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Therapeutic options for human papillomavirus-positive tonsil and base of tongue cancer

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The incidences of human papillomavirus-positive (HPV^+) tonsillar and base tongue squamous cell carcinomas (TSCC and BOTSCC) have increased in recent decades. Notably, HPV^+ TSCC and BOTSCC have a significantly better prognosis than their HPV-negative counterparts when treated with current surgical options, radiotherapy, or intensified chemoradiotherapy. However, a cure is not achieved in 20% of patients with HPV^+ TSCC/BOTSCC. Meanwhile, cured patients often present with severe chronic side effects. This necessitates novel tailored alternatives, such as targeted therapy, immune checkpoint inhibitors (ICIs), and treatment de-escalation, together with better follow-up. Current precision medicine therefore focuses on detecting predictive and driver cancer genes to better stratify patient treatment, provide those with poor prognostic markers targeted therapy, and select those with favorable markers for de-escalated therapy. Moreover, detecting cell-free HPV DNA (cfHPV DNA) in plasma before and after treatment has been attempted to improve follow-up. In this context, this perspective discusses the significance of optimally defining HPV^+ status, which requires HPV DNA and $p16^{INKa}$ overexpression, using prognostic markers, such as high $CD8^+$ T-cell counts and HPV E2 mRNA expression, tumor size, and following cfHPV DNA for patient selection for specific therapies. Clinical trials with ICI with/without chemotherapy, targeted therapy with specific inhibitors—such as phosphoinositide 3-kinase and fibroblast growth factor receptor inhibitors—or immune therapy with various HPV-based vaccines for treating recurrences have yielded promising results.

Keywords: base of tongue cancer, checkpoint inhibitors, head and neck cancer, human papillomavirus (HPV), immune therapy, oropharyngeal cancer, targeted therapy, tonsillar cancer

Introduction

Human papillomavirus (HPV) is a known risk factor for oropharyngeal squamous cell carcinoma (OPSCC) and is also responsible for the significant increase in the incidences of tonsillar and base tongue squamous cell carcinomas (TSCC and BOTSCC), the two major OPSCC subsites [1–20]. HPV^+ TSCC and BOTSCC have with surgery and/or radiotherapy (RT) better disease-free survival (DFS) than corresponding HPV-negative (HPV^-) cancer due to smoking and alcohol consumption [2–4, 9, 10, 16, 21, 22]. Despite the increased treatment intensity from adding induction chemotherapy (CT), concurrent chemoradiotherapy (CRT), or employing cetuximab—an epidermal growth factor receptor (EGFR) inhibitor—instead of CT, all result in more side effects, and survival rates remain mainly unchanged, with 20% of cases recurring [23–26]. Moreover, following HPV^+ TSCC/BOTSCC recurrence, the prognosis is

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dismal, with less than 6% survival rate, similar to those in HPV⁻ TSCC/BOTSCC [27]. The high and increasing incidence of OPSCC (Fig. 1) necessitates optimizing treatment for this growing group of patients [6–20, 24, 28]. Although HPV vaccination offers advantages, its distribution among both girls and boys remains limited. As a result, it may take decades for the rates of HPV⁺ OPSCC to decrease [29]. To improve survival and quality of life, better prognostication, tailoring, and new therapies are urgently needed [24–26]. Thus, current research focuses on the significance of correctly defining HPV⁺ status, revealing prognostic markers, and identifying common mutations and driver genes, all of which may be useful for initiating targeted or de-escalated therapies [30–58]. Monitoring tumor cell-free HPV DNA (cfHPV DNA) using droplet digital (dd) polymerase chain reaction (PCR) in plasma to improve follow-up is also being pursued [59–63]. This perspective deals with the importance of defining HPV⁺ status adequately, using prognostic markers, tumor size, and cfHPV DNA for patient selection for specific therapies. The promising results from clinical trials with immune checkpoint inhibitors (ICIs) and various targeted therapies are also discussed.

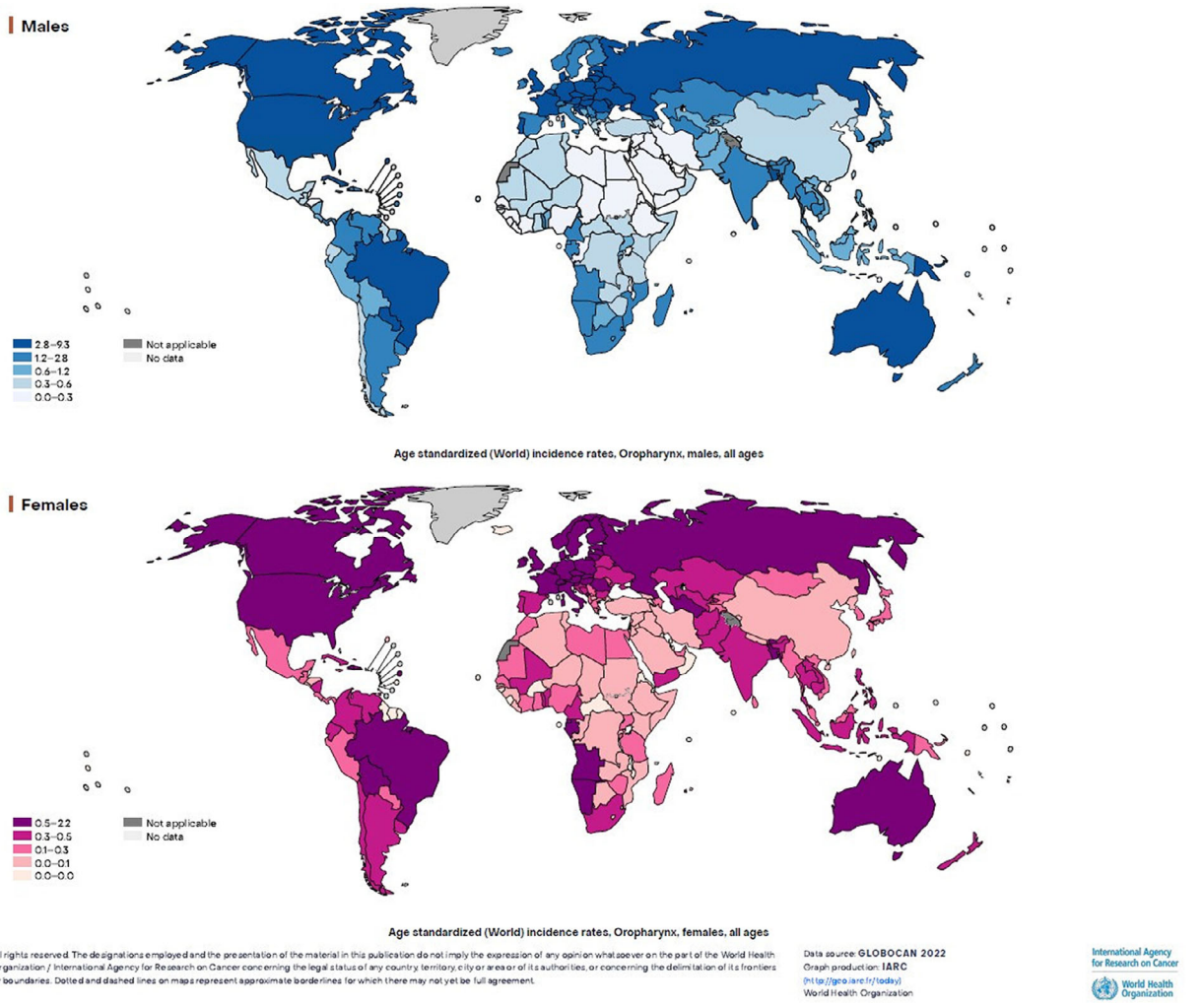

Fig. 1 *Age standardized incidences for oropharyngeal cancer for 2022, for males and females from GLOBCAN (graph production: the International Agency for Research on Cancer [IARC]) used with permission [28].*

