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After reading the article “The Role of Human Papillomavirus in Nongenital Cancers,” the learner should be able to:
1. Review available epidemiological and molecular evidence concerning the role of human papillomavirus (HPV) in nongenital cancers.
2. Describe the prognostic and therapeutic implications of HPV positivity in nongenital cancers.

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The Role of Human Papillomavirus in Nongenital Cancers

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Human papillomavirus (HPV), one of the most common sexually transmitted diseases worldwide, has an established role in the pathogenesis of genital malignancies such as cervical cancer. The virus has also been implicated in the oncogenesis of nongenital cancers including head and neck malignancies (specifically oropharyngeal cancers) as well as anal cancer. There is less clarity regarding its role in lung and esophageal cancers. Worldwide, the incidence and prevalence of HPV-associated oropharyngeal cancer has been increasing over time. These patients have improved outcomes compared with those with HPV-negative oropharyngeal cancers, and there is continued interest in designing treatments specifically for this HPV-positive subgroup. Clinicians continue to gain an understanding of HPV in anal cancers and the risk factors associated with infection and progression to malignancy. This has potential implications for the eventual screening of high-risk groups. While HPV vaccination is currently approved for the prevention of cervical cancer, it also has potential in the prevention of all HPV-associated malignancies. In this review, current understanding of the role of HPV in nongenital cancers is discussed, as well as future implications for treatment and prevention. CA Cancer J Clin 2013;63:57-81. ©2012 American Cancer Society.

Keywords: human papillomavirus, head and neck cancer, oropharyngeal cancer, anal cancer, lung cancer, esophageal cancer, oncogenesis, prognosis, treatment, prevention

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Introduction

An increasing number of malignancies are directly or indirectly the result of viral infection. Progress in this area of cancer research has in large part occurred through analysis of cell signaling and growth control pathways that may be altered by viral oncopgenes.1

Human papillomavirus (HPV) is currently one of the most common sexually transmitted infections worldwide, with the majority of individuals who engage in sexual activity becoming infected at some point in their lifetime.2 There are more than 130 HPV types identified and these have been classified into low- or high-risk groups according to their potential for oncogenesis based on persistent infection.3 Based on data involving cervical HPV infection, the majority of individuals who become infected with HPV will have an asymptomatic course, with clearance of the virus occurring in 90% of them within 1 or 2 years; the other 10% will have persistent infection and an increased risk at developing cancer. Of these 10%, about one-half will develop malignancy by year 30 of persistent high-risk HPV infection.4

This association between HPV and genital cancers has been well established, with evidence suggesting an infectious etiology dating as far back as 1842, when an Italian physician, Dr. Rigoni-Stern, noted a positive association between sexual activity and the cervical cancer mortality rate.5 In 1907, Ciugetto observed the contagious etiology of genital warts using cell-free filtration experimentation, and suspected a microscopic germ as the cause.6 It would not be until 1978 when Della Torre et al in Italy and Laverty et al in Australia first demonstrated HPV particles in cervical condylomatous lesions.7,8 In 1980, Gissmann and zur Hausen were able to isolate HPV DNA from genital warts, which later led to the replication of
HPV genotype 16 (HPV-16) by Durst et al and HPV-18 by Boshart et al using DNA hybridization experiments in 1983 and 1984, respectively.9-11 Through the mid-1980s, this led to the direct demonstration of viral proteins, their interaction with human keratinocytes leading to immortalization, and their interaction with retinoblastoma and p53, and the direct demonstration in the early 1990s that these viral proteins are responsible for cervical carcinoma.12-19 More recently, we have become aware of nongenital cancers associated with HPV infection. Through the integration of viral DNA into the human genome, expression of oncoproteins, and inactivation of the tumor suppressor genes, HPV evolves to transform its site of infection with damaging unchecked proliferation and oncogenesis. In the United States, it is estimated that approximately 25,000 HPV-associated cancers occur annually (Table 1),20 and HPV is responsible for up to 90% of anal cancers, 65% of vaginal cancers, 50% of vulvar cancers, 35% of penile cancers, and 45% to 90% of oropharyngeal cancers.21-23 We discuss the role of HPV infection in nongenital cancers including those of the head and neck, anus, esophagus, and lung.

**HPV Virology and Oncogenesis**

HPVs are small, nonenveloped double-stranded DNA viruses. The HPV has a diameter of 55 nanometers and a genome consisting of a double-stranded circular DNA of approximately 8000 nucleotide base pairs associated with histones. This genome is enclosed in an icosahedral capsid shell comprised of major and minor capsid proteins. The genome encodes for early structural genes (E1-E8) and late structural genes (L1 and L2). The late-coding regions produce structural proteins while the early-coding regions, especially E6 and E7, are responsible for malignant transformation.24,25

HPV types are often referred to as “low-risk” or “high-risk” based on their potential for oncogenesis. The HPV types that are high risk include HPV-16, -18, -31, -33, -35, -39, -45, -51, -52, -56, -58, -59, -68, -73, and -82. The “low-risk” HPV types cause more benign lesions affecting the anogenital areas, such as genital warts (condylomata), low-grade squamous intraepithelial lesions (SILs) of the cervix, and laryngeal papillomas. These low-risk types include HPV-6, -11, -40, -42, -43, -44, -53, -54, -61, -72, and -81.26

High-risk HPV-16 infection has been shown to be more prevalent than any other high-risk HPV type in most regions of the world.3,22,27 Those HPV-infected populations who are also positive for the human immunodeficiency virus (HIV) have a much higher rate of HPV-16–positive tumors than most HIV-negative populations. Consequently, the incidence of high-risk HPV malignancies seems to be amplified by HIV immune suppression. The effect of antiretroviral therapy on the incidence of HPV-related cancers has been examined in cervical cancer. In 2000, the International Collaboration on HIV and Cancer published findings examining the relationship between highly active antiretroviral therapy (HAART) and the incidence of cancer in HIV-positive adults. They found no significant change in the incidence of cervical cancer since HAART was introduced.28 Recently, however, a prospective trial in South African women infected with HIV and receiving HAART demonstrated increased regression and a decreased

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**TABLE 1. Cancers Attributable to High-Risk HPV Infection in the United States From 2004 to 2007**

| ANATOMIC AREA         | AVERAGE ANNUAL NO. OF CASES* | HPV-ASSOCIATEDb | HPV-16/18–ASSOCIATEDb |
|-----------------------|-------------------------------|-----------------|-----------------------|
| Cervix                | 11,845                        | 11,370          | 9000                  |
| Vagina                | 714                           | 460             | 400                   |
| Vulva                 | 3062                          | 1560            | 1350                  |
| Anus and rectum (women)| 2977                          | 2770            | 2590                  |
| Oropharynx (women)    | 2306                          | 1450            | 1380                  |
| Total (women)         | 20,903                        | 17,610          | 14,720                |
| Penis                 | 1000                          | 360             | 310                   |
| Anus and rectum (males)| 1618                          | 1500            | 1410                  |
| Oropharynx (males)    | 8936                          | 5630            | 5360                  |
| Total (males)         | 11,553                        | 7490            | 7080                  |

HPV indicates human papillomavirus.

*Data adapted from Watson M, Saraiya M, Ahmed F, et al. Using population-based cancer registry data to assess the burden of human papillomavirus-associated cancers in the United States: overview of methods. Cancer. 2008;113(suppl 10):2841-2854.

Data adapted from Gillison ML, Chaturvedi AK, Lowy DR. HPV prophylactic vaccines and the potential prevention of noncervical cancers in both men and women. Cancer. 2008;113(suppl 10):3036-3046.

Table reprinted from: Hariri S, Dunne E, Saraiya M, Unger E, Markowitz L. Human papillomavirus. In: Manual for the Surveillance of Vaccine-Preventable Diseases. 5th ed. Atlanta, GA: Centers for Disease Control and Prevention; 2011:8.25
The incidence of HPV-related cervical lesions compared with women not receiving HAART. Further work in understanding HPV-related transformation as it relates to HIV coinfection is needed.

HPV establishes infection only within the stratified epithelia of the skin, oral cavity, and anogenital tract. To date, the bulk of HPV identification and sequencing has been sorted in the alpha and beta genera. Generally, the genus alpha exhibit a mucosal tropism while the beta HPV infect cutaneous cells. The virus gains access to keratinocytes of the skin and oral mucosa through wounding of the stratified epithelium. Viral DNA replication is intimately coupled with keratinocyte differentiation in infected squamous epithelium. HPV is maintained in the epithelial cells that undergo DNA replication under the control of both viral and host proteins. As these infected keratinocytes differentiate in the epithelium, viral DNA synthesis occurs.

Once HPV infection has taken place, additional accumulating changes can lead to transformation. These changes are a multistage complex interaction of the host immune system in combination with the expression of 2 viral oncogenes, E6 and E7, followed by a series of epigenetic changes occurring in dysplastic lesions. In the normal viral life cycle, the genome replicates as episomal in early and low-grade SILs, the HPV genome is consistently retained in the epimonal state. In high-grade SILs, the viral genome can remain episomal or may be integrated into the host chromosomes. The E6 oncogene product associates with E6-associated protein (E6-AP) with E3 ubiquitin ligase activity. This association between E6 and E6-AP induces the rapid degradation of tumor suppressor p53 through the ubiquitin-proteasome pathway.

E7 competes with the E2F transcription factor for binding to the retinoblastoma tumor suppressor gene (pRb). This leads to inactivation of retinoblastoma family of tumor suppressor proteins, the release of E2F, and DNA synthesis through activation of the S-phase in the cell cycle. Inactivation of pRb, a negative regulator of the cyclin–dependent kinase inhibitor p16, leads to p16 upregulation. E7 is able to elude the normally inhibitory effects of p16 by stimulating the S-phase genes cyclin E and cyclin A. This ultimately leads to the inactivation of checkpoints and regulatory pathways that transform the cells. Together, these functions of E6 and E7 promote cellular transformation.

E6 and E7 proteins of high-risk HPVs have transforming activity that leads to independent or synergistic immortalization of the cell. In contrast, low-risk HPVs have weak immunization activity. This difference is believed to correlate with the ability of E7 proteins to induce the degradation of the Rb gene rather than just their affinity for the Rb gene. High-risk HPV E6 and E7 proteins can also induce mitotic abnormalities through mitotic spindle checkpoints, while this is not seen in low-risk HPV.

Our current understanding of HPV oncogenesis has come mostly from studies involving cervical cancer with extrapolation to other anatomic sites. Further study of HPV and host protein–protein interactions are needed to elucidate other steps in oncogenesis that account for why a small minority of infected patients develop malignancy while most infected patients clear the virus without issue.

The Role of HPV in Head and Neck Cancer

Introduction
Head and neck squamous cell carcinoma (HNSCC) arises in the oral cavity, nasopharynx, oropharynx, larynx, and hypopharynx. While it accounts for only 3% to 4% of all cancer diagnoses in North America and Europe, HNSCC is the sixth most common malignancy worldwide. While tobacco and alcohol had long been recognized as risk factors for head and neck cancer, in 1983, Syrjanen et al raised the possibility of HPV also having an etiological role in HNSCC when HPV structural proteins were detected by immunohistochemistry (IHC) in 6 of 8 oral squamous cell carcinomas (OSCCs). Since this observation, further basic science, clinical, and epidemiologic research has led to our current understanding of the role of HPV in head and neck cancer. It remains an ongoing area of investigation, with exciting implications for the care of this subgroup of patients with head and neck cancer.

Epidemiology

HPV Prevalence in Head and Neck Cancer
HPV is found in 23% to 35% of all HNSCC biopsies worldwide, with the majority of HPV-positive cancers arising in the oropharynx, where HPV has been detected in 45% to 90% of cases, most commonly in the lingual and palatine tonsils or base of the tongue. HPV has also been detected to a significantly lesser degree in the oral cavity and larynx. Similar to cervical cancer, HPV-16 accounts for most cases, with this HPV type found in 68% to 87% of cases of HPV-positive head and neck cancer worldwide. Other HPV types that have been found more rarely in HNSCC include HPV-18, -31, -33, -35, -45, -51, -52, -56, -58, -59, and -68.

The epidemiology of head and neck cancer has been changing over the last 4 decades. In the United States, while the incidence of tobacco-associated head and neck cancers has been decreasing, likely due to a decrease in tobacco use, the incidence and prevalence of HPV-related HNSCCs have been increasing.
Chaturvedi analyzed data from Surveillance, Epidemiology, and End Results (SEER) registries from 1973 to 2004 to compare the incidence of HPV-related and HPV-unrelated OSCCs. Patients were classified as having HPV-related disease if their documented primary anatomic site was strongly associated with HPV. This included the oropharynx, base of the tongue, lingual and palatine tonsils, or Waldeyer ring. The incidence of HPV-related OSCCs significantly increased annually by 0.8% over this time period, especially for base of the tongue and tonsillar cancers, while HPV-unrelated OSCCs significantly decreased annually by 1.85% after 1983. By age group, a significant increase over the time period in HPV-related OSCC was seen in patients aged 40 years to 49 years and 50 years to 59 years, but not in patients aged younger than 40 years or older than 60 years. In addition, age at diagnosis significantly decreased from 1973 to 2004, by 0.5 years per decade, while it significantly increased for HPV-unrelated OSCCs. The rising incidence of HPV-related OSCCs was seen predominantly in white men, while the incidence in black men initially increased from 1973 to 1987 before decreasing significantly between 1988 and 2004. 

The incidence decreased in women over time. A noted limitation to this study was that HPV-related cases were assumed to be positive for HPV based on anatomic location. However, analysis of the HPV status of tissue from the SEER residual tissue repositories program between 1984 and 2004 also showed a significant increase in the prevalence of HPV in oropharyngeal cancers from 16% to 72% over this time period. In the United States, the annual number of all oropharyngeal cancers is already greater than the number of cervical cancer cases and is expected to be approximately 3 times higher by the year 2030 (Fig. 1). If current trends continue, it is projected that the annual number of HPV-positive oropharyngeal cancers will also surpass the annual number of cervical cancers in the United States by the year 2020.

