Abstract

The past two decades have been witness to a steadily increasing incidence of oropharynx cancer, specifically related to human papillomavirus (HPV), primarily affecting middle-aged Caucasian men, in North America and Europe. The ever-increasing incidence, now clearly an epidemic, of this unique clinicopathologic entity demands new perspectives in diagnosis and staging and presents unique challenges in clinical research, given the excellent prognosis afforded by chemoradiation for the majority of these patients. To reduce the morbidity of late toxicity in survivors without compromising the high rates of survival currently enjoyed, and simultaneously address the poor prognosis of those with recurrence, it is critical to capitalize on the viral etiology and translate discoveries in genomics, target/drug discovery, viral oncogenesis, and immunobiology to improved outcomes for patients. Herein, we review ongoing and planned clinical research for HPV-related oropharynx cancer, the basis for which is constituted by prior clinical observations, knowledge of the genomic alterations and altered biology associated with HPV-related oncogenesis, and hope that molecularly targeted and immunomodulatory therapies can be harnessed.
Because of its origin in the epithelium lining the lymphoid crypts of the palatine and lingual tonsils, visualization is problematic and there is no known precursor lesion or effective screening procedure. Early in primary tumor evolution, spread to cervical lymph nodes occurs and patients commonly present with a neck mass and a subtle or occult primary tumor, constituting advanced tumor–node–metastasis (TNM) stage.

Despite late-stage presentation, with multilevel adenopathy, HPV⁻ OPC is more responsive to treatment than HPV⁺ head and neck squamous cell carcinoma (HNSCC). Clinical trials demonstrate that despite very advanced stage, patients with HPV⁺ OPC have an excellent prognosis with chemoradiation (6, 7). Increased chemo- and radiosensitivity in these tumors is most likely multifactorial, with contributions from lowered expression of EGFR, engagement of immune mechanisms, p16-induced transcriptional effects, and a low level of wild-type p53 protein that can affect apoptosis (8, 9). Tobacco exposure does not increase risk for HPV⁻ OPC but does confer a negative impact on prognosis based on retrospective analyses of clinical trial data and a case series (10–12). In contrast to HPV⁺ HNSCC, the AJCC/UICC TNM staging system is not prognostic and revision of the current staging system is needed (13, 14).

Pathogenesis and molecular profiling

HPV infection and subsequent integration of the viral genome into the nuclei of the specialized epithelial cells lining the lingual and palatine tonsillar crypts promotes tumorigenesis through upregulation of expression of the early viral oncoproteins E6 and E7 (15, 16). The overarching effect of these proteins is to promote genomic instability and disturb cell-cycle control. E6 promotes ubiquitin-mediated p53 degradation, leading to deregulation of G1–S and G2–M checkpoints (17). E7 similarly ubiquitinates retinoblastoma (Rb), stimulating E2F target transactivation and premature entry into S-phase (18–20).

Although next-generation sequencing data are available on relatively few HPV⁺ HNSCC tumors, combined data indicate genomic alterations both shared and distinct from HPV⁻ HNSCC (21–24). For example, HPV⁺ tumors rarely harbor alterations in TP53 or CDKN2A, display frequent mutation/amplification of PIK3CA, and have copy number gains in E2F1 and TRAF3. A perspective on potential therapeutic approaches based on genomic alterations is provided by Hammerman and colleagues (25).

On the Horizon

Because of their good prognosis, relative youth, and overall health, patients with HPV⁺ OPC have a higher probability of morbidity from late effects of aggressive chemoradiation. Thus, deintensification strategies represent a current major clinical research theme in curative-intent treatment. On the basis of, in part, prior nonclinical data suggesting that the immune response elicited by cisplatin/radiation is key to the high control rates for HPV⁺ OPC and the clear opportunity to target nonself viral antigens, a major theme in current research is capitalizing on recent advances in immunotherapy. Targeted therapies are being studied as well, but with few exceptions, trials include both HPV⁺ and HPV⁻ HNSCC.