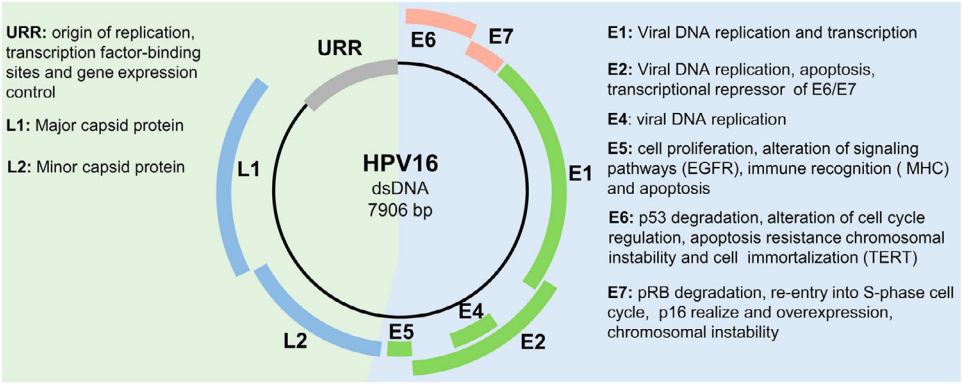

Fig. 2 *Genome structure of human papillomavirus type 16 (HPV16) and proteins. The HPV genome includes a long control region (LCR), an early gene region encoding the E1, E2, E4, E5, E6, and E7 early proteins, and a late gene region encoding L1 and L2 major capsid proteins. E, early region protein; EGFR, epidermal growth factor receptor; HPV16, human papillomavirus type 16; L, Late region protein; MHC, major histocompatibility complex; p53, tumor protein 53; pRB, retinoblastoma protein; TERT, catalytic subunit telomerase reverse transcriptase; URR, upstream regulatory region. Source: This figure has been used from Ref. [65] with permission from Elsevier.*

HPV and OPSCC, TSCC/BOTSCC

HPVs have a circular double-stranded DNA genome of 7900 kb, encoding the regulatory E1–E2, E4–E7 proteins and the viral capsid proteins L1 and L2 (Fig. 2), with a 52–55 nm virion [24, 64, 65]. In high-risk HPVs responsible for developing various cancers, E6, E7, and sometimes E5 are viewed as oncogenes [64]. E6 binds to and degrades p53, inhibiting DNA damage control, cell repair, and apoptosis, whereas E7 binds to Rb and abrogates and deregulates cell cycle control, leading to $p16^{INK4a}$ overexpression ($p16^+$) [64]. E5 also displays some transforming abilities, but importantly, it can reduce the efficiency of the immune system by decreasing the expression of major histocompatibility complex (MHC) antigens [24, 66]. HPV^+ OPSCC shares similar characteristics to cervical cancer (CC), the best known HPV^+ cancer, is thereby mainly aneuploid, has wild-type *TP53*, exhibits $p16^+$, often has chromosome 3q amplification, and presents early lymph node metastasis [5, 24, 64–70].

Definition of HPV^+ status in OPSCC and its consequences upon estimating prognosis

The gold standard for confirming active HPV infection is based on the presence of HPV E6 and E7 mRNA expression; however, because this is not usually tested, $p16^+$ ($p16^+$ in >70% of all OPSCC cells by immunohistochemistry [IHC]) has been adapted as a surrogate marker for HPV^+ [71, 72]. Unfortunately, $p16^+$ does not always correlate to the presence of HPV DNA^+ or HPV E6/E7 mRNA expression [71, 72]. Although this discrepancy is low (mainly <10%) in TSCC/BOTSCC with a high HPV prevalence, it is high (reaching almost 50%) in other OPSCC subsites with a lower HPV prevalence, and this remains a challenge [24, 72–75]. Fortunately, in TSCC/BOTSCC and often in other OPSCC subsites, the combined presence of HPV DNA^+ and $p16^+$ is virtually equivalent to the gold standard [71, 72]. This is of substantial significance because HPV $DNA^+/p16^+$ OPSCC has better DFS than HPV $DNA^+/p16^-$ OPSCC and HPV $DNA^-/p16^+$ OPSCC and considerably better DFS than HPV $DNA^-/p16^-$ OPSCC (Table S1) [24, 72, 76–79].

The American Joint Committee on Cancer (AJCC) 8th edition downstaged HPV^+ OPSCC with lymph node metastasis in the neck region compared with HPV^- OPSCC because the former had a better prognosis. However, HPV^+ status was defined here by $p16^+$ as a surrogate marker for active HPV infection, which is of concern [24, 80, 81]. We strongly recommend that when considering the active HPV^+ status in clinical trials, HPV DNA^+ and $p16^+$ should be assayed (Table S1) [24, 76–79].

OPSCC Treatment

Primary treatment. Most HPV^+ OPSCC present with lymph node metastasis upon diagnosis [23–26]. Treatment comprises RT and subsequent surgery, if needed, or vice versa [2–4, 23–26].

Based on the TNM stage, patients may receive RT of up to 70 Gray (Gy), with concomitant CT and, in selected cases, possibly EGFR blockers [2–4, 23–26]. In patients with residual lymph node metastases following treatment, therapy is complemented by neck-node surgery [23–26]. In patients with advanced/unresectable/non-curable tumors, ICIs—that is, programmed cell death protein 1 (PD-1) blockers with CT or alone—have been tested, and better effects than CT alone or CT together with EGFR blockers have been reported [24].

Relapse. Upon relapse, the prognosis is very poor; re-irradiation or salvage surgery is possible, and immunotherapy with PD-1 blockers has been attempted with/without (w/wo) CT, although the responses remain limited [24–27, 82, 83]. New targeted options have also been developed [84].

Side effects. Acute side effects include difficulties in swallowing and eating, nausea, mucositis, considerable weight loss, systemic infections, and fatigue [23–26]. CRT, followed by neck-node surgery, due to persisting lymph node metastasis following the initial primary therapy, has been linked to more side effects—such as increased fibrosis and stiffness of the neck—which can aggravate difficulties in swallowing, and some patients have reduced shoulder mobility [23–26, 85–89]. Chronic side effects include xerostomia, changes in taste, continuous difficulties in swallowing and talking, trismus, and a worsening hearing deficit, which together affect social interaction and frequently lead to depression [23–26, 85–89]. A late effect requiring reconstructive surgery is radio-osteonecrosis [23–26, 85–89].