A shift in epidemiology has also been noted in other developed nations internationally. A parallel increase in both the incidence of tonsillar or base of the tongue SCC and the percentage of HPV-positive cases has been demonstrated in Sweden. Hammarstedt et al analyzed 515 patients diagnosed with primary tonsillar SCC between 1970 and 2002 in Stockholm, using the Swedish cancer registry. A total of 203 patients had tissue available to confirm HPV status. The age-standardized incidence of tonsillar cancer increased by 2.8-fold between 1970 and 2002, and an increase was seen in both men and women. When the incidence of HPV-positive and HPV-negative tonsillar SCC was compared, the incidence of HPV-positive cancers increased over time, doubling per decade since 1970, while the incidence of HPV-negative tumors decreased since the 1990s, paralleling a decrease in smoking in Sweden. There was also a significant increase over this time period in the percentage of patients with HPV-positive tonsillar cancers, increasing significantly by 2.9-fold from 23% in 1970 to 1979 compared with 68% in 2000 to 2002. This percentage continued to increase to 79% when data up to the year 2007 were evaluated. Similarly, a significant increase in base of the tongue SCCs in Sweden was noted between 1970 and 2007, with an increase in the prevalence of HPV-positive cases from 58% in 1998 to 2001 to 84% in 2006 to 2007. Similar trends of increasing incidence and prevalence of HPV-positive oropharyngeal SCCs have been noted in Australia and Canada.

**Risk Factors**

Increased numbers of vaginal, oral, and oral-anal sex partners have been associated with an increased risk of developing HPV-associated HNSCC. This is likely due to an increased risk of exposure to the virus as the persistence of oral HPV infection increases the risk of eventual oropharyngeal cancer. Therefore, numerous studies have examined whether changes in sexual behavior...
may be able to explain the increase in the incidence of HPV-positive cases. Over time in both the United States and abroad, the age at sexual debut is decreasing and the number of sexual partners is increasing. In addition, sexual practices are changing, with oral sex being performed more by men and women that are currently aged 30 years to 49 years compared with older generations. In France for example, the lifetime prevalence of oral sex among women and men increased from 51% to 91% and from 55% to 94%, respectively, from 1970 to 2006. Therefore, changes in sexual practices over past decades may explain why the incidence of HPV-positive cases continues to increase.

There is conflicting evidence regarding the relationship between cigarette smoking and HPV and the risk of HNSCC. Some studies have suggested an additive or synergistic effect while others have not. Marijuana smoking, however, has been associated with HPV-positive HNSCC. There are also conflicting results concerning the relationship between alcohol, HPV, and the risk of HNSCC. The risk of oropharyngeal cancer is also increased by 2-fold in patients with HPV-16 seropositivity.

 Oral HPV Infection: Prevalence and Risk Factors

Compared with HPV-associated HNSCC, the epidemiology, transmission, and natural history of oral HPV infections are less well understood. In a cross-sectional study that involved 5579 healthy adults in the United States between 2009 to 2010, oral rinse samples were collected to detect HPV in the DNA of oral exfoliated cells. The overall prevalence was 6.9% and 3.7% for all oral HPV infections and high-risk HPV types, respectively. HPV-16 was the most prevalent HPV type, with an overall prevalence of 1%, and accounted for 20% of all high-risk HPV types identified. A combined analysis of 18 studies that evaluated oral HPV infection in healthy adults internationally revealed a similar prevalence of oral HPV. In the United States, a significantly higher prevalence of oral HPV was found in men compared with women (10.1% vs 3.6%), including in multivariate analysis. In this same study, black race was associated with a trend toward an increased prevalence of oral HPV infection in univariate analysis; however, this trend disappeared after controlling for differences in sexual behavior. The prevalence of oral HPV-16 infection is increased in developing nations compared with developed nations (4.3% vs 0.7%).

An increasing number of lifetime sexual partners has been independently associated with an increased prevalence of oral HPV infection and a stronger association has been seen when just high-risk oral HPV infections are considered. In a case-controlled study performed by D’Souza et al, the odds of oral HPV infection were significantly increased among subjects who reported greater than 25 lifetime vaginal sex partners or greater than 10 oral sex partners. In addition, greater than 6 recent (within the last 12 months) oral sex partners increased the odds of infection. It is unclear whether open-mouthed kissing increases the risk of oral HPV. Kreimer et al examined the relationship between oral HPV status and HIV infection. Patients who were positive for HIV had a significantly higher prevalence of all oral HPV infections (25.3% vs 7.6%; P < .001) and oral high-risk HPV infections (13.7% vs 4.5%; P < .001) compared with HIV-negative patients. In another study, the odds of an oral HPV infection were significantly increased in HIV-positive patients compared with those who were negative for HIV after controlling for sexual behavior, and were associated with a lower CD4 count. This suggests that HPV positivity and the degree of immunosuppression may increase the risk of oral HPV infection. Oral HPV prevalence is also increased in current tobacco smokers.

Comparatively, little is known about the natural history of oral HPV infection. D’Souza et al evaluated oral HPV status by collecting oral rinse samples twice during a 6-month interval in 182 women (123 of whom were HIV positive and 59 of whom were HIV negative). Approximately 55% of HIV-positive and 60% of HIV-negative patients tested positive for HPV both at baseline and at 6 months. HPV infections at baseline were more likely to persist at 6 months in current smokers and those with CD4 counts below 500 cells/µL, and the odds of persistent oral HPV infections increased significantly with increasing age. A 6-month sampling interval appears to be appropriate for future natural history studies of oral HPV infection. The transformation of benign tissue of the oropharynx to SCC involves the progression from normal epithelium to dysplasia, in situ carcinoma, and then invasive carcinoma. The HPV status of dysplastic lesions of the oral cavity and oropharynx was evaluated in a meta-analysis that included 22 studies. The prevalence of HPV-16/18 in oral cavity and oropharyngeal dysplastic lesions was 24.4%, and the odds of detecting HPV in dysplastic lesions in men was significantly higher compared with women (odds ratio [OR], 2.44; 95% confidence interval [95% CI], 1.26%–4.74% [P = .0008]). In 10 studies, HPV status was analyzed in benign, dysplastic, and invasive carcinoma lesions. The odds of detection of HPV in both dysplastic lesions and invasive carcinoma were 3 times as high as in tissue without dysplasia or cancer. There was no difference in the odds of detection between mild, moderate, and severe dysplasia. Their data suggest that, similar to cervical cancer, HPV is found in early precancerous lesions of the head and neck as well.

The continued study of the natural history of oral HPV has important implications. Screening has led to a substantial decrease in the morbidity and mortality from cervical cancer.
As our understanding of the natural history of asymptomatic oral HPV infection improves, the eventual development of screening measures and interventions may become possible.

**Pathogenesis**

Evidence for a causal relationship of HPV in the oncogenesis of oropharyngeal SCC comes from epidemiologic and molecular studies. The current model for the pathogenesis of HPV-associated HNSCC follows that of cervical cancer. As discussed in the previous section, oncogenesis involves the ubiquitin-mediated degradation of p53 by early-coding region E6,39,82,83 and the E7-induced inactivation of pRb. Loss of negative feedback as a result of downregulated pRb leads to upregulation of p16.16,40,41 Actions of E7 also allow for evasion of the normal tumor suppressor effects of p16.42,43 These actions collectively result in the inactivation of apoptotic pathways, disruption in the cell cycle, and activation of cellular proliferation that leads to oncogenesis.16,17,41,84,85 Evidence that HPV is directly oncogenic is strengthened by the observation that short hairpin RNA-mediated inhibition of HPV-16 E6 and E7 expression leads to restoration of the p53 and Rb tumor suppressor pathways and results in apoptosis.36,87 HPV-positive HNSCC is associated with wild-type p53, low pRb levels, and p16 overexpression, whereas tobacco- and alcohol-associated HNSCC are associated with mutated p53; high pRb levels; and, as a result of point mutations, promoter methylation, and homozygous deletion, low expression of p16 (Table 2).87-90

In HPV-associated HNSCC, the HPV genome can be found integrated in the host genome or in its episomal form.91 The reported percentage of tumors that contain the episomal form of the HPV genome varies from 35% to 100%, with the HPV genome found in mixed episomal and integrated form in 17% to 35% of cases.91-94 In tonsillar cancers specifically, the HPV genome has similarly been found in its episomal form in 40% to 100% of cases.91,92 How the HPV virus remains in the tissue in its episomal form and stays transcriptionally active has not been fully elucidated, but may involve the HPV E2 protein binding episomal HPV to cellular mitotic spindles.95

Gillison et al analyzed the clinical, pathological, and molecular characteristics of 253 patients with newly diagnosed or recurrent HNSCC. They evaluated tissue samples for the presence of the HPV genome using polymerase chain reaction (PCR), Southern blot hybridization and in situ hybridization (ISH), and sequencing of the viral E6 coding region and the p53 gene. They found that in HPV-positive tumors, HPV-16 accounted for 90% of HPV types and that HPV-16 DNA was integrated in 57% of patients found to be positive for HPV-16 on PCR (12 of 18 patients with oropharyngeal and none of the 3 patients with nonoropharyngeal HNSCC available for analysis). HPV DNA was located within the tumor cell nuclei of preinvasive and invasive lesions and tumor implants in regional lymph nodes, but not in the surrounding stroma or nondysplastic epithelium. There was a significant inverse relationship between HPV status and p53 mutation noted in the oropharyngeal subset, with a mutation found in 67% of HPV-negative but only 10% of HPV-positive patients. In a logistic regression analysis, HPV-positive patients had distinct characteristics of poorly differentiated histology, location in the oropharynx, and improved survival. This provides evidence that in the subset of patients with oropharyngeal HNSCC, HPV has an etiologic role in producing a distinct phenotype.49

D’Souza et al performed a case-control study in which 100 patients who were newly diagnosed with oropharyngeal cancer and seen in the outpatient otolaryngology clinic at Johns Hopkins Hospital were compared with 200 patients seen in the same clinic for benign reasons with no history of cancer. They found that a high lifetime number of oral sex or vaginal sex partners, seropositivity for the HPV-16 L1 capsid protein (a valid measure of HPV-16 exposure), and oral HPV infection were associated with the development of oropharyngeal SCC. HPV-16 DNA was found in 72% of tumor specimens from the 100 cancer patients, and 57% of these patients were seropositive for HPV-16 L1 compared with only 7% of control patients.66 This study adds further evidence to the etiologic role of HPV in that increased risk and exposure to HPV are associated with the development of oropharyngeal cancer compared with a control population. Other studies have shown that in patients with oropharyngeal cancer, HPV DNA is localized to tumor cell nuclei,87 is integrated87,96 and transcriptionally active96-98 and is specifically found in malignant tissue rather than adjacent tissue.99 While HPV has been found in nonoropharyngeal HNSCC, a causal role is less well established.

**HPV Testing**

HPV testing is recommended for patients with SCC of the oropharynx and can also be useful in the workup of patients with head and neck cancer of an unknown primary tumor (nccn.org). There are numerous techniques used to
test for HPV in tissue samples of patients with oropharyngeal cancers. These include type-specific DNA ISH, immunohistochemical detection of the surrogate biomarker p16, PCR for HPV DNA, real-time PCR assays to quantify viral load, detection of serum antibodies directed against HPV epitopes such as E6 and E7, or the detection of HPV E6/E7 mRNA. Each test has some limitations and the sensitivity and specificity vary between tests. Nonquantitative HPV DNA PCR is generally considered the gold standard in terms of sensitivity, and is perhaps most widely applied in studies. However, it is technically more challenging, and can not distinguish relevant transcriptionally active HPV from inactive HPV. Type-specific hybridization allows for localization of the HPV genome to the nuclei, which suggests the HPV may be more etiologically relevant. There are varying sensitivities and specificities and some discordance between tests. For example, 7% discordance between HPV-16 ISH and p16 IHC has been observed. As a result, some cancers are negative using HPV-16 ISH and positive for p16 expression. This may represent the minority of HPV-associated tumors that are positive for other HPV types, or a disruption of the pRb pathway that is unrelated to HPV. Given the limitations of individual tests, algorithms are being evaluated to most accurately detect biologically relevant HPV. Smeets et al evaluated 48 formalin-fixed, paraffin-embedded HNSCC specimens to evaluate the best algorithm to detect biologically relevant HPV infection, defined as the presence of HPV E6 oncogene expression in the specimen. They reported that using an algorithm of testing first for the expression of p16 by immunochemistry and confirming with PCR in positive cases yielded a sensitivity and specificity near 100%. Further research on a larger scale is needed to define the best algorithm for the detection of HPV that is biologically relevant.

Clinical and Pathologic Features
HPV-associated HNSCCs have distinct clinical and pathologic features and an improved prognosis. Patients with HPV-positive HNSCC are typically younger by approximately 10 years, male, and more likely to be non-smokers and nondrinkers. Although not pathognomonic, HPV-positive tumors more frequently are poorly differentiated and non-keratinizing, and exhibit basoid morphology compared with HPV-negative tumors. For example, in one study that evaluated 253 HNSCC specimens, HPV was found in 75% and 82% of all poorly differentiated and basaloid tumors, respectively. Clinically, HPV-positive patients usually present with TNM stage III and stage IV disease, with an earlier T category but more advanced N category, with cystic and multilevel lymph node metastasis.

Prognosis
Despite its presentation at a later stage, HPV-associated oropharyngeal SCC has been shown to be more responsive to therapy and to have a better outcome than HPV-negative tumors. These findings were initially shown in smaller retrospective studies. This led to confirmation in retrospective analysis of large clinical trials and in prospective studies. In the Radiation Therapy Oncology Group (RTOG) 0129 trial, 743 patients with stage III and stage IV HNSCC (without distant metastasis) were randomized to receive either concurrent cisplatin with accelerated fractionation radiotherapy or standard fractionation radiotherapy. Results of this study showed no difference between these 2 approaches in overall survival (OS) and progression-free survival (PFS). In patients with oropharyngeal SCC (n = 433), HPV status was determined and 63.8% of these patients tested positive for HPV DNA. Compared with HPV-negative oropharyngeal tumors, HPV-positive patients had a significantly improved 3-year OS rate (82.4% vs 57.1%; P < .001) and PFS rate (73.7% vs 43.4%; P < .001). Local or regional recurrence was significantly lower in the patients with HPV-positive cancers; however, there was no difference in distant metastasis at 3 years. In multivariate analysis, HPV status was a significant predictor of outcome, with HPV-positive patients having a 58% reduction in the risk of death (hazard ratio [HR], 0.42; 95% CI, 0.27-0.66) and a 51% reduction in the risk of relapse or death (HR, 0.49; 95% CI, 0.33-0.74). Similarly in another retrospective analysis of a large prospective phase 3 chemoradiation trial, patients with HPV-positive oropharyngeal cancer, as detected by p16 overexpression, had an improved 2-year OS and failure-free survival, as well as lower rates of locoregional failure. In multivariate analysis, increased p16 expression was a significant predictor of improved OS. In TAX 324, a randomized open-label phase 3 trial comparing induction with 3 cycles of docetaxel, cisplatin, and fluorouracil versus the combination of cisplatin and fluorouracil followed by chemoradiation using carboplatin, patients receiving the docetaxel, cisplatin, and fluorouracil regimen had improved OS and PFS. The authors retrospectively evaluated tumor HPV-16 status and outcome in the subjects with oropharyngeal cancer. They found that in the 111 patients with oropharyngeal cancer and pathology available for analysis, those who were HPV positive had a significantly greater 5-year PFS (78% vs 28%) and OS (82% vs 35%), with an 80% reduction in mortality (HR, 0.2; 95% CI, 0.1 to 0.38 [P < .0001]) compared with HPV-negative patients, independent of treatment. HPV-positive patients also had less total and locoregional failures.

