Review Article
The nonavalent vaccine: a review of high-risk HPVs and a plea to the CDC

Ariel Yusupov[1], Daniel Popovsky[1], Lyaba Mahmood[1], Andrew S Kim[1], Alex E Akman[1], Hang Yuan[2,3]

[1]Georgetown University School of Medicine, Washington, DC, USA; [2]Department of Pathology, [3]Center for Cell Reprogramming, Georgetown University Medical Center, Washington, DC, USA

Abstract: Two of the leading strategies to prevent cervical cancer are prophylactic human papillomavirus (HPV) vaccination and routine Papanicolaou (Pap) testing. However, regardless of being vaccinated with first-generation (bivalent and quadrivalent) HPV vaccines at the recommended dosing schedule, many women are still found to have low- and high-grade cervical intraepithelial lesions. Studies have shown that this is largely due to: (1) first-generation vaccines only protecting against 70% of high-risk HPV types that cause cervical cancer (HPVs 16/18) and (2) vaccinated women being more prone to infection with non-protected high-risk HPV types than unvaccinated women. Fortunately, the FDA recently approved a nonavalent vaccine that protects against 5 additional high-risk HPV types that cause 20% of cervical cancers (HPVs 31/33/45/52/58), which is the only HPV vaccine currently available in the United States. Although the Advisory Committee on Immunization Practices (ACIP) recommends the nonavalent vaccine in men and women up to the age of 45 years, it does not recommend the nonavalent vaccine in those previously vaccinated with 3 doses of bivalent or quadrivalent vaccine, deeming them "adequately vaccinated". As this population is most at risk, this review serves to provide background and argue for a change in their recommendation.

Keywords: HPV, Gardasil 9, nonavalent, second generation, vaccine, papillomavirus, CDC, ACIP, false positive, cervical cancer

Introduction

Human papillomaviruses (HPVs) are the most prevalent sexually transmitted infections and cause 99.7% of cervical cancers [1, 2]. Although over 205 HPV types have been isolated, a relatively small number of types--deemed high-risk HPVs--have been attributed to cervical cancer and subsequently found to also cause the majority of anal, vaginal, oropharyngeal, vulvar, and penile cancers [3, 4]. The discovery that preventing infection with high-risk HPV can prevent these cancers has propelled the development of HPV vaccines and a worldwide push for prophylactic immunizations and timely cervical screenings.

Of all the high-risk HPV types, HPVs 16/18 contribute the most to the development of cervical and oropharyngeal cancers, at up to 70% and 90%, respectively [5, 6]. The United States is of particular concern, as it not only has the highest proportion of HPV-induced oropharyngeal cancers (OPCs), but is also where the oropharynx has now superseded the cervix as the most prevalent site of HPV-related cancers, with the majority (95%) of HPV-positive OPCs being caused by HPV 16 [5]. Thus, two (first-generation) vaccines were developed to immunize against these two HPV types: a bivalent vaccine, Cervarix, which targets only HPV types 16 and 18 and a quadrivalent HPV vaccine, Gardasil, which targets HPVs 16/18 and additionally HPV types 6 and 11, which cause non-malignant genital condylomata (warts) and recurrent respiratory papillomatosis [7].

However, although the first-generation vaccines are effective in immunizing against their targeted HPV types, women are still exposed to the other high-risk HPVs which cause 30% of cervical cancer. Thus, over a decade after the development of the first HPV vaccine (Gardasil), the FDA approved the second-generation nonava-

lent HPV vaccine (Gardasil 9) that protects against an additional five high-risk HPVs (HPV 31/33/45/52/58) not covered by the bivalent or quadrivalent vaccines. The vaccine has been shown to be highly immunogenic, with the potential to prevent an additional 18.3-20% of cervical cancers [4, 7, 8].

The Center for Disease Control and Prevention (CDC) Advisory Committee on Immunization Practices (ACIP) has recommended that vaccination be given at age 11 or 12 for boys and girls who have not been previously vaccinated before age 26 and made specific recommendations for the vaccination of victims of sexual abuse, transgender persons, and those with primary or secondary immunocompromising conditions [9]. The ACIP, however, does not recommend nonavalent vaccination in those previously fully vaccinated with the bivalent and quadrivalent vaccines, as they consider them adequately vaccinated [9]. The omission of this group is particularly concerning because not only are they not adequately vaccinated against the other 20% of high-risk HPVs, but studies have also shown that vaccinated women have a significantly higher prevalence of infection with the high-risk HPV types not covered by the vaccine when compared to unvaccinated women; vaccinated women are more prone and predisposed than unvaccinated women to non-HPV 16/18 high-risk HPVs, such as HPVs 31/33/45/52/58 [10]. In order to have the best possible clinical outcome for this vulnerable population, it is imperative that the ACIP recommends revaccination in these individuals with the nonavalent vaccine.

