CTCAE), QoL	NCT03623646
	N=180; T1N2a-N2cM0, T2N1-2cM0, T3N0-2cM0 (AJCC 7th) or stage I/II excluding T1N0-1 and T2N0 (AJCC 8th)	50-66 Gy IMRT with nivolumab and ipilumumab	Dose limiting toxicity, CR rate, PFS (primary outcomes); grade 3 AEs, patient tolerability, clinical CR, acute and chronic AEs, acute toxicities, late toxicities, swallowing, pattern of failure, OS	NCT03799445
	N=180; locoregionally advanced, intermediate risk and non-metastatic (AJCC 8th)	70 Gy RT with cisplatin (100mg/m^2) or durvalumab IV (1500 mg) + adjuvant durvaluamb (1500 mg) or durvalumab + adjuvant durvalumab/trememlimumab (third arm closed to accrual)	3-year event-free survival (primary outcome); FACT-HN score, local regional failure, distant metastasis-free survival, OS, cost-effectiveness, toxicities	NCT03410615
	N=40; stage III (AJCC 8th) or 'matted lymph nodes'	Nivolumab (240 mg/m^2) before and concurrent with RT (70 Gy)/carboplatin (AUC 1)/paclitaxel (30 mg/m^2) and adjuvant nivolumab (480 mg/m^2)	PFS (primary outcome); progression, OS, acute/late toxicity incidence	NCT03829722
	N=82; Stage I/II/III (AJCC 8th)	Cohort I: SBRT with durvalumab IV followed by TORS and modified radical neck dissection then adjuvant durvalumab IV Cohort II: SBRT with trememilumab IV and durvalumab	PFS, incidence of AEs (primary outcomes); OS, primary tumour control, distant recurrence rate, locoregional control, contralateral neck failure, subclinical lymph node involvement, objective response, AEs, short/long-term QoL	NCT03618134

		IV followed by TORS and modified radical neck dissection then adjuvant durvalumab IV		
	N=20; any stage	Durvalumab IV followed by surgical resection within 3-17 days	Immune effector concentration, immune-regulatory miR responses, systemic immune response, regulatory response (primary outcomes); incidence of AEs, tumour volume, standardized uptake volume	NCT02827838
HARE-40	N=44; minimum 12 months post-treatment, no clinical evidence of disease or palliative intention-to-treat	HPV vaccine +/- Anti-CD40	Dose Limiting Toxicity	NCT03418480
	N=100; tumour progression or recurrence on standard of care therapy	HB-201 intravenous administration, 3+3 design dose determination	Recommended phase 2 dose	NCT04180215
	N=194; PD-L1 positivity	ISAS101b 3 times plus cemiplimab every 3 weeks (up to 24 months) or placebo plus cemiplimab	Overall response rate, treatment-related adverse events, duration of response	NCT03669718
	N=27; ECOG </= 1; incurable disease	Utomilumab plus ISA101b	Overall response rate, adverse events, PFS	NCT03258008
	N=711; early-stage, non-smoking associated disease	Image-guided RT or IMRT over 6 fractions/week with concurrent cisplatin vs. reduced dose image-guided RT or IMRT over 5 fractions/week with concurrent cisplatin vs. reduced dose image-guided RT or IMRT with nivolumab	PFS, QOL, locoregional failure, distant failure, OS , adverse events	NCT03952585
	N=180; stage II or III	Up to 3x1010 E7 TCR T-cells followed by standard treatment at time of maximum tumour response	Fraction who achieve success	NCT04015336
	N=15; stage I-IV	ADXS11-001 followed by robot-assisted resection vs. standard of care	HPV-specific T-cell response rate, any grade 3 or 4 toxicity	NCT02002182

	N=744; >/= 10 pack-year smoking history, stage T1-2N2-N3 or T3-4N0-3 OR < 10 pack-years, stage T4N0-N3 or T1-2N2-3	Cisplatin and IMRT followed by nivolumab once weekly for 12 months vs. cisplatin and IMRT followed by observation with potential cross-over to receive nivolumab over 12 months	PFS, OS, negative FDG PET	NCT03811015
	N=135; intermediate risk factors	45 or 50 Gy RT in 25 fractions; concurrent biweekly nivolumab (240mg) followed by monthly nivolumab for 6 doses (480 mg)	PFs, PEG tube dependence	NCT03715946

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