De-escalation. The better prognosis of HPV$^+$ OPSCC, together with the severe physical and mental side effects of treatment, has initiated efforts to perform quality-of-life studies and treatment de-escalation by reducing RT or CT [56, 57, 85–94]. In a phase II study on HPV$^+$ OPSCC (*E 1308)* using HPV16$^+$ and/or p16$^+$ stages I–III OPSCC, a favorable response to induction CT was correlated with a low 1-year failure rate, upon reduced RT dose in patients with <T4, <N2c, and ≤10 pack-year smoking history [90]. Moreover, these patients experienced fewer side effects and improved swallowing and nutritional status [92]. In the non-inferiority RTOG 1016 trial, the Phase III clinical trial (De-ESCALaTE HPV trial) and subsequent studies randomizing patients to cetuximab or cisplatin together with concurrent intensified RT revealed that cetuximab was inferior to cisplatin [56, 90–94]. Notably, however, RT de-escalation was associated with a worse prognosis in HPV$^+$ OPSCC patients with phosphatidylinositol-4,5-bis-phosphate 3-kinase catalytic subunit alpha (*PIK3CA)* mutations than in those with wild-type *PIK3CA* [54].

Moreover, a similar tendency was observed when administering only RT and not CRT to HPV$^+$ OPSCC patients with normal MHC class I expression [35]. These findings imply that various biomarkers may be significant for de-escalation, and one should not solely depend on having a small tumor volume and being a never-smoker, as is currently being done [24, 35, 54]. Several prognostic clinical characteristics, biomarkers, and algorithms have been developed. However, to our knowledge, these have not been applied in clinical settings [90–94]. Reliable prognostic markers, prognostic algorithms, and better follow-up are warranted and significant for initiating and following up de-escalated treatments or for introducing targeted therapy for patients with unfavorable prognostic markers [24, 49, 53, 58].

A potential approach for improving regular follow-ups

Recently, monitoring cfHPV DNA in plasma at diagnosis and follow-up has been used to determine the burden of HPV$^+$ CC and HPV$^+$ OPSCC at given time points [59–63, 95–103]. Detection of cfHPV DNA in the plasma of HPV$^+$ OPSCC patients showed high sensitivity (>80%–100%), and relapse was detected approximately 3–6 months earlier than in current clinical follow-ups [59–63, 98–100, 102]. A high sensitivity (97.2%) in one of these studies could be attributed to the fact that in the study, all included patients had HPV DNA$^+$/p16$^+$ OPSCC, thereby excluding false HPV$^+$ OPSCC (HPV DNA$^-$/p16$^+$ OPSCC) [61]. Concordance was also high between studies; that is, most (although not all) HPV$^+$ OPSCC patients were cfHPV DNA-positive (cfHPV DNA$^+$) in plasma at diagnosis, and the median value often correlated with TNM stage and decreased and/or disappeared (cfHPV DNA$^-$) upon treatment [59–63, 98–100, 102]. Plasma cfHPV$^+$ DNA generally recurred a few months before clinical recurrence in CC and HPV$^+$ OPSCC [59–63, 98–100, 102]. However, in some cases, cfHPV DNA$^+$ recurred and was present for months without traces of a relapse. Therefore, the method should be cautiously used [61, 63, 98, 99, 102].

Determining cfHPV DNA has also been examined for enhancing decision-making when a lymph node has not decreased in size according to positron emission-computed tomography (PET-CT) 3 months following therapy [101–103]. Absence of cfHPV DNA$^-$ in plasma would indicate an inflammation rather than a poor response, and an immediate neck-node surgery could be postponed, and the PET-CT could be repeated with a relevant time interval [100–104]. Neck-node surgery could thereby be avoided in ~60% of the patients with a false-positive PET-CT result [104].

Moreover, monitoring cfHPV DNA with ddPCR could be used to improve follow-up, such as following the effects of de-escalated treatment or when applying new therapeutic options. Such attempts have been initiated in at least one of the novel therapeutic options presented below.

To summarize, the monitoring of cfHPV DNA could be of use at regular follow-ups due to its high sensitivity; for complementing PET-CT after treatment; as well as for following the efficacy of de-escalation or novel therapies. However, caution is still warranted because the various protocols have not been compared or used extensively, and the potential pitfalls—such as, for example, that we are dealing with an infectious agent—have yet to be determined [63, 103].

Prognostic and targetable biomarkers in HPV$^+$ and HPV$^-$ OPSCC

To improve follow-up and therapy, a search for prognostic and targetable markers has been conducted. When differentiating between HPV$^+$ and HPV$^-$ OPSCC, 3q amplification frequently observed in the former was not correlated with prognosis, whereas *TP53* mutations sometimes worsened prognosis [24, 61, 68, 105]. Clinical parameters, viral characteristics, and markers detectable using IHC have been explored, and studies of the immune system and molecular targets have been conducted, as described below.

Clinical parameters and viral characteristics in HPV$^+$ OPSCC and prognosis.

Smoking and age. Nonsmokers with HPV DNA$^+$ OPSCC or p16$^+$ OPSCC were initially suggested to have a better outcome than smokers, but by defining HPV$^+$ status as HPV DNA$^+$/p16$^+$, the impact of smoking becomes ambiguous [21, 24, 76, 77, 106]. Younger patients have better outcomes than older; however, whether this is due to the former receiving stronger treatment remains to be determined [49].

TNM stage. HPV$^+$ OPSCC differs from HPV$^-$ OPSCC in that it has early lymph node metastasis in the neck, yet a potentially good prognosis [2–4, 24, 49]. In HPV$^+$ OPSCC, patients with T1–T2 tumors generally fared better than those with T3–T4 tumors, irrespective of whether the tumors had lymph node metastases [49]. This led to the reclassification of the stage of HPV$^+$ OPSCC in AJCC 8th ed., that is, downstaging tumors with the presence of lymph node metastasis, but with the drawback of p16$^+$ alone used as a surrogate marker for HPV$^+$.

HPV physical state, viral load, and expression of viral genes. HPV episomal or integrated status, viral load, and protein expression have been investigated in OPSCC, and an episomal genome and high viral load have been suggested to be beneficial; however, later data were inconsistent [30, 38, 107, 108]. The prognostic role of HPV16, E2, E5, and E7 mRNA and their influence on MHC class I expression have also been investigated [109–111]. HPV16 E2, E5, and E7 mRNA did not influence MHC class I expression in those studies, but like CC, the absence of E2 mRNA was associated with a worse prognosis [109–111]. In summary, E2 mRNA expression was a prognostic marker, whereas viral load, episomal state, and E5 and E7 mRNA expression were not.

Immune cell markers, MHC antigens, stem cell markers, and HPV$^+$ OPSCC prognosis. Cellular markers have been examined—for example, by using IHC on immune and stem cells in HPV$^+$ OPSCC—and some were correlated to prognosis [31–42, 45, 48, 54, 55, 112–119].