Prevalence of high-risk HPVs in certain cancers

Although it has been established that high-risk HPVs contribute to virtually all cervical cancers, they also contribute to several other life-threatening diseases. In a study in which archival tissue for cancers from 2,670 case patients were tested for HPV, it was observed that 90.6% of cervical (98.8% in situ), 68.8% of vulvar (97.1% in situ), 75.0% of vaginal, 91.1% of anal, 63.3% of penile, 30-90% of oropharyngeal (depending on region), 82.0% of tonsillar, 70.0% of base of tongue, 32.0% of oral cavity, and 20.9% of laryngeal cancers were positive for HPV [4, 5].

The most common HPV type found in all the cancers studied is HPV 16, and, secondly, HPV 18; mutually, HPVs 16 and 18 are responsible for 47.9-70% of all genital cancers in both men and women [4]. Interestingly, HPV 16/18 is detected more predominantly in cervical cancer from women 35 years of age or younger at the time of diagnosis compared to women 65 years of age or older; this age trend is seen for other cancer sites as well-including vaginal, oropharyngeal, and vulvar cancers [4]. While many of the cancers discussed can be attributed to HPVs 16 and 18, HPVs 31/33/45/52/58 also play a significant role, contributing to 16.2% of cervical, 20.7% of vulvar, 24.4% of vaginal, 8.9% of anal, 14.3% of penile, and 7.7% of oropharyngeal cancers [4]. Vaccination against these HPV types has the potential to substantially compound the anti-cancer protective effects of the first-generation HPV vaccines, which highlights the importance of the nonavalent and further HPV vaccines [4].

An unexplained phenomenon

One of the most concerning issues regarding women who have been vaccinated against HPVs 16 and 18--the two most malignant HPV types--is that despite being fully vaccinated with the bivalent or quadrivalent vaccine before sexual activity, many women still present with abnormal cervical cells positive for non-HPV 16/18 high-risk HPV types [11]. This is most likely because the first-generation vaccines only protect against two high-risk HPVs, HPV types 16 and 18, with little cross-protection to other high-risk types, which cause up to an additional 30% of cervical cancers [12].

In addition, vaccinated women have a higher risk of contracting non-HPV 16/18 high-risk HPV types and are more prone to cervical cancers not covered by the first-generation vaccines than unvaccinated women [10]. One may speculate that this phenomenon is a result of vaccinated women being more likely to engage in unprotected sex--due to a feeling of protection against HPV--than unvaccinated women; however, several studies have thoroughly concluded that this is not the case [13-15]. The mechanism as to why women vaccinated against HPVs 16 and 18 have a higher chance of infection with non-HPV 16/18 high-risk HPV types has yet to be determined, but what is clear is that there is an exigent need of protection for this population of women.

One possible explanation: false positive Paps

Although the most likely reason women vaccinated against HPVs 16 and 18 are still presenting with abnormal cervical cells in the clinic is that they have been infected with a non-HPV 16/18 high-risk HPV type, one must also consider the limitations of current testing procedures. The most commonly used screening method for low- and high-grade cervical intraepithelial lesions is the Papanicolaou (Pap) smear test [16]. Although consequentially important and credited with detecting countless cervical cancers in their most early stages, the Pap smear test has also been found to register false positives [17]. It has been suggested that this due to the unique staining procedure used in the popular ThinSmear Pap test, where the Periodic Acid Schiff stain causes cells to mimic the abnormal lesions of high-risk HPV, while not actually being caused by a high-risk HPV. In addition, increased levels of progesterone/estrogen, such as from use of oral contraceptives, induce glycogen production which cause cervical cell samples to appear like those of low-grade intraepithelial lesions seen with high-risk HPV, which can lead to a misdiagnosis of Atypical Squamous Cells of Undetermined Significance (ASCUS) [17]. This morphological change can affect the ability of clinicians to properly identify true HPV-induced lesions and neoplasms. To prevent future misdiagnoses, a more reliable screening method, such as liquid based monolayer cytology, may be implemented [18]. It is imperative for physicians to properly make a distinction between false positive ASCUS and a true cervical lesion. Nonetheless, false positives only account for a small percentage of abnormal results, and the most likely reason for the increase in abnormal Pap tests in vaccinated women remains to be due to inadequate coverage of first-generation vaccines [11].

Background on HPV, genetics, and phylogeny

In order to understand the effect of an HPV vaccine and its potential cross protection against other HPV serotypes, it is vital to understand the structure and relationships between HPV types. Human papillomaviruses (HPVs) are non-enveloped double-stranded circular DNA viruses whose genome can be functionally separated into two segments: an Early (E) segment, which makes up about two-thirds of the genome and activates immediately upon host infection, and a Late (L) segment that comprises the last third of the genome and activates during the "late" phase [19]. The E segment contains seven functional open reading frames (ORFs), designated E1 to E8, which code for host transfection, replication, and regulation--with the exception of E3, which is absent or dysfunctional in all papillomaviruses; the L segment has two ORFs, L1 and L2, which code for the major and minor viral capsid proteins, respectively [3, 19].