Locoregional approaches to reduce acute and late toxicity

To reduce toxicity from chemoradiation, either radiation dose must be reduced or systemic therapy altered. Strategies under investigation include substitution of cetuximab for cisplatin with radiation, selection of low-risk patients for reduced-dose radiation, and integration of transoral robotic surgery (TORS) for early-stage tumors (Table 1). Although a detailed discussion is beyond our scope herein, these clinical trials should provide insight on how best to optimize therapeutic index and reduce debilitating late effects in this young population.

Reversing immune evasion

The majority of individuals who develop HPV infection mount an HPV-specific CD4⁺ and CD8⁺ T-cell response to viral epitopes and ultimately clear the infection. Immune evasion is required for persistence of infection that can lead to carcinogenesis, and the

Table 1. Locoregional approaches to reduce acute and late toxicity in HPV-related OPC

<table>
<thead>
<tr>
<th>Trial/NCT#</th>
<th>Phase</th>
<th>Eligibility</th>
<th>Treatment</th>
<th>N</th>
<th>Primary endpoint</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>RT0G1066</td>
<td>III</td>
<td>● T1–2 N2a–N3/T3–4 N0–N3</td>
<td>Cisplatin vs. cetuximab + IMRT 70 Gy</td>
<td>990</td>
<td>5-y OS</td>
<td>Completed accrual</td>
</tr>
<tr>
<td>NCT0302854</td>
<td></td>
<td>● p16⁺ OPC</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ECOG 1508</td>
<td></td>
<td>● Stage III–IVA/b OPC</td>
<td>Cisplatin, paclitaxel, cetuximab + cetuximab + IMRT 45 Gy (CR) vs. 60 Gy (PR)</td>
<td>50</td>
<td>2-y PFS</td>
<td>Completed accrual</td>
</tr>
<tr>
<td>NCT0084083</td>
<td></td>
<td>● HPV⁺ by ISH and/or p16⁺</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quarterback</td>
<td>III</td>
<td>● Stage III–IVA/b OPC or CUP</td>
<td>TPF. If response, randomization to 56 Gy vs. 70 Gy IMRT + carboplatin</td>
<td>365</td>
<td>3-y PFS</td>
<td>Recruiting</td>
</tr>
<tr>
<td>NCT0706939</td>
<td></td>
<td>● HPV⁺ (PCR and p16)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OPTIMA</td>
<td></td>
<td>● Stage III–IVA/b</td>
<td>Nab-paclitaxel + carboplatin → IMRT 50 Gy vs. 45 Gy + CTX vs. 75 Gy +CTX (risk-based)</td>
<td>61</td>
<td>2-y PFS</td>
<td>Recruiting</td>
</tr>
<tr>
<td>NCT02258659</td>
<td></td>
<td>● HPV⁺ HNSCC</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ADEPT</td>
<td></td>
<td>● T1–4a N+ OPC p16⁺ S/P TORS with ECE</td>
<td>IMRT 60 Gy + weekly cisplatin</td>
<td>496</td>
<td>2-y DFS/LRC</td>
<td>Recruiting</td>
</tr>
<tr>
<td>NCT0168741</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NRG-HN002</td>
<td>II</td>
<td>● T1–2 Ni-2b/T3 N0–2b p16⁺ OPC</td>
<td>IMRT 60 Gy + weekly cisplatin</td>
<td>296</td>
<td>2-y PFS</td>
<td>Recruiting</td>
</tr>
<tr>
<td>NCT02254278</td>
<td></td>
<td>● &lt;10 pack-years smoking</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ORATORI</td>
<td>II</td>
<td>● T1–2, NO-1 (&lt;3 cm) or N2b</td>
<td>TORS + neck dissection vs. IMRT + CTX</td>
<td>68</td>
<td>1-y QDL</td>
<td>Recruiting</td>
</tr>
<tr>
<td>NCT01590355</td>
<td></td>
<td>(up to 2 nodes ≤ 3 cm) OPC</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E331I7</td>
<td></td>
<td>● Stage III, IVA/b</td>
<td>TORS → low risk → observation, intermediate → IMRT 50 Gy vs. 60 Gy, high → 66 Gy + cisplatin</td>
<td>377</td>
<td>2-y PFS</td>
<td>Recruiting</td>
</tr>
<tr>
<td>NCT0898494</td>
<td></td>
<td>● HPV⁺ p16⁺ OPC</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: CTX, chemotherapy; CUP, carcinoma of unknown primary; DFS, disease-free survival; ECE, nodal extracapsular extension; IC, induction chemotherapy; IMRT, intensity-modulated radiotherapy; LRC, locoregional control; NCT, National Clinical Trial; OS, overall survival; PFS, progression-free survival; QOL, quality of life; S/P, status post.
HPV oncoproteins likely play a role in this process (26, 27). Among other mechanisms, the programmed death-1 and programmed death ligand 1 (PD-1/PD-L1) pathway appears to be a key contributor to immune resistance of HPV-related OPC (28). PD-L1 is expressed on tonsillar crypt tissue and on both tumor cells and CD68+ tumor-associated macrophages, localizing to areas of lymphocyte fronts (28). Furthermore, CD8+ tumor-infiltrating lymphocytes (TIL) express high levels of PD-1. Moreover, high expression of PD-L1 in HPV-related oropharynx cancer and PD-1 expression in TILs has been confirmed by multiple groups (29, 30). These data suggest that the PD-1/PD-L1 pathway facilitates HPV infection, and subsequent to tumor development, immune evasion.