The immune system, cells, and factors. The numbers of CD8$^+$ lymphocytes infiltrating or surrounding HPV$^+$ OPSCC were generally higher than those in HPV$^-$ OPSCC, and having high CD8$^+$ cell counts, a high CD8$^+$/FoxP3$^+$ ratio, or a low CD4$^+$/CD8$^+$ ratio correlated with better prognosis, independent of HPV status [33, 34, 45, 48, 112]. In addition, high infiltration of CD68$^+$ CD163$^+$ M2 macrophages correlated with poor clinical outcomes in HPV$^+$ head and neck cancer squamous cell carcinoma (HNSCC), where most cases were OPSCC [113, 115]. The survival benefit of PD-1 expression has also been studied in HPV$^+$

OPSCC, and although $CD8^+$ density in the stroma was correlated with improved survival, $CD68^+$ and $CD68^+$ $PD\text{-}L1^+$ levels in the stroma were not associated with patient outcome [48]. However, overall survival (OS) was favorable in patients with HPV^+ OPSCC infiltrated by $CD8^+$ and $CD68^+$ immune cells with high programmed death-ligand 1 (PD-L1) expression [116]. Since then, additional studies have been conducted on the correlation between PD-L1 blockers and PD-1 expression, some of which are discussed below. To summarize, high $CD8^+$ counts within or surrounding the tumor were repeatedly a favorable prognostic factor, whereas the roles of $CD68^+$ macrophages and PD-L1 expression warrant further studies.

MHC antigen expression. In vitro, HPV16 E5 and E7 were shown to downregulate MHC class I antigen expression, which was also often shown to be absent or low in vivo in HPV^+ OPSCC [35, 108, 114, 117–119]. Moreover, in one HPV^+ OPSCC study, absent/low MHC class I antigen expression was unexpectedly a positive prognostic factor, whereas MHC class II expression did not influence prognosis [35]. In another study, however, only MHC class II antigen expression influenced prognosis [114]. Later, the Cancer Genome Atlas (TCGA) revealed high mRNA levels of MHC class I in CC and HPV^+ OPSCC, which was suggested to be attributed to an interferon-gamma response stimulating an increased MHC antigen expression [120]. Notably, HPV^+ OPSCC has been shown to be RT sensitive, and RT can affect E5 mRNA and MHC class I antigen expression and responses to these tumors in vivo [121–124]. In summary, due to the complexity of determining MHC class I and II antigen expression in HPV^+ OPSCC, MHC antigen expression should presently not be used for prognostication.

Studies on stem cells, suppressor genes, or other markers. Stem cell-, tumor suppressor-, and other markers have also been investigated as potential prognostic markers for HPV^+ OPSCC [31, 32, 39, 40, 54, 55, 58]. Low Bcl2, CD44 intensity, or low CD98 expressions were associated with a better prognosis irrespective of HPV status, whereas among the LRIG1–3 tumor suppressor genes, only high LRIG1 expression correlated with better survival [31, 32, 39]. Furthermore, psoriasin and FGF 11 expression levels have prognostic significance in HPV^+ BOTSCC and HPV^+ OPSCC [54, 55]. In conclusion, some markers were more specific for HPV^+ or HPV^- OPSCC, whereas others were relevant for both.

Markers potentially useful for targeted therapy or prognostication in HPV^+ OPSCC. The reduced cost of molecular technology has enhanced its use in HPV^+ OPSCC.

DNA sequencing, mutation analysis, and potential for targeted therapy. Although HPV^+ and HPV^- OPSCC differ, both have driver gene mutations that could be targeted [37, 46, 47, 51, 125–135]. Common HPV^+ OPSCC mutations are found in *PIK3CA*, Notch homolog 1 translocation-associated (*NOTCH1*), and fibroblast growth factor receptor 3 (*FGFR3)*, whereas HPV^- OPSCC has mutations in *TP53* and cyclin-dependent kinase inhibitor 2A/B [37, 46, 47, 51, 126]. Some of these mutations influence prognosis, but reports are not concordant. For example, mutant *TP53* is rarely observed in HPV^+ OPSCC, and its prognostic value is unclear [24, 51, 131, 134]. Likewise, having a mutated *FGFR3* was associated with poor outcomes in HPV^+ OPSCC (as shown in CC in one report), although this was not always the case [51, 130–132, 136]. Mutated *PIK3CA* showed a variable prognostic value, but with de-intensified RT, it was associated with a poor outcome [37, 51, 57, 125, 133]. Nevertheless, irrespective of their predictive value, some mutations can enhance tumor sensitivity to targeted therapy. Two mutant *p53* reactivating compounds—APR-246 and COTI-2—are currently used in clinical trials, but their effects warrant further investigation [137, 138]. Moreover, the Food and Drug Agency (FDA) has approved the phosphoinositide 3-kinase inhibitor (PI3K inhibitor, alpelisib [BYL719]) targeting *PIK3CA* for clinical use for advanced breast cancer and the FGFR inhibitor, erdafitinib (JNJ-42756493), for advanced bladder cancer. Both could potentially be useful for some HPV^+ OPSCC cases [37, 51, 139–141]. In conclusion, DNA sequencing provides valuable data on specific mutations eligible for potential targeted therapies.

MicroRNA expression in HPV^+ OPSCC in relation to clinical outcome. MicroRNA (miR) expression has been examined in HPV^+ OPSCC. Only a few miRNAs have been repeatedly found to be overexpressed or downregulated in HPV^+ OPSCC—with 9, 155, and 163b being overexpressed, and miR-31 and 193b being downregulated [36, 52, 142–146]. In one study, miR-142-3p, 146a, and 26b

overexpression correlated with better prognosis, whereas miR-31, 24, and 193b overexpression presented worse survival [144]. In another study, a better outcome was observed upon miR-155 overexpression, and the opposite was found for miR-185 overexpression, whereas miR-193b had no prognostic value [52]. In conclusion, data on miR expression have varied widely, both in relation to HPV$^+$ OPSCC and outcomes.

The transcriptome in HPV$^+$ and HPV$^-$ TSCC, BOTSCC, and OPSCC. Reports on the transcriptomes of HPV$^+$ and HPV$^-$ OPSCC vary. Some studies have explored HPV mRNA expression in HNSCC, whereas others have explored all types of mRNA [109, 118, 119, 147–149]. There are differences between HPV$^+$ and HPV$^-$ OPSCC in terms of immune responses, apoptosis, proliferation, and the cell cycle. However, their possible prognostic value remains unknown [118, 119, 124, 147–149].

Proteomics in HPV$^+$ and HPV$^-$ OPSCC. Reverse-phase protein array profiling of 137 proteins revealed disparities between HPV$^+$ and HPV$^-$ OPSCC, for example, in the PI3K/protein kinase *B (AKT)*/mammalian target of rapamycin (mTOR) receptor kinase pathways [125]. Protein expression in HPV$^+$ OPSCC associated with *PIK3CA* mutations was correlated with mTOR activation, suggesting the potential use of mTOR inhibitors in HPV$^+$ OPSCC with *PIK3CA* mutations [125]. The expression of 167 immune-related proteins in fresh frozen material from 42 HPV$^+$ and 17 HPV$^-$ OPSCC cases in association with normal tissue was assessed using Olink multiplex immunoassays, and some proteins associated with hypoxia and angiogenesis tended to be associated with better outcomes in HPV$^+$ TSCC/BOTSCC [150]. Notably, high vascular endothelial growth factor A expression correlated with poor prognosis, indicating that angiogenesis-associated proteins could be targeted in HPV$^+$ OPSCC [150].

The microbiome in HPV$^+$ and HPV$^-$ OPSCC. A low diversity of microbiota species has been observed in HNSCC patients compared with healthy individuals, and a different bacterial taxonomy could potentially discriminate oral samples of OPSCC patients from those of healthy individuals [151, 152]. Whether shifts in the oral microbial composition can enhance HPV$^+$ OPSCC progression or manipulating it can improve clinical outcomes remains to be determined.