The major capsid coding L1 segment is highly conserved and has been used to create two phylogenetic trees based on its sequence homology between HPVs--of which a >10% difference distinguishes a unique HPV type and a >60% sequence identity classifies an HPV supergroup--and has led to the typing of over 205 HPVs and division into 5 supergroups, or, more recently, genera [3, 20-23]. The A supergroup (or Alphapapillomavirus genus) comprises virtually all of the HPVs infecting the genital tract, with those of the A9 (HPVs 16/31/33/35/52/58/67) and A7 (HPVs 18/39/45/59/68/70) species causing at least 89.5% of cervical cancers [3, 12, 20]. Using a Bayesian algorithm and the L1 sequences of 189 HPVs, a phylogenetic tree was generated and has shown that the HPVs most closely related to HPV 16 are HPVs 31/35, followed by HPV 52, and then HPVs 33/58; the same tree has shown that HPV 45 is closest to HPV 18 [22].

Interestingly, when the potent E6 oncogene of 28 HPVs is aligned and analyzed instead of L1 in the construction of a phylogenetic tree, the mucosal high-risk HPVs all fall into one closely related subgroup, with two branches: one containing HPVs 16, 31, 33, 35, 51, 52, 56 and 58 (HPVs 31 and 35 closer to HPV 16 than HPVs 33/52/58) and the second containing HPVs 18, 39, 45 and 68 (HPV 45 much closer to HPV 18 than HPVs 39 and 68), which correspond to the A9 and A7 species of the Alphapapillomavirus genus, respectively [12, 20, 24]. These members of the A9 and A7 species that share high E6 sequence homology cause 71.9% and 17.4% of cervical cancers, respectively, with HPV 16 and HPV 18 each alone contributing 58.7% and 12.2%, respectively [12, 24]. This in itself suggests that E6 plays a critical role in development of high-risk mucosal lesions.

Due to the unavailability of specific E6 gene sequence homology data in the literature, the E6 gene sequences of A9 and A7 HPVs were acquired from the PapillomaVirus Episteme (PaVE) database and aligned using the Nucleotide Basic Local Alignment Search Tool (nucleotide BLAST®). The BLAST results have shown that HPVs 31, 33, 35, 52, and 58 share at least 70.5% E6 gene sequence homology to HPV 16, whereas HPV 45 shares 83.6% E6 gene sequence homology to HPV 18 (HPVs 39 and 68 each share 73.1% and 73.71%, respectively).

When the amino acid sequence of the L1 segment is evaluated, HPVs 31, 33, 35, 52, and 58, which together cause 11.9% of cervical cancers, share at least 80% sequence homology to HPV 16, whereas HPVs 45, which causes 4.7% of cervical cancers, shares 88% sequence homology to HPV 18 [12].

Before this review, most studies have focused on L1 sequence homology between HPV types. Although such information is critical when designing an effective HPV vaccine and predicting potential cross protection, the evaluation of E6 gene sequence homology may provide insight as to why certain HPV strains may have high L1 sequence homology, yet not be as highly pathogenic. Our BLAST analysis of the A9 and A7 HPVs suggest that E6 sequence analysis and homology to high-risk HPV types (such as HPV 16/18) may be a good screening tool for other potential high-risk HPVs.

Mechanism of HPV infection

One of the most interesting qualities of HPV is that a certain HPV type has preferential binding to cutaneous or mucosal tissues, which has great implications for whether an HPV type is high- or low-risk. In addition, understanding of the mechanism of HPV infection can provide future insights into cervical cancer treatment and prevention, such as E1/E2 or E6/E7 protein inhibitors [25].

The L1 and L2 capsid proteins are both essential for the binding and entry of HPV into target cells [26]. L1 regions of the capsid will bind to certain heparan sulfate proteoglycan sequences (HSPGs) and induce a conformational change in the capsid which exposes the N-termini of L2 proteins to extracellular enzymes [26].

Subsequent cleavage of these termini is believed to expose binding sites for endocytic receptors, although the specific pathway for internalization and intracellular trafficking is still contested [26]. After 8-12 hours of the initial binding, viral DNA complexes with L2 proteins, leaves the endosome, and enters the nucleus, where it localizes to either ND10 bodies or promyelocytic leukemia (PML) oncogenic domains and undergoes transcription [26]. The whole process of infection is quite slow, requiring 12-24 hours for the onset of transcription, and may help to explain the efficacy of vaccines, as the viral capsid is left exposed to antibodies for quite some time [26]. Interestingly, although HSPGs are widespread throughout the body, HPVs exclusively infect and replicate inside basal cells of stratified epithelium [26]. Furthermore, the L1 proteins are not specific for these basal cells but rather the underlying basement membrane (BM), which means that infection is only possible when the BM is exposed, normally either through physical or chemical trauma [26].

