Oropharyngeal cancer: clinical implications of the HPV connection

In addition to cervical cancer, the most widely established human papillomavirus (HPV)-associated malignancy, HPV has been implicated in the development of a subset of oropharyngeal cancers [1, 2]. Several lines of evidence support the causative role of HPV in oropharyngeal squamous cell carcinomas (OSCC). According to a workshop hosted by The Cancer Etiology Branch of the National Cancer Institute entitled ‘Validation of a Causal Relationship: Criteria to Establish Etiology’, four types of evidence are necessary to establish a causal relationship in cancer: epidemiological, molecular pathological, experimental, and evidence derived from animal studies [3]. There is strong epidemiological and molecular pathology evidence indicating that HPV16 is associated with a subset of OSCC [4–7]. Rampias et al. [8], by showing that E6 and E7 oncogene repression restores p53 and retinoblastoma tumor suppressor pathways and induces apoptosis in HPV16+ oropharyngeal cancer cell lines, provided experimental evidence that HPV16 is causally associated with a subset of OSCC. In addition, it has become clear that HPV-positive OSCC is defined by unique patient demographics and disease characteristics. HPV-positive patients tend to be younger with less exposure to tobacco and alcohol. The cancers are increasing in incidence; almost exclusively confined to the palatine tonsil and base-of-tongue; tend to present with lower T-stage and higher N-stage; and have been strongly epidemiologically linked with sexual activity, especially oral sex.

In this issue of *Annals of Oncology*, Posner et al. [9] report findings from retrospective analysis of TAX 324 study that contribute to the substantial body of evidence that HPV-positive OSCC is associated with a better prognosis than HPV-negative OSCC. TAX 324 [10] was a randomized trial of 501 patients with head and neck squamous cell carcinoma (all of whom had stage III or IV nonmetastatic unresectable disease or were candidates for organ preservation) to receive either taxotere, platinum, 5FU (TPF) or platinum, 5FU (PF) induction chemotherapy, followed by chemoradiotherapy (CRT) with weekly carboplatin therapy and radiotherapy for 5 days per week. Of 264 patients with oropharyngeal cancer, 111 had assessable pretreatment biopsies that were retrospectively examined for HPV DNA status by PCR for HPV16. There was insufficient size to demonstrate a treatment effect but a substantial impact on survival was demonstrated regardless of therapy assignment. Overall survival (OS) and progression-free survival were substantially better for HPV DNA+ patients (OS, hazard ratio = 0.20, P <0.0001). Local regional failure was less frequent in HPV DNA+ patients (13% versus 42%, P = 0.0006). At 5 years, 82% of HPV DNA+ patients were alive as opposed to 35% of HPV-negative patients (P <0.0001). The size of the sample—one 111 patients with OSCC and known HPV status, of whom 50% had HPV DNA+ tumors—limited the ability of the authors to control for other prognostic variables and to conclude that HPV status was a critical independent prognostic indicator. In addition, the authors defined HPV positivity based on the presence of HPV DNA in tumor tissue. HPV is ubiquitously present in humans and HPV DNA detection by itself does not prove causal association. The detection of E6 and E7 mRNA would be the definitive proof of HPV transcriptional activity but it is often not feasible in formalin-fixed paraffin-embedded (FFPE) tissue. Taken together, PCR for HPV DNA detection is not the optimal assay to determine HPV association.