In a retrospective analysis using SEER data that included patients who had undergone various treatments, HPV-positive patients had a significantly better long-term...
OS (median, 131 months vs 20 months; \(P < .001\)) and after adjusting for age, advanced stage of disease, lack of surgery or radiotherapy, receipt of chemotherapy, and diagnosis in earlier calendar periods, HPV-positive patients had a 69% reduction in their risk of death compared with HPV-negative patients. In patients treated with radiation, the difference in OS between HPV-positive and HPV-negative patients was greater compared with those who did not receive radiation. This study also showed that while OS has significantly improved since 1984 for patients with HPV-positive oropharyngeal cancers, there has been no significant change in OS in HPV-negative patients over this same time period.\(^{48}\) Thus, it appears that the improvement in outcomes seen in all patients with oropharyngeal cancer over the last 2 decades may be solely because of improved responses in the increasing cohort of HPV-positive patients.

Improved outcomes for HPV-positive patients have also been shown in small prospective trials.\(^{112,113}\) Outcome based on HPV status was tested in a prospective phase 2 clinical trial that included 96 patients with stage III or stage IV HNSCC of the oropharynx or larynx. Patients received induction chemotherapy with carboplatin and paclitaxel followed by chemoradiation with paclitaxel. HPV-positive patients had a significantly higher response rate after induction chemotherapy (82% vs 55%; \(P = .01\)) and at the completion of chemoradiation (84% vs 57%; \(P = .007\)), and a longer 2-year survival compared with the HPV-negative group (95% vs 62%; \(P = .005\)). They also had a lower risk of progression after adjustment for tumor stage, Eastern Cooperative Oncology Group performance status, and age (adjusted HR, 0.36; 95% CI, 0.15–0.85 [\(P = .02\)].\(^{112}\)

While improved prognosis has been shown repeatedly for patients with locally advanced disease who are treated with chemotherapy and radiation, data on patients treated primarily with surgery alone are conflicting. Licitra et al found that in patients undergoing surgical resection followed by adjuvant radiotherapy, HPV-positive patients showed a significantly improved 5-year OS rate (79% vs 46%; \(P = .0018\)).\(^{114}\) However, in a retrospective analysis of 102 patients also treated primarily with surgery at the Mayo Clinic, no significant survival difference was seen between HPV-positive and HPV-negative patients.\(^{115}\) In addition, 2 retrospective studies in patients with oropharyngeal cancer who were treated with transoral robotic surgery have also shown no difference in outcome according to HPV status.\(^{116,117}\) It is unknown if HPV-positive patients with metastatic disease have a better outcome.

**HPV, Prognosis, and Race**

Research has suggested that differences in outcomes based on HPV status may partially explain observed racial disparities in patients with locally advanced head and neck cancer. Numerous previous studies have shown a worse outcome for black patients with head and neck cancer compared with white patients, including after adjusting for age and disease stage at diagnosis as well as treatment.\(^{118–120}\) Settle et al analyzed OS by race in patients with stage III and stage IV head and neck cancer from a single tertiary institution and from the TAX 324 trial. In both groups, black patients had a significantly worse OS compared with white patients. However, analysis of the data from the single institution showed that the disparity was caused entirely by a large difference in outcome in patients with oropharyngeal cancer, and there was no difference in OS between races in patients with nonoropharyngeal cancers. HPV status was able to be examined in the TAX 324 trial. All white patients had a significantly longer median OS compared with all black patients in this trial (70.6 months vs 20.9 months; \(P = .03\)); however, a significantly higher percentage of white patients were HPV positive compared with black patients (34% vs 4%; \(P = .0004\)). Upon further analysis, while white HPV-positive patients had an improved OS compared with white HPV-negative patients, white HPV-negative patients had a similar median OS compared with all black patients (30.1 months vs 20.9 months; \(P = .56\)). Therefore, an increase in HPV-positive oropharyngeal cancers in white patients and, as a result their better outcome, may explain disparities seen by race between black and white patients in this cohort.\(^{121}\) Other studies have also shown a significantly worse outcome in black patients with oropharyngeal cancer and a decreased prevalence of HPV in black patients with HNSCC.\(^{122}\) Reasons for a difference in the prevalence of HPV-associated oropharyngeal cancers between races are unclear, but may be partially explained by differences in sexual practices.\(^{123}\)

**Defining a Subgroup of HPV-Positive Oropharyngeal Cancers With the Best Prognosis**

Several studies have attempted to better define the prognosis of patients with HPV-positive oropharyngeal cancers based on clinical characteristics, smoking status, and biomarkers. In the RTOG 0129 study discussed above, recursive partitioning analysis showed that HPV status was the most important determinant of OS followed by tobacco use and then lymph node or tumor stage. This study identified low-, intermediate-, and high-risk groups based on HPV status, pack-years smoking, and staging. The low-risk group, which included HPV-positive patients with a fewer than 10 pack-year history of smoking or a greater than 10 pack-year smoking history with N0 to N2a disease had the best 3 year OS rate of 93% compared with 70.8% for the intermediate-risk group, which included HPV-positive patients who had a greater than 10 pack-year smoking history and higher lymph node
classification (N2b-N3). This indicates that tobacco smoking and stage of the cancer have an impact on prognosis in patients with HPV-positive tumors.

Weinberger et al analyzed the relationship between HPV DNA status, p16 expression, and outcome in 79 patients with oropharyngeal SCC who were treated with radiation or surgery plus adjuvant radiation. Patients who were both positive for HPV-16 DNA and had high p16 expression had a better OS and disease-free survival in univariate and multivariate analyses compared with patients who were HPV positive but had low p16 expression, or who were HPV negative and had low p16 expression. The HPV-positive group with low p16 expression had a similar outcome to the HPV-negative group. These patients with HPV-positive tumors with high p16 expression also had significantly lower p53 and Rb expression than the other groups. The authors suggest that it was the subgroup of patients with HPV DNA-positive oropharyngeal SCC with increased expression of p16 who appear to have HPV-induced carcinogenesis, which may account for the better outcome seen in this group (Fig. 2).

Epidermal growth factor receptor (EGFR) overexpression has been associated with a more aggressive phenotype and worse prognosis in patients with HNSCC. Reimers et al analyzed 106 patients with disease ranging from stage I to stage IVc to understand the relationship between p16 and EGFR expression. There was a trend toward an inverse relationship between p16 and EGFR expression, with tumors that expressed p16 having low EGFR expression. However, this relationship did not reach statistical significance. They found that patients with tumors that expressed p16 but not EGFR had a significantly higher 5-year disease-free and OS then patients whose tumors overexpressed EGFR but not p16. In a phase 2 prospective clinical trial involving 66 patients with stage III to IV HNSCC of the oropharynx who were treated with induction chemotherapy with cisplatin and fluorouracil followed by chemoradiation and then adjuvant paclitaxel, an increased HPV copy number on PCR was significantly associated with treatment response and disease-specific survival. This improvement in outcome with increased HPV copy number was still significant in multivariate analysis after adjusting for sex, smoking status, staging, age, and primary tumor site. This trial was then analyzed further to evaluate the relationship between the expression of p16, EGFR, and p53 and outcome. There was a significant association between increased HPV copy number and p16 expression, and those patients whose tumors expressed p16 had a significantly better response to induction chemotherapy and chemoradiation as well as improved OS and disease-specific survival. EGFR expression alone was associated with a worse outcome, and was inversely associated with both HPV copy number and p16 expression. As combined markers, a higher HPV copy number or p16 expression

![FIGURE 2. Three Different Classes of Tumors in Head and Neck Squamous Cell Carcinoma (HNSCC). p16, protein 16; p53, protein 53; HPV, human papillomavirus; ETOH, alcohol. Reprinted with permission from Weinberger PM, Yu Z, Haffty BG, et al. Molecular classification identifies a subset of human papillomavirus-associated oropharyngeal cancers with favorable prognosis. J Clin Oncol. 2006;24:736-747. ©2012 American Society of Clinical Oncology.]
combined with low EGFR expression was associated with significantly better OS and disease-specific survival. This relationship was maintained after controlling for age, sex, smoking status, primary tumor site, and T and N classification.128

These studies highlight that we are beginning to understand that not all HPV-positive HNSCCs behave the same and these patients likely include both those who have truly HPV-driven oncogenesis and those in whom HPV superinfests an already mutated cancer cell.129 Continued work is needed to firmly define the subgroup of HPV-positive patients with the best prognosis.

Potential Reasons for an Improved Prognosis

The reasons for an improved outcome and response to therapy in patients with HPV-positive HNSCC are unclear. Theories include increased sensitivity to radiation and chemotherapy; differences in the role of the host immune system; and, in patients who do not smoke tobacco, the absence of field cancerization.130

Increased response to radiation and chemotherapy in HPV-associated HNSCC may result from E6-induced degradation of p53 rather than the inactivating mutations in p53 that are found more frequently in patients with HPV-negative HNSCC. Therefore, an intact p53-mediated apoptotic response to chemotherapy and radiation may account for the better efficacy of treatment in patients with HPV-positive tumors.131,132 Analysis of oropharyngeal SCC cell lines has also suggested that isoforms of E6 may increase radiation-induced cell death independent of the p53 pathway.133 However, this would not explain why HPV-positive patients may have a better outcome after surgery alone.134

Further outcome differences may be due to the field cancerization effect. This hypothesis states that head and neck tumors develop within a field of abnormal tissue as a result of long-term exposure to carcinogens, which can lead to subsequent local recurrences or second primary tumors.134,135 In patients with HPV-negative HNSCC, surgical margins have been found to contain abnormal tissue, and histopathologically normal tissue around the tumor has been found to have genetic abnormalities, including microsatellite alterations and p53 mutations.134,136,137 In contrast, a field cancerization effect has not been observed in HPV-positive tumors, where HPV viral DNA integration is confined to the neoplastic and dysplastic tissue only.138,139

The role of the immune system in relation to an improved prognosis in patients with HPV-positive HNSCC is an area of ongoing study. Spanos et al studied the effect of cisplatin and radiation in a mouse model of HPV-positive and HPV-negative HNSCC.140 They found that there was an increase in response to both radiation and cisplatin in those with HPV-positive compared with HPV-negative HNSCC. However, an improved response in HPV-positive HNSCC was seen in immune-competent but not in immune-incompetent mice. In addition, adoptive transfer of immune cells into immune-incompetent mice improved response to cisplatin, adding further evidence to the role of the immune system.140 Wansom et al retrospectively evaluated pretreatment levels of peripheral blood T-cell subsets in patients with HPV-positive and HPV-negative stage III and IV oropharyngeal cancer.141 These patients were undergoing treatment with induction chemotherapy followed by either chemoradiation or surgery depending on response as part of a prospective phase 2 clinical trial. HPV-positive patients had a significantly higher mean percentage of CD8 T-cells compared with HPV-negative patients. HPV-positive smokers similarly had significantly higher CD8 T-cell levels compared with HPV-negative smokers. A higher percentage of CD8 cells in the peripheral blood were significantly associated with improved response to induction chemotherapy and chemoradiation. There was also a trend toward better OS with higher CD8 levels.141 The same group then evaluated the relationship between T-cell subsets in the tumor microenvironment of pretreatment biopsies, HPV status, and outcome. There were no significant differences in the types of T-cell infiltrates noted in the biopsy specimens between HPV-positive and HPV-negative patients. There also was no correlation with T-cell subsets and EGFR expression or p53 mutation status and no significant differences in T-cell infiltrates by tumor stage or response to therapy. In the entire cohort, a lower CD4/CD8 ratio and higher mean sum of CD4 and CD8 infiltrates were associated with improved OS and remained significant after controlling for HPV status as well as EGFR expression or smoking status. Thus, while local immune responses may be predictive of better outcome, they may be independent of HPV status or other known independent predictors of improved outcome.142 The relationship between the immune system, HPV status, and outcome remains an interesting area of ongoing research.

HPV in Nonoropharyngeal Head and Neck Cancer

HPV has also been detected to varying degrees in other anatomic sites in patients with head and neck cancer including the oral cavity, hypopharynx, larynx, paranasal sinuses, and nasopharynx.23,143 For example, the frequency of HPV detection in patients with cancers of the oral cavity has ranged from less than 10% to upward of 90% in some studies.144-146 From a combined analysis of 60 studies with a total of 4195 patients, the weighted prevalence of HPV in oral cavity carcinomas was 20.2%, and the most common HPV type detected was HPV-16.143 While there are very limited data on paranasal sinus and nasopharyngeal cancer,
there have been some retrospective studies evaluating outcome in patients with HPV-positive oral cavity and laryngeal cancers. In oral cavity tumors, 2 studies showed only a trend toward improved survival,\textsuperscript{147,148} while another study found no association with HPV status and outcome.\textsuperscript{149} No differences in outcome have been seen in patients with HPV-negative laryngeal cancer.\textsuperscript{150-152} A meta-analysis of nonoropharyngeal cancers showed no difference in the risk of death between HPV-positive and HPV-negative tumors.\textsuperscript{153} While further study is needed in patients with nonoropharyngeal primary tumors, it is unclear whether HPV has an etiologic role, and at this time data do not suggest that HPV portends an improved prognosis at these other sites.

