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EDITORIAL

Human Papillomavirus and Prognosis of Oropharyngeal Squamous Cell Carcinoma: Implications for Clinical Research in Head and Neck Cancers

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Oropharyngeal squamous cell carcinomas are etiologically heterogeneous, with one subset attributable primarily to human papillomavirus (HPV) infection and another to alcohol and tobacco use. These subsets are clinically and molecularly distinct, and these distinctions extend to patient prognosis.[1] In this issue of the *Journal of Clinical Oncology*, Licitra et al[2] report that patients with HPV DNA–positive oropharyngeal cancers have an approximately 60% reduction in 5-year mortality when compared with patients with HPV-negative tumors, who have a considerably worse prognosis. These data corroborate several previous single-institutional, retrospective analyses that reported similarly improved disease-specific and overall survival in HPV-positive patients.[3-8]

Retrospective analyses, such as those performed in this study, must be interpreted with caution because of limited quality of retrospectively collected data, sample size constraints, and the potential for residual confounding. However, in a prospective, population-based, observational study, the association between HPV status and prognosis strengthened after adjustment for potential confounders.[9] Indeed, tumor HPV status was as important to patient prognosis as tumor stage. Therefore, the association between tumor HPV status and prognosis is now sufficiently strong and consistent such that its impact on clinical research involving head and neck cancer patients must be considered.

As for any cancer prognostic biomarker with potential clinical utility, research involving HPV tumor status is strongly dependent on the performance of the assay used for HPV detection as well as the definition of a positive test.[10] Classification of a tumor as HPV positive is more complicated than would appear at first glance. Licitra et al[2] detected HPV 16 DNA in 17 (19%) of 90 oropharyngeal cancers and reported evidence for viral integration into the tumor cell genome. The investigators used an assay that measures viral copy number in tumors that has limited sensitivity for viral integration and assumes that a specific region of the viral genome (the E2 region) is deleted during integration into the host cell genome.[11,12] Although viral integration occurs in the majority of cervical cancers, as demonstrated via sensitive detection of the viral-human genome fusion construct (mRNA or DNA integration site),[13] integration is neither as necessary nor specific to invasive disease as was once believed.[12] Viral oncogene expression by a high-risk HPV type is essential for transformation and is also necessary for the maintenance of the transformed phenotype.[14] However, even viral oncogene (E6 and E7) expression—considered the gold standard for establishing the HPV etiology of a tumor—can be detected in young women with a high-risk, cervical HPV infection and normal cervical cytology.[15] The ideal test for clinical classification of a tumor as HPV positive would use clinical, formalin-fixed, and paraffin-embedded tumor samples; would specifically and sensitively detect transcriptionally active, high-risk HPV DNA types (16, 18, 31, 33, 35) found in head and neck cancer cell nuclei; would provide evidence for viral integration; and would demonstrate a copy number sufficient for a viral-tumor clonal association.

Although a combination of tests can now be used, no single test provides all of the required information, and each has its limitations. Commercially available in situ hybridization assays for HPV DNA are currently close to this ideal, but do not demonstrate oncogene transcription and may also have limited sensitivity for some HPV types. It is therefore important that the strengths and limitations for assays used in research for HPV detection be clarified, that investigators consider use of more than a single assay, and that the definition of a positive result be defined carefully in each study.

The association between tumor HPV status and survival may affect our interpretation of other potential head and neck cancer prognostic biomarkers.[10] For example, the effect of mutations in the tumor suppressor protein p53 on head and neck cancer prognosis currently is unclear,[16] and may be confounded significantly by tumor HPV status. In accordance with previous reports, Licitra et al[2] found tumor HPV status to be inversely associated with p53-inactivating mutations, consistent with functional inactivation of p53 by the HPV E6 oncoprotein. The data from Licitra et al[2] also suggest that p53 mutation status had no impact on survival among HPV-negative patients. In addition to the inverse association with p53-inactivating mutations, deletions of p16 were less common among HPV-positive tumors in the study by Licitra et al.[2] This finding is in agreement with previous reports of a correlation between strong p16 staining by immunohistochemistry and HPV DNA–positive tumors.[17,18] Whether the enhanced p16 staining is