Using a gene expression signature developed in melanoma, Saloura and colleagues found that approximately half of HPV+ tumors showed a high T-cell infiltrated phenotype (TCIP), compared with HPV− tumors (31). TCIP was correlated with intensity of CD8+ T-cell infiltration, expression of immune-related markers, including PD-L1 and CTLA-4, and a mesenchymal gene expression signature. Approximately half of HPV+ HNSCC resected tumors showed significantly higher levels of IFNγ and IL-17+ CD8+ T lymphocytes, myeloid dendritic cells, and proinflammatory cytokines (32). In patients with HPV+ OPC, HPV-specific T-cell responses were reduced, PD-1 expression was increased on CD4+ T cells, and myeloid-derived suppressor cells increased, following chemoradiation (33). All of these data provide rationale for investigation of checkpoint inhibition in HPV+ OPC in all settings of treatment, with the hope not only of improving efficacy of treatment in high-risk patients but also of reducing late effects through integration in deintensification approaches.

Simultaneously with the development of checkpoint inhibition, there is renewed interest in therapeutic HPV vaccines. The E6 and E7 early oncoproteins, universally expressed in established HPV-related malignancies, are the basis for many vaccines, including approaches with live vectors, peptides/proteins, nucleic acids, and whole cells, as reviewed by others (34, 35). Below we discuss novel HPV vaccines, checkpoint inhibition, co-stimulatory agonists, and adoptive T-cell transfer (Table 2).