**Therapeutic Implications**

Given the different clinical behavior, treatment responses, and outcomes of HPV-positive oropharyngeal cancer, current and future studies will need to control for HPV status and there is also much interest in creating treatment regimens specifically for HPV-positive patients. Clinical trials are being developed to determine if there are regimens that can be used that maintain efficacy while decreasing the toxicity associated with multimodality treatment. RTOG 1016, a phase 3 multicenter clinical trial, is a noninferiority study comparing weekly cetuximab-based chemoradiation with standard-dose cisplatin-based chemoradiation in patients with HPV-positive oropharyngeal cancer, with OS as its primary outcome. A similar comparison is being done internationally in the De-ESCALaTE HPV trial (Determination of Epidermal growth factor receptor inhibitor [cetuximab] versus Standard Chemotherapy [cisplatin] early And Late Toxicity Events in Human Papillomavirus-positive oropharyngeal SCC), where the primary objective is to compare acute and late toxicity.

The use of immunotherapy designed to enhance immune-mediated action against the E6/E7-specific antigen is another area of continued investigation. E6 and E7 are ideal targets because they contribute to oncogenesis and tumor progression but are not found on normal cells. Harris et al demonstrated a reduction in tumor growth with a radiolabeled monoclonal antibody to HPV-16 E6 in a tumor model in nude mice.\textsuperscript{154} Vaccination with a DNA vaccine, in which DNA is injected intradermally via a gene gun, allowing for direct delivery of DNA to dendritic cells for the priming of antigen-specific T cells, has shown promise in a tumor model.\textsuperscript{155} Phase 1 clinical therapeutic vaccine trials are currently enrolling patients.

The specific targeting of viral carcinogenesis is also being studied. One area of interest is the disruption of E6 and E7 with its ubiquitin ligase partner. The HPV-16 E6 protein with E6-associated protein (E6AP) forms an ubiquitin ligase that leads to ubiquitin-mediated degradation of p53.\textsuperscript{39,82,83} Compounds have been found that in cell culture assays selectively inhibit the interaction between E6 and E6AP, interfering with HPV 16-E6–mediated degradation of p53.\textsuperscript{156} In cervical cancer cells, a lead compound, RAMB1, inhibited ubiquitin-mediated degradation and when combined with a lysosome inhibitor, chloroquine, synergistically triggered cell death.\textsuperscript{157} This work is still in its early stages but may provide the preliminary data needed to eventually develop clinically useful pharmacologic agents. Another target area under investigation is the ubiquitin/proteasome system needed for host cell proliferation. However, studies combining bortezomib, a ubiquitin/proteasome system inhibitor at the 20S catalytic site, with radiation have not shown positive results. A phase 1 trial that combined bortezomib and cetuximab with intensity-modulated radiotherapy in patients with either stage IV, residual, or recurrent disease was terminated early when 5 of 6 patients with a favorable prognosis progressed within 1 year. Four of the 5 patients who progressed were HPV positive. The authors suggested that the poorer-than-expected outcome may be related to an antagonistic effect of proteosome inhibitors and chemotherapy– or radiation-induced EGFR degradation and antiproliferative effects.\textsuperscript{158} An untoward protective effect against radiation in HNSCC by proteosome inhibition has also been suggested in a phase 1 trial in which radiation was combined with bortezomib alone in patients with recurrent HNSCC.\textsuperscript{159}

The increasing knowledge and investigation into therapies specifically for HPV-associated head and neck cancers presents an exciting time for the field. However, our current understanding of the improved prognosis in HPV-positive patients also highlights the need to investigate alternative therapies targeted at HPV-negative patients whose prognosis is poor.

**The Role of HPV in Anal Intraepithelial Neoplasia and Anal Cancer**

**Introduction**

Anal cancer commonly refers to cancers arising in the mucosal lining of the anal canal, with the majority being SCC. Anal cancers account for approximately 2.2% of gastrointestinal tract malignancies in the United States, with 6230 cases newly diagnosed each year.\textsuperscript{27} Historically, it was thought that conditions leading to chronic irritation or inflammation of the perianal area such as hemorrhoids, fissures, and fistulae contributed to the development of anal cancer.\textsuperscript{160} Over the past 2 decades, significant progress has been made in understanding the pathogenesis of these tumors. Several epidemiologic and clinical studies have identified a
causal association between HPV and anal cancer. Anogenital HPV infection is the most common sexually transmitted disease in the United States and has been implicated in the pathogenesis of anal intraepithelial neoplasia (AIN) and anal cancer, similar to what has been described in cervical intraepithelial neoplasia (CIN) and cervical cancer.\textsuperscript{161-163} New understanding of the pathogenesis and risk factors associated with the transmission of HPV can make AIN and anal cancers potentially preventable.

**Epidemiology**

**Prevalence of Anal Cancer**

Although anal cancer is rare, the incidence is increasing by approximately 2\% per year among both men and women in the general population.\textsuperscript{164} Recent data from the SEER database showed that the annual percentage change for cancer of the anus, anal canal, and anorectum was a 2.2\% increase (2.6\% for males and 2.0\% for females) between 1975 and 2009 (seer.cancer.gov/statfacts/html/anus.html). Historically, the annual rate of invasive anal cancer has been higher in women than men\textsuperscript{164-166}; however, the rate of in situ disease has increased more significantly over time in men compared with women, decreasing the gender-based difference in incidence.\textsuperscript{164} Among women, anal cancer incidence is higher in the oldest age group (aged older than 65 years) and in patients aged 50 years to 64 years. However, men had a slightly higher rate among patients aged 20 years to 49 years.\textsuperscript{164} As far as racial variation is concerned, anal cancer in females has been found to be highest among whites, whereas rates in males were highest among blacks.\textsuperscript{167}

**HPV Prevalence in Anal SCCs and AIN**

A global systematic literature review of HPV type distribution in anal cancer and AIN revealed that the crude HPV prevalence in invasive anal cancer is 71\%, and is 78\% specifically in SCC.\textsuperscript{168} Most patients (85\% of those who were HPV positive) were positive for HPV-16, and fewer (7\%) were positive for HPV-18. Other HPV subtypes that were isolated included HPV-33, -31, and -45. The prevalence of invasive anal cancer was highest in Europe (80\%) followed by North America (77\%), and was lowest in Asia (57\%). The prevalence of HPV for high-grade and low-grade anal SIL (ASIL) was 91\% and 88\%, respectively, with low-grade ASIL more commonly associated with HPV-6 and -11, which are considered low-risk subtypes.\textsuperscript{168} Epidemiologic studies and recent data from the Centers for Disease Control and Prevention (CDC) have shown that up to 93\% of anal SCCs are associated with HPV infection, predominantly oncogenic HPV-16 and HPV-18.\textsuperscript{167,169,170} Similarly, in a large population-based case-control study, 88\% of patients with anal cancer were found to be positive for HPV, 73\% of which was HPV-16, whereas no patients with rectal cancer in the control sample demonstrated HPV positivity.\textsuperscript{129}

**Risk Factors**

Risk factors for the development of HPV-associated AIN and anal cancers include anal HPV infection,\textsuperscript{161,171,172} women with previous HPV-related cervical and/or vulvar disease,\textsuperscript{173} a previous history of HPV-related cancers,\textsuperscript{174} high-risk sexual practices including receptive anal intercourse,\textsuperscript{162,163,175} HIV infection,\textsuperscript{176,177} and chronic immunosuppressive states as seen in solid-organ transplant recipients receiving immunosuppression.\textsuperscript{178-181}

**Anal HPV Infection**

There are several lines of evidence that have established anal HPV infection as a risk factor in the pathogenesis of AIN and anal cancer. The prevalence of AIN and anal cancer correlates well with observed patterns of HPV infection.\textsuperscript{169,182,183} In addition, HPV DNA has been detected in AIN and anal cancer tissues.\textsuperscript{161,167,169,170,184,185} Using the PCR technique, Palefsky et al identified 29 individual HPV subtypes and 10 grouped HPV subtypes from the anal canal of men who have sex with men (MSM), both with and without HIV infection.\textsuperscript{170} HPV-53, -58, -61, and -70 were commonly isolated in anal samples, but have been rarely reported in cervical samples. These types are thought to be related to intermediate-high-risk HPV types. The isolation of HPV-32, an oral HPV subtype, was seen in a small percentage of anal samples and points toward transmission by oral-anal intercourse. In another study, HPV-31/33/35 were detected in those who reported a history of anal condyloma within the past year.\textsuperscript{186} Simultaneous testing of the oral cavity once again revealed the presence of these HPV subtypes, indicating possible transmission through the oral-anal route.\textsuperscript{186} The range of HPV subtypes was similar in both HIV-positive and HIV-negative men. However, infection with multiple HPV types was found in 73\% of HIV-positive and 23\% of HIV-negative men.\textsuperscript{170}

HPV infection is also a common link that explains the statistically significant association between the development of anal cancers in women with a prior history of CIN\textsuperscript{187,188} or cervical/vulvar cancers,\textsuperscript{189} and also between anogenital cancers and second primary oropharyngeal cancers, suggesting a genital-anal as well as a genital-oral transmission.\textsuperscript{174}

In a case-control study of 29,648 women registered in the Danish Cancer Registry, Melby et al showed that individuals with anal cancer were 5.2 times as likely as those with colon cancer and 3.6 times as likely as those with stomach cancer to have had a prior diagnosis of CIN.\textsuperscript{189}
In addition, data from the US tumor registries obtained from the SEER database showed that women with a prior history of cervical cancer were at an increased risk of developing anal cancer (relative risk [RR], 4.6). The relative risks were also increased for cancers of the oral cavity (RR, 2.2), larynx (RR, 3.4), lung (RR, 3.0), and vagina (RR, 5.6), among others.190

In another population-based epidemiological study using the SEER database of men with an index oral cavity/pharyngeal or anogenital cancer, Sikora et al showed that the standardized incidence ratio (SIR) was elevated for both anogenital cancer following oral cavity/pharyngeal cancer (SIR, 1.9; 95% CI, 1.2-2.7) and oral cavity/pharyngeal cancer following a primary anogenital cancer (SIR, 3.0; 95% CI, 2.1-4.2), with an increase in SIR being most pronounced for tonsillar cancer following the diagnosis of anal cancer (SIR, 8.4; 95% CI, 2.7-19.6). There was no increased risk of oral cavity/pharyngeal or anogenital cancer following HPV-unrelated cancers or vice versa.174

**High-Risk Sexual Behavior as a Risk Factor for HPV-Associated Anal Cancers**

Changes in sexual practices, such as receptive anal intercourse and multiple sexual partners, are thought to be responsible, in part, for the rising incidence of anal cancer.191 Early epidemiologic surveys showed a rising incidence of anal cancer in MSM, implicating sexual practices in the development of anal cancer.192,193 An early population-based case-control study by Daling et al demonstrated that, in men, homosexual contact (RR, 50), genital warts (RR, 27), and gonorrhea (RR, 27) conferred a higher risk for the development of anal cancer and among women, the risk of anal cancer was strongly associated with genital warts (RR, 32.5), infection with herpes simplex virus type 2 (RR, 4.1), and *Chlamydia trachomatis* (RR, 2.3), thereby demonstrating a link between sexual activity and anal cancer.162 These findings were corroborated in another case-control study of 126 patients with anal cancer and 372 population controls in the San Francisco bay area that showed that the RR of anal cancer was elevated for men with a history of homosexual activity.166

Subsequent studies have helped to further establish the relationship between anal cancer and receptive anal intercourse.191,194-196 A large population-based case-control study using the national cancer registries in Denmark and Sweden was conducted by Frisch et al to evaluate the role of sexual practices and venereal diseases in the development of anal cancers, with a particular focus on the role of HPV.163 The study demonstrated that, in women, there was a statistically significant trend toward an association between the number of male sexual partners and the risk of anal cancer; the RR was highest for those with 10 or more sexual partners (RR, 4.5). In addition, women with anal warts, genital warts, gonorrhea, cervical neoplasia, or HIV or those who had intercourse with partners with a history of sexually transmitted diseases were found to have a significantly higher risk of developing anal cancers, with the RR being the highest for those with anal and genital warts (RR, 11.7 and RR, 4.6, respectively). The study also reported an increased risk of anal cancer among women who were aged younger than 30 years at the time of first receptive anal intercourse and had had 2 or more anal intercourse partners.

Among heterosexual men, a significantly increased risk of anal cancer was seen in those with 10 or more sexual partners or a history of anal warts, syphilis, or hepatitis on multivariate analysis. These findings demonstrated that sexual behavior is a key risk factor for the development of anal cancer in both men and women.

It is presumed that anal intercourse is one of main mechanisms by which HPV is introduced into the anal canal. However, in the large case-control study by Frisch et al detailed above, the majority of men and women with anal cancer in the study population did not practice anal intercourse.163 Therefore, other modes of anal transmission of HPV should be considered.

**HIV as an Associated Risk for HPV-Related Anal Cancer**

The incidence of HPV-associated anal infections, including preinvasive and invasive anal cancer, is higher in HIV-positive individuals, which cannot be explained on the basis of sexual practices alone.197,198 The relationship between HIV, HPV, and anal cancer has been studied extensively, particularly in MSM. Anal cancer incidence is higher in MSM than in heterosexual males.174,176,199,200 The risk is even greater among HIV-positive MSM.199,201,202

A meta-analysis of 53 studies, which included HIV-positive and HIV-negative MSM, found that HIV-positive MSM have a higher prevalence of high-risk anal HPV subtypes (74% vs 34%) and anal cancer (45.9 vs 5.1 per 100,000 men) than HIV-negative MSM.199 Similarly, in a large multicohort study including data from 13 cohorts (34,189 HIV-infected and 114,260 HIV-uninfected individuals) in the North American AIDS Cohort Collaboration on Research and Design (NA-ACCORD), a significantly higher incidence of AIN and anal cancer was found in HIV-infected MSM and heterosexual men and women compared with non-HIV–infected individuals.202 The incidence rates for HIV-positive MSM was over 80 times higher than in HIV-negative individuals.202 In addition, several population-based studies have shown an increasing incidence of anal cancer in the HAART era.201-205

In a prospective observational study using data from the US Military History Study, data collected from 4506 HIV-positive men showed a statistically significant 5-fold rise in
anal cancer incidence rates from 11 per 100,000 person-years in the pre-HAART era (1985-1995) to 55 per 100,000 person-years in the era of HAART (after 1995) and continued to rise to 128 per 100,000 person-years during 2006 through 2008, showing that antiretroviral therapy did not confer any protection against the development of anal cancers. Furthermore, patients with a history of HIV infection for over 15 years demonstrated a 12-fold higher rate of anal cancer compared with those with history of HIV infection of fewer than 5 years, suggesting that longer exposure to HPV is associated with an increased risk of anal cancer. As mentioned previously, Palefsky et al showed that infection with multiple HPV types was found in 73% of HIV-positive and 23% of HIV-negative men and was associated with lower baseline CD4 counts. This is important because it has been shown that, among HIV-positive men, persistent infection with one or more HPV subtypes increases the likelihood of developing high-grade AIN (HG-AIN). Immune status can greatly influence the detection of HPV. In a study from the Danish Cancer Registry, it was found that there is an inverse relationship between the detection of HPV and the CD4/CD8 ratio. Detection of HPV was noted to be 7.3% among subjects with CD4/CD8 ratios above 1.0 and increased to 35.3% among those with a ratio below 0.4 (P = .003), showing a strong correlation between HPV detection and immunosuppression.