Different types of HPV will display different L1 proteins, which in turn determine their primary points of infection as different epithelia will display differences in both HSPG concentration and patterns [26, 27]. Notably, at physiological pH (7.4), the L1 capsid protein of mucosal HPV types is positively charged and that of cutaneous HPV types is negatively charged [27]. Since heparan sulfate (HS) is highly negatively charged and much more prominent in mucosal than cutaneous tissues, investigators have suggested that the binding of HPVs to target tissues is charge-dependent [28]. The positively charged L1 proteins of mucosal types are more apt to target and bind to HS-coated mucosal than HS-depleted cutaneous tissues; however, HPV tropism requires further study, as secondary receptors are too likely involved [27].

The oncogenic effects of high-risk HPVs are attributed largely to oncoproteins E6 and E7, both of which have multiple mechanisms through which they exhibit their oncogenic behavior [29]. While expression of E6/E7 is initially regulated by E2, a transcriptional regulator in the HPV genome, expression of E2 halts as the virus life cycle progresses, allowing for continuous, unregulated expression of E6/E7 [29]. The E7 protein has been shown to either inactivate

or inhibit retinoblastoma tumor suppressor protein (pRB), pocket proteins p107 and p130, and CDK inhibitors p21 and p27 resulting in subsequent deregulation of cell growth and cell cycle transitions [29]. While inactivation of these factors would normally trigger a cell death response, HPV E6 complexes with cellular E6-AP protein to induce degradation of p53, a key regulator of the apoptotic pathway [29]. HPV E6 has also been shown to upregulate expression of telomerase, which further adds to the longevity of infected cells [29].

First-generation HPV vaccines

The first-generation HPV vaccines, which have been in circulation for some time, consist of the bivalent (Cervarix) and quadrivalent (Gardasil) vaccines [7]. In a study (Papilloma TRIal against Cancer In young Adults, or PATRICIA) of young women aged 15-25 years--9,319 randomized participants who received at least one vaccine dose (Total Vaccinated Cohort, or TVC) and 5,822 who tested negative for high-risk HPV (TVC-naïve)--the efficacy of the bivalent vaccine against cervical intraepithelial lesions (CIN), graded CIN1-CIN3, was investigated [30]. In the TVC group, vaccine efficacy against CIN1-CIN3 lesions that were HPV 16/18-related was 33.6-55%; protection against CIN1-CIN3 lesions impartial to specific HPV was 21.7-33.4% [30]. In the TVC-naïve group, vaccine efficacy against CIN1-CIN3 lesions that were HPV 16/18-related was 96.5-100% and significantly lower for CIN1-CIN3 lesions impartial to specific HPV [30, 31]. This can be because the bivalent vaccine protects against 1-year persistent infection with non-HPV 16/18 high-risk HPV types only 49.0% [30].

Although both first-generation vaccines contain an aluminum-based adjuvant, one of the interesting differences between them is that the bivalent vaccine also contains monophosphoryl lipid A (part of its ASO4 formulation), which augments the immune response via activation of Toll-like receptor 4 [32-35]. Although this modification has resulted in slightly higher antibody titers in the bivalent vs quadrivalent vaccines, the differences in the clinic are insignificant, as both protect against HPV 16/18-related neoplasia with greater than 90% efficacy [35].

In addition to protecting against HPV 16/18, which cause 70% of cervical cancers, the quadrivalent vaccine protects against HPVs 6 and 11, which cause 90% of genital warts and 95% of recurrent respiratory papillomatosis [7]. In a combined analysis of the FUTURE I and FUTURE II trials, vaccine efficacy of the quadrivalent vaccine against low-(CIN1) and high-grade (CIN2-CIN3/AIS [adenocarcinoma in situ]) lesions was investigated in 17,622 and 20,583 women, respectively, aged 16-26 years [36, 37]. After 3 year follow up, vaccine efficacy against HPV 16/18-related CIN2-3/AIS in women who were negative for HPV 16/18 at the time of vaccination was 98-100%; vaccine efficacy against CIN2-CIN3/AIS in women, regardless of previous exposure or HPV type, was 18% [37]. The quadrivalent vaccine is 96% effective in preventing HPV 16/18-related CIN1 and 30% effective in preventing CIN1 irrespective of HPV type in those who were HPV 16/18-negative at the time of vaccination [36]. As mentioned previously, this is likely because the bivalent and quadrivalent vaccines only protect against HPV 16/18, which cause 66.2%% of invasive cervical cancers, with poor cross protection to other high-risk HPV types [4].

Cross protection of first-generation vaccines

Since the most popular HPV vaccines (bivalent and quadrivalent) are virus-like particles (VLPs) that mimic the major capsid proteins of HPVs 16 and 18, due to the sequence similarity of their L1 segment to other high-risk HPVs, it has been hypothesized that the vaccines will offer cross protection to closely related high-risk HPVs not covered by the vaccines [12]. For practicality, the study focused on the cross protection of those HPVs that show at least 80% L1 amino acid sequence homology to HPVs 16 and 18 and at least 2% contribution to cervical cancer, which refined to five HPVs: 31, 33, 45, 52, and 58 [12]. The indication of cross protection was established based on the reduction in CIN1-CIN3/AIS lesions and incidence of HPV infection in 17,622 women who tested negative for all mucosal high-risk HPV types on day 1 of vaccination with quadrivalent vaccine [12].