Table 2. Immunotherapy approaches in HNSCC, including HPV-related OPC

<table>
<thead>
<tr>
<th>Trial/NCT#</th>
<th>Phase</th>
<th>Eligibility</th>
<th>Target</th>
<th>Treatment</th>
<th>N</th>
<th>Primary endpoint</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCT01693562</td>
<td>II</td>
<td>Stage III/IVa/b HNSCC</td>
<td>Vaccine</td>
<td>ADXS11-001 → TORS</td>
<td>30</td>
<td>HPV E6/E7-specific CD8+ CTL</td>
<td>Recruiting</td>
</tr>
<tr>
<td>NCT02110082</td>
<td>I</td>
<td>Solid tumors (HNSCC cohort)</td>
<td>Anti-PD-1</td>
<td>Pembrolizumab 10 mg/kg Q2W or 200 mg Q5W</td>
<td>224</td>
<td>ORR, AEs</td>
<td>Accrual completed</td>
</tr>
<tr>
<td>NCT01693562</td>
<td>II</td>
<td>Solid tumors (HNSCC eligible)</td>
<td>Anti-PD-1</td>
<td>Pembrolizumab</td>
<td>150</td>
<td>ORR, AEs</td>
<td>Recruiting</td>
</tr>
<tr>
<td>KEYNOTE-040</td>
<td>III</td>
<td>Platinum-refractory HNSCC</td>
<td>Anti-PD-1</td>
<td>Pembrolizumab</td>
<td>180</td>
<td>PFS, OS</td>
<td>Recruiting</td>
</tr>
<tr>
<td>KEYNOTE-055</td>
<td>III</td>
<td>Platinum-refractory HNSCC</td>
<td>Anti-PD-1</td>
<td>Pembrolizumab</td>
<td>48</td>
<td>36m PFS</td>
<td>Recruiting</td>
</tr>
<tr>
<td>KEYNOTE-012</td>
<td>I</td>
<td>Solid tumor (HNSCC cohort)</td>
<td>Anti-PD-1</td>
<td>Pembrolizumab</td>
<td>180</td>
<td>PFS, OS</td>
<td>Recruiting</td>
</tr>
<tr>
<td>NCT02290209</td>
<td>II</td>
<td>Locoregional recurrence or second primary HNSCC</td>
<td>Anti-PD-1</td>
<td>Pembrolizumab</td>
<td>760</td>
<td>ORR, AEs</td>
<td>Recruiting</td>
</tr>
<tr>
<td>NCT0135562</td>
<td>II</td>
<td>Solid tumors (HNSCC eligible)</td>
<td>Anti-PD-L1</td>
<td>Pembrolizumab</td>
<td>966</td>
<td>PFS, OS</td>
<td>Recruiting</td>
</tr>
<tr>
<td>NCT0280628</td>
<td>II</td>
<td>HPV-related cancer</td>
<td>Anti-PD-L1</td>
<td>Pembrolizumab</td>
<td>264</td>
<td>ORR, AEs</td>
<td>Recruiting</td>
</tr>
<tr>
<td>NCT0280628</td>
<td>II</td>
<td>HPV-related cancer</td>
<td>Anti-PD-L1</td>
<td>Pembrolizumab</td>
<td>66</td>
<td>2 y PFS, AEs</td>
<td>Recruiting</td>
</tr>
<tr>
<td>NCT0280628</td>
<td>II</td>
<td>HPV-related cancer</td>
<td>Anti-PD-L1</td>
<td>Pembrolizumab</td>
<td>28</td>
<td>In development</td>
<td>Recruiting</td>
</tr>
<tr>
<td>NCT0280628</td>
<td>II</td>
<td>HPV-related cancer</td>
<td>Anti-PD-L1</td>
<td>Pembrolizumab</td>
<td>104</td>
<td>AEs</td>
<td>Recruiting</td>
</tr>
<tr>
<td>NCT01836029</td>
<td>II</td>
<td>HPV-related cancer</td>
<td>Anti-PD-L1</td>
<td>Pembrolizumab</td>
<td>73</td>
<td>Recruiting</td>
<td>Recruiting</td>
</tr>
<tr>
<td>NCT01836029</td>
<td>II</td>
<td>HPV-related cancer</td>
<td>Anti-PD-L1</td>
<td>Pembrolizumab</td>
<td>61</td>
<td>ORR, duration of response</td>
<td>Recruiting</td>
</tr>
</tbody>
</table>

Abbreviations: AEs, adverse events; CRC, colorectal cancer; CTL, cytotoxic T lymphocyte; 5-FU, 5-fluorouracil; ORR, overall response rate; R/M, recurrent or metastatic.

*Overall trial population, not HNSCC-specific.
**Immunotherapy for locoregional disease.** Listeria-based vaccines act both as natural adjuvants and as HPV-specific vaccines, which elicit a powerful, antigen-specific, cell-mediated immune response. ADXS11-001 is a live attenuated Listeria monocytogenes organism that secretes an antigen-adjuvant fusion protein consisting of a fragment of the protein listeriolysin fused to HPV16 E7 (36, 37). Clinical trial data from patients with cervical cancer provide proof that vaccine therapy alone can be effective for a subset of patients with refractory HPV-related cancer (38). ADXS11-001 is being studied in patients with TORS-eligible HPV+ OPC (Table 2).