Whether HIV has a direct role in the pathogenesis of anal epithelial abnormalities remains unclear. Immunosuppression caused by HIV/acquired immunodeficiency syndrome (AIDS) can facilitate the progression to HG-AIN, as demonstrated in a prospective cohort study of homosexual men that showed that HIV-induced immunosuppression was an independent risk factor for the development of HG-AIN after adjusting for HPV subtype, level of HPV detection, and number of positive HPV tests. In addition, there is an increased risk of progression from low-grade AIN to HG-AIN in HIV-positive men compared with HIV-negative men (RR, 2.4). Approximately 62% of HIV-positive and 36% of HIV-negative men with low-grade AIN at baseline progressed to HG-AIN during a 2-year follow-up period. This risk is increased in HIV-positive men with CD4 counts less than 200 (RR, 3.1).

**Chronic Immunosuppression as a Risk Factor for HPV-Associated Anal Cancers**

Impaired cell-mediated immunity is an important risk factor for the development of certain malignancies, including anogenital cancers. Anogenital malignancies seen in immunocompromised individuals are strongly associated with HPV positivity and usually demonstrate a different clinical course as they tend to occur at earlier ages, involve multiple sites, and are less responsive to standard therapy. A 100-fold increase in the incidence of anogenital cancers has been reported for renal transplant recipients compared with the general population. These included in situ cancer in one-third of patients and invasive cancers involving the anus, perianal skin, and external genitalia in both sexes. The presence of HPV-16 DNA has been reported in 47% of anal biopsies from renal allograft recipients compared with 12.4% of controls, making transplant recipients a high-risk group for the development of anal neoplasia. A 10-fold increase in the risk of anal cancer was also demonstrated in a national cohort study from Sweden of 5931 organ transplant recipients. Furthermore, in a meta-analysis of 7 studies of individuals with HIV/AIDS (n = 444,172) and 5 studies of transplant recipients (n = 31,977), a significantly increased incidence of all HPV-related malignancies was seen in both populations, suggesting that immunodeficiency was the common risk factor.

**Pathogenesis of HPV-Associated Anal Cancer**

The pathogenesis of HPV-associated AIN and SCC of the anal canal parallels that of CIN and cervical cancer. Anal cancer may be preceded by AIN or ASIL. AIN can be classified into low-grade AIN, which correlates with grade 1 AIN, and high-grade AIN, which correlates with grade 2 and 3 AIN. The natural history of AIN is not well understood. It is suspected that low-grade AIN may undergo spontaneous regression or progress to high-grade AIN, similar to what has been demonstrated in CIN. As described previously, the risk factors for this progression include infection with high-risk HPV subtypes, HIV, and low CD4 counts. High-grade AIN is felt to rarely regress and can progress to anal cancer. The progression rate from high-grade AIN to invasive cancer has been reported to be between 9% and 13% which is similar to the rate of transformation of CIN to cervical cancer. Similar to cervical and head and neck cancers, the oncogenesis of HPV-related anal cancers is mediated through E6 and E7 viral oncoproteins, causing inactivation of tumor suppressor genes as described in the head and neck cancer section. In addition, based on clinical and experimental data, a molecular biology model for the pathogenesis of anal SCC has been proposed by Gervaz et al. According to this model, while HPV infection is an initiating event, HPV integration into the host genome is the key step that ultimately leads to the transformation of AIN to invasive cancer. A consistent early event that occurs in anal carcinogenesis is loss of heterozygosity at 11q, which occurs independent of HIV status. In HIV-negative individuals, progression to invasive carcinoma is said to require additional losses of alleles in the region of tumor suppressor genes such as 5q (APC), 17p (p53), and 18q (DCC). In patients who are HIV positive, there is evidence of persistent HPV infection and more rapid progression of...
AIN to anal cancer. Therefore, it was proposed that, in HIV-positive patients, HPV-induced microsatellite instability, rather than chromosomal instability, is the mechanism through which there is progression to cancer.

**Clinical and Pathologic Features**

The squamocolumnar junction within the anal canal is anatomically and embryologically similar to that of the cervix. It is this region that is thought to be most susceptible to the oncogenic effects of HPV within the cervix. Similarly, histopathologic manifestations of HPV infection are most apparent at the anal transition zone, where the squamous epithelium of the anal canal transitions into the columnar epithelium of the rectum.

HPV infection of the anal canal can be latent, subclinical, or clinical. Latent HPV infection occurs in the presence of apparently normal tissue. Latent infection is thought to be the most common form of anogenital HPV infection and can only be detected by the presence of HPV DNA in the absence of macroscopic or histologic abnormalities in the surrounding tissue. Subclinical HPV infection such as AIN can only be detected with anal cytology or through high-resolution anoscopy and clinical HPV infection can be manifested in the form of anal condyloma or anal cancer. As mentioned previously, the natural history of HPV infection is complex and can follow a fluctuating course.

Anal cancers can be of various histologic subtypes; however, the association between HPV and anal cancer is specific to SCCs, which include basaloid, cloacogenic, and transitional cell types that account for 85% of all anal cancers in the United States. In a study comparing the properties of HPV-positive and HPV-negative anal carcinoma, it was found that HPV-positive tumors were more likely to occur in the anal canal than in the perianal region and to show a mixed squamous and basaloid appearance.

**Screening for AIN**

The value of screening for anal cancer precursor lesions in an at-risk population is not completely established. Screening strategies and the optimal management of abnormal test results remains a topic of debate. As such, there are no formal national guidelines to direct screening practices. However, some specialists recommend screening HIV-positive individuals based on indirect evidence from various epidemiologic studies. Screening involves the evaluation of anal exfoliative cytology obtained by a swab. If abnormalities are identified on cytology (atypical cells of undetermined significance, atypical squamous cells—cannot exclude high-grade squamous intraepithelial lesion, low-grade AIN, or high-grade AIN), anoscopy is warranted for further evaluation. The New York State Department of Health AIDS Institute recommends a baseline anal Papanicolaou (Pap) test be performed and repeated annually thereafter for MSM, patients with history of anogenital warts, and women with abnormal cervical or vulvar histology.

A systematic review evaluated the indirect evidence available in the literature regarding the usefulness of anal Pap test screening in HIV-positive patients. The indirect evidence supporting screening includes the high incidence of anal cancer among HIV-positive patients, particularly HIV-positive MSM, the availability of a screening modality with accuracy similar to that of the cervical Pap test, the presence of treatment modalities for high-grade dysplasia, the morbidity and mortality associated with anal cancer diagnosis, and studies showing the cost-effectiveness of the screening strategy. However, limitations include the lack of randomized trials demonstrating improved survival with screening, a lack of epidemiological data showing a decrease in anal cancer risk with screening as seen in cervical cancer, a lack of studies evaluating the various treatment strategies available for the treatment of AIN, and the uncertain natural history of AIN.

In a study assessing anal cytology versus histology and high-resolution anoscopy in 395 HIV-positive and HIV-negative individuals, it was found that anal cytology performed similarly to cervical cytology in a clinical setting. The sensitivity of anal cytology was dependent on HIV status (76% in HIV-positive vs 59% in HIV-negative individuals; \( P = .009 \)). Among HIV-positive individuals, the sensitivity was much higher at 90% when the CD4 cell count was 400 cells/μL or less. However, in another cross-sectional study of 401 HIV-positive men, anal cytology and HPV detection were found to have a high sensitivity but low specificity for detecting high grade AIN and it is proposed that high-resolution anoscopy is required for the optimal detection of high-grade anal dysplasia.

**Prognosis**

Since the great majority of anal cancers are associated with HPV infection, with only a small percentage of tumors being HPV negative, there are limited studies evaluating the prognostic relevance of HPV positivity in anal cancers. While the favorable prognosis of HPV-positive tumors compared with HPV-negative tumors is well established in patients with HNSCC, its prognostic significance in anal cancer remains to be conclusively established. Thus far no difference has been noted between HPV-positive and HPV-negative tumors with regard to prognosis. In a retrospective study, Yhim et al evaluated 47 patients with anal SCC who were treated with combined chemoradiotherapy for outcomes and determined the HPV status of their tumors, including HPV-16 and
to its small sample size and retrospective nature. Another Chinese study evaluated the association between HPV infection and esophageal cancer and related precursor lesions. A total of 702 patients underwent endoscopy with adequate cytologic and endoscopic examination. A multivalent HPV hybridization probe was used on all samples. HPV positivity was identified in 13% of subjects without squamous dysplasia, 8% with mild dysplasia, 7% with moderate dysplasia, 16% with severe dysplasia, and none of the subjects with invasive cancer. Another recent study pooled over 1500 serum samples from patients with esophageal cancer. Centralized multiplex serology was applied to each sample to detect circulating antibodies against 28 HPV antigens. They found nominal statistical significance associated with esophageal SCC and HPV-16 E6, HPV-33 L1, HPV-6 E6, HPV-6 L1, and HPV-11 L1. The authors concluded the serologic evidence was limited in showing an association between esophageal SCC and HPV in the populations studied. While the possibility of a small subset of patients having esophageal carcinoma based on the above data may exist, a definite conclusion on HPV being a risk factor in esophageal SCC oncogenesis cannot be made based on this study.

While some studies have suggested a possible association, current evidence does not implicate a causal role for HPV in a significant percentage of esophageal cancers, or reveal differences between patients with HPV-positive and HPV-negative disease.

The Role of HPV in Lung Cancer

Lung cancer is the leading cause of cancer death worldwide, causing over 1,350,000 deaths per year. While tobacco smoke is the primary culprit responsible for lung cancer, a distinct subset of lung cancer patients are considered never-smokers, or people who have smoked fewer than 100 cigarettes in their lifetime. Globally, lung cancer in never-smokers makes up an estimated 15% to 20% of cases in men and over 50% in women. There are major geographic differences in Asia, where 61% of East Asian women and 83% of South Asian women with lung cancer are nonsmokers. Contrast in one analysis with the United States, an estimated 19% of women with lung cancer were never-smokers. A viral etiology has been theorized as playing a role in the development of lung cancer in never-smokers, with a
Several more recent studies have suggested HPV as a risk factor and that it synergistically acts with tobacco to cause lung cancer. While their association is interesting, we currently do not have clear indications of HPV as a direct cause of lung cancer.240

A recent extensive meta-analysis suggested the wide variability in HPV detection rates in lung cancer was more likely due to the geographic location of the study and histologic subtype of lung cancer, as opposed to the technique used for HPV detection. A conclusion on different etiologies based on geography could not be made by regression meta-analysis, however. The possible pathogenesis of lung cancer among smokers, nonsmokers, males, and females could not be undertaken given the failure to control for smoking history and gender. This extensive analysis highlighted the need for prospective cohort studies to better evaluate the role of HPV oncogenesis in lung cancer.241 Currently it is not clear whether HPV infection has a direct role in the oncogenesis of lung cancer.

### HPV Vaccines

To date, 2 vaccines have been developed and approved for use against HPV. Gardasil is a quadrivalent vaccine that targets HPV-6, -11, -16, and -18. The bivalent vaccine Cervarix targets HPV-16 and -18. Randomized clinical trials evaluating Gardasil have shown efficacy in preventing HPV-related cervical intraepithelial lesions of grade 2 or worse in 98% to 100% of HPV-naive treated females. The quadrivalent vaccine efficacy, however, was much lower at about 34% to 44% when sexually active females were included at analysis.242 A randomized clinical trial evaluating Cervarix has shown efficacy in preventing HPV-related cervical intraepithelial lesions of grade 2 or worse in 93% of HPV-naive treated females. Like the quadrivalent vaccine, the bivalent vaccine’s efficacy was much lower at 30% when including sexually active females.243

The timing of immunization is important to maximize the efficacy of the vaccine, with the data showing vaccination before sexual debut as the most effective at preventing HPV-related disease. Of note, the efficacy of either HPV vaccine in preventing AIN has not yet been studied in

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**TABLE 3. ACIP-Recommended Age Groups, Schedule, Dosages, and Route of Administration for HPV Vaccines**

| TARGET POPULATION | ACIP RECOMMENDATION | VACCINE | SCHEDULE | DOSAGE, ROUTE |
|-------------------|---------------------|---------|----------|---------------|
| Females           | Routine at age 11 or 12 y and catch-up through age 26 y | Quadrivalent or bivalent vaccine | 0, 1 to 2, and 6 mo | 0.5 μL, intramuscular injection |
| Males             | May be given to males aged 9 to 26 y | Quadrivalent | 0, 1 to 2, and 6 mo | 0.5 μL, intramuscular injection |

ACIP indicates Advisory Committee on Immunization Practices; HPV, human papillomavirus.