After 1 dose and a mean follow up of 3.6 years in 2068 patients, quadrivalent vaccination reduced the incidence of HPV 31/33/45/52/58

infection by 25.0%, with the highest reductions (46.2% and 28.7%, respectively) observed for HPVs 31 and 33 [12]. When HPV 31 was removed from composite end points using post-hoc analysis, reduction of HPV infection for HPV 33/45/52/58 was only 14.9% [12].

In the analyses of reduction of cervical intraepithelial lesions in 9296 patients over a course of 3.6 years, quadrivalent vaccination reduced the incidence of CIN1-CIN3/AIS caused by HPVs 31/33/45/52/58 by 29.2%; when detection of HPV DNA was restricted to cervix and anal swab samples to limit bias, the reduced incidence of HPV 31/33/45/52/58-related CIN1-CIN3/AIS was 23.1% [12]. They noted that cross protection was most robust in the A9 species (HPVs 31/33/35/52/58), with a combined reduction of CIN1-CIN3/AIS by 31.9%, whereas that for the A7 species was not statistically significant [12].

An identical study focused rather on women who were sexually active, than generally HPV-naïve, and did not exclude those already infected with HPV or HPV-related disease [38]. The efficacy of cross protection was moderately inferior to the HPV-naïve population and found that quadrivalent vaccination reduced the incidence of HPV 31/33/45/52/58 infection by 17.7% and CIN1-CIN3/AIS by 18.8% [38]. In addition, they observed that the reduction in CIN2-CIN3/AIS (higher grade lesions) was not statistically significant; further, in placebo studies investigating coinfection with homologous HPV types, they observed that cross protection was not additive [38]. Thus, in both HPV-naïve and sexually active women who are vaccinated against HPVs 16 and 18, although HPV 16/18 major capsid proteins share greater than 80% sequence homology to HPVs 31/33/45/52/58, cross protection against these HPV types is poor. Furthermore, since HPVs 31, 33, 45, 52, and 58 contribute to 15.2-20% of cervical cancers, it is imperative that both HPV-naïve and sexually active women are vaccinated against these five (and hopefully more) HPV types, regardless of being fully protected against HPVs 16 and 18.

The second-generation HPV vaccine

As mentioned previously, the first-generation vaccines are not adequately immunoprotective due to their protection against only two HPV types and, although conceptually one may assume cross protection to other high-risk HPV types due to their high L1 sequence homology to HPVs 16/18, that is simply not the case. Fortunately, a new nonavalent vaccine, which protects against an addition five non-HPV 16/18 high-risk HPVs that contribute the most to cervical cancer has been recently approved by the FDA [7].

When the nonavalent vaccine (9vHPV) was compared to the quadrivalent (4vHPV) vaccine, the 9vHPV vaccine was 96.3% more effective in reducing the incidence of CIN2-3/AIS due to HPV 31/33/45/52/58 and 95.7% more effective in reducing the incidence of persistent infection of HPV 31/33/35/52/58 [39]. In addition, nearly 100% of participants were seropositive to all 9HPVs in the administered nonavalent vaccine and had equivalent Geometric Mean Titers (GMT, which represents the average antibody titer for a study group) for HPVs 6/11/16/18 to those administered the quadrivalent vaccine [39]. It is also important to mention the limitations of this study: there was no placebo control, low-grade lesions were not investigated, and neither was 9vHPV protection against HPV 16/18-related cervical intraepithelial lesions [39]. A study that remedied some of these limitations investigated the potential impact of the nonavalent vaccine on low- and high-grade cervical intraepithelial lesions caused by HPVs 16/18/31/33/45/52/58 and found that the nonavalent vaccine has a greater than 80% increased impact on low-grade CIN and 50% for high-grade lesions when compared to the bivalent or quadrivalent vaccines [8]. They confirmed that the switch from bivalent and quadrivalent to nonavalent vaccine would increase the prevention of cervical cancers from 66.2% to up to 90% [4, 8]. In addition, long-term cohort studies have shown that the quadrivalent vaccine does not show evidence of waning immunity and its effectiveness remains above 90% for at least 10 years, which suggests the nonavalent vaccine will also provide long-term immunity [39, 40].

Safety profile of HPV vaccines

In exploring the efficacies of HPV vaccines, assessing the side effects and comparing the safety profiles associated with them is imperative. Injection-site tenderness is the most com-

mon local reaction, observed in 93.8% of participants who receive the bivalent HPV vaccine and 86.3% of participants who receive the quadrivalent HPV vaccine [41]. However, there is a significant difference in the severe tenderness caused by the bivalent and quadrivalent HPV vaccines, observed in 24% of individuals who received the bivalent HPV vaccine and 6.9% of individuals who received the quadrivalent HPV vaccine [41]. Though both groups experience fatigue in similar proportions for the first two doses of their respective vaccine, 11.5% of individuals who receive the bivalent HPV vaccine and 3% of individuals who receive the quadrivalent vaccine report moderate fatigue at dose 3 [41]. Evidence suggests that the relative increase of the side effects experienced with bivalent HPV vaccines is due to its adjuvant [41].