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indicative of inactivation of tumor suppressor retinoblastoma protein function by the viral oncoprotein E7, as in the cervical cancer model,[19] or is a characteristic of the tonsillar crypt epithelium susceptible to transformation by HPV[20]—or a combination of both—remains to be clarified. Whether the impact of tumor HPV status on patient prognosis depends on additional molecular classification (for example, p53 mutation status or p16 expression) also must be determined in a study sufficiently powered to address this question. Such a study would have to account for the strong inverse and direct correlations among HPV status, p53 inactivating mutations, and p16 expression, respectively. The current literature indicates that intratumoral high-risk HPV genomic DNA is the most important prognostic factor, and the data from Licitra et al[2] corroborate this conclusion.

In the study by Licitra et al,[2] smoking-related second primary tumors were observed primarily among patients with HPV-negative and/or p53 mutation–containing tumors. This finding is consistent with the observations that p53 mutations in head and neck cancers occur largely as a consequence of smoking,[21] and that HPV-positive tumors are more likely to arise in nonsmokers.[22] Although the risk of second smoking-related cancers appears less in patients with HPV-associated tumors, the risk for other HPV-associated cancers (such as cervical and anal cancer) is elevated in this patient population, who are expected to survive their head and neck cancer.[23] Screening strategies for second primary tumors in patients with HPV-positive tumors should therefore include anogenital cytology in combination with HPV detection, as is currently recommended for the general population.[24,25]

There are several settings in which tumor HPV status could alter interpretation of clinical trials in head and neck cancer patients. HPV-positive tumors arise predominantly from the lingual and palatine tonsils within the oropharynx. Therefore, randomized clinical trials with treatment arms that are unbalanced by primary tumor site and/or HPV status may lead to erroneous therapeutic conclusions. Differences in the proportion of HPV-associated disease among trials may also lead investigators to favor one therapeutic approach inappropriately over another. Furthermore, recent improvements in locoregional control of head and neck cancer may be attributable in part to a gradual shift in the etiology of the underlying disease. Tonsillar cancer incidence rates, a possible surrogate for HPV-associated disease, increased significantly in the United States from 1973 through 2001, whereas the incidence at other oral sites declined or remained unchanged.[26,27]

Clearly, the impact of tumor HPV status on prognosis should be evaluated in prospective clinical trials. In the meantime, additional insight into the effect of tumor HPV status on treatment response, survival outcomes, and other therapeutic inferences could be gained from completed trials with access to archived tumor specimens. These studies will help to shed light on the reasons for the improved survival among HPV-positive tumors, which might include reduced comorbidity, improved responsiveness to radiation and chemotherapy, immune surveillance to viral tumor-specific antigens, and the absence of field cancerization in these patients who tend to be nonsmokers.[28] Despite the current lack of effective HPV-specific targeted therapeutics, HPV status could affect clinical decision making through risk stratification: for example, the risks and benefits of intensive induction chemotherapy, concomitant chemoradiotherapy, and adjuvant chemoradiotherapy should be evaluated separately for HPV-positive and HPV-negative patients. Perhaps the current American Joint Committee on Cancer staging system for oropharyngeal cancer will also need to be modified to reflect different prognoses for patients with HPV-positive and HPV-negative tumors. Classification of tumor HPV status should be performed to avoid the introduction of potential confounding by HPV status in clinical trials by methods based on in situ hybridization or polymerase chain reaction. Until a prospectively validated clinical assay becomes available, investigators could also consider anatomic site-specific trials or use of surrogate markers of HPV status, including smoking status, primary tumor site, and p16 immunohistochemistry for stratification of patients in clinical trials.

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Author's Disclosures of Potential Conflicts of Interest

The author indicated no potential conflicts of interest.