The molecular differences between HPV- and tobacco-associated OSCC can assist in the development of biomarkers to determine HPV transcriptional activity in FFPE. The molecular signatures between HPV-associated and tobacco-associated OSCC demonstrate well-identified differences. HPV-associated cases express the viral E6 and E7 oncoproteins. Each of these proteins exerts its tumor promoting function via perturbation of various cellular regulatory mechanisms of whom the best known are the p53 and retinoblastoma (pRb) tumor suppressors. E6 binds and degrades p53 tumor suppressor protein, while the gene encoding p53 remains wild type in almost all HPV-associated tumors. E7 binds and degrades the pRb tumor suppressor protein. To the contrary, in HPV-negative OSCC, abrogation of p53 and pRb tumor suppressor pathways occurs via mutational inactivation of p53 gene and the p16 tumor suppressor, a component of the pRb tumor suppressor network, respectively. Most HPV-associated tumors, including HPV-associated OSCC, express p16 and p16 overexpression has been associated with HPV oncogenic potential in premalignant cervical lesions. There are also other molecular differences between HPV-positive and HPV-negative OSCC. For example, E6 and E7 oncoproteins activate Wnt signaling in HPV16-positive oropharyngeal cancer cell lines, whereas this pathway is not activated in HPV-negative OSCC [11]. p16 protein expression by immunohistochemistry (IHC) has been demonstrated to be a robust indirect indication of HPV transcriptional activity in FFPE OSCC where HPV mRNA detection is often not feasible [2]. In addition, HPV in situ hybridization (ISH) can be used to determine HPV association in FFPE tissue; virus integration into host DNA provides a punctate staining pattern, whereas the episomal form is associated with diffuse staining pattern.

Recently, two international phase III studies comparing chemoradiotherapy regimens in locally advanced head and neck squamous cell cancer reported on retrospective analyses of
survival in relation to HPV status [12, 13]. Ang et al. [12] reported findings from retrospective analysis of the association between tumor HPV status and survival among patients with locally advanced OSCC who were enrolled in a randomized trial comparing cisplatin combined with accelerated fractionation radiotherapy or standard fractionation radiotherapy. The authors found that 63.8% of 323 patients with OSCC had HPV-positive tumors assessed by ISH for HPV16; these patients had better 3-year OS rates (82.4% versus 57.1% among patients with HPV-negative tumors; \( P < 0.001 \)) and, after adjustment for age, race, tumor and nodal stage, tobacco exposure, and treatment assignment, had a 58% reduction in the risk of death. p16-positive status was also a strong prognostic indicator as p16-positive patients had a 67% reduction in the risk of death. Recursive partitioning analysis showed that the HPV status of the tumor was the major determinant of OS, followed by the number of pack-years of tobacco smoking (\( \leq 10 \) versus \( > 10 \)). This analysis classified patients with OSCC into three categories with respect to the risk of death: low risk, with a 3-year rate of OS of 93.0%; intermediate risk, with a 3-year rate of 70.8%; and high risk, with a 3-year rate of 46.2%. Rischin et al. [13] studied the association between p16 status determined by IHC and outcome in a subset of 465 patients with locally advanced HNSCC enrolled in a randomized phase III study comparing concurrent radiotherapy and cisplatin with or without tirapazamine. p16-positive tumors compared with p16-negative tumors were associated with better 2-year OS and failure-free survival. After adjustment for performance status, hemoglobin and T- and N-stage p16 retained independent prognostic significance.

Patients with HPV-associated OSCC are more likely to achieve partial or complete responses to therapy, even after adjustment for differences in tumor stage. Eastern Cooperative Oncology Group E2399 study [14], a phase II study of two cycles of induction chemotherapy with paclitaxel and carboplatin followed by concomitant weekly intravenous paclitaxel and standard fractionation radiation therapy, was analyzed prospectively for tumor HPV status in relation to outcome measures. Compared with patients with HPV-negative tumors, patients with HPV-positive tumors had higher response rates after induction chemotherapy and after chemoradiation treatment.