The nonavalent HPV vaccine also exhibits a greater likelihood to induce adverse effects related to the injection site such as pain, swelling, and pruritus when compared to the quadrivalent HPV vaccine [39]. Of the women who receive the nonavalent HPV vaccine, 90.7% experience adverse effects related to the injection site in contrast to 84.9% of the women who receive the quadrivalent HPV vaccine [39]. However, this is anticipated because nonavalent HPV vaccines have a greater amount of HPV virus-like particle antigens and aluminum hydroxyphosphate sulfate (AAHS), which is often utilized in vaccines to potentiate an immune response to an antigen [39]. Both the quadrivalent and nonavalent HPV vaccines demonstrate similar frequencies of systemic adverse events, such as headache, pyrexia, fatigue and nausea: 55.8% of women who receive the nonavalent HPV vaccine experience systemic adverse events versus 54.9% of women who receive the quadrivalent HPV vaccine [39]. The nonavalent vaccine is thus very promising as it possesses the potential to increase the prevention of cervical cancer from approximately 70% to 90% while maintaining a safety profile similar to that of the quadrivalent HPV vaccine [39].

Gardasil 9 in those previously vaccinated

As of late 2016, the nonavalent vaccine (9vHPV) is the only available vaccine in the United States [9]. The ACIP currently recommends routine vaccination at 11 or 12 years of age and, for those not yet vaccinated with three doses at the recommended dosing schedule of any of the three HPV vaccines, recommends catching up with the nonavalent vaccine up to 45 years of age [9, 42]. Although the bivalent (2vHPV) and quadrivalent (4vHPV) vaccines only cover HPVs 16 and 18, which cause less than 70% of cervical cancers, the ACIP considers those who have been vaccinated with 2vHPV and 4vHPV on an appropriate dosing schedule "adequately vaccinated" and does not recommend revaccination with 9vHPV, regardless of the fact that they are not protected against HPVs 31/33/45/52/58, which cause 20% of cervical cancers [8, 9, 12]. In addition, when highlighting the fact that cross protection of the 2vHPV and 4vHPV vaccines is poor against non-HPV 16/18 HPV types and also the finding that women who were previously vaccinated with first-generation vaccines are more prone to infection with non-HPV 16/18 high-risk HPVs than unvaccinated women, it is clear that the population previously vaccinated with 2vHPV or 4vHPV is in fact not adequately vaccinated [10, 12].

Some have suggested that this recommendation is due, in part, to the geometric mean antibody titers (GMTs) of HPVs 31/33/45/52/58 in 9vHPV recipients who have been previously vaccinated with 4vHPV being lower than in subjects who have never been vaccinated [7, 43, 44]. They concluded, however, that these lower GMTs are not likely due to immune interference by antibodies from previous immunizations with 4vHPV, because GMTs were high for HPVs 6/11/16/18 [7].

The study (NCT01047345), which investigated the effect of the nonavalent vaccine in women aged 12-26 years who previously received 3-doses of 4vHPV at least one year prior, had a primary objective to assess safety and a secondary objective show that 9vHPV is immunogenic to HPVs 31/33/45/52/58 [43]. Both objectives were met: the nonavalent vaccine was safe and 98% of subjects who received 9vHPV were seropositive for HPV types 31/33/45/52/58 after 3 doses [43]. In addition, serum analysis was conducted using competitive Luminex Immunoassay (cLIA) and showed geometric mean antibody titers (GMTs) of HPVs 31, 33, 45, 52, and 58 were 260.0 milli-Merck Units/mL (mMU/mL), 175.2 mMU/mL, 97.4 mMU/mL, 264.1 mMU/mL, and 269.6 mMU/

mL, respectively, and for HPVs 6, 11, 16, and 18 were 2207.4 mMU/mL, 2077.4 mMU/mL, 1824.0 mMU/mL, and 11,192.8 mMU/mL, respectively, at month 7 [43]. The disparity between GMTs of HPVs 6/11/16/18 and HPVs 31/33/45/52/58 is expected in this population, because the 9vHPV is eliciting a memory response to HPVs 6/11/16/18, which is much stronger than primary responses [43]. This is further evidenced by the fact that those who were seropositive at baseline to HPV 31, 33, 45, 52, or 58 had a more pronounced anti-HPV response (higher generated GMTs) to that particular HPV type, similar to that of unvaccinated individuals [43]; the same observation has been directly observed in those seropositive for HPVs 6/11/16/18 prior to 4vHPV vaccination in a different study [45].