Addition of immunotherapy to definitive concurrent chemoradiotherapy is also a promising strategy given the recently reported data regarding immunosuppression, broad and HPV-specific, following treatment (33). This approach is most valid for patients with high-risk HPV+ OPC given their relatively poor prognosis. There is particular interest in combining cetuximab with immunotherapy given preclinical data indicating it may initiate adaptive immune responses to EGFR and other tumor antigens, resulting in primed CD8+ T cells and affecting antibody-dependent cellular cytotoxicity (ADCC; ref. 39). Trials combining checkpoint inhibition with cetuximab and radiotherapy in HPV+ OPC are ongoing or planned (Table 2).

**Immunotherapy for recurrent/metastatic disease.** Tissue analysis from the HNSCC cohort of the anti–PD-1 pembrolizumab phase Ib trial revealed that PD-L1 expression (≥1%) in stroma or tumor was present in 78% of patients with HNSCC but PD-L1 positivity did not differ by HPV status (40, 41). Response rate to pembrolizumab directly was correlated with PD-L1 expression but was similar between HPV+ and HPV− patients (~20%). An ongoing phase II trial of pembrolizumab in refractory HNSCC will accrue 150 patients and should provide a reasonable estimate of response rate in HPV+ OPC. This agent will also be studied with reirradiation (Table 2). A phase III trial of anti–PD-1 nivolumab versus investigator’s choice therapy is ongoing in platinum-refractory metastatic/recurrent HPV-related and unrelated HNSCC and a similarly designed trial is planned with pembrolizumab (Table 2). The response rate to anti–PD-L1 MEDI4736 was surprisingly lower in HPV+ OPC (4%, 1 of 23), compared with HPV− HNSCC (19%, 4 of 21) in an expansion cohort of a phase I/II trial (42). Accrual continues in this cohort to determine the difference in overall response rate (ORR) based on HPV status.

Results of pembrolizumab and MEDI4736 trials are at odds with the prediction that greater efficacy would be observed in HPV+ OPC based on higher expression of PD-L1 and greater degree of T-cell infiltration at diagnosis (28, 31). Thus, the tumor microenvironment in recurrent/refractory HPV+ OPC may be altered to reduce dependence on the PD-L1/PD-1 pathway for immune evasion. Tissue and blood correlates from these trials should provide information about the immunophenotype of recurrent HPV+ and HPV− cancer, and more importantly, responders versus nonresponders, giving a rationale for immunotherapy combinations to address resistance to PD-1 inhibition. Toll-like receptors (TLR) are critical to initiate innate and adaptive immune responses to pathogen-associated molecular patterns. The TLR agonist VTX-2337 results in synergistic effects with chemotherapy and increased ADCC with monoclonal antibodies in nonclinical models (43–45). A randomized phase II trial of platinum, 5-fluorouracil, and cetuximab ± VTX-2337 is ongoing for patients with recurrent HNSCC, stratified by HPV status (Table 2).

Two trials, both in the planning stages, combine immune activation with Listeria monocytogenes and checkpoint inhibition. Given PD-L1 expression in HPV+ OPC and the heavy T-cell infiltration, the use of combined PD-1 blockade with vaccination is supported by preclinical data (30). In a murine syngeneic model with TC-1 lung fibroblasts transfected with HPV16 E6/E7 and HRAS, increased efficacy of PD-1 blockade was observed when preceded by an HPV E7 peptide vaccine. Furthermore, in HPV+ OPC tumors, infiltrating PD-1+ T cells frequently expressed activation markers and were functional after PD-1 inhibition, although TIM-3 was also expressed, consistent with an exhausted phenotype.

A synthetic long peptide HPV16 vaccine consisting of 13 overlapping peptides from the HPV16 oncoproteins E6 and E7 (ISA101) with adjuvant montanide, developed by Kenter and colleagues, proved effective in causing regression of high-grade vulvar premalignancy (VIN) in 15 of 19 patients and durable complete disappearance of disease in 9 of 19 patients (46). Importantly, complete clinical responses (CR) were correlated with stronger IFNγ-induced T-cell responses and all patients with complete responders developed HPV16-specific immunity. Although this vaccine did not have single-agent activity in patients with recurrent HPV16-related gynecologic cancers (47), a rationale exists to pursue combination approaches given the efficacy in VIN. ISA101/adjuvant montanide and the anti–PD-1 antibody nivolumab will be studied in a phase II “basket” trial for patients with recurrent HPV16-related cancers (Table 2).