Table reprinted from: Hariri S, Dunne E, Saraiya M, Unger E, Markowitz L. Human papillomavirus. In: Manual for the Surveillance of Vaccine-Preventable Diseases. 5th ed. Atlanta, GA: Centers for Disease Control and Prevention; 2011:8.20

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particular focus on HPV. Cheng et al hypothesized that HPV-16 and HPV-18 may be associated with the development of lung cancer based on the high prevalence of p53-negative immunostains in lung tumors in females compared with lung tumors in men. This study examined 141 patients with lung cancer and 60 noncancer controls. Tissue samples were analyzed for HPV-16 and HPV-18 by PCR and ISH. Approximately 55% of the 141 patients with lung cancer had HPV-16 or HPV-18 compared with about 27% of the 60 noncancer controls. The specimens found to be positive for HPV by ISH were also uniformly located in lung tumor cells, but not in the adjacent nontumor cells. Further stratification by gender, age, and smoking status suggested a high prevalence of HPV-16 and HPV-18 in nonsmoking female lung cancer patients aged older than 60 years.228 However, in a series including white patients, no increased incidence of HPV positivity or a clear association with HPV and lung cancer was noted. In this study, Bohlmeyer et al analyzed 34 lung SCC tissue specimens for HPV by PCR and ISH. Two were positive by PCR for HPV-18, one of which was from a 45-year-old female smoker and the other from a 65-year-old male smoker. ISH failed to detect HPV-6, -11, -16, -18, -31, -33, or -35.230 Koshiol et al evaluated 399 patients newly diagnosed with lung cancer. About 50% of the patients were current smokers and about 20% were women. Two patients were positive for HPV-16 at low copy number by type-specific primers for HPV-16 and HPV-18; however, with additional testing, both were negative for high-risk HPV. Broad-range HPV-type testing was performed on all the tumor specimens and was negative.230 At present, the data remain conflicting as several other studies have supported the direct role of HPV in lung cancer,231-234 while others have refuted its causality.230,235-238

Most recently a study examining p16 expression in lung SCCs revealed that about one-third were positive for p16. However, the samples were negative for both high-risk and low-risk HPV subtypes by ISH, indicating p16 was not a reliable indicator for HPV infection in lung SCC like it is in cervical and oropharyngeal SCCs.239

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**HPV in Nongenital Cancers**
females, but a benefit is anticipated as the majority of anal cancers are related to HPV-16 and HPV-18. Neither vaccine treats or accelerates the clearance of preexisting vaccine-type HPV infections or related disease. For females, the American Cancer Society recommends the HPV vaccine be offered to patients aged 11 years to 18 years, with the earliest immunization allowed at age 9 years.244 For males, the CDC Advisory Committee on Immunization Practices recommends use of the quadrivalent vaccine for those ages 11 years or 12 years, but the vaccine can be administered as early as age 9 years (Table 3).245 A catch-up vaccination is also recommended for males aged 13 years to 21 years who have not been vaccinated or had an incomplete 3-dose series. Permissive use of the quadrivalent vaccine in males is also supported for those aged 22 years to 26 years who have not been vaccinated.245

New data suggest that the current recommended dosing schedule of 3 injections may not be needed to confer immunity to HPV-16 and HPV-18. Data from the National Cancer Institute-sponsored Costa Rica Vaccine Trial revealed that many of the 7153 women missed 1 or more of 3 prescribed doses of a randomly assigned HPV-16/18 vaccine versus controls. Vaccine efficacy was evaluated by HPV DNA testing for newly detected HPV-16 or HPV-18 infections that persisted at least 1 year. The estimated vaccine efficacy against infection with HPV-16 and HPV-18 was similar regardless of whether the woman received 1, 2, or all 3 doses.246 However, it is still not known whether the 3-dose regimen confers a longer duration of infection at this time, or whether these data can be extrapolated to Gardasil.

A nested analysis of about 4200 women from the Costa Rica trial indicated that vaccine efficacy against anal HPV-16 and HPV-18 measured one time at 4 years after vaccination was about 84%. This was comparable to the vaccine efficacy for cervical HPV-16 and HPV-18 of about 88%.247 While this suggests strong protection against anal HPV, further study and evaluation of these women over time will be required to evaluate the efficacious prevention of anal cancer.

In the United States, HPV vaccination has been available since 2006. The administration of the vaccination occurs mainly through primary care providers. A publicly funded program, the CDC’s Vaccines for Children, provides the vaccine at no charge to children aged 18 years or younger who are uninsured or who meet eligibility criteria. The CDC tracks vaccination coverage among adolescents aged 13 years to 17 years through the National Immunization Survey-Teen (NIS-Teen). In the United States, HPV vaccination coverage increased from 44.3% to 48.7% between 2009 and 2010 among females who received 1 or more doses of the vaccine, and from 26.7% to 32.0% for those with 3 or more doses of the vaccine. At least 24 weeks must elapse between the first and third doses of the HPV vaccine to complete the series. Among females who initiated the HPV series, 94.3% met the minimum period needed for completion. Of these, 69.6% received 3 or more doses. Among adolescent males, 1.4% received 1 or more doses of the HPV vaccine.248 In Baltimore, the city where we practice, a relatively small percentage of eligible women have elected to receive the vaccine as a primary prevention for cervical cancer. A significant percentage of women who initiate vaccination do not complete the recommended 3-dose regimen. Young adult women are the least likely to complete the 3-dose regimen, as are women in minority groups.249

To date, the US Food and Drug Administration has approved Gardasil in males for the prevention of genital warts and AIN.245

In Canada, HPV vaccination has been available since 2006. Since 2007, public health agencies have delivered school-based HPV vaccination programs, and all provinces and territories had publicly funded programs in place by 2009. HPV vaccination has been offered free of charge to girls aged 9 years to 15 years. Series coverage varied nationally among jurisdictions that reported, with a range of 80% to 85% reported in the Atlantic (eastern) provinces to 51% in Ontario after the first year of the program. There are no publicly funded programs in Canada to vaccinate boys, but the vaccine is available to boys and men who wish to pay for it.250

Conclusions

Worldwide, HPV is felt to be responsible for more than 550,000 new cases of human cancer annually.22,251 A clear association has already been established for the role of HPV in cervical oncogenesis, and it is implicated in vaginal, vulvar, and penile cancers.252 It has also become apparent that the effect of HPV is more far-reaching than originally thought, with a strong link between infection and the development of anal and oropharyngeal cancers, with the incidence of the latter increasing rapidly. There is continued ongoing investigation into its role in the development of esophageal and lung cancers.

Although important strides have been made in our understanding of HPV in nongenital cancers, significant questions still remain. Future basic science, epidemiologic, and clinical research will hopefully continue to improve our understanding of the natural history of the virus, its oncogenesis, and the potential clinical implications. Our current knowledge base serves as a platform for exciting new research into screening as well as targeted therapies that will hopefully improve outcomes in those with HPV-associated malignancies. While we hope for improved therapeutic modalities in the future, perhaps the greatest potential lies in the ability to prevent the development of these malignancies through widespread vaccination. Like other diseases of the early 20th century that ravaged man before vaccination, HPV has the potential to be eradicated, and with it, HPV-related malignancy.
References

1. Klingelhütz AJ, Roman A. Cellular transformation by human papillomaviruses: lessons learned by comparing high-risk and low-risk viruses. Virology. 2012;424:77-98.

2. Baseman JB, Koutsy LA. The epidemiology of human papillomavirus infections. J Clin Virol. 2005;32(suppl 1):S16-S24.

3. zur Hausen H. Papillomaviruses in the causation of human cancers—a brief historical account. Virology. 2009;384:260-265.

4. Schiffman M, Wentzensen N, Wacholder S, Kinney W, Gage JC, Castle PE. Human papillomavirus testing in the prevention of cervical cancer. J Natl Cancer Inst. 2011;103:368-383.

5. Gasparini R, Panatto D. Cervical cancer: from Hippocrates through Rigoni-Stern to zur Hausen. Vaccine. 2009;27(suppl 1):A4-A5.

6. Cifio G. Innesto positivo con fitratro di verruca volgare. Giorn Ital Mal Venereol. 1907;48:12-17.

7. Della Torre G, Ploitti S, de Palo G, Rilke F. Viral particles in cervical condylomatosus lesions. Tumori. 1978;64:549-553.

8. Laverty CR, Booth N, Hills E, Cossart Y, zur Hausen H. Detection of viral DNA from human genital warts (Condylomata acuminata). EMBO J. 1980;25:605-609.

9. Durst M, Gissmann L, Ikenberg H, zur Hausen H. A papillomavirus DNA from a cervical carcinoma and its prevalence in cancer biopsy samples from different geographic regions. Proc Natl Acad Sci U S A. 1983;80:3812-3815.

10. Boshart M, Gissmann L, Ikenberg H, Kleinheinz A, Scheurlein W, zur Hausen H. A new type of papillomavirus DNA, its papillomavirus biotypes and in cell lines derived from cervical cancer. EMBO J. 1984;3:1151-1157.

11. Schwarz E, Freeke U, Gissmann L, et al. Structure and transcription of human papillomavirus sequences in human cervical carcinoma cells. Nature. 1985;314:111-114.

12. Yee C, Krishnan-Hewlett I, Baker CC, Schlegel R, Howley PM. Presence and expression of human papillomavirus sequences in human cervical carcinoma cell lines. Am J Pathol. 1985;119:361-366.

13. Durst M, Dzairleva-Petrusevskua RT, Boukamp P, Fusenig NE, Gissmann L. Molecular and cytogenetic analysis of immortalized human primary keratinocytes obtained after transfection with human papillomavirus type 16 DNA. Oncogene. 1987;1:251-256.

14. Pirisil L, Yasumoto S, Feller M, Doniger J, DiPaolo JA. Transformation of human fibroblasts and keratinocytes with human papillomavirus type 16 DNA. J Virol. 1987;61:1061-1066.

15. Dyson N, Howley PM, Munger K, Harlow E. The human papillomavirus type 16 E7 oncoprotein is able to bind to the retinoblastoma gene product. Science. 1992;243:934-937.

16. Wernes BA, Levine AJ, Howley PM. Association of human papillomavirus types 16 and 18 E6 proteins with p53. Science. 1990;248:76-79.

17. von Knebel Doeberitz M, Rittmüller C, zur Hausen H, Durst M. Inhibition of tumorigenicity of cervical cancer cells in nude mice by HPV E6-E7 anti-sense RNA. Int J Cancer. 1992;51:831-844.

18. Butel JS. Viral carcinogenesis: revelation of molecular mechanisms and etiology of human disease. Cancer. Carcinogenicity of human papillomavirus. 1989;75:403-441.

19. Hoffmann R, Hirt B, Bechtold V, Beard P, Raj K. Different modes of human papillomavirus DNA replication during maintenance. J Virol. 2006;80:4431-4439.

20. Kadaja M, Isok-Paas H, Laos T, Ustav E, Ustav M. Mechanism of genomic instability in cells infected with the high-risk human papillomaviruses. PLoS Pathog. 2009;5:e1000397.

21. Alazawi W, Pett M, Arch B, et al. Changes in cervical keratinocyte gene expression associated with integration of human papillomavirus 16. Cancer Res. 2002;62:6959-6965.

22. Kraus R, Woerner SM, Ridder R, et al. Detection of high-risk cervical intraepithelial neoplasia and cervical cancer by amplification of transcripts derived from integrated papillomavirus oncogenes. Cancer Res. 1999;59:6132-6136.

23. Scheffer M, Huibregtse JM, Vierstra RD, Howley PM. The HPV-16 E6 and E6-AP complex functions as a ubiquitin-protein ligase in the ubiquitination of p53. Cell. 1993;75:495-505.

24. van Knebel Doeberitz M, Rittmüller C, zur Hausen H. Papillomaviruses in the causation of human cancers—a brief historical account. Virology. 2009;384:260-265.

25. Durst M, Gissmann L, Ikenberg H, zur Hausen H. A papillomavirus DNA from a cervical carcinoma and its prevalence in cancer biopsy samples from different geographic regions. Proc Natl Acad Sci U S A. 1983;80:3812-3815.

26. Boshart M, Gissmann L, Ikenberg H, Kleinheinz A, Scheurlein W, zur Hausen H. A new type of papillomavirus DNA, its papillomavirus biotypes and in cell lines derived from cervical cancer. EMBO J. 1984;3:1151-1157.

27. Schwarz E, Freeke U, Gissmann L, et al. Structure and transcription of human papillomavirus sequences in human cervical carcinoma cells. Nature. 1985;314:111-114.

28. Yee C, Krishnan-Hewlett I, Baker CC, Schlegel R, Howley PM. Presence and expression of human papillomavirus sequences in human cervical carcinoma cell lines. Am J Pathol. 1985;119:361-366.

29. Durst M, Dzairleva-Petrusevskua RT, Boukamp P, Fusenig NE, Gissmann L. Molecular and cytogenetic analysis of immortalized human primary keratinocytes obtained after transfection with human papillomavirus type 16 DNA. Oncogene. 1987;1:251-256.

30. Piri sil L, Yasumoto S, Feller M, Doniger J, DiPaolo JA. Transformation of human fibroblasts and keratinocytes with human papillomavirus type 16 DNA. J Virol. 1987;61:1061-1066.

31. Dyson N, Howley PM, Munger K, Harlow E. The human papillomavirus type 16 E7 oncoprotein is able to bind to the retinoblastoma gene product. Science. 1992;243:934-937.

32. Wernes BA, Levine AJ, Howley PM. Association of human papillomavirus types 16 and 18 E6 proteins with p53. Science. 1990;248:76-79.

33. von Knebel Doeberitz M, Rittmüller C, zur Hausen H, Durst M. Inhibition of tumorigenicity of cervical cancer cells in nude mice by HPV E6-E7 anti-sense RNA. Int J Cancer. 1992;51:831-844.

34. Butel JS. Viral carcinogenesis: revelation of molecular mechanisms and etiology of human disease. Cancer. Carcinogenicity of human papillomavirus. 1989;75:403-441.

35. Hoffmann R, Hirt B, Bechtold V, Beard P, Raj K. Different modes of human papillomavirus DNA replication during maintenance. J Virol. 2006;80:4431-4439.

36. Kadaja M, Isok-Paas H, Laos T, Ustav E, Ustav M. Mechanism of genomic instability in cells infected with the high-risk human papillomaviruses. PLoS Pathog. 2009;5:e1000397.

37. Alazawi W, Pett M, Arch B, et al. Changes in cervical keratinocyte gene expression associated with integration of human papillomavirus 16. Cancer Res. 2002;62:6959-6965.

38. Kraus R, Woerner SM, Ridder R, et al. Detection of high-risk cervical intraepithelial neoplasia and cervical cancer by amplification of transcripts derived from integrated papillomavirus oncogenes. Cancer Res. 1999;59:6132-6136.