It is also important to note one of the most critical limitations in the study (NCT01047345): the women were vaccinated with all three doses within a 6 month period [43]. Previous studies have shown that immunogenicity of women given 3 doses of the nonavalent vaccine in a 6 month period even in HPV-naïve women results in incredibly low antibody titers [46]. When the nonavalent vaccination schedule is shifted from three doses at months 0, 2, and 6 to two doses at months 0 and 12, the GMTs increase by 3.47-/5.07-/4.54-/3.69-/3.7-/6.31-/1.96-/3.08-/4.98-fold for HPVs 6/11/16/18/31/33/45/52/58, respectively [46]. Thus, it is almost certain that have the investigators modified their nonavalent revaccination study in women previously vaccinated with 4vHPV and increased the time period between vaccinations with 9vHPV, GMTs for all 9 HPV types would be significantly higher.

At first glance, however, to someone unfamiliar with the limitations in the study and what constitutes normal or protective titer values, it may appear that the antibody response to HPVs 31/33/45/52/58 is relatively poor compared to HPVs 6/11/16/18 in previously vaccinated individuals and they may suggest there is no point in vaccinating these individuals with 9vHPV. However, the literature, two other clinical trials, and close investigation of the results suggest otherwise [34, 35, 43, 44, 47].

When comparing the GMTs of day 1 (prior to first dose) to month 7 (one month post-dose 3) in the above study, 9vHPV vaccination resulted in a 6.3-, 7.2-, 10.5-, 14.8-fold increase in GMTs for HPVs 6, 11, 16, and 18, respectively, and a 63.4-, 43.8-, 32.5-, 88.0-, and 67-fold increase in GMTs for HPVs 31, 33, 45, 52, and 58, respectively, which confirms that revaccination with 3 doses of 9vHPV in those previously vaccinated with 3 doses of 4vHPV at least one year prior is highly immunogenetic [43]. In addition, the GMTs of HPV 31, 33, 45, 52, and 58 are comparable to unvaccinated women between the ages of 16-26, which was investigated in two other clinical trials: in study 7 (NCT01651949), GMTs were 570.1 mMU/mL, 322.0 mMU/mL, 185.7 mMU/mL, 335.2 mMU/mL, and 409.2 mMU/mL, respectively, and in study 2 (NCT00943722), GMTs were 753.9 mMU/mL, 466.8 mMU/mL, 272.2 mMU/mL, 419.6 mMU/mL, and 590.5 mMU/mL, respectively [44].

An alternative explanation to the relatively lower titers for HPVs 31/33/45/52/58 compared to HPVs 6/11/16/18 in previously vaccinated individuals is the theory of Original Antigenic Sin [48]. The theory states that if an individual has been previously exposed to and developed immune memory to a certain antigen, subsequent exposures to a new, related, but slightly different antigen will result in a preferential memory response to the original antigen, rather than mount a strong primary and secondary response to the new epitope [35, 48]. According to the theory, the nonavalent vaccine may result in a preferential activation of a memory response to HPVs 6/11/16/18 and blunt a primary response to the closely related HPV 31/33/45/52/58 L1 proteins. However, this is very likely not the case, as 9vHPV vaccination results in a 32.5 to 88-fold increase in GMTs for HPV 31/33/45/52/58 and only a 6.3 to 14.8-fold increase in GMTs for HPVs 6/11/16/18 in those previously vaccinated with 4vHPV, which heavily contradicts the theory's assumption of preferential activation of HPVs 6/11/16/18. As mentioned previously, the relatively higher titers for HPVs 6/11/16/18 are likely only due to a memory response and a primary and secondary response to HPVs 31/33/45/52/58 is highly evident [43].

It is important to stress that lower immunogenicity cannot be interpreted as lower protective efficacy against HPV 31/33/45/52/58-related cervical cancers, because there exists no esta-

blished GMT value that defines a minimum needed for protection; any vaccine-induced serum antibodies may represent levels that are above the threshold for protection [35, 43]. Thus, given that there are no contraindications of administration of the nonavalent vaccine to prior recipients of the quadrivalent vaccines and the GMTs for HPVs 31/33/45/52/58 are several-fold higher than that induced during natural infection, many have recommended revaccination with the nonavalent vaccine in those previously vaccinated with any first-generation vaccine [35, 43, 47].

Closing argument

If lower GMT values are of concern to the ACIP/ CDC, it is confusing as to why they extended their recommendation for the nonavalent vaccine in men and women up to the age of 45 [42]. Studies have shown that the Geometric Mean Titers (GMTs) of HPV-naïve individuals vaccinated with quadrivalent vaccine is inversely proportional to age, with GMTs in the low hundreds (mMU/mL) after the age of 23 [45]. The study actually omitted the GMT data points for the studied 24-26 age group, because of trivial values [45]. This steep decline in GMT is also observed in study 2 (NCT009437-22), which compared GMT at month 7 of the 9-15 age group to the 16-26 age group who were naïve to the studied HPV types prior to receiving the 3 doses of 9vHPV [44, 49]. The decline in GMTs was 47.1%, 45.3%, 49.2%, 61.7%, 60.2%, 52.6%, 61.5%, 56.4%, and 54.2% for HPVs 6, 11, 16, 18, 31, 33, 45, 52, and 58, respectively, from the 9-15 age group to the 16-26 age group [44, 49]. This finding confirms that, like the quadrivalent vaccine, the GMT at 7 months of the nonavalent vaccine is also inversely proportional to age and suggests that GMTs will decline to low hundred levels past the age of 24-26 [45]. In addition, 11 other studies, performed in 6 different countries, have all found that vaccine efficacy lowers with increasing age, 7 of which have found no statistically significant effect in the oldest studied age group--two between 23-27 years [42]. Thus, it is logically inconsistent for the ACIP to extend the age range to 26-45 years--who the aforementioned literature suggests almost certainly will have lower GMTs (even if HPV-naïve at time of vaccination)--yet not recommend 9vHPV vaccination to those already vaccinated with 4vHPV due to lower immunogenicity, which stu-