Costimulation of T cells is required for T-cell proliferation, differentiation, and survival. Costimulatory agonistic antibodies in planned and ongoing trials include those targeting OX-40 (CD134) and 4-1BB (CD137) receptors. The latter is of particular interest as recent data in a murine syngeneic model of tonsillar epithelial cells transfected with HPV16 E6/E7 indicate that activation of the 4-1BB pathway can enhance efficacy of cisplatin alone and with radiation (48). Phase I trials include evaluation of monotherapy and combinations with checkpoint inhibitors, and in some cases, expansion cohorts for patients with refractory HNSCC. Of particular interest is a phase Ib trial of uralumab (4-1BB agonist) plus cetuximab in patients with HNSCC and colorectal cancer (Table 2), based on synergy of the combination in murine xenografts (49). It will be important for these trials to prospectively assess HPV status to identify differences in outcomes associated with viral carcinogenesis.

Adaptive T-cell transfer (ACT) has significant potential in HPV-related cancers, given the ability to target viral epitopes, new insight into variables influencing T-cell recognition, memory, and differentiation, and new technologies reviewed earlier (50, 51). The efficacy of checkpoint inhibitors and hope of greater impact with combinatorial approaches contributes to resurgence of interest in this approach, which has previously only been an option for selected tumors/patients at a few centers. Both preclinical and clinical research toward HPV-specific ACT supports further development in this area. Recent data demonstrate successful reactivation and expansion of HPV16 E6- and E7-specific T cells from patients with cervical cancer and OPC, with promise for treatment of HPV16-related cancers (52). Using TILs obtained from surgical resection, Stevanović and colleagues (53) demonstrated proof of concept that HPV-specific ACT can be highly effective in treatment of refractory cervical cancer (Table 2). TILs selected for E6 and E7 reactivity and expanded in vitro were
infused, preceded by lymphocyte depletion, and followed by high-dose bolus aldesleukin. Of the 6 patients who showed peripheral T-cell HPV reactivity, 1 partial response (PR) and 2 CRs were observed; the latter responses were associated with prolonged HPV-reactive T cells following treatment. Patients with CRs remained free of recurrence 18 and 11 months posttreatment at the time of report. With inclusive eligibility for all HPV16-related cancers, this trial continues to accrue patients. A similar trial involves administration of autologous T cells engineered to express the HPV16 E6 T-cell receptor in patients with all HPV16-related cancers (Table 2). ACT as given above has promise but requires prolonged hospitalization to manage acute toxicity and is logistically challenging. Combination approaches with ACT and new immunotherapeutics may limit the need for aggressive conditioning/boosting while simultaneously increasing efficacy (54).

Molecularly targeted therapy

**Background.** There are no validated predictive markers for cetuximab, a monoclonal antibody to EGFR, the only targeted agent approved for HNSCC. Although high expression of EGFR is a well-documented poor prognostic marker, numerous studies demonstrate that EGFR expression or amplification is not predictive of benefit (55, 56). Retrospective data suggest that patients with p16+ OPC derive greater benefit from cetuximab/radiation compared with patients who have p16- OPC, but further prospective evidence is required (57). In addition, retrospective analysis of a randomized trial of platin/5-fluorouracil ± cetuximab showed that benefit from cetuximab was independent of p16 status (58). Although EGFR is not amplified and expression is low in HPV+ OPC, it is notable that the HPV E5 oncoprotein enhances EGFR signaling and is directly correlated with EGFR expression and survival (59). Identification of a biomarker predicting benefit for cetuximab, dependent or independent of HPV, is probable based on tumor correlating data that should be forthcoming from completed or ongoing trials of cetuximab in HNSCC and HPV+ OPC, including RTOG 1016 and ECOG 1308 (Tables 1 and 3).

Until recently few HPV+ OPC cell lines were available, and thus preclinical research was limited. Now approximately 10 validated HPV+ OPC cell lines are in use, and data are emerging (9). Attempts to establish HPV+ patient-derived xenografts are also under way. Given data that immune response can influence response to cytotoxins and radiation in HPV+ OPC, it is important that human cell line and xenograft data with molecularly targeted drugs be validated in immunocompetent animal models. Molecular profiling data point to rational targets for therapy of HPV+ OPC, including PI3K, FGF, and DNA damage response pathways (21–24).