Concluding remarks on prognostic and targetable biomarkers in HPV$^+$ OPSCC. There are several reports regarding prognostic and targetable markers in HPV$^+$ OPSCC. However, because many of the studies have been performed in small cohorts with limited numbers of patients, some caution is required, and therefore, only a few are emphasized below.

Numerous numbers of reports suggest and concur that having a low T-stage (I/II vs. III/IV); having tumors that are both p16$^+$/HPV DNA$^+$ versus only being p16$^+$ or HPV DNA$^+$; and having tumors with either infiltrating/surrounding high CD8$^+$ counts as well as expressing HPV16 E2 mRNA 16 are favorable prognostic markers [43, 45, 49, 53, 58, 71–79, 109, 111]. Moreover, there is concordance that some specific mutations such as, for example, *PIK3CA, FGFR3*, and *NOTCH1*, are common in HPV$^+$ OPSCC, suggesting that inhibitors targeting these genes or their affected pathways could be of potential therapeutic value [37, 46, 47, 51, 126].

Younger age and not being a smoker can also be regarded as prognostic favorable factors, but these parameters are more complex due to performance status and because upon better diagnostics, the prognostic value of smoking is not as clear cut [43, 49, 106].

Novel therapeutic options for HPV$^+$ OPSCC with ICI and targeted therapies

Novel therapeutic options for HPV$^+$ OPSCC patients who do not respond to primary therapy or have relapsed remain urgent. With more data on the common mutations in HPV$^+$ OPSCC and HPV, new approaches have been proposed.

ICI clinical trials in HNSCC. Upon the failure of primary CRT or recurrent/metastatic (R/M) HPV$^+$ OPSCC, ICI has been introduced in some clinics as the standard of care (SOC) for selected patients. Nevertheless, most clinical trials have been conducted in R/M HNSCC cohorts, where details for R/M HPV$^+$ OPSCC are not shown. Of note, when ICI w/wo other drugs was compared with SOC in these trials, the latter did not include ICI (Tables 1 and 2) [83, 152–165].

ICI w/wo CT or CTLA-4 inhibitors—some early studies. Phase III studies on various ICIs—such as nivolumab, CHECKMATE-141 (NCT02105636), and pembrolizumab

Table 1. *Updates on therapy of head and neck cancer with immune checkpoint inhibitors (PD-1 with/without [w/wo] CTL-4) inhibitors and chemotherapy (CT) versus, for example, CT and epidermal growth factor receptor (EGFR).*

Drugs	Mechanism of action	Trial	Status	Main results	References
Nivolumab vs. single-agent systemic therapy	PD-1 inhibitor	NCT02105636 (CHECKMATE-141)	Completed	Median OS: 7.5 months vs. 5.1 months (HR 0.70, $p = 0.01$)[a], response rate 13.3% vs. 5.8%	[151, 152]
Pembrolizumab vs. single-agent systemic therapy	PD-1 inhibitor	NCT02252042 (KEYNOTE-040)	Completed	Median OS: 8.4 months vs. 6.9 months (HR 0.80, $p = 0.0161$)[a]	[153]
Durvalumab w/wo tremelimumab vs. single-agent systemic therapy	PDL-1 inhibitor w/wo.CTLA-4 inhibitor	NCT02369874 (EAGLE)	Completed	No OS benefit	[155]
Pembrolizumab w/wo platinum and 5-fluorouracil vs. EXTREME regimen	PD-1 inhibitor w/wo chemotherapy	NCT02358031 (KEYNOTE-048)	Completed	Improved OS (2–3 months) in pembrolizumab w/wo platinum and 5-fluorouracil groups	[161, 162]
Pembrolizumab and carboplatin and paclitaxel	PD-1 inhibitor + chemotherapy	NCT04489888 (KEYNOTE-B10)	Completed	ORR: 43%, OS: 12.1 months, complete respone: 7%	[158]
Durvalumab w/wo tremelimumab vs. EXTREME regimen	PD-1 inhibitor w/wo CTLA-4 inhibitor vs. chemotherapy	NCT02551159 (KESTREL)	Completed	No OS benefit	[159]

Abbreviations: CTLA-4, cytotoxic T-lymphocyte associated antigen 4; HR, hazard ratio; ORR, objective response rate; OS, overall survival; PD-1, programmed death-1.
[a]For PD-L1 positive tumours.

Table 2. *Phosphoinositide 3-kinase (PI3K)/mammalian target of rapamycin (mTOR) or fibroblast growth factor receptor (FGFR) inhibitors, with/without (w/wo) immune checkpoint inhibitors or chemotherapy or other drugs in head and neck cancer.*

Drugs	Mechanism of action	Trial	Status	Main results	References
Alpelisib w/o, Paclitaxel	PI3K inhibitor, cytostatic	NCT02051751	Completed	Frequent Grade 3–4 toxicity	[165]
Alpelisib w cisplatin and radiotherapy	PI3K inhibitor, cytostatic radiotherapy	NCT02537223	Completed	Tolerable toxicity	[166]
Bimiralisib	PI3K/mTOR inhibitor	NCT03740100	Terminated	Set ORR 30% not met (ORR: 7%)	[167]
Everolimus (adjuvant) vs. placebo	PI3K/mTOR inhibitor vs. placebo	NCT01111058	Terminated	No PFS benefit	[168]
Erdafitinib	FGFR inhibitor	NCT01703481	Completed	Promisning ORR and PFS in specific subgroups (urothelial-and cholagiocarcinoma	[169]

Abbreviations: ORR, objective response rate; PFS, progression-free survival.

KEYNOTE-040 (NCT02252042)—have reported survival benefits compared to CT or RT without ICI, whereas with durvalumab EAGLE (NCT02369874), this was not the case (Table 1) [153–156, 158].

More specifically, with a 24.2-month minimum follow-up with nivolumab (CHECKMATE-141), a survival advantage, increased durability of response, and much less toxicity than SOC was observed [154, 155]. For pembrolizumab (KEYNOTE-040), 247 patients were allocated randomly to pembrolizumab, whereas 248 were allocated to SOC. The primary endpoint of increased OS resulted in a median of 8.4 months in the former compared to 6.9 months in the SOC arm, respectively, and survival was 37% and 26.5%, respectively, in the first year [156].

In contrast, EAGLE—a randomized open-label phase III study that administered durvalumab w/wo tremelimumab (an antibody against cytotoxic T-lymphocyte-associated antigen 4 [CTLA-4])—showed similar OS to SOC treatment, and in part, this could be due to an unexpectedly good outcome in patients treated with SOC [158]. Notably, the authors reported increased mortality in the initial stages in the immunotherapy arm, but those who survived had an increased duration of response and superior survival at 2 years. The authors also reported reduced toxicity to durvalumab relative to the SOC arm [158].

In summary, the effect of ICI on R/M HNSCC is limited by the proportionally low number of patients who respond and the necessity to manage autoimmune toxicities, resulting in high treatment costs [83]. However, we anticipate that the capacity to mitigate autoimmune toxicities will likely improve.

Furthermore, the effect of ICI cannot be entirely based on high PD-1 expression in tumors or various immune cells [48, 158, 163]. The limited number of responses has not spurred an investigation into why most patients with HNSCC do not respond to ICI. Instead, studies have focused on biomarkers to better select patients who will benefit the most from ICI. Moreover, while searching for markers for better patient selection, additional combinations of ICI w/wo CT, CTLA-4, targeted therapy, or preventive vaccines have been pursued. Some of these efforts are discussed below.