dies have not only shown the GMTs are comparable, if not higher, to unvaccinated individuals aged 16-26, but also the investigators (and further literature) insist is sufficient for adequate protection against HPVs 31/33/45/52/58 [43, 47].

If the argument against recommendation of the nonavalent vaccine to those already fully vaccinated is not due to lower immunogenicity against HPVs 31/33/45/52/58, but instead that those already fully vaccinated are more likely to be exposed to those HPV types, eliminating their prophylactic effect, the same argument applies for those aged 27-45, which have been shown to have higher prevalence of HPV infection at time of vaccination [42]. Thus, this argument would also be logically inconsistent.

A substantial amount of literature supports revaccination with the nonavalent vaccine in women previously vaccinated with first generation vaccines [35, 43, 47]. The only literature found that objects to such a practice is a review [50], which has made several erroneous claims without scientific support (such as that "Cervarix remains equivalent to Gardasil9 in the prevention of HPV infections and precancers of any HPV type"), is heavily biased towards Cervarix (which is a concern, given the author has been given grants by the manufacturer, yet excludes any disclaimers in their review), and is inaccurate in its analysis of immunogenicity and benefits of nonavalent revaccination. The same author has previously made unsubstantiated claims that Gardasil only has protection that lasts five years (it does not--the vaccine is effective up to 9+ years following vaccination [51]) and argued against HPV vaccination, because "ninety-five percent of women who are infected with HPV never, ever get cervical cancer" and should get Pap smears instead of the vaccine [52]. Such language undermines the fact that cervical cancer is the second-leading cause of cancer deaths in women worldwide and dismisses the substantial amount of other cancers that result from high-risk HPVs, all of which has been shown to be effectively prevented with HPV vaccination [4, 5]. It also overestimates the accuracy of Pap smears, which we have investigated in this review, as well as ignores the fact that, even with early detection, cervical cancer treatment is gruesome and results in infertility in many women [53].

A study that the ACIP has cited in its guidance for nonavalent revaccination--and likely influenced their lack of recommendation--investigated cost-effectiveness of administration of 3 doses of 9vHPV in women previously vaccinated 4vHPV; they concluded that the average cost per quality-adjusted life-year (QALY) gained by 9vHPV revaccination exceeded $100,000 in both studied models [54]. However, their model is significantly flawed in that it ignores several key variables: 1. additional 9vHPV vaccination in previous 4vHPV recipients would likely prolong duration of protection against HPVs 6/11/16/18; 2. Those previously vaccinated with first-generation vaccines have been shown to be more prone to non-HPV 16/18 high-risk HPV types and are in more need of protection against those additional HPV types (and 9vHPV vaccination) than HPV-naïve individuals; 3. A 2-dose 9vHPV schedule has been shown to be non-inferior to 3-dose schedule [55]. There are several other limitations to the study, which is why they admit their "findings do not allow for firm conclusions as to whether additional 9vHPV vaccination can be considered cost-effective by conventional standards" [54]. Incorporation of the aforementioned variables will undoubtedly increase QALY and significantly lower cost-per-QALY, likely markedly below $50,000.

When considering nonavalent revaccination of quadrivalent vaccinated individuals, one must remember the NCT01047345 study's limitations and that the nonavalent vaccine remains the only FDA approved vaccine that protects against the 5 high-risk HPV types that cause 20% of cervical cancers [7]. One modification to alleviate concerns is shifting to a 2-dose (0 and 12 months) vaccination regiment, which will not only vastly increase immunogenicity to all 9 HPV types when compared to a 6 month 3-dose regiment, but will also increase cost effectiveness [46]. Furthermore, when considering the finding that those who were previously vaccinated are more prone to infection with the other non-covered high-risk HPV types and the fact that cross protection of HPVs 16/18 to related high-risk HPVs is poor, we believe it is imperative that the ACIP and other professional societies recommend revaccination with the nonavalent vaccine to those who have previously received the bivalent or quadrivalent HPV vaccines.

Disclosure of conflict of interest

None.

Address correspondence to: Ariel Yusupov, Georgetown University School of Medicine, Washington, DC, USA. Tel: 347-323-8021; E-mail: ay428@georgetown.edu

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