39. Scheffer M, Huibregtse JM, Vierstra RD, Howley PM. The HPV-16 E6 and E6-AP complex functions as a ubiquitin-protein ligase in the ubiquitination of p53. Cell. 1993;75:495-505.

40. van Knebel Doeberitz M, Rittmüller C, zur Hausen H. Papillomaviruses in the causation of human cancers—a brief historical account. Virology. 2009;384:260-265.
51. Munoz N, Bosch FX, de Sanjose S, et al; International Agency for Research on Cancer Multicenter Cervical Cancer Study Group. Epidemiologic classification of human papillomavirus types associated with cervical cancer. N Engl J Med. 2003;348:518-527.

52. Chaturvedi AK, Engels EA, Anderson WF, Gillison ML. Incidence trends for human papillomavirus-related and -unrelated oropharyngeal squamous cell carcinomas in the United States. J Clin Oncol. 2008;26:612-619.

53. Byrson AM, Peters ES, Coughlin SS, et al. Breast cancer prevalence and human papillomavirus-associated cancers of the oropharynx and oral cavity in the US, 1998-2003. Cancer. 2008;113(suppl 10):2901-2909.

54. Frisch M, Hjalgrim H, Jaeger AB, Biggar RJ. The role of a cellular protein human papillomavirus type 16 or 18. EMBO J. 1991;10:4129-4135.

55. Shiboski CH, Schmidt BL, Jordan RC. Changing patterns of tonsillar squamous cell carcinoma in the United States: 1999 through 2008. CA Cancer J Clin. 2010;60:260-263.

56. Auluck A, Hislop G, Bajdik C, Poh C, Gillison ML, D’Souza G, Westra W, et al. Human papillomavirus infection. A meta-analysis. 1985-2010. Oral Oncol. 2011;47:1048-1054.

57. Scheffner M, Huibregtse JM, Howley PM. Identification of a human ubiquitin-conjugating enzyme that mediates the E6-AP-dependent ubiquitination of p53. Proc Natl Acad Sci U S A. 1994;91:8797-8801.

58. Huibregtse JM, Scheffner M, Howley PM. A cellular protein mediates association of p53 with the E6 oncprotein of human papillomavirus types 16 or 18. EMBO J. 1991;10:4129-4135.

59. Thomas M, Pirone D, Banks L. The role of the E6-p53 interaction in the molecular pathogenesis of HPV. Oncogene. 1999;18:7690-7700.

60. Li Y, Nichols MA, Shay JW, Xiong Y. Transcriptional repression of the D-type cyclin-dependent kinase inhibitor p16 by the retinoblastoma susceptibility gene product pRb. Cancer Res. 1994;54:6078-6082.

61. Ramplas T, Sasaki C, Weinberger P, Pysri A. E6 and e7 gene silencing and transformed phenotype of human papillomavirus 16-positive oropharyngeal cancer cells. J Natl Cancer Inst. 2009;101:412-423.

62. Halkamp HC, Speel EJ, Haesevoets A, et al. A subset of head and neck squamous cell carcinomas exhibits integration of HPV-16/18 DNA, and overexpression of p16INK4A and p53 in the absence of mutations in p53 exons 5-8. Int J Cancer. 2003;107:394-400.

63. Pysri A, Gourin PC, Vermerken JB. Human papillomavirus-related head and neck tumors: clinical and research implication. Curr Opin Oncol. 2009;21:201-205.

64. Boyle JO, Hakim J, Koch W, et al. The incidence of p53 mutations increases with progression of head and neck cancer. Cancer Res. 1993;53:4477-4480.

65. Reed AL, Caliano J, Cairns P, et al. High frequency of p16 (CDKN2/MTS-1/INK4A) inactivation in head and neck squamous cell carcinomas. Cancer Res. 1996;56:3630-3633.

66. Mellin H, Dahlgren L, Munck-Wikland E, et al. Human papillomavirus type 16 is episomal and a high viral load may be correlated to better prognosis in tonsillar cancer. Int J Cancer. 2005;115:152-158.

67. Koskinen WJ, Chen RW, Leivo I, et al. Prevalence and physical status of human papillomavirus in squamous cell carcinomas of the head and neck. Int J Cancer. 2003;107:401-406.

68. Liu B, Lu Z, Wang P, Basang Z, Rao X. Prevalence of high-risk human papillomavirus types (HPV-16, HPV-18) and their physical status in primary laryngeal squamous cell carcinoma. Neoplasma. 2010;57:594-600.

69. Badaracco G, Rizzo C, Mafera B, et al. Molecular analyses and prognostic relevance of HPV in head and neck tumours. Oncol Rep. 2007;17:931-939.

70. Van Tine BA, Dao LD, Wu SY, et al. Human papillomavirus (HPV) origin-binding protein associates with mitotic spindles to enable viral DNA partitioning. Proc Natl Acad Sci U S A. 2004;101:4030-4035.

71. Wietl T, Schwarz E, Enders C, Flechtenmacher C, Bosch FX. Involvement of intact HPV16 E6/E7 gene expression in head and neck cancers with unrelated p53 status and perturbed pRb cell cycle control. Oncogene. 2002;21:1510-1517.
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97. van Houten VM, Snijders PJ, van den Brekel MW, et al. Biological evidence that human papillomaviruses are etiologically involved in a subgroup of head and neck squamous cell carcinomas. *Int J Cancer*. 2001;93:232-235.

98. Balz V, Scheckenbach K, Gotte K, Bockmuhl U, Petersen I, Bier H. Is the p53 inactivation frequency in squamous cell carcinomas of the head and neck underestimated? Analysis of p53 exons 2-11 and human papillomavirus 16/18 E6 transcripts in 123 unselected tumor specimens. *Cancer Res*. 2003;63:1188-1191.

99. Begum S, Gillison ML, Ansari-Lari MA, Shah K, Westra WH. Detection of human papillomavirus in cervical lymph nodes: a highly effective strategy for localizing site of tumor origin. *Cancer Res*. 2003;63:6469-6475.

100. Marur S, D’Souza G, Westra WH, Forastiere AA. HPV-associated head and neck cancer: a virus-related cancer epidemic. *Lancet Oncol*. 2010;11:791-798.

101. Smeets SJ, Hesselink AT, Speel EJ, et al. Improved survival of patients with human papillomavirus-positive head and neck squamous cell carcinoma in a prospective clinical trial. *J Natl Cancer Inst*. 2008;100:261-269.

102. Worden FP, Kumar B, Lee JS, et al. Chemoselection as a strategy for organ preservation in advanced oropharyngeal cancer: response and survival: a phase II/III study using a novel agent associated with HPV16 copy number. *J Clin Oncol*. 2008;26:3138-3146.

103. Begum S, Cao D, Gillison M, Zahurak M, et al. Three synchronous HPV-associated head and neck cancer patients: biological and clinical implications. *Clin Cancer Res*. 2005;11:5694-5699.

104. Brennan JA, Mao L, Hruban RH, et al. Molecular characterization of histopathological staging in squamous-cell carcinoma of the head and neck: long-term results of the TAX 324 randomised phase 3 trial. *Lancet Oncol*. 2011;12:153-159.
papillomavirus-related head and neck cancer. *Arch Otolaryngol Head Neck Surg.* 2009;135:1137-1146.

141. Wansom D, Light E, Worden F, et al. Correlation of cellular immunity with human papillomavirus 16 status and outcome in patients with advanced oropharyngeal cancer. *Arch Otolaryngol Head Neck Surg.* 2010;136:1267-1273.

142. Wansom D, Light E, Thomas D, et al; UM Head Neck SPORE Program. Infiltrating lymphocytes and human papillomavirus-16–associated oropharyngeal cancer. *Laryngoscope.* 2012;122:121-127.

143. Isayeva T, Li Y, Maswah D, Brandwein-Gensler M. Human papillomavirus in non-oropharyngeal head and neck cancers: a systematic literature review. *Head Neck Pathol.* 2012;6(suppl 1):S104-S120.

144. Braakhuis BJ, Snijders PJ, Keune WJ, et al. High-risk human papillomavirus in oral squamous cell carcinoma of young patients. *Int J Cancer.* 2012;130:1726-1732.

145. Elango KJ, Suresh A, Erode EM, et al. Role of human papilloma virus in oral tongue squamous cell carcinoma. *Asian Pac J Cancer Prev.* 2011;12:889-896.

146. Bouda M, Gorgoulis VG, Karstrinakis NG, et al. "High risk" HPV types are frequently detected in potentially malignant and malignant oral lesions, but not in normal oral mucosa. *Mod Pathol.* 2000;13:644-653.

147. Kaminagakura E, Villa LL, Androoli MA, et al. High-risk human papillomavirus in oral squamous cell carcinoma: clinical correlates and 5-year survival. *Br J Oral Maxillofac Surg.* 2007;45:116-122.

148. Smith EM, Rubenstein LM, Haugen TH, et al. Human papillomavirus-16 status and tumor progression associated with enhanced EGFR signaling with bortezomib, and taxotere for head and neck cancer. *Clin Cancer Res.* 2011;17:5575-5564.

149. Morris JC, Citron DE, Nottingham L, Rudy SF, et al. Phase I study of proteasome inhibitor bortezomib concurrent with re-irradiation therapy (re-RT) for recurrent squamous cell carcinoma of the head and neck (SCCHN) [abstract]. *J Clin Oncol.* 2010; 28:155. Abstract 2603.

150. Buckwalter JA, Jurayj MN. Relationship of tobacco, and alcohol: a case for multi-factor disease. *JAMA.* 2010;303:1336-1342.

151. Stephen JK, Chen KM, Havard S, Harris RD, et al. Age-related prevalence of anal high-grade squamous intraepithelial lesions among HIV-positive and HIV-negative homosexual and bisexual men. *AIDS.* 1998;12:495-503.

152. Frisch M, Biggar RJ, Engels EA, Goedert JJ; AIDs-Cancer Match Registry Study Group. Association of cancer with AIDs-related immunosuppression in adults. *JAMA.* 2001:285:1736-1745.

153. Penn I. Cancers of the anogenital region in renal transplant recipients. Analysis of 65 cases. *Cancer.* 1986;58:611-616.

154. Ogubiyi OA, Schofield JH, Raftery AT, et al. Prevalence of anal human papillomavirus infection and intraepithelial neoplasia in renal allograft recipients. *Br J Surg.* 1994;81:365-367.

155. Grulich AE, van Leeuwen MT, Falster MO, Vajdic CM. Incidence of cancers in people with HIV/AIDS compared with immunosuppressed transplant recipients: a meta-analysis. *Lancet.* 2007;370:59-67.

156. Palefsky JM, Schofield JH. Early detection and treatment of anal Neoplasia in renal allograft recipients. *Cancer.* 1989;81:1726-1731.

157. Smith EM, Rubenstein LM, Haugen TH, et al. Human papillomavirus type distribution in anal cancer and anal intraepithelial lesions. *Int J Cancer.* 2009;124:2375-2383.

158. Kiviat NB, Critchlow CW, Holmes KK, et al. Detection of human papillomavirus DNA and the polymerase chain reaction. *Retrovirology.* 1994;1:49-59.

159. Holmes F, Borek D, Owen-Kummer M, et al. 'High risk' HPV types are frequent among HIV-infected women. *Gastroenterology.* 1988;95:107-111.

160. Holly EA, Whittemore AS, Aston DA, Ahn DK, Greenspan JS. Detection of human papillomavirus DNA in anal intraepithelial neoplasia and anal cancer. *Cancer Res.* 1991;51:1014-1019.

161. Palefsky JM, Holly EA, Gonzales J, Berline J, Ahn DK, Greenspan JS. Human papillomavirus DNA in normal oral mucosa. *Int J Cancer.* 1987;314:1350-1358.

162. Johnson LG, Madeleine MM, Newcomer LM, Schwartz SM, Daling JR. Anal cancer incidence and survival: the surveillance, epidemiology, and end results experience, 1973-2000. *Cancer.* 2004;101:281-288.

163. Holmes F, Borek D, Owen-Kummer M, et al. Anal intraepithelial lesions among HIV-positive and HIV-negative homosexual and bisexual men. *AIDS.* 1998;12:495-503.

164. Palefsky JM, Holly EA, Raftery AT, et al. Prevalence of anal human papillomavirus infection and intraepithelial neoplasia in renal allograft recipients. *Br J Surg.* 1994;81:365-367.

165. Frisch M, Glimelius B, van den Brule AJ, et al. Analytically transmitted infection as a cause of anal cancer. *N Engl J Med.* 1987;317:975-977.

166. Daling JR, Weiss NS, Hislop TG, et al. Sexual practices, sexually transmitted diseases, and the incidence of anal cancer. *N Engl J Med.* 1997;337:1350-1358.

167. Frisch M, Glimelius B, van den Brule AJ, et al. Sexually transmitted infection as a cause of anal cancer. *N Engl J Med.* 1997;337:1350-1358.

168. Holmes F, Borek D, Owen-Kummer M, et al. Anal intraepithelial lesions among HIV-positive and HIV-negative homosexual and bisexual men. *AIDS.* 1998;12:495-503.

169. Palefsky JM, Holly EA, Gonzales J, et al. Human papillomavirus infection and anal carcinoma in women. *AIDS.* 1998;12:495-503.

170. Ogunbiyi OA, Scholefield JH, Raftery AT, et al. Prevalence of anal human papillomavirus infection and intraepithelial neoplasia in renal allograft recipients. *Br J Surg.* 1994;81:365-367.

171. Ching-Hong PV, Wittinghoff E, Cranston RD, et al. Age-related prevalence of anal cancer precursors in intrarectal and anal mucosa in a cohort study in Sweden. *Br J Cancer.* 2003;89:1221-1227.

172. Adami J, Gabel H, Lindelof B, et al. Cancer risk following organ transplantation: a critical review of a cohort study in Sweden. *Br J Cancer.* 2003;89:1221-1227.

173. Grulich AE, van Leeuwen MT, Falster MO, Vajdic CM. Incidence of cancers in people with HIV/AIDS compared with immunosuppressed transplant recipients: a meta-analysis. *Lancet.* 2007;370:59-67.

174. Palefsky JM, Holly EA, Hogeboom CJ, et al. Age-related prevalence of anal cancer precursors in intrarectal and anal mucosa in a cohort study in Sweden. *Br J Cancer.* 2003;89:1221-1227.