Patients with HPV-associated OSCC are younger, nonsmokers, have better performance status, and are less likely to have serious comorbidities than patients with HPV-negative cancers in the majority of studies. Posner et al. [9] found that HPV DNA+ patients were younger, had T1/2 tumors and performance status of zero. Ang et al. [12] also found that HPV-positive oropharyngeal cancer was more common among nonsmokers and was associated with younger age, white race, better performance status, absence of anemia, and smaller primary tumors. However, Ang et al. [12] estimate that only about one-tenth of the prognostic difference between the two groups can be attributed to these favorable prognostic factors. Given this finding, how might we explain the relative contributions of differences in disease biology and treatment response to the prognosis for patients with HPV-associated OSCC? One potential explanation is that HPV-associated cancer is inherently less aggressive than tobacco-induced cancer. The conclusion across studies is that HPV-associated OSCC has a favorable outcome independent of treatment selection, as long as it is within the current standard of care. It has been shown for example that even when surgery is the only treatment modality used, OSCC patients with HPV-positive tumors have a better prognosis than those with HPV-negative tumors, after adjustment for tumor stage [15]. Another hypothesis is that molecular features of HPV-positive tumors may contribute to their better response to treatment with radiation and chemotherapy. At the molecular level, it has been shown that p53 and pRb tumor suppressor pathways in HPV-positive cancer cells are active but dormant. Laboratory data indicate that treatment of HPV-positive cells with standard chemotherapeutic agents induces down-regulation of HPV E6 and E7 viral oncoprotein expression and reactivation of tumor suppressor pathways [16]. On the contrary, tobacco-induced cancers bear mutations that might confer a more resistant phenotype. Other gene and protein expression differences might also explain the different clinical behavior of the two subtypes.

How can we translate these laboratory and clinical observations into patient benefit? Presently, HPV-associated OSCC are treated similarly to stage-matched tobacco-associated ones. However, as mentioned, these patients are younger with fewer tobacco and alcohol-associated comorbidities. Thus, one could argue that the burden of late toxicity is higher in HPV-positive individuals since younger healthier patients are more likely to be productively employed and long-term toxicity, that impairs daily function, would be expected to exert a higher societal and economic price. Research efforts aiming to reduce the intensity of radiation or chemoradiation are ongoing with the underlying hypothesis that cure rates could be maintained while long-term toxicity could be abrogated. These studies offer a sanguine outlook for patients with HPV-positive OSCC in the near future but a cautionary note must be sounded to earnestly pursue this strategy within the confines of carefully conducted and supervised prospective clinical studies. It would be tragic if non-evidence-based shifts in management standards crept into our therapy for eminently curable patients and this hypothesis turned out to be incorrect.

Moreover, HPV-associated OSCC is a distinct disease entity and its treatment or prevention may entail intrinsically different approaches than tobacco-induced OSCC. For example, in HPV-positive cancers, strategies to decrease the expression of E6 and E7 oncogenes might increase cure rates [8]. For tobacco-induced cancers, selective targeting of cancer cells bearing p53 mutation with ONYX-015, an E1B-55kDa gene-deleted adenovirus engineered to selectively replicate in and lyse p53-deficient cancer cells, combined with conventional chemotherapy might improve therapeutic effect [17]. Prevention strategies involve either risk reduction through modification of exposure to the various causative agents or the development of screening methods. HPV vaccines may be another strategy to prevent HPV-associated OSCC. One HPV vaccine (Gardasil, Merck & Co., Inc., Collegeville, PA) targets...
HPV subtypes 6, 11, 16, 18, and another vaccine (Cervarix, GlaxoSmithKline, Research Triangle Park, NC) subtypes 16 and 18. Several randomized placebo-controlled trials in human volunteers showed that these two vaccines decrease the incidence of persistent genital HPV16 and HPV18 infections and associated moderate-to-high grade cervical intraepithelial neoplasia CIN2/3 [18, 19]. However, the impact of these vaccines on the incidence of persistent oropharyngeal HPV infection has not been studied. Evidence from animal models immunized against HPV16 has demonstrated reduction in the development of HPV oral lesions [20]. It is not clear, however, whether persistent oral HPV infection can lead to premalignant changes in oropharynx, as it does in cervical carcinoma. The natural history of oral HPV has not been studied and routine screening for HPV-associated OSCC is not recommended.

In the future, OSCC should no longer be considered as a single disease entity for the purpose of clinical trials. HPV-associated OSCC represents a distinct biological and clinical entity. Targeting HPV or reducing the intensity of radiotherapy or CRT include research areas that are actively being pursued in order to improve therapeutic results.

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disclosure

The authors declare no conflict of interest.

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