**PI3K pathway.** The PI3K pathway is most commonly activated in HPV+ OPC, with approximately 50% incidence (PIK3CA mutation/amplification and PTEN loss combined). Furthermore, preclinical data indicate resistance to EGFR inhibitors in HNSCC PIK3CA-mutant cell lines, supporting a rationale for combination with cetuximab (60). Thus far, data with PI3K inhibitors alone in patients with PI3K+ mutant solid tumors do not show dramatic efficacy, supporting combination therapy as the major strategy in early-phase clinical trials (61–64). PX866, a pan-class I PI3K antagonist, has been studied in combination with cetuximab or docetaxel, in two randomized phase II trials. No benefit in clinical efficacy endpoints was observed in either trial (65, 66). In the trial with cetuximab, neither HPV status (13 patients each arm) nor PI3KCA mutation status (8 patients) appeared to influence efficacy. Results from trials with BKM120, another pan-class I PI3K antagonist, and BYL719, a p110α-specific inhibitor, are summarized in Table 3.

**DNA damage response.** DNA repair proteins, such as PARP, are upregulated; mutations in DNA repair genes (e.g., BRCA1/2) are present; and p53 and pRb are downregulated in HPV-related OPC, providing a rationale for investigation of PARP inhibitors and inhibitors of the DNA damage checkpoint kinases, Chk1/2 and Wee-1 (67).

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**Table 3.** Targeted therapy in HNSCC including HPV-related OPC

<table>
<thead>
<tr>
<th>Trial/NCT#</th>
<th>Phase</th>
<th>Eligibility</th>
<th>Target</th>
<th>Treatment</th>
<th>N</th>
<th>Primary endpoint</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCT02113878</td>
<td>Ib</td>
<td>Stage III-IVa/b HNSCC</td>
<td>PI3K inhibitor</td>
<td>BKM120 + cisplatin + IMRT</td>
<td>46</td>
<td>MTD</td>
<td>Recruiting</td>
</tr>
<tr>
<td>NCT02282371</td>
<td>Ib</td>
<td>Stage III-IVa/b HNSCC</td>
<td>PI3K inhibitor</td>
<td>BYL719 + cetuximab + IMRT</td>
<td>18</td>
<td>RP2D</td>
<td>Recruiting</td>
</tr>
<tr>
<td>NCT02298595</td>
<td>I/II</td>
<td>Resectable HPV+ OPC</td>
<td>PI3K inhibitor</td>
<td>BYL719 + paclitaxel + cisplatin → TORS or TLM ± IMRT ± cisplatin</td>
<td>62</td>
<td>ORR after IC</td>
<td>Pending activation</td>
</tr>
<tr>
<td>NCT0171541</td>
<td>I/II</td>
<td>Stage IVa/b HNSCC</td>
<td>PARP inhibitor</td>
<td>Carboplatin + paclitaxel + veliparib → IMRT + chemo</td>
<td>110</td>
<td>ORR after IC</td>
<td>Recruiting</td>
</tr>
<tr>
<td>NCT01169284</td>
<td>I/II</td>
<td>Platinum-refractory HNSCC</td>
<td>PI3K inhibitor</td>
<td>BKM120 + cetuximab</td>
<td>30</td>
<td>Measurement of tumor pEGFR</td>
<td>Recruiting</td>
</tr>
<tr>
<td>NCT01602315</td>
<td>I/II</td>
<td>Platinum-refractory HNSCC</td>
<td>PI3K inhibitor</td>
<td>BYL719 + cetuximab</td>
<td>202</td>
<td>PFS</td>
<td>Recruiting</td>
</tr>
<tr>
<td>NCT0115790</td>
<td>I</td>
<td>Refractory solid tumors (HNSCC cohort)</td>
<td>Chk1 inhibitor</td>
<td>LY2606368</td>
<td>150</td>
<td>RP2D, AEs, ORR</td>
<td>Recruiting</td>
</tr>
<tr>
<td>NCT02124148</td>
<td>Ib</td>
<td>Solid tumors</td>
<td>Chk1 inhibitor</td>
<td>LY2606368 + cisplatin or cetuximab</td>
<td>70</td>
<td>MTD</td>
<td>Recruiting</td>
</tr>
<tr>
<td>NCT02196356</td>
<td>I</td>
<td>HNSCC treatment-naive (first line)</td>
<td>Weel inhibitor</td>
<td>Cisplatin ± Mk-1775</td>
<td>82</td>
<td>ORR</td>
<td>Recruiting</td>
</tr>
</tbody>
</table>