ICI w/wo CT or CTLA-4 inhibitors versus CT and cetuximab and the search of markers for better patient selection in HNSCC. More trials comparing ICI w/wo a CTLA-4 inhibitor, or CT, or CT w/wo cetuximab have also been conducted in R/M HNSCC [159, 160, 164, 165].

In the KEYNOTE-048 (NCT02358031) phase III study, the effects of pembrolizumab w/wo CT versus cetuximab and CT were examined in R/M HNSCC (Table 1) [160, 164]. Based on its efficacy

and safety, pembrolizumab-CT (platinum and 5-fluorouracil) has been suggested as an appropriate first-line treatment for R/M HNSCC, whereas pembrolizumab alone was suggested as an appropriate first-line treatment for PD-L1-positive R/M HNSCC [164]. An enhanced effect of pembrolizumab or pembrolizumab-CT was found with increasing PD-L1 expression. However, although the latter was informative, the authors concluded that other predictive markers were needed for HNSCC with low PD-L1 expression [164]. Furthermore, although pembrolizumab w/wo CT had an OS benefit versus cetuximab-CT in R/M HNSCC, neither improved the progression-free survival (PFS) [164]. Upon 4-year follow-up, first-line pembrolizumab and pembrolizumab-CT continued to show a benefit in outcome versus cetuximab-CT in R/M HNSCC, but there were no details of HPV^+ OPSCC [160, 164].

In a phase IV study, KEYNOTE-B10 (NCT04489888), on R/M HNSCC with first-line pembrolizumab plus carboplatin and paclitaxel, the latter combination was shown to improve the objective response rate (ORR) in R/M HNSCC (Table 1) [161]. More specifically, 101 patients—regardless of PD-L1 status—received treatment, and upon a median follow-up of 18.9 months (range, 9.1–27.0), 49 (49%) patients had an ORR (95% CI, 38.4–58.7), including 7 patients (7%) with a complete response (CR). In the 101 patients, Grades 3–5 and serious treatment-related adverse events were observed in 76 (75%) and 27 (27%) patients, respectively. The authors suggested that pembrolizumab plus carboplatin and paclitaxel has promising antitumor activity and a manageable safety profile in first-line R/M HNSCC [161].

In a phase III study, KESTREL (NCT02551159), three markers were examined for possible associations with responsiveness in R/M HNSCC patients randomized to receive durvalumab without tremelimumab versus patients receiving SOC, according to the EXTREME regimen (Table 1) [162]. Blood and tumor samples from patients were analyzed for PD-L1, blood tumor mutational burden (bTMB) in circulating tumor DNA, and the neutrophil-to-lymphocyte ratio (NLR). Durvalumab w/wo tremelimumab showed durable responses and reduced treatment-related adverse effects compared with the EXTREME regimen; however, PD-L1 expression and NLR did not select for better OS for durvalumab monotherapy or durvalumab plus tremelimumab versus the EXTREME regimen. Notably, in the bTMB $\geq$16 mut/Mb subgroup, OS hazard ratios (95% confidence interval) for durvalumab monotherapy and durvalumab plus tremelimumab versus the EXTREME regimen were 0.90 (0.48–1.72) and 0.69 (0.39–1.25), respectively. CRs were 8.6% with durvalumab plus tremelimumab and 4.3% with the EXTREME regimen ($\geq$16 mut/Mb subgroup). The authors concluded that bTMB was potentially useful for identifying patients with R/M HNSCC who could benefit from durvalumab w/wo tremelimumab versus the EXTREME regimen [162].

However, in another study, when NLR and C-reactive-protein-to-albumin ratio (CAR) were examined as potential prognostic response factors in pembrolizumab-containing regimens as a first-line treatment for R/M HNSCC, a low NLR was found to be a positive prognostic factor [165]. Specifically, 74 patients with R/M HNSCC received pembrolizumab w/wo CT, and patient characteristics, tumor response, OS, PFS, and laboratory findings were reviewed. Correlations between NLR, CAR, and survival were investigated, with the 1-year OS rates being 60.4% and 18.1%, respectively, and in a multivariate analysis, a low NLR (<5) was associated with better OS and PFS. Therefore, the authors suggested that a low NLR could be a prognostic factor for OS and PFS in patients with R/M HNSCC receiving a pembrolizumab-containing regimen [165].

There was a discrepancy in NLR between the two studies. However, in the former study, pembrolizumab was combined with a CTLA-4 inhibitor, whereas in the latter, it was combined with CT [162, 165].

ICI w/wo CT or CTLA-4—some conclusions. Using ICI w/wo CT on R/M HNSCC showed slight benefits, although whether HPV^+ OPSCC patients are more or less responsive to ICI compared with other HNSCC patients remains to be determined [159–161, 164, 165]. Combining pembrolizumab with a CTLA-4 inhibitor showed that a high bTMB was a positive predictive response factor, whereas for ICI with CT, a low NLR was linked to a better prognosis, indicating that further studies on predictive response factors are necessary [162, 165]. Nonetheless, several side effects are associated with ICI—including fatigue, gastrointestinal toxicity, skin reactions, decreased appetite, coughing, and serious autoimmune complications [153–165]. Moreover, as ICI is often combined with other drugs

with other side effects, the variability in side effects can be immense.

PI3K/mTOR or FGFR inhibitors alone or w/wo ICI, CT, or other drugs.

PIK3CA and inhibitors against PI3K/mTOR w/wo CT, CRT, or ICI. *PIK3CA* encodes the p110α protein, a catalytic subunit of PI3K commonly mutated in human cancers, including HPV$^+$ OPSCC [46, 51, 166]. Some clinical studies on PI3K inhibitors have been performed in HNSCC (Table 2). However, these were not HPV$^+$ OPSCC-specific, and more experience with PI3K inhibitors has been obtained from breast cancer therapy [166–174].

In a small phase I study (NCT02051751), the PI3K inhibitor alpelisib (BYL719) and paclitaxel were administered to patients with human epidermal receptor 2 (HER2)-negative breast cancer, R/M HNSCC, and other solid cancers to confirm the safety and tolerability of the combination of BYL719 and paclitaxel and to dose-escalate BYL719 (Table 2) [168]. However, alpelisib plus paclitaxel has a challenging safety profile in patients with advanced solid tumors, and the study was closed following the completion of the dose-finding phase [168]. In another phase I study (NCT02537223) of nine HNSCC patients (7/9 with p16$^+$ cancer) with untreated locally advanced tumors with no metastasis, oral alpelisib was administered daily at 200 and 250 mg with cisplatin 100 mg/m^2 IV every 3 weeks and standard fractionation RT 70 Gy in 35 fractions (Table 2) [170]. The OS was 77.8%, and the lower dose of alpelisib had a tolerable safety profile with the administered CRT combination.

In a different phase Ib/II study, alpelisib was combined with cetuximab in R/M HNSCC. However, the authors reported no PFS benefit with the alpelisib–cetuximab combination, although it did have moderate activity in cetuximab-resistant patients with a consistent safety profile [169].