175. Beckmann AM, Daling JR, Sherman KJ, et al. Human papillomavirus infection and anal cancer. *Int J Cancer.* 1989;43:1042-1049.

176. Zaki SR, Judd R, Coffield LM, Greer P, et al. Analysis of inhibitors to papillomavirus type 16 E6 protein based on three-dimensional structures of interacting proteins. *Antiviral Res.* 1995;27:165-173.

177. Ancooosh RK, Khan SR, Subiblnivong T, et al. Stressing the ubiquitin-proteasome system without 20S proteolytic inhibition selectively kills cervical cancer cells. *PLoS One.* 2011;6:e22115.

178. Argiris A, Duong AG, Kumar M, et al. Early tumor progression associated with enhanced EGFR signaling with bortezomib, and taxotere for head and neck cancer. *Clin Cancer Res.* 2011;17:5575-5564.

179. Morris JC, Citron DE, Nottingham L, Rudy SF, et al. Phase I study of proteasome inhibitor bortezomib concurrent with re-irradiation therapy (re-RT) for recurrent squamous cell carcinoma of the head and neck (SCCHN) [abstract]. *J Clin Oncol.* 2010; 28:155. Abstract 2603.

180. Buckwalter JA, Jurayj MN. Relationship of tobacco, and alcohol: a case for multi-factor disease. *JAMA.* 2010;303:1336-1342.

181. Adami J, Gabel H, Lindelof B, et al. Cancer risk following organ transplantation: a critical review of a cohort study in Sweden. *Br J Cancer.* 2003;89:1221-1227.

182. Chin-Hong PV, Wittinghoff E, Cranston RD, et al. Age-related prevalence of anal cancer precursors in intrarectal and anal mucosa in a cohort study in Sweden. *Br J Cancer.* 2003;89:1221-1227.
neoplasia: possible parallel. Lancet. 1989;2:765-769.

188. Edgren G, Sparen P. Risk of anogenital cancer after diagnosis of cervical intraepithelial neoplasia: a prospective population-based study. Lancet Oncol. 2007;8:311-316.

189. Melbye M, Sprogel P. Aetiological parallel between anal cancer and cervical cancer. Lancet. 1991;338:657-659.

190. Rabbkin CS, Biggar RJ, Melbye M, Curtis RE. Second primary cancers following anal and cervical carcinoma: evidence of shared etiological factors. Am J Epidemiol. 1992;136:54-58.

191. Frisch M, Melbye M, Moller H. Trends in incidence of anal cancer in Denmark. BMJ. 1993;306:419-422.

192. Daling JR, Weiss NS, Kloppenstein LF, Cochran LE, Chew WH, DaiFuku R. Correlates of homosexual behavior and the incidence of anal cancer. JAMA. 1982; 247:1988-1990.

193. Peters RK, Mack TM. Patterns of anal carcinoma by gender and marital status in Los Angeles County. Br J Cancer. 1983;48:629-636.

194. Melbye M, Rabbkin C, Frisch M, Biggar RJ. Changing patterns of anal cancer incidence in the United States, 1940-1989. Am J Epidemiol. 1994;139:772-780.

195. Critchlow CW, Surawicz CM, Holmes KK, et al. Prospective study of high grade anal squamous intraepithelial neoplasia in a cohort of homosexual men: influence of HIV infection, immunosuppression and human papillomavirus infection. AIDS. 1995;9:1255-1262.

196. Rabbkin CS, Yellin F. Cancer incidence in a population with a high prevalence of infection with human immunodeficiency virus type 1. J Natl Cancer Inst. 1994;86:1711-1716.

197. Frisch M, Biggar RJ, Goedert JJ. Human papillomavirus-associated cancers in patients with human immunodeficiency virus infection and acquired immunodeficiency syndrome. J Natl Cancer Inst. 2000;92:1500-1510.

198. Critchlow CW, Hawes SE, Kuypers JM, et al. Effect of HIV infection on the natural history and outcome of anal human papillomavirus infection. AIDS. 1998;12:1177-1184.

199. Machalek DA, Poynten M, Jin F, et al. Anal human papillomavirus infection and anal neoplastic lesions in men who have sex with men: a systematic review and meta-analysis. Lancet Oncol. 2012;13:487-500.

200. Palefsky JM, Gonzales J, Greenblatt RM, Ake DH, Hollander H. Anal intraepithelial neoplasia and anal papillomavirus infection among homosexual males with group IV HIV infection. JAMA. 1990;263:2911-2916.

201. D’Souza G, Wiley DJ, Li X, et al. Incidence and epidemiology of anal cancer in the multicenter AIDS cohort study. J Acquir Immune Defic Syndr. 2008;48:491-499.

202. Silverberg MJ, Lau B, Justice AC, et al; North American AIDS Cohort Collaboration on Research and Design (NA-ACCORD) of IeDEA. Risk of anal cancer in HIV-infected and HIV-uninfected individuals in North America. Clin Infect Dis. 2012;54:1026-1034.

203. Bower M, Powles T, Newsom-Davis T, et al. HIV-associated anal cancer: has highly active antiretroviral therapy reduced the incidence or improved the outcome? J Acquir Immune Defic Synr. 2004;37:1563-1565.

204. CRM-Cianflone NF, Hullsiek KH, Marconi VC, et al; Infectious Disease Clinical Research Program HIV Working Group. Anal cancers among HIV-infected persons: HAART is not slowing rising incidence. AIDS. 2010;24:535-543.

205. Piketty C, Selinger-Leneman H, Grabar S, et al; FHFD-AHNS CO 4. Marked increase in the incidence of anal cancer among HIV-infected patients despite treatment with combination antiretroviral therapy. AIDS. 2008;22:1203-1211.

206. Sillman FH, Sedlis A. Anogenital papillomavirus infection and neoplasia in immunodeficient women: an update. Dermatol Clin. 1991;9:353-369.

207. Arends MJ, Buckley CH, Wells M. Aetiology, pathogenesis, and pathology of cervical neoplasia. J Clin Pathol. 1998;51:96-103.

208. Gervaz P, Hirschel B, Morel P. Molecular biology of squamous cell carcinoma of the anus. Br J Surg. 2006;93:531-538.

209. Palefsky JM. Anal human papillomavirus infection and anal cancer in HIV-positive individuals: an emerging problem. AIDS. 1994;8:283-295.

210. Handsfield HH. Clinical presentation and natural course of anogenital warts. J Med. 1997;102:16-20.

211. Joseph DA, Miller JW, Wu X, et al. Understanding the burden of human papillomavirus-associated anal cancers in the US. Cancer. 2008;113(suppl 10):2892-2900.

212. Williams GR, Lu QL, Love SB, Talbot IC, et al. The natural history of anal human papillomavirus infection. Dis Esophagus. 2004;1711-1716.

213. Sun S, Schiller JH, Gazdar AF. Lung cancer in never smokers—a different disease. Nat Rev Cancer. 2007;7:778-790.

214. Wakelee HA, Chang ET, Gomez SL, et al. Lung cancer incidence in never smokers. J Clin Oncol. 2007;25:472-478.

215. Cheng YY, Chiou HL, Sheu GT, et al. The association of human papillomavirus 16/18 infection with lung cancer among nonsmoking Taiwanese women. Cancer Res. 2001;61:2799-2803.

216. Koshiol J, Rotunno M, Gillison ML, et al. InterSCOPE Collaboration. InterSCOPE study: associations between esophageal squamous cell carcinoma and human papillomavirus serological markers. J Natl Cancer Inst. 2010;104:147-158.

217. Subramanian J, Govindan R. Lung cancer in never smokers: a review. J Clin Oncol. 2007;25:561-570.

218. Sun S, Schiller JH, Gazdar AF. Lung cancer in never smokers—a different disease. Nat Rev Cancer. 2007;7:778-790.

219. Cheng YY, Chiou HL, Sheu GT, et al. The association of human papillomavirus 16/18 infection with lung cancer among nonsmoking Taiwanese women. Cancer Res. 2001;61:2799-2803.

220. Bower M, Powles T, Newsom-Davis T, et al. HIV-associated anal cancer: has highly active antiretroviral therapy reduced the incidence or improved the outcome? J Acquir Immune Defic Synr. 2004;37:1563-1565.

221. El-Serag HB, Hollier JM, Gravitt P, Alsaaraj A, Younes M. Human papillomavirus and the risk of Barrett’s esophagus [published online ahead of print August 14, 2012]. Dis Esophagus. doi: 10.1111/j. 1442-2050.2012.01392.x.

222. Sitas F, Egger S, Urban MJ, et al; InterSCOPE Collaboration. InterSCOPE study: associations between esophageal squamous cell carcinoma and human papillomavirus serological markers. J Natl Cancer Inst. 2010;104:147-158.

223. Sun S, Schiller JH, Gazdar AF. Lung cancer in never smokers—a different disease. Nat Rev Cancer. 2007;7:778-790.

224. Wakelee HA, Chang ET, Gomez SL, et al. Lung cancer incidence in never smokers. J Clin Oncol. 2007;25:472-478.

225. Cheng YY, Chiou HL, Sheu GT, et al. The association of human papillomavirus 16/18 infection with lung cancer among nonsmoking Taiwanese women. Cancer Res. 2001;61:2799-2803.

226. Sun S, Schiller JH, Gazdar AF. Lung cancer in never smokers—a different disease. Nat Rev Cancer. 2007;7:778-790.

227. Wakelee HA, Chang ET, Gomez SL, et al. Lung cancer incidence in never smokers. J Clin Oncol. 2007;25:472-478.

228. Koshiol J, Rotunno M, Gillison ML, et al. Assessment of human papillomavirus in lung squamous cell carcinomas in Western China. J Natl Cancer Inst. 2011;103:501-507.

229. Yu Y, Yang A, Hu S, Yan H. Correlation of HPV-16/18 infection of human papillomavirus with lung squamous cell carcinoma in Western China. Oncol Rep. 2009; 21:1627-1632.

230. Yu Y, Yang A, Hu S, Zhang J, Yan H. Significance of human papillomavirus 16/18 infection in advanced squamous cell carcinoma with p53 mutation in lung carcinomas [published online ahead of print December 16, 2011]. Clin Respir J. doi: 10.1111/j. 1752-699X.2011.00277.x.

231. Klein F, Amin Korb WF, Petersen I. Incidence of human papillomavirus in lung cancer. Lung Cancer. 2009;65:13-18.

232. Cheng YY, Wu MF, Wang J, et al. Human papillomavirus 16/18 E6 oncoprotein is expressed in lung cancer and related with p53 inactivation. Cancer Res. 2007;67:10686-10693.

233. Park MS, Chang YS, Shin JH, et al. The prevalence of human papillomavirus infection in Korean non-small cell lung cancer.
236. Aguayo F, Anwar M, Koriyama C, et al. Human papillomavirus-16 presence and physical status in lung carcinomas from Asia. Infect Agent Cancer. 2010;5:20.

237. Iwakawa R, Kohn T, Enari M, Kiyono T, Yokota J. Prevalence of human papillomavirus 16/18/33 infection and p53 mutation in lung adenocarcinoma. Cancer Sci. 2010;101:1891-1896.

238. Simen-Kapeu A, Surcel HM, Koskela P, Pukkala E, Lehtinen M. Lack of association between human papillomavirus type 16 and 18 infections and female lung cancer. Cancer Epidemiol Biomarkers Prev. 2010;19:1879-1881.

239. Doughter EE, Katzenstein AL. The relationship between p16 expression and high-risk human papillomavirus infection in squamous cell carcinomas from sites other than uterine cervix: a study of 137 cases. Hum Pathol. 2012;43:327-332.

240. Syrjanen K, Silvoniemi M, Salminen E, Vasankari T, Syrjanen S. Detection of human papillomavirus genotypes in bronchial cancer using sensitive multimetric assay. Anticancer Res. 2012;32:625-631.

241. Syrjanen K. Detection of human papillomavirus in lung cancer: systematic review and meta-analysis. Anticancer Res. 2012;32:3235-3250.

242. FUTURE II Study Group. Quadrivalent vaccine against human papillomavirus to prevent high-grade cervical lesions. N Engl J Med. 2007;356:1915-1927.

243. Paavonen J, Naud P, Salmeron J, et al; HPV PATRICIA Study Group. Efficacy of human papillomavirus (HPV)-16/18 AS04-adjuvanted vaccine against cervical infection and precancer caused by oncogenic HPV types (PATRICIA): final analysis of a double-blind, randomised study in young women. Lancet. 2009;374:301-314.

244. Saslow D, Castle PE, Cox JT, et al. American Cancer Society Guideline for human papillomavirus (HPV) vaccine use to prevent cervical cancer and its precursors. CA Cancer J Clin. 2007;57:7-28.

245. Centers for Disease Control and Prevention (CDC). Recommendations on the use of quadrivalent human papillomavirus vaccine in males–Advisory Committee on Immunization Practices (ACIP), 2011. MMWR Morb Mortal Wkly Rep. 2011;60:1117-1123.

246. Kreimer AR, Gonzalez P, Katki HA, et al; CVT Vaccine Group. Proof-of-principle evaluation of the efficacy of fewer than three doses of a bivalent HPV16/18 vaccine. J Natl Cancer Inst. 2011;103:1444-1451.

247. Kreimer AR, Gonzalez P, Katki HA, et al; CVT Vaccine Group. Efficacy of a bivalent HPV 16/18 vaccine against anal HPV 16/18 infection among young women: a nested analysis within the Costa Rica Vaccine Trial. Lancet Oncol. 2011;12:862-870.

248. Centers for Disease Control and Prevention (CDC). National and state vaccination coverage among adolescents aged 13 through 17 years–United States, 2010. MMWR Morb Mortal Wkly Rep. 2011;60:3767-3772.

249. Colucci R, Hryniuk W, Savage C. HPV Vaccination Programs in Canada: Are We Hitting the Mark? Report Card on Cancer in Canada, 2008. Toronto, Ontario, Canada: Cancer Advocacy Coalition of Canada; 2008.

250. Parkin DM. The global health burden of infection-associated cancers in the year 2002. Int J Cancer. 2006;118:3030-3044.

251. Parkin DM, Bray F, Chapter 2: The burden of HPV-related cancers. Vaccine. 2006;24(suppl 3):S3/11-S3/25.