**Table 3.** Targeted therapy in HNSCC including HPV-related OPC

**Locoregional disease**

**Recurrent/metastatic disease**

**Abbreviations:** IC, induction chemotherapy; MTD, maximum tolerated dose; RP2D, recommended phase II dose; TLM, transoral laser microsurgery.

*Overall trial population, not HNSCC-specific.
Results from studies in HPV+ HNSCC cell lines indicate that levels of PARP may be predictive for response to single-agent veliparib (ABT888), a PARP inhibitor, and that PARP inhibition combined with either cetuximab or radiation enhanced cytotoxicity, compared with veliparib alone, through inhibition of DNA repair (68, 69). Veliparib is combined with neoadjuvant paclitaxel and carboplatin in a phase I/II trial (Table 3). Because of myelosuppression observed with PARP inhibitors and chemotherapy, combination with cetuximab and radiation may prove more feasible in HPV-related OPC.

The Chk1/2 kinases play a critical role in genomic surveillance and may be particularly important in mediating cell-cycle arrest in response to DNA damage when normal p53 is absent. Inhibition of Chk1/2 with AZD7762 reversed cisplatin resistance, mediated by the absence of functional p53, causing mitotic cell death in HPV+ HNSCC cell lines (70). Because of downregulation of p53, it is reasonable to assume that this would be an effective strategy for HPV+ OPC as well. LY2606368, a Chk1 inhibitor, is being studied in two clinical trials that include HNSCC cohorts (HPV status assessed retrospectively; Table 3).

Wee-1 kinase inactivates cyclin-dependent kinase, allowing DNA damage–induced G2–M arrest. Preclinical data support the efficacy of AZD1775, targeting Wee-1, to enhance effects of cisplatin and radiation in HPV+ HNSCC cell lines (71). Notably, this effect was associated with enhanced apoptosis mediated by reduced MC1-1 and XIAP protein expression and is distinct in this effect was associated with enhanced apoptosis mediated by reduced MC1-1 and XIAP protein expression and is distinct in mechanism of cisplatin sensitization of HPV+ cell lines. A randomized phase II trial of cisplatin + AZD1775 is ongoing with HPV status assessed retrospectively (Table 3).

FGFR signaling. Preclinical data demonstrate the potential of targeting FGFR signaling in HNSCC, and as is the case with PI3K, evidence exists that EGFR activation may confer resistance (72, 73). FGFR inhibitors are currently being studied in broad phase I trials (e.g., NCT01962532), and their evaluation in molecularly defined subsets of HNSCC, including somatic mutations in FGFR2/3 (most relevant for HPV+ OPC), FGFR2/3 fusions, and FGFR amplification, is awaited.

Summary

Many exciting new approaches are emerging in the treatment of HPV+ OPC translating recent advances in immunobiology/therapeutics and molecular profiling to the clinic. In view of the unique biology and clear improvement in prognosis, it is important that trial designs allow for separate evaluation of patients with HPV+ OPC or prospective documentation of HPV status, and stratification in randomized studies. In trials accruing patients with recurrent cancer, pretreatment tumor tissue, as opposed to tissue archived at diagnosis, will be integral to understanding clinical outcomes and effecting progress.

Authors’ Contributions

Conception and design: E. Massarelli, B.S. Glisson Development of methodology: B.S. Glisson Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): E. Massarelli Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): R. Ferrarotto, B.S. Glisson Writing, review, and/or revision of the manuscript: E. Massarelli, R. Ferrarotto, B.S. Glisson Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): B.S. Glisson Study supervision: B.S. Glisson

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References


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