Alpelisib has also been combined with pembrolizumab in two case reports [173]. The combination of alpelisib and pembrolizumab had temporary success in a female aged 69 years and a male aged 58 years, both with HPV$^+$ OPSCC and *PIK3CA* mutations [173]. Both patients had advanced disease, several metastases, and unsuccessful therapeutic attempts and responded with partial tumor or metastasis regression to alpelisib combined with pembrolizumab. The responses eventually weakened, and both patients had to cease therapy owing to disease severity and major side effects, such as hyperglycemia due to alpelisib and Grade 3 immune-related hepatitis due to pembrolizumab in the female patient [173].

In another study, the efficacy of the PI3K/mTOR inhibitor, bimiralisib, was examined in eight patients with *NOTCH1*-mutant R/M HNSCC that had progressed during CT and ICI (NCT03740100) (Table 2) [171]. The ORR was 17%, and survival in these heavily pretreated patients was better than in those undergoing historical SOC [171].

The FDA-approved mTOR inhibitor, everolimus, has also been used in clinical phase II trials (NCT01111058, NCT01051791, NCT01283334). One study (NCT01111058) where patients were randomized to receive placebo (n = 24) or everolimus (n = 28) reported no significant differences in PFS or OS when considering the whole cohort. However, there was an improvement in PFS for patients with p16$^-$ cancer and in patients with mutant *TP53* tumors (Table 2) [172].

In summary, alpelisib or mTOR inhibitors are rarely used for HPV$^+$ OPSCC, but they may provide therapeutic benefits when combined with other drugs [173]. Common side effects include hyperglycemia, rash, diarrhea, and sometimes pneumonitis and interstitial lung disease, and deaths have been reported. However, recently, the clinical management of these side effects has improved [174].

FGFR inhibitors and solid tumors. FGFR has also been targeted in several solid tumors, especially urothelial carcinoma (UC), where *FGFR3* gene alterations are frequent and some effects have been observed. However, HNSCC is not a primary FGFR target cancer group [141, 175–177]. Nevertheless, FGFR inhibitors may be of interest for HPV$^+$ OPSCC, where *FGFR3* mutations are relatively common [52]. In a phase I study (NCT01703481) of patients with solid tumors, the safety, pharmacokinetics, and pharmacodynamics of the FGFR inhibitor erdafitinib (JNJ-42756495) were evaluated (Table 2) [141]. JNJ-42756493 administered at 10 mg on a 7-days-on/7-days-off schedule yielded clinical responses, showed pharmacodynamic biomarker activity, and had a manageable safety profile [141].

It has been implied that ICIs have decreased efficacy in UC patients with *FGFR3* mutations. Therefore, a meta-analysis was performed in 1963 UC patients to evaluate the prognosis and response to ICIs in those w/wo *FGFR* alterations in their tumors [177]. UC patients with *FGFR3* mutations receiving maintenance ICI or second-line ICI following first-line therapy, rather than first-line or neoadjuvant ICI, were more likely to have worse OS than those without *FGFR3* alterations [177]. The authors suggested that this was due to an immunosuppressive effect on the microenvironment by *FGFR3* alterations, and that this effect could be counteracted by combining FGFR inhibitors and ICI rather than using ICI monotherapy alone [177]. Whether this is relevant to HPV^+ OPSCC remains unclear.

To summarize, FGFR inhibitors have been used in UC and demonstrated beneficial effects but also included side effects such as hyperphosphatemia, constipation, decreased appetite, stomatitis, dry mouth, elevated creatinine, asthenia, and fatigue. Therefore, due to toxicity, many treatments have been terminated [141, 175–177].

Combinations of specific inhibitors. To avoid the development of resistance against PI3K and FGFR inhibitors, they are often combined with CT [170, 176]. In breast cancer, anti-HER2 or cyclin-dependent kinases 4 and 6 (CDK4/6) inhibitors can be combined with PI3K/mTOR inhibitors [178]. Moreover, PI3K and FGFR inhibitors can be combined [179]. Other specific inhibitors and combinations exist, however, as discussed later.

Several inhibitor combinations have been explored experimentally [179–184]. We have previously shown that combining PI3K with FGFR or CDK4/6 inhibitors and others in vitro in HPV^+ OPSCC cell lines w/wo the corresponding specific mutations induced synergistic effects and drug doses could be reduced considerably [179–182]. These data were encouraging because reducing drug doses could reduce potential side effects.

Very recently, the PI3K and FGFR inhibitors alpelisib and infigratinib were combined in patients with *PIK3CA*-mutant advanced solid tumors, w/wo *FGFR1–3* alterations with a primary endpoint as the maximum tolerated dose and secondary endpoints as the pharmacokinetics and response [178]. Alpelisib and infigratinib were administered at full single-agent doses. However, the high rate of dose interruption or reduction suggested that long-term tolerability was challenging, and evidence of synergistic activity was not observed [178]. On the other hand, to clearly observe synergistic effects, sometimes suboptimal doses are required [179–182]. The latter presents an important issue that ultimately has to be addressed clinically.

HPV targeting therapeutic agents. Currently, data on the HPV genome and the immunotherapeutic use of its products are abundant [64, 65, 84]. Targeting HPV16 remains a primary focus, as it accounts for approximately 90% of all HPV^+ OPSCC cases. HPV therapeutic vaccines can be based on peptides, viral vectors, nucleic acids, or cellular products and mainly target E6 and E7 oncoproteins, whereas prophylactic HPV vaccines target the L1 major virus capsid protein [64, 65, 84]. There are also efforts to activate HPV-specific T cells or administer adoptive cell therapy (ACT)—mainly targeting E6 or E7—to boost the immunity of these approaches. They are often administered with immunostimulatory agents—for example, ICI or interleukin 2 (IL-2) [84]. Some clinical trials are listed in Table 3.

Peptide-based vaccines alone or combined with ICI or other agents. *ISA101b* includes 12 synthetic long peptides that cover the complete and near-complete sequences of E6 and E7 viral proteins, respectively [84]. It induces HPV16-specific $CD4^+$ and $CD8^+$ T-cell activity and results in regression in a few patients with HPV16 high-grade vulvar intraepithelial neoplasia [185]. In patients with CC, the vaccine was administered following hysterectomy and did not exceed Grade 2 toxicity. However, although immune responses were obtained, whether the vaccine had any clinical effect remains undetermined [186].

In a single-arm phase II study, ISA101b was combined with nivolumab in 24 patients with $HPV16^+$ tumors, of which 22 were HNSCCs, and the initial overall response rate was 33% [190]. Notably, eight patients—all with HPV^+ OPSCC—achieved initial responses: two with CR and six with a partial response (PR). However, the combined treatment increased side effects, including fatigue, diarrhea, and hepatotoxicity [190]. Positive effects remained after long-term follow-up in NCT02426892 (Table 3) [191]. However, further studies are warranted to determine whether ICIs combined with ISA101b are better than ICI alone.

Table 3. *Phosphoinositide 3-kinase (PI3K)/mammalian target of rapamycin (mTOR)or fibroblast growth factor receptor (FGFR) inhibitors, with/without (w/wo) immune checkpoint inhibitors or chemoradiotherapy or other drugs in head and neck or cervical cancer.*

Drugs	Mechanism of action	Trial	Status	Main results	References
ISA101b + nivolumab	E6 and E7 peptide immunization (HPV 16) with PD-1 inhibitor	NCT02426892	Completed	ORR: 33% well-tolerated	[185]
PDS010 w/wo standard of care chemoradiotherapy	E6 and E7 peptide immunization cytostatic	NCT04580771 IMMUNOCERV	Ongoing	Preliminary: 36 months OS: 84.4%, PFS: 74.9%	[186]
GX-1884 with pembrolizumab	E6 and E7 DNA-based vaccine	NCT03444376	Completed	ORR: 35%, durable respone	[187]
BNT113 w/wo pembrolizumab	E6 and E7 mRNA-based vaccine with ICI	NCT04534205 (AHEAD MERIT)	Ongoing	Preliminary: ORR 40%, well tolerated	NA***
E7 TCR cells with IL-2	TCR-engineered T cells targeting E7 with IL-2 boostering	NCT02858310	Ongoing		[188]
TILs selected for E6 and E7 reactivity with aldesleukin	TILs targeting E6 and E7 with IL-2 boostering	NCT01585428	Completed	ORR: 28% (cervical-), 18% (non-cervival-camcer)	[189]

Abbreviations: E, early protein; IL-2, interleukin-2; NA, not applicable; PD-1, programmed death-1; PFS, progression-free survival; ORR, objective response rate; OS, overall survival; TCR, T-cell receptor; TILs, tumor-infiltrating lymphocytes.
***https://www.annalsofoncology.org/article/S0923-7534(24)02457-8/fulltext

PDS0101 contains six HPV16 E6/E7 peptides condensed in immunogenic cationic lipid nanoparticle [192]. In a single-arm phase I study, IMMUNOCERV (NCT04580771), 49 patients with advanced CC received SOC, and 17 participated in a clinical trial combining PDS0101. The patients were followed for cfHPV DNA in plasma to monitor recurrence-free survival (RFS) (Table 3) [192]. The authors found that cfHPV DNA levels significantly changed during CRT, cfHPV DNA at follow-up predicted RFS, and the delivery of the therapeutic HPV vaccine PDS010 with CRT was linked to a rapid cfHPV DNA decline [192].

Virus-vector-based vaccines. PRGN-2009, HB201, HB202, and TG400 are all viral-based vaccines [84]. PRGN-2009 is a recombinant gorilla-adenovirus vector-based vaccine with deletions in the HPV16 E1 and E4 regions, and it contains 35 non-HLA-restricted epitopes of HPV16 and HPV18 [187]. HB201 and HP202 are attenuated arenavirus vectors of the lymphocytic choriomeningitis virus and Pichinde virus, respectively, engineered to express the HPV16 E6/E7 fusion protein [193]. TG4001 is a modified virus of Ankara-based vaccine containing a plasmid with modified E6, E7, and IL-2 to stimulate immune responses [193]. These virus vector-based vaccines can potentially generate antigen-specific $CD8^+$ T-cell responses and are often combined with ICI. However, their potential use is limited [187, 193].

Nucleic acid-based vaccines. MEDI0457 and GX-188E are DNA-based vaccines, whereas BNT113 is RNA-based [183]. MEDI0457—which has two plasmids encoding the E6 and E7 proteins, respectively, and a plasmid encoding IL-12 DNA—has been previously tested, for example, together with ICI [194]. GX-1884 also encodes the E6/E7 antigens together with a tissue plasminogen activator signal sequence to enhance immunogenicity and the Fms-like tyrosine kinase-3 ligand to recruit and induce maturation of dendritic cells [195]. It has also been tested with pembrolizumab in patients with recurrent advanced CC (NCT03444376) with accompanying Grades 3–4 side effects but had some positive activity in an interim analysis (Table 3) [193]. BNT113 comprises synthetic messenger RNAs (mRNA) encoding E6 and E7, encapsulating liposomal formations [84]. BNT113 was beneficial for cervical intraepithelial lesions [189] and has been included in the clinical trial AHEAD MERIT (NCT04534205) recruiting HPV^+ R/M HNSSC, expressing PD-L1 where pembrolizumab w/wo BNT113 was compared for safety, tolerability, and effects (Table 3).

Adoptive cell therapy (ACT). ACT has been tested against several cancers but has been hampered by its limited efficacy and significant toxicity when used against various cellular antigens [196]. Three different T-cell therapies—namely, cell therapy with tumor-infiltrating lymphocytes (TILs), T-cell engineered lymphocyte T (TCR-T) cells, and chimeric antigen T cells—are potentially useful in this context [196–198]. For CC and HPV^+ OPSCC, the advantage is that HPV codes for proteins and antigens that do not induce autoimmunity. In a phase I/II study (NCT02858310) using E7 TCR cells, patients with CC received a single dose of E7 TCR, followed by a high-dose IL-2 infusion (Table 3) [197]. OR was observed in 6/12 CC cases (including three CRs) in patients with CC previously receiving ICI therapy. Prolonged persistence of the E7 TCR has been observed in some patients. Grades 3–4 toxicities were related to CT conditioning or IL-2 therapy. Phase II of this study was underway at the time this article was written. There have also been studies in CC, where TILs have been established from tumor fragments and expanded using IL-2, and in one phase II study (NCT01585428), HPV^+ OPSCC patients with HPV16 or HPV18 positive tumors were included (Table 3) [198]. In that study, TIL cultures were required to have $\geq$3 HPV oncoprotein reactivities, and the OOR was 28% when both CR and PR were included.

In conclusion, ACT is potentially useful for HPV^+ OPSCC—especially against viral antigens—and may be enhanced by ICI and/or therapeutic vaccines by boosting immune-responsive TILs. However, timing of the various administrations could be important.

Concluding remarks

The incidences of HPV^+ TSCC and BOTSCC continue to increase, and despite their relatively good prognosis, this does not apply to all patients. CRT is likely an overtreatment for some patients, but for those with recurrences, the prognosis remains poor; therefore, better tailored treatment remains warranted. To achieve this, reliable prognostic markers are needed to predict whether the cancer is easily treatable, if the patient is likely to have a therapy-resistant tumor or has a high risk of

recurrence. Thus, potential predictive markers can be used more readily.

These markers should be integrated into the decision to de-escalate treatment instead of relying solely on a small tumor volume. Furthermore, the monitoring of cfHPV DNA in plasma should be performed to ensure that cfHPV DNA decreases readily. In patients with large tumors and poor prognostic markers, a different strategy should be adopted to intervene at an earlier stage. There are several possibilities, such as using ICI alone, targeted therapies with specific inhibitors w/wo CT or ICI, or establishing TILs w/wo prior stimulation of the immune system with ICI, therapeutic vaccines, or both. Additionally, monitoring cfHPV DNA should assist in tracking the tumor's or metastasis's responsiveness to the selected treatments.

Numerous new therapeutic options present clear challenges, accompanied by various side effects and knowledge gaps in managing their effects. Similar to the early challenges of hematopoietic stem cell transplantation with major side effects—which are currently manageable—in the treatment of solid tumors, there is a need to improve the management of side effects. Better prognostication and follow-up, as well as earlier intervention for high-risk patients and better management of side effects, are important to increase the survival and quality of life of patients.

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Conflict of interest statement

The authors have no conflicts of interest to declare.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. List of publications in support of the importance of assaying for presence of human papillomavirus (HPV) DNA and p16INK4a (P16) for the presence of anactive HPV infection in oropharyngeal squamous cell carcinoma, with presentation of their Methods, Results and Conclusions, respectively. ■

